# CHAPTER 1

# Olfactory system in mammals: structural and functional anatomy

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## 1.1 Introduction

The survival and reproductive success of living organisms, including human beings, depends on the detection of sensory stimuli. Living organisms do not eat or reproduce with whatever is available; instead, they show considerable selectivity by taking advantage of their chemical and physical senses. In this regard, the sense of smell and its capacity to detect myriad of odorant molecules is of critical importance for humans and most animal species. This sense significantly contributes to the identification of food and assessment of its palatability, as well as to the detection of chemical compounds carrying specific information concerning dangers, social interactions and reproductive behaviours. In mammals, these diverse roles are accomplished by a complex olfactory system. The primary tissue responsible for sensing volatile odorants is the olfactory epithelium (OE) which is localized in the nasal cavity. Sensory neurons residing in the OE convey olfactory information to the olfactory bulb (OB) which, in turn, transfers this information towards multiple higher cortical regions collectively referred to as the olfactory cortex. Other olfactory subsystems such as the vomeronasal organ coexist with the main OE in many species. These subsystems are separate entities that are dedicated to distinct functional roles.

The principal aim of this review is to gather the results of very recent as well as major studies on the processing of olfactory information by the olfactory system and to highlight its plasticity. We first describe the physiology of the main OE and the molecular mechanisms of odorant detection. We then show how endogenous and exogenous factors may induce different forms of plasticity of the OE. We also outline the main features of other olfactory subsystems. Next, we examine how the olfactory signal generated at the peripheral level is transformed at the first processing center in the brain, the OB. Finally, we provide an overview of the

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higher olfactory pathways involved in the processing of olfactory information and we consider the pathways that shape odour perception.

## 1.2 Organization and function of the peripheral olfactory system

### 1.2.1 Physiology of the peripheral olfactory system

Stimulation of the olfactory system begins when odorant molecules are detected by the olfactory neuroepithelium located in the upper part of the nasal cavity. The odorant molecules can reach the epithelium by two pathways: *via* the nose (orthonasal olfaction) and *via* the mouth (retronasal olfaction). Odorants perceived by the orthonasal pathway originate from the external world whereas odorants perceived retronasally emanate from food or drink (aroma compounds) (see Chapter 13 for more details on these pathways).

The nose and the nasal cavity are separated into two halves along the midline by a cartilaginous structure called the nasal septum. The lateral wall of each nasal cavity is typically shaped by three bony protuberances termed the inferior, middle and superior turbinates. Animals can have more turbinates, for example, the rat has four. The turbinates and the septum are covered with an epithelium. Depending on its location, this epithelium is either nonsensory (respiratory) or sensory (olfactory). The nonsensory respiratory portion of the nasal cavity warms, cleans and humidifies the inspired air.

There is widespread acknowledgement that the human OE is located in the superior region of the nasal cavity, predominantly on the dorsal side of the nasal vault, the septum, and the superior turbinate. However, recent studies have reported a more extending distribution of OE on the middle turbinate (Escada et al. 2009). Actually, the location of the OE is variable among people. Besides, its organization is thought to change over time: ageing induces conversion to or ingrowth of respiratory epithelium and loss of olfactory neurons (Nakashima et al. 1991). Environmental compounds or pathophysiological processes such as infection or inflammation can also modify the distribution of OE. The OE in the adult has therefore a non-contiguous and patchy distribution. Globally, the human olfactory region covers between 1 and 2 $cm^2$ in each cavity (Moran et al. 1982). This area is modest relative to those of other vertebrates such as rodents and dogs (Gross et al. 1982, Harkema 1991).

In the superior part of the nasal cavity, a horizontal bone, called the cribriform plate of the ethmoid, separates the OE from the brain. The cribriform plate is a highly perforated bone: the perforations provide access for the olfactory nerve bundles to the OB. This is the only site in the body where the central nervous system is in direct contact with the outer surface. The nerves serving the olfactory region are called the first cranial nerves or the olfactory nerves. They concentrate

multiple axons of olfactory neurons located in the lamina propria. These axons convey the nerve impulse generated by the odorant detection into the OB.

### 1.2.2 Structure of the olfactory epithelium

The human OE has a structure similar to that of other vertebrates (Morrison and Costanzo 1992). It is a pseudo-stratified columnar epithelium that lies on a dense connective tissue, the lamina propria. Together, the OE and the lamina propria form the olfactory mucosa (OM). The human OE is about 60 μm in height and has a slight yellow-brownish colour. It is composed of several distinct cell types, notably olfactory sensory neurons (OSNs), sustentacular cells (a type of nonsensory supporting cells), microvillar cells, two types of stem cells (horizontal basal cell and globose basal cell) as well as Bowman's glands and duct cells (Figure 1.1B).

Vertebrate OSNs are slender and bipolar neurons spread in the epithelium with a density of $10^6$-$10^7$ per $cm^2$. Their cell bodies are generally located within the lower two thirds of the neuroepithelium. At the apical surface of the epithelium, about 10-25 cilia protrude from the OSN dendrites (Morrison and Costanzo 1992). These olfactory cilia float in the mucus which covers the epithelial surface and their plasma membrane contains the olfactory receptors (ORs). On the opposite side, the axons of OSNs penetrate through the basement membrane into the lamina propria where they are ensheathed by the olfactory ensheathing cells (OECs) (Figure 1.1B). OSNs and OECs together with fibroblasts form the olfactory nerve bundles. Serous glands called olfactory glands or Bowman's glands, bundles of the accessory olfactory nerve (surrounded by accessory OECs), as well as trigeminal nerve bundles (surrounded by Schwann cells) are also located within the lamina propria. The olfactory nerve bundles project through the cribriform plate towards the OB where the OSNs' axons synapse with mitral/tufted cells and interneurons (Figure 1.1A).

Stem cells divide to give rise to sustentacular cells and immature OSNs which mature and migrate apically. In rodent OE, there are two kinds of stem cells: horizontal basal cells and globose basal cells (GBCs). These two types are morphologically and functionally distinct. In humans, however, only one basal cell type has been reported. These human basal cells morphologically resemble the GBCs in the rat (Hahn et al. 2005).

Additional cell types, called microvillar cells, have also been described in the olfactory neuroepithelium of vertebrates. These cells, which are located near the epithelial surface, are flask shaped and have an apical tuft of microvilli extending into the nasal cavity. They provide trophic factors such as neuropeptide Y (NPY) to the OE under the control of odorant or trigeminal nerve stimulation, or both (Montani et al. 2006). Microvillar cells might therefore play a role in the regulation of cellular homeostasis in the OE.

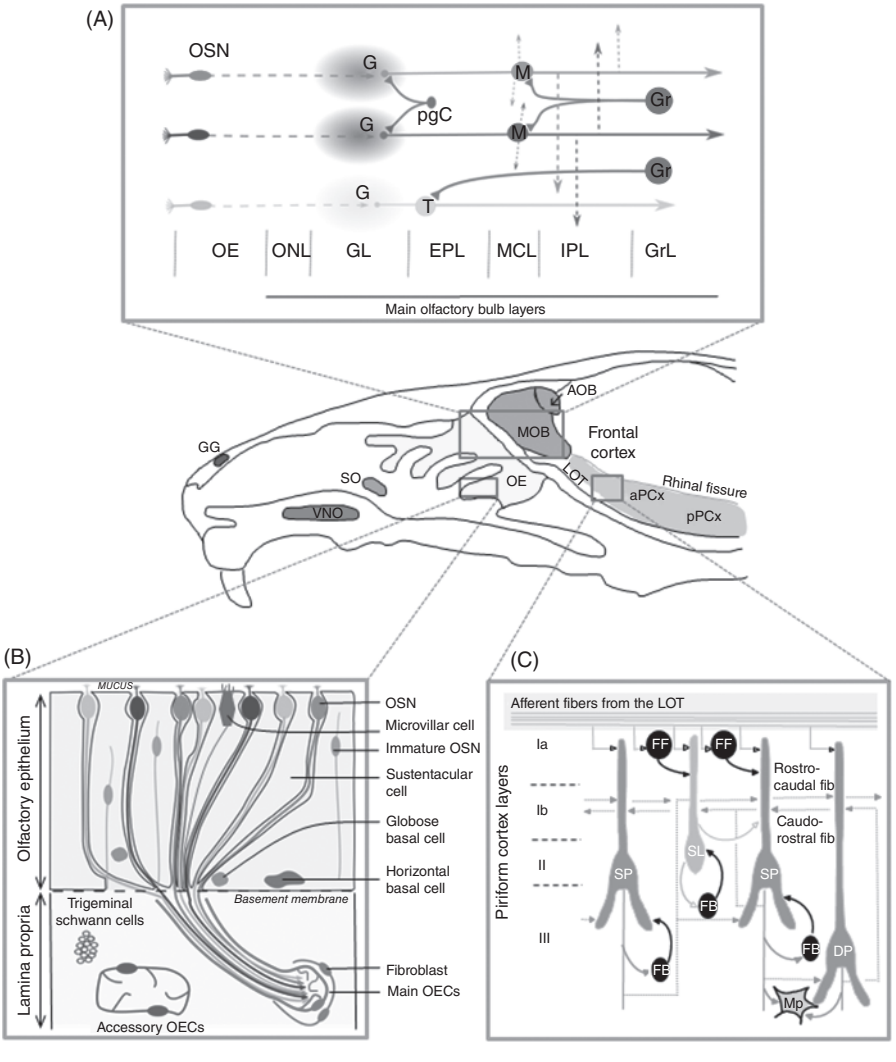


**Figure 1.1** Schematic drawing of the rodent olfactory system (sagittal cross section through the nasal region of the head, lower jaw is not shown). Inset **A** shows the different cell layers observed in the olfactory bulb and the neuronal connections. Inset **B** represents the various cell types and structures located in the olfactory mucosa. Inset **C** schematizes the connectivity of glutamatergic neurons in the PCx (Source: Adapted from Ekberg and St John 2014, Haberly 2001). Abbreviations: AOB, accessory olfactory bulb; aPCx, anterior piriform cortex; DP, deep pyramidal cells; EPL, external plexiform layer; FB, feedback interneurons; FF, feed forward interneurons; G, glomeruli; GG, Grueneberg ganglion; GL, glomerular layer; Gr, granule cells; GrL, granular cell layer; IPL, internal plexiform layer; LOT, lateral olfactory tract; M, mitral cells; MCL, mitral cell layer; Mp, multipolar cells; OB, olfactory bulb; OE, olfactory epithelium; ONL, olfactory nerve layer; pgC, periglomerular cells; pPCx, posterior piriform cortex; OSN, olfactory sensory neuron; SL, semilunar cells; SO, septal organ; SP, superficial pyramidal cells; T, tufted cells. A colored version of this figure can be found in the online version of this chapter.

Like other epithelia, the peripheral OE constantly regenerates itself throughout life. OSNs only live for 1-3 months after which they undergo apoptosis and are replaced by new neurons originating from basal cells (Mackay-Sim and Kittel 1991). The continuous turnover of OSNs protects the OE against damage induced by environmental factors that can result in cell death. This replenishment after damage is critical to maintain the functional integrity of the OE.

### 1.2.3 Molecular mechanisms of odorant detection

The airborne odorants diffuse into the aqueous nasal mucus before reaching olfactory cilia where ORs are localized. In the mucus, proteins called odorant-binding proteins (OBPs) are thought to carry odorants, which are commonly hydrophobic molecules, through the mucus towards the ORs (Heydel et al. 2013). In addition to the solubilisation of odorants, OBPs may have other functional roles. Recent studies have revealed that OBPs directly interact with ORs thus modulating their function (Vidic et al. 2008) or contribute to the clearance of odorants from the microenvironment of the receptor (Strotmann and Breer 2011).

Binding of odorants to specific ORs is a key event that induces olfactory signaling. ORs were first identified from rats in 1991 by Linda Buck and Richard Axel (Buck and Axel 1991) who received the Nobel Prize in 2004 for this discovery. These authors revealed that OR genes belong to a large multigene family that encode G protein coupled receptors (GPCRs). Further studies confirmed that OR genes constitute the largest multigene family in mammals. Comparison of diverse genome sequences showed that the numbers of OR genes vary greatly among species (Niimura 2012). Rats and mice have 1,400–1,700 OR genes in their genomes, cows and horses have higher numbers (2,200-2,600) and recently, it has been reported that the genome of African elephants contains more than 4,200 OR genes (Table 1.1). Compared with other mammals, primates tend to have smaller numbers of OR genes (600-800). A fraction of mammalian OR genes has been shown to be pseudogenes (i.e., genes that are not functional). The fraction of OR pseudogenes varies widely among species (Niimura et al. 2014). In human genome, more than half (52%) of the entire set of OR genes are pseudogenes, leading to 396 intact (potentially functional) OR genes (Matsui et al. 2010).

An important feature of OSNs is the fact that each cell expresses only one allele of a single OR gene: this has been proven through extensive studies in the mouse OSNs (Chess et al. 1994, Malnic et al. 1999, Serizawa et al. 2004). OSNs expressing the same OR would be distributed randomly within one of four circumscribed zones in the OE (Ressler et al. 1993, Vassar et al. 1993). However, some studies suggest that OR gene expression zones broadly overlap rather than bear sharp zonal boundaries (Iwema et al. 2004, Miyamichi et al. 2005). All OSNs expressing the same OR in turn converge upon spatially invariant glomeruli in the OB, the

**Table 1.1** Numbers of OR genes in the genome sequence from 13 placental mammalian species (Source: Adapted from Niimura et al. 2014). An intact gene was defined as a sequence starting from an initiation codon and ending with a stop codon that did not contain any disrupting mutation. A pseudogene was defined as a sequence with a nonsense mutation, frameshift, deletion within conserved regions, or some combination thereof. A truncated gene represents a partial sequence of an intact gene.

| Species | Total number | Intact genes | | Truncated genes | | Pseudogenes | |
|---|---|---|---|---|---|---|---|
| | | number | % | number | % | number | % |
| **Human** | 821 | 396 | *48.2* | 0 | *0* | 425 | *51.8* |
| **Chimpanzee** | 813 | 380 | *46.7* | 19 | *2.34* | 414 | *50.9* |
| **Orangutan** | 821 | 296 | *36.1* | 37 | *4.51* | 488 | *59.4* |
| **Macaque** | 606 | 309 | *51.0* | 17 | *2.81* | 280 | *44.7* |
| **Marmoset** | 624 | 366 | *58.7* | 27 | *4.33* | 231 | *36.9* |
| **Mouse** | 1,366 | 1,130 | *82.7* | 0 | *0* | 236 | *17.3* |
| **Rat** | 1,767 | 1,207 | *68.3* | 52 | *2.94* | 508 | *28.7* |
| **Guinea pig** | 2,162 | 796 | *36.8* | 26 | *1.20* | 1,340 | *62* |
| **Rabbit** | 1,046 | 768 | *73.4* | 22 | *2.10* | 256 | *24.5* |
| **Horse** | 2,658 | 1,066 | *40.1* | 23 | *0.87* | 1,569 | *59* |
| **Dog** | 1,100 | 811 | *73.7* | 11 | *1.00* | 278 | *25.3* |
| **Cow** | 2,284 | 1,186 | *51.9* | 41 | *1.80* | 1,057 | *46.3* |
| **Elephant** | 4,267 | 1,948 | *45.7* | 89 | *2.09* | 2,230 | *52.3* |

site of the first synaptic relay in olfactory sensory processing (Mombaerts et al. 1996, Ressler et al. 1994, Vassar et al. 1994). Thus, activation of specific ORs by an odorant elicits a characteristic pattern of activity in the OB.

The OR functionality was demonstrated through a number of *in vitro* and *in vivo* studies. Odorants may be recognized by multiple ORs, and one OR may recognize multiple odorants (Kajiya et al. 2001, Malnic et al. 1999). This implies that different odorants are recognized by different combinations of ORs. This scheme of combinatorial coding is now widely admitted to explain how odorants are encoded at the peripheral level. However, some ORs (such as the human receptor OR7D4) have been shown to bind to a limited number of structurally related odorants (Keller et al. 2007). ORs can therefore be classified into two groups: ORs that are broadly tuned and ORs that are narrowly tuned. However, the way a receptor can recognize an odorant still remains poorly understood and further studies are necessary to investigate the physicochemical laws that govern OR-ligand interactions.

ORs belong to the class-A of the GPCR family that includes a number of diverse membrane receptors. Bovine rhodopsin or β2-adrenergic receptors, class-A GPCRs whose structural features have been widely investigated, were used as templates to perform homology modeling. These experiments indicated that ORs fold into quite similar tertiary structures, consisting of seven trans-membrane (7-TM) helices connected by extra-cellular and intra-cellular

loops (Baud et al. 2011, Singer 2000). The 7-TM helices form a bundle in which a pocket is dedicated to odorant binding. Studies combining molecular modeling and site-directed mutagenesis helped specifying the nature of the binding sites of some ORs (Baud et al. 2011, Gelis et al. 2012, Katada et al. 2005, Launay et al. 2012). The binding pockets were predicted to be located between TM3, TM5 and TM6 and the main amino acids in contact with the ligands could be identified. For a given OR, the binding mode differs from one odorant to another but some amino acids, all hydrophobic, are involved in binding whatever the ligand (Charlier et al. 2012).

In the cilia of OSNs, ORs are coupled to a specific G-protein called $G_{olf}$. When a cognate ligand binds to an OR, this interaction activates the $G_{\alpha olf}$ subunit which elevates intracellular cAMP through type III adenylate cyclase enzymatic reaction. Binding of cAMP to the cyclic nucleotide-gated channel allows influx of cations, mainly calcium, into OSNs. Elevation of intracellular calcium induces the opening of the calcium-gated chloride channel that produces an efflux of chloride ions to amplify cellular depolarization (Kleene 2008). The cAMP pathway is thought to be the main signalization pathway involved in peripheral olfactory transduction. However, some studies suggest the involvement of cAMP-independent signaling pathways, including guanylate cyclase and phospholipase C (PLC) signaling, in olfactory transduction (Lin et al. 2004, Meyer et al. 2000). Recently, it has been demonstrated that a subset of mouse OSNs located in the most ventral zone of OE can mediate both the phospholipase C signaling pathway and the cAMP pathway upon binding to structurally similar ligands (Yu et al. 2014). In consequence, some ORs could possess conformational plasticity leading to preferential interactions with different downstream elements, depending on ligand that binds to the OR.

### 1.2.4 Plasticity of the olfactory epithelium

Several endogenous and exogenous factors induce different forms of plasticity at the OE level.

#### 1.2.4.1 Development and ageing

Evidence has been accumulated that the peripheral olfactory system is functional before birth. Behaviour studies have shown that prenatal olfactory experience provoked by odorants present in the amniotic fluid contributes to postnatal preferences and behaviours such as suckling and feeding (Logan et al. 2012, Schaal et al. 2000). In mouse embryonic development (lasting 19 days from conception), the OE is fully formed at embryonic day 10 (E10) and at around E14, multiple short cilia can be observed on neuron dendrites (Cuschieri and Bannister 1975). Several works reported that ORs and components of the main olfactory signaling pathway (such as protein $G_{olf}$, adenylate cyclase III and cyclic nucleotide-gated ion channels) are expressed in the OE at the same stage

(Saito et al. 1998, Schwarzenbacher et al. 2005). In line with these observations, electrophysiological studies performed in the OE or individual OSNs of rodents gave evidence for odorant responses at E16 (Gesteland et al. 1982, Lam and Mombaerts 2013). These recordings indicate that late-stage mouse embryos possess functional OSNs and the ability to detect odorants.

A handful of studies have addressed the OR expression profile throughout life. Newborn rats express fewer OR genes than adult and ageing rats, and generally at a lower level (Rimbault et al. 2009). However, a small subset of OR genes are expressed specifically or even overexpressed in newborns. In C57B6L/N mice raised under well-controlled conditions, the majority of OR gene expression (58.4%) remained stable throughout life while 32.8% presented downward profiles and 7.2% upward profiles (Khan et al. 2013). A recent study performed in human OE supports these results. In samples collected from individuals aged from 39 to 81 years, authors showed that the expression of most OR genes is stable with age. However the expression level of a small number of ORs significantly decrease or increase (Verbeurgt et al. 2014). The overall conclusion of these studies is that OR gene expression in mammal OE seems rather stable throughout life.

Nevertheless, decline of olfactory function is common in elderly humans. This decreased sensitivity with ageing has been postulated to be due partly to structural and cellular changes occurring in the OE rather than OR expression level. These changes are probably also associated with alterations occurring in the central components of the olfactory system. Studies on animal models and human biopsies support a gradual degradation of the OE that could account for olfactory loss. A significant age-related loss of OSNs in the affected areas, which results in a thinner epithelium (Rosli et al. 1999), and a strong reduction in the sensitivity of human OSNs (Rawson et al. 2012) have been demonstrated. In aged mice, OSNs expressing a defined OR exhibit a lower density while the functional properties of these neurons did not change (Lee et al. 2009). These peripheral changes might contribute to poor odour discrimination and identification in the elderly.

#### 1.2.4.2 Nutritional and metabolic status

In mammals, odour perception also depends closely on nutritional status. Fasting results in an increased ability to detect odours, some of which are food-related. Meanwhile, satiety with one type of food reduces the ability to detect the odour specially associated with that food type (Mulligan et al. 2002, O'Doherty et al. 2000). Recently published works suggest that the olfactory system is intimately linked with the endocrine systems that regulate energy balance. Hunger and satiety status are signaled by blood-circulating peptide hormones. Receptors for metabolically important hormones such as ghrelin, orexins, NPY, insulin, leptin, and cholecystokinin have been shown to be expressed in the OM (Palouzier-Paulignan et al. 2012). These molecules have access to the OM

through the peripheral circulation but the local production of insulin within this tissue has also been reported (Lacroix et al. 2008). Using an *ex vivo* intact epithelium preparation, Savigner and colleagues (Savigner et al. 2009) showed that bath perfusion of insulin or leptin, both anorexigenic factors, decreased the odorant response. These peptides also reduced the odorant-induced activity in the OM in well-fed animals (Lacroix et al. 2008, Savigner et al. 2009). Conversely, NPY, an orexigenic peptide, increases the electrophysiological response of OSNs to odorants in fasted adult rats (Negroni et al. 2012). In addition, the expression of metabolic hormone receptors in mammal OM can be regulated by nutritional status. An overexpression of insulin, leptin and NPY receptors has been observed in OM from fasted rats (Baly et al. 2007, Lacroix et al. 2008, Negroni et al. 2012).

Chronic energy imbalance can also alter the sensitivity of the peripheral olfactory sensory system. A limitation of the duration of daily food intake was found to provoke a modulation of olfactory-mediated behaviours regarding food odours in rats (Badonnel et al. 2012). This restriction was accompanied by a slight decrease in insulin receptor expression in the OM, suggesting that this hormone could be part of this process. Olfactory dysfunctions were also reported in mice fed a high-fat diet for 24 weeks (Thiebaud et al. 2014). Marked loss of OSNs and their axonal projections and a concomitant reduction in electro-olfactogram amplitude were observed in these mice. These structural and functional changes at the OM level could evoke dysfunctions in olfactory driven behaviour. Taken as a whole, these different studies demonstrate that nutritional and metabolic state can modulate olfactory perception by regulating the sensitivity of the peripheral olfactory system.

#### 1.2.4.3 Exogenous compounds

Embedded in the epithelium lining of the nasal cavity, OSNs are continuously exposed to environmental factors such as odorant molecules or non-odorant volatile chemicals. These exogenous compounds can modify OSNs' properties or even induce damage to the OM in case of long-term and high-level exposition.

The effects of odorant enrichment on OSNs have been analyzed in a number of studies. Olfactory stimulation generally promotes the survival of OSNs (Francois et al. 2013, Watt et al. 2004). It also induces higher sensitivity of the epithelium to the odorant used for the exposure, suggesting an increase in the target OR expression (Wang et al. 1993). The effects of odorant exposure on a specific OSN population diverge depending on the population considered: odorant exposure either increases survival of OSNs (Francois et al. 2013, Watt et al. 2004) or decreases the number of OSNs (Cadiou et al. 2014, Cavallin et al. 2010). However, in spite of a decrease in OSN number, exposed target neurons were found to respond to their ligand with higher sensitivity, broader dynamic range, faster rise time, and shorter responses (Cadiou et al. 2014). This suggests that

neurons could take part in the compensation of their lower density by sending more information to the OB. Through this form of plasticity, OSNs can adapt to their environment.

Exposure to volatile compounds, including chemicals, solvents and environmental contaminants, may induce various lesions in the OE such as inflammation, necrosis, atrophy and proliferation (for reviews, see Gaskell 1990, Harkema et al. 2006). Global necrosis of the OE has been observed after exposure to irritants such as chlorine and sulfur dioxide. In contrast, cell-specific toxicity may occur in the OE. Notably, it has been reported that inhalation of acetone selectively damages progenitor cells of the OE in mice (Buron et al. 2009). Intranasal administration of satratoxin-G, a mycotoxin produced by the black mold *Stachybotrys chartarum* that grows in water-damaged housings, induces widespread apoptosis in OSNs. This apoptosis was associated with an acute, neutrophilic rhinitis in the nasal airways of Rhesus monkeys (Carey et al. 2012). Several metals, such as cadmium, have also been associated with olfactory function impairment in exposed workers (Gobba 2006). Experimentally, cadmium instillation resulted in an important but recoverable cell loss in mouse OE (Bondier et al. 2008). Accumulation of cadmium in the mice OB has also been observed in the same study, suggesting that cadmium can be transported through the OSN axons to the OB. Like cadmium, a number of metals and other chemicals (Minn et al. 2002), as well as pathogenic microbes (Dando et al. 2014), can enter the brain *via* the olfactory pathway. Among other causes, this phenomenon is suspected to contribute to the development of a number of neurodegenerative diseases, most notably Alzheimer's and Parkinson's diseases (Prediger et al. 2012).

### 1.2.5 Subsystems in the main olfactory epithelium

We are presenting here three subsystems coexisting with the main OE (MOE) in many mammals: the vomeronasal organ (VNO), the Grueneberg ganglion (GG) and the septal organ (SO) also called Masera organ (Figure 1.1). In humans however, these subsystems are either nonfunctional, for example the VNO, or do not appear to exist (GG and SO).

#### 1.2.5.1 The vomeronasal organ

The VNO was described two hundred years ago by Ludvig von Jacobson (Trotier and Doving 1998) in many mammalian species, especially rodents. In rodents, the VNO is a bilateral tubular structure located ventrally on the nasal septum. A bilayer chemosensory neuroepithelium covers the medial wall of the VNO. The epithelium comprises thousands of microvillar sensory neurons (VSNs for Vomeronasal Sensory Neurons) whose axons project to the accessory OB (AOB). During development, the VNO can be observed in humans, but then shows many signs of regression and even absence of epithelial neurons or nerve fibers that would allow neural information to be transported to the brain (Trotier 2011).

At least three types of vomeronasal receptors (VNRs) have been described, mainly in rodents. All VNRs belong to the GPCR family. The first type, V1r, is associated with G proteins of the Gi type and is only expressed in the apical neuroepithelium (Dulac and Axel 1995). It projects to the anterior portion of the AOB. In humans, only five members of this family have an intact open reading frame (Rodriguez and Mombaerts 2002). They are expressed only in the main OE but their function is unknown (Rodriguez et al. 2000). The second type, V2r, is expressed in the basal neuroepithelium with G proteins of the Go type and VSNs expressing V2rs project to the posterior portion of the AOB (Dulac and Torello 2003, Halpern and Martinez-Marcos 2003). Few mammalian species maintain a functional family of these receptors, which are not present and functional in humans (Shi and Zhang 2007). Signal transduction in V1r and V2r expressing neurons relies on a PLC-mediated pathway involving a G protein (Gi or Go) leading to production of secondary messengers. These messengers eventually open transient receptor potential cation channels (TRPC2) localized in the microvilli of the sensory neurons. The VNRs of the third type belong to the family of formyl peptide-like receptors (FPR) (Liberles et al. 2009, Riviere et al. 2009). These receptors are present in all mammals and are generally expressed in the immune system. Their expression is related to olfaction only in rodents.

Historically, the VNO has been considered as an organ specific for the detection of social cues such as pheromones. However, since the discovery of different types of receptors detecting pheromones in the MOE and others detecting general odorants in the VNO, there is some overlap of ligands of the MOE and the VNO (Ma 2007). The main difference between the MOE and the VNO is that odorants can access to the chemosensory neurons in the VNO only once they are dissolved in the mucus and drawn into the lumen of the organ. This represents an active process involving vasoconstriction of sinuses or blood vessels. The process allows the characterization of very heavy nonvolatile molecules such as peptides.

Relatively few ligands able to activate vomeronasal neurons have been identified. Some of these ligands are volatile compounds, such as 2-heptanone, a ligand of V1rb2 (Boschat et al. 2002). Most ligands however are heavy molecules, including short peptides. The ESP (exocrine-gland-secreting peptides) family is secreted by different glands and is present in tears, nasal mucus and saliva (Kimoto et al. 2005). Some members of this family are involved in sexual behaviours (Haga et al. 2010). Major histocompatibility complex-related peptides are also mentioned (Leinders-Zufall et al. 2004). Several ligands connected to inflammation or pathogens such as the peptide fMLF have been associated with specific FPRs (Riviere et al. 2009). Other potential ligands of VNRs are generated in urine, either volatiles or non-volatiles such as steroids, or peptides involved in aggressive behaviours (Ibarra-Soria et al. 2014). The receptors involved in the detection of these ligands are not identified at the moment.

### 1.2.5.2 The septal organ and the grueneberg ganglion

These two subsystems were primarily described in rodents, and there is so far no description of them in humans and other primates. Their role in olfaction was described only recently.

The SO (or Masera organ) is a small area of olfactory epithelium isolated from the main OE in the respiratory epithelium. It is present in the nasal cavity of many species of mammals particularly rodents (Ma 2010). There is no evidence of the presence of a Masera organ in humans. The position of the SO in the nasal cavity is unique: located ventrally just behind the vomeronasal organ and near the choana, it is located on the pathway of the air during breathing at rest. This anatomical situation suggested that the SO may have a role to alert the animal of the presence of an odorant.

The SO appears during embryonic development and reaches its maximum development in young adults. Its epithelium has many similarities with that of the MOE: it contains many ciliated olfactory neurons but also some rare microvillar cells (Ma 2007). However, there are some structural differences with the MOE (number of layers, morphology of OSNs). Neurons in the SO express the canonical transduction cascade of the main OE (Grosmaitre et al. 2007, Ma et al. 2003). The expression levels of the 120 receptors identified is very characteristic: a small group of receptors covers approximately 95% of these cells. But the rule of one-receptor gene expressed in one neuron remains valid. The axons of the SO neurons target a small group of glomeruli called "septal glomeruli" and a large group of weakly stained glomeruli receiving axons from both SO and MOE (Levai and Strotmann 2003).

SO neurons respond to a large number of odorants either in electroolfactogram (Marshall and Maruniak 1986) or in single cell recordings (Grosmaitre et al. 2007, Ma et al. 2003): the SO neurons seem to be generalists, due to the expression of broadly tuned odorant receptors (Grosmaitre et al. 2009). These physiological results confirm the alerting role of the SO as an odorant detector leaving the discrimination task to the MOE.

The Grueneberg ganglion (also spelled Grüneberg ganglion, GG) was at first thought to be part of the peripheral nervous system without any connection with olfaction (Grüneberg 1973). This group of cells is located at the dorsal tip of the nasal cavity and close to the opening of the naris. Recently, clues from gene-targeted mice suggested a role in olfaction (Munger et al. 2009). Although they express olfactory marker proteins, the GG cells have a very different morphology from that of classical OSNs: i) their shape is ovoid without a dendrite but with short cilia directly connected to the soma; ii) they are combined and attached to each other in grape-like clusters; iii) they are separated from the nasal cavity by a keratinized epithelial layer made of glial cells. These clusters of cells appear during the embryonic development and reach their maximum at the perinatal stage, suggesting a role in the mother-infant interactions. The one-neuron one-receptor rule also applies to GG neurons. The majority of

GG neurons express a VNR; some express a classical OR and others a trace amine-associated receptor (Fleischer and Breer 2010). The putative transduction pathway proteins depend on the receptor expressed, but the majority seems to use a cGMP pathway (Fleischer and Breer 2010).

GG neurons project their axon to the OB into glomeruli located in the necklace glomeruli area. This suggests that GG neurons are involved in mother-pups interactions. This role was also supported by the detection by these neurons of a decrease in temperature through the isolation of pups from the mother enhancing the response to specific odorants (Fleischer and Breer 2010). In adult, GG neurons were shown to respond to an alarm pheromone (Brechbuhl et al. 2008) with structural similarities to predator chemosensory signals (Brechbuhl et al. 2013).

To date, the question of the role of the GG is not fully resolved: at an early age, GG neurons respond to a drop in temperature, and specific odorant molecules. At adult age, they respond to an alarm pheromone emitted by conspecifics in distress. This does not completely unveil the role of the GG while strongly suggesting its involvement in the mother-pup relationship.

## 1.3 Anatomical and functional organization of the main olfactory bulb

### 1.3.1 Background

OSNs send their axons to OB where they synapse onto second order neurons at the level of glomeruli which are neuropilar subunits. The number of glomeruli in the mice OB has been estimated to 1,810 (Royet et al. 1988). As the total number of OSNs in the mice OE is about $5.10^6$ unilaterally (Kawagishi et al. 2014), it can be calculated that a single glomerulus receives converging inputs from 2,760 OSN axons. In the human OB, the number of glomeruli varies between individuals; the average number of glomeruli has been estimated to 5,500 (Maresh et al. 2008). At the level of the OB, olfactory signals are processed by interneurons (periglomerular cell, granular cells, external tufted cells, short axon cells, Van Gehuchten cells, Blanes cells) before being exported to higher centers of the brain by output neurons (mitral cells, tufted cells). The main interneurons are the periglomerular cells and the granular cells. The periglomerular cells are small cells which surround glomeruli. They have highly arborizing dendrites in one glomerulus and may extend towards 3-6 glomeruli where they synapse onto apical trunk of projecting neurons and other granular cells. The granular cells are also small cells; they have a large apical dendrite which projects onto tufted and mitral cells but they do not have axons.

The overall organization of rodent OB is laminated (for review see Greer et al. 2008). From the periphery to the center of the bulb, the following layers can be found (Figure 1.1A): the olfactory nerve layer (ONL) which is composed of

the mass of axons projecting from the OE, the glomerular layer (GL) where the glomeruli are located, the external plexiform layer (EPL) which contains the tufted neuron cell bodies, the mitral cell layer (MCL) which contains the cell bodies of mitral cells, the internal plexiform layer (IPL) where the axons migrating to the cortex fasciculate, the granular cell layer (GrL) which contains the granular cell bodies, and the rostral migratory stream layer (RMS). A comparable laminar organization is found in the human OB. However, it is less rigorous in the segregation of cell populations and also often lacks the circumferential organization of layers found in rodents and the medial-lateral symmetry of the rodent OB (Maresh et al. 2008).

Mitral cells are the most prominent population of output neurons. They have a single apical dendrite which invades a single glomerulus where it arborizes. About 20–25 mitral cells project into each glomerulus. The axon extends from the basal pole of the neuron and joins other axons forming the lateral olfactory tract (LOT). Mitral cells also have secondary dendrites which extend laterally in the EPL. Tufted cells are the second population of output neurons in number. Their shapes are similar to those of mitral cells but they are thought to mediate parallel circuits for processing olfactory information. The cortical target of mitral and tufted cells differs. Tufted cells project to the most rostral part of the olfactory cortex and the more medial olfactory tubercle while mitral cells distribute broadly throughout the olfactory cortex.

The synaptic organization of microcircuits in the OB is highly complex and far from being fully understood. The OB exhibits a circuitry that supports extensive inhibitory lateral interactions before the information is transmitted to the rest of the brain (Gire et al. 2013). This lateral inhibition is mainly due to the large population of interneurons.

### 1.3.2 The architecture of the olfactory bulb supports its function

As mentioned above, all OSNs expressing the same OR send their projection axon into the OB in a very limited number of glomeruli (~2 in each OB) (for review see Mombaerts 2004). The spatial position of a given glomerulus is not randomly distributed in an area corresponding in size to the equivalent of 30 glomeruli (Mombaerts 2006). Such invariant organization among different individuals of the same species strongly suggests a highly wired organization of the topography of projection from OE to OB. What are the consequences of this organization in term of odorant coding? Each OSN can detect a range of odorants and a given odorant can activate different OSNs. The activation of OSNs by odorants is therefore a combinatorial event (Kajiya et al. 2001, Malnic et al. 1999). Nevertheless, each OSN is thought to have its own specificity in terms of odorant sensitivity. Then, the organization of OSN projection onto the bulb raises the question of a functional topological organization between OE and OB. At least there is

an anatomical topology which could be named "receptoro-topy" (Murthy 2011) since all OSNs expressing the same OR project onto the "same" glomeruli. Does such anatomical topology support functional topology? If odotopy which refers to the activation of subset of glomeruli by a given odorant is generally accepted, the chemotopy which refers to activation of subset of glomeruli according to properties of odorant molecules is more controversial (Murthy 2011).

### 1.3.3 The overall architecture of the olfactory system is genetically determined

The relatively stereotyped organization of the olfactory system has been further supported by the discovery of the "receptoro-topy" between OE and OB. From the developmental point of view, one can wonder what are the mechanisms underlying such an invariant organization. In other words, what are the mechanisms allowing all the OSNs expressing the same OR to converge onto one or two glomeruli in specified location in the OB? The mechanisms involved appear to be complex and combinatorial. First, it has been known for long now that there is a correspondence between the position of the OSN in the OE and the dorsoventral position of the glomerulus where they converge into at the OB level (Astic et al. 1987). Second, the guidance of OSN along the dorso-ventral axis appears to be dependent of 2 sets of repulsive ligand/receptor pairs, that is, Slits/Robo2 and Sema3F/Nrp2 (Takeuchi et al. 2010). Third, concerning the antero posterior-position of the glomeruli, the pre-targeted axon sorting is due to classic axon guidance molecules such as Np1 and Sema3A (Sakano 2010). These are thought to be under the control of intracellular signaling involving cAMP (Col et al. 2007, Zou et al. 2007). The axon sorting along the antero-posterior axis occurs before the OB, at the level of the axons bundles (Imai et al. 2009) and is independent of stimulus driven activity (Nakashima et al. 2013).

### 1.3.4 The fine architecture of the olfactory system is environmentally determined

Even in invertebrates, non-programmed activity-dependent factors are involved in the development of the nervous system. In the mammalian olfactory system, this is true both at the level of the OE and the OB. For instance, since the 1980's, it is known that naris closure induce a reduction of 10% in the number of OSNs in the OE (Farbman et al. 1988). At the level of the OB, the convergence of all OSNs expressing the same OR to one or two glomeruli sets up progressively during post-natal development. This convergence establishes gradually and takes place differently depending on the OR. Finally, this convergence is activity-dependent since olfactory deprivation prevents its typical organization (Zou et al. 2004), even if this latest statement has been controversial (Lin et al. 2000). Nevertheless, the involvement of neuronal activity by the way of competition mechanisms between active and inactive neurons has been beautifully demonstrated (Zhao

and Reed 2001). More recently, it has been shown that ligand driven activity of OSNs appears to control the glomerular segregation and that this is mediated by $G_{olf}$ transducing cascade (Nakashima et al. 2013).

### 1.3.5 In the olfactory system, development never ends

The OE has been the first place where continuous neurogenesis has been demonstrated (Hinds et al. 1984). This peripheral neurogenesis is thought to be linked to the fact that OSNs are continuously exposed to external air and particularly to toxic components. This exposure induces an increased neuronal death which should be compensated by neurogenesis in order to keep the system functional. Later, another nest of adult neurogenesis has been demonstrated in the brain in the sub-ventricular zone. From there, 3 migratory pathways send newborn cells in different directions. Among them, the rostral migratory pathway forwards new cells to the OB. While migrating, the newborn cells differentiate into neurons and integrate the OB circuits. They appear to mature mainly as granule cells.

There is a controversy regarding the status of an intact rostral migratory stream in the human OB. However, a wider consensus exists showing that newly differentiated neurons are found in the adult human OB, even among the elderly. This indicates that the human OB is a dynamic structure with a capacity for plasticity throughout life (Maresh et al. 2008). Such neurogenesis is meaningful regarding the plasticity of the olfactory system in response to changes in the olfactory environment. For example, an exposure to an odorant-enriched environment increases significantly both the number of newborn neurons integrated in the circuits and the learning performances (Rochefort et al. 2002). Conversely, a naris closure induces a reduction in the number of new neurons integrated into the OB circuits (Gheusi et al. 2000, Gheusi and Rochefort 2002).

## 1.4 Central odour processing

### 1.4.1 The primary olfactory cortex

In rodents, the primary olfactory cortex consists in brain areas that are direct targets of the OB. The bulbar outputs are conveyed by the LOT. The LOT is a myelinated fiber tract reaching diverse brain structures (Figure 1.2): the anterior olfactory nucleus, the tenia tecta, the olfactory tubercle, the anterior and posterior piriform cortex, the nucleus of the lateral olfactory tract, the anterior cortical amygdaloid nucleus, the posterolateral cortical amygdaloid nucleus, and the lateral entorhinal cortex (Haberly 2001). The LOT appears as a non-homogeneous tract. Thus axons from bulbar neurons (mitral/tufted cells) seem to be located in distinct parts of the LOT, suggesting different pathways to send information to higher olfactory regions (Nagayama et al. 2010). Each area of the primary

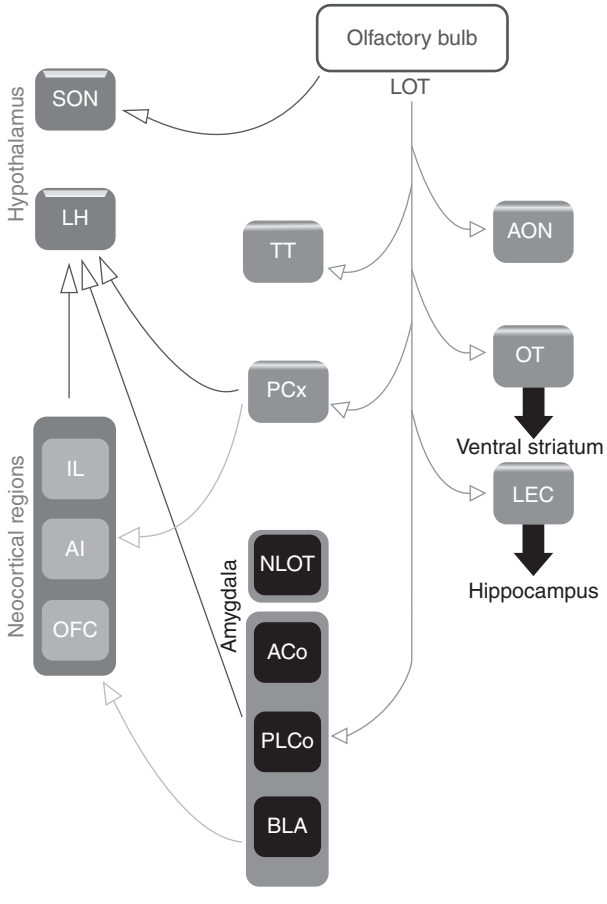


**Figure 1.2** Schematic illustration of the main anatomical efferents arising from the main olfactory bulb in rodents. The primary cortex consists in regions receiving bulbar outputs conveyed by the LOT. The piriform cortex relays the olfactory information to several neocortical areas involved in complex processes such as multisensory integration, flavour perception and decision-making. It is also connected with the lateral hypothalamus that plays a role in feeding behaviour. Within the amygdala, bulbar efferents primarily target the superficial cortical nuclei (Aco, PLco) which are connected to deep nuclei such as BLA. The NLOT is located in the rostral part of the amygdala. Unlike other parts of olfactory amygdala, it does not project directly to the hypothalamus. Olfactory information is also transmitted to reward circuit through the olfactory tubercle efferents reaching the ventral striatum. The entorhinal cortex, as a gateway to hippocampus, allows olfactory-related mnesic processes. Abbreviations: Aco, anterior cortical nucleus of amygdala; AI, agranular insular cortex; AON: anterior olfactory nucleus; BLA, basolateral amygdala; IL, infralimbic cortex; LEC, lateral entorhinal cortex; LH, lateral hypothalamus; LOT, lateral olfactory tract; NLOT, nucleus of the lateral olfactory tract; OFC, orbitofrontal cortex; OT, olfactory tubercle; PCx, piriform cortex; PLco, posterolateralcortical nucleus of amygdala; SON, supraoptic nucleus; TT, tenia tecta. A colored version of this figure can be found in the online version of this chapter.

olfactory pathway is believed to process odours and to interpret the activity map originating from the OB in different manners.

In humans, the organization is not completely similar to rodents since the olfactory cortical areas that are direct targets of the OB are less developed. In the human brain, the main region receiving bulbar information through the lateral olfactory tract consists of the piriform cortex which is located at the junction of the inferior frontal and temporal lobes (Chen et al. 2010, Zatorre et al. 1992).

#### 1.4.1.1 The piriform cortex

In rodents, the piriform cortex (PCx) is the largest cortical area of the olfactory cortex. It is located on the ventrolateral surface of the brain close to the LOT (Figure 1.1C). The PCx is divided into anterior (aPCx) and posterior (pPCx) subdivisions which differ in their anatomical features. In contrast to cortical regions in other sensory systems, it does not receive sensory inputs *via* the thalamus but direct synaptic inputs from the OB. Mitral cells have been shown to terminate in rather broad patches in the PCx (Buonviso et al. 1991) and collaterals are distributed to wide areas of the PCx (Nagayama et al. 2010). Axonal projections of mitral/tufted cells from the OB to the PCx appears to be sparsely distributed and overlapping (Stettler and Axel 2009). Individual cortical neurons in highly restricted areas of the PCx receive direct inputs representing glomeruli that are distributed throughout the OB with no apparent topographical organization (Ghosh et al. 2011, Miyamichi et al. 2011, Sosulski et al. 2011). No marked topography in odour-evoked activity has been demonstrated in the PCx and the spatial organization pattern of activity induced by odour in the OB is not conserved (Illig and Haberly 2003).

***Cellular types and cytoarchitecture***

The PCx is a trilaminar paleocortex (Figure 1.1C). The upper part of the layer I (layer Ia) contains afferent fibers from the LOT. The layer II contains the principal PCx glutamatergic neuronal types which are superficial pyramidal (SP) cells. The SP cells are characterized by well-developed dendritic trees and are the target of more associational excitatory inputs (Bekkers and Suzuki 2013, Suzuki and Bekkers 2011). Besides SP, another glutamatergic cells, the semilunar (SL) cells, are also found in the layer II (Suzuki and Bekkers 2006). The SL cells receive stronger afferent excitatory bulbar inputs and weak associational inputs than SP cells. In the layer III, deep pyramidal (DP) cells and multipolar (Mp) cells are observed (Protopapas and Bower 2000). DP cells have a rather similar connectivity as SP cells.

As in other cortices, synaptic inhibition is observed in the PCx. Indeed the PCx contains GABAergic interneurons that are present across all layers and provide feed-forward and feedback inhibition of the principal cells (Suzuki and Bekkers 2012). Thus, neurogliaform and horizontal interneurons in the layer Ia receive LOT inputs and provide feed-forward inhibition of the distal apical

dendrites of SL and SP cells. Feedback inhibitory interneurons are restricted to deeper associational layers and involve a variety of interneurons such as bi-tufted, soma targeted fast-spiking, axons targeting chandelier, dendrite targeting regular-spiking and deep neurogliaform cells (Larriva-Sahd 2010).

A main feature of the PCx is a dense network of associational fibers. Indeed, the SP cells give rise to massive axon collaterals that form synapses on other pyramidal cells across wide areas of the PCx and form an extensive circuitry of recurrent connections (Johnson et al. 2000). Thus, each pyramidal cell makes a small number of synaptic contacts on a large number (more than 1000) of other cells at various locations within the PCx (Johnson et al. 2000). The network of intrinsic connections can enhance or suppress bulbar inputs and subsequently influence the recruitment of PCx principal neurons by afferent bulbar inputs (Franks et al. 2011). This might allow detection of temporally patterned OB inputs, shaping odour-evoked responses and encoding odorant identity.

Both PCx subdivisions are not similar regarding the organization and intra-cortical connectivity. The pPCx receives dense connections from the aPCx and has more recurrent connections, suggesting an associative role. On the contrary, the aPCx receives more afferent inputs from the OB and less associational inputs. In aPCx, the strength of inhibitory connections onto pyramidal cells is different along its rostro-caudal axis. Thus, pyramidal cells located at more caudal level of aPCx receives greater inhibition than cells at rostral location (Luna and Pettit 2010).

Besides intrinsic cortico-cortical fibers, the pyramidal cells give rise to extrinsic associational fibers which are restricted to layers Ib, II and III and connect the PCx with other regions of the primary cortex. In addition, commissural fibers originating from the aPCx layer II can reach the contralateral pPCx.

***Role in olfactory processing***

In the environment, odours mainly result from the perception of odorant mixtures. The PCx is assumed to build odour representations from sensory fragment allowing perceptual stability and behavioural adaptability even if changes in mixture components occur (Wilson and Sullivan 2011). The PCx receives convergent inputs from random collections of glomeruli and, as a result, it might be expected that odour representation mainly depends on its behavioural significance. The extensive network of excitatory association fibers support the view that PCx might construct unitary odour objects from the chemical components processed at earlier stages of the olfactory system. This auto associative network might allow complex processes such as pattern completion (possibility to fill the gap for partial inputs) and pattern discrimination (possibility to extract information from background) (Bekkers and Suzuki 2013, Chapuis and Wilson 2012, Uchida et al. 2014).

In rodents, PCx subdivisions have been shown to differ in their role regarding coding of odorant identity and odour quality. The process of familiarization differentially involves both subdivisions. As odorant mixtures become more

familiar, aPCx neurons show habituation and develop enhanced ability to discriminate those mixtures from their environment (Wilson 2000). In the pPCx, more broadly tuned neurons might encode odorant qualities of the stimuli (Kadohisa and Wilson 2006). In humans, data from functional magnetic resonance imaging suggest that the PCx also shows a functional heterogeneity along its rostro-caudal axis (Gottfried et al. 2002).

Odour representation in the PCx has been shown to be modified through experience. Plasticity occurs at the level of the associated connections (Stripling and Galupo 2008). Long-lasting modifications of neuronal activity and synaptic efficiency have been shown to occur in various learning contexts (Barkai and Saar 2001, Martin et al. 2004). The PCx may mediate different learned behaviour in the absence of sensory inputs (Choi et al. 2011). It may function as an associative area rather that a classical primary sensory cortex (Barkai et al. 1994), synthesizing features from olfactory cues and linking them with other brain functions.

#### 1.4.1.2 Other regions of the primary olfactory cortex

***The anterior olfactory nucleus***

The anterior olfactory nucleus **(**AON) is placed in the olfactory peduncle (i.e. the region connecting the OB with the basal forebrain). It consists in several subdivisions. The AON is reciprocally connected to both the ipsi- and contralateral regions of OB and PCx. There is a highly topographic axonal projection of mitral/tufted cells on the AON (*pars externa*) following the glomerular map. As a consequence, the AON maintains a dorso-ventral topography (Miyamichi et al. 2011, Yan et al. 2008). Regarding the PCx, dense functional connections from the AON exist with the aPCx in comparison with the pPCx (Hagiwara et al. 2012). In view of its connections, the AON is in position to broadly influence the central processing of odour information by preprocessing the bulbar inputs before sending to other cortical areas (Kay and Brunjes 2014).

The AON contains two main neuronal populations: excitatory projection neurons and inhibitory interneurons (at least 5 classes of inhibitory cells were observed). It has been proposed that the AON provides feed-forward modification of information from the OB to the PCx. The AON is also assumed to participate to the localization of odour sources by comparing the ipsi-nostril to contra-nostril inputs of the same odorant category (Kikuta et al. 2010).

***The olfactory tubercle***

Together with the accumbens, the olfactory tubercle (OT) is referred as the ventral striatum. It is assumed to be involved in the induction of appetitive and fearful motivated behaviours (Ikemoto 2007, Wesson and Wilson 2011). Thus, food odour information processed in the OT appears to influence the dopaminergic circuits for reward expectation (Giessel and Datta 2014).

*The anterior and posterolateral cortical amygdaloid nuclei*

The anterior cortical amygdaloid nucleus projects to melanin-concentrating hormone-containing neurons in the lateral hypothalamus. This suggests a role in the modulation of feeding behaviours (Niu et al. 2012). The posterolateral cortical amygdaloid nucleus has relationships with the ventral striatum which might play a role in processing the reinforcing properties of olfactory stimuli (Ubeda-Banon et al. 2007). Trans-synaptic tracing has shown that neurons from the cortical amygdala mainly receive inputs from the dorsal OB (Miyamichi et al. 2011). Mice lacking OSNs that project to the dorsal OB lose their innate avoidance for odour from predator urine and spoiled food, suggesting that cortical amygdala nuclei may preferentially process olfactory information that directs innate behaviours.

*The lateral entorhinal cortex*

The lateral entorhinal cortex (LEC) receives direct input from OB and piriform cortex. The LEC has multiple reciprocal connections with hippocampus, amygdala and perirhinal cortex (Kerr et al. 2007). It also projects back to the PCx and OB (Agster and Burwell 2009). As a result, it is thought to play a crucial role in the olfactory memory and modulation of odour processing (Chapuis et al. 2013). The LEC exerts an inhibitory effect on PCx responses to the OB stimulations (Mouly and Di Scala 2006) and this might participate to the modulation of olfactory learning and memory (Wirth et al. 1998). To sum up, the major role of LEC is mnesic, cognitive and multimodal processing of olfactory cues (Martin and Ravel 2014).

## 1.4.2 Beyond the primary olfactory cortex

Besides regions of the primary olfactory cortex, the olfactory inputs reach various brain areas. These areas are thought to mediate complex functions related to the integration of sensory cues with behaviour, emotional or motivational significance, multisensory association and memory.

### 1.4.2.1 The basolateral amygdala

The superficial amygdala nuclei (anterior and posterolateral) relay the olfactory inputs coming from the OB to deeper amygdala nuclei such as the basolateral nucleus (BLA) (McDonald 1998). The BLA is a major area for odour-taste associations, i.e. flavour integration. This nucleus further plays a role in emotional learning involving olfaction such as conditioned odour aversion (Sevelinges et al. 2009), taste-potentiated odour aversion (Dardou et al. 2007, Shionoya and Datiche 2009) but also socially transmitted food preference (Wang et al. 2006) or conditioned flavour learning (Lienard et al. 2014). The BLA shares

extensive reciprocal connections with the orbitofrontal cortex and these regions likely contribute to both mnesic and affective processes (Cardinal et al. 2002). The orbitofrontal cortex and BLA play partially overlapping roles in the use of incentive information that supports normal discrimination performance.

#### 1.4.2.2 The hippocampus

The LEC is the gateway for olfactory inputs to the hippocampus which is a major region for formation of associative memories. A wide range of evidence indicates that this pathway sustains olfactory mnemonic processing (Gold et al. 2011, Raineki et al. 2010).

#### 1.4.2.3 The orbito-frontal cortex

The PCx has reciprocal connections with the orbito-frontal cortex (OFC) which is a multimodal cortical area with neurons responding to several types of sensory cues including the olfactory ones (Rolls 2012). The OFC receives convergent inputs from olfactory and gustatory cortices; both sensory modalities can be combined to give rise to the sensation of flavour. Neurons from the OFC are also believed to encode reward expectancy and to link sensory representations to behavioural inputs (Mainen and Kepecs 2009, Schoenbaum et al. 2003).

#### 1.4.2.4 The hypothalamus

Several regions from the primary olfactory cortex (at least the anterior olfactory nucleus, the piriform cortex, the olfactory tubercle and the anterior cortical nucleus of the amygdala) have been shown to project onto the lateral hypothalamus (Barone et al. 1981, Price et al. 1991). A direct projection from the main OB to the supraoptic nucleus of the rat has also been reported (Smithson et al. 1989). Olfactory inputs further target vasopressin neurons from the paraventricular hypothalamic nucleus (Bader et al. 2012). Together, these connections with hypothalamic nuclei might allow to affect feeding, reproductive activity, and autonomic reflexes triggered by olfactory signals (Palouzier-Paulignan et al. 2012).

## 1.5 Conclusion

In the last three decades, our knowledge about the functional architecture of the primary and accessory olfactory systems in mammals, including humans, has grown rapidly. The discovery of OR genes by Buck and Axel (Buck and Axel 1991) has been a major step in deciphering the molecular mechanisms that govern odorant coding at the peripheral level. This led to propose the concept of combinatorial scheme for odour coding. The OB is the first central relay, where olfactory inputs are spatially organized, noise-filtered, and sharpened. A number of studies support the existence of a coarse topographic map from the receptor

level to this first stage of processing (named "receptoro-topy") but how such a map translates to a functional olfactory map continues to be difficult to resolve (Murthy 2011). The topographic connectivity between OSNs and OB glomeruli is retained in projections to the amygdala and anterior olfactory nucleus, but lost in the projections to piriform cortex. One step beyond, the network of central connections participate to complex integration processes such as recognition of odours and odour-guided decisions, allowing adaptive behaviours crucial for survival (food intake, maternal bonding, etc … ).

A notable feature of the olfactory system is the continual neurogenesis that occurs during adulthood in the OE (from a locally dividing pool of progenitor cells) and in the OB (from cells born in the sub-ventricular zone). Adult neurogenesis modulated by olfactory inputs has been reported in the olfactory cortex and in brain structures related to emotion (amygdala), reward (striatal system), learning and memory (hippocampus and entorhinal cortex) (Arisi et al. 2012). Recent works demonstrated that several internal and external factors can also modulate olfactory signals throughout the processing pathways. The different levels of the olfactory system are therefore dynamic structures with features reflecting innate and environmental as well as developmental influences. As a consequence, it can be assumed that the structural and functional properties of the olfactory system may slightly differ among individuals and change over time. Future studies are therefore needed to better assess the impact of aging and nutrition on the olfactory function and more specifically how they shape the encoding of odour information.

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