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Encoding Olfactory Signals via Multiple Chemosensory Systems

Minghong Ma
Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA

ABSTRACT Most animals have evolved multiple olfactory systems to detect general odors as well as social cues. The sophistication and interaction of these systems permit precise detection of food, danger, and mates, all crucial elements for survival. In most mammals, the nose contains two well described chemosensory apparatuses (the main olfactory epithelium and the vomeronasal organ), each of which comprises several subtypes of sensory neurons expressing distinct receptors and signal transduction machineries. In many species (*e.g.*, rodents), the nasal cavity also includes two spatially segregated clusters of neurons forming the septal organ of Masera and the Grueneberg ganglion. Results of recent studies suggest that these chemosensory systems perceive diverse but overlapping olfactory cues and that some neurons may even detect the pressure changes carried by the airflow. This review provides an update on how chemosensory neurons transduce chemical (and possibly mechanical) stimuli into electrical signals, and what information each system brings into the brain. Future investigation will focus on the specific ligands that each system detects with a behavioral context and the processing networks that each system involves in the brain. Such studies will lead to a better understanding of how the multiple olfactory systems, acting in concert, offer a complete representation of the chemical world.

KEYWORDS Main olfactory epithelium, vomeronasal organ, septal organ, Grueneberg ganglion, signal transduction, odorant receptor

INTRODUCTION

The ability to detect myriad chemicals in the surrounding environment and to make appropriate behavioral adjustments is critical for the survival of most organisms. Many species (from worms and insects to mammals) have developed sophisticated chemosensory systems to detect the chemical cues signaling the preserves of potential food, danger, and mates (Bargmann, 2006). The mammalian olfactory, gustatory, and trigeminal systems, which are principally responsible for the sense of smell, taste, and pain/touch, respectively, are all involved in chemical senses. This review focuses only on the olfactory system, especially on the peripheral mechanisms underlying chemical detection. For the rodent, which is the best-understood model system, there are two major olfactory apparatuses in the nasal cavity: the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). Traditionally, MOE and VNO

Address correspondence to Dr. Minghong Ma, Department of Neuroscience, University of Pennsylvania School of Medicine, 215 Stemmler Hall, 3450 Hamilton Walk, Philadelphia, PA 19104. E-mail: minghong@mail.med.upenn.edu

are considered as detectors for general odors (small organic molecules) and specific social cues (such as pheromones, ranging from small organic molecules to large peptides), respectively. New studies have challenged the clear functional separation between these two systems (Brennan and Zufall, 2006; Dulac and Wagner, 2006; Meredith, 1998; Shepherd, 2006), and have revealed a more complicated organization of the mammalian nose (Breer *et al.*, 2006).

Most sensory neurons in the MOE are ciliated olfactory sensory neurons (OSNs), which express G-protein–coupled odorant receptors (GPCRs) and utilize the cAMP cascade to transform chemical energy into electrical signals (Firestein, 2001; Frings, 2001). Some ciliated OSNs express distinct receptors or signal transduction machineries, including trace amine associated receptors (TAARs) (Liberles and Buck, 2006), transient receptor potential channel M5 (TRPM5) (Lin *et al.*, 2007), and guanylyl cyclase type D (GC-D) (Fulle *et al.*, 1995; Juilfs *et al.*, 1997). In addition, the MOE contains nonciliated, microvillar cells composed of heterogeneous subpopulations, including non-neuronal epithelial cells expressing TRPM5 (Lin *et al.*, 2007) and sensory neurons transducing signals via a distinct pathway involving phospholipase C (PLC $\beta 2$) and another TRP channel (TRPC6) (Elsaesser *et al.*, 2005). Furthermore, the apical and basal compartments of VNO comprise sensory neurons expressing two classes of vomeronasal receptors (V1Rs and V2Rs, respectively) and project to different portions of the accessory olfactory bulb (Dulac and Torello, 2003; Halpern and Martinez-Marcos, 2003). Finally, two spatially segregated clusters of neurons form the septal organ of Masera (Rodolfo-Masera, 1943) and the Grueneberg ganglion (Gruneberg, 1973), which likely serve chemosensory functions (Figure 1).

In the last few years, considerable progress has been made in understanding the molecular organization of each olfactory system and in linking the chemoreceptors to the activity of the olfactory sensory neurons. Although the exact role each system plays remains elusive, cutting-edge technology applied in modern neuroscience from various disciplines (genetics, molecular biology, biochemistry, cell biology, physiology, imaging, anatomy, and behavior) has contributed to our rapidly expanding knowledge in this area and holds great hopes for the future studies. Ultimately, olfactory perception will be traced from the sensory neurons (receptors) to the processing centers in the brain and to behavior.

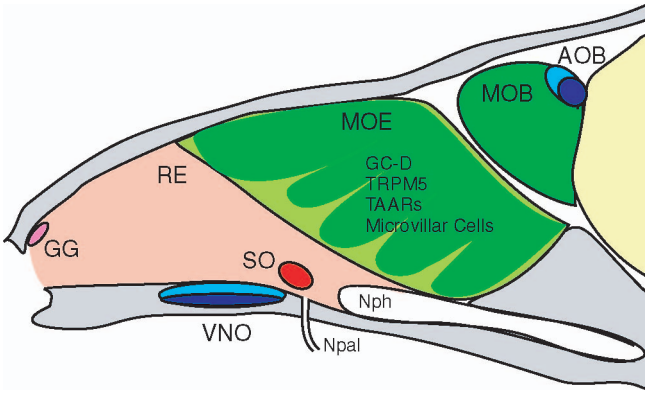

FIGURE 1 **The rodent nose contains multiple chemosensory systems. The olfactory sensory neurons in the main olfactory epithelium (MOE) project to the main olfactory bulb (MOB). The apical and basal sensory neurons in the vomeronasal organ (VNO) send axons to the anterior and posterior accessory olfactory bulb (AOB), respectively. The MOE also contains cells expressing guanylyl cyclase D (GC-D), transient receptor potential channel M5 (TRPM5) or trace amine associated receptors (TAARs). GC-D neurons project to the necklace glomeruli in the caudal MOB and TRPM5 positive neurons to the ventral MOB. The neurons in the septal organ (SO) (surrounded by the respiratory epithelium, RE) project to the ventroposterior MOB and those in Grueneberg ganglion (GG) to specific glomeruli in the caudal MOB. Npal = Nasopalatine duct. Nph = nasopharyngeal duct. The trigeminal system and the terminal nerve (or nervus terminalis) are not shown.**

Recent progress concerning each of the chemosensory organs and subsystems mentioned above will be summarized in this review.

I. CHEMORECEPTION VIA THE MAIN OLFACTORY EPITHELIUM

I.1 Anatomical Organization of the Main Olfactory System

The MOE is a pseudostratified neuroepithelium located in the posterior nasal cavity lining the cartilaginous turbinates and septum. In rodents, the MOE harbors 6 to 10 million OSNs, the principal sensing cells for odor molecules. The OSN axons form the olfactory nerve bundles that cross the cribriform plate and project to the main olfactory bulb (MOB) (Figure 2). The axon terminals of the sensory neurons make synaptic contacts with the dendritic arbors of second-order neurons in specialized structures called glomeruli, which are distributed as a layer under the surface of the bulb. The projection neurons in the MOB form the lateral olfactory tract and project to the primary olfactory cortex, including the anterior olfactory nucleus (AON), the olfactory tubercle (OT), the piriform cortex (Pir), and the entorhinal cortex (Ent) (Figure 2) (Haberly, 2001; Lledo *et al.*,

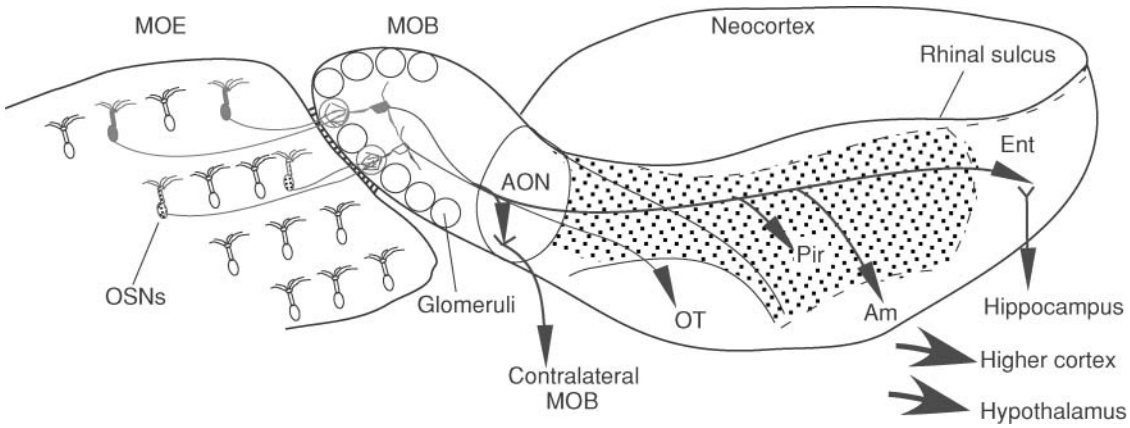

FIGURE 2 **The main olfactory system sends information to diverse regions in the brain. The schematic rodent brain is rotated upward by 45° to provide the ventrolateral view. The olfactory sensory neurons (OSNs) expressing a certain receptor are scattered in a broad band (zone) in the MOE, but their axons often converge onto two glomeruli and synapse with neurons in the main olfactory bulb (MOB). The projection neurons (mitral/tufted cells) send their axons in the lateral olfactory tract and project to the anterior olfactory nucleus (AON), the olfactory tubercle (OT), the piriform cortex (*Pir, dotted area*), the amygdala (*Am*) and the entorhinal area (*Ent*). Olfactory information is subsequently relayed to the higher cortex and the hypothalamus.**

2005). Olfactory information is subsequently relayed to the higher cortex for cognitive processing, which underlies perception of smell. The MOB is also connected with some limbic structures, including the nucleus of lateral olfactory tract, the anterior cortical nucleus, and the posterolateral cortical nucleus of amygdala (Am), presumably involved in the emotional aspects associated with odors.

Aside from the massive inputs to the higher cortex, the main olfactory system (via the connections from the cortical and limbic structures mentioned above) also carries the chemosensory information to the hypothalamus, a key regulator in behaviors such as feeding, aggression, and reproduction. The medial preoptic area of the hypothalamus contains most of the neurons secreting gonadotropin-releasing hormone (GnRH, also named LHRH for luteinizing hormone-releasing hormone), which plays a critical role in controlling the reproductive endocrine status. The GnRH neurons are shown to receive inputs from the main olfactory system in two recent studies using genetic approaches (Boehm *et al.*, 2005; Yoon *et al.*, 2005). Indeed, ablating key signal transduction elements in the MOE leads to impaired sexual and aggressive behaviors (Mandiyan *et al.*, 2005; Wang *et al.*, 2006). In addition, several physiological and imaging studies have demonstrated that the MOE and the MOB respond to both general odors and social cues (Lin *et al.*, 2005; Spehr *et al.*, 2006a; Wang *et al.*, 2006; Xu *et al.*, 2005). The emerging picture is that the main olfactory system functions in conscious perception as well as in subconscious processing of olfactory cues. These findings may explain why innate reproductive behaviors in organisms without a functional VNO (such as humans) can be affected by social semiochemicals including pheromones (Bhutta, 2007; Brennan and Zufall, 2006; Wysocki and Preti, 2004).

I.2 Odorant Receptors and Topographical Subdivisions

The OSNs in the MOE express more than 1000 G-protein–coupled seven-transmembrane odorant receptors (ORs). These receptors form the largest subfamily of GPCRs and the encoding genes account for ~3% of the total genes in the mammalian genome (Mombaerts, 2004). Each OSN stably expresses one functional odorant receptor (with few exceptions), which determines its response profile and its glomerular target in the MOB. Although the OSNs expressing a particular receptor are scattered in a broad band (zone) in the MOE, their axons typically coalesce onto two glomeruli (one medial and one lateral) in each bulb (Mombaerts, 2006). The medial and lateral glomeruli receive segregated inputs from the OSNs with the same receptor identity located in the medial and lateral aspects of the olfactory epithelium, respectively (Levai *et al.*, 2003; Schoenfeld and Knott, 2004).

Based on distinct OR expression patterns, the MOE was originally divided into four circumscribed zones (Ressler *et al.*, 1993; Vassar *et al.*, 1993). More recent studies suggest that ORs are expressed in multiple, overlapping zones arranged along the dorsomedial (center) to ventrolateral (periphery) axis, which correspond to the dorsal/ventral positioning of glomeruli in the olfactory

bulb (Iwema *et al.*, 2004; Miyamichi *et al.*, 2005). One exception to the zonal expression is the clustered distribution of the OSNs expressing the OR37 receptors (four functional members, OR37A, B, C, and E) in a small patch of the olfactory epithelium. The neurons expressing OR37 receptors project to a small region in the ventral bulb, with each receptor mainly corresponding to a single (instead of two) glomerulus (Strotmann *et al.*, 2000; Strotmann *et al.*, 2004). Within the olfactory epithelium, the numbers of OSNs expressing different ORs can vary tremendously. One extreme example is that half of the OSNs in the septal organ express a single receptor MOR256-3 (Section III).

The functional consequences of the zonal receptor expression are under active investigation. Such arrangement may render the ligand-receptor binding subject to modification by the physicochemical properties (stability, volatility, and water solubility) of the odorants during odor sampling (Kent *et al.*, 1996; Schoenfeld and Cleland, 2006; Scott, 2006). In general, hydrophilic molecules (which are highly absorptive and more retained in the aqueous mucus) preferentially encounter the OSNs in the dorsomedial region where the airflow reaches first, while hydrophobic molecules (which are less absorptive) tend to reach the ventrolateral region, supported by a numerical model of the nasal cavity in rats (Yang *et al.*, 2007). Consequently, the airflow rate in the nostril has a stronger effect on the responses induced by more hydrophilic molecules (Kent *et al.*, 1996; Scott *et al.*, 2006). Interestingly, the dorsomedial zone (zone 1) contains most of the fish-like class I ORs (Tsuboi *et al.*, 2006; Zhang *et al.*, 2004), which are presumably tuned to water-soluble molecules. Electroolfactogram (EOG) recordings demonstrate that the dorsal region is more responsive to hydrophilic odorants, while the ventral region is more responsive to hydrophobic odorants (Scott *et al.*, 2000). Matching the OR types with the physicochemical features of the odorants that each region tends to encounter may increase the efficiency of odor detection and contribute to the peripheral coding mechanisms.

I.3 Signal Transduction Mediated by the cAMP Pathway

The olfactory sensory neurons are responsible for detecting odor molecules and transforming the chemical energies into electrical signals. These bipolar neurons have a thin axon and a thick dendrite with a bulb-like end (called dendritic knob) bearing 10 to 20 cilia, which contain the odorant receptor proteins and associated signal transduction machineries (Figure 3). Binding of odor molecules to odorant receptors activates a series of events, which eventually lead to generation of action potentials sending the neural code to the olfactory bulb.

The ligand-bound OR activates an olfactory-specific G protein (G_{olf}), which in turn activates the adenylyl cyclase type III (ACIII). The cyclase catalyzes the production of cAMP, a second messenger that directly opens a cyclic nucleotide-gated (CNG) channel. This nonselective cation channel allows Na^+ and Ca^{2+} to flow into the cell, which depolarizes the cell membrane (Firestein, 2001; Frings, 2001). G protein-mediated

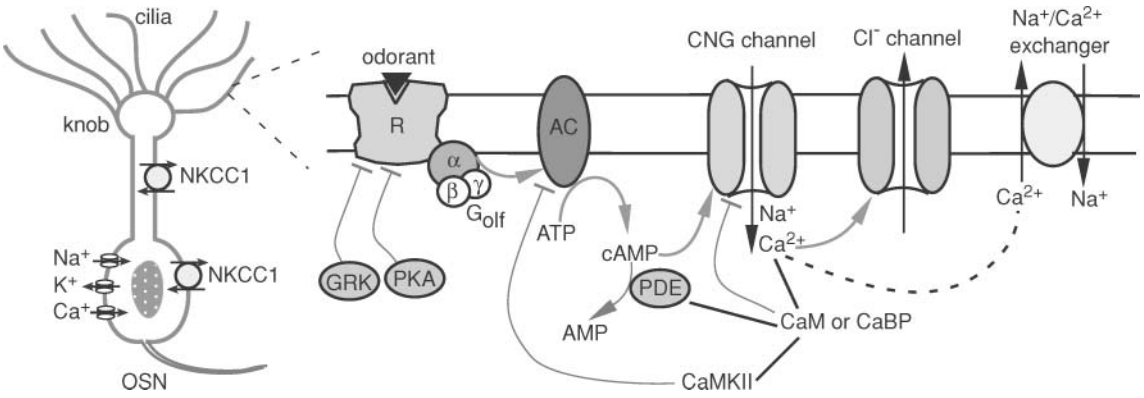

FIGURE 3 **The cAMP pathway underlies signal transduction in the olfactory sensory neurons. Within the cilia of an olfactory sensory neuron (OSN), binding of odor molecules to odorant receptors (*R*) triggers a cascade of enzymatic activity that leads to channel opening, thus transducing chemical energy into an electrical signal. G_{olf}, olfactory specific G protein. AC, adenylyl cyclase (type III). CNG channel, cyclic nucleotide gated channel. CaM = calcium-calmodulin. CaBP = calcium-binding protein. CaMKII = calmodulin kninase II. PDE = phosphodiesterase. PKA = protein kinase A. GRK = G protein coupled receptor kinase. NKCC1 = Na^+-K^+-$2Cl^-$ cotransporter.**

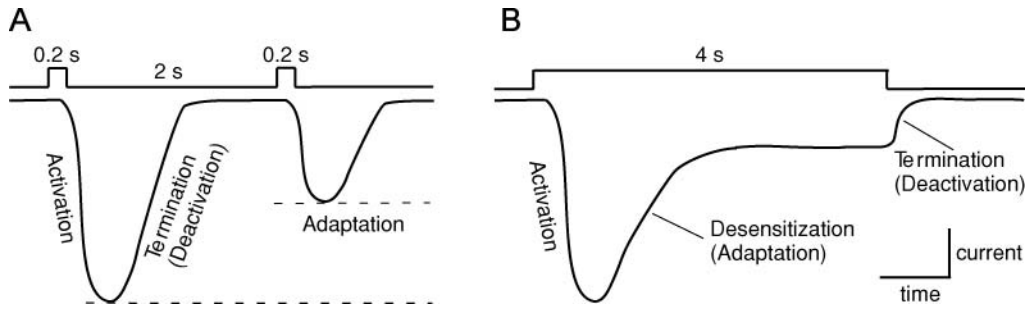

FIGURE 4 **Odor-induced transduction currents show fast adaptation and desensitization under different stimulation paradigms. The currents are schematically drawn based on patch-clamp recordings from vertebrate OSNs under voltage clamp mode with a holding potential close to the resting membrane potential (−60 mV). (*A*) Fast (or short-term) adaptation is induced by two brief odor pulses. The second pulse induces a smaller response than the first one when the inter-pulse interval is within a certain range. (*B*) Prolonged odor exposure causes desensitization (or adaptation) when the stimulation stays on, which is distinct from termination (or deactivation) when the stimulation is turned off.**

signal transduction generally yields substantial amplification. For example, one photoisomerized rhodopsin (photoreceptor in the retinal rod) activates many G protein molecules (transducin), and each G protein molecule leads to hydrolysis of many cGMP molecules. In contrast, a ligand-bound OR has a low probability of activating one G protein molecule (Bhandawat *et al.*, 2005), which may contribute to the overall limited cAMP production (Takeuchi and Kurahashi, 2005). The low amplification in the enzymatic cascade in the OSNs seems to be compensated by an unusual outward Cl^- current (activated by Ca^{2+} ions), which nonlinearly amplifies the transduction current carried by the CNG channel (Kurahashi and Yau, 1993; Lowe and Gold, 1993). The OSNs maintain a relatively high intracellular Cl^- concentration via Na^+-K^+-$2Cl^-$ cotransporters (NKCC1) located in the soma and dendrite (Kaneko *et al.*, 2004; Reisert *et al.*, 2005). The Ca^{2+} ions entered through the CNG channel (and subsequently via voltage-gated Ca^{2+} channels and internal Ca^{2+} stores) are eventually removed from the cell, presumably through the Na^+/Ca^{2+} exchangers (Pyrski *et al.*, 2007). The genes encoding the key elements in the cAMP pathway, including G_{olf}, ACIII, and CNG channel subunits have been cloned (Bakalyar and Reed, 1990; Dhallan *et al.*, 1990; Jones and Reed, 1989). Knocking out any of these genes dramatically reduces odorant-induced EOG signals recorded in the MOE and causes severe olfactory deficits at the behavioral level, strongly supporting the vital role played by the cAMP cascade in chemoreception (Belluscio *et al.*, 1998; Brunet *et al.*, 1996; Wong *et al.*, 2000). A recent report suggests that bestrophin-2 may function as the Ca^{2+}-activated Cl^- channel in the mouse OSNs (Pifferi *et al.*, 2006b). Further studies on the response properties of OSNs with disrupted bestrophin-2 function would help to confirm this finding.

The odorant-activated cAMP cascade in the OSNs, like all other G-protein mediated signal transduction pathways, is subject to negative feedback regulation, which contributes to response termination (or deactivation) and adaptation (Figure 4). Feedback regulation in this pathway can occur at multiple sites, and Ca^{2+} plays a critical role in many processes (Figure 3) (Matthews and Reisert, 2003; Zufall and Leinders-Zufall, 2000). The best studied site is the CNG channel (Pifferi *et al.*, 2006a), a heterotetramer assembled by three homologous subunits CNGA2:CNGA4:CNGB1b in a 2:1:1 stoichiometry (Zheng and Zagotta, 2004). Opening of the CNG channel upon odor stimulation leads to influx of Ca^{2+}, which in turn inhibits the CNG current by reducing the apparent binding affinity for cAMP (Kramer and Siegelbaum, 1992; Zufall *et al.*, 1991), mediated via a direct interaction between the channel and Ca^{2+}-calmodulin (Ca-CaM) (Chen and Yau, 1994; Liu *et al.*, 1994). Such inhibition is believed to play a dominant role in fast adaptation during repeated odor stimulation (Figure 4A), because similar adaptation can be reproduced by photorelease of caged cAMP or 8-Br-cAMP (a hydrolysis-resistant analog of cAMP), placing the action site downstream of cAMP formation (Boccaccio *et al.*, 2006; Kurahashi and Menini, 1997). Ca-CaM mediated inactivation of the CNG channel critically depends on the modulatory subunits, CNGA4 and CNGB1b, and lacking either one results in a much slower inhibition of the cAMP-induced CNG current in the presence of Ca-CaM (Bradley *et al.*, 2004; Bradley *et al.*, 2001; Michalakis *et al.*, 2006; Munger *et al.*, 2001). Consistent with the impaired odor adaptation in physiological recordings, CNGA4-null mice display strong deficits in

olfactory tasks with a background odor (Kelliher *et al.*, 2003). Because CNGA4 and B1b subunits are also required in ciliary trafficking of properly assembled CNG channels (Jenkins *et al.*, 2006; Michalakis *et al.*, 2006), it would be interesting to investigate the functional consequences of selective deletion of the CaM binding domain in the CNG channel (Song *et al.*, 2007).

Aside from the CNG channel, almost every key component in the cAMP pathway has been proposed to participate in some forms of negative feedback regulation. For instance, the ACIII activity is inhibited by phosphoralytion via Ca^{2+} activated CaM-dependent protein kinase II (CaMKII), which potentially attenuates the olfactory signal (Wei *et al.*, 1998). Inhibition of CaMKII reduces the amount of adaptation induced by sustained odor stimuli, but not by brief stimuli in a patch clamp study (Leinders-Zufall *et al.*, 1999), suggesting that down-regulation of ACIII activity becomes more important during prolonged odor stimulation. Consistent with this finding, the response to photorelease of caged cAMP, which mimics an odor response in a brief pulse, shows a slower and less profound decay than an odor response in a long pulse (Takeuchi and Kurahashi, 2002). Another target for Ca-CaM-dependent feedback regulation is phosphodiesterase 1C (PDE1C, isoform 2), which degrades cAMP and cGMP upon activation by Ca-CaM and is highly expressed in the cilia of OSNs (Borisy *et al.*, 1992; Yan *et al.*, 1995). A potent PDE blocker, 3-isobutyl-1-methylxanthine (IBMX), is widely used to activate the cAMP cascade in the vertebrate OSNs, suggesting that there are resting cyclase and PDE activities in these neurons. The exact role that PDE1C plays in olfactory signal transduction is still elusive and studies in the PDE1C null mice will shed light on this issue (Cygnar and Zhao, 2007). A Ca-CaM independent, cAMP-specific PDE (PDE4A) is highly expressed in most ciliated OSNs (Cherry and Davis, 1995; Juilfs *et al.*, 1997). Antibody staining indicates that PDE4A protein is abundant in the axons and dendrites, but absent from the cilia, and its function in signal transduction is undetermined. Additional mechanisms potentially involved in termination or adaptation of the olfactory signal include desensitization of the odorant receptors via phosphorylation (Boekhoff and Breer, 1992; Dawson *et al.*, 1993; Peppel *et al.*, 1997; Schleicher *et al.*, 1993), internalization of the odorant receptors mediated by β-arrestin2 (Mashukova *et al.*, 2006), down-regulation of ACIII activity by RGS2 (regulator of G-protein signaling, surprisingly acting on ACIII in the OSNs) (Sinnarajah *et al.*, 2001), depletion of Cl^- ions within the cilia (Lindemann, 2001), and activation of a carbon monoxide/cGMP cascade leading to a form of long-lasting adaptation (Zufall and Leinders-Zufall, 1997). The feedback processes mentioned above are likely activated under different odor stimulation paradigms (the duration ranges from a fraction of a second to minutes, hours or even longer) to ensure proper function of the sensory neurons under all conditions.

The cAMP cascade in the OSNs is also subject to modulation by other messengers or molecules. The CNG channel can be down-regulated by phosphatidylinositol 3,4,5-trisphosphate (PIP_3) via a direct interaction (Brady *et al.*, 2006; Zhainazarov *et al.*, 2004). Consistent with this finding, blocking the activity of phosphatidylinositol 3-kinase, which is essential for generating 3-phosphoinositides, significantly enhances odorant-induced calcium signals in some OSNs (Spehr *et al.*, 2002). Another molecule that modulates olfactory signaling is olfactory marker protein (OMP), expressed at high levels in all mature OSNs. OMP-null mice exhibit a higher odor detection threshold than wild-type mice (Youngentob and Margolis, 1999), and display altered odor responses in EOG recordings, *i.e.*, a smaller and slower response and a reduced recovery from adaptation (Buiakova *et al.*, 1996; Ivic *et al.*, 2000), although the molecular targets are yet ambiguous. In addition, odor-induced responses can be modified by ATP via the purinergic receptors expressed in OSNs (Hegg *et al.*, 2003), by dopamine via the D2 receptors expressed in mature OSNs (Coronas *et al.*, 1997; Hegg and Lucero, 2004; Koster *et al.*, 1999), and by adrenaline via its action on the voltage-gated channels (Kawai *et al.*, 1999). Considering the new findings that there are subsets of OSNs expressing distinct receptors (*e.g.*, TAARs) or transduction machineries (*e.g.*, TRPM5), olfactory signaling in the MOE is more complicated than previously appreciated.

I.4 Odor Response Profiles of Individual OSNs with Defined Receptors

A combination scheme is used by the OSNs expressing different ORs to encode odor quality and intensity (Buck, 2004; Firestein, 2001). Although a single sensory neuron expresses only one OR type, it can respond to multiple odorants with different thresholds

and dose-response relations. This is probably because a single receptor can broadly, yet selectively, bind to multiple odorants via relatively weak hydrophobic and van der Waals interactions (Katada *et al.*, 2005). Conversely, a single odorant can be recognized by multiple receptors that detect different molecular features. A given odorant at a higher concentration elicits stronger responses in individual neurons and recruits more receptors/neurons (Ma and Shepherd, 2000), presumably with lower affinities to that odorant. As a result, both odor quality and intensity are encoded by combinations of multiple receptors/neurons (Malnic *et al.*, 1999). A combinatorial strategy built on ~1000 receptors gives the olfactory system an almost unlimited capability of odor detection and discrimination. So far, the receptor-ligand relation has only been characterized for a small number of ORs, partly due to the technical difficulties in heterologous expression of mammalian ORs (Mombaerts, 2004). Identification of molecular chaperones and other factors involved in functional expression of OR proteins brings new hopes to efficiently deorphan this large family of GPCRs (Neuhaus *et al.*, 2006; Saito *et al.*, 2004; Shirokova *et al.*, 2005; Von Dannecker *et al.*, 2006). Most deorphaned mammalian ORs preferably respond to a few related odorants from a list of up to several hundreds, such as rat I7 to octanal (Araneda *et al.*, 2000; Zhao *et al.*, 1998), suggesting that ORs are relatively selective. However, physiological recordings indicate that single olfactory sensory neurons can be broadly tuned. For example, a large portion of olfactory sensory neurons in rats responds to multiple odorants with distinct chemical structures in an *in vivo* study (Duchamp-Viret *et al.*, 1999). It remains to be determined how the expressed ORs along with the cellular properties define the specificity and sensitivity of individual OSNs.

To understand how odor information is encoded in the olfactory epithelium, it is crucial to study the response profiles of individual OSNs with defined receptors. Intermingling of ~1000 types of OSNs in the MOE precludes such investigation in wild-type animals. Generation of OR gene targeted mouse lines has allowed genetic tagging and subsequent visualization of the OSNs expressing an endogenous OR (Mombaerts *et al.*, 1996). The response profiles of dissociated M71 or M72-expressing OSNs, tagged with green fluorescent protein (GFP), are characterized by monitoring the odorant-induced calcium signals in the cell bodies (Bozza *et al.*, 2002; Feinstein *et al.*, 2004). While the dynamic range of individual neurons to the ligands acetophenone and benzaldehyde is narrow (one order of magnitude), the overall sensitivity of M71 cells varies over two orders of magnitude. The heterogeneity in the responses of OSNs expressing the same OR is further confirmed in a patch clamp study (Grosmaitre *et al.*, 2006). Odor-induced transduction currents are directly measured under voltage clamp mode in GFP-tagged MOR23 cells situated in an intact, nondissociated olfactory epithelium (Figure 5A, B). All green cells respond to lyral, a known ligand for MOR23 (Touhara *et al.*, 1999), but with profound differences in response kinetics and sensitivity (Figure 5C, D), which would be uneasily explained by varying levels of receptor expression that follows a "normal" distribution. Besides the receptor protein level, intrinsic variations may exist in the enzymatic activity of the transduction cascade and the number and size of cilia, which could

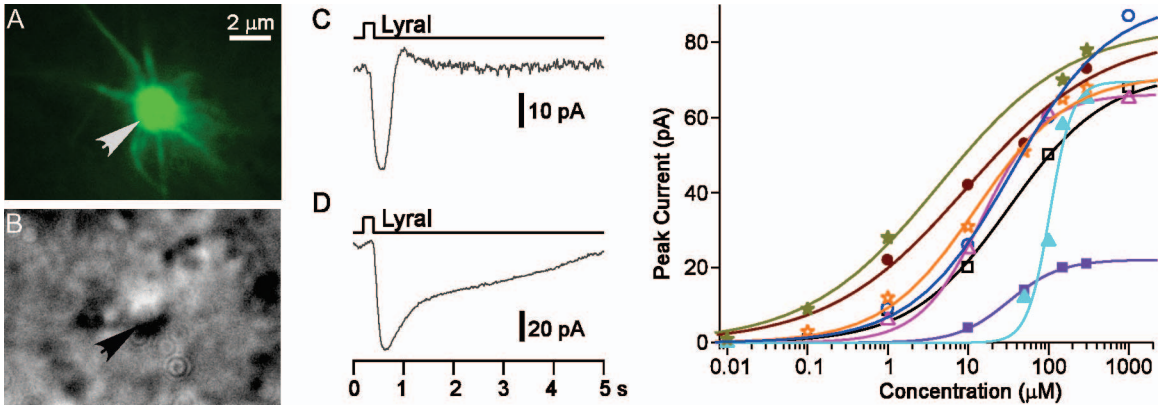

FIGURE 5 **Individual MOR23 cells vary in response kinetics and sensitivity to lyral. (*A*, *B*) Visualization of GFP-positive MOR23 cells in the intact epithelium under fluorescent (*A*) and transmitted light (*B*). (*C*, *D*) Two MOR23 cells display different response kinetics to brief pulses of 300 μM lyral under voltage clamp mode with a holding potential of −50 mV. (E) The dose-response curves of the peak transduction current from eight cells are fitted with Hill equations. Modified from (Grosmaitre *et al.*, 2006).**

result from the asynchronous maturation of these neurons and other unknown factors. Consequently, a single glomerulus, which receives inputs from a few thousand OSNs with heterogeneous responses, could display heterogeneity of the intraglomerular activity (Wachowiak *et al.*, 2004). It remains to be determined whether a glomerulus is as sensitive as the most sensitive OSNs that innervate it or whether new features emerge from convergence of heterogeneous inputs. A recent study demonstrates that individual glomeruli with a defined OR exhibit a different response profile and higher sensitivity than the corresponding OR characterized in a heterologous system and in isolated OSNs with the same OR (Oka *et al.*, 2006). Certainly, one cannot rule out that some of the discrepancies are related to the differences between the intact epithelium and dissociated cells or heterologous expression (see below).

Another unexpected finding in the MOR23 study is that cells in the intact epithelium respond to lyral with a very broad dynamic range, which covers ~3 log units of lyral concentration from threshold to saturation (Figure 5E). These observations are in sharp contrast with the dose-response relationships of dissociated amphibian or mouse OSNs, where the transduction currents or the calcium signals saturate within one to two log units (Bozza *et al.*, 2002; Firestein *et al.*, 1993; Reisert and Matthews, 1999; 2001). The dynamic range of the OSNs in the intact epithelium may depend, to some extent, on the microenvironment in which these neurons reside (the mucus as well as the supporting cells), in addition to the OR-ligand interaction and signal amplification. For instance, the mucus contains complex components such as odorant binding proteins, which may change the odorant concentrations that reach the cilia of the OSNs. When the odorant concentration is low, OBPs may help the odorant molecules to accumulate and deliver them to the cilia. But when the concentration is high, the mucus may act as a diffusion barrier and odorant binding proteins as buffers to reduce the concentration that reaches the cilia. Patch clamp recordings from labeled M71 cells in the intact epithelium have revealed a dynamic range that is wider than that of dissociated M71 cells, but narrower than that of MOR23 cells (unpublished data, Grosmaitre X.). Furthermore, washing out the mucus has a dramatic effect in the glomerular responses to certain odorants in an *in vivo* imaging study (Oka *et al.*, 2006). These studies highlight the importance of the peri-receptor events in odorant reception, which may have been underestimated in the past.

I.5 TAAR-Expressing OSNs in Amine Detection

In a large-scale search for additional GPCRs expressed in the olfactory epithelium, Liberles and Buck (2006) have discovered a second class of chemosensory receptors, the trace amine-associated receptors (TAARs). Originally identified in the brain as amine receptors (Borowsky *et al.*, 2001), TAARs share some sequence similarities with receptors of classical biogenic neurotransmitters, such as serotonin and dopamine, but not with odorant receptors. The TAAR family is found in all vertebrates, including fish (>100 members in zebrafish), amphibians (6 members in frog), rodents (15 members in mouse), and humans (5 members) (Gloriam *et al.*, 2005; Hashiguchi and Nishida, 2007; Liberles and Buck, 2006). The mouse TAARs are divided into nine subtypes (TAAR1 to 9) with each subtype containing one member, except for TAAR7 (five highly related members) and TAAR8 (three members). Eight of the nine subtypes (TAAR2-9) are primarily expressed in the olfactory epithelium in similar patterns as odorant receptors, *i.e.*, each TAAR member is detected in a small subset of OSNs scattered in discrete zones and is not coexpressed with other TAARs or odorant receptors. Some TAARs are present in the Grueneberg ganglion (Section IV). The TAAR-positive OSNs in the MOE coexpress $G_{\alpha\ olf}$, suggesting that these receptors transduce signals via the canonical cAMP pathway. In a heterologous expression system, interestingly but not surprisingly, several TAAR members are found to detect specific amines, including three that are present in the urine. One member (TAAR5) is activated by urine from sexually mature males only, but not from females or prepubescent males (Liberles and Buck, 2006). The current data support that the TAAR-expressing OSNs in the main olfactory system are involved in detecting social cues. Further studies on the central targets of these neurons and the behavioral deficits of knocking out these genes will help to unveil the specific function this subsystem serves.

I.6 TRPM5-Positive OSNs Transducing Chemical Signals via Dual Pathways

The transient receptor potential (TRP) ion channels are found to regulate the electrical signals in sensory cells responding to different stimuli, including touch, pain, temperature, sound, pheromones, and taste

(Peterlin *et al.*, 2007; Ramsey *et al.*, 2006). The TRP proteins are six-transmembrane (6-TM) cation-permeable channels, and opening of these channels leads to depolarization of the cell membrane. On the basis of sequence homology, mammalian TRP proteins can be grouped into six subfamilies: TRPC, TRPV, TRPM, TRPA, TRPP, and TRPML, which are activated by diverse mechanisms (Ramsey *et al.*, 2006). The GPCR-activated phospholipase C (PLC) pathway is shown to modulate some TRP channels, including TRPM5 originally identified in taste receptor cells (Perez *et al.*, 2002; Zhang *et al.*, 2003). In the TRPM5-GFP transgenic mice (the TRPM5 promoter drives GFP expression), GFP cells are surprisingly found in the olfactory epithelium (Lin *et al.*, 2007). Aside from a subpopulation of microvillar cells, TRPM5 expressing cells comprise many OMP-positive ciliated OSNs and most of them coexpress CNGA2. These neurons are also positively stained by antibodies against PLC $\beta 2$ and G protein $\gamma 13$ subunit, two components involved in taste transduction (Huang *et al.*, 1999), suggesting coexistence of the cAMP and PLC-TRPM5 pathways in individual OSNs. The TRPM5-positive OSNs are predominantly located in the ventrolateral areas of the olfactory epithelium and project to the ventral regions of the olfactory bulb, activated by exposure to mouse urine and putative pheromones (Lin *et al.*, 2007). With intact cAMP signaling, TRPM5 null mice do not show reduced odor or pheromone responses in EOG recordings from the MOE. However, in CNGA2 knockout mice, the PLC-TRPM5 pathway may mediate the residual odor or pheromone-induced responses (Lin *et al.*, 2004). It is undetermined whether the same ligand-receptor binding activates these two distinct transduction cascades in single OSNs.

I.7 GC-D Neurons Innervating the Necklace Glomeruli

A small subset of ciliated OSNs in the MOE clearly defines a unique chemosensory subsystem with distinct signal transduction machineries and central targets in the olfactory bulb. Unlike TAAR or TRPM5 expressing OSNs, these neurons do not express the key elements in the cAMP-signaling pathway, such as G_{olf}, ACIII, CNGA2, PDE1C, and PDE4A. Instead, they express type D guanylyl cyclase (GC-D), a cGMP-stimulated phosphodiesterase PDE2, and a cGMP-specific CNG channel previously identified in the retinal cone photoreceptors (Fulle *et al.*, 1995; Juilfs *et al.*, 1997; Meyer *et al.*, 2000). GC-D neurons are broadly distributed in the central region of the main olfactory epithelium, but their axons only innervate about 12 glomeruli in the main olfactory bulb (Juilfs *et al.*, 1997). These glomeruli are part of the "necklace glomerular complex," which apparently contains discrete subtypes and encircles the caudal main olfactory bulb (Ring *et al.*, 1997). The chemoreceptors expressed in GC-D neurons are not identified, but GC-D itself is speculated to interact with olfactory cues, because it shares sequence similarities with other guanylyl cyclases, known transmembrane receptors for peptides (Fulle *et al.*, 1995). It is unresolved how the proposed cGMP pathway mediates signal transduction in GC-D neurons, but this process is evidently independent of the cAMP pathway. Genetic deletion of CNGA2 causes a dramatic reduction of tyrosine hydroxylase expression (a measurement of the afferent activity to the olfactory bulb) in the majority of periglomerular (PG) neurons, but spares the PG cells associated with the PDE2-positive necklace glomeruli (Baker *et al.*, 1999). New studies shed light on the potential ligands that stimulate GC-D neurons. These neurons specifically respond to some components of urine, and the sensory responses are eliminated in GC-D knockout mice (Leinders-Zufall *et al.*, 2007). In addition, GC-D neurons coexpress carbonic anhydrase II (an enzyme that catalyzes the rapid conversion of carbon dioxide to bicarbonate and proton) and may serve as CO_2 sensors for rodents (Hu *et al.*, 2007). It would be interesting to know if detection of specific pheromones and CO_2 is integrated at the cellular and behavioral level.

II. CHEMORECEPTION VIA VOMERONASAL ORGAN

Besides the MOE, the rodent nose possesses a well developed VNO, a bilateral blind-ending tube encased within bony capsules in the ventral nasal septum (Figure 1). Lining the medial wall of the VNO is the sensory neuroepithelium, which harbors microvillar sensory neurons whose axons project to the accessory olfactory bulb (AOB). The projection neurons in the AOB directly send axons to the limbic system, including the medial amygdaloid nucleus, the posteromedial amygdaloid cortical nucleus, the bed nucleus

of the stria teminalis, and the nucleus of the accessory olfactory tract. These structures are, in turn, connected with several hypothalamic nuclei, such as the medial preoptic area, the ventromedial hypothalamus, and the premammilary and supraoptic nuclei (Dulac and Wagner, 2006; Meredith, 1998). Traditionally, the VNO is considered a specialized organ for detecting social cues (*e.g.*, pheromones) and involved in modulating behaviors such as aggression and mating. Recent findings indicate that the VNO readily detects small organic molecules including volatile odors that elicit responses in the MOE, suggesting a greater overlap of the ligands between the two systems in chemoreception (Brennan and Zufall, 2006; Dulac and Wagner, 2006; Spehr *et al.*, 2006b).

The accessory olfactory system clearly comprises at least two separate subsystems with different chemoreceptors and central targets. The sensory neurons in the apical neuroepithelium express vomeronasal receptor V1Rs (~150 members in the mouse) with G_i proteins and project to the anterior portion of the AOB, while the neurons in the basal neuroepithelium express V2Rs (~150 members forming a different gene family than V1Rs) with G_o proteins and project to the posterior portion of the AOB (Dulac and Torello, 2003; Halpern and Martinez-Marcos, 2003). The anterior and posterior parts of the AOB are then connected with many common targets as well as differentially innervated areas in the amygdala (Mohedano-Moriano *et al.*, 2007). Although both V1Rs and V2Rs belong to the GPCR superfamily, they share little sequence similarities with each other or with the ORs expressed in the MOE. Unlike ORs and V1Rs, V2Rs have a very large N-terminal extracellular domain, a feature shared by metabotropic glutamate and $GABA_B$ receptors (Dulac and Torello, 2003). Individual V2Rs are coexpressed with V2R2 receptor, a unique V2R member present throughout the basal layer (Martini *et al.*, 2001). Proper function of V2Rs also depends on selective association with M10 and M1 families of major histocompatibility complex molecules (Loconto *et al.*, 2003). Not surprisingly, the V1R-expressing neurons are better tuned to small organic molecules (Del Punta *et al.*, 2002; Leinders-Zufall *et al.*, 2000), while the V2R-expressing neurons tend to respond to larger peptides (Kimoto *et al.*, 2005; Leinders-Zufall *et al.*, 2004). The sensory neurons in the VNO bind appropriate ligands with high sensitivity and specificity, and a given ligand at a higher concentration does not recruit more receptors/neurons (Leinders-Zufall *et al.*, 2000). This is in sharp contrast with the combinatorial strategy used by the sensory neurons in the main olfactory system (Section I.4.).

Signal transduction in both V1R and V2R expressing neurons relies on a PLC-mediated pathway. Via different G proteins, ligand-binding in the VNO sensory neurons activates PLC, which results in production of phosphatidylinositol-3-phosphate (IP3) and diacylglycerol (DAG), followed by generation of arachidonic acid. These processes eventually lead to the opening of a TRP cation channel (TRPC2) localized in the microvilli of the sensory neurons (Liman *et al.*, 1999; Lucas *et al.*, 2003). The critical role that TRPC2 plays in pheromone detection in the VNO is further confirmed by targeted gene deletion. TRPC2 null mice exhibit reduced electrophysiological responses to urines and pheromones recorded in the VNO as well as impaired aggressive and mating behaviors (Leypold *et al.*, 2002; Stowers *et al.*, 2002). However, detection of some major histocompatibility peptides by the basal zone is apparently independent of TRPC2-mediated transduction (Kelliher *et al.*, 2006).

In addition to pheromones, some VNO neurons are responsive to volatile odorants, which may trigger instinctive behaviors (Sam *et al.*, 2001). This finding is further supported by a study using ACIII knockout mice, in which odor detection via the main olfactory epithelium is severely impaired (Trinh and Storm, 2003). The ACIII null mice still detect certain odorants, which elicit electrical responses in the VNO. It is unclear whether the volatile odorants can directly bind to V1Rs or V2Rs and consequently activate the same PLC-TRPC2 pathway. Identification of certain ORs in a subset of VNO neurons provides an alternative mechanism underlying detection of volatile odorants by the VNO (Levai *et al.*, 2006). The OR-positive VNO neurons lack ACIII and Golf, but instead express G_i and TRPC2, and project to the anterior part of the AOB. These neurons seem to form a distinct subpopulation within the apical zone of the VNO. Taken together, current evidence indicates that certain compounds (both odorants and pheromones) are processed in parallel by the main and accessory olfactory system, suggesting that these compounds may serve both as common odors leading to olfactory perception and as social cues to trigger innate behaviors.

III. SEPTAL ORGAN OF MASERA

III.1 Odorant Receptors and Signal Transduction in the Septal Organ

The septal organ is a small island of olfactory neuroepithelium lying bilaterally at the ventral base of the nasal septum near the entrance of nasopharynx (Figure 1). It is also named as the "Organ of Masera" after the first detailed description by Rodolfo-Masera (1943). The basic architecture in the MOE is maintained in the septal organ, which contains both ciliated OSNs (the dominant type) and microvillar cells (Miragall *et al.*, 1984). However, some morphological differences are observed between these two systems. The septal organ contains fewer layers of OSNs (two to three layers), compared with the MOE (six to eight layers in most regions) (Ma *et al.*, 2003; Weiler and Farbman, 2003). Furthermore, the septal organ OSNs have flattened somata, shortened dendrites and larger dendritic knobs, representing one of the rare differences described among the otherwise uniform morphology of ciliated OSNs in the MOE (Ma *et al.*, 2003). Most septal organ neurons express G_{olf} and ACIII, suggesting that signal transduction is essentially mediated by the canonical cAMP pathway (Section I.3.), which is further supported by patch clamp recordings from individual OSNs in this region. Odorant responses are mimicked by an adenylyl cyclase activator and a phosphodiesterase inhibitor, and these responses are blocked by an adenylyl cyclase inhibitor and eliminated in CNGA2 null mice (Grosmaitre *et al.*, 2007; Ma *et al.*, 2003).

The odorant receptors expressed in the septal organ are well described. Using different degenerate primers in a cDNA cloning approach, two groups have collectively identified more than 120 candidate OR genes in the mouse septal organ (Kaluza *et al.*, 2004; Tian and Ma, 2004). However, the expression levels of individual OR genes vary dramatically, verified by Affymetrix genechips covering all the mouse olfactory receptor genes (a high-density oligonucleotide array suitable for monitoring the expression of a large number of genes simultaneously) (Zhang *et al.*, 2004) and *in situ* hybridization (a method of detecting transcribed mRNAs of certain genes by specific antisense RNA probes). The septal organ mainly expresses only a few abundant receptors, with the most predominant OR (MOR256-3 or SR1) in nearly 50% of the cells and the nine most predominant ORs (MOR256–3, 244–3, 235–1, 0–2, 256–17, 236–1, 160–5, 122–1, and 267–16) together in ~95% of the cells. The unusually high density of MOR256-3 cells in the septal organ raises the question whether a single cell expresses one OR type. Experiments with combined OR probes in double *in situ* hybridization reiterate the one cell-one receptor tenet (with very few exceptions) (Figure 6A). Comparable evidence has been missing from the MOE due to the vast number of ORs expressed in any given region. All nine abundant septal organ ORs are also expressed in the most ventrolateral zone of the MOE, even though the relative abundance does not match that in the septal organ (Tian and Ma, 2004). Identification of a few abundant ORs, plus many scarce ones in the septal organ, is consistent with its projection pattern to the main olfactory bulb (Giannetti *et al.*, 1992; Levai and Strotmann, 2003; Ma *et al.*, 2003). Neurotracing experiments demonstrate that the septal organ OSNs send axonal fibers mainly to the posterior, ventromedial olfactory bulb and target onto a few densely labeled glomeruli, plus many (up to 150) lightly labeled ones (Levai and Strotmann, 2003). The densely labeled glomeruli receive inputs mainly (if not exclusively) from the septal organ (so called "septal" glomeruli), while the lightly labeled glomeruli receive mixed inputs from the septal organ and the MOE.

Although the septal organ primarily covers a small fraction of the receptor repertoire, surprisingly, individual sensory neurons are broadly tuned to diverse chemicals, demonstrated by patch clamp recordings (Figure 6B) (Grosmaitre *et al.*, 2007). These neurons are extremely sensitive to several randomly chosen odorants with a nanomolar threshold and a wide dynamic range, which covers 3 to 4 log units of concentration from threshold to saturation (Figure 6C). These results suggest that the septal organ, situated in the air path, may serve as a general odor detector by responding to many odorants with high sensitivity, thus supporting an alerting role. An alternative and complementary hypothesis is proposed that the septal organ functions as a "mini-nose" in surveying food odors as well as social cues (Breer *et al.*, 2006).

III.2 Mechanical Sensitivity of OSNs in the Septal Organ and the MOE

The mammalian OSNs are recently found to detect two distinct sensory modalities transmitted by chemical and mechanical stimuli. As revealed in patch-clamp recordings, most septal organ neurons respond not only to odorants, but also to mechanical stimuli

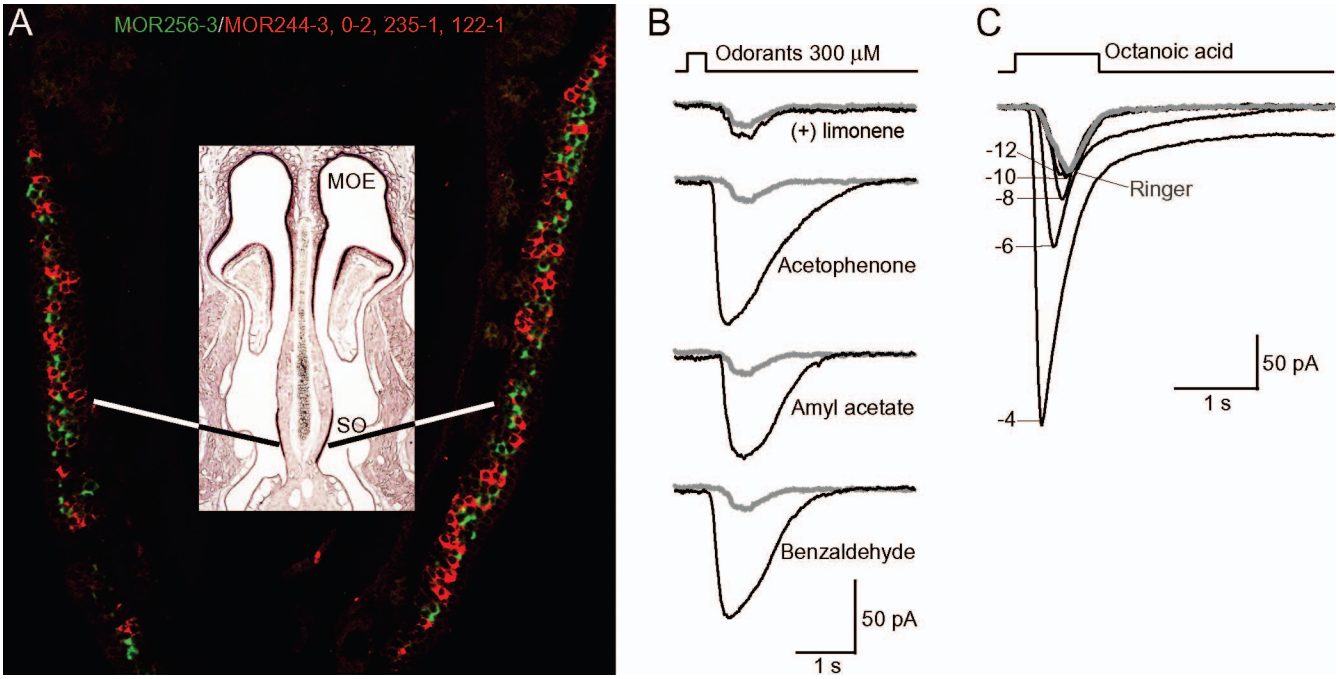

FIGURE 6 **The septal organ neurons predominantly express a few odorant receptors and respond to a wide range of odorants. (*A*) A single neuron expresses only one receptor gene demonstrated by double *in situ* hybridization. The section is hybridized with fluorescein-labeled MOR256-3 antisense RNA probe (green) and a mixture of four Digoxigenin-labeled probes (*red*) including MOR244–3, 0–2, 235–1, and 122–1. The middle inset indicates the location of the septal organ (SO) in a coronal section labeled by an OMP antisense probe. MOE, main olfactory epithelium. The image appears in color online. (*B*) A single neuron responds to multiple odorants (except (+) limonene) at 300 μM, recorded using perforated patch clamp technique. Inward currents are elicited by odor and Ringer puffs under voltage-clamp mode with a holding potential of -60 mV. (*C*) A single neuron responds to octanoic acid puffs at different concentrations (10^{-12} to 10^{-4} M) under voltage-clamp mode with a holding potential of -60 mV. The gray trace indicates the response induced by puffing Ringer. Image in (*A*) is provided by Tian H. and recordings in (*B*) and (*C*) are taken from (Grosmaitre *et al.*, 2007).**

delivered by pressure ejections of odor-free Ringer solution (Figures 6 and 7). The mechanical responses directly correlate with the pressure intensity and similar mechanosensitivity also exists in ~50% of the neurons in the main olfactory epithelium attached to the nasal septum. The responses occur with relatively long delays and are completely blocked by an adenylyl cyclase inhibitor, suggesting involvement of cAMP as a second messenger (Figure 7A). Elimination of mechanosensitivity in the OSNs from CNGA2 knock-out mice further supports this notion (Figure 7B). Both chemical and mechanical responses of the OSNs are likely mediated by a shared cascade involving cAMP and the CNG channel (Grosmaitre *et al.*, 2007). Identification of the mechanical sensor in the OSNs in future studies will address the issue of whether the mechanosensitivity is tied to certain OR types.

The mechanosensitivity found in the OSNs is especially interesting, because these neurons are situated in the nostril and constantly experience episodic pressure changes carried by the airflow. One possible role of the mechanosensitivity is that when the air flows faster in the nose, such as during a powerful sniff, it can enhance the firing probability and frequency of individual OSNs weakly stimulated by odorants. In addition to the critical roles in odor delivery and sampling (Section I.2.; Verhagen *et al.*, 2007), sniffing may increase the overall sensitivity of the olfactory system via the mechanosensitivity of the sensory neurons (Grosmaitre *et al.*, 2007). A second role the mechanosensitivity of the OSNs may play is to synchronize the rhythmic activity (theta-band oscillation) in the olfactory bulb with the breathing cycles, even in the absence of odorants, priming the system for processing odor information. In CNGA2null mice, the OSNs fail to exhibit odorant and mechanical responses (Figure 7B), and the coupling between the bulb rhythmic activity and respiration is drastically reduced (Figure 7C). Therefore, the mechanosensitivity of the OSNs, in addition to episodic access to odorants, may cause the respiration-coupled, odorant-induced activity in the olfactory epithelium (Chaput, 2000), the olfactory bulb (Adrian, 1951; Cang and Isaacson, 2003; Luo and Katz, 2001; Macrides and Chorover, 1972; Onoda and Mori, 1980; Philpot *et al.*, 1997; Spors *et al.*,

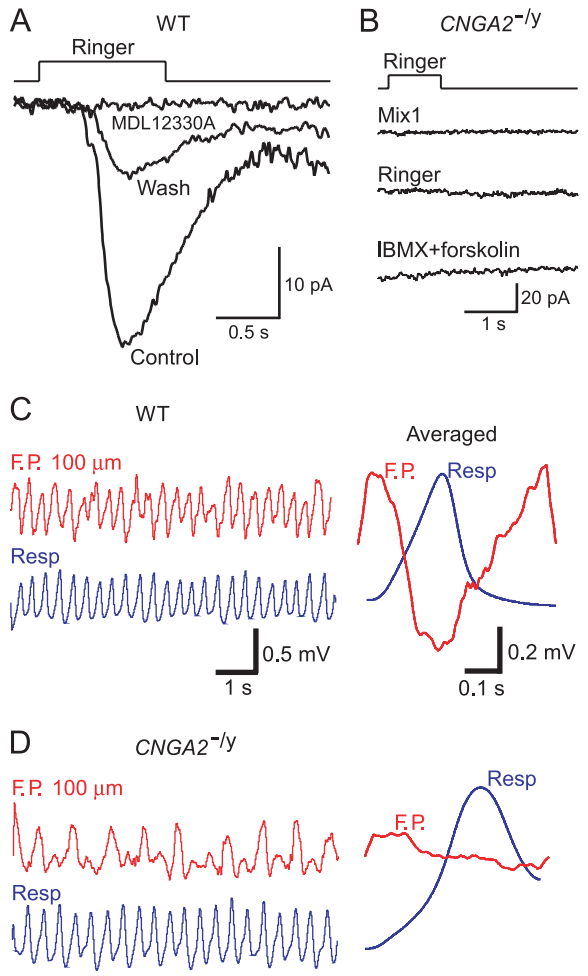

FIGURE 7 **The cAMP mediated mechanosensitivity of the OSNs may drive the olfactory bulb activity to synchronize with the breathing cycles. (*A*) The inward currents induced by Ringer puffs under voltage-clamp mode are reversibly blocked by 50 μM MDL12330A, an adenylyl cyclase inhibitor. (*B*) Both odorant and mechanical responses are eliminated in *CNGA2*$^{-/y}$ mice. The holding potential is −60 mV in (*A*) and (*B*). (*C*, *D*) Oscillatory field potentials (F.P.) in the olfactory bulb are recorded (at a depth of 100 μm from the surface) in wild-type (WT) (C) or CNGA2$^{-/y}$ mice (*D*). Traces marked with Resp indicate the respiratory rhythm. The averaged field potential within one respiratory cycle is shown in the right column of each panel. To calculate the averaged traces, the field potentials within individual respiratory cycles are truncated, normalized to the same length, and then averaged over 200 sec. Modified from Grosmaitre *et al.* (2007).**

2006; Ueki and Domino, 1961), and the olfactory cortex (Fontanini *et al.*, 2003; Rennaker *et al.*, 2007). The mechanosensitivity of OSNs provides new insights into the important roles played by sniffing in olfactory perception (Mainland and Sobel, 2006). In fact, sniffing alone is sufficient to induce activities in the olfactory cortex in human subjects (Sobel *et al.*, 1998). It would be critical to determine how the "odor maps" along the olfactory pathway are modified under different breathing and sniffing patterns.

IV. GRUENEBERG GANGLION

The Grueneberg (also spelled Gruneberg) ganglion is located bilaterally at the anterior tip of the nasal septum (Figure 1), which contains clusters of neurons, unlike the pseudostratified epithelium in the MOE, the VNO and the septal organ. This ganglion is originally thought to be nonsensory and part of the terminal nerve system (Gruneberg, 1973). However, recent studies have disclosed that these neurons express OMP, a marker for mature chemosensory neurons, albeit they lack the typical chemoreceptive structures such as cilia or microvilli. The axons of these neurons fasciculate into several nerve bundles and terminate in ~10 glomeruli in the caudal MOB, the same region as the necklace glomeruli (the central targets of GC-D neurons, Section I.7.) (Fleischer *et al.*, 2006a; Fuss *et al.*, 2005; Koos and Fraser, 2005; Roppolo *et al.*, 2006; Storan and Key, 2006). The majority of OMP-positive neurons in this organ expresses V2r83, a V2R subtype sharing 99% amino acid sequence identity with V2R2 and likely encoded from the same gene as V2R2 (a receptor expressed in all basal VNO cells, Section II). The remaining OMP-positive cells (V2r83-negative) express several subtypes of TAARs (Fleischer *et al.*, 2006b; Fleischer *et al.*, 2007). Certain ORs are transiently expressed in the Grueneberg ganglion. For instance, MOR256-17 is expressed in very few cells at the embryonic stages, but its expression disappears at postnatal stages (Fleischer *et al.*, 2006b). Although the potential ligands and signal transduction cascades have not been identified, most (if not all) cells in the Grueneberg ganglion coexpress G_o and G_i (Fleischer *et al.*, 2006b). Expressing V2r83 and TAARs in the Grueneberg ganglion suggest that these neurons may play a role in detecting pheromone-like molecules. The number of OMP-positive cells is particularly high in perinatal stages, followed by a decline in postnatal development, suggesting a more important function of this organ in newborns (Fleischer *et al.*, 2007; Fuss *et al.*, 2005). Since a subset of the necklace glomeruli (the "modified glomerular complex") in rodent pups is activated during suckling behavior (Greer *et al.*, 1982; Teicher *et al.*, 1980), it is tempting to link the Grueneberg ganglion with newborn behaviors.

V. CONCLUSIONS

Organization of the mammalian nose is more complicated than previously appreciated. Besides the four physically segregated apparatuses (MOE, VNO, septal

organ, and Grueneberg ganglion), both MOE and VNO consist of several subsystems. More subsystems may still emerge with availability of new molecular markers and detailed functional analysis. Future studies along the following lines will shed light on how the mammalian nose, via detection of common odors and social cues, influences behaviors such as feeding, mating, and aggression. First, genetic labeling of each subtype of the sensory neurons combined with physiological recordings at the cellular level will help to define the precise "odor space" each subsystem responds to. Such studies will provide new insights into the molecular and cellular mechanisms underlying signal transduction, which are still ambiguous for many of the subsystems. Second, investigation of the neural network beyond the olfactory bulb each subsystem is involved in will offer important functional implications. This may require sophisticated neurotracing techniques combined with genetic approaches for most subsystems. Finally, surgical removal (*e.g.*, the septal organ and the Grueneberg ganglion) or genetic ablation (*e.g.*, GC-D, TRPM5 or TAAR expressing neurons) combined with appropriate behavioral tests will help dissect out the exact role(s) each subsystem serves. This approach may be complicated by the built-in redundancy in the olfactory system, *i.e.*, multiple subsystems can process the same cues in a parallel fashion, and may require combined deletion of more than one system to unveil the behavioral deficits. Taken together, these studies will lead to a better understanding of the complex interplay among the multiple chemosensory systems as well as of the way information processing occurs from the nose to the brain.

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