Subsystem Organization of the Mammalian Sense of Smell

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Abstract

The mammalian olfactory system senses an almost unlimited number of chemical stimuli and initiates a process of neural recognition that influences nearly every aspect of life. This review examines the organizational principles underlying the recognition of olfactory stimuli. The olfactory system is composed of a number of distinct subsystems that can be distinguished by the location of their sensory neurons in the nasal cavity, the receptors they use to detect chemosensory stimuli, the signaling mechanisms they employ to transduce those stimuli, and their axonal projections to specific regions of the olfactory forebrain. An integrative approach that includes gene targeting methods, optical and electrophysiological recording, and behavioral analysis has helped to elucidate the functional significance of this subsystem organization for the sense of smell.

INTRODUCTION

MOS: main olfactory system

MOE: main olfactory epithelium

OSN: olfactory sensory neuron

MOB: main olfactory bulb

Semiochemical: a chemosensory stimulus that communicates information between animals

To many, the nose appears to be a unitary organ with a single sensory role: to detect odors. However, the role of olfaction is broad in humans and other mammals. The sense of smell helps to identify food, to assay its quality, and to enhance its flavor. The activation of nasal chemosensory cells warns of potential toxins or pathogens. The olfactory system even detects information about reproductive status, gender, and genetic identity. In all these cases, the activation of chemosensory cells in the nasal cavity initiates a process of neural recognition that can influence behaviors, hormonal state, and mood.

How does the olfactory system accomplish so many diverse tasks? It has become increasingly clear that the concept of a single olfactory system is grossly oversimplified, even wrong. The olfactory system is actually composed of a number of subsystems, some well known and others only recently characterized (**Figure 1**) (1, 2). These subsystems may be anatomically segregated within the nasal cavity, and they each make distinct neural connections to regions of the olfactory forebrain. They are clearly distinguished by the receptors they express and the signaling mechanisms they employ to detect and transduce chemosensory stimuli. And they respond, sometimes quite specifically, to a plethora of diverse molecules that range from volatile odors to peptides and proteins. In this review, we discuss a number of olfactory subsystems in the mammalian nose. In particular, we emphasize exciting recent results that elucidate the chemosensory selectivity and transduction machinery of these subsystems. Those readers interested in a more comprehensive discussion of the main or accessory olfactory systems are encouraged to consult any of a number of reviews (e.g., References 3–12).

MAIN OLFACTORY SYSTEM

The main olfactory system (MOS) (**Figure 2**) consists of

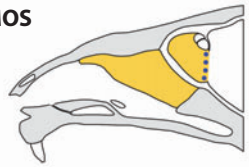
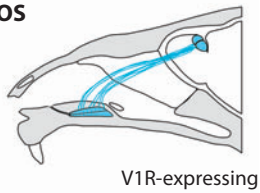
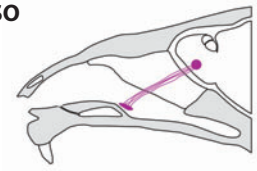
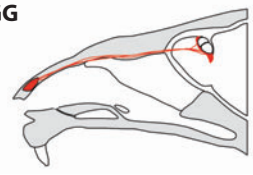
1. the main olfactory epithelium (MOE), which contains ciliated olfactory sensory neurons (OSNs), microvillar cells of uncertain function, sustentacular cells that may serve a glia-like role, and populations of progenitor/stem cells that replenish this regenerating tissue;
2. the main olfactory bulb (MOB), a region of the forebrain innervated by axons of the OSNs and serving as the first processing center of olfactory information; and
3. higher olfactory centers that receive direct or indirect information from the MOB.

It is well established that the MOS is a broadly tuned odor sensor; it responds to thousands of volatile chemicals carrying information about the quality of food and the presence of pathogens, prey, predators, or potential mates (6). There is a growing recognition that the MOS is also responsive to semiochemicals that may elicit specific behaviors or hormonal responses (12). This functional heterogeneity suggests that the MOS contains several olfactory subsystems that serve specific chemosensory roles.

Indeed, distinct subpopulations of OSNs can be defined by their expression of specific chemosensory receptors, enzymes, and ion channels (7). Although the anatomical divisions of these OSN subpopulations are not clearly

Figure 1

Chemosensory subsystems in the mouse nose. Each is composed of heterogeneous cell populations. Abbreviations used: ACIII, adenylyl cyclase type III; AOB, accessory olfactory bulb; AOS, accessory olfactory system; CAII, carbonic anhydrase type II; CNG, cyclic nucleotide–gated channel; GG, Grueneberg ganglion; IP_3R3, inositol 1,4,5-trisphosphate receptor 3; MOB, main olfactory bulb; MOS, main olfactory system; N.D., not determined; OR, odor receptor; OSN, olfactory sensory neuron; PDE, phosphodiesterase; PLC, phospholipase C; TAAR, trace amine–associated receptor; V1R, type 1 vomeronasal receptor; V2R, type 2 vomeronasal receptor; TRPC2/6, transient receptor potential channel types C2 and C6; TRPM5, transient receptor potential channel type M5; VSN, vomeronasal sensory neuron.

System	Principal target	Signal transduction components	Stimuli
MOS 			
Canonical OSNs	Glomeruli in general MOB	ORs, ACIII, $G\alpha_{olf}$, PDE1C2, PDE4A, CNGA2, CNGA4, CNGB1b	Volatile and nonvolatile (?) odor ligands
TAAR-expressing neurons	N.D.	TAARs, $G\alpha_{olf}$	Volatile amines
GC-D^+ neurons	Necklace glomeruli (dark circles, above)	GC-D, PDE2, CNGA3, CAII	Uroguanylin, guanylin, cues in urine, CO_2
TRP-expressing cells	TRPM5: glomeruli in medial, ventral, and lateral MOB TRPC2: N.D. TRPC6: restricted to MOE	TRPM5, Gγ13, CNGA2, PLCβ2 TRPC2 TRPC6, IP_3R3, PLCβ2	TRPM5: 2,5-dimethylpyrazine, 2-heptanone TRPC2: N.D. TRPC6: lilial, volatile odor ligands
V1R-expressing cells	N.D.	V1Rs	N.D.
AOS 			
V1R-expressing VSNs	Rostral AOB	V1Rs, $G\alpha_{i2}$, TRPC2, PDE4A	Volatile phermones
V2R-expressing VSNs: H2-Mv^- H2-Mv^+	Anterior part of caudal AOB Posterior part of caudal AOB	V2Rs, $G\alpha_o$, TRPC2 V2Rs, $G\alpha_o$, TRPC2, H2-Mv	Genetically encoded ligands (peptides, proteins)
OR-expressing VSNs	Rostral AOB	ORs, $G\alpha_{i2}$, TRPC2	General odor ligands (?)
SO 			
Canonical OSNs	Posterior part of the ventromedial MOB	ORs, ACIII, $G\alpha_{olf}$, CNGA2	Volatile odors
GC-D^+ neurons	Necklace glomeruli (?)	GC-D, PDE2	N.D.
GG 	Dorsocaudal MOB, near AOB and necklace glomeruli	TAARs V2R83, $G\alpha_o$, $G\alpha_{i2}$, ORs, $G\alpha_{olf}$	Alarm signal
Trigeminal system			
Solitary chemosensory cells α-gustducin$^+$	Trigeminal nerve (ethmoid nerve)	T2Rs, α-gustducin, TRPM5, PLCβ2 or PLCγ13	General odor ligands (lilial, citral, geraniol), CO_2

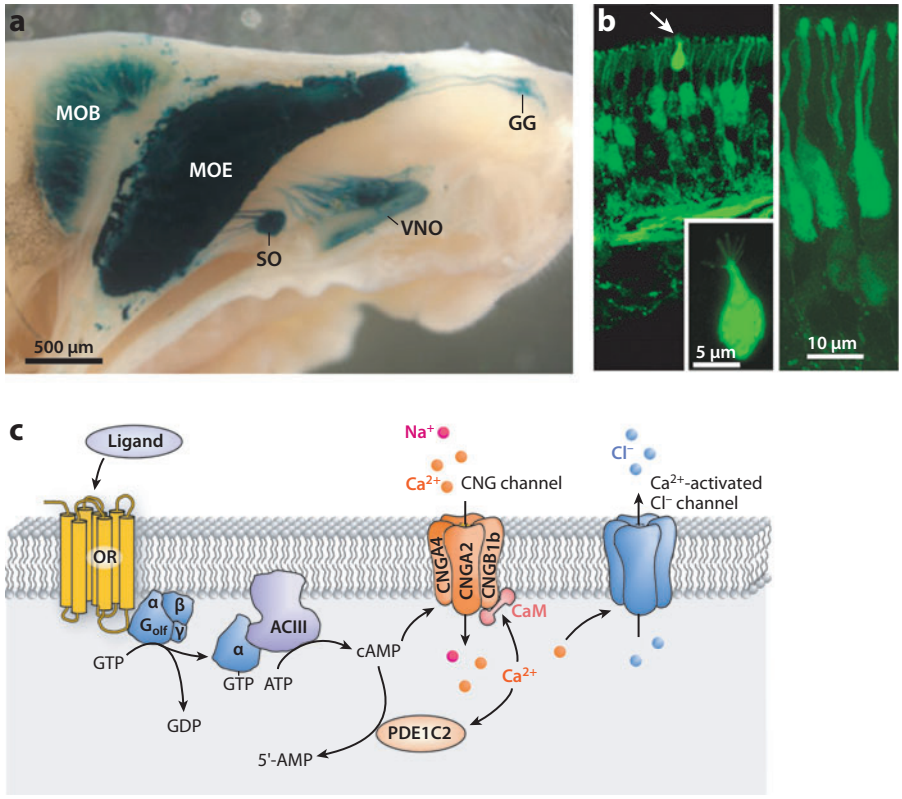


Figure 2

Organization of the sense of smell in mice. (*a*) Sagittal whole-mount view of the nasal cavity and forebrain of an *Omp-IRES-tau:LacZ* mouse (168) stained blue with X-Gal. Abbreviations used: MOB, main olfactory bulb; MOE, main olfactory epithelium; SO, septal organ of Masera; VNO, vomeronasal organ; GG, Grueneberg ganglion. Modified and reprinted from Reference 159 with permission from Wiley. (*b*) TRPM5 promoter–driven green fluorescent protein reveals microvillar (*arrowhead* and *inset*) and ciliated (*right panel*) TRPM5$^+$ MOE cells. Modified and reprinted with permission from Reference 93. Copyright 2007, National Academy of Sciences. (*c*) Schematic representation of the cAMP second messenger cascade of canonical olfactory sensory neurons (OSNs). Abbreviations used: ACIII, adenylyl cyclase type III; CaM, calmodulin; CNGA2/A4/B1b, cyclic nucleotide–gated channel types A2, A4, and B1b; OR, odor receptor; PDE, phosphodiesterase. Odor activation of one of many hundred OR family members expressed in canonical OSNs initiates a cAMP-mediated signaling cascade that results in the depolarization of these cells.

delineated within the MOE, each subpopulation displays unique patterns of MOB innervation. For example, canonical OSNs express components of an adenosine 3′:5′-cyclic monophosphate (cAMP)-mediated signaling cascade to transduce odors (6), whereas MOE neurons expressing the guanylyl cyclase GC-D employ a guanosine 3′:5′-cyclic monophosphate (cGMP)-mediated sensory transduction mechanism (13–16). These two subpopulations of MOE sensory neurons make distinct connections in the MOB (7), suggesting that these OSN subpopulations and their MOB targets are parts of specialized olfactory subsystems, each of which may process different subsets of chemosensory information.

Canonical Olfactory Sensory Neurons

A primary role of the MOS is to detect a wide array of odorants. Canonical OSNs are critical for this task. Each canonical OSN expresses one

of hundreds of distinct OR-type odor receptors, utilizes a cAMP-mediated cascade to transduce odor stimuli, and sends odor information to the MOB. Thus, canonical OSNs serve as the general chemosensory cells of the MOS.

Cellular and molecular organization. Canonical OSNs are bipolar, ciliated neurons within the MOE. These cilia are the site of odor transduction and contain the receptors, enzymes, and ion channels required for this process. Each OSN sends a single, unbranched axon to the MOB, where the axon makes synaptic contact with interneurons and projection neurons within 1 of up to 2000 glomeruli (6).

The functional identity of these sensory cells is most clearly defined by their expression of OR-type odor receptors (17) (**Figure 2*c***). ORs are members of the G protein–coupled receptor (GPCR) superfamily (8, 17). Although >1000 intact OR genes have been identified in mice (and ~350 in humans) (18–21), the vast majority have been defined as odor receptors on the basis of sequence homology alone. Thus, most ORs await functional annotation through deorphaning and localization to OSNs.

It appears that each canonical OSN expresses only a single OR gene (8). Those OSNs expressing a particular OR are restricted to only part of the MOE, although they are randomly distributed throughout that zone (8). Axons of OSNs expressing the same OR converge on a few glomeruli (usually two) within the MOB. This precise pattern of axonal targeting and convergence is influenced by many molecular factors, with the OR itself playing a critical, although as-yet-undefined, role in the process (8).

It was recognized more than two decades ago that most odors are transduced by a G protein–coupled, cAMP-mediated signaling mechanism (**Figure 2*c***) that includes a stimulatory G protein, adenylyl cyclase, and a cAMP-gated ion channel (22–26). Three of the key molecules in the cascade were soon cloned: the G protein subunit $G\alpha_{olf}$ (27), a calcium-sensitive adenylyl cyclase (type III, or ACIII) (28), and the olfactory cyclic nucleotide–gated (CNG) channel subunit CNGA2 (29). Two other subunits of the olfactory channel, CNGA4 (30, 31) and CNGB1b (32), were identified several years later. The protein(s) responsible for a Ca^{2+}-activated chloride conductance important for amplification of the odor response (33–35) has not been definitively identified, although intriguing candidates have been proposed (e.g., Reference 36). Proteins that may modulate the transduction cascade have also been identified: The guanine-exchange factor Ric-8B contributes to amplification of the odor signal through its actions on G proteins (37, 38), whereas two cyclic nucleotide phosphodiesterases (PDEs), PDE1C2 (39) and PDE4A (40), most likely participate in the adaptation or the termination of odor responses. Canonical OSNs also express olfactory marker protein (OMP) (41), which is critical for modulating the amplitude and kinetics of the odor response (42).

Chemoreceptive properties. Most OSNs recognize multiple odorants (6). ORs themselves are broadly tuned (8, 43, 44), and multiple ORs can respond to the same odorant, although usually with different efficacies (8). Thus, odors are encoded through use of a combinatorial strategy (5, 45). OSNs expressing the same OR converge on different sets of MOB glomeruli such that the activation of a group of glomeruli represents the presence of an individual odorant (46). The convergent innervation of individual glomeruli by OSNs expressing the same OR argues that the glomerulus, not the receptor, is the fundamental unit of odor coding.

All the ORs deorphaned to date respond to volatile odorants of diverse chemical classes (e.g., References 8, 43, 44, 47, and 48). However, not all odorants activate ORs; some odorants are antagonists (47, 49, 50). For example, undecanal inhibits the ability of bourgeonal to activate the human OR17-4, a receptor implicated in both olfactory function and sperm chemotaxis (49, 51), whereas an oxidatively dimerized isoeugenol derivative, but not isoeugenol itself, inhibits eugenol-dependent activation of mOR-EG (e.g., References 47 and

G protein–coupled receptors (GPCRs): characterized by seven transmembrane domains and the ability to couple ligand binding to G protein–mediated intracellular signaling. Most mammalian chemosensory receptors are GPCRs

Odorant: a compound that functions as an odor. Odors can be single odorants or mixtures of odorants

52). The potential for the presence of both agonist and antagonist odorants in complex odor mixtures provides an additional layer of complexity to the coding of odor stimuli.

It appears that interindividual differences in odor sensitivity and perception, including specific anosmias, are due largely to differences in the complement of OR genes. For example, human variation in sensitivity to, and perception of, the steroid odor androstenone (53) is associated with allelic variations of the OR7D4 receptor that affect receptor efficacy (54). The presence of large numbers of segregating pseudogenes in the olfactory genome (55) may also contribute to perceptual differences between individuals, such as differential sensitivity to isovaleric acid (56). The prevalence of interindividual differences in odor sensitivity and perception indicates that defining "normal" olfactory function may be problematic.

Signaling mechanisms. The molecular mechanisms of olfactory signaling are understood in some detail (6–8). The transduction process is initiated upon odor activation of one of many hundred OR family members. Members of this class of chemosensory receptor confer selective odor responsiveness on either OSNs (e.g., References 44 and 57) or heterologous cells (e.g., Reference 43). For example, OSNs expressing the mouse OR M71 respond to acetophenone and benzaldehyde. However, if the M71 receptor gene is replaced with a receptor gene encoding a well-characterized rat OR, I7, the odor selectivity of this same cell population is changed: These OSNs now respond to the I7 ligand octanal, but not to acetophenone or benzaldehyde (57). Thus, the OR dictates the stimulus tuning of the OSN.

Although ligands have been identified for several ORs, little is known about the key molecular determinants for ligand binding and selectivity, ligand-induced conformational changes, or G protein coupling (but see References 43, 44, 48, 50, 57, and 58). Thus, our understanding of the members of the largest GPCR family lags far behind that of many other receptors more amenable to structure-function analyses, such as rhodopsin and the β-adrenergic receptor (59). The biggest hurdle to more systematic structure-function studies is the ability to isolate large quantities of purified ORs. Unfortunately, the small number of OSNs expressing each OR proteins in the MOE makes biochemical purification of native ORs unrealistic. ORs express poorly in heterologous cells, even in the presence of chaperones or fusion tags geared to facilitate membrane targeting (43, 60), making in vitro strategies problematic as well. Overcoming the technical hurdles of OR expression and purification is critical if we are to understand how ORs recognize odors.

The preeminent role of a cAMP-mediated signaling cascade for the transduction of odors by canonical OSNs was confirmed by the characterization of gene-targeted mice in which each of the principal signaling molecules was deleted. $G\alpha_{olf}$ null mice exhibit a pronounced reduction in MOE responses, as assayed by electroolfactogram (EOG), to a variety of volatile odors (61). However, these responses are not completely abolished, possibly as a result of the expression of the partially redundant $G\alpha_s$ in the MOE (62). Similarly, the role of ACIII in odor transduction was confirmed by characterizing ACIII null mice (63): EOG responses to a number of odorants are completely absent in ACIII null mice, which also display deficits in olfaction-dependent learning.

The contribution of individual CNG channel subunits to the odor response is more complex. CNGA2 is necessary for the formation of a functional olfactory CNG channel in vitro, whereas the CNGA4 and CNGB1b subunits increase channel sensitivity to cyclic nucleotides (64). Indeed, deletion of *Cnga2* by gene targeting in mice (65) abolishes EOG responses to most odors tested, although responses to the volatile semiochemicals 2-heptanone and 2,5-dimethylpyrazine (66) and to the natriuretic peptides uroguanylin and guanylin (16) are maintained (see below). Both *Cnga4* and *Cngb1* null mice show reduced channel and OSN sensitivity to cyclic nucleotides (67, 68). *Cnga4* null mice also exhibit reduced

behavioral sensitivity to odors (69) (behavioral tests of *Cngb1* null mice have not been reported).

The olfactory CNG channel is the principal site of Ca^{2+}/calmodulin (Ca^{2+}/CaM)-mediated odor adaptation in OSNs (64, 67, 70). Although CNGA2 homomeric channels are desensitized in vitro in the presence of Ca^{2+}/CaM, the specific contribution of the CNGA2 subunit to the mechanisms of odor adaptation is unresolved (64). *Cnga4* null mice show slower Ca^{2+}/CaM-mediated channel desensitization and defects in cellular odor adaptation to repeated or prolonged stimulation of OSNs (67). These mice are also profoundly impaired in their ability to discriminate olfactory stimuli in the presence of a background odor (69). Odor adaptation is also perturbed in the MOE of *Cngb1* null mice (68), although mutation of the Ca^{2+}/CaM binding site of this subunit affects adaptation to prolonged, but not repeated, odor stimulation (71). Together, these results indicate that all three subunits of the olfactory CNG channel play important, but distinct, roles in the process of odor adaptation.

Olfactory Sensory Neurons Expressing Trace Amine–Associated Receptors

Not all OSNs express OR-type odor receptors. Recently, a small family of trace amine–associated receptors (TAARs) has been identified in a subpopulation of OSNs that do not express ORs. These receptors, and the OSNs that express them, may be responsive to semiochemicals such as pheromones.

Cellular and molecular organization. Activation of the MOE by atypical olfactory stimuli such as volatile pheromones (66) and major histocompatibility complex (MHC)-related peptides (72) suggested that some OSNs might express members of other GPCR families. A systematic screening of OSN-enriched cDNA led to the amplification of several members of the TAAR family, a group of GPCRs more closely related to serotonin and dopamine receptors than to ORs (73, 74). Eight of nine mouse *Taar* genes are expressed in nonoverlapping subsets of OSNs in the MOE. TAAR-expressing OSNs do not appear to express ORs, although they do express $G\alpha_{olf}$ (74). Therefore, although TAAR-expressing OSNs may utilize a cAMP-mediated signaling cascade to transduce odors, they are distinct from the canonical OR-expressing OSNs.

Like canonical OSNs, $TAAR^+$ OSNs expressing the same receptor are zonally restricted within the MOE, although randomly distributed within those zones. Individual TAARs are expressed sparsely in the MOE (~1/1000 OSNs), again similar to the canonical OSNs (74). However, although TAAR-expressing OSNs share many similarities with canonical OSNs, it remains unknown if they observe the same properties of glomerular convergence, or if they preferentially target regions of the MOB. Therefore, it is unclear if there is combinatorial coding of TAAR ligands in the MOS.

Chemoreceptive properties. Although recordings from TAAR-expressing OSNs have not been reported, heterologous expression of olfactory TAARs has identified a number of ligands (74). As predicted (73), mouse TAARs (mTAARs) respond to biogenic amines, but not to related alcohols or amino acids (74). Of note are β-phenylethylamine (an mTAAR4 ligand), isoamylamine (an mTAAR3 ligand), and trimethylamine (an mTAAR5 ligand). Consistent with a role in detecting species-specific, urine-derived pheromones and other social cues, mTAAR5 responds to highly diluted (1/30,000) adult male mouse urine, but not to female urine, prepubescent male urine, or human male urine (74). Urine from either BALB/c or C57BL/6 mice, which vary in MHC haplotype, was equally effective, indicating that the mTAAR5 ligand is not related to MHC-linked genetic identity. Together, these results suggest that at least some TAARs may act as pheromone receptors. However, the conservation of TAARs in fish, chicken, mouse, and human (74–76) suggests that they serve a more general role as well.

Pheromones: a subclass of semiochemicals used for intraspecies communication and that elicit a behavioral or hormonal change in the recipient animal

SO: septal organ of Masera

Signaling mechanisms. Little is known about signaling in TAAR-expressing OSNs. TAARs are coexpressed with $G\alpha_{olf}$, suggesting that they use the same transduction cascade as do canonical OSNs (74). Although TAARs can couple to cAMP signaling pathways in vitro (73, 74), this coupling has not been demonstrated in MOE neurons.

GC-D-Expressing Chemosensory Neurons and the Necklace Glomeruli

OSN subpopulations can also be differentiated on the basis of transduction mechanisms. GC-D$^+$ neurons, which express the receptor guanylyl cyclase GC-D, utilize a cGMP-mediated cascade to transduce chemosensory stimuli, including two natriuretic peptide hormones, uroguanylin and guanylin.

Cellular and molecular organization. The mammalian receptor guanylyl cyclases are a small family of peptide and orphan receptors (77). They contain three functional domains: an extracellular receptor domain, an intracellular regulatory domain, and an intracellular catalytic domain that generates the second messenger cGMP. One family member, GC-D, was identified by homology cloning from rat MOE (13). GC-D$^+$ neurons compose less than ~0.1% of MOE neurons, exhibit a typical OSN bipolar morphology, and can be found singly or in clusters within the MOE (13, 14, 78). The highest density of GC-D$^+$ neurons is in the dorsal recesses of the ectoturbinates, although they are also found on the endoturbinates and septum and in the septal organ of Masera (SO) (13, 78, 79).

GC-D$^+$ neurons do not express many of the transduction proteins found in canonical OSNs and implicated in cAMP-mediated olfactory transduction, including ACIII, $G\alpha_{olf}$, CNGA2, PDE1C2, and PDE4A (14, 15) (**Figure 3*c***). However, these cells do express the cGMP-sensitive channel subunit CNGA3 and the cGMP-stimulated PDE2 (14, 15), suggesting that GC-D$^+$ neurons utilize a cGMP-mediated signaling cascade to transduce chemosensory stimuli. PDE2 is localized throughout the GC-D$^+$ neurons, which permits the glomerular targets of GC-D$^+$ neurons to be easily identified (14). PDE2 immunolabeling of a small number of cholinesterase-positive glomeruli that ring the caudal MOB (14) indicated that GC-D$^+$ neurons specifically innervate a subset of atypical glomeruli known as the necklace glomeruli (80). Genetic labeling of GC-D neurons through gene targeting has supported this interpretation (16, 78, 81) (**Figure 3*a***).

Chemoreceptive properties. The chemosensory stimuli to which GC-D$^+$ neurons respond were only recently determined. Two natriuretic peptides, uroguanylin and guanylin (82), elicited responses in the MOE of *Cnga2* null mice (16) (**Figure 3*b***), indicating that the response to these stimuli was, as expected (14, 15, 83), independent of a canonical cAMP-mediated transduction cascade. These two peptides are highly effective stimuli: In EOG recordings from wild-type mice, $K_{1/2}$ values were as low as 66 pM. GC-D$^+$ neurons respond to stimulation with uroguanylin, guanylin, or dilute urine with an increase in action potential frequency and a rise in intracellular Ca^{2+} (16). However, two volatile semiochemicals found in urine that elicit c-fos activation in PDE2-positive glomeruli, 2-heptanone and 2,5-dimethylpyrazine (66), fail to activate GC-D$^+$ neurons themselves (16). GC-D$^+$ neurons are heterogeneous in their stimulus tuning: Although approximately one-half of GC-D$^+$ neurons respond to both peptides, the remainder respond to either uroguanylin or guanylin alone (16). Thus, GC-D$^+$ neurons function as receptors for uroguanylin, guanylin, and components of urine.

A contemporaneous study reached a very different conclusion: that GC-D neurons are sensitive CO_2 sensors (81). GC-D$^+$ neurons express the CO_2-catalyzing enzyme carbonic anhydrase type II (CAII) (81). Stimulation of labeled GC-D$^+$ neurons in a gene-targeted mouse expressing enhanced green fluorescent protein (EGFP) under the control of the *Gucy2d*

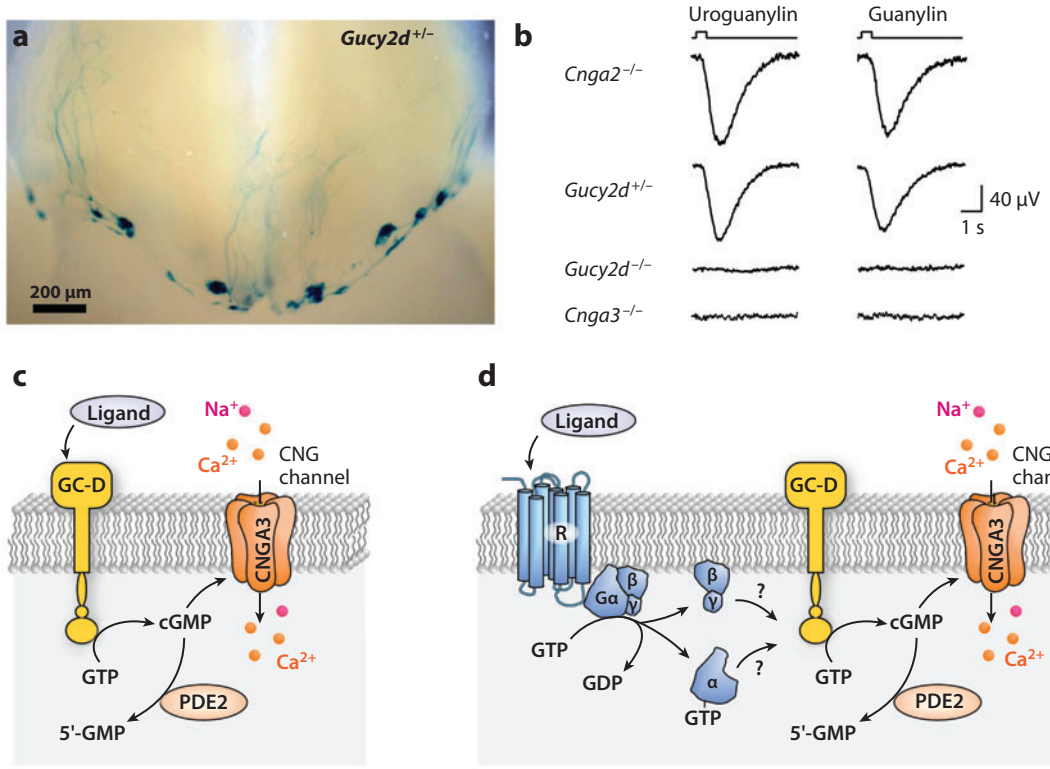


Figure 3

GC-D-expressing neurons and the necklace glomeruli. (*a*) Whole-mount X-Gal staining of paired olfactory bulbs from *Gucy2d-Mapt-lacZ* $^{+/-}$ mice showing stained necklace glomeruli (*ventral view*). (*b*) Stimulus-evoked field-potential responses to guanylin family peptides in the main olfactory epithelium (MOE) of *Cnga2*, *Gucy2d*, or *Cnga3* null mice. Panels *a* and *b* are reprinted with permission from Reference 16. Copyright 2007, National Academy of Sciences. (*c*,*d*) Possible signal transduction mechanisms in GC-D$^+$ neurons. (*c*) GC-D itself may serve as the chemosensory receptor for these cells, or (*d*) GC-D activity may be modulated by an unknown GPCR (R). CNGA3 channel, cyclic nucleotide–gated channel type A3; Gα, -β, -γ, unknown G protein α, β, and γ subunits; PDE2, phosphodiesterase type 2.

promoter with CO_2 elicits a rise in intracellular Ca^{2+} that is blocked by a CA inhibitor. Olfactory bulb neurons associated with the necklace glomeruli are activated by CO_2 stimulation of the MOE (81). *Car2* null mice, which lack CAII, show reduced behavioral responses to CO_2 as compared with wild-type mice.

Further experiments are needed to resolve these two distinct functions. Some insights may be had from the observation that, although the *Gucy2d* ortholog is a pseudogene in most primates (including humans), it is intact in some prosimians and in dog, mouse, rat, and tree shrew (84). Thus, a common chemoreceptive role of GC-D$^+$ neurons may be retained in these mammals.

Signaling mechanisms. GC-D$^+$ neurons utilize a cGMP-mediated transduction cascade. They are unresponsive to the adenylyl cyclase activator forskolin but are stimulated by the membrane-permeant 8-bromo-cGMP or by PDE inhibitors (which elevate intracellular cyclic nucleotides) (16). Also, the CNG channel inhibitor l-*cis*-diltiazem attenuates uroguanylin and guanylin responses in the MOE (16). In contrast to the mammalian phototransduction mechanism, which responds to sensory

stimulation with a decrease in intracellular cGMP and hyperpolarization of photoreceptor cells (85), GC-D$^+$ neurons respond to sensory stimulation with GC-D-dependent increases in intracellular cGMP and Ca^{2+}, membrane depolarization, and an increase in action potential firing (16). Thus, GC-D$^+$ neurons utilize an excitatory, cGMP-mediated signaling cascade to transduce peptide and urine stimuli. The mechanism by which CAII-dependent hydrolysis of CO_2 leads to action potential firing in GC-D$^+$ neurons remains unknown.

The nature of the peptide receptor in GC-D$^+$ neurons is also unclear. GC-D itself is an excellent candidate: Other members of the receptor guanylyl cyclase family respond to natriuretic peptides (82), and deletion of *Gucy2d* in mice abolishes responses to uroguanylin, guanylin, and urine (16). However, the three distinct tuning profiles of GC-D$^+$ neurons suggest that subpopulations of GC-D$^+$ neurons express distinct receptors, receptor complexes, or common receptors with distinct modifications or variants. Consistent with this view, rat GC-D responds to uroguanylin, but not to guanylin, in heterologous cells (86).

TRP Channel–Expressing Olfactory Sensory Neurons

The expression of several transient receptor potential (TRP) channel family members in subpopulations of cells in the MOE indicates further functional diversity in the MOS. However, it remains unclear whether olfactory TRP channels function as chemosensory receptors or transduction effectors, or whether they play some other role.

Cellular and molecular organization. Members of the TRP channel superfamily are structurally diverse and are found in numerous tissues (87). Three TRP channel isoforms, TRPC2, TRPC6, and TRPM5, are expressed in distinct subsets of MOE cells. TRPC2 is found in a small number of cells (<1%) in the adult and embryonic rat MOE; in adult MOE these cells appear restricted to the basal layers, suggesting that they are immature neurons (88). However, it is unclear if these cells innervate the central nervous system (CNS) or even if they are chemosensory neurons.

Expression of TRPC6 is restricted to a population of bipolar microvillar cells in the apical MOE that extend a process to, but not through, the basal lamina (89). The restriction of these cells to the MOE suggests that they may play a local role in chemosensory processing. Expression of neuropeptide Y in some TRPC6$^+$ cells suggests a role in development and/or regeneration (90, 91). TRPC6$^+$ cells express two components of a phosphoinositide (PI) signaling cascade, the inositol 1,4,5-trisphosphate receptor 3 (IP_3R3) and the effector enzyme phospholipase C β2 (PLCβ2), but do not express ACIII, CNGA2, or OMP, three key markers of canonical OSNs (89).

TRPM5 is expressed in two morphologically distinct cell types within the MOS: a population of solitary microvillar cells innervated by trigeminal nerve fibers (92; see below) and a large group of ciliated MOE neurons (93) (**Figure 2*b***). The ciliated TRPM5$^+$ OSNs are enriched in the ventrolateral MOE and project axons to the ventral, lateral, and medial MOB (93). Surprisingly, these neurons express components of both PI- and cAMP-mediated signaling cascades, including PLCβ2, the G protein subunit Gγ13, and the CNGA2 channel subunit (93). These neurons also express OMP (93).

Chemoreceptive properties. Some odorants, such as lilial and lyral, stimulate the production of IP_3 in MOE membrane preparations (e.g., Reference 94). However, the dependence of canonical OSN odor responses on an intact cAMP-mediated signaling cascade brings into question the relevance of IP_3-mediated odor transduction in the MOE (4, 61, 63, 65). TRPC6$^+$ cells respond to mixtures of IP_3-mediated odors and to the single odorant lilial (10 μM) with a rise in intracellular Ca^{2+} (89). If this Ca^{2+} increase is dependent on IP_3, such responses may at least partially account for the earlier biochemical results showing

odor-dependent IP_3 increases in whole-MOE membrane preparations.

$TRPM5^+$ OSNs may respond to pheromones or other semiochemicals. For example, mouse urine, the putative pheromone 2,5-dimethylpyrazine, and the social cue (methylthio)methanethiol (MTMT) each elicit increased c-fos expression in periglomerular cells associated with MOB glomeruli receiving $TRPM5^+$ neuron innervation (93). The degree to which $TRPM5^+$ OSNs vary in their stimulus selectivity is unknown and awaits functional characterization of individual $TRPM5^+$ neurons.

Signaling mechanisms. The different subfamilies of TRP channels, including TRPC and TRPM isoforms, display distinct physiological properties and modes of activation (87). Both TRPC2 and TRPC6 are diacylglycerol-sensitive cation channels (87). Odor transduction in $TRPC6^+$ microvillar cells may be mediated by PI signaling; odor responses in these cells, which also express PLCβ2 and IP_3R3, are at least partially independent of extracellular Ca^{2+} (89).

Odor transduction in $TRPM5^+$ OSNs may be more complex. They express components of both PI- and cAMP-mediated signaling cascades, but the role each cascade plays in cellular responses to odors is unclear. For example, EOG responses to the semiochemical 2,5-dimethylpyrazine, but not to the environmental odor lilial, are reduced in *Trpm5* null mice in the presence, but not in the absence, of an adenylyl cyclase inhibitor (93). Thus, both pathways may be required for the transduction of certain stimuli by $TRPM5^+$ OSNs.

ACCESSORY OLFACTORY SYSTEM

In addition to the MOS, many mammals possess an accessory olfactory system (AOS) (10, 95–98) (**Figure 4**). The AOS consists of

1. the vomeronasal organ (VNO), a chemoreceptive structure situated at the base of the nasal septum, which houses the microvillar vomeronasal sensory neurons (VSNs);
2. the accessory olfactory bulb (AOB), a region of the forebrain that receives synaptic input from the VNO and serves as the first processing center of vomeronasal information; and
3. higher olfactory centers that receive direct or indirect information from the AOB.

The AOS has attracted a great deal of attention over the past ten years because of a growing recognition of this system's essential role in chemical communication and the regulation of social behaviors (10, 95, 96, 99, 100). However, the traditional distinction that the MOS detects only volatile, environmental odorants and the AOS detects only nonvolatile pheromones is no longer valid. Both systems have considerable overlap in terms of the stimuli they detect and the effects that they mediate. Furthermore, both systems are capable of recognizing a wide variety of chemical signals and structures (10, 12).

CRANIAL NERVE ZERO: THE TERMINAL NERVE

The terminal nerve, or nervus terminalis, is present in all vertebrates whether or not they have a vomeronasal organ. Although the terminal nerve was first suggested to function as a chemosensory system in its own right, increasing evidence now points to a centrifugal, modulatory role (166). As the most anterior vertebrate cranial nerve, the terminal nerve extends between hypothalamic nuclei and the nasal cavity, reaching deep into the lamina propria surrounding the olfactory epithelium. The nerve consists of fibers that are highly heterogeneous in terms of neurochemically distinct phenotypes, including the expression of neuropeptide Y, gonadotropin-releasing hormone (GnRH), tyrosine hydroxylase, nitric oxide synthase, and several other molecules (167). With respect to neuropeptide Y, which plays an important role in the control of appetite and feeding and is released by stress, terminal nerve–derived peptide appears to modulate olfactory sensory neuron activity in a context-dependent manner, at least in lower vertebrates (166).

AOS: accessory olfactory system

VNO: vomeronasal organ

VSN: vomeronasal sensory neuron

AOB: accessory olfactory bulb

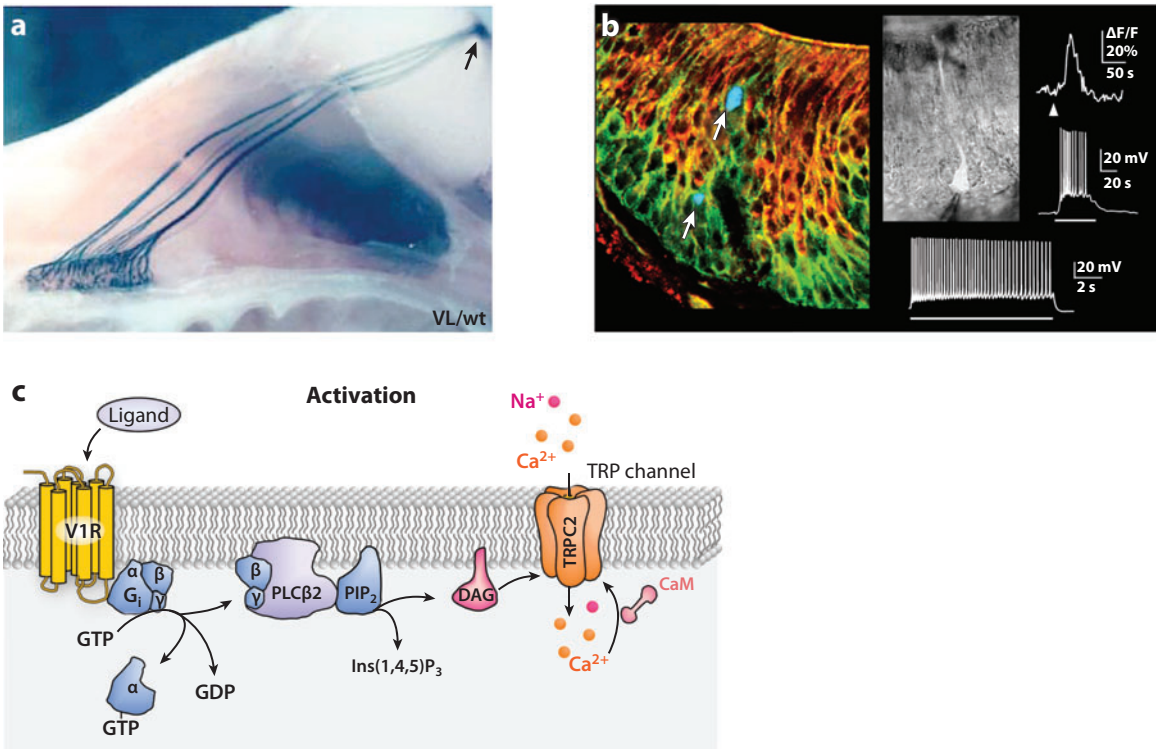


Figure 4

The accessory olfactory system. (*a*) Whole-mount X-Gal staining of a heterozygous *V1r2-IRES-tau-lacZ* (VL/wt) mouse reveals the distribution and axonal projection pattern of a subpopulation of vomeronasal sensory neurons (VSNs) expressing a single V1R. The AOB is indicated by the arrow. Reprinted from Reference 107, with permission from Elsevier. (*b*) Imaging and recording from identified VSNs. (*Left panel*) Confocal Ca^{2+} imaging in vomeronasal organ (VNO) slices showing VSNs responding to MHC class 1 peptides (*cyan*, *arrows*) are superimposed onto a protein expression map indicating the two epithelial layers (*red*, $V1R^+$ VSNs; *green*, $V2R^+$ VSNs). (*Right panel*, *counterclockwise from upper left)* Patch-clamp recording from a gene-targeted basal VSN expressing the V2R1b receptor (*white cell*). Current injection produces sustained action potential discharges that show spike-frequency adaptation (*bottom trace*). Ligand-induced responses in single VSNs, obtained by whole-cell current clamp recording (*middle trace*) or Ca^{2+} imaging (*upper trace*). Reprinted and modified from References 112, 118, and 120, with permission from AAAS, Elsevier, and the American Physiological Society, respectively. (*c*) Signal transduction mechanisms in $V1R^+$ VSNs. Activation of a V1r receptor initiates a G protein–coupled phospholipase C signaling cascade that results in Ca^{2+} entry and depolarization of the cells. Abbreviations used: CaM, calmodulin; DAG, diacylglycerol; $Ins(1,4,5)P_3$, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol-4,5-bisphosphate; PLCβ2, phospholipase C type β2; TRPC2, transient receptor potential channel canonical type 2.

The detection of molecular cues by the mouse VNO is mediated by independent subsystems that originate from VSN subpopulations residing in nonoverlapping apical and basal zones of the VNO neuroepithelium. VSNs of these two subdivisions are molecularly and functionally distinct. VSNs of the apical layer express members of the *V1r* family of vomeronasal receptor genes, whereas VSNs of the basal layer express members of the *V2r* family (8, 95). This spatial segregation correlates with the differential expression of two G protein subunits, $G\alpha_{i2}$ and $G\alpha_o$ (101, 102) (**Figure 4*b***), and is maintained at the level of the AOB: VSN axons from the apical layer synapse in the anterior half of the AOB, whereas VSN axons from the basal layer synapse in the posterior half of the AOB. This segregation is at

least partly maintained in higher levels of the CNS: Anterior and posterior divisions of the AOB each project to specific areas of the amygdala (103).

V1R-Expressing Vomeronasal Sensory Neurons

V1R$^+$ VSNs are narrowly tuned sensory neurons that detect a range of small natural ligands present in the urine of conspecifics. Each V1R$^+$ VSN expresses one V1R-type receptor, utilizes a phospholipase C (PLC)-mediated signaling cascade to transduce molecular cues, and sends information to the anterior aspect of the AOB. Thus, V1R$^+$ VSNs play a crucial role in chemical communication between members of the same species.

Cellular and molecular organization. The spatial segregation of the VNO into molecularly defined subsystems has important consequences for the sensing of structurally and functionally distinct sets of chemical stimuli and ultimately for the regulation of distinct sets of behavioral repertoires. V1R$^+$ VSNs are characterized by the expression of a given member of the V1R family, which consists of class A (rhodopsin-like) GPCRs (8, 95, 104). Database mining identified 308 *V1r* genes in the mouse genome, of which 191 appear to be intact (105). *V1r* genes are classified into 12 families, each containing between 1 and 30 members (105, 106). Transcription of *V1r* genes occurs in a monogenic and monoallelic manner (107). Humans have five intact *V1r* genes, at least some of which are expressed in the MOE (106). It is not yet clear whether V1R$^+$ MOE cells are displaced VSNs or whether they represent a unique cell type. Of all vertebrates surveyed thus far, the semiaquatic platypus has the largest *V1r* repertoire, with 270 intact genes and 579 pseudogenes (108). Several groups have used gene-targeted mouse lines, in which V1R$^+$ VSNs coexpress cellular markers, to determine the pattern of axonal projections to the AOB (107, 109). The results are complex and reveal a fundamentally different wiring logic as compared with canonical OSN-MOB projections. Individual mitral cells in the anterior AOB seem to receive information from multiple glomeruli associated with distinct, but possibly closely related, V1R$^+$ VSN populations (11, 110). This would indicate a considerable degree of integration of information at the level of the AOB.

Chemoreceptive properties. High-resolution fluorescence imaging techniques have been developed to investigate the activity of potential pheromone ligands in large neuronal populations of the VNO neuroepithelium (111). An acute coronal tissue slice preparation of the mouse VNO enables superimposition of neuronal activation maps onto protein or gene expression maps to identify the molecular identity of responsive VSNs (111, 112). Systematic analysis of the detection capabilities of individual VSNs established that V1R$^+$ VSNs function as highly sensitive pheromone detectors that recognize small organic pheromones present in the urine of conspecifics, such as the testosterone-dependent volatiles (*R*,*R*)-3,4-dehydro-*exo*-brevicomin (DHB) and (*S*)-2-*sec*-butyl-4,5-dihydrothiazole (SBT) (111). Neuronal responses showed highly selective tuning properties, and their specificity did not broaden as the stimulus concentration was increased (111). These functional properties predict that ablation of *V1r* genes would cause discrete deficits in the ability of the VNO to detect specific molecules. Indeed, this was observed following deletion of a cluster of 16 *V1r* genes (113). These *V1r*-deficient mice failed to show VSN responses to specific pheromonal cues, including 6-hydroxy-6-methyl-3-heptanone, *n*-pentylacetate, and isobutylamine (113). These mice also displayed alterations in social behaviors such as maternal aggression, thus establishing a role of V1Rs as pheromone receptors (113). In an alternative approach, green fluorescent protein–tagged VSNs that express the *V1rb2* gene responded to 2-heptanone, a response that was absent when the *V1rb2* gene was deleted (114). This indicates that V1Rb2 is a receptor for 2-heptanone, a urinary constituent that has a

primer pheromonal effect extending the length of female estrous cycles (114).

Other imaging approaches used to analyze population responses in the VNO have produced contrasting findings with respect to the spatial distribution of responses to dilute urine in apical and basal VSNs (115, 116). One study concluded that urine responses are essentially confined to $V1R^+$ VSNs (116), whereas others showed evidence that subsets of $V2R^+$ VSNs respond to dilute urine or molecular cues present in urine (112, 115, 117). A complete understanding of chemoreception in $V1R^+$ VSNs will require the identification of ligand-receptor pairs for the entire V1R family.

Signaling mechanisms. Stimulation of $V1R^+$ VSNs elicits action potentials and elevates intracellular Ca^{2+} (111, 114). In patch-clamp recordings from identified $V1Rb2^+$ VSNs, depolarizing currents of only a few picoamperes are sufficient to produce repetitive firing (118). Low-threshold, regenerative Ca^{2+} spikes are responsible, in part, for driving action potential firing (118). There is good evidence that a PLC-mediated signaling cascade underlies primary signal transduction in these neurons (119) (**Figure 4*c*,*d***), but genetic proof is required to firmly establish a critical role of $G\alpha_{i2}$ and PLC subtypes for the signal transduction mechanism. A key target of PLC activity is a 42-pS diacylglycerol-sensitive cation channel present in VSN dendrites (120). Activation of the diacylglycerol-sensitive channel is strongly impaired in mice exhibiting a targeted deletion of the transient receptor potential channel TRPC2 (120). *Trpc2*$^{-/-}$ mice also reveal a striking reduction in the electrical response to pheromonal ligands that activate $V1R^+$ cells (120, 121). Together, these results establish a direct link between PLC activity, gating of a TRPC2-dependent cation channel by diacylglycerol, and the sensory response in $V1R^+$ VSNs. Despite these advances, very little is known about the molecular architecture and subunit composition of the TRPC2 channel. Like the olfactory CNG channel, the TRPC2 channel is subject to strong modulation by Ca^{2+}/CaM feedback, offering a powerful mechanism for pheromone adaptation in these cells (122). Spike-frequency adaptation of action potential bursts provides a second mechanism for regulating the temporal response properties of $V1R^+$ VSNs (118). Thus, multiple mechanisms exist in VSNs to mediate pheromone adaptation, in contrast to the previous belief that VSNs lack any form of sensory adaptation (123).

V2R-Expressing Vomeronasal Sensory Neurons

$V2R^+$ VSNs represent a second major class of sensory neurons in the VNO. Only very recently has it become possible to obtain functional information from these neurons. These investigations indicate that $V2R^+$ VSNs detect several families of peptide and protein pheromones that are critical for chemical communication and the regulation of social behaviors.

Cellular and molecular organization. *V2r* genes are class C GPCRs, characterized by a long extracellular N terminus (8, 3, 95). Nearly 300 *V2r* genes have been identified in the mouse genome, of which 61–120 are putatively functional (124, 125). Presently, all V2Rs are orphan receptors. They are grouped, according to sequence homology, into four families: A, B, C, and D (125, 126). $V2R^+$ VSNs show combinatorial coexpression of different V2Rs (126). Hence, these neurons seem to be an exception to the one neuron–one receptor rule for chemosensory cells.

$V2R^+$ VSNs express another multigene family, termed *H2-Mv*, containing nonclassical MHC class 1 genes (127, 128). Initially, it was thought that *H2-Mv* genes might function as subunits in a native receptor complex with V2Rs and might be required as escort molecules in the transport of V2Rs to the cell surface (128–130). However, a substantial fraction of mouse $V2R^+$ VSNs do not express any of the nine *H2-Mv* genes (131). These results reveal a novel compartmentalization of the basal layer of the

VNO neuroepithelium, with at least two distinct neuronal subpopulations: one expressing *H2-Mv* genes and the other not. $V2R^+$/H2-Mv^- cells are localized in the upper sublayer of the basal layer, i.e., the middle layer of the VNO neuroepithelium, a subdomain organization that is maintained at the level of the AOB (131). Whether this organizational feature underlies specific aspects of chemosensory processing is yet to be determined.

Chemoreceptive properties. $V2R^+$ VSNs respond to several families of nonvolatile peptide and protein pheromones that require direct physical contact between the nose and the stimulus source for effective transmission (112, 117, 132). Fluorescence imaging of identified $V2R^+$ VSNs in intact VNO tissue slices revealed a vast family of antigenic peptides—the MHC class 1 peptides—as sensory stimuli for these cells (112, 115) (**Figure 4*b***). It is not yet known whether VSN detection of MHC peptide ligands correlates with the expression of *H2-Mv* genes. Such MHC peptides, which are crucial in the context of immune surveillance, carry information about the genetic makeup of an individual (133). Hence, the sensing of MHC peptides can potentially serve as a self-referent genetic recognition mechanism whereby individuals compare their own MHC type with those of conspecifics (134). Indeed, behavioral studies in mice have shown that VSN detection of MHC peptides mediates the formation of a persistent memory that is required for mate recognition in the context of selective, odor-induced pregnancy termination (the Bruce effect) (112). Interestingly, MHC peptide ligands are also detected by cAMP-sensitive OSNs of the MOE (72). These results illustrate that the same molecular cues can be processed by distinct olfactory pathways (72) and likely with distinct functional consequences.

Additional stimuli for some $V2R^+$ VSNs have been identified. A male-specific, 7-kDa peptide called ESP1, which is secreted from the extraorbital lacrimal gland, functions as a sensory cue for these neurons (132). ESP1 is encoded by a gene from a multigene family consisting of 38 members in mice (135). Field-potential recordings show that ESP1 elicits an electrical response in the VNO, whereas c-Fos activity measurements indicate that this response occurs in $V2R^+$ VSNs (132, 135, 136). The exact role of ESP family peptides in mouse communication is still unclear, but the observation that ESP expression patterns differ between strains suggests that such expression patterns may transmit strain-specific information (135).

A third group of nonvolatile chemosensory stimuli detected by VSNs of the basal layer consists of the major urinary proteins (MUPs) (117). MUPs represent another polygenic and highly polymorphic set of proteins thought to be involved in multiple aspects of chemosensory communication, including identity recognition (137) and the induction of ovulation (138). New work (117) indicates that MUPs also act as male-male aggression pheromones that specifically stimulate $G\alpha_o^+$ VSNs.

Signaling mechanisms. Few data are available on the signaling properties of identified $V2R^+$ VSNs, although it is clear that these VSNs respond to sensory stimulation with action potential generation (112, 135) and intracellular Ca^{2+} elevation (112, 115, 117, 132) (**Figure 4*b***). Patch-clamp analysis of gene-targeted VSNs expressing the V2R1b receptor, which are H2-Mv^- (131), has investigated the mechanisms underlying action potential firing (118). These cells are capable of maintaining low-frequency persistent firing for tens to hundreds of seconds (**Figure 4*b***). This is interesting because long-term potentiation at the mitral-to-granule cell synapse in the AOB, which is thought to underlie pheromonal learning in the context of the Bruce effect, is effectively triggered by low-frequency, 10-Hz pulses applied for extended periods of time. Specific coupling of L-type voltage-gated Ca^{2+} channels and large-conductance Ca^{2+}-activated K^+ channels mediates persistent firing in $V2R1b^+$ VSNs (118).

The TRPC2 channel is widely expressed in apical and basal layers of the VNO (88). Gene

knockout studies firmly established this channel's critical role for VSN activation by urine and $V1R^+$ neuron–specific stimuli (121, 139), as well as for the regulation of a variety of social behaviors (121, 139, 140). Ca^{2+} responses to MUPs are reduced in basal VSNs of *Trpc2*$^{-/-}$ mice (117). It came as a surprise, therefore, that deletion of *Trpc2* does not significantly influence the transduction of MHC peptides by $V2R^+$ VSNs (141). Likewise, memory formation in the context of the Bruce effect remains intact in *Trpc2*$^{-/-}$ mice, despite a requirement for a fully functional VNO (141). Whether these findings reflect a TRPC2-independent transduction mechanism is not yet clear. Alternatively, Ca^{2+} flux through residual channels in *Trpc2*$^{-/-}$ VSNs (120) may be sufficient to drive a secondary amplification mechanism and thus produce an excitatory response in these cells, not unlike the Ca^{2+}-activated Cl^- conductance of canonical OSNs (see above). In any case, considerable differences in terms of transduction and signaling mechanisms appear to exist between distinct VSN populations, and systematic studies comparing the signaling properties of molecularly defined VSN subsets will be required to address these questions.

OR-Expressing Vomeronasal Sensory Neurons

An additional neuronal subpopulation in the VNO is defined by the expression of members of the OR gene family (142). RT-PCR analysis suggests that at least 44 different OR genes are expressed in the mouse VNO. OR^+ cells in the VNO also express TRPC2 and $G\alpha_{i2}$, are located in the apical layer, and project their axons to distinct glomeruli of the anterior subdomain of the AOB (142). On the basis of their dendritic morphology, i.e., the absence of cilia, OR^+ cells in the VNO resemble typical VSNs.

The biological role of these cells is presently unclear, although they may be responsible for the detection of environmental odorants by the VNO (142). It has been known since the 1970s that the VNO can detect a range of odorants that do not exhibit any known pheromonal functions (143–147). Like OSNs, odor-sensitive VSNs are activated by more than one odorant mixture (145), indicating that the breadth of tuning of these VSNs differs from that of narrowly tuned $V1R^+$ VSNs. However, it remains unknown whether odor-detecting VSNs are involved in the stimulation of innate behavioral responses.

SEPTAL ORGAN OF MASERA

Cellular and Molecular Organization

In addition to the MOE and VNO, mice and rats possess a small, isolated patch of sensory epithelium known as the septal organ of Masera (SO), which lies near the base of the nasal septum at the entrances to the nasopalatine ducts (**Figure 2*a***). Despite its discovery decades ago, the functional role of the SO is still enigmatic. The SO sensory epithelium is composed of 1–3 layers of ciliated OSNs, compared with 6–8 layers in most regions of the MOE (1, 79, 148). The SO expresses 50–80 genes of the OR family, all of which are expressed in the MOE as well (149, 150). Greater than 90% of the SO cells express one of only nine ORs; there is no evidence that a single cell expresses more than one OR (149, 150). Like canonical OSNs, the vast majority of SO OSNs express OMP, ACIII, and $G\alpha_{olf}$ (79). A small subset of SO OSNs express GC-D and PDE2 (78, 79). Whether additional OSN subpopulations found in the MOE also exist in the SO is not yet known.

OSNs in the SO project to a small subset of glomeruli in the MOB (1, 79, 151). These glomeruli are located in the posterior, ventromedial aspect of the bulb. Some glomeruli appear to be innervated exclusively by SO OSNs, whereas others seem to receive axonal input of OSNs from both the MOE and the SO (151).

Chemoreceptive Properties

Early field-potential recordings first demonstrated that SO OSNs respond at relatively low concentrations to several general odorants, including pentylacetate (152). More recently,

patch-clamp recordings from individual knobs of SO OSNs have shown that these cells produce a sensory current in response to stimulation with known concentrations of diverse odorants, not unlike canonical OSNs (79, 153).

Signaling Mechanisms

Involvement of the cAMP second messenger system in SO odor transduction is supported by pharmacological evidence (79) and by genetic deletion of *Cnga2* (153). SO OSNs appear to serve dual functions as odor detectors and mechanical sensors because they respond to both chemical stimuli and mechanical stimuli (153). Remarkably, the mechanical responses appear to be mediated by the same cAMP-dependent pathway that is employed for signal transduction by chemical stimuli (153). The mechanical sensitivity of OSNs is also observed in the MOE and has been hypothesized to serve at least two functions: (*a*) to modulate sensory responses of OSNs with respect to airflow and (*b*) to synchronize rhythmic activity in the olfactory bulb with respiration (153).

GRUENEBERG GANGLION

Cellular and Molecular Organization

First described in 1973 (154), the Grueneberg ganglion (GG) was rediscovered just a few years ago (155–159). It consists of OMP^+ cells located at the dorsal tip of the nasal cavity, close to the opening of the naris. Its biological function is unknown. At a light-microscopic level, cells of the GG do not seem to possess prominent dendrites, cilia, or microvilli and lack direct access to the nasal lumen (1). Therefore, GG cells may detect gaseous or other highly membrane-permeant stimuli. Several chemosensory receptors found in the main and accessory olfactory systems, including the vomeronasal receptor V2R83 (160) and several TAARs (161), have been reported. However, it is unclear how sensory stimuli might access these receptors. GG cells project along the nasal septum and the medial surface of the MOB to reach the dorsal regions of the caudal MOB, near the AOB (155, 159). This region overlaps somewhat with that occupied by the necklace glomeruli, which are innervated by $GC\text{-}D^+$ neurons (see above). However, GG cells do not express GC-D (78), and the relationship between the caudal glomeruli innervated by GC-D neurons and the GG cells is unclear.

GG: Grueneberg ganglion

Chemoreceptive Properties

Physiological recordings from GG cells have not been reported. Consequently, no sensory stimuli detected by these cells are known, and the potential sensory function of the GG subsystem remains elusive (see note added in proof).

Signaling Mechanisms

On the basis of the expression of molecular markers, the GG appears to comprise cells of MOE-like and VNO-like molecular phenotypes (160). Signaling molecules found in canonical OSNs, such as ACIII and $G\alpha_{olf}$, are expressed by a few GG cells during the prenatal and perinatal stages (160). An antibody that recognizes members of the V2R2 family (126) labels a considerable number of cells in the GG, as do antibodies against $G\alpha_o$ and $G\alpha_i$ (160). By contrast, another study (158) concluded that the GG is unlikely to express ORs, V1Rs, V2Rs, or other typical elements of OSN and VSN signal transduction cascades.

TRIGEMINAL SYSTEM

Nasal chemosensation depends not only on the sense of smell but also on the activity of branches of the trigeminal nerve, which provide sensory innervation to the epithelia of the head (162). Inhalation of noxious or irritating chemical stimuli activates the trigeminal system and triggers protective reflexes such as apnea or sneezing. It was thought that receptors for trigeminal irritants are located exclusively on free nerve endings within the nasal epithelium (92, 93, 163, 164). However, new

work has identified a large population of solitary chemosensory cells in mice and rats that reach the surface of the nasal epithelium, form synaptic contacts with trigeminal afferent nerve fibers, and respond to odorous irritants at high concentrations (92, 93, 163, 164). These cells were first characterized by the expression of T2R-type taste receptors, PLCβ2, and the G protein α-gustducin (163). More recently, these cells were also shown to contain TRPM5 (92, 93, 164) (**Figure 2*b***). Ca^{2+} imaging of dissociated, genetically labeled $TRPM5^{+}$ solitary chemosensory cells demonstrated that they respond directly to a panel of volatile odorants at relatively high concentrations (92). It is not yet clear whether TRPM5 is directly involved in this response (92). Together, this work provides a new strategy for dissecting the mechanisms underlying the perception of irritating odors.

CONCLUSIONS

The cellular, molecular, and functional diversity of olfactory subsystems begs the question of why they are needed. The answer is most certainly multifaceted. Perhaps the most obvious reason for separate subsystems is that, because they express distinct families of chemosensory receptors, they expand the repertoire of chemicals that can be detected. The need for diverse transduction mechanisms is less clear but likely reflects both the coupling properties of the receptor itself and the kinetic and modulatory requirements associated with different classes of stimuli.

Some subsystems likely subserve species-specific roles. Although humans can respond quite well to diverse environmental odors and even to some semiochemicals, humans lack several olfactory subsystems present in lower mammals, including the entire AOS and $GC\text{-}D^{+}$ neurons of the MOS. These subsystems are particularly attuned to stimuli that are present in urine and glandular secretions and that may have been supplanted by many of the visual cues utilized for communication by higher primates.

Subsystems can also confer meaning to a stimulus through their connections to the CNS. The most dramatic example comes from mice in which olfactory signaling through the dorsal MOE and MOB has been disrupted (165). These mice no longer avoid odors associated with predators or spoiled food, even though they can still detect the stimuli, which stimulate OSNs in multiple regions of wild-type mouse MOE. Thus, the same odorant can evoke distinct perceptions, depending on the subset of OSNs it activates. The distinct CNS connections of the MOS and AOS further support a model in which olfactory subsystems help animals to extract information about the meaning of a stimulus, not just its identity.

SUMMARY POINTS

1. The mammalian olfactory system contains a diverse array of subsystems. These vary in the stimuli to which they respond, the cell types and molecules they employ to detect and transduce stimuli, and the connections they make with the CNS.
2. The use of gene targeting in mice has provided essential tools with which to identify, differentiate, and characterize olfactory subsystems.
3. The view that the main olfactory system is only a general odor sensor and the accessory olfactory system only a detector of semiochemicals such as pheromones is not valid.
4. Olfactory subsystems can allow for parallel processing of stimuli, thereby providing a means to extract different types of information from a single chemosensory cue.

FUTURE ISSUES

1. Although it is clear that olfactory subsystems utilize distinct molecular mechanisms to detect and transduce chemosensory stimuli, these mechanisms remain poorly understood. Identification of the key molecular players for each subsystem will provide invaluable tools for dissecting their biological function.
2. Elucidating the specific behavioral and physiological roles of each olfactory subsystem is required to understand how these subsystems work together to represent the sensory environment.
3. Little is known about how information from each olfactory subsystem is integrated within higher brain centers. Of particular interest is the question of how olfactory information is integrated with taste, somatosensory, and hormonal inputs, all of which can critically impact perception and motivation.
4. Although humans detect a complex olfactory world, they lack at least some subsystems present in rodents (e.g., the AOS). An understanding of the repertoire of human olfactory subsystems is needed if we are to understand fully the extent of the human chemosensory world.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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RELATED RESOURCES

1. Olfactory Receptor Database (ORDB) (**http://senselab.med.yale.edu/ordb/default.asp**)
2. The Human Olfactory Receptor Data Exploratorium (HORDE) (**http://bioportal.weizmann.ac.il/HORDE/**)
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NOTE ADDED IN PROOF

After acceptance of this manuscript, Brechbühl et al. (2008) reported that cells of the Grueneberg ganglion (GG) mediate alarm pheromone detection in mice. These chemicals, which remain unidentified in mammals, may signal stress, injury, or the presence of predators. This report also found that stimuli may gain access to GG neurons and their cilia though a keratinized epithelium that is permeable to hydrophilic substances.

Brechbühl J, Klaey M, Broillet M-C. 2008. Grueneberg ganglion cells mediate alarm pheromone detection in mice. *Science* 321:1092–95

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