Leading Edge
Review

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Olfactory Perception: Receptors, Cells, and Circuits

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Remarkable advances in our understanding of olfactory perception have been made in recent years, including the discovery of new mechanisms of olfactory signaling and new principles of olfactory processing. Here, we discuss the insight that has been gained into the receptors, cells, and circuits that underlie the sense of smell.

Introduction

Animals in their natural environments are immersed in odors. These odors are rich in information, and animals have evolved sophisticated olfactory systems to detect and interpret them. The ability to encode the identity and intensity of odors can allow an animal to locate food sources, thereby permitting survival, to identify mates, promoting reproduction, and to avoid predators, averting death.

Olfactory systems have evolved great sensitivity and discriminatory power. A single molecule of a female moth pheromone is believed sufficient to elicit a response from a male antennal neuron (Kaissling and Priesner, 1970). Honeybees can distinguish between many pairs of structurally similar odorants (Laska et al., 1999), and mice can likewise distinguish between many pairs of enantiomers (compounds that are mirror images of each other) (Laska and Shepherd, 2007).

The olfactory system is like the visual and auditory systems in that it detects and discriminates a wide range of stimuli. Odors differ from light and sounds, however, in that odors can not be classified by a simple parameter such as wavelength or frequency. The complexity of odorant identity poses a challenge that has been met through the use of a large number of diverse odor receptors. The multiplicity of receptors allows detection of a vast number of odors. Discrimination depends on combinatorial coding and on circuit-level interactions at multiple steps of olfactory processing, and it can be enhanced by olfactory learning.

The functional organization of the olfactory system is remarkably similar in organisms ranging from insects to mammals, suggesting that it represents an extremely good solution to some difficult problems. Thus, principles elucidated in one experimental organism often apply to many others. In both insects and mammals, odorants bind to receptors in the cilia or dendrites of olfactory receptor neurons (ORNs), each of which expresses one or a small number of receptor types. In both kinds of animals, ORNs that express the same odor receptor send axons to the same glomeruli, spheroidal structures that consist of ORN axon terminals and the dendrites of second order neurons. The glomeruli form the antennal lobe of the insect brain, or its mammalian equivalent, the olfactory bulb. In both of these centers, the olfactory signals are processed and relayed to higher centers of the brain.

Recent discoveries have provided new understanding of how the identity and intensity of odors are first encoded in the olfactory organs and how they are subsequently decoded in the central nervous system. Studies in the past few years have identified new odor receptor families, new signaling mechanisms, and even a new mammalian olfactory organ. Analysis of the insect antennal lobe, the mammalian olfactory bulb, and higher brain regions has led to a better understanding of how olfactory signaling is shaped by circuit-level interactions between neurons. It has also shed light on the fascinating question of how olfactory stimuli such as pheromones elicit innate behaviors.

In this review, we focus on recent insight gained into the molecules, cells, and circuits that underlie olfactory perception. Particular attention is paid to mammals and insects, in which illuminating advances have recently been made. We first consider the primary molecular sensors of odorants, the odorant receptors, and the neurons in which they are expressed, with a view to understanding how the initial pattern of sensory input is generated by an olfactory stimulus. We then examine how this input is transformed at the first processing center in the brain, the antennal lobe, or olfactory bulb. Next, we consider the processing that occurs at higher centers in the brain and how it relates to perception. Finally, we discuss olfactory perception in the context of odor space.

A Multiplicity of Olfactory Organs

Both mammals and insects rely upon multiple olfactory organs (Figure 1). In each organ, odors partition into an aqueous fluid that bathes the cilia or dendrites of sensory neurons. However, the organs differ in location and numerical complexity, in the receptors that they express, and in the targets of their neurons within the central nervous system. To what extent do these anatomical and molecular differences underlie functional differences, either in the kinds of stimuli that the organs encode or in the behaviors that they drive?

In mammals, the main olfactory epithelium lies in the dorsal nasal cavity, and its sensory neurons send projections to glomeruli in the main olfactory bulb. A wide variety of volatile odorants partition from the air into the fluid surrounding the

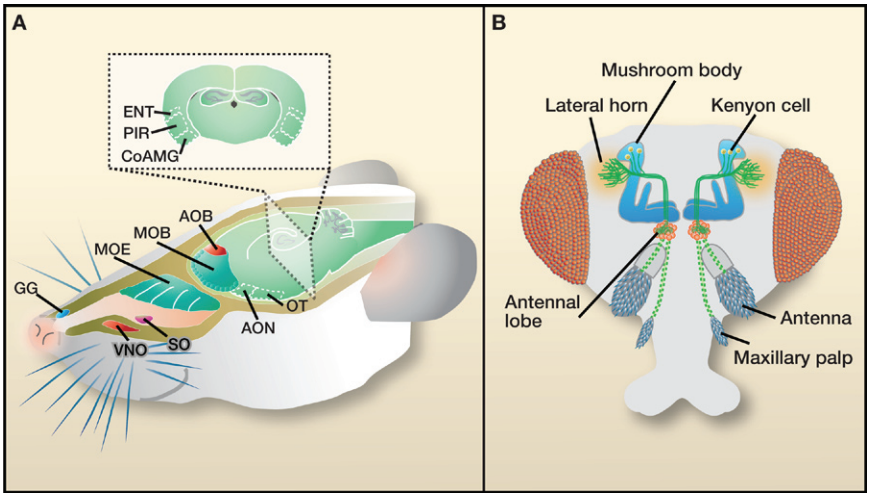

Figure 1. Olfactory System Anatomy

(A) Sagittal view of a rodent head, showing four olfactory organs: the main olfactory epithelium (MOE), vomeronasal organ (VNO), Grueneberg ganglion (GG), and septal organ of Masera (SO). Olfactory receptor neurons (ORNs) in the MOE, GG, and SO all project to the main olfactory bulb (MOB), whereas the VNO neurons project to the accessory olfactory bulb (AOB). Olfactory information is further processed in higher brain regions, such as the anterior olfactory nucleus (AON), the olfactory tubercle (OT), entorhinal cortex (ENT), piriform cortex (PIR), and cortical amygdala (CoAMG). Inset: coronal section of the brain.

(B) Frontal view of a *Drosophila* head. There are two pairs of olfactory organs: the third antennal segments and maxillary palps. Olfactory information is first relayed to the antennal lobe, which contains multiple glomeruli. Subsequent processing takes place at the lateral horn of the protocerebrum and Kenyon cells in the mushroom body. Connectivity has been simplified for clarity.

cilia of ORNs, where they are detected by odor receptors. The mammalian vomeronasal organ lies just below the ventral nasal cavity. Its sensory neurons express different receptors, and they project to glomeruli in the neighboring accessory olfactory bulb. Functional experiments have demonstrated that the vomeronasal organ is sensitive to a variety of pheromones, molecules that are released by an individual and that induce innate behaviors in conspecific animals (Table 1).

In recent years, it has become clear that there is functional overlap between the main olfactory epithelium and the vomeronasal organ. Certain pheromones have been found to activate neurons in the main olfactory system, and the activity of this system has been found necessary for several sexual and social behaviors that likely depend on pheromones (Lin et al., 2004; Luo et al., 2003; Mandiyan et al., 2005; Spehr et al., 2006; Wang et al., 2006; Xu et al., 2005). Likewise, some general odorants not known to act as pheromones have been found to activate the accessory olfactory system and modulate behavior in the absence of a functional main olfactory system (Sam et al., 2001; Trinh and Storm, 2003; Xu et al., 2005).

A third mammalian organ, the septal organ of Masera (SO), also contains sensory neurons that express odor receptors (Table 1) (Kaluza et al., 2004; Tian and Ma, 2004). The SO was recently shown to respond to multiple volatile odorants that are also detected by the main olfactory epithelium (Grosmaitre et al., 2007; Ma et al., 2003). Interestingly, a subset of ORNs from both the SO and the main olfactory epithelium may respond to mechanical pressure and thus may report changes in air pressure induced by sniffing (Grosmaitre et al., 2007).

Recently, another mammalian organ was found to subserve olfaction: the Grueneberg ganglion contains sensory neurons that express olfactory receptors (Fleischer et al., 2006; Fleischer et al., 2007). Moreover, these neurons are activated by volatile alarm pheromones and are required for a freezing behavior in mice, indicating a role in pheromonal signaling (Brechbuhl et al., 2008).

Insects also rely on multiple, distinct organs for olfaction (Figure 1B). Adult *Drosophila* contain two olfactory organs, the antenna and the maxillary palp. Both contain sensory hairs, or sensilla, that house the dendrites of up to four ORNs, but ORNs from the different organs project to glomeruli in different regions of the antennal lobe. Although these organs respond to overlapping sets of odorants, the maxillary palp lies close to the labellum, the main taste organ of the head, and there is evidence that olfactory input via the maxillary palp enhances taste-mediated behaviors (Shiraiwa, 2008). Other insect olfactory organs include the labial pits of moths, which respond to CO_2 and some odorants (Bogner et al., 1986).

Further increasing the extent of anatomical diversity, insect olfactory sensilla fall into different morphological types known as basiconic, trichoid, and coeloconic sensilla (Table 2). Whereas basiconic sensilla are found on both the antenna and the maxillary palp in *Drosophila*, trichoid and coeloconic sensilla are located exclusively on the antenna and may serve distinct chemosensory functions. Whereas basiconic ORNs respond to general odorants, trichoid neurons respond poorly to most odorants but respond to pheromones (Clyne et al., 1997; Hallem and Carlson, 2006; van der Goes van Naters and Carlson, 2007). Recent studies have demonstrated that activation of specific trichoid neurons is both necessary and sufficient to mediate the stereotyped courtship behavior elicited by the *Drosophila* pheromone 11-*cis*-vaccenyl-acetate (cVA) (Ha and Smith, 2006; Kurtovic et al., 2007). This functional division among sensillar types appears to be evolutionarily conserved, as other insects also detect pheromones with trichoid sensilla (de Bruyne and Baker, 2008). Coeloconic ORNs express a distinct class of olfactory receptors that are likely to underlie the strong response of these neurons to a variety of amines and carboxylic acids (Benton et al., 2009; Yao et al., 2005).

In summary, mammals and insects each receive olfactory input via multiple organs. Although there is some degree of overlap in the kinds of stimuli to which the organs of a species are sensitive, there is increasing evidence that different olfactory organs are functionally distinct and that their wiring to different targets in the brain may underlie differences in the behavioral output that they drive. An intriguing direction for future olfactory research is to define the functional roles of the different olfactory organs in a variety of odor-driven behaviors.

Table 1. Ligands and Functions for Mammalian Olfactory Organs and Receptors

Organ	Receptors	Ligands	Origin	Proposed Functions
MOE	ORs	general odors	food, environment	odor recognition, discrimination, attraction/repulsion
		MHC class I peptides	urine, bodily secretions	social recognition of other strains
	TAARs	volatile amines	urine	stress response, gender recognition, acceleration of female puberty onset
	GC-D	CO_2 (bicarbonate)	atmosphere	avoidance behavior
		peptide hormones (uroguanylin and guanylin)	urine	salt/water homeostasis, detection of cues related to hunger, satiety, or thirst
VNO	V1Rs	volatile pheromones, sulfated steroids	urine	conspecific recognition, male sexual behavior, maternal aggression, regulation of female estrous cycles, stress level indicator
	V2Rs	MHC class I peptides	urine, bodily secretions	mate recognition in the context of pregnancy block (Bruce effect)
		exocrine gland-secreting peptides (ESPs)	tears from specific genders or strains	information about gender and individual identity, conspecific recognition
		major urinary protein (MUP) complex	male urine	male aggression
		sulfated steroids	female urine	indication of stress levels
	Formyl Peptide Receptors	formyl peptides	gram-negative bacteria	indication of pathogenicity or health status
		CRAMP, lipoxin, uPAR peptides	immune system	indication of pathogenicity or health status
GG	TAARs, V2r83	alarm pheromones	Stressed conspecifics	avoidance of dangerous situations
SO	ORs	general odors	food, environment	alerting role or "mini-nose"

Abbreviations: MOE, main olfactory epithelium; OR, olfactory receptor; GG, Grueneberg ganglion; SO, septal organ of Masera; TAARs, trace amine-associated receptors; GC-D, receptor guanylyl cyclase; VNO, vomeronasal organ; MHC, major histocompatibility complex.

Odor Receptors: An Expanding Roster of Dynamic Gene Families

Whereas vision depends on a handful of related receptors, olfaction relies on large numbers of receptors that belong to multiple families. The dimension and diversity of the receptor repertoire have likely arisen to facilitate the detection and discrimination of the vast number of odorants that are encountered by animals in their environments. New families of odor receptors have recently been discovered in both mammals and insects, and their functional roles are currently being explored.

A variety of odor receptor families are expressed in mammalian ORNs (Table 1). Mammalian genomes typically contain ~250–1200 functional OR genes (Niimura and Nei, 2007), which are the predominant receptors of the main olfactory epithelium and the SO (Table 1). A minority of ORNs express the trace amine-associated receptors (TAARs), some of which respond to volatile amines found in urine and are likely to act in the detection of social cues (Fleischer et al., 2007; Liberles and Buck, 2006). There are ~15 TAAR genes in the mouse, and TAARs are found in all vertebrate genomes examined thus far (Hashiguchi and Nishida, 2007). The vomeronasal organ expresses receptors of the V1R family (~200 genes in the mouse, excluding pseudogenes) and the V2R family (~100) (Touhara and Vosshall, 2009). One V2R gene and some TAAR genes are expressed in the Grueneberg ganglion. Recently, another family of vomeronasal organ receptors has been discovered: the formyl peptide receptor-like proteins (approximately seven) (Liberles et al., 2009; Riviere et al., 2009). These receptors respond to molecules related to disease and inflammation and may identify pathogens or report the health status of the animal. All five classes of mammalian receptors are predicted to contain seven transmembrane domains and have either been shown to signal via G proteins or are likely to do so based on their sequence similarity to known G protein-coupled receptors (GPCRs).

Insect olfaction is also mediated by receptors of multiple classes (Table 2). Insect genomes contain 60–340 members of the phylogenetically distinct insect Odor receptor (Or) family (Touhara and Vosshall, 2009). In addition, a few members of the Gustatory receptor (Gr) family are expressed in olfactory organs, where some have been found to mediate response to CO_2 (Jones et al., 2007; Kwon et al., 2007; Suh et al., 2004). Recently, another family of ~60 receptors called IRs (Ionotropic receptors) has been identified, of which several are expressed in ORNs of coeloconic sensilla (Benton et al., 2009). Ors and Grs are predicted to contain seven transmembrane domains, whereas IRs are related to ionotropic glutamate receptors and are predicted to contain three transmembrane domains and a pore loop.

Table 2. Ligands and Functions for Insect Olfactory Organs and Receptors

Organ	Sensilla	Receptors	Ligands	Origin	Proposed Functions
Antenna	Basiconic (ab1∼ab10)	Ors	many volatile compounds, food odors	food, environment	odor recognition, discrimination, attraction/repulsion
		Gr21a and Gr63a	CO_2	atmosphere, stressed flies	avoidance behavior
	Coeloconic (ac1∼ac4)	Or35a	many volatile compounds, food odors	food, environment	unknown
		IRs	volatile amines, carboxylic acids, a few food odors	food, environment	unknown
		unknown	humidity	environment	dessication avoidance
	Trichoid (at1∼at4)	Ors	*cis*-vaccenyl acetate	male genitalia, recently mated females	deterrent for courting males and mated females, aggregation pheromone
		Ors	cuticle extracts	male or female flies	gender and conspecific detection
Maxillary Palp	Basiconic (pb1∼pb3)	Ors	many volatile compounds, food odors	food, environment	taste enhancement

The evolutionary dynamics of odor receptors shows a great deal of fluidity. The Or families of insects in particular show great diversity. Within an insect species, many pairs of receptors show little sequence identity, and between some species of the same order, such as *Drosophila* and *Anopheles*, it is difficult to identify orthologous pairs of receptors (Hill et al., 2002). This divergence is likely to reflect rapid evolution. There are intriguing questions concerning the mechanisms by which receptor repertoires have evolved to meet the ecological needs of the species. As a species adapts to a new environment or food source, as in the evolution of human host-seeking behavior in mosquitoes, do new clades of receptors arise via duplication and divergence to sense new odors, such as human odors in the case of mosquitoes, or do the extant receptors adapt? Now that functional assays for insect odorant receptors are available, such questions can be addressed by systematic analyses of receptor repertoires.

In mammals, it is clear that OR families have evolved in part through both expansion and pseudogenization (a process by which mutations render genes nonfunctional). For example, 15%–78% of ORs are pseudogenes, and all human V2R genes have been pseudogenized (Touhara and Vosshall, 2009). The evolutionary forces leading to gene pseudogenization and expansion can be seen in mammalian ORs, which fall into two classes. Aquatic vertebrate genomes nearly exclusively contain older class I ORs that are generally tuned toward water-soluble odorants, whereas the genomes of terrestrial vertebrates contain both class I and class II ORs, which are tuned toward hydrophobic odors (Freitag et al., 1998; Saito et al., 2009). In the dolphin, an aquatic mammal, the class II receptors have been pseudogenized (Freitag et al., 1998).

There is also widespread genetic variation among mammalian OR repertoires within species, including single-nucleotide polymorphisms and copy number variation. Such natural genetic variation contributes to perceptual differences within human populations. Humans vary in their perception of specific odors. An individual may be anosmic, or insensitive, to a particular odor. An odor may be perceptible, but with an altered detection threshold. In some cases, an odor may acquire an altered perceptual quality. Two recent studies showed that differences in sensitivity to androstenone, a steroid, and isovaleric acid, which has a sweaty odor, can be attributed at least in part to polymorphisms in two specific OR genes (Keller et al., 2007; Menashe et al., 2007).

The role of individual members of a receptor repertoire in perception can be examined prospectively through mutational studies in *Drosophila*. Loss of odor receptors has been shown to cause a reduction in behavioral or electrophysiological responses to specific odorants (Dobritsa et al., 2003; Jones et al., 2007; Kreher et al., 2008; Kurtovic et al., 2007; Semmelhack and Wang, 2009). However, the deletion of an individual *Drosophila* Or does not necessarily eliminate the behavioral response to odorants it detects (Elmore et al., 2003; Keller and Vosshall, 2007). A simple interpretation of the residual response is that it is mediated by other receptors of the repertoire with partially overlapping function. Consistent with this explanation, a recent analysis of the response to ethyl acetate reveals that the response to high concentrations depends primarily on one receptor, whereas the response to low concentrations depends primarily on another (Kreher et al., 2008).

The Primary Representation of an Odor

The roles of individual receptors in olfactory perception raise interesting questions about how an odor is encoded by an entire receptor repertoire. The primary representation of an odor lies in the differential activities of the population of odor receptors. Although this representation is transformed at successive levels of olfactory circuitry, ultimately the perception and discrimination of odors is founded upon the profile of receptor activity. Insight into the nature of this primary representation has come from systematic analysis of the responses of receptor repertoires to panels of odors (Hallem and Carlson, 2006; Kreher et al., 2008; Malnic et al., 1999; Saito et al., 2009; Xia et al., 2008).

Three basic principles emerge from such analysis. First, individual odorants activate subsets of receptors. This finding supports a model of combinatorial coding, in which most odor-

ants are identified not by the activation of a single receptor, but by the pattern of receptors that are activated. Second, individual receptors are activated by subsets of odorants. Receptors vary in their breadth of tuning: some are broadly tuned, responding to many odors, whereas others are narrowly tuned, responding to few. The receptors lie along a smooth continuum of tuning breadths. Broadly tuned receptors are most sensitive to structurally similar odorants. Third, higher concentrations of odorants elicit activity from greater numbers of receptors. Thus, odor intensity as well as odor identity is represented by the number of activated receptors.

The primary representations of odors can vary in their temporal dynamics. An individual odorant can elicit a response of short duration from some ORNs and a long-lasting response from others. Likewise, an individual ORN can give a short response to some odors and a long response from others (Hallem et al., 2004). Analysis in an in vivo expression system, in which odor receptors are misexpressed in a mutant, "empty" *Drosophila* neuron that lacks an endogenous receptor, suggests that the termination dynamics of a neuron are dictated primarily by the receptor, as opposed to the cellular environment in which it operates (Hallem et al., 2004).

In addition to activation, receptors exhibit another mode of response: inhibition. Odor-induced reduction of basal ORN activity has been documented in both vertebrates and invertebrates (for review, see Reisert and Restrepo, 2009). Analysis of the responses of *Drosophila* receptors to panels of odorants has shown that an individual odorant can activate some receptors and inhibit others, whereas an individual receptor can be activated by some odorants and inhibited by others. The existence of two response modes may add a degree of freedom to odor coding (de Brito Sanchez and Kaissling, 2005).

Perhaps the most biologically interesting form of receptor inhibition, however, is the ability of certain odorants to antagonize the response of receptors to activating odorants (Oka et al., 2004). This kind of antagonism may be essential to the coding of natural odors, which consist not of a single monomolecular species but of complex mixtures of molecules. We note that odor antagonism may also be of direct practical importance. For example, 1-hexanol has been found to inhibit the response of *Drosophila* CO_2 receptors, and it is possible that compounds that inhibit the CO_2 response of mosquitoes or other human-seeking insect pests could be useful in their control (Kwon et al., 2007; Lu et al., 2007; Turner and Ray, 2009).

The primary representations of different odors can be distinguished largely because of a key principle of mammalian and insect odor receptor expression: individual ORNs express only one or a small number of receptors. Thus the signaling of specific ORNs reflects the activity of specific odor receptors. This pattern of organization is in sharp contrast to that of the mammalian taste system, in which multiple bitter receptors are coexpressed in the same neurons (Mueller et al., 2005), impeding the discrimination of different bitter compounds.

Major questions remain concerning the initial representations of odorants. One critical direction for the field is to analyze the coding of odorant mixtures, which, although more difficult to study, is more representative of the problems that the olfactory system has evolved to solve. The temporal dynamics of odor representations need more attention; an impediment to such analysis is that airborne odors are more difficult to deliver with temporal precision than are visual or acoustic stimuli. We note that flies engineered to express only a single Or are still capable of odor discrimination, which may reflect the salience of temporal differences in the responses elicited by different odors (DasGupta and Waddell, 2008).

Translating Chemical Signals to Electrical Signals

What are the molecular events through which chemical signals—odors—are converted into electrical signals in the ORNs? The mechanisms have evolved under pressure to provide sensitivity, faithful temporal representation of odor stimuli, and a means of adaptation. Mechanisms of olfactory signal transduction have recently been reviewed in detail (Kato and Touhara, 2009; Nakagawa and Vosshall, 2009). Different types of ORNs in various olfactory organs use different signaling mechanisms. Here, we will highlight several areas in which recent progress has been particularly rapid or that offer particular opportunities for progress.

The olfactory systems of terrestrial animals face a challenge: most airborne odorants are hydrophobic, but ORNs must operate in an aqueous environment. To reach the receptors, therefore, hydrophobic odorants must traverse an aqueous fluid. In both mammals and insects, this fluid contains high concentrations of odorant binding proteins (OBPs), which are believed to solubilize and transport odorants. Mammals contain a few distinct OBPs, but insects contain remarkable numbers: in *Drosophila* there are 51 diverse members of the OBP family, a number comparable to the number of Ors (for review, see Pelosi et al., 2006). The crystal structures of some insect OBPs have been solved (Sandler et al., 2000), and the remarkable size and diversity of the insect OBP family have attracted much interest in their functional roles in olfaction.

Do OBPs confer odor specificity upon ORNs? When *Drosophila* Ors were expressed individually in the empty neuron system, the Ors conferred odor specificities that matched those of the ORNs from which they originated, suggesting that the Or is sufficient to endow the ORN with its odor specificity, at least in many cases (Hallem et al., 2004). Could pheromone reception be an exceptional case? A recent study found evidence that an OBP called LUSH, which binds the *Drosophila* pheromone cVA, interacts directly with the receptor of the cVA-sensitive ORN: a mutant LUSH protein, designed to mimic the cVA-bound conformation of LUSH, could elevate the activity of the ORN in the absence of cVA (Laughlin et al., 2008). The generality of this result is not yet clear, given that the binding protein for the silkmoth pheromone bombykol is not essential for the activation of the bombykol receptor, either in *Xenopus* oocytes (Nakagawa et al., 2005) or in the empty neuron system (Syed et al., 2006). However, the latter study found evidence that the presence of the bombykol OBP enhances sensitivity. Perhaps the development of an "empty sensillum" system, a mutant sensillum containing no endogenous OBPs, would provide a useful laboratory in which to examine physiologically in vivo the functions of the 51 members of the *Drosophila* OBP family.

After an odorant reaches a receptor, what happens? In the case of mammalian ORs, elegant structure-function analysis has provided evidence that the odorant binds to a pocket sur-

rounded by transmembrane domains 3, 5, and 6 of the receptor (Katada et al., 2005), a conclusion supported by a recent computational analysis (Saito et al., 2009). Binding is apparently mediated largely by hydrophobic and van der Waals interactions, and is looser than for many other GPCRs, which often bind their ligands via ionic or hydrogen bonds (Katada et al., 2005). The looseness of OR-odorant binding is consistent with the finding that odorant dwell times are extremely short (<1 ms) (Bhandawat et al., 2005). Loose interactions are also in agreement with the broad tuning of many odorant receptors, and they enhance combinatorial coding.

Although physiological and computational analyses have been very informative, our understanding of OR-ligand interactions and olfactory transduction would be enhanced enormously by the determination of the structure of an OR. The crystal structure of rhodopsin was extremely useful in understanding the conformational change it undergoes upon activation, and a comparable structure for an OR, although an immense challenge, would provide a great advance to the field of olfaction.

The activation of a mammalian odor receptor leads to a concatenation of events: the activation of a G protein, the activation of adenylyl cyclase, the elevation of cyclic AMP (cAMP) levels, the opening of a cyclic-nucleotide gated channel, the influx of Ca^{2+}, and the opening of a Ca^{2+} activated Cl^- channel, recently identified as Anoctamin2 (Stephan et al., 2009). The Cl^- influx provides the major amplification step in olfactory transduction, which is unique to vertebrate ORNs and is enabled by a chloride transporter that maintains a high Cl^- concentration in the cilia (Reisert et al., 2005). By contrast, in phototransduction amplification occurs via the activation of many G proteins by a single activated rhodopsin molecule, which does not occur in olfaction, consistent with the short odorant dwell time (Bhandawat et al., 2005). We note that in the olfactory system, mutation of the G protein, the adenylyl cyclase, and the cyclic-nucleotide channel have all been shown to have severe effects on olfactory function, suggesting that cAMP-dependent signaling is the dominant transduction mechanism in the main olfactory epithelium (for review, see Touhara and Vosshall, 2009).

To ensure faithful temporal representation of odor stimuli, expeditious signal termination is required. Interestingly, the key termination mechanisms reported in phototransduction—phosphorylation of the receptor, arrestin binding to the receptor, and RGS protein-mediated inactivation of the G protein—have not been shown to play a major role in the rapid termination of an olfactory signal. Rather, Ca^{2+}-mediated feedback inhibition of the cyclic-nucleotide gated channel, activation of phosphodiesterase, and inhibition of the adenylyl cyclase, along with extrusion of Ca^{2+}, have been implicated (for review, see Bradley et al., 2005). Perhaps the receptor is not a target for signal termination in olfaction because of the short odorant dwell time.

The acuity of olfactory perception relies upon adaptation. Adaptation allows extension of the dynamic range of ORNs such that they are informative over a broader range of odorant concentrations, and it enables an animal to detect new scents above a background odor. Common mechanisms may contribute to both adaptation and termination. However, the details remain to be elucidated. For instance, although the cyclic-nucleotide-gated channel and a phosphodiesterase (PDE1C) had been believed to play a key role in adaptation and termination, respectively, recent studies have not supported these notions (Cygnar and Zhao, 2009; Song et al., 2008). Determination of the mechanisms of adaptation will require more detailed electrophysiological studies of ORNs in genetically-manipulated animals.

Insect olfactory transduction has been the focus of much recent attention. Insect odor receptors have seven transmembrane domains and have long been assumed to be GPCRs like their counterparts in mammals and in the nematode *C. elegans*. However, in contrast to mammals and worms, no G protein mutant has been found to suffer a severe loss of olfactory function. Moreover, the topology of the insect Ors is inverted relative to GPCRs (Benton et al., 2006; Smart et al., 2008), such that the N terminus is intracellular, and each Or appears to form a heteromultimer with one particular Or family member, Or83b (Benton et al., 2006; Neuhaus et al., 2005).

Two recent studies show that insect odor receptors can act as ionotropic receptors: a canonical Or, together with Or83b, can form a ligand-gated cation channel (Sato et al., 2008; Wicher et al., 2008). Both studies examine heterologous cells expressing an Or with Or83b and observe an odorant-induced, rapidly developing, transient inward current. Both groups find the rapid, inward transient to be independent of G protein signaling; however, one of the groups also observes a second, slower and larger component to the odorant-induced inward current (Wicher et al., 2008). This second component is slower both in onset and decay kinetics and is sensitive to inhibition by the GDP analog GDP-βS, as if insect odor receptors can also function as metabotropic receptors that signal via a G protein-mediated pathway. Interestingly, this metabotropic pathway produces cyclic nucleotides, and Or83b is activated directly by cyclic nucleotides. The observation of this second component of the current led to the proposal of a two-step signaling model. Upon odorant-binding, the ligand-gated Or/Or83b channel complex would produce a fast, inward current, followed by a larger and slower metabotropic cyclic nucleotide-gated current.

Taken together, these results provide strong evidence that Ors can act as ligand-gated ion channels. A detailed understanding of their role in G protein signaling will require further analysis of the nature of their interactions with the G protein. Understanding of both signaling modes would benefit enormously from structural analysis of the receptor. Above all, conclusions from the studies in heterologous cell systems will need to be confirmed in fly ORNs. It will also be of interest to compare the signaling mechanism of Ors with those of IRs and of the Grs that act in carbon dioxide signaling.

Transformation of Olfactory Signals at the First Processing Center

The primary representation of an odorant is distributed among a large number of ORNs. This representation is transformed into a secondary representation at the first processing center, the olfactory bulb in mammals and the antennal lobe in insects. The secondary representation is distributed among a much smaller number of output cells,

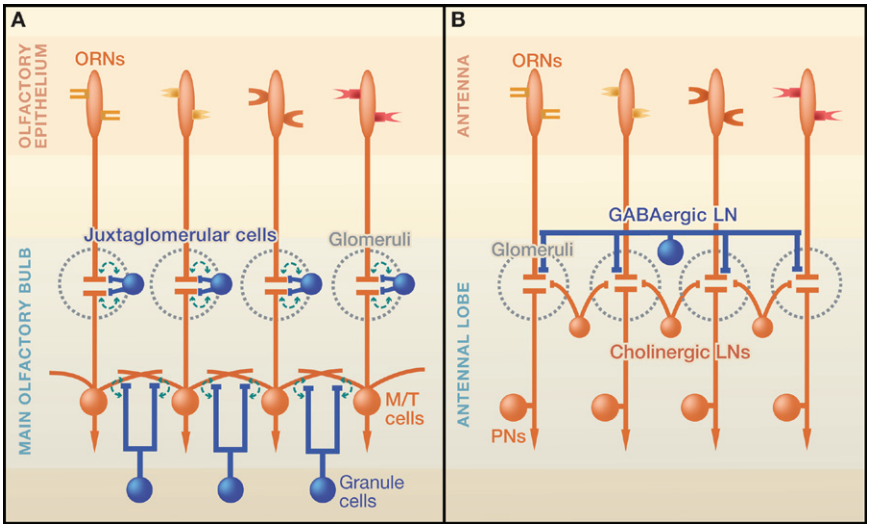

Figure 2. Olfactory Bulb and Antennal Lobe Circuitry

Excitatory neurons are shown in orange and inhibitory neurons in blue.

(A) Olfactory receptor neurons (ORNs) in the olfactory epithelium that express different olfactory receptors project axons to separate glomeruli (dashed outlines) in the olfactory bulb where they synapse on mitral and tufted (M/T) cells, whose apical dendrite is usually localized to a single glomerulus. Juxtaglomerular cells (blue) contribute to intraglomerular inhibition. In the glomerulus, ORNs form synapses on juxtaglomerular cell dendrites, which in turn inhibit ORN axon terminals. Reciprocal synapses are also found between juxtaglomerular cell and M/T cell dendrites. Reciprocal synapses are formed between the dendrites of granule cells and M/T cells. M/T cells excite granule cells, which respond by inhibiting M/T cells. Due to the lateral spread of M/T secondary dendrites, granule cells contact multiple M/T cells associated with different glomeruli, and thus can mediate both intra- and interglomerular inhibition.

(B) In *Drosophila*, ORNs expressing the same olfactory receptors in the antenna or maxillary palp synapse on projection neurons in a single glomerulus, analogous to the olfactory bulb. GABA-releasing local neurons (LNs) presynaptically inhibit ORN axon terminals in multiple glomeruli, mediating interglomerular inhibition. Excitatory cholinergic LNs mediate interglomerular excitation.

which transmit information to higher regions of the brain. The organization of the center that accomplishes this signal transformation is surprisingly similar in mammals and insects (Figure 2).

In most mammals and insects examined, ORNs that express the same receptor converge upon one or two glomeruli (see also Maresh et al., 2008; for review, see Wilson and Mainen, 2006). The convergence ratio is high, on the order of 50 ORNs per glomerulus in *Drosophila* and 5000 ORNs per glomerulus in rodents. In the glomerulus, the axon terminals of ORNs form synapses with the dendrites of the output neurons, which are called mitral and tufted (M/T) cells in mammals and projection neurons in insects. Most individual M/T cells and projection neurons receive direct excitatory input from only one type of ORN, expressing one type of odor receptor. The high convergence ratios of ORNs to projection neurons may allow for the integration and amplification of weak signals; they may also allow for the averaging of stronger signals, which should lead to a higher signal-to-noise ratio in the projection neurons, that is, a higher ratio of response strength to variance.

The activities of M/T cells and projection neurons are also regulated by interneurons that allow communication within and between glomeruli. In the mammalian olfactory bulb, these interneurons lie in two layers: interneurons called juxtaglomerular cells lie in the glomerular layer, and granule cells lie in a deeper layer called the external plexiform layer (Figure 2A). Juxtaglomerular cells receive direct excitatory input from ORN axons and form inhibitory synapses onto ORN axons within the same glomerulus. Granule cells form inhibitory synapses onto M/T cells of multiple glomeruli and mediate interglomerular information transfer (for reviews, see Shepherd et al., 2007; Wilson and Mainen, 2006). In the insect antennal lobe, interneurons called local neurons connect glomeruli and are primarily inhibitory (Figure 2B) (Wilson and Mainen, 2006).

The circuitry of each of these processing centers and the computations that they perform have been analyzed through anatomical, electrophysiological, and imaging studies (for reviews, see Laurent, 2002; Wilson and Mainen, 2006). Here, we will focus on the cellular basis of the transformations that occur in these centers, with special attention to recent advances made in the antennal lobe of *Drosophila*.

An ideal means of analyzing these transformations is to compare the response profiles of presynaptic ORNs to their postsynaptic M/T cell or projection neuron partners. This approach is conceptually simple but technically difficult. The complexity of vertebrate olfaction makes it very difficult to carry out such analysis systematically.

Drosophila is an attractive system in which to carry out such analysis because of its numerical simplicity and defined organization. In the fly there are 18 defined types of sensilla in the antenna and three in the maxillary palp, and the ORNs they contain have been functionally analyzed through single-unit electrophysiology (de Bruyne et al., 1999; de Bruyne et al., 2001; Hallem et al., 2004; van der Goes van Naters and Carlson, 2007; Yao et al., 2005) (Table 2). The odor response profiles of ~35 ORN classes have been defined in systematic studies. The odor response profiles of most of the antennal Or receptors have been analyzed in considerable detail (Hallem and Carlson, 2006). Receptor-to-neuron maps have been constructed, and a glomerular projection map has been generated (Couto et al., 2005; Fishilevich and Vosshall, 2005; Hallem et al., 2004). The glomeruli have stereotyped locations and can be genetically labeled via ORNs or PNs using the promoter-*GAL4*/*UAS*-reporter system.

Projection neurons innervating one particular glomerulus, DM2, respond to a broader range of odorants than their presynaptic ORNs in a patch-clamp analysis (Wilson et al., 2004b). Similar results obtained for six other glomeruli suggest that this broadening of range represents a general principle of olfac-

tory processing in the antennal lobe. Moreover, weak ORN responses, but not strong ones, are amplified in projection neurons, a process called nonlinear amplification (Bhandawat et al., 2007). However, all of these glomeruli received input from ORNs that respond to a number of general odors. Examination of a specialized glomerulus that responds to a *Drosophila* pheromone does not reveal a comparable broadening (Schlief and Wilson, 2007).

What mechanism underlies the broadening of receptive range observed in most glomeruli? Lateral excitatory inputs mediated by a class of cholinergic local neurons may make a minor contribution to this broadening (Olsen et al., 2007; Root et al., 2007; Shang et al., 2007). Interestingly, however, the broader tuning widths and nonlinear amplification among projection neurons are mainly due to strong ORN-projection neuron synapses (Kazama and Wilson, 2008). Weak presynaptic ORN activity is sufficient to trigger robust neurotransmitter release at this synapse and cause substantial projection neuron responses, thereby producing amplification. Strong ORN activity leads to depletion of synaptic neurotransmitter, explaining why strong ORN responses are not amplified proportionally in projection neurons. The strong synapses between ORNs and projection neurons are attributable to the presence of numerous synaptic vesicle release sites and a high release probability (Kazama and Wilson, 2008). High probabilities of vesicle release have also been found in the mammalian olfactory bulb (Murphy et al., 2004), which likely reflects the same principle of information processing.

Odor perception may be enhanced by this nonlinear amplification in interesting ways (for review, see Masse et al., 2009). The fly encounters an enormous range of odorant concentrations in its natural environment, and the ability to evaluate odor intensity may facilitate navigation. ORNs respond to odors over a range as wide as eight orders of magnitude (Hallem and Carlson, 2006), which raises questions about how such a wide range can be efficiently conveyed via the relatively narrow firing frequency ranges of ORNs and projection neurons (approximately two orders of magnitude). One mechanism is through gain control: by altering the relationship between the input firing rate of ORNs and the output firing rate of projection neurons, nonlinear amplification prevents saturation of projection neuron responses when ORN responses are high (Kazama and Wilson, 2008). Another interesting consequence of the physiology of the ORN-projection neuron synapse is that it may emphasize the initial phase of an ORN response, given that the first spikes produce a larger effect on projection neurons. Such emphasis may also aid navigation: moth projection neurons have been shown to respond quickly to changes in odor concentration (Vickers et al., 2001).

A second mechanism of gain control arises from lateral inhibitory communications among glomeruli, mediated by GABAergic local neurons (Olsen and Wilson, 2008). The site of inhibition is presynaptic, that is, at the ORN axon terminals. For an individual glomerulus, the strength of the lateral inhibition that it receives is proportional to the total ORN activity across the antenna. Thus, high levels of overall ORN activity downregulate projection neuron activity and prevent their saturation. The extent of downregulation, however, may vary for different glomeruli (Root et al., 2008).

The mammalian olfactory bulb also exhibits presynaptic inhibition, mediated primarily by intraglomerular connections (McGann et al., 2005). Many juxtaglomerular cells receive direct excitatory input from ORN axons and form inhibitory synapses onto ORN axons within the same glomerulus, thereby providing intraglomerular feedback inhibition that may extend the dynamic range of the glomerulus. The strength of inhibition appears independent of ORN activity (Pirez and Wachowiak, 2008), suggesting a different mechanism from that of *Drosophila*. Interglomerular inhibition occurs mainly on M/T cells and is mediated by granule cells in the external plexiform layer (for review, see Wilson and Mainen, 2006).

It will be interesting to determine how inhibition may contribute to other aspects of odor perception. Does the activation of one glomerulus inhibit surrounding glomeruli in such a way as to enhance odor discrimination? To resolve this question, it will be helpful to have more detailed maps of the patterns of lateral inhibition and to have a better understanding of the functional organization of glomeruli. Many studies have provided evidence for a coarse chemotopy, in the sense that the location of glomeruli is related to the chemical nature of the odors that activate them (for review, see Johnson and Leon, 2007). However, recent large-scale studies have not found clear evidence for fine-scale chemotopic maps (Hallem and Carlson, 2006; Soucy et al., 2009), and the relationship between function and topography remains an intriguing issue.

We note finally that odor-evoked oscillations in electrical activity have been found in the olfactory bulb of mammals, as well as in the antennal lobe of locusts, bees, and moths: the activities of populations of neurons are synchronized in an oscillatory pattern (for review, see Laurent, 2002). Disruption of this oscillatory network affects the ability of honeybees to discriminate similar odorants (Stopfer et al., 1997), consistent with a function for oscillatory mechanisms in enhancing olfactory acuity.

Circuitry and Coding in Higher Brain Regions

In mammals and insects, the second order neurons—M/T cells and projection neurons—innervate multiple higher brain regions. In these regions, the olfactory information is integrated with information from other sensory modalities, information from past experience, and information concerning the animal's behavioral state, to shape olfactory perception and to instruct behavior.

In mammals, M/T cells synapse directly on pyramidal neurons in the olfactory cortex. The olfactory cortex contains several distinct regions, including the piriform cortex, the olfactory tubercle, the anterior olfactory nucleus, and certain parts of the amygdala and entorhinal cortex. Unlike other sensory systems, olfactory signals are not relayed through the thalamus before reaching the cortex. Olfactory cortical neurons form dense reciprocal connections with neurons from other regions of the olfactory cortex. They also form connections with other regions such as the orbitofrontal cortex, thalamus, and hypothalamus, allowing them to act as sites of integration.

Odor coding has been analyzed in pyramidal neurons of the piriform cortex and has been found to be sparse: an odor stimulus elicits responses from only a small fraction of spa-

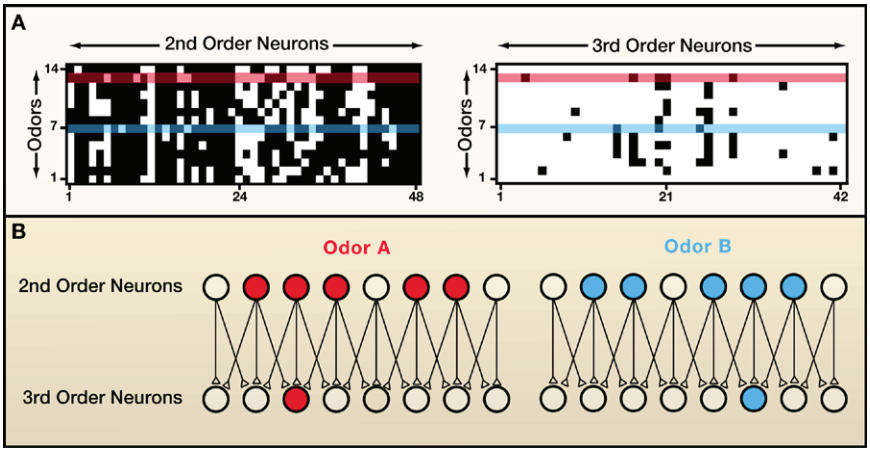

Figure 3. Transformation of Odor Representations

(A) Responses of 48 second order neurons (projection neurons, PNs) and 42 third order neurons (Kenyon cells) in locusts to a panel of 14 odors. Black squares indicate activation of a neuron by an odor; white squares indicate lack of response or inhibitory responses. PNs respond to many odors, in contrast to Kenyon cells, which respond to only a few (sparse coding). Additionally, similar activation patterns of second order neurons, e.g., odors 7 (blue) and 13 (red), result in highly divergent activation patterns of third order neurons, a process termed "decorrelation." This decorrelation is thought to make these odors easier to discriminate. Adapted from Perez-Orive et al. (2002). Reprinted with permission from AAAS.

(B) A simplified illustration of how sparsening and decorrelation of responses occur in third order neurons. Odors A and B each activate multiple second order neurons (colored circles), with similar patterns of activated neurons. However, due to the requirement of synchronized inputs from multiple second order neurons (coincidence detection), many fewer third order neurons are activated (sparsening) with more distinct activation pattern (decorrelation).

tially dispersed neurons, and these responses consist of few action potentials (Figure 3) (Illig and Haberly, 2003; Litaudon et al., 2003; Poo and Isaacson, 2009). Moreover, each neuron responds to only a limited number of odors (Litaudon et al., 2003; Poo and Isaacson, 2009). Thus, cortical pyramidal neurons appear to be much more narrowly tuned than their presynaptic neurons.

What is the underlying basis of this reduction in tuning width? Unlike M/T cells, whose activity is driven primarily by a single type of odor receptor, pyramidal cortical neurons receive synapses from multiple M/T cells that carry output from multiple glomeruli and thus multiple odor receptors (Franks and Isaacson, 2006). The pyramidal neurons act as coincidence detectors: they only fire action potentials when a certain subset of M/T cells is synchronously active. The coincident activity of several presynaptic M/T cells is required to overcome widespread inhibition mediated by local interneurons, inhibition that is odor evoked (Poo and Isaacson, 2009). Given that an individual odor is unlikely to activate the precise combination of odor receptors necessary for an individual pyramidal neuron to fire, very few pyramidal neurons fire, and hence coding is sparse. This form of processing is likely to enhance the capacity of the system to discriminate structurally similar odorants. Even if two odorants activate very similar subsets of glomeruli in the olfactory bulb, their representations in cortical regions are distinct; they are decorrelated as a result of the requirement for coincidence detection (Figure 3B).

In *Drosophila* and many other insects, projection neurons innervate the lateral horn of the protocerebrum and the mushroom bodies, where they synapse on neurons known as Kenyon cells. Coding in the mushroom body is remarkably similar to that in the piriform cortex. Kenyon cells receive inputs from multiple glomeruli and are more narrowly tuned than their presynaptic projection neurons; their sparse coding also depends on coincidence detection, and on global inhibition (Lin et al., 2007; Perez-Orive et al., 2002; Tanaka et al., 2004; Turner et al., 2008; Wang et al., 2004).

An individual olfactory stimulus may elicit different percepts depending on prior experience and olfactory learning. In rodents, it is well established that neural representations of odors in higher brain regions as well as the olfactory bulb are dictated not only by odorant structure, but also by experience (Wilson et al., 2004a). Recent functional magnetic resonance imaging studies on humans have found evidence for experience-based changes in the piriform cortex (Li et al., 2008; Li et al., 2006). The anatomical basis of olfactory learning in this brain region may involve an extensive system of association fibers that connects neurons within the same and different olfactory cortical regions and whose synaptic strengths can be modified by olfactory experience (Wilson et al., 2004a). Olfactory learning can enhance odor discrimination and may be important to the survival of many organisms. In humans, changes in cortical neuronal activity due to olfactory learning are correlated with improved discrimination of similar odorants (Li et al., 2008).

In many animals, certain odors exhibit behavioral responses that appear not to be learned, but rather to be innate. These responses are reminiscent of the innate responses to sweet and bitter substances that are driven by the gustatory system. In addition to responses to pheromones, which are innate, both insects and mammals can be innately attracted to or repelled by certain nonpheromonal odorants through a mechanism that depends on the activation of specific glomeruli or glomerular subsets (Kobayakawa et al., 2007; Semmelhack and Wang, 2009; Suh et al., 2007).

Innate responses are believed to be mediated by hardwired circuits that link specific ORNs to specific neurons in higher centers, and evidence to support this notion comes from genetic labeling studies in *Drosophila.* These studies reveal that axons of projection neurons from the same glomerulus show stereotyped projections to the lateral horn (Marin et al., 2002; Wong et al., 2002). Moreover, projection neurons associated with food odors target a different region of the lateral horn than do projection neurons associated with pheromones, which may underlie the difference in

innate behaviors elicited by these two classes of odorants (Jefferis et al., 2007). Interestingly, projection neurons sensitive to the pheromone cVA have sexually dimorphic projections, suggesting a mechanism by which the pheromone evokes different behaviors in males and females (Datta et al., 2008). Anatomical and physiological analysis did not find such a high level of stereotypy in the mushroom body (Marin et al., 2002; Murthy et al., 2008; Tanaka et al., 2004), consistent with the finding that the structure of the mushroom body is influenced by experience and that it plays a central role in learning (Heisenberg, 2003).

Regulation of the First Processing Center by Higher Centers

Thus far, we have considered the flow of olfactory information from the receptors to the first processing center to higher brain regions, that is, the "bottom-up" pathway. However, odor perception is not a simple feedforward process in mammals: there is also a "top-down," or centrifugal, pathway that provides feedback and other forms of regulation. Ultimately, odor perception is shaped by the interaction of the two pathways.

Higher brain regions regulate olfactory bulb activity in a manner that is influenced by learning, by anticipation of an odor or a reward, and by behavioral states including hunger (for review, see Rinberg and Gelperin, 2006). The centrifugal fibers that effect this regulation have been divided into two classes based on their location of origin. One class originates in cortical regions, primarily the olfactory cortex. The other class originates from brain regions containing neurons that release neuromodulators.

The centrifugal fibers that arise in areas of the olfactory cortex, including the piriform cortex and the anterior olfactory nucleus, provide feedback to the olfactory bulb. These fibers release the excitatory neurotransmitter glutamate and form synapses primarily on subsets of granule cells. Such fibers regulate the lateral inhibition of M/T cells and can undergo experience-dependent synapse strengthening (Balu et al., 2007; Gao and Strowbridge, 2009). Interestingly, whereas the targets of ORNs and M/T cells are ipsilateral, subsets of centrifugal fibers from the anterior olfactory nucleus receive input from specific glomeruli and send feedback projections to isofunctional glomeruli in the contralateral olfactory bulb (Yan et al., 2008). This pattern of organization suggests a role for these fibers in coordinating signal processing between the two brain hemispheres, a possibility that is of interest in light of recent evidence that rats use internasal comparisons to locate odor sources (Rajan et al., 2006).

Neuromodulatory centrifugal fibers originating in other regions selectively target granule, M/T, and/or juxtaglomerular cells and release norepinephrine, serotonin, or acetylcholine. Disruption of these inputs influences olfactory-driven behaviors. For example, the blocking of norephinephrine receptors prevents rat pups from learning to associate tactile stimuli with specific odors, an ethologically important form of associative olfactory learning (Sullivan et al., 1992). More recent investigations have focused on the circuit-level effects of neuromodulators. For example, the pairing of an odor with norepinephrine release leads to a long-lasting decrease in M/T cell responses specific to that odor (Shea et al., 2008), whereas serotonin release nonselectively reduces odor-evoked ORN synaptic activity due to an increase in juxtaglomerular cell-mediated presynaptic inhibition (Petzold et al., 2009). Through such effects on signal processing in the olfactory bulb, the centrifugal neuromodulatory fibers have been suggested to influence odor discrimination, to provide a means of gain control, and to generate local experience-dependent changes in the olfactory bulb. It will be particularly interesting to learn more about the mechanisms through which these fibers are activated.

In the insect antennal lobe, centrifugal inputs are largely uncharacterized. Moths provide an interesting exception: serotonergic fibers increase behavioral sensitivity of male moths to some sex pheromones by supplying centrifugal input from the protocerebrum to antennal projection neurons and local neurons (Kloppenburg and Mercer, 2008). In *Drosophila*, serotonin was recently shown to excite both projection and local neurons and to increase ORN presynaptic inhibition; it is proposed to suppress weak ORN responses (Dacks et al., 2009). An integrated analysis of the anatomy, physiology, and behavioral effects of centrifugal inputs in insects seems likely to be a fruitful avenue of future research.

We note finally another interesting modulator of olfactory activity in mammals: sniffing. Changes in sniffing frequency affect odor intake into the nasal cavity and thus regulate the magnitude and temporal dynamics of ORN activation. Sustained high-frequency sniffing depresses activity in ORNs responsive to the presented odor, perhaps as a result of OR adaptation (Verhagen et al., 2007). Increases in the rate of sniffing are often observed when mammals encounter novel odorants and when they anticipate odor presentation; higher sniffing rates may enhance discrimination (Kepecs et al., 2007).

Odor Spaces

Olfaction can be thought of as a series of transformations. Information about the structure of an odorant is transformed into a succession of neural representations and is ultimately transformed into a perception. A long-term goal of the field is to understand the rules governing each transformation. Ultimately, one would like to be able to predict each transformation. If a chemist synthesizes a new molecule, one would like to be able to predict its activity profile across an odor receptor repertoire, the activities of glomeruli and of higher centers, and whether the molecule will evoke a fruity or a musky odor, or whether it will elicit attraction or repulsion.

There are major challenges to meeting this goal. First, predictive ability is limited by the biological complexity of odor perception. In many cases, the relationship between odor structure, neural representations, and perception is not strictly deterministic, owing to the role of experience and other factors. Second, prediction depends on identifying parameters that adequately describe the odorants, the neural representations, and the perception.

Odor structure cannot be described by a simple variable such as wavelength. It is therefore difficult to compare two odorant structures quantitatively: is the structure of hexyl hexanoate more closely related to that of methyl hexanoate

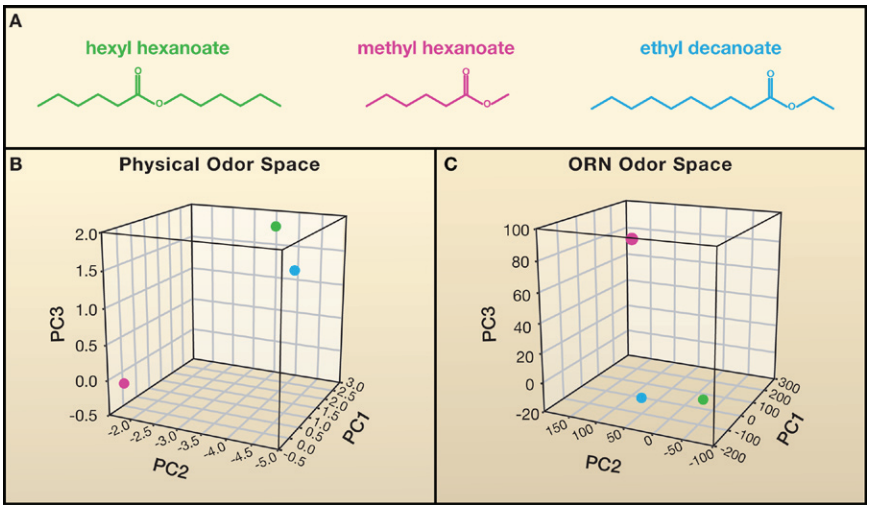

Figure 4. Representations of Odor Space

(A) Chemical structures of three odorants. It is difficult to compare the degree of relatedness between odorants by visual inspection.

(B and C) A physical odor space (B) constructed using 32 optimized descriptors of odorant structure (Haddad et al., 2008), for the odor panel used in Hallem and Carlson (2006). The Euclidean distance between hexyl hexanoate (green) and ethyl decanoate (blue) is smaller than the distance of either odorant to methyl hexanoate (magenta), indicating that hexyl hexanoate and ethyl decanoate are more structurally similar. The first three principal components (PC) are shown. Similarly, hexyl hexanoate and ethyl decanoate map closer to each other than to methyl hexanoate in a neural odor space (C), based on the functional data reported in Hallem and Carlson (2006).

or ethyl decanoate (Figure 4A)? Odorants vary in an indeterminate number of parameters, including carbon-chain length, molecular weight, and polarity. Recently a multidimensional, physicochemical odorant space was devised to describe odorant structure: 1664 molecular descriptors for more than 1500 odorants were used to construct a 1664-dimensional odor space, in which each dimension represents one feature of odorant structure (Haddad et al., 2008). An odorant can be mapped to a unique location in this space according to its values for each descriptor (Figure 4B). The physicochemical relationship between two odorants can then be quantitated as the Euclidean distance between them in this space.

This physical odor space is useful in predicting odor perception. Principle component analysis reveals a correlation between odorant structure, as defined in the space, and its perceived pleasantness among humans (Khan et al., 2007). Further analysis led to the development of a simpler, optimized odor space based on 32 of the descriptors, chosen after analysis of the functional responses these odorants elicited in several experimental systems (Haddad et al., 2008). Interestingly, a subsequent study of odor responses elicited from a set of human and mouse ORs yields a slightly different set of optimized descriptors. The optimized descriptors may differ for ORs of different species (Saito et al., 2009). Much of the variation in OR response could be explained by a relatively small subset of descriptors.

Another kind of olfactory space is a neural odor space, which illustrates how a particular odorant is represented in the olfactory system. A neural space can be constructed from direct measurements of odor responses from neurons of the system or, in the case of ORNs, from responses of odor receptors in an expression system, provided that the expression system faithfully represents the activities of receptors in their endogenous neurons (Hallem et al., 2004). Constructing such a space is at present a major undertaking, but is more feasible in systems that are numerically simpler. For example, in an analysis of the *Drosophila* antennal receptor repertoire, most of the Ors expressed in the antenna could be examined. Of these, ~75% were found to yield responses and were systematically tested with 110 odorants (Hallem and Carlson, 2006). An odor space was then constructed in which each axis represents the response of one receptor. Each odorant was then mapped to a position in this space based on the response magnitude for each receptor. In such a space, two odorants will map close together if they elicit similar responses patterns across the receptor repertoire (Figure 4C). Are two odorants that are close in this neural space also close in perceptual quality? Analysis of the odor receptor repertoire of *Drosophila* larvae, along with an accompanying behavioral analysis, provides support for this notion (Kreher et al., 2008).

Analogous neural spaces can be constructed for each successive level of processing, and the distribution of odorants in each successive space is likely to differ from that of its predecessors. The nonlinearity of the ORN-projection neuron transformation, for example, acts to generate a broader distribution of odorants in projection neuron space than in ORN space (Bhandawat et al., 2007). It will be interesting to determine whether the relative positions of odorants at successive levels of processing provide more accurate predictions of perceptual relationships, such as perceived odor similarity. In interpreting such spaces, however, it is important to consider that certain innate olfactory behaviors may be mediated by small subsets of the neurons, rather than the activation patterns of the entire set of neurons (Semmelhack and Wang, 2009). Although combinatorial coding underlies odor perception, it may not be essential for the initiation of many odor-induced innate behaviors.

An interesting problem for future research is to determine how neural spaces have evolved to meet the ecological needs of the species. Different species rely on different odorants as cues, and it seems likely that neural odor spaces have undergone changes in structure to promote the detection and discrimination of particular subsets of odorants.

Conclusion

Olfactory perception has a more diverse basis than previously appreciated—entire new families of receptor genes and a new signal transduction mechanism have recently been discovered. The molecular, cellular, and anatomical diversity of the signal-

ing systems may reflect the immense variety of signals that are detected and encoded. It will be of great interest to gain further insight into how the distinct features of different signaling systems subserve their biological functions.

New insight has also been gained into the mechanisms by which signals are processed in the glomeruli and in higher brain regions. Despite their evolutionary distance, the parallels between insect and mammalian olfactory circuitry are striking, perhaps reflecting similar challenges in extracting critical olfactory information. Further understanding of olfactory processing will surely benefit from more detailed maps of neural circuitry. Just as the discovery of new receptor genes provides a new dimension to our knowledge of how environmental signals are transduced, the delineation of the circuitry should provide new insight into how olfactory representations are transformed into a succession of neural representations.

Ultimately, understanding of olfactory perception requires a higher-order biological perspective. Although the mechanisms by which odors are coded and decoded can be powerfully deconstructed through molecular and physiological analysis, the significance of these mechanisms must be examined in behaving animals of a wide variety of species.

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