How We Smell: The Molecular and Cellular Bases of Olfaction

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Three models for the perception of odor molecules are suggested for the first time by experimental data. These studies illustrate how the nose may smell. Moreover, they suggest additional role(s) for odor receptors within and outside the olfactory system.

An idealist is one who, on noticing that a rose smells better than a cabbage, concludes that it will also make better soup.

H. L. Mencken (1880–1956)
A Book of Burlesques, Sententiae (1920)

Olfaction is the sense responsible for the perception of odors and, together with audition and vision, provides information for analyzing the surroundings. In association with taste, it allows the subtle detection and discrimination of a myriad of scents that are disseminated in the air by odoriferous plants or animals and present in various foods. The sense of smell has multiple functional roles: it influences emotional states, i.e., enthusiasm or attention, and sexual behavior. It provides social information about the family, the clan, or outsiders in animals without language skills; it allows the control of food hygiene by warning against rotten food after memorizing associations with unpleasant odors; and, finally, it activates salivary and gastric secretions in response to pleasant odors.

Only volatile substances have an odor, which, in the air, may be associated with particulate matter. Several thousand odors can be distinguished by the sense of smell, but it is very difficult to categorize them based on their chemical structure or molecular shape. A trained human can recognize several classes of compounds, such as ethereal (wine), aromatic (camphor, menthol), balsamic (violet, vanilla), alliaceous (garlic, iodine), ambrosial (amber, musk), empyreumatic (tar, coffee), which defines an acrid odor contracted by an organic matter when subjected to the action of a strong heat, hircinnic (sweat), repulsive (pyridine, opium), or nauseous (feces, putrefaction).

One of the most remarkable features of olfaction is the very high sensitivity or the low threshold of detection. Certain odors in the air are already detected, although not identified (“I smell something”), at a concentration as low as $4 \times 10^{-15}$ g/l and are readily identified at $2 \times 10^{-13}$ g/l. By comparison, one of the most potent taste molecules, quinine, is not detectable in water below 4 mg/l, whereas table salt is not detected in water below 1 g/l even by the finest palate. However, the olfactory system is subject to a rapid habituation which takes place within 1 min by the desensitization of the odor receptors at the periphery, and to a slower adaptation, starting usually after 1 min, of neuronal pathways in the central nervous system. Therefore, the olfactory system is always ready for the detection of novel odor molecules, but once they have been recognized, adaptation takes place and the odor signal is no longer perceived in a conscious manner.

Cellular structures involved in smell perception

Olfactory molecules are detected either by a pseudostatified sensory neuroepithelium, bathed by a mucus produced by the Bowman’s glands, located in the olfactory conchae of the nasal fossae (in the upper tract of the respiratory airways) in vertebrates (Fig. 1), or by the antennae in insects. In humans, the olfactory epithelium covers a surface of about 3 cm² and is composed of three main cellular types: the olfactory sensory neurons (also called olfactory receptors), the sustentacular or supporting cells, and the basal epithelial or stem cells.
More than 20,000 odors are thought to be detected.

A preparation of isolated olfactory neurons has been extensively used to characterize their electrophysiological responses to odor stimulation. The olfactory neurons (~10⁵ in humans, ~10⁶ in rodents) are bipolar and possess a dendritic knob ending with several cilia that are bathed in the mucus. More than 20,000 odors are thought to be detected by odor-receptor proteins enriched in the ciliary membranes. The initial chemical signal is thereby transformed into electrical impulses propagated along a single axon that projects directly, without relay, to specialized structures (the glomeruli) in the olfactory bulbs (Fig. 2). The olfactory neurons are directly exposed not only to odor molecules but also to xenobiotics and toxic substances from the external environment. Olfactory neurons degenerate with a lifetime (turnover) of 30-40 days. The dying neuron is replaced by a newly differentiated stem cell, which eventually expresses the same cellular properties in terms of odor sensitivity, odor preference, and axonal projection as the previous neuron (5).

As opposed to the cellular basis of olfaction (see above), little was known, until recently, about the molecular basis for the sensitivity and specificity of the sense of smell. What signal transduction pathways are induced by odors? What types and how many different odor receptors exist? Below I present a summary of the observations that increased the understanding of olfaction by the cloning and investigation of a large family of putative odor-receptor genes. For the transduction mechanisms involved in olfaction, the reader may consult a recently published review (1).

The putative odor-receptor gene family

With a very sensitive technical approach called reverse transcription-polymerase chain reaction (RT-PCR) using highly degenerated primers obtained from the seven transmembrane domain (7-TMD) class of receptors (8), an interesting observation was made using cDNA derived from the olfactory epithelium (4). It was possible to amplify ~30-50 different 7-TMD receptor sequences that define an entire novel subfamily of genes (4). The proteins derived from these coding sequences have been called either 1) odor, odorant, or olfactory receptors; 2) putative olfactory receptors; or even 3) orphan receptors, because none of these proteins has been shown to bind individual odor molecules with a high and specific affinity.

Genomic DNA analysis and PCR amplification have provided estimates for the size of the putative odor receptor (OR) gene family repertoire of ~20 genes in birds, ~50 in fish, ~200 in Caenorhabditis elegans, and ~500-1,000 in rodents or humans. These observations may correlate with the performance indexes for the efficacy of the different olfactory systems: high discrimination capabilities in mammals, but low performances in birds and fish. The vast majority of the OR genes are expressed, as observed by in situ hybridization, in olfactory neurons. So far, the spatial distributions of tested OR transcripts on the surface of the olfactory epithelium are widespread and are random in birds and fish but more restricted (or zonal) with a bilateral symmetry in rodents (11). Although widespread and randomly distributed at the periphery (in ~1,000–10,000 sensory neurons in the mouse olfactory epithelium), transcripts for a particular OR are restricted to only two discrete places in the mouse olfactory bulbs, which correspond to one pair of glomeruli of ~900 pairs of glomeruli. The convergence of axonal projections for neurons expressing the same OR gene to a fixed position in the brain may represent the molecular explanation for the high sensitivity of the olfactory system (Fig. 2). Indeed, these results are consistent with theoretical models in which sensitivity is proportional to the convergence ratio (a large convergence ratio results in a high sensitivity system). These data also suggest that convergent processing of odor signals from the periphery to the brain occur through a “hard-wired” system. Discrimination capabilities, however, could rely on the intrinsic binding affinities of odor molecules for their receptors: if high and specific, the activation of a single receptor by a given odor would result in the activation of a single pair of glomeruli; if low and unspecific, a...
given odor could activate several receptors, which, in turn, could simultaneously activate several pairs of glomeruli.

Models to study olfaction: molecular, neural assembly, and genetic

For rodents, a “molecular approach” model, derived from the data presented above in the preceding paragraph in which a topographic map of OR activation encodes odor quality in the olfactory bulb, has been proposed by Richard Axel (2) at Columbia University and is supported by the following observations. 1) Olfactory neurons that express a particular type of OR are randomly distributed within a zone in the olfactory epithelium. 2) Their axonal projections converge to a pair of specialized regions (glomeruli) in the olfactory bulbs. In this model, a given odor would therefore be identified by a characteristic pattern of activity in the glomeruli (see Fig. 2).

A very different model has been proposed by Gilles Laurent at Caltech, who has developed a “network approach” to study neural assemblies and olfactory coding in the antennal lobe system in the locust brain. In insects, odor molecules elicit oscillatory waves of activity (15) but not individual glomerulus activation as proposed by the first model. Each odor activates a different sequence of spatial patterns of ~100 projection neuron cells and evokes temporal sequences of firing in dynamic ensembles of transiently synchronized neurons. The oscillatory synchronization depends on inhibitory interactions within the olfactory network, thus providing direct support for a cellular component in odor coding and processing in the brain.

In a third approach, Cory Bargmann and associates (12) at University of California San Francisco have developed a genetic approach to investigate odor responses in the nematode C. elegans, which possesses 14 types of chemosensory neurons and responds to dozens of odors. Genetically engineered mutants deficient for the detection of one or several odors have been produced and analyzed. Among “important” olfactory candidate genes, more than 40 highly divergent receptors have been found, but they

FIGURE 2. Convergence of axonal projections from olfactory epithelium (OE) to olfactory bulb (OB). Sensory neurons that express a particular type of odorant receptor (A, B, or C) in OE converge, respectively, with their axonal projections to the glomeruli A’, B’, or C’ within the OB. Cribriform bone separates peripheral sensory epithelium from the brain. Sensitivity is enhanced when convergence ratio is high. This model also suggests that a convergent transmission of odorant signals by receptors from periphery to brain occurs through a hard-wired system and that a given odor would be identified by the characteristic pattern of activity of a small number of glomeruli.
show no sequence homology, only structural homology, with vertebrate OR proteins. At least 11 of those *C. elegans* receptor genes are expressed in small subsets of chemosensory neurons, and a single neuron can express up to 4 different OR genes (13). A receptor gene called *odr-10* is expressed in one of the sensory neurons and encodes a potential odorant receptor. Indeed, *odr-10* mutants lacking a functional *odr-10* receptor are unable to detect the odor diacetyl. These genetic studies provide for the first time an “in vivo” model for the specific interaction between a receptor of the 7-TMD protein family and an odor ligand.

The combination of these three models, derived from different approaches not mutually incompatible, provides for the first time a working frame to study the molecular, cellular, and genetic bases of the sense of smell. Indeed, the sense of smell is integrative (responds to a mixture of odors) rather than analytical (detection of individual odor). Therefore, the activation of ORs in the periphery of glomeruli and of neural assemblies in the central nervous system is likely to represent the odor-coding processing.

**Additional role(s) for the ORs**

If the coding sequence of the OR gene A that is normally expressed by neurons projecting to a fixed glomerulus A’ in the bulb (see Fig. 2) is replaced by the coding sequence of the OR gene B (projecting normally to B’; see Fig. 2), then the newly made projections are found near position B’, not in the bulbar position A’ (7). This type of study is referred to as a “receptor swap” experiment. Because the point of convergence is dramatically changed when B replaces the OR gene A, it suggests an additional role for the OR proteins in the guidance process of axonal projections during the development of the olfactory system. Moreover, OR transcripts are sometimes detected at the end of the olfactory axon located in the central nervous system (14). This guidance hypothesis is supported by other studies showing early expression of the OR genes in the mouse at embryonic day 10 (8), as early as embryonic day 10 in the chick (9), and 24–30 h after fertilization in the zebrafish (3). Moreover, OR-positive cells in the chick are also found during early development along the olfactory nerve and between the olfactory placode (the precursor site of the future olfactory epithelium) and the telencephalic vesicles, which will give rise much later to the olfactory bulb (9). Taken together, the data suggest that unknown guidance ligands (because the primordium of the olfactory bulb or the bulb itself is unlikely to produce specific odor molecules) might be detected by early OR proteins. The gene expressions for the ligand and the receptor must be temporally coordinated; otherwise, no signal will be transmitted.

**Unsolved questions**

Some members of the OR gene family, indistinguishable from those expressed in olfactory neurons, have been found to be expressed in tissues unrelated, in appearance, to olfaction. Without a clear function, OR-positive cells are observed 1) in the tongue (gustatory receptor, tongue morphogenesis), 2) in sperm cells (spermotaxis, sperm maturation), 3) in the developing rat heart (6) (cardiac morphogenesis), and 4) in the avian notochord (10), a nonneuronal tissue responsible for the induction of the differentiation of the floor plate (neural tube) by contact-dependent signal(s) and of motoneurons by diffusion of signal(s). In the latter case, although the chick OR gene *COR7b* is expressed in the sensory neurons of the olfactory epithelium (9, 10), it may also be involved in the detection of unknown nonodorant signals in a way similar to the OR present in the bulb.

Despite several attempts to reconstitute odor-induced responses with ORs, all the classical in vitro systems failed to produce functional ORs. Therefore, data addressing the specificity or the affinity of the odor/receptor complex are still missing. Thus a final model on how odor molecules are detected, discriminated, and encoded remains mostly hypothetical, because the binding affinities of odor molecules to their receptors are lacking. The missing data could dramatically affect the proposed models. For example, if each OR protein is activated by only one odor molecule (high-affinity binding and high specificity), then the “hard-wired” model will be validated. Alternatively, if odorant binding affinities or the ligand specificities are weak for ORs, then a “cell population”-based model will be favored, also suggesting that odor processing occurs first in the olfactory epithelium. It also remains to be seen whether odor processing in the brain of vertebrates is similar to that of insects.

**Concluding remarks and perspectives**

In comparison with vision and audition, olfaction is still relatively poorly understood despite certain new advances. The use of molecular probes to identify the expression of OR genes (2, 4, 7), of electrophysiological approaches to record the firing of neuron assemblies (15), and of genetic mutant analyses in *C. elegans* is of great interest because knowledge of how odor mole-
molecules are detected in the olfactory epithelium and then discriminated, encoded, and processed in the brain is supported by experimental data for the first time. How an olfactory neuron “chooses” to express only a few OR genes among the large repertoire of OR genes and how an odorant epitope map that represents the odor signature is generated in the olfactory epithelium and analyzed in the olfactory bulb or in the brain remain to be discovered. Olfaction provides a unique opportunity to study a sensory neural coding scheme responsible for the conscious perception of a myriad of chemical stimuli within the context of other environmental signals.

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References