Olfaction: From Odorant Molecules to the Olfactory Cortex
Anna Menini, Laura Lagostena, and Anna Boccaccio
Sector of Neurobiology, International School for Advanced Studies, Scuola Internazionale di Studi Superiori Avanzati, 34014 Trieste, Italy

How do we smell? Our knowledge of how odor information from the environment is perceived has greatly advanced since the discovery of ~1,000 genes for odorant receptors in the mammalian genome. From the combination of molecular-genetic, electrophysiological, and optical imaging studies a better understanding of how we smell is emerging.

The olfactory system detects and discriminates among a large number of structurally diverse odorant molecules that carry information about the environment. Although humans recognize fewer molecules than other mammals, it seems that we can discriminate among ~10,000 different odors. How do we accomplish this incredible task? What is an odor? What is the odor of a flower or that of our favorite food? Several types of volatile odorant molecules detach from a flower or from the food, even though we perceive them as a unique odor. Let's try to follow which events volatile molecules of diverse molecular structure are causing in the vertebrate olfactory system. Many of these volatile molecules will reach the inside of the nose when air is inhaled. In the nasal cavity, molecules will interact with the olfactory epithelium, where olfactory sensory neurons have receptors to which several volatile molecules can bind and elicit an electrical signal that is transmitted to the brain. This is the origin of odor perception: the chemical interaction of odorant receptors with volatile molecules is transformed into electrical signals that will carry information about the external world to the brain (4, 12). The axons of the olfactory sensory neurons from the nasal cavity send information to second-order neurons in the olfactory bulb, which in turn project to the olfactory cortex and then to other brain areas (Fig. 1).

It was only after the discovery in 1991 of a large multigene family for odorant receptors (3) that several specific questions could be answered. How many odorant receptor genes are there? How many odorant receptors are expressed per olfactory sensory neuron? Is there a pattern of expression of odorant receptor genes in the olfactory epithelium? How many transduction mechanisms are there? Where do odorant sensory neurons expressing the same odorant receptor project their axons in the olfactory bulb? How are odorant receptors targeted in the olfactory cortex?

How many odorant receptors are there?

In the mouse genome there are ~1000 genes encoding different types of odorant receptors (2, 3, 14). Humans have a similar number of odorant receptor genes, although a large fraction of them appear to be pseudogenes and only between 300 and 400 are functional genes. Odorant receptor genes constitute the largest gene family in the vertebrate genome, and they allow the discrimination between a large number of odors. The basic organization of the olfactory system and of the odorant receptors is similar in mice and in humans, and therefore the experimental information about the functioning of the olfactory system that has been recently obtained in the mouse is likely to apply also to humans.

Odorant receptors belong to the superfamily of G protein-coupled receptors and have the same general structure with seven hydrophobic membrane-spanning regions, but they differ in their amino acid sequence, especially in the third, fourth, and fifth transmembrane regions, which may form the ligand-binding pocket for odorant molecules (Fig. 2C). Odorant receptors are located in the cilia of olfactory sensory neurons, and the binding of odorants activates a transduction cascade that leads to the production of action potentials, as described later (Fig. 3).

How many types of odorant receptor genes are expressed in each olfactory sensory neuron? An individual olfactory sensory neuron expresses only one type of odorant receptor gene, out of ~1,000 possibilities. Moreover, the olfactory epithelium is divided into four spatially distinct zones of gene expression, but olfactory sensory neurons expressing the same odorant receptor are almost randomly distributed within one of these four zones. The functional significance of this zone division is unknown. The important notion that each olfactory sensory neuron expresses only one type of odorant receptor gene has been established by using various experimental approaches, such as in situ hybridization studies and analysis of receptor expression in single olfactory sensory neurons using the polymerase chain reaction (2, 14).

Electrophysiological recordings from individual sensory neurons have shown that they respond to odors in different ways (5). Some olfactory sensory neurons can detect several odors, and a given odorant can activate neurons with various odorant specificity (Fig. 2).

Soon after the discovery of odorant receptors, several laboratories tried to determine which odorant molecules bind to each receptor by trying to functionally express odorant receptors in heterologous cell lines. Unfortunately most of these attempts failed, probably because odorant receptors did not reach the plasma membrane. Therefore, other techniques have been used to find the odorant specificity of various receptors (8, 11, 19). However, until now only a small number of odorant receptors...
has been linked to molecules that can activate them. In general, it has been well established that one single odorant activates several types of receptors. Moreover, all of the investigated receptors may be activated by several different odorants. However, one odorant activates a unique combination of receptors. Therefore, the odorant receptor family is used in a combinatorial way, with the great advantage of allowing the olfactory system to recognize an enormous number of odorants (2).

How many olfactory transduction mechanisms?

How is the binding of an odorant molecule to receptors converted into an electrical signal? (Fig. 3). The olfactory sensory neurons are bipolar neurons with a single dendrite that terminates in a knob from which 10–20 fine cilia originate. These cilia are immersed in the nasal mucus and are the sites where the entire transduction mechanism occurs. Although two possible transduction cascades have previously been proposed, involving the production of cAMP or of IP₃, there is converging evidence for only one common pathway of intracellular signaling. The binding of odorants to odorant receptors in the cilia causes, via G protein activation of adenylyl cyclase, the production of a cyclic nucleotide, cAMP, which directly opens ionic channels in the plasma membrane. An inward transduction current is carried by Na⁺ and Ca²⁺ ions. Olfactory sensory neurons maintain an unusually high intracellular concentration of Cl⁻ ions, and the increase in the internal concentration of Ca²⁺ causes the opening of Ca²⁺-activated Cl⁻ channels that produce an efflux of Cl⁻ from the cilia, contributing to the olfactory neuron depolarization. The depo-
Captonization spreads passively to the dendrite and soma of the olfactory neuron, triggering action potentials that are conducted along the axon to the olfactory bulb (4, 12).

The described molecular mechanisms of the olfactory transduction cascade have several important physiological consequences. One is that the amplification of the transduction cascade allows the production of an electrical quantal event even by the binding of a single odorant molecule (13). Another one is that it is already at the level of the transduction process that the physiological process of adaptation to odorants occurs. Indeed, the initial response of an olfactory sensory neuron to an odorant stimulus is followed by a period of reduced responsiveness. It has been shown that, in combination with calmodulin, Ca²⁺ mediates odorant adaptation through a negative feedback, by desensitizing cAMP-gated channels (9, 12).

Where do olfactory sensory neurons project their axons?

Action potentials elicited by the binding of odorants as a consequence of the transduction cascade travel along the axon of olfactory sensory neurons and reach the olfactory bulb. Axons of all of the olfactory sensory neurons expressing a particular odorant receptor converge to only two anatomically discrete synaptic units, called glomeruli, in the olfactory bulb. In mice there are ~2,000 glomeruli, and their localization is roughly conserved among individuals. Therefore the olfactory bulb is topographically organized, with individual glomeruli representing a single type of odorant receptor. The convergence of axons of olfactory sensory neurons expressing a given odorant receptor has been experimentally demonstrated at single-axon resolution by using transgenic mice in which the expression of a given odorant receptor gene was linked to the expression of the green fluorescent protein. It has been shown that all and only the axons of neurons expressing that particular odorant receptor were converging to their target glomeruli (14, 18).

Each type of odorant receptor seems also to participate in the process that continuously maintains the specific targeting of the axons of olfactory sensory neurons. Indeed, it is well known that olfactory sensory neurons undergo a cycle of degeneration and regeneration every few weeks, and therefore the organization of the olfactory system needs to be reconstituted often (16).

In addition to the analysis of axon projections in the olfactory bulb, functional analysis has been performed in many laboratories by using a variety of imaging techniques, such as intrinsic imaging, calcium indicators, voltage-sensitive dyes, and functional magnetic resonance. As expected, the activity induced by individual odorants consisted of the combinatorial activation of several glomeruli, similar to that observed for odorant receptors. Therefore, it is now well established that odorant information is spatially represented in the olfactory bulb (1, 6, 7, 15). Recent studies have suggested that tempo-
eral aspects of responses to odorants also play an important role in olfaction, although further investigation is necessary to understand the correlation between spatial and temporal aspects.

In the olfactory bulb a complex processing of olfactory information is also taking place. Indeed, each glomerulus contains the axons of several thousands of olfactory sensory neurons (each expressing the same odorant receptor) and the dendrites of ~50 mitral and tufted cells, which are the main input-output neurons of the olfactory bulb. These neurons are activated by olfactory sensory neurons, but odorant information is further processed by the activity of inhibitory interneurons, periglomerular cells, and granule cells (10, 17).

How are odorant receptors targeted in the olfactory cortex?

What happens next? Mitral and tufted cells from glomeruli in the olfactory bulb transmit signals to pyramidal neurons in the olfactory cortex. The olfactory cortex is composed of several anatomically distinct areas: the piriform cortex, olfactory tubercle, anterior olfactory nucleus, and specific parts of the amygdala and entorhinal cortex. Previous anatomic studies and recent genetic approaches indicate that the topographical organization of odorant information in the olfactory bulb is not present in the olfactory cortex. Instead, odorant receptors seem to be mapped to multiple, discrete clusters of cortical neurons (Fig. 4). Indeed, a genetic approach has been recently developed to visually follow where the signals from neurons expressing a chosen odorant receptor are targeted in the olfactory cortex (20). A plant lectin, barley lectin, was used as neuronal tracer because it was found that when expressed in olfactory sensory neurons it is transported through the axons to the olfactory bulb, transferred through synapses to second-order neurons in the bulb, and then transferred from those neurons to third-order neurons in the olfactory cortex. The preparation of transgenic mice in which barley lectin was co-expressed only in olfactory sensory neurons with a chosen odorant receptor allowed visualization by histochemical techniques of neurons in the olfactory cortex that receive input from that odorant receptor. Only two odorant receptors, M5 and M50, expressed in distant zones of the olfactory epithelium were studied in different transgenic mice. Immunostaining of nose and brain sections to visualize neurons that contained barley lectin revealed labeled neurons in the corresponding zones of the olfactory epithelium, in the glomeruli, and in the mitral and tufted neurons. It was found that mitral cells carrying input from M5 or M50 form synapses in most olfactory cortical areas and are organized in discrete clusters. Moreover, by comparing the location of neuronal clusters in different animals, it was found that there is a stereotyped map of sensory inputs in the olfactory cortex where odorant receptors are targeted to multiple, discrete clusters of cortical neurons (20).

Conclusions

Molecular genetics studies have shown how sensory inputs are organized in the olfactory bulb, and functional imaging studies have given an overview of the activity of glomeruli in response to odorants. Therefore it is now well established that odorant information is encoded spatially in the olfactory bulb and that the quality of an odorant is determined not only by a single odorant receptor but by the combination of a number of odorant receptors, each recognizing a specific molecular feature of the odorant molecule. In the olfactory cortex, inputs from an odorant receptor are targeted to multiple clusters of cortical neurons in various olfactory cortical areas. The combination of molecular genetics with functional imaging at the level of the olfactory cortex will improve our understanding of how we smell.

Our work is supported by grants from NATO, the Italian Ministero dell’Istruzione, dell’Università e della Ricerca, and from the European Union.

References