UNRAVELING THE SENSE OF SMELL

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by

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INTRODUCTION

The subject of my lecture is the sense of smell, one of the five senses through which we perceive the world. Through the sense of smell, humans and other mammals can perceive a vast number and variety of chemicals in the external world. It is estimated that humans can sense as many as 10,000 to 100,000 chemicals as having a distinct odor. All of these "odorants" are small, volatile molecules. However, they have diverse structures and somehow those different structures are perceived as having different odors (Figure 1).

The sense of smell is mediated by the olfactory system, a system that is characterized by exquisite sensitivity and discriminatory power. Even a slight change in the structure of an odorant can change its perceived odor. For example, the close relative of a chemical that is perceived as pear can have the scent of an apple. In addition to odorants, the olfactory system detects pheromones, chemicals that are released from animals and act on members of the same species, stimulating hormonal changes or instinctive behaviors, such as mating or aggression. The olfactory system also detects predator odors, which can elicit innate fear responses.

Over the past 16 years, our work has focused on two questions. First, how do mammals detect so many different environmental chemicals? And second, how does the brain translate those chemicals into diverse odor perceptions and behaviors?

Odorants are initially detected by olfactory sensory neurons, which are located in the olfactory epithelium lining the nasal cavity (Figure 2). These neurons transmit signals to the olfactory bulb of the brain, which then relays those signals to the olfactory cortex. From there, olfactory information is sent to a number of other brain areas. These include higher cortical areas thought to be involved in odor discrimination as well as deep limbic areas of the brain, which are thought to mediate the emotional and physiological effects of odors. In contrast to odorants, pheromones are detected primarily in the vomeronasal organ, or VNO, a separate olfactory structure in the nasal septum.

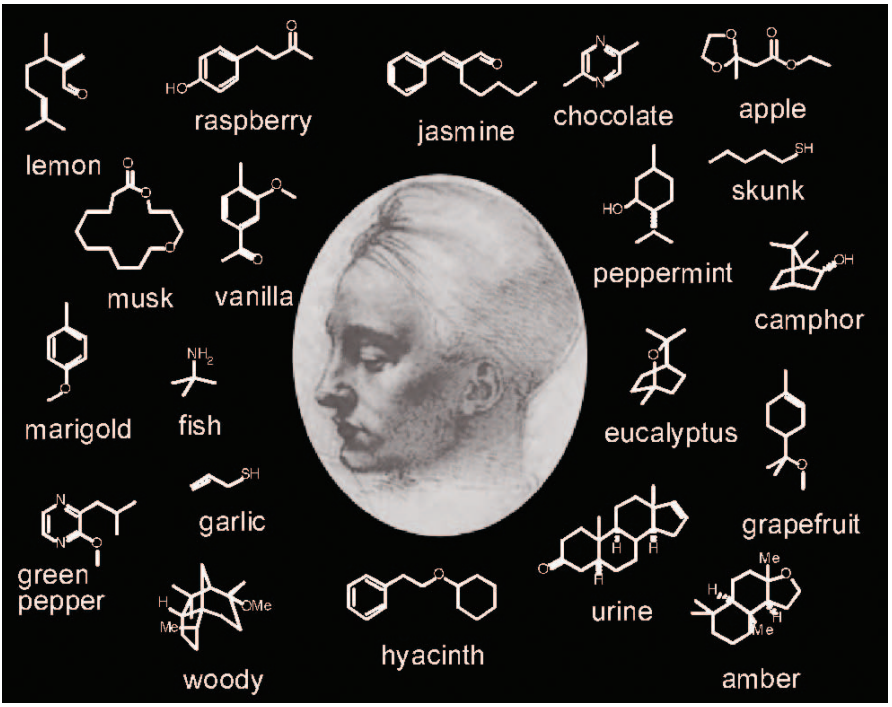

Figure 1. Humans and other mammals perceive a vast number of chemicals as having distinct odors.

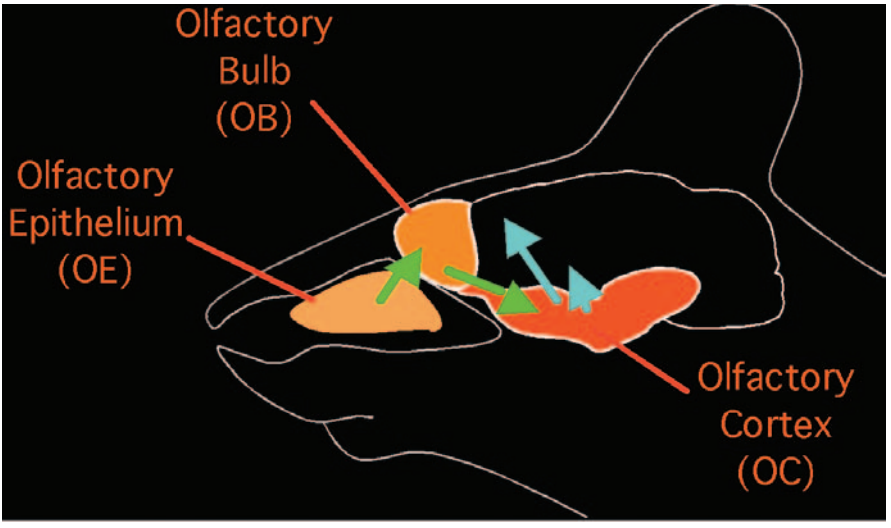

Figure 2. The olfactory pathway. Odorants are detected by olfactory sensory neurons in the olfactory epithelium. Signals generated in those neurons are relayed through the olfactory bulb to the olfactory cortex and then sent to other brain areas.

From VNO neurons, signals are relayed through the accessory bulb to the medial amygdala and then the hypothalamus, areas implicated in hormonal and behavioral responses to pheromones.

The olfactory epithelium contains millions of olfactory sensory neurons. It also contains supporting cells and a basal layer of stem cells. Olfactory sensory neurons are short-lived cells that are continuously replaced from the stem cell layer. At the surface of the epithelium, each neuron extends cilia into the nasal lumen, allowing it to come in contact with odorants dissolved in the nasal mucus. Each neuron communicates with the brain via a single axon that it extends to the olfactory bulb.

ODORANT RECEPTORS

In our initial experiments, Richard Axel and I asked how it is that these neurons detect odorants. Beginning in 1965 with the work of Robert Gesteland (Gesteland *et al.*, 1965), numerous electrophysiological studies had shown that different olfactory sensory neurons are depolarized, or activated, by different odorants. John Amoore proposed that these neurons had odorant receptor proteins that varied in their affinity for different odorants (Amoore, 1970; Amoore, 1977). In the mid 1980s, hints started to emerge about signal transduction in the cilia of the olfactory neurons. Doron Lancet and Sol Snyder and their colleagues showed that odorants induce GTP-dependent increases in adenylyl cyclase activity in the cilia, suggesting the involvement of intracellular G proteins (Pace *et al.*, 1985; Sklar *et al.*, 1986), and Randy Reed identified Gαolf, a G protein that could mediate this response and was highly expressed in olfactory sensory neurons (Jones and Reed, 1989).

In 1988, Richard Axel and I embarked on a search for odorant receptors. The strategy we devised was based on three assumptions. First, odorant receptors would be selectively expressed in the olfactory epithelium. Second, since odorants vary in structure, there would be a family of varied, but related receptors, and those receptors would be encoded by a multigene family. And third, odorant receptors would be related to other types of receptors that interact with intracellular G proteins. By 1989, molecular cloning had revealed the structures of about 20 of these G protein-coupled receptors, or "GPCRs". All of these receptors had seven potential transmembrane domains and they shared a few amino acid sequence motifs.

On the basis of these assumptions, we set out to search for a family of GPCRs expressed in the rat olfactory epithelium (Buck and Axel, 1991). To do this, we first used PCR (the polymerase chain reaction) to look for receptors expressed in the olfactory epithelium that were related to known GPCRs. We designed 11 degenerate oligonucleotide primers that matched amino acid sequences in transmembrane domains 2 and 7 of known GPCRs. We then used these primers in all 30 pairwise combinations to amplify related sequences in cDNA prepared from rat olfactory epithelium RNA. From the 30 PCR reactions, we obtained 64 different PCR products in the appropriate size range. Each of these appeared as a distinct band using agarose gel electrophoresis.

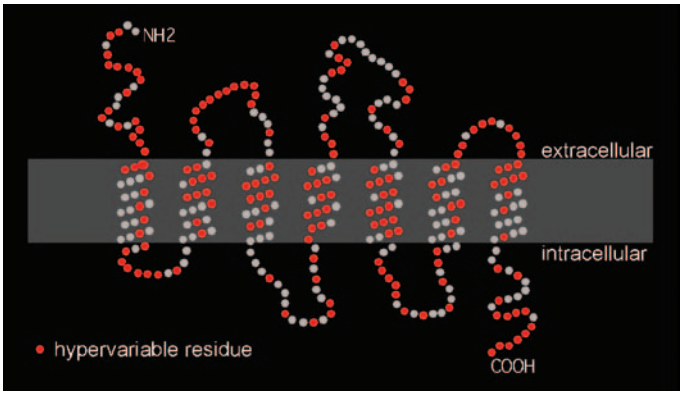

Figure 3. Topology of an odorant receptor in the membrane. Individual amino acid residues are indicated by balls. Red balls indicate residues that were hypervariable among ten odorant receptors. Adapted from Buck and Axel, 1991.

We then asked whether any of the 64 PCR products contained multiple members of a multigene family. To do this we cut the DNA in each PCR product with a restriction enzyme. Most of the bands were cut into a small number of fragments that added up to the original in size. However, one band, #13, was cut into a large number of fragments, suggesting that it might contain multiple members of a multigene family. When we cloned and sequenced five of the DNAs in #13, we found what we had been looking for. All five encoded novel proteins with the hallmarks of GPCRs. Moreover, all five were related, but each one was unique.

Using these DNAs as probes, we isolated a series of related cDNAs from an olfactory epithelium cDNA library. We initially examined the proteins encoded by ten of the cDNAs. All ten proteins had the seven potential transmembrane domains characteristic of GPCRs. In addition, they had several amino acid sequence motifs seen in other GPCRs. However, the ten receptors all shared sequence motifs not seen in any other GPCRs, indicating that they were members of a novel receptor family.

Figure 3 shows a model of one of these receptors in the membrane with individual amino acids represented as balls. Red balls indicate amino acids that were especially variable among the ten receptors. Importantly, though related, the ten olfactory receptors varied extensively in amino acid sequence. This hypervariability was consistent with an ability of the receptors to interact with odorants with different structures.

Consistent with the selective expression of these receptors in the olfactory epithelium, a mixed olfactory receptor DNA probe hybridized to RNA from the olfactory epithelium, but not other tissues. Moreover, enriching for olfactory sensory neurons also enriched for receptor RNAs, suggesting that the receptors were expressed predominantly or exclusively by olfactory sensory neurons.

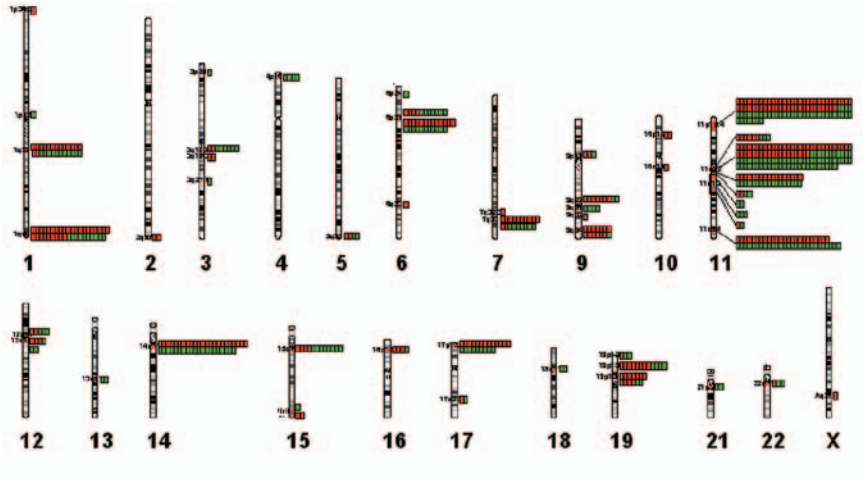

Figure 4. The chromosomal distribution of human odorant receptor genes. Intact receptor genes are shown in red and pseudogenes in green. Adapted from Malnic *et al.*, 2004.

On Southern blots of genomic DNA, single receptor probes hybridized to multiple bands, and a mixed receptor probe hybridized to a large number of bands. This further indicated that the receptors we had found were encoded by a large multigene family. Genomic library screens indicated that the multigene family contained in excess of 100 members. In later studies, we obtained evidence for about 1000 different olfactory receptor genes in mouse.

On the basis of these results, we concluded that the receptor family we had identified coded for odorant receptors (ORs) expressed by olfactory sensory neurons in the nose (Buck and Axel, 1991). Subsequent studies showed that homologous families of odorant receptors are present in vertebrate species ranging from fish to humans (reviewed in Mombaerts, 1999). In 1991, after publishing our work on odorant receptors (Buck and Axel, 1991), I left Richard Axel's lab to join the faculty of Harvard Medical School.

A decade later, the sequencing of human and mouse genomes made it possible to determine the number of OR genes in these species. This was done by Lancet and Zozulya for human (Glusman *et al.*, 2001; Zozulya *et al.*, 2001) and by Firestein and Trask for mouse (Young and Trask, 2002; Zhang and Firestein, 2002), and in my lab it was done for both species by Bettina Malnic and Paul Godfrey (Godfrey *et al.*, 2004; Malnic *et al.*, 2004). These studies indicate that humans have about 350 different ORs and mice have about 1000. This indicates that roughly 1–5% of the genes in the genome are devoted to the detection of odorants. OR genes are highly distributed across the genome. In our studies of the human genome, we found OR genes on 21 different chromosomes and at 51 different chromosomal loci, where they are found singly or in clusters (Figure 4) (Malnic *et al.*, 2004).

In the mid 1990s, two additional families of receptors were found in the olfactory system. These receptors, called V1Rs and V2Rs, are unrelated to ORs in protein sequence, but both types have the characteristic seven transmembrane structure of GPCRs. V1R and V2R genes are selectively expressed in the VNO, suggesting that they might be pheromone receptors. Both receptor families have more than 100 members. The V1R family was identified in 1995 by Dulac and Axel (Dulac and Axel, 1995), and the V2R family was identified in 1997 by Hiroaki Matsunami in my lab and also by the laboratories of Catherine Dulac and Nicholas Ryba (Herrada and Dulac; Matsunami and Buck, 1997; Ryba and Tirandelli, 1997).

ORGANIZATION OF ODORANT RECEPTORS IN THE OLFACTORY EPITHELIUM

The discovery of odorant receptors explained how the olfactory system detects a vast array of chemicals in the external world. It also did something else that was important: it provided a set of molecular tools to explore how the nervous system translates chemical structures into odor perceptions. This is what we set out to do in my lab at Harvard.

In the mouse, we found evidence for as many as 1000 different OR genes. We first asked how information from different ORs is organized in the olfactory epithelium (Ressler *et al.*, 1993). In these experiments, Kerry Ressler, a graduate student in the lab, and Susan Sullivan, a postdoctoral fellow, hybridized labeled OR gene probes to sections through the mouse nose (Figure 5). These studies showed that the olfactory epithelium has distinct spatial zones that express nonoverlapping sets of OR genes (Figure 6). Each OR gene is expressed in about 1/1000 neurons and those neurons are randomly scattered within one zone. Similar findings were made in Richard Axel's lab in rat (Vassar *et al.*, 1993). The OR expression zones form stripes that extend along the anterior posterior axis of the nasal cavity.

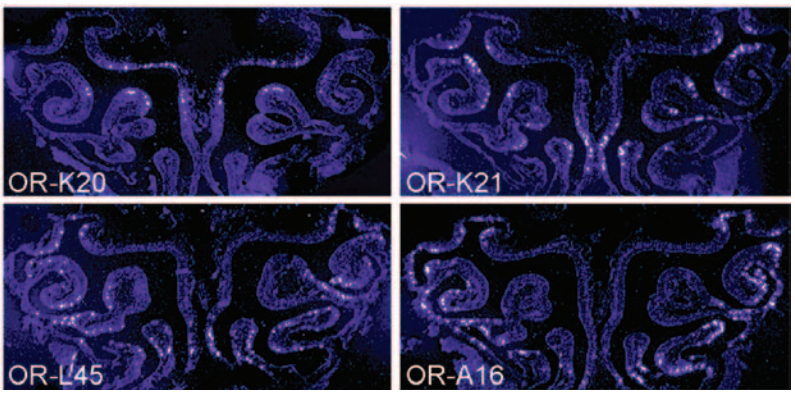

Figure 5. Expression patterns of odorant receptor genes in the mouse olfactory epithelium. Tissue sections through the mouse nose were hybridized to four different receptor gene probes. Adapted from Ressler *et al.*, 1993; Sullivan *et al.*, 1996.

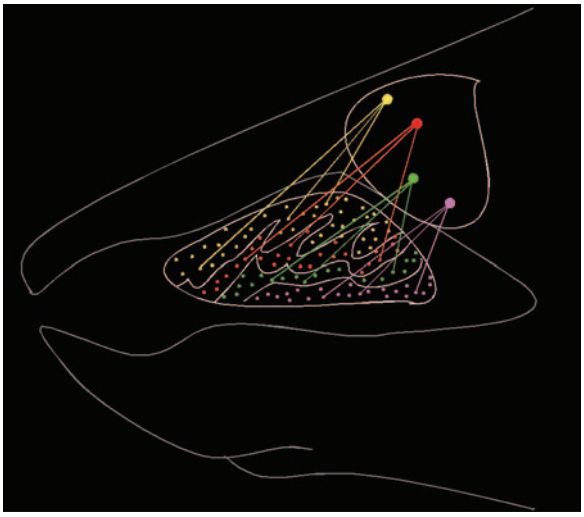

Figure 6. The organization of odorant receptor inputs in the olfactory epithelium and olfactory bulb. Sensory neurons expressing the same receptor are scattered within one epithelial zone, but their axons converge in specific glomeruli in the olfactory bulb. Adapted from Ressler *et al.*, 1993; Ressler *et al.*, 1994; Sullivan *et al.*, 1996.

These findings told us two important things. First, input from one type of OR is highly distributed in the epithelium. Therefore, neurons with receptors for one odorant, for example a strawberry odorant, must be interspersed with neurons that have receptors for another odorant, such as a lemon one. Second, each neuron may express only one OR gene. We later confirmed this by examining gene expression in single neurons (Malnic *et al.*, 1999). Thus, in the nose, inputs from different ORs are segregated in different neurons, and the information that each neuron transmits to the brain is derived from a single receptor type.

COMBINATORIAL RECEPTOR CODES FOR ODORS

In later studies, we asked how the OR family encodes the identities of different odorants. To explore this question, we searched for ORs that recognize specific odorants (Malnic *et al.*, 1999). This work was done by Bettina Malnic in the lab in collaboration with Takaaki Sato and Junzo Hirono at the Life Electronics Research Center in Japan. We first exposed single mouse olfactory sensory neurons to a series of odorants, using calcium imaging to visualize their responses. We then isolated each responsive neuron and used RT (reverse transcriptase)-PCR to determine the OR gene it expressed. In every case, we identified only one expressed OR per neuron, confirming that each neuron expresses a single OR gene.

For test odorants, we used four different classes of n-aliphatic odorants with different functional groups and carbon chains ranging in length from 4 to 9 carbon atoms. Each neuron was imaged as it was exposed sequentially to

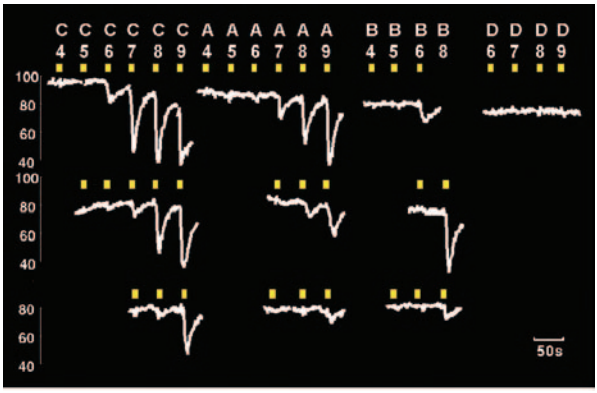

Figure 7. Responses of a single olfactory sensory neuron to different odorants. Fluorescence emission was monitored during sequential exposure of a Fura-2 containing neuron to a series of odorants (C4-D9). Responses to lower odorant concentrations are shown below. Adapted from Malnic *et al.*, 1999.

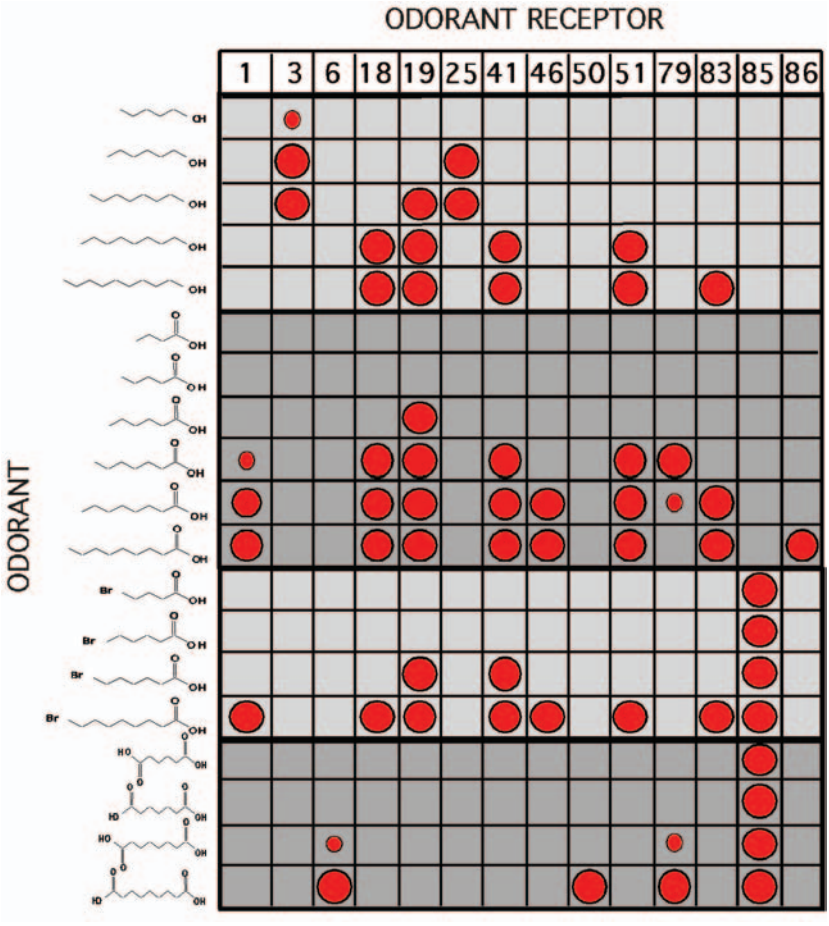

Figure 8. Odorant receptors are used combinatorially to detect odorants and encode their identities. The recognition profiles of individual odorant receptors to a series of odorants were determined by calcium imaging and single cell RT-PCR. The sizes of circles reflect response intensity. Adapted from Malnic *et al.*, 1999.

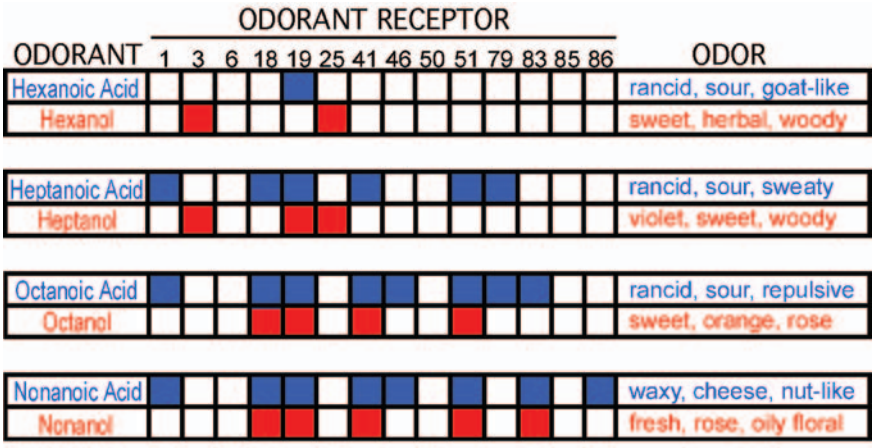

Figure 9. Closely related odorants with different perceived odors are detected by different combinations of receptors. Adapted from Malnic *et al.*, 1999.

different odorants (Figure 7). If a response was seen, the neuron was retested with a lower concentration of the same odorant.

Figure 8 shows the response profiles of 14 neurons and therefore the recognition properties of the ORs expressed in those neurons. These data make three important points. First, each OR can recognize multiple odorants, something previously shown by Stuart Firestein for one rat OR (Zhao *et al.*, 1998). Second, each odorant can be detected by multiple different ORs. And finally, and most importantly, different odorants are recognized by different combinations of ORs.

These results indicated that ORs are used combinatorially to encode odor identities (Malnic *et al.*, 1999). Different odorants are detected and thereby encoded by different combinations of ORs. However, each OR serves as one component of the codes for many odorants. Different odorants have different "receptor codes". Given the number of possible combinations of 1000 different ORs, this combinatorial coding scheme could allow for the discrimination of an almost unlimited number of odorants. Even if each odorant were detected by only three ORs, this scheme could potentially generate almost one billion different odor codes.

These studies also provided insight into several puzzling features of human odor perception (Malnic *et al.*, 1999). Changing the structure of an odorant even slightly can alter its perceived odor. Sometimes the change in odor can be dramatic. The aliphatic acids and alcohols that we used in our studies are excellent examples of this phenomenon (Figure 9). All of the acids have unpleasant odors, such as rancid, sour, or sweaty. In contrast, all of the alcohols have pleasant odors, such as herbal, woody, or orange. In our studies, pairs of acids and alcohols that differed by a single functional group invariably had different receptor codes (Figure 9). This indicates that even a slight change

in the structure of an odorant can alter its receptor code, and thereby change its perceived odor.

Our studies showed that a change in the concentration of an odorant can also change its receptor code. At higher concentrations, additional ORs were invariably recruited into the odor response. This may explain why changing the concentration of an odorant can alter its perceived odor.

A STEREOTYPED MAP OF ODORANT RECEPTOR INPUTS IN THE OLFACTORY BULB

These studies indicated that, in the nose, different odorants are detected by different combinations of ORs and that the different OR combinations ultimately generate different odor perceptions. How is this accomplished? How does the brain translate an odorant's combinatorial receptor code into a perception?

Each olfactory sensory neuron in the olfactory epithelium sends a single axon to the olfactory bulb of the brain. Here the sensory axon enters a spherical structure called a glomerulus, where it synapses with the dendrites of bulb neurons. The mouse olfactory bulb has about 2000 glomeruli, each of which receives input from several thousand olfactory sensory neurons. Each sensory neuron synapses in only one glomerulus. Similarly, each mitral cell in the bulb receives input from a single glomerulus. Mitral cells are relay neurons that transmit signals to the olfactory cortex.

In the bulb, we found something very different from what we had seen in the nose (Ressler *et al.*, 1994). Here, single OR probes labeled OR mRNA in sensory axons in only a few glomeruli, and those glomeruli were located at only two spots, one on either side of the bulb (Figure 10A). We found that different OR probes labeled different glomeruli, and surprisingly, those glomeruli had nearly identical locations in different individuals (Figure 10B). These findings were made by Kerry Ressler and Susan Sullivan in my lab. In independent experiments in rat, Vassar and Axel obtained similar results, though individual OR probes generally labeled a larger number of glomeruli at more locations in the bulb in their studies (Vassar *et al.*, 1994).

Our studies in mouse indicated that the axons of thousands of sensory neurons with the same OR converge in only 2-4 glomeruli, each of which is likely to be dedicated to one OR (Figure 10) (Ressler *et al.*, 1994). They further indicated that sensory information that is broadly organized into 4 zonal sets in the nose is transformed in the bulb into a stereotyped sensory map (Figure 6). In this map, inputs from different ORs are targeted to different glomeruli and the bulb neurons associated with those glomeruli. Remarkably, this map is virtually identical in different individuals. The olfactory epithelium and bulb have one important thing in common, however. At both sites, inputs from different ORs are segregated. Each sensory neuron in the epithelium, and each glomerulus and relay neuron in the bulb, appears to be dedicated to only one type of OR.

The structure of the bulb map is likely to be important in at least two respects. First, it is likely to maximize sensitivity to low concentrations of odorants. Signals from 5000 or so neurons with the same OR converge on

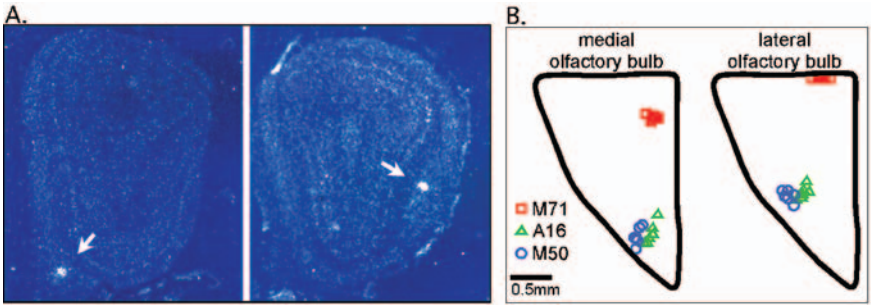

Figure 10. The olfactory bulb has a stereotyped map of OR inputs. A. A single OR gene probe hybridized to sensory axons in only 1-2 glomeruli on either side of the olfactory bulb. B. Different OR probes (A16, M50, M71) hybridized to different glomeruli and those glomeruli had similar locations in six different bulbs.

2–4 glomeruli and about 50 mitral cells, allowing a high degree of signal integration. Second, the bulb map is likely to be important for the stimulation of odor memories. Sensory neurons in the epithelium are short lived and are continuously replaced. However, the bulb map remains constant over time. Thus the neural code for an odor remains intact, assuring that odorants can elicit distant memories.

ODOR CODING IN THE OLFACTORY EPITHELIUM AND BULB

Given our finding that each odorant is recognized by a combination of ORs (Malnic *et al.*, 1999), these results imply that the code for an odor in the nose is a dispersed ensemble of neurons, each expressing one OR component of the odorant's receptor code (Figure 11). In the bulb, the code is a specific

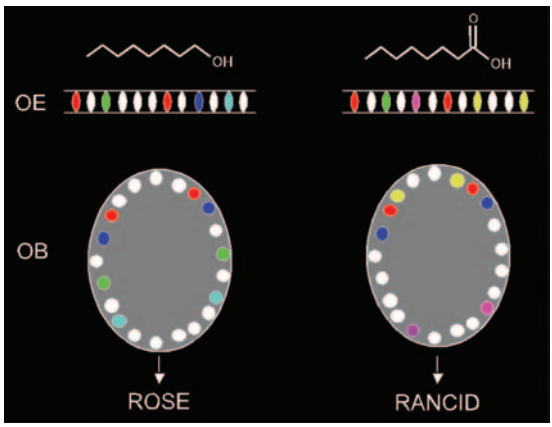

Figure 11. Odor coding in the olfactory epithelium and olfactory bulb. In this schematic, inputs from different ORs that recognize an odorant are indicated by different colors. In the olfactory epithelium (OE), the code for an odorant is a dispersed ensemble of neurons, each expressing one component of the odorant's receptor code. In the olfactory bulb (OB), it is a specific combination of glomeruli whose spatial arrangement is similar among individuals. Partially overlapping combinations of OR inputs generate distinct odor perceptions.

combination of glomeruli that receive inputs from those ORs and have a similar spatial arrangement in different individuals. This arrangement is consistent with many studies of odor-induced activity in the epithelium and bulb, beginning in the 1950s with the studies of Lord Adrian, who discovered that different mitral cells in the rabbit bulb respond to different sets of odorants (Adrian, 1950; Adrian, 1956; Buck, 1996).

STEREOTYPY, DIVERGENCE, AND CONVERGENCE IN OLFACTORY CORTEX

What happens to this information at higher levels of the nervous system to ultimately generate diverse odor perceptions?

Mitral cell relay neurons in the bulb extend axons to the olfactory cortex, a large area that stretches along the ventral lateral part of the brain. The olfactory cortex is composed of a number of distinct anatomical areas, at least some of which are likely to have different functions. The largest area is the piriform cortex, which itself has morphologically distinct anterior and posterior halves.

In the 1980s, Lewis Haberly and others showed that a tracer placed in one small region of this cortex would back-label mitral cells in many parts of the bulb (Haberly, 1998). This clearly indicated that the organization of sensory information in the olfactory bulb could not be recapitulated in the cortex. But how olfactory information is organized in the olfactory cortex was a mystery.

We were initially interested in three questions regarding the olfactory cortex. First, do different areas of the olfactory cortex, which may have different functions, receive signals derived from different subsets of ORs or, alternatively, does each area receive input from the entire OR repertoire? Second, is input from one OR scattered in the cortex, as in the nose, is it targeted to unique, stereotyped sites, as in the bulb, or is it organized in some other way? And finally, given that each odorant is recognized by multiple ORs, are inputs from different ORs combined in individual cortical neurons, or are they segregated in different neurons as in the nose and bulb?

To determine how OR inputs are organized in the cortex, we first asked whether it would be possible to trace neural circuits genetically. In those studies, Lisa Horowitz, a graduate student in the lab, made transgenic mice that expressed a plant protein, barley lectin, in all olfactory sensory neurons in the nose. In those mice, the expression of barley lectin, or BL, was controlled by the promoter of the OMP gene, a gene that is selectively expressed by olfactory sensory neurons.

Using BL-specific antibodies, we detected BL in olfactory sensory neurons in the olfactory epithelium, glomeruli and relay neurons in the bulb, and also in neurons in the olfactory cortex (Horowitz *et al.*, 1999). This indicated that BL produced by olfactory sensory neurons in the nose could travel across two synapses to label connected neurons first in the olfactory bulb and then in the olfactory cortex.

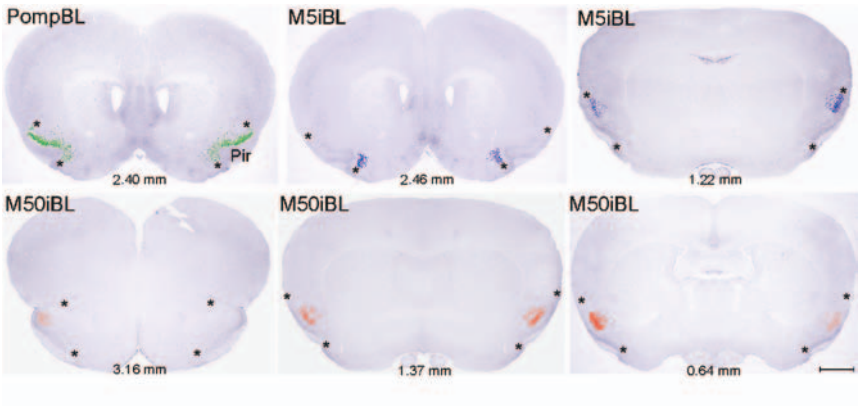

Figure 12. Inputs from one OR are targeted to 2–3 clusters of neurons in the anterior piriform cortex. Coronal sections through the anterior piriform cortex of mice in which barley lectin was expressed in all olfactory sensory neurons (PompBL) or only in neurons expressing the M5 (M5iBL) or M50 (M50iBL) odorant receptor. Asterisks indicate the outer limits of the piriform cortex (Pir). Distance from an anterior-posterior landmark is shown in mm. (Adapted from Zou *et al.*, 2001).

Having developed a genetic method for charting neural circuits, we were able to move to the next step. That was to ask how inputs from individual ORs are organized in the cortex.

Our goal was to coexpress BL with only one of the 1000 different OR genes. To do this, Lisa Horowitz and Jean-Pierre Montmayeur in our lab altered individual OR genes by inserting, just 3' to their coding regions, an IRES sequence followed by a BL coding sequence. Using gene targeting in embryonic stem cells, Zhihua Zou, a postdoctoral fellow in the lab then made "knockin" mice that contained an altered allele of either the M5 or M50 OR gene (Zou *et al.*, 2001). In these knockin mice, BL was produced only in neurons that expressed the M5 or M50 OR.

In the olfactory cortex, the axons of bulb neurons branch and form synapses in layer Ia with the dendrites of pyramidal neurons located in layers II and III. In PompBL mice, which express BL in all olfactory sensory neurons (Horowitz *et al.*, 1999), we saw labeled neurons in layers II and III throughout the olfactory cortex (Figure 12). In the M5 and M50 knockin mice, we also detected labeled cortical neurons, but they were located in distinct clusters (Figure 12) (Zou *et al.*, 2001). Moreover, the clusters appeared to have similar locations in different individuals.

In each knockin strain, we detected 2–3 clusters of labeled neurons in the anterior piriform cortex (Figure 12). Most of these clusters were bilaterally symmetrical in the left and right brain. We also found clusters of labeled neurons in several other areas of the olfactory cortex. In each cluster, the high-

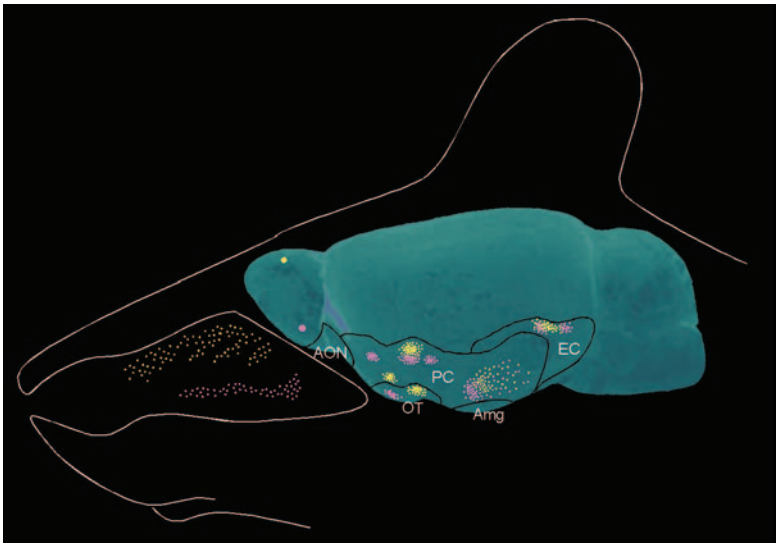

Figure 13. The olfactory cortex has a stereotyped map of OR inputs. Organization of inputs from the M5 (yellow) and M50 (pink) odorant receptors in the olfactory epithelium, bulb, and cortex. Black lines and abbreviations indicate different areas of the olfactory cortex. AON, anterior olfactory nucleus; PC, piriform cortex; OT, olfactory tubercle; Amg, olfactory nuclei of amygdala; EC, lateral entorhinal cortex.

est density of labeled neurons was in the center, but even in the center, only about half of the resident pyramidal neurons were labeled for the BL tracer.

Detailed analysis of the clusters in the anterior piriform cortex revealed that they had similar locations and similar dimensions in different individuals and, in most cases, they were bilaterally symmetrical (Zou *et al.*, 2001). The clusters had different locations in the two knockin strains, but one of the M5 clusters appeared to partially overlap with one of the M50 clusters.

These results showed that the olfactory cortex has a stereotyped map of OR inputs (Figure 13). In this map, signals derived from one type of OR are targeted to several loose clusters of cortical neurons. The clusters of neurons that receive input from a particular OR are found at specific locations, which are virtually identical among individuals.

These studies clearly indicated that input from one OR diverges to multiple areas of the olfactory cortex. This divergence of OR inputs may allow a parallel processing of OR signals in which signals from the same ORs are combined or modulated in different ways prior to transmission to other brain regions that have different functions.

We found that in the anterior piriform cortex, the clusters of neurons that receive input from one OR occupy about five percent of the total area along the anterior-posterior and dorsal-ventral axes (Zou *et al.*, 2001). In PompBL mice, which express BL in all olfactory sensory neurons, there were about

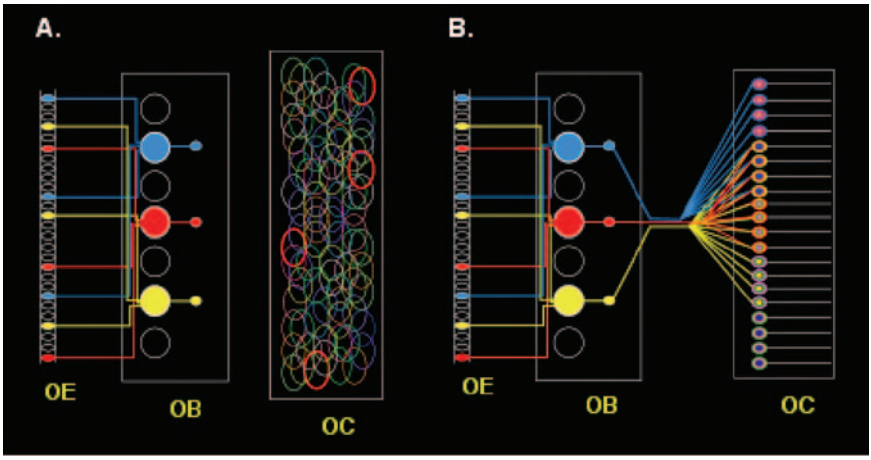

Figure 14. Schematic diagrams showing the organization of odorant receptor inputs in the olfactory epithelium (OE), olfactory bulb (OB), and olfactory cortex (OC). Inputs from different ORs are segregated in different neurons and glomeruli in the OE and OB. In contrast, it appears that different receptor inputs overlap extensively in the OC (A) and that single cortical neurons receive signals from a combination of receptors (B).

180,000 BL-labeled neurons in the anterior piriform cortex. If each cortical neuron received input from only one of 1000 different ORs, one might expect to see about 180 labeled neurons in this area each knockin mouse. However, we detected about 4000–6000 BL-labeled neurons in the anterior piriform cortex in each knockin strain (Zou *et al.*, 2001).

These results indicated that the map of OR inputs in the olfactory cortex is markedly different from that in the olfactory bulb. First, while inputs from different ORs are spatially segregated in different glomeruli in the bulb, they are likely to overlap extensively in the cortex (Figure 14A). Second, while signals from different ORs are segregated in different neurons in both the nose and bulb, each cortical neuron is likely to receive signals derived from multiple different ORs (Figure 14B). Since each odorant is recognized by a combination of ORs, this may permit an initial integration of multiple components of an odorant's receptor code that is critical to the generation of diverse odor perceptions.

These findings raise the possibility that neurons in the olfactory cortex function as coincidence detectors that are activated only by correlated combinatorial inputs from different ORs. For example, in a simple model, signals from different ORs that recognize vanillin would be targeted to partially overlapping locations in the cortex, but the only neurons activated by vanillin would be those that receive coincident signals derived from more than one of the vanillin ORs.

In sensory systems, environmental stimuli are deconstructed and then reconstructed in the brain to create perceptions. The organization of OR inputs seen in the olfactory cortex may serve as an initial step in the reconstruction of an odor image from its deconstructed features.

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