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Odor Perception

Unraveling the Sense of Smell (Nobel Lecture)**

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From the Contents

Biography

SEATTLE

I was born in 1947 in Seattle, Washington, a city surrounded by mountains, forests, and the sea. My mother was the daughter of Swedish immigrants who had come to the US in the late nineteenth century while my father's family had Irish roots on one side and ancestors extending back to the American Revolution on the other. I was the second of three children, all girls. My mother was a homemaker who was exceptionally kind and witty and loved word puzzles. My father was an electrical engineer who, at home, spent much of his time inventing things and building them in our basement. It may be that my parents' interest in puzzles and inventions planted the seeds for my future affinity for science, but I never imagined as a child that I would someday be a scientist.

During my childhood, I did the things that girls often do, such as playing with dolls. I was also curious and easily bored though, so I frequently embarked on what were to me new adventures. Aside from school and music lessons, my life was relatively unstructured and I was given considerable independence. I learned to appreciate music and beauty from my mother and my father taught me how to use power tools and build things. I spent a lot of time with my maternal grandmother, who told me magical stories about her childhood in Sweden and, to my delight, taught me how to sew clothes for my dolls. I was fortunate to have wonderfully supportive parents who told me that I had the ability to do anything I wanted with my life. They taught me to think independently and to be critical of my own ideas, and they urged me to do something worthwhile with my life, in my mother's words, to "not settle for something mediocre". I realize now that I internalized those lessons and that they have influenced my work as a scientist.

I received my undergraduate education at the University of Washington, which was only a few miles from our home. I had always wanted to have a career in which I would help others, so I initially decided to major in psychology, thinking that I would become a psychotherapist. Over time, my interests expanded and I entertained a variety of different career possibilities. However, none seemed ideal and I was reluctant to embark on something that might prove to be inappropriate. Over the next several years, I intermittently traveled, lived on a nearby island, and took more classes in Seattle. I finally found my direction when I took a course in immunology, which I found fascinating. I would be a biologist.

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DALLAS

In 1975, I began graduate school in the Microbiology Department at the UT Southwestern Medical Center in Dallas. The department had recently undergone an expansion in the area of immunology, making it a major center in this still young area and a stimulating place to learn. I had done a small amount of research at the University of Washington, first in psychology with Walter Makous and then in immunology with Ursula Storb, but it was in Texas that I truly learned to be a scientist. I had a wonderful thesis advisor, Ellen Vitetta, who demanded excellence and precision in research, habits that I believe are important to learn as a student. For my thesis, I compared the functional properties of subsets of B lymphocytes that differed in the class of cell-surface immunoglobulin that they used as antigen receptors. In this work and much of my subsequent work, I thought in terms of molecules and the molecular mechanisms underlying biological systems, and sought to gain insight into those mechanisms in my experiments.

NEW YORK

In 1980, I moved to Columbia University in New York City to do postdoctoral work in immunology with Benvenuto Pernis. As a graduate student, I had become fascinated with the unexplained requirement for major histocompatibility complex (MHC) proteins in immune responses, a mystery that was later solved. I decided to explore this puzzle, focusing on class II MHC proteins found on the surface of B lymphocytes. I found that, contrary to expectation, the MHC proteins rapidly accumulated inside these cells when they were activated. My further experiments indicated that they were being internalized from the cell surface and were probably being recycled to it. It was known that antigens are endocytosed with antigen receptors and then degraded. One possibility raised by the internalization and apparent recycling of MHC molecules was that, following internalization, they might be targeted to a specialized microenvironment where they could interact with degraded antigen. The MHC–antigen complexes might then be exported to the cell surface for co-recognition by T-helper cells.

By this time, it had become clear to me that to study molecular mechanisms underlying biological systems, which is what interested me, I needed to learn the recently developed techniques of molecular biology. To this end, I moved to the laboratory of Richard Axel, at Columbia University. Richard had begun to work in the area of neuroscience several years earlier through collaboration with Eric Kandel, who was also at Columbia. Their collaboration had focused on molecular studies on the nervous system of Aplysia, a sea snail. This was the model organism that Eric had used in many of his studies of learning and memory, for which he received a Nobel Prize in 2000. Perhaps not surprisingly, I was interested in searching for genes encoding neuronal cell surface receptors. However, at that time, Richard wanted to continue studying Aplysia, so I agreed to a project in which I would try to develop a technique for cloning genes expressed in one Aplysia neuron, but not another. After spending a short time learning molecular techniques from Jim Roberts, a student in the lab, I started my Aplysia project. Eric Kandel's group showed me how to isolate giant Aplysia neurons that had been assigned names and could be identified by their locations and, within a relatively short time, I began to uncover genes that were differentially expressed among Aplysia neurons.

While studying a neuropeptide gene expressed in neuron number R15, I discovered that the gene was also expressed in some other neurons, but that its primary transcript was alternatively spliced in different neurons to give different polyproteins. The two polyproteins could generate two different combinations of peptides in different neurons, thus suggesting a way to produce physiological or behavioral programs with partially overlapping components. While working on the neuropeptide gene, I encountered numerous technical challenges that increased my knowledge of molecular biology and honed my abilities. During this period, I learned a lot of molecular biology from Richard and other members of his lab. I also got to know Eric Kandel, who has continued to be a wonderful source of inspiration and encouragement for me over the years.

From my first introduction to neuroscience, I had been fascinated by the brain's cellular and connectional diversity. In parallel with my Aplysia experiments, I sporadically tried to find a way to scan the genome for genes that had undergone gene rearrangement or gene conversion in neurons, thinking that genes that showed this characteristic might be involved in the generation of neuronal diversity. One method that I devised showed promise in Drosophila, but was not sensitive enough for the much larger genome of a mammal, which is what interested me. Nonetheless, these efforts were a great source of creative enjoyment for me as I proceeded with the more mundane task of searching for minute alternative exons in the Aplysia genome.

I was grateful that Richard was tolerant of my high-risk endeavors. He was an unusual mentor in that he gave people in his lab extensive independence in charting their own course once they had established themselves. During this time, I had many colleagues at Columbia with whom I enjoyed long discussions about science. Among these were George Gaitanaris, who has remained a close friend over the years, as well as Tom Jessell and Jane Dodd, neuroscientists from whom I learned a great deal about neural development.

As I was nearing the end of my Aplysia project, I read a paper that changed my life. It was a 1985 publication from Sol Snyder's group that discussed potential mechanisms underlying odor detection. This was the first time I had ever thought about olfaction and I was fascinated. How could humans and other mammals detect 10000 or more odorous chemicals, and how could nearly identical chemicals generate different odor perceptions? In my mind, this was a monumental puzzle and an unparalleled diversity problem. It was obvious to me that the first step to solving the puzzle was to determine how odorants are initially detected in the nose. This meant finding odorant receptors, a class of molecules that had been proposed to exist, but had not been found. I decided that

this is what I had to do as soon as my neuropeptide work was completed.

In 1988, I embarked on a search for odorant receptors, staying on in Richard's lab for this purpose. In a recent commentary in the journal *Cell*, I described what was known about odor detection at that time and the approaches that I tried in the quest to find the elusive odorant receptors. In short, it was known that odorants depolarize, and thereby activate, olfactory sensory neurons in the nose. Although there were varied proposals as to what kind of molecules might interact with odorants, there was compelling evidence that olfactory transduction involved G-protein-induced increases in cAMP. After trying several different approaches, I identified the odorant receptor family by designing experiments based on three assumptions: First, since odorants vary in structure and can be discriminated, there would be a family of varied, but related odorant receptors, which would be encoded by a multigene family. Second, odorant receptors would be at least distantly related to the relatively small set of G-protein-coupled receptors whose sequences were known at that time. Finally, odorant receptors would be selectively expressed in the olfactory epithelium, where olfactory sensory neurons are located. It took some time to devise and develop the methods I used in my search, but in the end they succeeded. Looking at the first sequences of odorant receptors obtained from rats, I was moved by nature's marvelous invention. This work showed that the rat has a multigene family that codes for in excess of one hundred different odorant receptors, all related, but each one unique. The unprecedented size and diversity of this family explained the ability of mammals to detect a vast array of diverse chemicals as having distinct odors. In 1991, Richard Axel and I published the identification of odorant receptors.

(Photo: Roland Morgan)

BOSTON

In 1991, I departed for Boston to be an assistant professor in the Neurobiology Department at Harvard Medical School. There, I was immersed in an environment in which I could broaden my understanding of the nervous system. I received excellent support from my chairman, Gerry Fischbach, as I set up my lab. I also acquired many excellent colleagues, including David Hubel, whose pioneering studies of the visual system with Torsten Wiesel (for which they received a Nobel Prize in 1981) had always been an inspiration to me. In 1994, I became an Investigator of the Howard Hughes Medical Institute, which has generously supported our work for the past 11 years. Over the next decade, I remained at Harvard, gradually rising through the ranks to become associate and then full professor. In 1994, I met Roger Brent, a marvelous intellect and fellow scientist who has been my partner and an important part of my life ever since.

The discovery of odorant receptors had explained how the olfactory system detects odorants. My next goal was to learn how signals from those receptors are organized in the brain to generate diverse odor perceptions. I was joined in this endeavor by a series of excellent students and postdoctoral fellows. The discoveries on the organization of the olfactory system that were cited by the Nobel Foundation were made over a period of ten years, during which I was a faculty member at Harvard.

The first question we asked was how odorant receptors are organized in the olfactory epithelium of the nose. This work was begun by Kerry Ressler, an MD/PhD student who came to the laboratory for a few months just as the equipment and supplies I had ordered began to arrive in January 1992. I had decided to switch from the rat to the mouse as a model organism because of the advantage of using isogenic inbred strains for dissecting a multigene family, and the possibility of generating transgenic mice. After cloning and sequencing a series of mouse odorant receptor genes, Kerry did our first in situ hybridization experiments to examine patterns of receptor gene expression. By June, Kerry had returned as a full-time student and Susan Sullivan had joined the lab as a postdoctoral fellow. At this point, we began to precisely analyze gene-expression patterns and to compare them in different individuals. Prior to the present era of digital photographs that can be stored and analyzed on a computer, this was painstaking work that involved displaying photographic slides on a desktop viewer and recording, on transparencies, the locations of individual labeled cells in different animals. Our studies showed that each receptor gene is expressed in about 1/1000 olfactory sensory neurons, that the olfactory epithelium has several spatial zones that express non-overlapping sets of odorant receptor genes, and that neurons with the same odorant receptor are randomly scattered throughout one zone. This indicated that signals derived from different odorant receptors are segregated in different sensory neurons and in the information they transmit to the brain. It further indicated that, in the olfactory epithelium, neurons that detect the same odorant are dispersed and those that detect different odorants are interspersed. Thus, there is a broad organization of sensory information into several zonal sets in the epithelium, but, overall, information is encoded in a highly distributed manner. We published these findings in 1993. Similar

observations in rats by Richard Axel and his colleagues were also reported that year.

Having determined how inputs from different odorant receptors are organized in the nose, we asked how they are arranged at the next structure in the olfactory pathway, the olfactory bulb. In the bulb, the axons of olfactory sensory neurons synapse in about 2000 spherical structures, called glomeruli. Kerry began to use retroviral vectors to investigate how the axons of neurons expressing specific receptors are organized in the bulb, but then we inadvertently found another way to address the question. While using in situ hybridization to identify a number of receptor genes expressed in each epithelial zone for chromosomal mapping studies, Susan found that, in one tissue section, a receptor probe labeled a single spot in the bulb, which proved to be a glomerulus. Using probes that recognized single receptor genes rather than subfamilies of related receptor genes, we found that each probe labeled odorant receptor mRNAs in sensory axons that were confined to one or a few glomeruli at only two sites, one on either side of the bulb. Different probes labeled different glomeruli and those glomeruli had virtually identical locations in different individuals. I still remember a meeting with Kerry and Susan in my office in which I asked Kerry how many sections separated different labeled glomeruli in different bulbs. All of us were stunned by his answer, because it provided the first hint that the bulb might have a stereotyped map of receptor inputs and we could not imagine how this could be generated given the organization of receptor gene expression in the epithelium. This mystery still has not been solved. These studies indicated that while thousands of neurons expressing the same receptor are highly dispersed in the epithelium, their axons all converge in a few specific olfactory bulb glomeruli. The result is a stereotyped map of receptor inputs in which signals derived from different receptors are segregated in different glomeruli and in the bulb projection neurons whose dendrites innervate those glomeruli. Remarkably, Bob Vassar in Richard Axel's lab had concurrently found that different receptor probes labeled different glomeruli in the rat bulb. Our two groups published these findings in 1994.

Several years later, we began to investigate how the receptor family and the patterning of receptor inputs encode the identities of different odorants. By using single-cell RT-PCR (reverse transcriptase–polymerase chain reaction), Bettina Malnic, a fellow in the lab, had been comparing gene expression in single olfactory sensory neurons. Her work demonstrated that each neuron expresses only a single receptor gene, something that we had previously suspected, but that needed to be verified. Bettina was initially focused on the identification of genes that might be involved in receptor gene choice or axon targeting in the bulb, but we decided to change course when Takaaki Sato visited our lab and told us about his calcium imaging studies of odor responses in the olfactory epithelium. This was the beginning of a highly successful collaboration in which Takaaki used calcium imaging to define the odor response profiles of individual neurons and Bettina then used RT-PCR to identify the receptor expressed by each responsive neuron. These studies demonstrated that the receptor family is used in a combinatorial manner. Different neurons are recognized, and thereby encoded, by different combinations of receptors, but each receptor is used as one component of the combinatorial receptor codes for many different odorants. These studies also provided explanations for several intriguing features of human odor perception, including how a slight change in the structure of an odorant can dramatically change its perceived odor quality.

As soon as we had determined how receptor inputs are organized in the olfactory bulb, we began to explore how they are arranged at the next structure in the olfactory pathway, the olfactory cortex. Lisa Horowitz, an MD/PhD student in the lab, initially investigated connections between the bulb and cortex using classical anatomical techniques. By depositing different tracers in the dorsal and ventral bulb, she determined that these areas project axons to the same regions of the cortex. In agreement with previous findings, this indicated that there could not be a point-to-point patterning of connections between the bulb and cortex. We decided to abandon traditional approaches and to instead ask whether we could chart neural pathways genetically by expressing a gene encoding a transneuronal tracer in olfactory sensory neurons. Lisa found that this was indeed possible. When she made transgenic mice that expressed barley lectin in all olfactory sensory neurons, the lectin crossed two synapses, labeling second-order neurons in the bulb, and then third-order neurons in the cortex. This work, which we published in 1999, opened the way to investigating a wide array of questions concerning neural circuits, including those that carry olfactory information.

We then went on to use the genetic tracer to examine how inputs from individual types of receptors are organized in the olfactory cortex. To do this we used gene targeting to generate mice that coexpressed barley lectin with a single receptor gene. Lisa, together with a fellow in the lab, Jean-Pierre Montmayeur, prepared the DNA constructs for gene targeting. Zhihua Zou, another fellow, then generated and analyzed mice that coexpressed the tracer with different receptor genes. The approach worked, but was difficult, with Zhihua investing almost a year in perfecting the conditions needed to detect minute amounts of the tracer in cortical neurons. These studies revealed that the olfactory cortex has a stereotyped map of receptor inputs, but one that is radically different from that in the bulb. The segregation of receptor inputs in different glomeruli and neurons in the bulb gives way in the cortex to a complex array of receptor inputs in which signals from different receptors partially overlap and single cortical neurons appear to receive signals from combinations of different receptors. This offers a means by which the individual components of an odorant's receptor code could be integrated at the level of single neurons. This could serve as an initial step in the reconstruction of an odor image from its deconstructed features, which are conveyed by the receptor elements of the receptor code. We published our findings on the cortex in 2001.

During the ten-year period at Harvard in which we did the work described above, my laboratory also investigated a

number of other questions. These included studies of the chromosomal organization of receptor genes and the evolution of the receptor gene family by Susan Sullivan, studies of the development of receptor gene expression patterns by Susan and Staffan Bohm, and bioinformatic studies by Bettina Malnic and Paul Godfrey that defined and compared the receptor gene repertoires of humans and mice. We also conducted a series of studies on the detection of pheromones in the vomeronasal organ, including studies by Emily Liman and Anna Berghard that revealed differences between transduction molecules involved in odor versus pheromone detection, the discovery of zonal patterns of transduction molecules likely to be involved in pheromone detection by Anna, analyses of vomeronasal responses to pheromones and odorants by Mehran Sam, and the discovery, by Hiroaki Matsunami, of a family of candidate pheromone receptors. During the latter part of this period, Hiroaki Matsunami, Jean-Pierre Montmayeur, and Stephen Liberles also began to explore the mechanisms underlying taste detection, in the process discovering candidate receptors for both bitter and sweet tastes, both of which were also found by other groups at about the same time.

SEATTLE

In 2002, I returned to Seattle to be a Member of the Division of Basic Sciences at the Fred Hutchinson Cancer Research Center and Affiliate Professor of Physiology and Biophysics at the University of Washington. I had always intended to someday return to the West Coast and had already stayed longer in Boston than I had anticipated. When Mark Groudine, then Director of the Basic Sciences Division at Fred Hutchinson, offered me a faculty position there, I gladly accepted. The Hutchinson Center had a reputation for cutting-edge science as well as a high level of collegiality, both of which were important to me. In addition, by moving to Seattle, I would be closer to my partner, Roger, who lived in Berkeley, and to my family and friends in Seattle.

In Seattle, we are continuing to explore the mechanisms underlying odor perception as well as the means by which pheromones elicit instinctive behaviors. We have also become interested in the neural circuits that underlie innate behaviors and basic drives, such as fear, appetite, and reproduction. We are currently developing molecular techniques to uncover those circuits and to define their composite neurons and the genes they express. In a different vein, we have developed a high-throughput approach in which we are using chemical libraries to identify genes that control aging and lifespan, our chief interest being whether there might be a central mechanism that determines lifespan and regulates the aging of cells throughout the body.

LOOKING BACK

Since Richard Axel and I published the discovery of odorant receptors in 1991, it has been immensely satisfying for me to see many laboratories using these receptors in a large-scale effort to dissect the mechanisms that underlie the sense of smell and the developmental processes that shape the organization of the olfactory system. Molecular approaches to studying olfaction have extended to other vertebrates as well as to invertebrate species, with Cori Bargmann's group discovering a large variety of chemosensory receptors in the nematode worm *C. elegans*, and several groups, including Richard Axel's, identifying families of odorant and taste receptors in the fruit fly *D. melanogaster*.

Looking back over my life, I am struck by the good fortune I have had to be a scientist. Very few in this world have the opportunity to do everyday what they love to do, as I have. I have had wonderful mentors, colleagues, and students with whom to explore what fascinates me, and have enjoyed both challenges and discoveries. I am grateful for all of these things and look forward to learning what nature will next reveal to us.

As a woman in science, I sincerely hope that my receiving a Nobel Prize will send a message to young women everywhere that the doors are open to them and that they should follow their dreams.

1. 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 10000 to 100000 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?

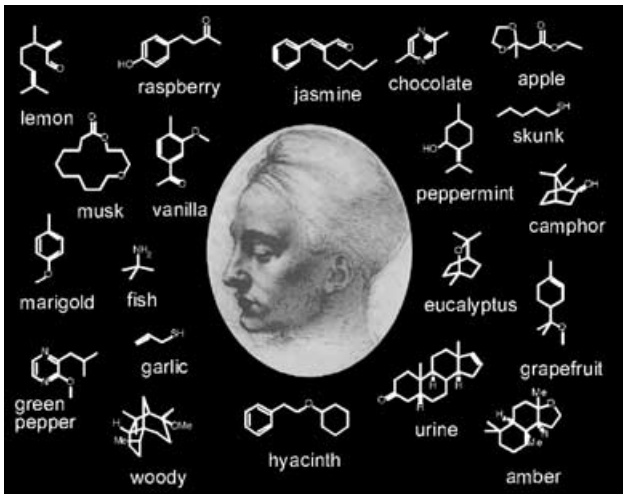

Figure 1. Humans and other mammals perceive a vast number of chemicals as having distinct odors.

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

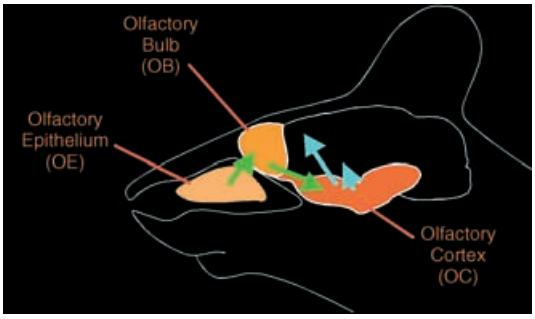

Figure 2. The olfactory pathway. Odorants are detected by olfactory sensory neurons in the olfactory epithelium. Signals generated in those neurons are relayed through the olfactory bulb to the olfactory cortex and then sent to other brain areas.

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 (VNO), a separate olfactory structure in the nasal septum. 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 through a single axon that it extends to the olfactory bulb.

2. 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,[1] 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.[2,3] In the mid 1980s, hints started to emerge about signal transduction in the cilia of the olfactory neurons. Doron Lancet, Sol Snyder, and their colleagues showed that odorants induce GTP-dependent increases in adenylyl cyclase activity in the cilia, thus suggesting the involvement of intracellular G proteins,[4,5] and Randy Reed identified G_{α}olf, a G protein that could mediate this response and was highly expressed in olfactory sensory neurons.[6]

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. 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 (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.[7] To do this, we first used the polymerase chain reaction (PCR) 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 by agarose gel electrophoresis.

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, no. 13, was cut into a large number of fragments, thus suggesting that it might contain multiple members of a multigene family. When we cloned and sequenced five of the DNAs in this band, 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.

By 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, thus 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 of different structures.

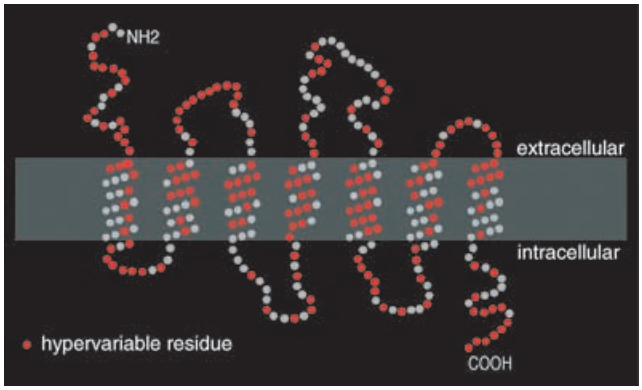

Figure 3. Topology of an odorant receptor in the membrane. Individual amino acid residues are indicated by balls. Red balls indicate residues that were hypervariable among ten odorant receptors. Adapted from Ref. [7].

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, thus suggesting that the receptors were expressed predominantly or exclusively by olfactory sensory neurons.

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 mice.

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.[7] Subsequent studies showed that homologous families of odorant receptors are present in vertebrate species ranging from fish to humans.[8] In 1991, after publishing our work on odorant receptors,[7] 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[9,10] and by Firestein and Trask for mouse,[11,12] and in my lab it was done for both species by Bettina Malnic and Paul Godfrey.[13,14] These studies indicate that humans have about 350 different ORs and mice have about 1000. This result indicates that roughly 1–5 % of the genes in the genome are devoted to the detection of odorants. Odorant receptor 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).[14]

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 domain structure of GPCRs. V1R and V2R genes are selectively expressed in the VNO, thus 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,[15a] 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.[15b,16,17]

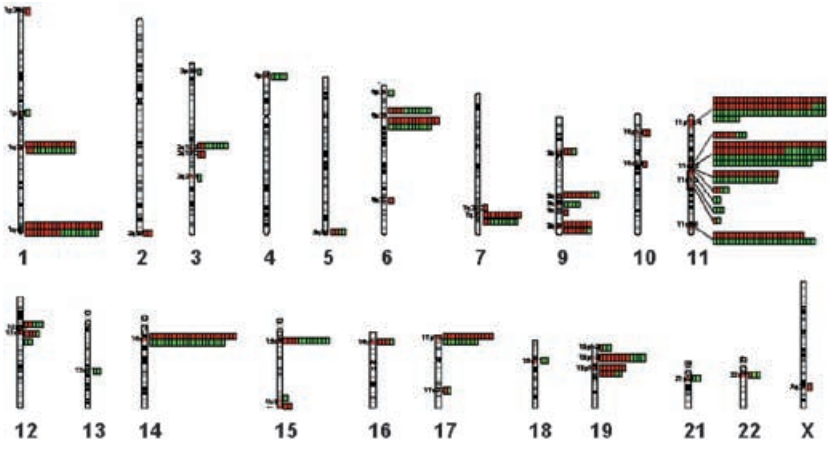

Figure 4. The chromosomal distribution of human odorant receptor genes. Intact receptor genes are shown in red and pseudogenes in green. Adapted from Ref. [14].

3. 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.[18] 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

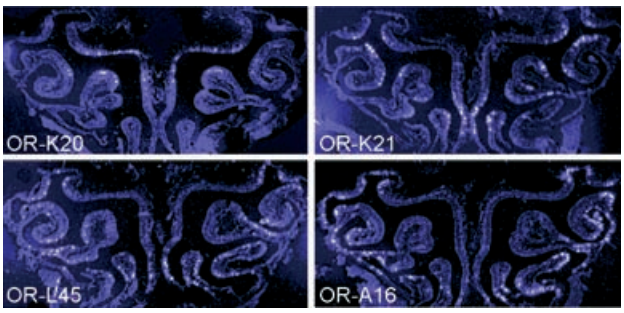

Figure 5. Expression patterns of odorant receptor genes in the mouse olfactory epithelium. Tissue sections through the mouse nose were hybridized to four different receptor gene probes. Adapted from Refs. [18, 30].

has distinct spatial zones that express non-overlapping 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 rats.[19] The OR expression zones form stripes that extend along the anterior–posterior axis of the nasal cavity.

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.[20] 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.

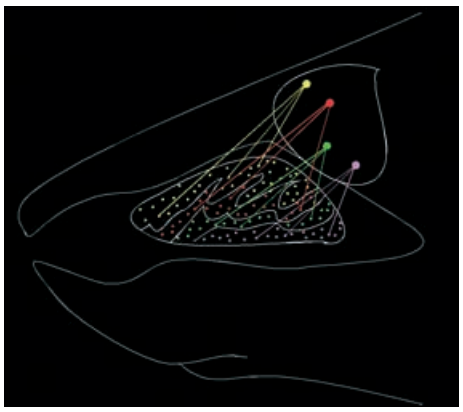

Figure 6. The organization of odorant receptor inputs in the olfactory epithelium and olfactory bulb. Sensory neurons expressing the same receptor are scattered within one epithelial zone, but their axons converge in specific glomeruli in the olfactory bulb. Adapted from Refs. [18, 22, 30].

4. 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.[20] This work was done by Bettina Malnic in my 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 reverse transcriptase–PCR (RT-PCR) to determine the OR gene it expressed. In every case, we identified only one expressed OR per neuron, thus confirming that each neuron expresses a single OR gene.

For test odorants, we used four different classes of linear aliphatic odorants with different functional groups and carbon chains ranging in length from four to nine carbon atoms. Each neuron was imaged as it was exposed sequentially to different odorants (Figure 7). If a response was seen, the neuron was retested with a lower concentration of the same odorant.

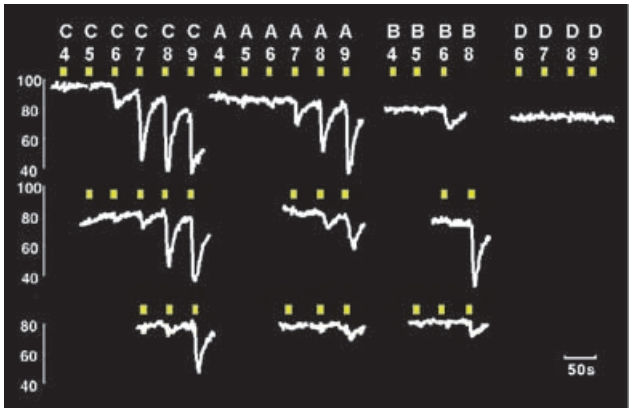

Figure 7. Responses of a single olfactory sensory neuron to different odorants. Fluorescence emission was monitored during sequential exposure of a neuron containing an indicator dye (Fura-2) to a series of odorants (C4–D9). Responses to lower odorant concentrations are shown below. Adapted from Ref. [20].

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.[21] Second, each odorant can be detected by multiple different ORs. Finally, and most importantly, different odorants are recognized by different combinations of ORs.

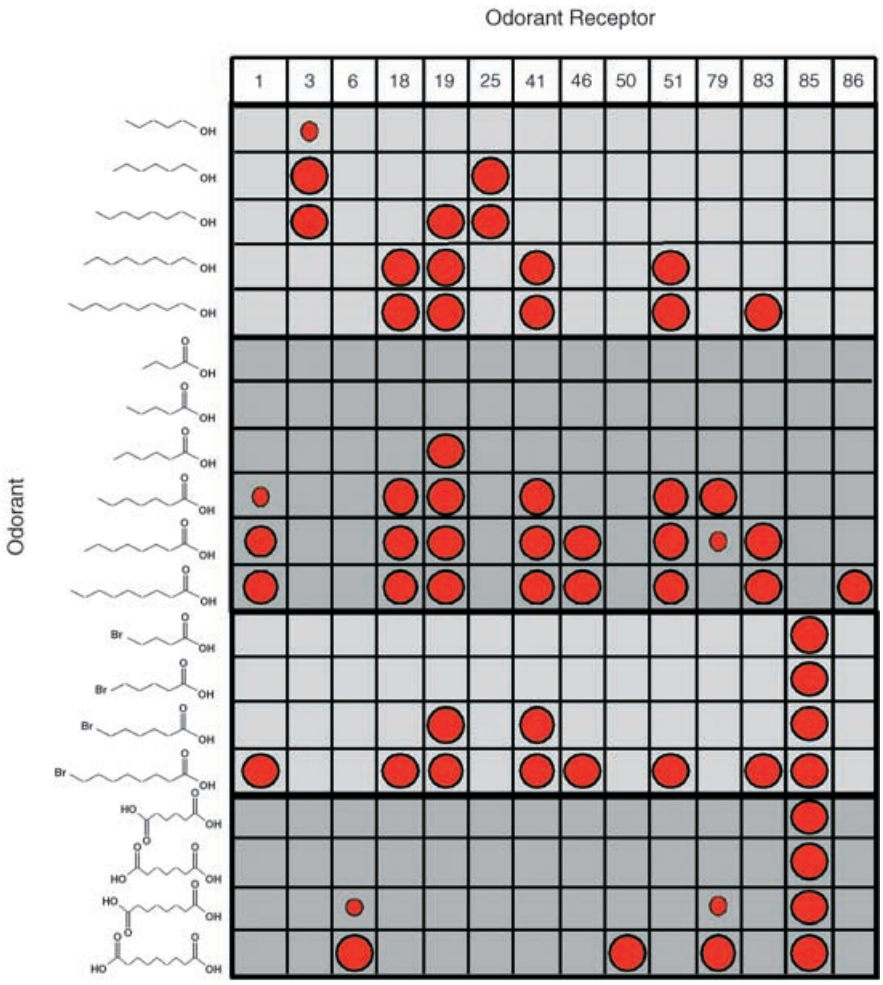

Figure 8. Odorant receptors are used combinatorially to detect odorants and encode their identities. The recognition profiles of individual odorant receptors to a series of odorants were determined by calcium imaging and single-cell RT-PCR. The sizes of circles reflect response intensity. Adapted from Ref. [20].

These results indicated that ORs are used combinatorially to encode odor identities.[20] 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.

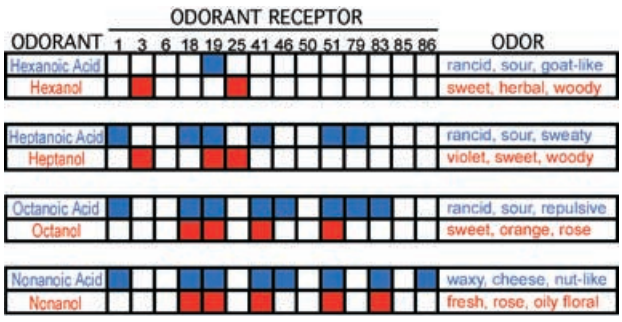

Figure 9. Closely related odorants with different perceived odors are detected by different combinations of receptors. Adapted from Ref. [20].

These studies also provided insight into several puzzling features of human odor perception.[20] 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).

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.

5. 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 combinations of ORs 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.[22] Here, single receptor probes labeled receptor 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 receptor 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 rats, Vassar and Axel obtained similar results, although individual receptor probes generally labeled a larger number of glomeruli at more locations in the bulb in their studies.[23]

Our studies in mice 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).[22] They further indicated that sensory information that is broadly organized into four 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.

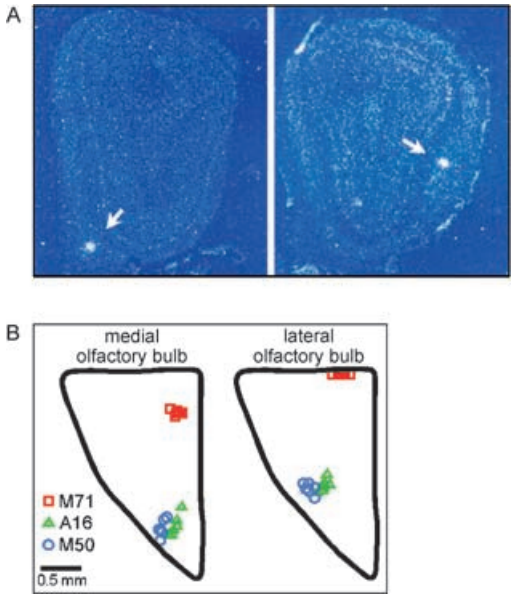

Figure 10. The olfactory bulb has a stereotyped map of OR inputs. A) A single OR gene probe hybridized to sensory axons in only one or two glomeruli on either side of the olfactory bulb. B) Different OR probes (A16, M50, M71) hybridized to different glomeruli and those glomeruli had similar locations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

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 2–4 glomeruli and about 50 mitral cells, thus 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.

6. Odor Coding in the Olfactory Epithelium and Bulb

Given our finding that each odorant is recognized by a combination of ORs,[20] 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 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.[24–26]

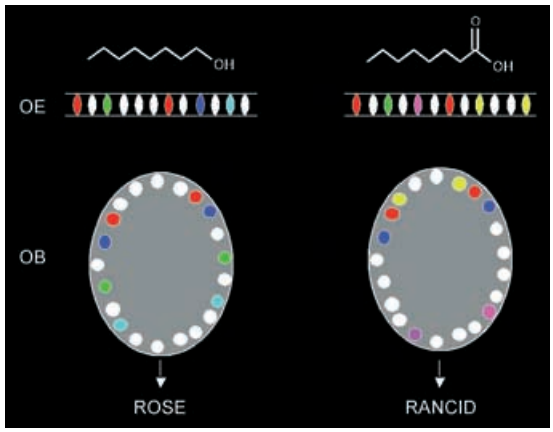

Figure 11. Odor coding in the olfactory epithelium and olfactory bulb. In this schematic representation, inputs from different ORs that recognize an odorant are indicated by different colors. In the olfactory epithelium (OE), the code for an odorant is a dispersed ensemble of neurons, each expressing 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.

7. 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 backlabel mitral cells in many parts of the bulb.[27] 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? 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?

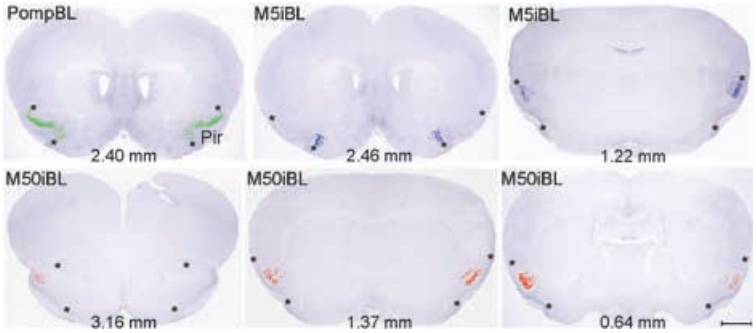

Figure 12. Inputs from one OR are targeted to two to three clusters of neurons in the anterior piriform cortex. Coronal sections through the anterior piriform cortex of mice in which barley lectin was expressed in all olfactory sensory neurons (PompBL) or only in neurons expressing the M5 (M5iBL) or M50 (M50iBL) odorant receptor. Asterisks indicate the outer limits of the piriform cortex (Pir). The distance from an anterior–posterior landmark is shown in mm. Adapted from Ref. [29].

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 (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.[28] 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.

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, 3′ to their coding regions, an internal ribosome entry site (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.[29] 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,[28] 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).[29] 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 highest density of labeled neurons was in the center, but even in the center, only about half of the resident pyramidal neurons were labeled with 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.[29] 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

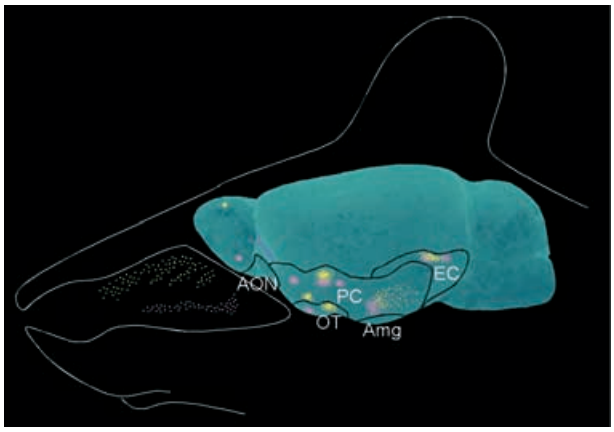

Figure 13. The olfactory cortex has a stereotyped map of OR inputs. Organization of inputs from the M5 (yellow) and M50 (pink) odorant receptors in the olfactory epithelium, bulb, and cortex. Black lines and abbreviations indicate different areas of the olfactory cortex. AON: anterior olfactory nucleus;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

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.[29] In PompBL mice, which express BL in all olfactory sensory neurons, there were about 180000 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 in each knockin mouse. However, we detected about 4000–6000 BL-labeled neurons in the anterior piriform cortex in each knockin strain.[29]

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).

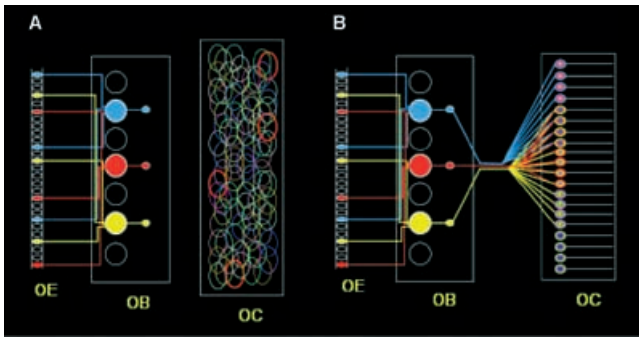

Figure 14. Schematic diagrams showing the organization of odorant receptor inputs in the olfactory epithelium (OE), olfactory bulb (OB), and olfactory cortex (OC). Inputs from different ORs are segregated in different neurons and glomeruli in the OE and OB. In contrast, it appears that different receptor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

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 receptor inputs seen in the olfactory cortex may serve as an initial step in the reconstruction of an odor image from its deconstructed features.

I would like to acknowledge the very talented students and postdoctoral fellows in my lab who did the experiments that I discussed today. Kerry Ressler and Susan Sullivan did all of the early studies on OR inputs in the olfactory epithelium and bulb. Hiroaki Matsunami identified and characterized the V2R family of candidate pheromone receptors. Bettina Malnic conducted the studies of OR specificities in collaboration with Takaaki Sato and Junzo Hirono at the Life Electronic Research Center in Japan. Bettina and Paul Godfrey defined the OR gene repertoires of humans and mice. Lisa Horowitz developed the genetic method for tracing neural circuits, and Zhihua Zou, in collaboration with Lisa and Jean-Pierre Montmayeur, conducted the studies of OR inputs in the olfactory cortex.

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