Odorant Receptors and the Organization of the Olfactory System

1. Odorants bind to receptors

2. Olfactory receptor cells are activated and send electric signals

3. The signals are relayed in glomeruli

4. The signals are transmitted to higher regions of the brain

Nasal epithelium

Olfactory receptor cells

Olfactory bulb
Scents and Sensibility: A Molecular Logic of Olfactory Perception (Nobel Lecture)**

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Keywords:

brain research · fluorescence · Nobel Lecture · olfactory cells · receptors

Biography

New York City is my world. I was born in Brooklyn, the first child of immigrant parents whose education was disrupted by the Nazi invasion of Poland. Although not themselves learned, my parents shared a deep respect for learning. I grew up in a home rich in warmth, but empty of books, art, and music. My early life and education were centered on the streets of Brooklyn. Stickball—baseball with a pink ball and broom handle—and schoolyard basketball were my culture. In stickball, a ball hit the distance to one manhole cover was a single, and four manhole covers, a home run, a “Nobel Prize”. My father was a tailor. My mother, although quick and incisive, did not direct her mind to intellectual pursuits and I had not even the remotest thought of a career in academia. I was happy on the courts. In those days, we worked at a relatively young age. At eleven, I was a messenger, delivering false teeth to dentists. At twelve, I was laying carpets, and at thirteen, I was serving corned beef and pastrami in a local delicatessen. Vladimir, the Russian chef, was the first to expose me to Shakespeare, which he recited as we sliced cabbage heads for coleslaw.

My local high-school had the best basketball team in Brooklyn, but the Principal of my grade school had a vision different from my own and insisted that I attend Stuyvesant High-School, far away in Manhattan. Stuyvesant High advertised itself as a school for intellectually gifted boys but had the worst basketball team in the city. I was unhappy about the prospect of attending, for it seemed antithetical to my self-image. Shortly after I entered, however, my world changed. I
embraced the culture and aesthetics of Manhattan. The world of art, books, and music opened before me and I devoured it. In school, I heard bits of an opera for the first time. I remember it distinctly, the Letter Duet from Mozart’s Marriage of Figaro. The next night I attended Tannhauser at the Metropolitan Opera and thus began a love affair, bordering on an obsession, that has had no end. Twice a week, I stood in line for standing room tickets at the Metropolitan Opera where I was exposed to a cult of similarly obsessed but far more knowledgeable afficionados who taught me the intricate nuances of this rich genre. The great Italian tenor, Franco Corelli, would serve us coffee as we waited, and the diva, Joan Sutherland, would invite us backstage.

On other days, I would read in a most beautifully appointed place, the Reading Room of the Central New York Public Library on 42nd Street. One passes the pair of sculpted lions, ascends a flight of stairs into a huge high-ceilinged room of impressive silence where I read incessantly without direction but with a new-found fascination that made up for years of illiteracy. I met a coterie of library dwellers, men and women of New York, who spent all of their days in the Reading Room. I did not know who they were or how they came to be there, but they had an insight and understanding of literature that amazed and still perplexes me, and they were my teachers. This was New York for me, a city of the culturally obsessed that opened up before me and framed my new world.

To support a seemingly extravagant life for a young high-school student, I ascended a flight of stairs into a huge high-ceilinged room of impressive silence where I read incessantly without direction but with a new-found fascination that made up for years of illiteracy. I met a coterie of library dwellers, men and women of New York, who spent all of their days in the Reading Room. I did not know who they were or how they came to be there, but they had an insight and understanding of literature that amazed and still perplexes me, and they were my teachers. This was New York for me, a city of the culturally obsessed that opened up before me and framed my new world.

To support myself in college, I obtained a job washing glassware in the laboratory of Bernard Weinstein, a Professor of Medicine at Columbia University. Bernie was working on the universality of the genetic code. The early sixties was a time shortly after the elucidation of the structure of DNA and the realization that DNA is the repository of all information and from which all information flows. The genetic code had just been deciphered and the central dogma was complete. I was fascinated by the new molecular biology with its enormous explanatory power. I was a terrible glassware washer because I was far more interested in experiments than dirty flasks. I was fired and was rehired as a Research Assistant and Bernie spent endless hours patiently teaching this scientifically naïve, but intensely interested young student. I was torn between literature and science. Dubious about my literary ambitions and fascinated by molecular biology, I decided to attend graduate school in genetics.

My plans were thwarted by an unfortunate war and to assure deferment from the military, I found myself a misplaced medical student at Johns Hopkins University School of Medicine. I entered medical school by default. I was a terrible medical student, pained by constant exposure to the politically fired student body, many the sons of Marxist immigrants. With this array of artistic faculty, Stuyvesant nourished my new and voracious appetite.

But old worlds die hard. I continued to play basketball in high-school and this led to a most memorable and humbling experience. I came onto the court as the starting center, and the center on the opposing team from Power Memorial High School lumbered out on the court, a lanky 7 foot 2 inch sixteen year old. When I was first passed the ball, he put his hands in front of my face, looked at me and asked, “What are you going to do, Einstein?” I did rather little. He scored 54 points and I scored two. He was the young Lew Alcindor, later known as Kareem Abdul Jabar, who went on to be among the greatest basketball legends, and I became a neurobiologist.

My decision to remain in New York and attend Columbia College revealed the provincial but endearing quality of my family. When I chose to accept a gracious scholarship offered by Columbia, my father was disappointed. It was a well-known fact that the brightest children of Brooklyn immigrants attended City College. My freshman year at Columbia, I lived with abandon. The opera, the arts, the freedom, the protest left little time for study. In the first semester, I met a student from Tennessee, Kevin Brownlee, who remains a dear friend and is now a Professor of Medieval French at the University of Pennsylvania. Brownlee urged me to redirect this intensity to learning. The world of the arts will remain, but my time at Columbia University was limited. Once again, a new world opened before me. With Kevin as my guide, I became a dedicated, even obsessed, student. My life was spent in a small room lined with volumes of Keats’ poetry at the Columbia Library and I immersed myself in my studies. The study of literature at Columbia in the sixties was exciting in the presence of the poet, Kenneth Koch, the critics, Lionel Trilling, Moses Hadas, and Jacques Barzun. It was largely chance, however, that led me to biology.

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the suffering of the ill and thwarted in my desire to do experiments. My clinical incompetence was immediately recognized by the faculty and deans. I could rarely, if ever, hear a heart murmur, never saw the retina, my glasses fell into an abdominal incision and finally, I sewed a surgeon’s finger to a patient upon suturing an incision. It was during this period of incompetence and disinterest that I met another extremely close friend, Frederick Kass, now a Professor of Psychiatry at Columbia University. Fred was an unusual medical student, a Texan with a degree in art history from Harvard, who remains a kindred spirit.

It was a difficult time, but I was both nurtured and protected by Howard Dintzis, Victor McCusick, and Julie Krevins, three professors at Johns Hopkins who somehow saw and respected my conflict. Without them, there is little question that I would not have been tolerated but they urged the deans to come up with a solution. I was allowed to graduate medical school early with an MD if I promised never to practice medicine on live patients. I returned to Columbia as an intern in Pathology where I kept this promise by performing autopsies. After a year in Pathology, I was asked by Don King, the Chairman of Pathology, never to practice on dead patients.

Finally, I was afforded the opportunity to pursue molecular biology in earnest. I joined the laboratory of Sol Spiegelman in the Department of Genticus at Columbia University. Spiegelman was a short, incisive, witty man with a tongue as sharp as his mind. Spiegelman was the first to synthesize infectious RNA in vitro and this led to a series of extremely interesting and clever experiments revealing Darwinian selection at the level of molecules in a test tube. Sol recognized the importance of the early RNA world in the evolution of life and had recently turned his laboratory to a study of RNA tumor viruses. An immediate bond formed between us, and Sol taught me how to think about science, to identify important problems, and how to effect their solution.

Although I felt a growing confidence in my abilities in molecular biology, I was naive in other areas of biology, notably biophysics. Importantly, I had a sense early in my career that my interest in biology was eclectic and that I would need a concomitantly broad background to embrace the different areas of biology without trepidation. I left to begin a second postdoctoral fellowship at the National Institutes of Health, working with Gary Felsenfeld on DNA and chromatin structure. Since I entered medical school to avoid the draft, I had a military obligation that was fulfilled by my years at the NIH and was endearingly termed a “yellow beret.” Gary was great, but the NIH was alien, a government reservation with a fixed workday. As a night person, I found it strange and at some level difficult since I arrived at noon after all the parking spaces were occupied, left at midnight and accumulated an increasing number of parking tickets. In the midst of a molecular hybridization reaction, I was arrested by two FBI agents (the NIH is a federal reservation) for 100 summonses for parking violations.

As a fellow in Felsenfeld’s lab studying how chromatin serves to regulate gene expression, I formed close friendships that continue to the present. On the beach at Cold Spring Harbor, I sat with Tom Maniatis and Harold Weintraub and talked about chromosome replication and gene expression and within a few hours a bond formed, a respect for one another and for one another’s thinking, that has lasted for thirty years. Hal, unfortunately, died ten years ago of a brain tumor, but his warmth, his creativity persist.

Sol Spiegelman invited me to return to Columbia as an Assistant Professor in 1974 at the Institute of Cancer Research. I was ecstatic to occupy a lab and office adjacent to his. Sol had many visitors in those years, and when he felt bored in a meeting he would excuse himself and hide in my office where we talked science until his visitors finally gave up and left. I was studying the structure of genes in chromatin and had the good fortune of participating in a revolution made possible by recombinant DNA technology. I spent a great deal of time with Tom Maniatis, who pioneered many of the techniques in recombinant DNA. Tom left Harvard for Caltech, because he was restricted from performing recombinant DNA experiments in Cambridge, Massachusetts. We learned how to cut and paste DNA, to isolate genes, and to analyze their anatomy down to the last detail. We recognized that to understand gene control and gene function, however, required a functional assay. Within months of establishing my own laboratory in 1974, Michael Wigler, my first graduate student along with Sol Silverstein, a Professor at Columbia, developed novel procedures that allowed DNA-mediated transformation of mammalian cells. Michael, even at this very early stage in his career, was conceptually and technically masterful and within a few years he devised procedures that permitted the introduction of virtually any gene into any cell in culture. He developed a system that not only allowed for the isolation of genes, but also for detailed analysis of how they worked. We now had a facile assay to study the sequences regulating gene expression as well as gene function.

Michael went off to the Cold Spring Harbor Laboratories and, simultaneous with Bob Weinberg at MIT, identified the mutant ras gene as the gene responsible for malignant transformation in many cancer cells. My laboratory went off in many directions, first identifying the regulatory sequences responsible for control of specific gene expression. At the same time, a research fellow, Dan Littman, now a Professor at NYU, joined the lab and was interested in two molecules that characterize the major classes of T cells. Dan, along with a student, Paul Maddon, succeeded in exploiting the gene transfer to isolate these two molecules. As often in science, serendipity heightened the interest in these molecules: we demonstrated that one of these receptors, CD4, was the high-affinity receptor for HIV, allowing attachment and infection of immune cells.

This early work on recombinant DNA was a period of enormous excitement, for it led to a revolution in both thinking and technology in biology. It provided a new tool for the study of fundamental problems and spurred a new and valuable industry: biotechnology. We, who were involved at its inception, were perhaps a bit haughty, aggressive, and proud, and were accused by many of playing “God.” As evidence, the press noted that “I baptized my first child, Adam.”
Recombinant DNA aroused a good deal of passion and hostility. The notion of tinkering with life was thought to endanger life and this cry became one of the major indictments of modern biology. These experiments raised endless debate because the idea that genes can be taken out of one organism and introduced into the chromosome of another is by itself upsetting. The very notion of the performance of recombinant DNA was linked with the mysterious and supernatural. This conjured up myths that elicited intense anxiety. Recombinant DNA, it was feared, would permit biologists to alter individual species as well as the evolution of species. This controversy emphasized the fact that advances in science may indeed bring harm as well as benefit. In the case of recombinant DNA, as Francois Jacob said, “Apocalypse was predicted but nothing happened.” In fact, with recombinant DNA, only good things happened. At a practical level, the ability to construct bacteria-replicating eucaryotic genes has allowed for the production of an increasingly large number of clinically important proteins. At a conceptual level, gene cloning has permitted a detailed look at the molecular anatomy of individual genes, and from a precise analysis of these genes we have deduced the informational potential of the gene and the way in which it dictates the properties of an organism.

At a personal level, the emergence of a new discipline, biotechnology, introduced me to a world outside of academia. This important excursion showed me that brilliance is not limited to universities. I met and remain very close to two dynamic leaders of technology development, Fred Adler and Joe Pagano. Despite disparate histories, we remain very close and they continue to fascinate me with lives quite different from that of a university professor.

In 1982, I began to think about the potential impact of the new molecular biology and recombinant DNA technology on problems in neuroscience. Molecular biology was invented to solve fundamental problems in genetics at a molecular level. With the demystification of the brain, with the realization that the mind emerges from the brain and that the cells of the brain often use the very same principles of organization and function as a humble bacterium or a liver cell, perhaps molecular biology and genetics could now interface with neuroscience to approach the tenuous relationship between genes and behavior, cognition, memory, emotion, and perception. This thinking was the result of a faculty meeting at which Eric Kandel and I overcame our boredom with administration by talking science. Eric was charismatically exuberant about his recent data that revealed a correlation between a simple form of memory in the marine snail Aplysia and cellular memory at the level of a specific synapse. Molecular biologists had encountered cellular memory before in the self-perpetuating control of gene expression. This led to the realization that this was the moment to begin to apply the techniques of molecular biology to brain function and I would attempt to recruit Eric Kandel as my teacher.

A courageous new postdoctoral fellow in my laboratory, Richard Scheller, now Director of Research for Genentech, was excited about embarking on an initial effort in molecular neurobiology in a laboratory with absolutely no expertise in neuroscience. Together with Richard and Eric, we set out to isolate the genes responsible for the generation of stereotyped patterns of innate behaviors. All organisms exhibit innate behaviors that are shaped by evolution and inherited by successive generations that are largely unmodified by experience or learning. It seemed reasonable to assume that this innate behavior was dictated by genes that might be accessible to molecular cloning. It was an exciting and amusing time, with myself unfamiliar with action potentials and Kandel uncomfortable with central dogma. Richard Scheller exploited the techniques of recombinant DNA to identify a family of genes encoding a set of related neuromolecules whose coordinated release was likely to govern the fixed action pattern of behaviors associated with egg laying. A single gene, the ELH gene, specifies a polypeptide that is cut into small biologically active peptides such that individual components of the behavioral array may be mediated by peptides encoded by one gene.

Watching the story unfold, observing the interface of molecular biology and neuroscience, provided great pleasure. More importantly, this collaboration formed the basis of a continuing relationship with Eric Kandel, with his incisive mind, inimitable laugh, and boundless energy. In 1986 neuroscience for me was made even richer when Tom Jessell came along. Tom joined the faculty at Columbia and was to occupy a lab adjacent to my own. Not surprisingly, the lab was not ready and I had the great pleasure of hosting Tom in my own laboratory, and this forged a long-lasting scientific and personal relationship. Jessell, the understated British scientist with a wry wit and piercing mind, joined a fellow in my laboratory, David Julius, now at the University of California at San Francisco, and together they devised a clever assay for the isolation of genes encoding the neurotransmitter receptors. These experiments, which might have been the last performed by the hands of Jessell, led to the isolation of genes encoding the seven transmembrane domain serotonin receptor 5HT1C, and more generally provided an expression system that permitted the identification of functional genes that encode receptors in the absence of any information on the nature of the protein sequence. With Kandel one floor above, and Jessell next door, there was no departure from neuroscience. I was surrounded and I did not want to escape. I was beginning to feel that neuroscience was indeed an appropriate occupation for a molecular biologist. To quote Woody Allen, a fellow New Yorker, “The brain is my second favorite organ.”

In the late 1980s I became fascinated in the problem of perception: how the brain represents the external world. I was struck by observations from animal behavior that what an organism detects in its environment is only part of what is around it and that part can differ in different organisms. The brain functions then not by recording an exact image of the world, but by creating its own selective picture. Biological reality will therefore reflect the particular representation of the external world that a brain is able to build, and a brain builds with genes. If genes are indeed the arbiters of what we perceive from the outside world, then it follows that an understanding of the function of these genes could provide
Odor Perception

1. Introduction

The image in the painting *La Bonne Aventure* is not a nose (Figure 1). It is a portrayal by the surrealist René Magritte of his own brain’s representation of the external world. It is a

Figure 1. The painting *La Bonne Aventure* (Fortune Telling), by René Magritte (1937) portrays a monumental nose. I have added the inscription “Ceci n’est pas un nez” (“This is not a nose”) in Magritte’s script to emphasize the tension between image and reality, a conflict inherent in much of his art as well as in the science of perception.

insight into how the external world is represented in the brain. Together with Linda Buck, a creative research fellow in the lab, we began to consider how the chemosensory world is represented in the brain. The problem of olfaction was a perfect intellectual target for a molecular biologist. How we recognize the vast diversity of odorous molecules posed a fascinating problem. We assumed that the solution would involve a large family of genes and Linda Buck devised a creative approach that indeed identified the genes encoding the receptors that recognize the vast array of odorants in the environment. Linda came to me with the experimental data late one night, exuberant, and I fell uncharacteristically silent. There were 1000 odorant receptor genes in the rat genome, the largest family of genes in the chromosome, and this provided the solution to the problem of the diversity of odor recognition. More importantly, the identification of these 1000 genes and their expression revealed an early and unanticipated logic of olfaction. Indeed, the subsequent use of these genes to manipulate the genome of mice has afforded a view of how the olfactory world could be represented in the brain and how genes shape our perception of the sensory environment. From that late night moment to the present, it has been a joy to watch this story unfold.

It is this work for which Linda Buck and I share the profound honor and good fortune of having been awarded the Nobel Prize in Physiology or Medicine. But there are, deeper, more human joys, two sons, Adam and Jonathan, my sister, Linda, a very close coterie of friends, and a new love. Watching, contributing to the growth of my children is not only moving but humbling and puts my intense life in science in perspective. Often this intensity, bordering on obsession, distracted me from fathering and this is a regret. But my sons have emerged from a frenetic teenage into very human college students, extremely unlikely to pursue a career in science. My sister remains a close and dedicated member of an increasingly small family. A new love, Cori Bargmann, a behavioral geneticist now at Rockefeller University, has entered my world. Her intensity for science hides a knowledge and passion for books, music, and art. I have learned much from her, but most importantly Cori has shown me how to combine intellectual intensity with humanity and warmth.

Finally, the Nobel Prize was awarded to me not as a man, but for my work, a work of science that derives from the efforts of many brilliant students as well as from the incisive teachings of devoted colleagues. I take equal pride in the science that has been accomplished in the laboratory as in the scientists that have trained with me and are now independently contributing to our understanding of biology. I therefore feel that I can only accept the Nobel Prize in trust, as a representative of a culture of science in my laboratory and at Columbia University. I am deeply grateful for this culture.

vignette that reveals a tension between image and reality, a tension that is a persistent source of creativity in art, brought to its culmination by the surrealists. The problem of how the brain represents the external world is not only a central theme in art but is at the very core of philosophy, psychology, and neuroscience. We are interested in how the chemosensory world is represented in the brain.

All organisms have evolved a mechanism to recognize sensory information in the environment and transmit this information to the brain where it then must be processed to create an internal representation of the external world. There are many ways for organisms to probe the external world: some smell it, others listen to it, many see it. Each species therefore lives in its own unique sensory world of which other species may be partially or totally unaware. A whole series of specific devices alien to human perception have evolved: biosonar in bats, infrared detectors in snakes, electroreceptive organs in fish, and a sensitivity to magnetic fields in birds. What an organism detects in its environment is only part of what is around it and that part differs in different organisms. The brain functions, then, not by recording an exact image of the world, but by creating its own selective picture—a picture largely determined by what is important for the survival and reproduction of the species.

Sensory impressions, therefore, are apprehended through the lens of the particular perceiving brain, and the brain must therefore be endowed with an a priori potential to recognize the sensory world.10 Our perceptions are not direct recordings of the world around us, rather they are constructed internally.
according to innate rules. Colors, tones, tastes, and smells are active constructs created by our brains out of sensory experience. They do not exist as such outside of sensory experience. Biological reality, I argue, therefore reflects the particular representation of the external world that a brain is able to build, and a brain builds with genes.

If our genes are indeed the arbiters of what we perceive from the outside world, then it follows that an understanding of the function of these genes could provide insight into how the external world is represented in our brain. But what can molecular biology really tell us about so elusive a brain function as perception? Molecular biology was invented to solve fundamental problems in genetics at a molecular level. With the demystification of the brain, with the realization that the mind emerges from the brain, and that the cells of the brain often use the same very simple principles of organization and function as a humble bacterium or a liver cell, molecular biology and genetics could now interface with neuroscience to approach the previously tenuous relationship between genes and behavior, cognition, memory, emotion, and perception.

Why would a molecular neuroscientist interested in perception choose to focus on the elusive sense of smell? In humans, smell is often viewed as an aesthetic sense, as a sense capable of eliciting enduring thoughts and memories. Smell, however, is the primal sense. It is the sense that affords most organisms the ability to detect food, predators, and mates. Smell is the central sensory modality by which most organisms communicate with their environment. Second, humans are capable of recognizing hundreds of thousands of different odors. For molecular neuroscientists studying the brain, the mechanism by which an organism can interact with the vast universe of molecular structures defined as odors provides a fascinating problem in molecular recognition and perceptual discrimination. Finally, the problem of perception necessarily involves an understanding of how sensory input is ultimately translated into meaningful neural output: thoughts and behavior. In olfaction, the sensory input is extremely well defined and consists of chemicals of precise molecular structure. The character of the input in olfaction is far simpler than that of a visual image, for example, which consists of contours, texture, color, movement, and form of confounding complexity. Representation of an olfactory image is simpler and reduces to the problem of how precisely defined chemical structures are transformed in brain space.

As molecular neurobiologists, Linda Buck and I approached olfactory sensory perception by dividing it into two problems: First, what mechanisms have evolved to allow for the recognition of the vast array of molecular structures we define as odors? Clearly, there must be receptors in the sensory neurons of the nose capable of associating with odor molecules. Do we have a relatively small number of “promiscuous” receptors, each capable of interacting with a large number of odorous molecules? Alternatively, olfactory recognition may involve a very large number of “chaste” receptors each capable of interacting with a limited set of odor molecules. The second problem is conceptually more difficult: how does the olfactory sensory system discriminate among the vast array of odorous molecules that are recognized by the nose? Put simply, how does the brain know what the nose is smelling? This question will ultimately require knowledge of how the different odors are represented and encoded in the brain.

2. A Large Family of Odorant Receptor Genes

We approached the problem of odor recognition directly by isolating the genes encoding the odorant receptors. The experimental design we employed to isolate these genes was based on three assumptions: 1) the odorant receptors were likely to belong to the superfamily of receptors, the G-protein coupled receptors (GPCRs), that transduce intracellular signals by coupling to GTP-binding proteins. 2) The large repertoire of structurally distinct, odorous molecules suggests that the odorant receptors themselves must exhibit significant diversity and are therefore likely to be encoded by a multigene family. 3) The expression of the odorant receptors should be restricted to the olfactory epithelium. Experimentally, we used the polymerase chain reaction (PCR) to amplify members of the GPCR gene superfamily expressed in olfactory sensory neurons. We then investigated whether any of the PCR products were indeed members of a large multigene family. We observed that restriction-enzyme cleavage of a single PCR band generated a set of DNA fragments whose molecular weight summed to a value significantly greater than that of the original PCR product. In this manner, we identified a multigene family that encodes a large number of GPCRs whose expression is restricted to the olfactory sensory neurons. The receptors were subsequently shown to interact with odors, translating the energy of odor binding into alterations in membrane potential.

The completed sequence of both the murine and human genome ultimately identified 1300 odorant receptors in the mouse and 500 in humans. If mice possess 20000 genes, then as much as 5% of the genome (one in 20 genes) encodes the odorant receptors. A large family of odorant receptors is observed not only in vertebrates, but in the far simpler sensory systems of invertebrates. A somewhat smaller but highly diverse family of about 80 odorant receptor genes has been identified in the Drosophila genome. The invertebrate, C. elegans, with only 302 neurons and 16 olfactory sensory neurons expresses about 1000 odorant receptor genes. These experiments provide a solution to the first question: we recognize the vast array of molecular structures defined as odors by maintaining in our genome a large number of genes encoding odorant receptors.

The observation that over 1000 receptors are required to accommodate the detection of odors suggests a conceptual distinction between olfaction and other sensory systems. Color vision in humans, for example, allows the discrimination of several hundred hues with only three different photoreceptors. These photoreceptors each have distinct but overlapping absorption spectra. Discrimination of color is thought to result from comparative processing of the information from these three classes of photoreceptors. Whereas three photoreceptors can absorb light across the entire visible spectrum, our data suggest that a small number of odorant receptors cannot recognize the full spectrum of distinct
molecular structures perceived by the mammalian nose. Rather, olfactory perception requires a large number of receptors, each capable of recognizing a small number of odorous ligands.

The large number of odorant receptor genes when compared with receptor numbers in other sensory systems perhaps reflects the fact that in vision and hearing the character of the sensory stimulus is continuously variable. Color is distinguished by quantitative differences in a single parameter, the wavelength of light. Similarly, one important parameter of hearing, the frequency of sound, is continuously variable. The diversity of chemical structures of odors do not exhibit continuous variation of a single parameter and therefore cannot be accommodated by a small number of receptors. Rather, the full spectrum of distinct molecular structures perceived by the olfactory system requires a large number of receptors, each capable of interacting with a small number of specific odorous ligands.

3. A Topographic Map in the Olfactory Bulb

We next turned to the question of olfactory discrimination: how does the brain know what the nose is smelling? The identification of a large family of receptor genes allowed us to pose this question in molecular terms. We could now ask how the brain knows which of the numerous receptors have been activated by a given odor. The elucidation of a mechanism by which the brain distinguishes the different combinations of receptors activated by different odors would provide a logic of odor discrimination. This problem was further simplified by the demonstration that an individual sensory neuron expresses only one of the 1000 receptor genes.[10,24] This observation emerged from single-neuron cDNA cloning experiments, and allowed us to translate the problem of how the brain determines which receptor has been activated to a far simpler problem: how does the brain know which neuron has been activated by a given odor? As in other sensory systems, an invariant spatial pattern of olfactory sensory projections could provide a topographic map of receptor activation that defines the quality of a sensory stimulus.

In other sensory systems, spatially segregated afferent input from peripheral sensory neurons generates a topographic map that defines the location of a sensory stimulus within the environment as well as the quality of the stimulus itself. Olfactory sensory processing does not extract spatial features of the odorant stimulus. Relieved of the requirement to map the position of an olfactory stimulus in space, we asked whether the olfactory system might employ spatial segregation of sensory input to encode a quality of an odorant. Robert Vassar in my lab and Kerry Ressler in Linda Buck’s lab therefore analyzed the spatial patterns of receptor expression in the olfactory epithelium by in situ hybridization and observed that cells expressing a given receptor are dispersed. When they performed in situ hybridization experiments to the bulb, the first relay station for olfactory sensory neurons in the brain, they observed that topographic order was restored.[27,28] Neurons expressing a given receptor, although randomly distributed in the epithelium, project to spatially invariant glomeruli in the olfactory bulb, thus generating a topographic map.

Peter Mombaerts, then a research fellow in the lab, developed a genetic approach to visualize axons from olfactory sensory neurons, thereby expressing a given odorant receptor as they project to the brain.[29] We modified receptor genes by targeted mutagenesis in the germ line of mice. These genetically altered receptor genes now encoded a bicistronic mRNA that allows the translation of receptor along with tau-lacZ, a fusion of the microtubule-associated protein tau with β-galactosidase. In these mice, olfactory neurons that transcribe a given receptor also express tau-lacZ in their axons, permitting the direct visualization of the pattern of projections in the brain (Figure 2).

We observe that neurons expressing a receptor project to only two topographically fixed loci, or glomeruli, in the bulb, thus creating mirror-image maps in each bulb. Neurons expressing different receptors project to different glomeruli. The position of the individual glomeruli is topographically defined and is similar for all individuals in a species (Figure 3). Individual odors could activate a subset of receptors that would generate specific topographic patterns of activity within the olfactory bulb such that the quality of an olfactory stimulus could be encoded by spatial patterns of glomerular activity.

The identification of an anatomic olfactory sensory map poses four questions. The first addresses the singularity of receptor gene choice. What mechanism assures that a sensory neuron expresses only a single receptor and then projects with precision to one of 1000 topographically fixed glomerular loci? Second, does the anatomic map translate into a functional map such that different odors elicit different patterns of activity? Third, can we relate specific spatial patterns of glomerular activity to specific behaviors? Finally how is the map read? How does the brain look down upon a spatial pattern of activity and associate this pattern with a particular odor?

4. Receptor Choice and the Topographic Map

The topographic map in the olfactory system differs in character from the orderly representation inherent in the retinotopic, tonotopic, or somatotopic sensory maps. In these sensory systems, the peripheral receptor sheet is represented in the central nervous system (CNS), such that neighboring relations in the periphery are preserved in the CNS (for reviews, see Refs. [30,31]). In this manner, peripheral receptor cells may acquire a distinct identity that is determined by their spatial position in the receptor sheet. Spatial patterning in the periphery can therefore endow individual neurons with positional information that directs their orderly representation in the brain.

The olfactory system, however, does not exhibit an orderly representation of receptor cells in the periphery.
Neurons expressing a given receptor are randomly dispersed within a given zone and order is restored in the bulb where neurons expressing a given receptor converge on discrete loci to create a topographic map. Olfactory neurons differ from one another not by virtue of their position in a receptor sheet, but rather by the nature of the receptor they express. The tight linkage between the choice of an odorant receptor and the site of axon convergence suggests a model in which the odorant receptor is expressed on dendrites, where it recognizes odorants in the periphery, and also on axons, where it governs target selection in the bulb. In this manner, an olfactory neuron would be afforded a distinct identity that dictates the nature of the odorant to which it responds as well as the glomerular target to which its axon projects. If the odorant receptor also serves as a guidance molecule, this leads to two experimental predictions. First, the receptor should be expressed on axons as well as on dendrites, and second, genetic modifications in the receptor sequence might alter the topographic map.

Figure 2. Convergence of axons from neurons expressing a given receptor. Odorant receptor loci were modified by homologous recombination in ES cells to generate strains of mice in which cells expressing a given receptor also express a fusion of the microtubule-associated protein tau with β-galactosidase. These photographs reveal neurons expressing either the M12 (left) or P2 (right) receptors along with their axons as they course through the cribriform plate to a single locus in the olfactory bulb. Neurons expressing different receptors converge on different glomeruli. The genetic modifications that assure the coordinate expression of receptor and tau-lacZ are shown beneath the photographs.

Figure 3. A Topographic map of olfactory sensory axons in the bulb. The picture reveals neurons expressing two modified P2 alleles: P2-IRES-tau-lacZ (red) or P2-IRES-GFP (green). These neurons send axons that co-converge on the same glomerulus in the olfactory bulb. Neurons expressing other receptors converge on different glomerular loci that are shown schematically. All nuclei are stained blue with TOTO-3. The relative positions of the different glomeruli are maintained in different mice, thus revealing an invariant topographic map in the olfactory bulb.
The first prediction was tested by Gilad Barnea, who generated specific antibodies against two odorant receptors and examined the sites of receptor expression on sensory neurons. Antibodies were raised against extracellular and cytoplasmic epitopes of the mouse odorant receptors MOR28 and MOR11-4. In the sensory epithelium, we observe intense staining in the dendritic knobs, the site of odor binding. In the olfactory bulb, antibody stains axon termini whose arbors are restricted to two glomeruli (Figure 4). Antibody-staining of the bulb from mice bearing the MOR28-IRES-tau-lacZ allele reveals that the glomeruli stained by antibodies to MOR28 also receives the tau-lacZ fibers. Thus, the receptor is expressed on both dendrites and the axons of sensory neurons.

In a second series of experiments performed by my student, Fan Wang, we provided genetic evidence suggesting that the receptor on axons is indeed a guidance molecule. We modified our gene-targeting approach to ask whether substitutions of the P2 receptor coding sequence alter the projections of neurons that express this modified allele. We replaced the coding region of the P2 gene with the coding regions of several other receptors, and examined the consequences on the formation of the topographic map. Substitution of the P2 coding region with that of the P3 gene, a linked receptor gene homologous to P2 and expressed in the same epithelial zone, results in the projection of axons to a glomerulus distinct from P2 that resides immediately adjacent to the wild-type P3 glomerulus. Other substitutions that replace the P2 coding sequences with receptor sequences expressed either in different zones or from different chromosomal loci also result in the convergence of fibers to glomeruli distinct from P2. These observations, along with recent experiments involving more extensive genetic modifications, provide support for the suggestion that the olfactory receptor plays an instructive role in axon targeting as one component of the guidance process.

How may the odorant receptors participate in the guidance process? In one model, the odorant receptor is expressed on the axon termini along with other guidance receptors where it recognizes positional cues elaborated by the bulb. Each of the 1000 distinct types of sensory neuron will therefore bear a unique combination of guidance receptors that define a code dictating the selection of a unique glomerular target. Such a model does not necessarily imply that there are 1000 distinct cues, each spatially localized within the bulb. Rather, a small number of graded cues may cause the differential activation of the different odorant receptors on axon termini. In this manner, the different affinities of individual receptors for one or a small number of cues, and perhaps different levels of receptor, might govern target selection. Such a model is formally equivalent to models of retinotopy in which a gradient of guidance receptors on retinal axons is matched by a positional gradient of guidance cues in the tectum (for a review see Ref. [31]).

5. The Singular and Stable Choice of Receptor

If the odorant receptor defines the functional identity of a sensory neuron and also determines the site of projection in
the brain, then the expression of a single receptor gene in a neuron is an essential feature in models of olfactory perception. This immediately poses the question as to what mechanism has evolved to assure the expression of a single receptor gene from the family of 1000 genes in the chromosome. One model for the control of olfactory receptor expression invokes the existence of 1000 different sensory neurons, each expressing a unique combination of regulatory factors that governs the choice of a different olfactory receptor gene. This deterministic model predicts that all olfactory receptor genes will contain different cis-regulatory sequences that are recognized by unique sets of transcription factors. An alternative, stochastic model of receptor gene selection suggests that all odorant receptor genes within a zone contain the same cis-regulatory information and are controlled by the same set of transcription factors. In this model, a special mechanism must exist to assure that only one receptor gene is chosen. Moreover, once a specific receptor is chosen for expression, this transcriptional choice must be stable for the life of the cell, because receptor switching after stable synapse formation would seriously perturb odor discrimination.

A series of transgene experiments performed by Ben Shykind in my own laboratory, as well as by other researchers, provide evidence for a mechanism of receptor choice that is stochastic. We have generated mice in which the endogenous P2 allele has been replaced with the P2-IRES-tau-lacZ allele. We have also introduced a randomly integrated P2-IRES-GFP transgene into the chromosome of this strain. In a deterministic model, we predict that a unique combination of transcription factors would activate both the endogenous and transgenic P2 alleles such that cells that express lacZ from the endogenous P2-IRES-tau-lacZ allele should also express GFP from the P2 transgene. Examination of the sensory epithelium in these mice, however, reveals a singularity of P2 expression. Cells that express the endogenous P2 allele never express the transgene. In a conceptually similar experiment, we generated transgenic mice that harbor an integrated array of multiple P2 transgenes that include P2-IRES-tau-lacZ and P2-IRES-GFP linked at the same chromosomal locus. In these strains, we also observe a singularity of transgene expression. Neurons that express the P2-IRES-tau-lacZ transgene do not express the linked P2-IRES-GFP gene. Taken together, these experiments provide support for a model in which receptor choice is not deterministic, rather it is stochastic.

Once a single receptor gene is chosen for expression, this transcriptional choice must be stable for the life of the cell because receptor switching after stable synapse formation would seriously perturb odor discrimination. In recent experiments, Ben Shykind in my lab along with the research groups of Randall Reed and Hitoshi Sakano devised genetic strategies that permit the analysis of the stability of receptor choice. We have employed a lineage tracer to map the fate of sensory neurons that express either an intact or a nonfunctional deletion of the MOR28 gene. Mature neurons that express an intact MOR28 receptor, but have not yet formed stable synapses in the brain, can switch receptor expression, albeit at low frequency. Thus, we observe that switching is an inherent property of wild-type receptor gene choice. Neurons that choose to express a mutant MOR28 receptor subsequently extinguish its expression and switch at

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**Figure 5.** A feedback model assuring the stable expression of a functional receptor. A) The transcriptional machinery (blue sphere) expresses only one of 1000 odorant receptor genes (in this case, R2). R2 encodes a functional receptor that elicits a feedback signal that leads to the stabilization of receptor choice (purple sphere). B) If the transcriptional machinery chooses the nonfunctional receptor R1, which is not competent to mediate feedback stabilization, switching occurs. The transcriptional machine is then free to select a second receptor for expression that will ultimately mediate feedback stabilization. This model provides a mechanism to assure that a neuron expresses a functional odorant receptor.
high frequencies to express alternate receptors such that a
given neuron stably transcribes only a single receptor gene. 
These observations suggest a mechanism of olfactory receptor 
gene choice in which a cell selects only one receptor allele but 
can switch at low frequency. Expression of a functional 
receptor would then elicit a signal that suppresses switching 
and stabilizes odorant receptor expression. Neurons that 
initially express a mutant receptor fail to receive this signal 
and switch genes until a functional receptor is chosen
(Figure 5).

The mouse genome contains 340 olfactory receptor 
pseudogenes, whereas the human genome contains 550 
pseudogenes, several of which continue to be transcribed.\[12,16\]
Expression of a pseudogene would result in the generation of 
sensory neurons incapable of odor recognition. A mechanism 
that allows switching provides a solution to the pseudogene 
problem such that if pseudogenes are chosen, another tran-
scriptional opportunity is provided, thus assuring that each 
neuron expresses a functional receptor. This model of serial 
monogamy assures that neurons will express a single receptor 
throughout their life. This feedback model in which expres-
sion of a functional odorant receptor suppresses switching to 
other olfactory receptor genes is reminiscent of one mecha-
nism of allelic exclusion in T and B lymphocytes.

6. Cloning a Mouse from an Olfactory Sensory 
Neuron

What mechanism assures that a single receptor gene is 
chosen stochastically in a sensory neuron? One model 
invokes DNA recombination of odorant receptor genes at a 
single active expression site in the chromosome. DNA 
recombination provides Saccharomyces cerevisiae\[41\] trypa-
nosomes,\[42\] and lymphocytes\[43\] with a mechanism to stochas-
tically express one member of a set of genes that mediate 
cellular interactions with the environment. One attractive 
feature shared by gene rearrangements in trypanosomes and 
lymphocytes is that gene choice is a random event, a feature 
of receptor gene selection in olfactory sensory neurons. 
However, efforts to demonstrate a recombination event 
involving receptor genes have been seriously hampered by 
the inability to obtain populations of neurons or clonal cell 
lines that express the same receptor. Kristin Baldwin in my 
laboratory, in collaboration with Rudy Jaenisch, Kevin 
Eggan, and Andy Chess at MIT, addressed this problem by 
generating ES cell lines and cloned mice derived from the 
nuclei of olfactory sensory neurons expressing the P2 receptor 
(Figure 6).\[44\] The generation of cloned mice from cells of the 
nose derives from an initial insight of Woody Allen in his 1978 
futuristic comedy, Sleeper. In this film, efforts are made to 
resurrect a totalitarian leader by cloning from his only 
surviving body part, his nose. Twenty-five years later, science 
successfully imitated art with the generation of mice cloned 
from a single sensory neuron from the nose.

We would predict that if DNA recombination accompa-
nies receptor gene choice, then the olfactory epithelium from 
cloned mice derived from a sensory neuron expressing the 
P2 gene should be clonal with respect to receptor expression,
such that all cells transcribe the rearranged P2 allele. Analysis 
of the sequence and organization of the DNA surrounding the 
P2 allele expressed in cloned mice revealed no evidence for 
either gene conversion or local transposition at the P2 locus. 
In addition, the pattern of receptor gene expression in the 
sensory epithelium of cloned mice was normal. Multiple 
odorant receptor genes are expressed without preference for 
the P2 allele transcribed in the donor nucleus (Figure 6). 
These data, along with similar experiments by Peter Mombaerts,\[45\] demonstrate that the mechanism responsible for 
the choice of a single odorant receptor gene does not involve 
irreversible changes in DNA. In a broader context, the 
generation of fertile cloned mice that are anatomically and 
behaviorally indistinguishable from wild-type indicates that 
the genome of a postmitotic, terminally differentiated olfac-
tory neuron can re-enter the cell cycle and be reprogrammed.
to a state of totipotency after nuclear transfer. The stochastic choice of a single olfactory receptor gene is therefore not accomplished by DNA recombination but rather by a rate-limiting transcriptional process, perhaps involving a single transcriptional machine capable of stably accommodating only one olfactory receptor gene.

7. Olfaction in the Fly: A Functional Map in the Antennal Lobe

The identification of an anatomic map in the olfactory bulb immediately poses the question as to whether this map provides a meaningful representation of odor quality that is translated into appropriate behavioral output. Recently, we have become interested in how the olfactory world is represented in the brain of the fruit fly. *Drosophila* provides an attractive system to understand the logic of olfactory perception. Fruit flies exhibit complex behaviors controlled by an olfactory system that is anatomically and genetically simpler than that of vertebrates. Genetic analysis of olfaction in *Drosophila* may therefore provide a facile system to understand the mechanistic link between behavior and the perception of odors. The recognition of odors in *Drosophila* is accomplished by sensory hairs distributed over the surface of the third antennal segment and the maxillary palp. Olfactory neurons within sensory hairs send projections to one of the multiple glomeruli within the antennal lobe of the brain. Leslie Vosshall and Allan Wong showed that most sensory neurons express only one of about 80 odorant receptor genes. Neurons expressing the same receptor project with precision to one or rarely two spatially invariant glomeruli in the antennal lobe, the anatomic equivalent of the olfactory bulb of mammals (Figure 7).

Leslie Vosshall and Allan Wong in my lab developed an isolated *Drosophila* brain preparation that is amenable to two-photon imaging and is responsive to odor stimulation for up to five hours. We expressed the calcium-sensitive fluorescent protein G-CaMP in primary olfactory sensory neurons and projection neurons. G-CaMP consists of a circularly permuted EGFP flanked at the N-terminus by the calcium-binding site of calmodulin and at the C-terminus by the M13 fragment of myosin light chain kinase. In the presence of calcium, calmodulin interacts with the M13 fragment and elicits a conformation change in EGFP. The resulting elevations in fluorescent intensity reflect changes in the intracellular calcium concentration, a presumed mirror of electrical activity. Moreover, the ability to express G-CaMP in genetically defined populations of neurons allowed us to determine with certainty the locus of neural activity. Odor-evoked changes in fluorescence intensity within the antennal lobe are monitored by a laser-scanning two-photon microscope.

This imaging technique has allowed us to measure the responsivity of 23 glomeruli to 16 different odors. A number of interesting features of the glomerular response to odors are revealed by these experiments. First, different odors elicit different patterns of glomerular activation and these patterns are conserved among different animals (Figure 8). At odor concentrations likely to be encountered in nature, the map is sparse and glomeruli are narrowly tuned. Second, the patterns of activity are insular, such that neighboring glomeruli do not necessarily respond together to a given odor. Each glomerulus visualized anatomically appears to be a functional unit. Third, the patterns of glomerular activity are qualitatively similar upon imaging either sensory or projection neurons. These observations suggest the faithful transmission of sensory input to higher brain centers. Fourth, we have coupled genetic experiments with imaging to demonstrate that the odor-evoked profile for a given glomerulus directly reflects the responsivity of an individual odorant receptor. This finding is consistent with previous molecular and anatomic studies that reveal that neurons that express only a single receptor in like axons converge on a single glomerulus. Thus these studies, along with other imaging approaches in insects, demonstrate that the anatomic map is indeed functional and suggests that each odor elicits a sparse pattern of glomerular activation that may confer a signature for different odors in the brain.

The anatomic organization in *Drosophila* is therefore remarkably similar to that of the olfactory system of mammals, suggesting that the mechanism of odor discrimination has been shared despite the 600 million years of evolution separating insects from mammals. This conservation may reflect the maintenance of an efficient solution to the complex problem of recognition and discrimination of a vast repertoire of odors in the environment. In both flies and mice, the convergence of like axons into discrete glomerular structures provides a map of receptor activation in the first relay station for olfactory information in the brain, such that the quality of an odorant may be reflected by spatial patterns of activity, first in the antennal lobe or olfactory bulb and ultimately in higher olfactory centers.

An understanding of the logic of odor perception requires functional analysis to identify odor-evoked patterns of activity in neural assemblies and ultimately the relevance of these patterns to odor discrimination. We have performed two-photon calcium imaging to examine the relationship between the anatomic map and the functional map in the antennal lobe. Jing Wang and Allan Wong in my lab developed an isolated *Drosophila* brain preparation that is amenable to two-photon imaging and is responsive to odor stimulation for up to five hours. We expressed the calcium-sensitive fluorescent protein G-CaMP in primary olfactory sensory neurons and projection neurons. G-CaMP consists of a circularly permuted EGFP flanked at the N-terminus by the calcium-binding site of calmodulin and at the C-terminus by the M13 fragment of myosin light chain kinase. In the presence of calcium, calmodulin interacts with the M13 fragment and elicits a conformation change in EGFP. The resulting elevations in fluorescent intensity reflect changes in the intracellular calcium concentration, a presumed mirror of electrical activity. Moreover, the ability to express G-CaMP in genetically defined populations of neurons allowed us to determine with certainty the locus of neural activity. Odor-evoked changes in fluorescence intensity within the antennal lobe are monitored by a laser-scanning two-photon microscope.

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Imaging experiments in vertebrates similarly reveal a functional representation of the anatomic map.\[56–58\]

8. Spatial Representations and Innate Behavior

All animals exhibit innate behaviors in response to specific sensory stimuli that are likely to result from the activation of developmentally programmed circuits. Allan Wong and Jing Wang in my lab, in collaboration with Greg Suh, David Anderson, and Seymour Benzer at Caltech, asked whether we can relate patterns of glomerular activity elicited by an odor to a specific behavior.\[59\] Some time ago Benzer observed that *Drosophila* exhibits robust avoidance to odors released by stressed flies. Gas chromatography and mass spectrometry identified one component of this “*Drosophila* stress odorant (DSO)” as CO\(_2\). Exposure of flies to CO\(_2\) alone also elicits an avoidance behavior at levels of CO\(_2\) as low as 0.1 % (Figure 9).

We therefore performed imaging experiments with the calcium-sensitive fluorescent indicator G-CaMP and two-photon microscopy to ask whether we could discern a pattern of glomerular activity in response to DSO and CO\(_2\). We first examined flies in which the G-CaMP indicator is driven in all neurons by the pan-neural activator Elav-Gal4. DSO activates only two glomeruli, DM2 and the Vglomerulus, whereas CO\(_2\) activates only the Vglomerulus. Activation of the Vglomerulus was detected at CO\(_2\) levels as low as 0.05 % and this glomerulus was not activated by any of 26 other odorants tested (Figure 9).

We demonstrated that axonal projections to the Vglomerulus originate from sensory neurons expressing the receptor GR21A.\[50\] We therefore performed calcium imaging with flies in which the UAS G-CaMP reporter was driven by a GR21A promoter Gal4 activator. CO\(_2\), as well as DSO, activated GR21A sensory termini in the Vglomerulus. We next asked whether the GR21A sensory neurons are necessary for the avoidance response to CO\(_2\). Inhibition of synaptic trans-

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**Figure 8.** Different odors elicit different patterns of glomerular activation that are conserved among different organisms. Two different flies (top and bottom panels) bearing the GH146-Gal4 and UAS-G-CaMP transgenes were exposed to three odors. Glomerular responses reveal different patterns of activity for the different odors that are conserved in different animals. The pre-stimulation images (left) shows the glomerular structure and the images on the right show the specific glomeruli schematically.

**Figure 9.** CO\(_2\) activates a single glomerulus and elicits avoidance behavior. A) Avoidance of air from stressed flies (CS) as well as of increasing concentrations of CO\(_2\). Inhibition of synaptic transmission in GR21A neurons that project to the Vglomerulus using shi\(^*\) blocks CO\(_2\) avoidance. Red and blue bars indicate avoidance behavior at the nonpermissive (28 °C) and permissive (21 °C) temperatures, respectively. B) Two-photon imaging in a strain harboring GR21A-Gal4 and UAS G-CaMP reveals robust activation of the Vglomerulus.
mission in the GR21A sensory neurons that innervate the Vglomerulus, using the temperature-sensitive shibire gene shi
t blocks the avoidance response to CO₂ (Figure 9). Inhibition of synaptic release in the vast majority of other olfactory sensory neurons or in projection neurons other than those that innervate the Vglomerulus had no effect on this behavior.

The identification of a population of olfactory sensory neurons innervating a single glomerulus that mediates robust avoidance to a naturally occurring odorant provides insight in the neural circuitry that underlies this innate behavior. These observations suggest that a dedicated circuit that involves a single population of olfactory sensory neurons mediates detection of CO₂ in Drosophila. The simplicity of this initial olfactory processing offers the possibility of tracing the circuits that translate odor detection into an avoidance response.

9. How is the Map Read?

Our experiments indicate that different odors elicit different patterns of glomerular activity within the antennal lobe and moreover that defined patterns of activity can be associated with specific behaviors. We can look at the pattern of activity in the fly antennal lobe with a two-photon microscope and discern, with a reasonable degree of accuracy, what odorant the fly has encountered in nature. Thus, we can determine with our eyes (and our brain) what odors the fly has encountered, but how does the fly brain read the sensory map?

A topographic map in which different odors elicit different patterns of activity in the antennal lobe suggests that these spatial patterns reflect a code defining odor quality. However, the mere existence of a map, whether anatomic or functional, does not prove that spatial information is the underlying parameter of an odor code. It has been suggested, for example, that the quality of an odor is reflected in temporal dynamics of a distributed ensemble of projection neurons. In this model, a given odor might activate a small number of glomeruli and a large ensemble of projection neurons such that different odors elicit different temporal patterns of activity in the same projection neuron. This temporal hypothesis in its simplest form postulates that the brain exploits circuit dynamics to create spatiotemporal patterns of neuronal activation to achieve a larger coding space. Whatever the code, patterns of activity in the antennal lobe must be translated by higher sensory centers to allow the discrimination of complex olfactory information. If odor quality is encoded by spatial patterns, we might expect that a representation of the glomerular map is retained in the protocerebrum.

We have begun to address the question of how the map in the antennal lobe is represented in higher olfactory centers by examining the pattern of projections of the neurons that connect the glomeruli to the protocerebrum. Allan Wong and Jing Wang randomly labeled individual projection neurons to visualize their processes that connect defined glomeruli with their targets in the mushroom body and protocerebrum. We have used an enhancer trap line in which Gal4 is expressed in a subpopulation of projection neurons along with the “FLP-out” technique to label single projection neurons with a CD8 GFP reporter. A similar experimental approach has been used to determine the lineage relationship of individual projection neurons and to examine their pattern of axonal projections. We observe that most projection neurons send dendrites to a single glomerulus. Projection neurons that receive input from a given glomerulus extend axons that form a spatially invariant pattern in the protocerebrum (Figure 10).

Projection neurons from different glomeruli exhibit patterns of axonal projections that are distinct, but often interdigitated (Figure 11). Our data reveal a striking invariance in the spatial patterns of axon arbors of projection neurons that
innervate a given glomerulus, a precision of connectivity that assures the specificity of information transfer.

The precision of projections of projection neurons reveals a spatial representation of glomerular activity in higher brain centers, but the character of the map differs from that observed in the antennal lobe. Axon arbors in the protocerebrum are diffuse and extensive, often extending over the entire dimension of the brain hemisphere (Figures 10, 11). This is in sharp contrast to the tight convergence of primary sensory axons, whose arbors are restricted to a small 5–10 μm spherical glomerulus. As a consequence, the projections from different glomeruli, although spatially distinct, often interdigitate. Thus, the point-to-point segregation observed in the antennal lobe is degraded in the second order projections to the protocerebrum. This affords an opportunity for the convergence of inputs from multiple different glomeruli essential for higher order processing. Third-order neurons in the protocerebrum might synapse on projection neurons from multiple distinct glomeruli, a necessary step in decoding the spatial representation of glomerular activity in higher brain centers, but the character of the map differs from that observed in the antennal lobe. Axon arbors in the protocerebrum might synapse on projection neurons from multiple distinct glomeruli, a necessary step in decoding the spatial patterns to allow the discrimination of odor and behavioral responses.

10. Concluding Remarks

These data suggest a model in which the convergence of information from deconstructed patterns in the antennal lobe are reconstructed by “cardinal cell assemblies” that sit higher up in a hierarchical perceptual system in the protocerebrum. Olfactory processing will initially require that the structural elements of an odor activate a unique set of receptors that in turn result in the activation of a unique set of glomeruli. The odorous stimuli must then be reconstructed in higher sensory centers that determine which of the numerous glomeruli have been activated. The identification of a spatially invariant sensory map in the protocerebrum that is dispersive affords an opportunity for integration of multiple glomerular inputs by higher odor neurons.

The elucidation of an olfactory map in both the olfactory bulb or antennal lobe and in higher olfactory centers leaves us with a different order of problems. Though we may look at these odor-evoked images with our brains and recognize a spatial pattern as unique and can readily associate the pattern with a particular stimulus, the brain does not have eyes. How does the brain perceive the olfactory image? How is the map read? How are spatially defined bits of electrical information in the brain decoded to allow the perception of an olfactory image? We are left with an old problem, the problem of the ghost in the machine.

Finally, how do we explain the individuality of olfactory perception? The innately configured representation of the sensory world, the olfactory sensory maps that I have described, must be plastic. Our genes create only a substrate upon which experience can shape how we perceive the external world. Surely the smell of a madeleine does not elicit in all of us that “vast structure of recollection” it evoked for Marcel Proust. For Proust, smell is the evocative sense, the sense that brings forth memory and associations with a richness not elicited by other sensory stimuli. Nowhere is this more apparent than in the eloquent words recalling the madeleine incident from “Remembrance of Things Past”.[6]

“... But when from a long distant past nothing subsists, after the people are dead, after the things are broken and scattered, still alone, more fragile but with more vitality, more unsubstantial, more persistent, more faithful, the smell and taste of things remain, poised a long time, like souls ready to remind us, waiting and hoping for their moment, amid the ruins of all the rest; and bear unfaltering in the tiny and impalpable drop of their essence, the vast structure of recollection.”

This lecture encompasses the efforts in my laboratory over the past 13 years to provide further insight into the molecular logic of olfactory sensory perception. I wish to thank the Howard Hughes Medical Institute, the National Institutes of Health, and the Mathers Foundation for their continued gracious support of our research. The Howard Hughes Medical Institute provided an opportunity to interface molecular biology with neuroscience and has consistently encouraged and supported the efforts of the laboratory in novel directions. It is this work for which Linda Buck and I share the profound honor and good fortune of having been awarded the Nobel Prize in Physiology or Medicine. This award was not made to me as a man but for my work, a science that derives from the efforts of many brilliant students and from the incisive teachings of my colleagues. I take equal pride in the science that has been accomplished in the laboratory and in the scientists that have trained with me and have contributed to our efforts. I therefore feel that I accept this prize in trust as a representative of a culture of science in my laboratory and at Columbia University, I am deeply grateful for this culture. Over the past 30 years, Columbia has provided an atmosphere that fosters intellectual rigor and creativity and at the same time is imbued with a spirit of warmth and collaboration.

Received: May 19, 2005


