A Nobel for Smell

Essay

David Julius[1],* and Lawrence C. Katz[2],*
[1]Department of Cellular
  and Molecular Pharmacology
University of California, San Francisco
San Francisco, California 94143
[2]Howard Hughes Medical Institute
  and Department of Neurobiology
Duke University Medical Center
Durham, North Carolina 27710

> We have a belief, founded on long-continued, personal observation, that there is more in the Nose than most owners of that appendage are generally aware. We believe that, besides being an ornament to the face, or a convenient handle to grasp an impudent fellow, it is an important index to its owner's character….It will not be contended that all the faculties and properties of the mind are revealed by the Nose….it rather reveals Power and Taste— Power or Energy to carry out ideas, and the Taste or Inclination which dictates or guides them.

> *Nasology, or Hints Towards a Classification of Noses*
> Eden Warwick, 1848

Humans share with most other animals an extraordinary ability to perceive and discriminate among thousands of different odorants representing a vast range of chemical space and configuration. The olfactory systems of humans and animals exhibit not only impressive discriminatory abilities but also an astonishing sensitivity to certain volatile molecules that exceeds even the best man-made devices (Buck, 2000). The specificity and sensitivity of this sense have fascinated scientists and laymen alike and driven neurobiologists to unlock the secrets of the molecular machinery enabling these capacities. This year's Nobel Prize in Physiology or Medicine, awarded to Linda Buck and Richard Axel, recognizes the discovery of the vertebrate odorant receptors (Buck and Axel, 1991), the keys that opened the door to understanding the molecular logic and organization of the olfactory system.

Some of the mysteries surrounding the process of olfaction resembled those pertaining to humoral immunity and the means by which antibodies recognize a seemingly endless variety of antigens, even those of subtly different shape or composition. Would olfactory sensory neurons (the primary receptor cells lining the inside of the nose) require a large number of different odorant receptors to enable fine distinctions? If so, would these receptors be encoded by distinct genes or, as in the case of the immune system, arise through some form of somatic or germline recombination or conversion? If olfactory neurons used a limited repertoire of receptors, how could the brain synthesize and interpret the resulting patterns of activity? At least one thing seemed certain—answers to these questions would not

*Correspondence: julius@cmp.ucsf.edu (D.J.), katz@neuro.duke.edu (L.C.K.)

emerge without a more detailed view of the molecular nature of odorant receptors themselves. Indeed, as the Nobel citation makes clear, Buck and Axel not only solved the mystery of the olfactory receptors but, in a remarkable series of subsequent experiments, outlined the basic organizational principles of the vertebrate olfactory system. More generally, the discovery of the olfactory receptors and the resulting body of work from the Buck and Axel labs as well as a number of others have provided a general framework for addressing one of the outstanding fundamental questions in neuroscience: how is the external world represented in the internal workings of the brain?

Humans are highly visual animals, so it is perhaps not surprising that so much effort has been devoted to understanding this system. Three Nobel prizes have been awarded over the past 90 years for fundamental advances in deciphering vision. Biochemical methods, innovations in the microelectrode, and new neuroanatomical techniques together outlined the transduction machinery involved in the detection of light and the neural pathways responsible for the perception and discrimination of shapes, colors, and motion. Insights into the detailed molecular underpinnings of vision followed, crowned by the cloning of G protein-coupled opsins and the elucidation of genetic mechanisms responsible for color vision and blindness (Nathans, 1987). For audition as well, our basic understanding of how sound waves are transduced into electrical signals, and how those signals are first ordered in the brain, emerged from sophisticated electrophysiological and biophysical measurements (Hudspeth, 1997). In contrast, olfaction is the first sensory system whose basic organizing principles were largely revealed by molecular approaches.

The power of chemistry and biochemistry, used so elegantly by George Wald and colleagues to solve the problem of phototransduction, was of little practical value in defining the front end machinery of olfactory transduction: the paucity of sensory neurons severely limited probing receptor composition by conventional pharmacological or biochemical purification schemes. Similarly, the basic tools of systems neurobiology —the microelectrode and anatomical tracers—that revealed the functional architecture of the mammalian visual system had limited utility when confronted with the baffling complexity of the olfactory system. Hubel and Wiesel's recordings from individual neurons in visual cortex yielded an elegant, orderly progression of neurons responding to lines of different orientations, and injections of tracers into the eye beautifully revealed the exquisite pattern of eye-specific ocular dominance columns in the visual cortex (Hubel and Wiesel, 1977). But conceptually similar approaches yielded conflicting models of the functional architecture of the olfactory epithelium and olfactory bulb and could not rigorously distinguish between several plausible models for how the first stages of olfactory processing were organized (Kauer, 1991; Shepherd and Firestein, 1991). In retrospect it is abundantly clear why these classical biochemical, electrophysiological, or anatomical techniques alone, no mat-

ter how deftly applied, could not decipher the underlying logic of olfaction.

These stumbling blocks humbled all approaches for identifying odorant receptors, save those relying on genetics. Classical work in olfaction (Amoore, 1967) had identified an intriguing array of "anosmias" in which individual humans were unable to perceive a specific odor. Thus, one promising avenue was to exploit forward genetic screens in model organisms, such as flies or worms, with the aim of identifying mutants exhibiting similar anosmias for specific chemo-attractants or repellents. The power of this approach rests with its ability to identify components of a signal transduction pathway without having to make assumptions about the nature of mechanisms or molecules involved. However, in the end the secrets of olfaction would be gleaned not from forward genetic screens, but from a reverse genetic analysis of the mammalian olfactory epithelium based on a specific model of how odorant detection works.

Physiological and biochemical experiments performed in the mid-1980s by Lancet, Snyder, Nakamura, and Gold suggested that odors excited olfactory sensory neurons by stimulating adenylyl cyclase and activating a cyclic nucleotide-gated ion channel (Nakamura and Gold, 1987; Pace et al., 1985; Sklar et al., 1986). This hypothesis was significantly strengthened by molecular studies of Reed and colleagues, who cloned genes encoding an olfactory-specific G_s protein and a cyclic AMP-gated channel (Dhallan et al., 1990; Jones and Reed, 1989). Together, these findings set the stage for a directed reverse genetic approach to identify what was presumed to be a family of G protein-coupled odorant receptors.

Buck and Axel's quest was facilitated by the timely emergence of powerful technology, in this case the demonstration that the polymerase chain reaction, when used in conjunction with degenerate oligonucleotide primers, could amplify DNA sequences encoding proteins of related structure, such as multiple members of a gene family. By the time they began their search for the odorant receptors, the sequences of several G protein-coupled receptors (GPCRs), such as the visual opsins, had been determined, providing an expanding template from which to design sets of degenerate PCR primers. Indeed Vassart and colleagues had already used this methodology to identify several new members of the G protein-coupled receptor superfamily (Libert et al., 1989).

Diligence, trial and error with degenerate primers, and one profoundly elegant and insightful trick made it all gel. Because all eukaryotic cells express GPCRs engaged in a wide range of cellular functions, many primer pairs would be expected to amplify sequences corresponding to receptors irrelevant to odorant detection. However, if one assumed that the olfactory epithelium expressed a relatively large and novel repertoire of GPCRs, then the relevant PCR product might appear as a uniform band on a gel, but it would actually consist of several related but distinct species. If so, then restriction digestion of these PCR products would reveal a ladder of bands whose sizes would sum to something significantly greater than that of the uncleaved material. This assumption allowed Buck to successfully identify a collection of PCR products representing the first five of what would turn out to be hundreds of novel, olfactory sensory neuron-specific receptors. Thus, what in retrospect seems like a simple component of the screen was the stroke of experimental brilliance that would lay the molecular logic of the nose before our eyes.

Indeed, the landmark paper cited by the Nobel committee (Buck and Axel, 1991) is still impressive in its scope and prescience. Based on a limited data set—18 sequenced putative receptors—but a remarkable string of logical analyses, Buck and Axel inferred that the size of the family must be very large (they were within a factor of 3, even with crude initial estimates). Even more impressively, they deduced the presence of multiple subfamilies (now numbering in the hundreds), predicted that each olfactory sensory neuron must select one or at most a few ORs to express from that large repertoire (and proposed mechanisms for how this might be accomplished), and articulated an explicit model for the first stages of sensory coding in the olfactory bulb.

A Revolution in Sensory Biology

The approach pioneered by Buck and Axel to uncover the ORs ushered in a new era in understanding a range of chemosensory systems. Perhaps most dramatic was the subsequent discovery (independently, by both Buck and Axel labs) of the pheromone receptors in the accessory olfactory system. This system is perhaps even more enigmatic than olfaction, as its peripheral sensory organ (the vomeronasal organ) appears to be absent in humans and generates percepts inaccessible to our own conscious experience. However, the discovery of a large and diverse collection of vomeronasal receptors (VRs) provides a unique opportunity to closely link molecular signaling pathways to innate behaviors in other mammals. In an impressive demonstration of this link, both the Axel and Dulac labs selectively silenced the vomeronasal organ by knocking out a transduction channel unique to these sensory neurons; male mice carrying this mutation appear to lose the ability to distinguish males from females and avidly attempt to copulate with other males. Moreover, Mombaerts and colleagues showed that genetic deletion of clusters of the VR genes lead to substantial decrements in maternal defensive behaviors. And the discovery that the sensory transduction channel in this system has become a pseudogene in humans, presumably rendering this ancient system nonfunctional, argues that over the course of recent evolution we (and our close primate cousins) have genuinely lost something on our way to becoming highly visual animals (Dulac and Torello, 2003; Mombaerts, 2004).

The significance of the discovery of these large families of GPCRs is magnified by the fact that the number of genes is so large—between one and three percent of the genome in various mammals (and, incredibly, in *C. elegans* as well). One could argue that the discovery of olfactory receptors would have been as significant had there been far fewer, but the contrast with the three receptors that subserve color vision implies a fundamental difference in coding strategy. It is unlikely that such a large gene family would be maintained if chemical cues of importance to rodents could be efficiently encoded by a substantially smaller number of receptors.

Interestingly, along with the loss of the pheromone receptors, roughly two-thirds of olfactory receptors genes have become pseudogenes during the course of human evolution—coincident with the emergence of trichromatic vision and our upright gait, which removes our nose from contact with the rich palette of odorants near the ground. Thus, formation and maintenance of this repertoire are likely to be determined by ethological niches, as well as by behavioral changes. Perhaps the de-emphasis of odorant perception in humans is related to enhanced social interactions such as individual recognition, which has largely become the province of our visual system.

In the retina, the absorbance spectra of various types of cones was predicted by psychophysics and by the various forms of color blindness. Nathan's elegant and groundbreaking analysis of the opsin genes related these observations to their core molecular mechanisms by demonstrating that the cloned receptors, when expressed in heterologous cells, could recapitulate the spectral characteristics of color vision. This has been problematic in the olfactory world because cloned odorant receptors are not readily expressed in cellular environments other than the olfactory sensory neuron, although a very recent finding by Matsunami and colleagues suggests that this obstacle may now be partly overcome (Saito et al., 2004). Thus, a comprehensive "de-orphaning" of olfactory receptors has not yet been achieved, limiting the analysis of ligand-receptor specificity or the use of pharmacological reagents for manipulating receptor function in vitro or in vivo. Despite heroic efforts over the past decade by several investigators, we currently have plausible ligands for only about 1% of the receptors.

Nonetheless, the fact that each olfactory sensory neuron expresses one and only one of the 1000 possible receptors has facilitated functional analyses of olfactory receptors in their native environment and provided initial insights into both ligand specificity and coding at the level of the olfactory periphery. Buck and her collaborators have utilized a combination of calcium imaging and single-cell PCR to generate two important insights: a given odorant receptor can recognize multiple odorants, and a single odorant can be recognized by multiple odorant receptors (Malnic et al., 1999). These observations suggest a combinatorial coding strategy whereby a given odorant activates a particular constellation of receptors and by extension generates action potentials in a particular subset of sensory neurons, producing a signature response at the level of the olfactory epithelium. Subtle alterations in odorant structure or even concentration can alter this constellation, consistent with the known contributions of these parameters to odor perception. Efficient heterologous expression of odorant receptors will obviously greatly expedite the process of matching ligands to receptors and elucidating the link between specific percepts and the activation of the olfactory epithelium.

Olfaction and Perception
In situ hybridization with probes directed against individual olfactory receptors demonstrated not only that each olfactory neuron expresses mRNA encoding a single receptor, but also that sufficient mRNA is present in the axon terminals of these neurons to visualize their projection to the olfactory bulb. Both the Buck and Axel labs independently observed that the mRNA for each receptor labels one or a few individual glomeruli in the bulb and that the positions of these glomeruli are similar among individuals (Ressler et al., 1994; Vassar et al., 1994). This implies that the axons of olfactory sensory neurons bearing the same receptor converge on a single glomerulus. Taking advantage of the fact that each olfactory sensory neuron expresses just a single receptor, Mombaerts and Axel adapted a powerful genetic tracing technique (pioneered by Thomas and colleagues in *Drosophila* [Callahan and Thomas, 1994]) to elegantly and convincingly demonstrate this convergence (Mombaerts et al., 1996). Perhaps even more astounding is the observation that establishment of specific connections to the bulb actually requires the production of receptor protein (Feinstein et al., 2004). Signal transduction via the cyclic AMP pathway does not seem to be required, as normal targeting is observed in mice whose olfactory sensory neurons lack downstream effectors, such as cyclic nucleotide-gated ion channels (Belluscio et al., 2002; Brunet et al., 1996). Understanding how ORs contribute to axonal guidance and identifying the structural elements of ORs that influence this process are topics of ongoing investigation.

The axons of tens of thousands of sensory neurons converge in each glomerulus onto the apical dendrites of perhaps 50 mitral cells, the principal neuron of the bulb carrying output to the rest of the brain. While the apical dendrites of mitral cells receive strong excitatory information from just one glomerulus, their extensive basal dendrites receive inhibitory input from a large and spatially dispersed set of inhibitory interneurons. Thus the proximal olfactory system is apparently designed to faithfully convey the activation of specific groups of receptor neurons to more central structures, while at the same time providing a potential substrate for interactions between different regions of the bulb. Are olfactory perceptions closely related to the initial transduction of odorants in the sensory periphery, or are they largely constructed by internal brain circuitry? At one extreme, an olfactory percept could result from the activation of just a single receptor population and its associated glomeruli. For example, the aroma of caramel can reportedly be recapitulated by a single molecular entity—maple furanone—at a threshold of 1 part in 10^{15}. Such extraordinary sensitivity may reflect high-affinity binding of a ligand to a unique receptor, with the logical extension that activation of a single receptor subtype could function as a "labeled line" for an odor percept. However, our entire range of olfactory experience cannot result from activation of just one set of receptors for each percept. Humans can distinguish far more than 300 odors (the estimated number of human olfactory receptors), and observations of Buck and colleagues described above also suggest that a combinatorial coding strategy is likely to pertain to mammalian olfaction. Accordingly, coactivation of cohorts of receptors, in defined combinations, could give rise to a far richer olfactory repertoire, as activation of each class of receptor could contribute partially to a range of olfactory percepts. Thus the smell of popcorn could result from the

coincident activation of a set of glomeruli innervated by olfactory sensory neurons sensitive to a palette of volatile molecules present in this mixture. The coincident activation of sensory neurons in the periphery, followed by the coordinated activation of the associated group of mitral cells might result in "binding," or signifying to the brain that this group of odorants belongs together. A different but related odor—say cotton candy—would result in activation of a different but partly overlapping set of glomeruli, conveying a distinct percept. In this way the problem of recognizing and distinguishing olfactory "objects" is not so very different from the problem faced by the visual system in recognizing and distinguishing visual objects that share some attributes but not others.

The question of how odor coding takes place, however, is by no means solved and is currently an area of vigorous debate and experimentation. Laurent and colleagues have articulated both theoretical and experimental arguments questioning the idea of an "identity code" based on combinatorial activation of receptors and their associated mitral cells. In this contrasting view of early olfactory coding, the interplay between receptors and the network of local excitatory and inhibitory connections constructs a distributed representation in which the synchronized firing of a substantial population of principal neurons is used to identify and discriminate odors (Laurent, 2002). Although the evidence contributing to this debate originally derived from very different experimental systems—mice on one hand, locusts on the other—the experimental focus has recently turned to *Drosophila*. The basic molecular and neuronal architecture of the *Drosophila* olfactory system is remarkably analogous to that of vertebrates but with the added power of a genetically tractable system. With the ability to use a wide range of experimental techniques—ranging from single-neuron electrophysiology to genetically encoded activity indicators and anatomical markers, genetic silencing, and activation of circuits—the fly may provide the resolution of these conflicting ideas. Ultimately, the ability to predictably manipulate behavior will provide the key test to any theory. Recently, for example, activation of a single receptor responsive to a unique odorant—carbon dioxide—was shown to be both necessary and sufficient to elicit avoidance behavior in flies, even when all local circuitry was blocked (Suh et al., 2004). Although it remains to be determined whether this represents a unique example of a highly specialized "labeled line," it does convincingly demonstrate that at least some odorants (and not only those involved in reproduction) are represented by the activation of a distinct, not distributed circuit. How the brain constructs and interprets these different patterns of output remains one of the enduring mysteries of both vision and olfaction. However, with the unique ability in the olfactory system to genetically manipulate the olfactory receptors—the basic "building blocks" of olfactory objects—understanding how olfactory perception takes place may inform us more generally about how the brain constructs sensory percepts.

A Gene Expression Puzzle

We now know that the logic of olfactory coding rests on the one neuron-one receptor rule; for not only does an olfactory neuron select just one among several hundred receptors for expression, but it also executes a process of allelic exclusion such that only the maternal or paternal gene is transcribed (Chess et al., 1994). And it does so despite the fact that the genome contains several dozen OR gene clusters scattered over many different chromosomes. Thus one of the most fascinating questions to emerge from the cloning of olfactory receptor genes concerns the processes whereby such a unique identity can be established by gene regulatory mechanisms. In lymphocytes a similar phenomenon of mutually exclusive, monoallelic expression controls the production of antigen receptor proteins through a mechanism involving irreversible rearrangements of genomic DNA. However, gene recombination or conversion events that produce irreversible rearrangements are unlikely to account for selective expression of olfactory receptor genes since mice cloned from a single mature olfactory sensory neuron express the normal repertoire of ORs (Eggan et al., 2004; Li et al., 2004). How then does an olfactory neuron come to express one and only one receptor gene in a monoallelic fashion?

One plausible explanation is suggested by the mechanism whereby mutually exclusive expression of red or green opsin genes occurs in cone cells of the retina. This process is mediated by an interaction between two *cis*-acting regulatory elements on the chromosome, namely, a locus control region and the promoter for the red or green pigment genes (reviewed in Serizawa et al. [2004]). This stochastic physical interaction results in the random expression of either the red or green visual pigment in a given cone cell. Recent studies from Sakano's group suggest that a similar mechanism governs monoallelic OR gene expression in the nose (Serizawa et al., 2003). If juxtaposition of a locus control region and a promoter occurs through a noncovalent process (such as DNA looping or bending), then an olfactory neuron could potentially reverse its choice and switch expression to another OR gene. Indeed, recent experiments from the Axel lab suggest that this can occur in immature sensory neurons that have not yet established axonal connections to glomeruli in the olfactory bulb (Shykind et al., 2004). However, the one neuron–one receptor rule would only have functional relevance with regard to coding if switching were to eventually cease before connections to the CNS are established. Indeed, this seems to be the case, but what regulates this stabilization of receptor choice? Recent studies from the Reed and Sakano labs suggest that this occurs through a negative feedback mechanism that is activated only once functional OR proteins are expressed (Lewcock and Reed, 2004; Serizawa et al., 2003). This implies that ORs generate a signal to suppress gene switching, but the nature of this inhibitory signal is currently unknown. Moreover, models involving negative selection have not been entirely ruled out. Clearly, there is still much to learn about this process, including the identification of specific *cis*- and *trans*-acting factors that participate in this fascinating regulatory mechanism.

The Future

The discovery of the olfactory receptors and their representation in the olfactory bulb opens the door to a great

many unanswered questions, including the properties of olfactory receptors themselves, the developmental patterning of olfactory sensory neuron central projections, and the mechanisms regulating the ongoing turnover of these neurons in adult life. Perhaps the greatest challenges lie, however, in understanding how the initial representations of odors are represented and integrated in the regions of olfactory cortex outside the olfactory bulb. The piriform cortex represents a substantial portion of the rodent's brain, yet its functional organization is almost completely enigmatic. Although genetic tracing experiments by Buck (Zou et al., 2001) have suggested that the output from the bulb is not uniformly represented in the cortex, we still have little insight into how the bulbar connections to the cortex are organized. At the level of individual neurons, cells in the cortex are even more selective than those in the bulb, but precisely what information they integrate, and how, remains unknown. Addressing these questions will almost certainly require an integration of molecular tracing techniques (it is unlikely that conventional neuroanatomy alone will be able to disentangle this complexity) with new tools to map the correspondence between anatomical connectivity and neuronal activity. An even greater challenge and opportunity will be to understand the relationship between connectivity patterns and olfactory-mediated behaviors. Do mammals, for example, like flies and worms, have distinct pathways that mediate attraction to certain odorants and repulsion from others? Are there separate pathways for particular behavioral repertoires, such as social recognition?

Finally, an enduring mystery relates to the intimate relationship between olfaction and memory. As Proust so evocatively describes, certain odors trigger recollections of a particular time, place, or person:

But when from a long-distant past nothing subsists, after the people are dead…taste and smell alone…remain poised a long time, like souls, remembering, waiting, hoping…and bearing unflinchingly the vast structure of recollection.

Remembrance of Things Past
Marcel Proust

In animals, even a single exposure to particular odors, such as those of a mate or of offspring, can create a persistent imprint of such odors. The olfactory system enjoys rich connectivity with the hippocampus and limbic systems, and dense innervation by neuromodulatory systems, but we know little about how and where this "vast structure of recollection" is created and maintained. Numerous natural behaviors in mice rely on the formation of long-term memories, providing an opportunity to reveal the distributed circuitry involved in instantiating particularly robust forms of memory such as olfactory imprinting.

The Nature of Scientific Inquiry
In an era in which we have seen the steady encroachment of numerical and statistical accounting procedures as proxies for evaluating scientific contributions at all levels of careers, the Buck and Axel paper serves as a bracing counterexample. We are increasingly inundated with the idea that science is a team sport, requiring the integration of disparate skills to make important new discoveries. The dual authorship of the Buck and Axel paper shows that great discoveries have at their core the genius and motivation of exceptional individuals with the confidence, courage, and conviction to venture into uncharted territory.

In the face of all the pressures to produce short-term, tangible evidence of ongoing accomplishments, a laboratory environment that genuinely encourages the "high-risk, high payoff" approach to scientific discovery remains a rarity. Those of us fortunate to be part of the Axel group in the early days of molecular neurobiology recall an atmosphere of intellectual excitement mixed with an element of creative chaos, one in which we were given the freedom and support (intellectual, philosophical, and financial) to take leaps of faith and develop innovative approaches to cloning and characterizing genes of significance to nervous and immune system function. Those of us outside the lab have marveled at the incredible string of outstanding individuals and discoveries that have flowed from this philosophy. In some circles this might be called "the vision thing," but this year's Nobel prize clearly shows how rewards go to those who follow their nose.

References

Amoore, J.E. (1967). Specific anosmia: a clue to the olfactory code. Nature *214*, 1095–1098.

Belluscio, L., Lodovichi, C., Feinstein, P., Mombaerts, P., and Katz, L.C. (2002). Odorant receptors instruct functional circuitry in the mouse olfactory bulb. Nature *419*, 296–300.

Brunet, L.J., Gold, G.H., and Ngai, J. (1996). General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. Neuron *17*, 681–693.

Buck, L.B. (2000). The molecular architecture of odor and pheromone sensing in mammals. Cell *100*, 611–618.

Buck, L., and Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell *65*, 175–187.

Callahan, C.A., and Thomas, J.B. (1994). Tau-beta-galactosidase, an axon-targeted fusion protein. Proc. Natl. Acad. Sci. USA *91*, 5972–5976.

Chess, A., Simon, I., Cedar, H., and Axel, R. (1994). Allelic inactivation regulates olfactory receptor gene expression. Cell *78*, 823–834.

Dhallan, R.S., Yau, K.W., Schrader, K.A., and Reed, R.R. (1990). Primary structure and functional expression of a cyclic nucleotide-activated channel from olfactory neurons. Nature *347*, 184–187.

Dulac, C., and Torello, A.T. (2003). Molecular detection of pheromone signals in mammals: from genes to behaviour. Nat. Rev. Neurosci. *4*, 551–562.

Eggan, K., Baldwin, K., Tackett, M., Osborne, J., Gogos, J., Chess, A., Axel, R., and Jaenisch, R. (2004). Mice cloned from olfactory sensory neurons. Nature *428*, 44–49.

Feinstein, P., Bozza, T., Rodriguez, I., Vassalli, A., and Mombaerts, P. (2004). Axon guidance of mouse olfactory sensory neurons by odorant receptors and the beta2 adrenergic receptor. Cell *117*, 833–846.

Hubel, D.H., and Wiesel, T.N. (1977). Ferrier lecture. Functional architecture of macaque monkey visual cortex. Proc. R. Soc. Lond. B. Biol. Sci. *198*, 1–59.

Hudspeth, A.J. (1997). How hearing happens. Neuron *19*, 947–950.

Jones, D.T., and Reed, R.R. (1989). Golf: an olfactory neuron spe-

cific-G protein involved in odorant signal transduction. Science *244*, 790–795.

Kauer, J.S. (1991). Contributions of topography and parallel processing to odor coding in the vertebrate olfactory pathway. Trends Neurosci. *14*, 79–85.

Laurent, G. (2002). Olfactory network dynamics and the coding of multidimensional signals. Nat. Rev. Neurosci. *3*, 884–895.

Lewcock, J.W., and Reed, R.R. (2004). A feedback mechanism regulates monoallelic odorant receptor expression. Proc. Natl. Acad. Sci. USA *101*, 1069–1074.

Li, J., Ishii, T., Feinstein, P., and Mombaerts, P. (2004). Odorant receptor gene choice is reset by nuclear transfer from mouse olfactory sensory neurons. Nature *428*, 393–399.

Libert, F., Parmentier, M., Lefort, A., Dinsart, C., Van Sande, J., Maenhaut, C., Simons, M.J., Dumont, J.E., and Vassart, G. (1989). Selective amplification and cloning of four new members of the G protein-coupled receptor family. Science *244*, 569–572.

Malnic, B., Hirono, J., Sato, T., and Buck, L.B. (1999). Combinatorial receptor codes for odors. Cell *96*, 713–723.

Mombaerts, P. (2004). Genes and ligands for odorant, vomeronasal and taste receptors. Nat. Rev. Neurosci. *5*, 263–278.

Mombaerts, P., Wang, F., Dulac, C., Chao, S.K., Nemes, A., Mendelsohn, M., Edmondson, J., and Axel, R. (1996). Visualizing an olfactory sensory map. Cell *87*, 675–686.

Nakamura, T., and Gold, G.H. (1987). A cyclic nucleotide-gated conductance in olfactory receptor cilia. Nature *325*, 442–444.

Nathans, J. (1987). Molecular biology of visual pigments. Annu. Rev. Neurosci. *10*, 163–194.

Pace, U., Hanski, E., Salomon, Y., and Lancet, D. (1985). Odorant-sensitive adenylate cyclase may mediate olfactory reception. Nature *316*, 255–258.

Ressler, K.J., Sullivan, S.L., and Buck, L.B. (1994). Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. Cell *79*, 1245–1255.

Saito, H., Kubota, M., Roberts, R.W., Chi, Q., and Matsunami, H. (2004). RTP family members induce functional expression of mammalian odorant receptors. Cell *119*, 679–691.

Serizawa, S., Miyamichi, K., Nakatani, H., Suzuki, M., Saito, M., Yoshihara, Y., and Sakano, H. (2003). Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. Science *302*, 2088–2094.

Serizawa, S., Miyamichi, K., and Sakano, H. (2004). One neuron-one receptor rule in the mouse olfactory system. Trends Genet. *20*, 648–653.

Shepherd, G.M., and Firestein, S. (1991). Making scents of olfactory transduction. Curr. Biol. *1*, 204–206.

Shykind, B.M., Rohani, S.C., O'Donnell, S., Nemes, A., Mendelsohn, M., Sun, Y., Axel, R., and Barnea, G. (2004). Gene switching and the stability of odorant receptor gene choice. Cell *117*, 801–815.

Sklar, P.B., Anholt, R.R., and Snyder, S.H. (1986). The odorant-sensitive adenylate cyclase of olfactory receptor cells. Differential stimulation by distinct classes of odorants. J. Biol. Chem. *261*, 15538–15543.

Suh, G.S., Wong, A.M., Hergarden, A.C., Wang, J.W., Simon, A.F., Benzer, S., Axel, R., and Anderson, D.J. (2004). A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila. Nature *431*, 854–859.

Vassar, R., Chao, S.K., Sitcheran, R., Nunez, J.M., Vosshall, L.B., and Axel, R. (1994). Topographic organization of sensory projections to the olfactory bulb. Cell *79*, 981–991.

Zou, Z., Horowitz, L.F., Montmayeur, J.P., Snapper, S., and Buck, L.B. (2001). Genetic tracing reveals a stereotyped sensory map in the olfactory cortex. Nature *414*, 173–179.