

SCENTS AND SENSIBILITY: A MOLECULAR LOGIC OF OLFACTORY PERCEPTION

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by

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INTRODUCTION

The image in the painting *La Bonne Aventure* is not a nose (Fig. 1). It is a portrayal by the surrealist René Magritte of his own brain's representation of the external world. It is a 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: bio-sonar in bats, infrared detectors in snakes, electrosensitive 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 (1). Our perceptions are not direct recordings of the world around us, rather they are constructed internally according to innate rules. Colors, tones, tastes, smells are active constructs created by our brains out of sensory experience. They do not exist as such outside of sensory experience (2). Biological reality, I argue, therefore

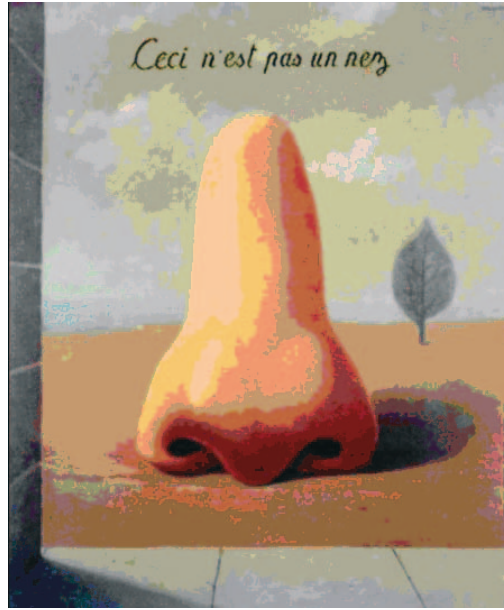


Figure 1. *La Bonne Aventure*. The painting *La Bonne Aventure* (Fortune Telling), by René Magritte (1937) portrays a monumental nose. I have added the inscription “Ceci n’est pas un nez” (This is not a nose) in Magritte’s script to emphasize the tension between image and reality, a conflict inherent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

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 very same 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 contour, 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 odorants? 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.

A LARGE FAMILY OF ODORANT RECEPTOR GENES

We approached the problem of odor recognition directly by isolating the genes encoding the odorant receptors (3). The experimental design we employed to isolate these genes was based on three assumptions: First, 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 (4,5,6,7). Second, 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. Third, 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 asked 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 (3). 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 (8,9,10,11).

The completed sequence of both the murine and human genome ultimately identified 1300 odorant receptors in the mouse (12,13) and 500 in humans (14,15,16). If mice possess 20,000 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 (17,18,19,50,67). The invertebrate, *C. elegans*, with only 302 neurons and 16 olfactory sensory neurons expresses about 1000 odorant receptor genes (20,21). These experiments provide a solution to the first question; we recognize the vast array of molecular structures defined as odorants 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 (22,23). 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.

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

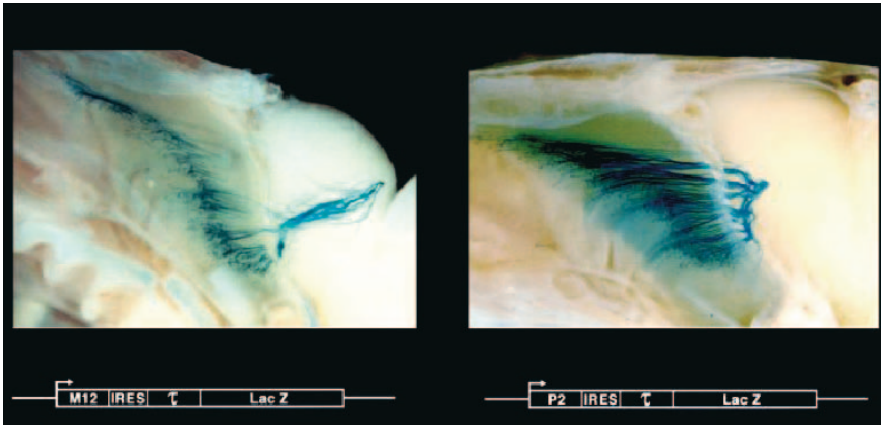


Figure 2. Convergence of Axons from Neurons Expressing a Given Receptor. Odorant receptor loci were modified by homologous recombination in ES cells to generate strains of mice in which cells expressing a given receptor also express a fusion of the microtubule associated protein, tau, with β -galactosidase. These whole mount 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 whole mount view. Reprinted from *Cell*, Vol 87, 1996, pp 675-686, Mombaerts *et al.*, with permission from Elsevier.

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 restricted to one of four broad but circumscribed zones (25,26).

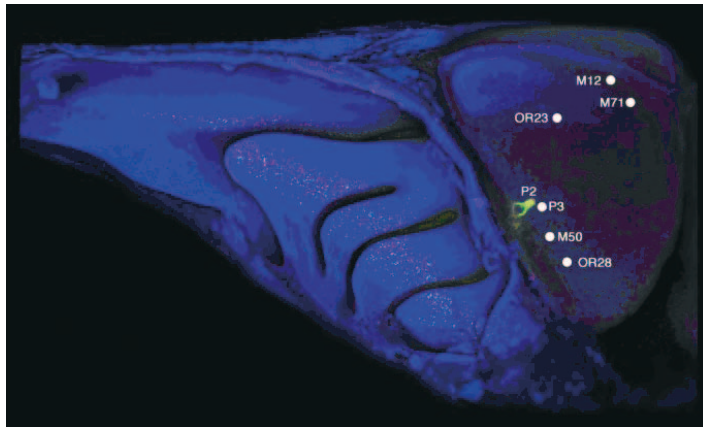


Figure 3. A Topographic Map of Olfactory Sensory Axons in the Bulb. A whole mount reveals neurons expressing two modified P2 alleles: P2-IRES-tau-lacZ (red) or P2-IRES-GFP (green). These neurons send axons that co-converge on the same glomerulus in the olfactory bulb. Neurons expressing other receptors 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 revealing an invariant topographic map in the olfactory bulb.

The overriding feature of this organization, however, is that within a zone neurons expressing a given receptor are not topographically segregated, rather they appear randomly 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 generating a topographic map.

Peter Mombaerts, then a fellow in the lab, developed a genetic approach to visualize axons from olfactory sensory neurons 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 encode 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 (Fig. 2).

We observe that neurons expressing a receptor project to only two topographically-fixed loci, or glomeruli, in the bulb 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 (Fig. 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 of with a particular odor?

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 neighbor relations in the periphery are preserved in the CNS (reviewed in 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.

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 (32). 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 den-

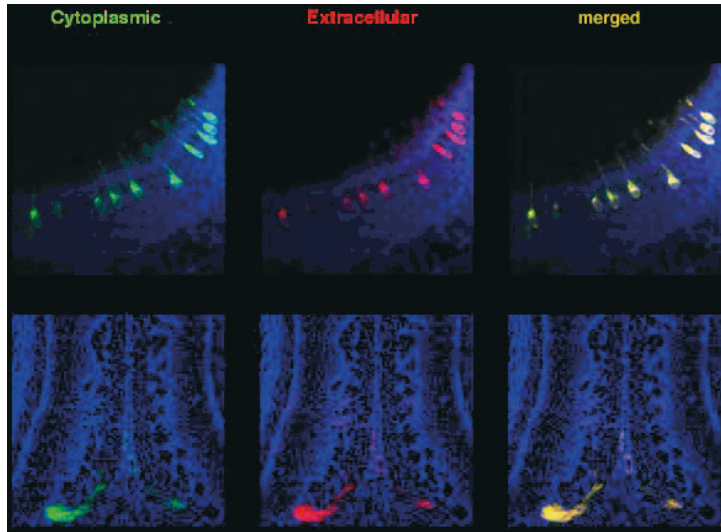


Figure 4. Odorant Receptor is Expressed on both Dendrites and Axons of Olfactory Sensory Neurons. The mouse sensory epithelium (upper panel) or olfactory bulb (lower panel) was stained with antibody to either an extracellular or cytoplasmic epitope of the MOR28 receptor. These experiments reveal the expression of odorant receptor in the cell body and dendrites in the epithelium as well as on axon termini within a defined glomerulus in the bulb. Antibody staining in the olfactory bulb coincides with the site of convergence of MOR28 axons. Adapted with permission from 32. Reprinted from *Science* 304,1468, 2004, with permission from *Science*.

dritic knobs, the site of odor binding. In the olfactory bulb, antibody stains axon termini whose arbors are restricted to two glomeruli (Fig. 4). Antibody staining of the bulb from mice bearing the MOR28-IRES-tau-lacZ allele reveals that the glomeruli stained by antibody 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 a 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 (33). 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 conver-

gence of fibers to glomeruli distinct from P2. These observations, along with recent experiments involving more extensive genetic modifications (34,35) provide support for the suggestion that the olfactory receptor plays an instructive role in axon targeting as one component of the guidance process.

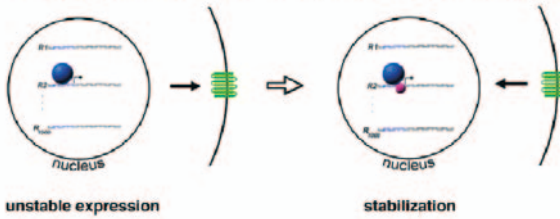
How may the odorant receptors participate in the guidance process? In one model, the odorant receptor is expressed on the axon termini along with other guidance receptors where it recognizes positional cues elaborated by the bulb. Each of the 1000 distinct types of sensory neuron will therefore bear 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 receptor on retinal axons is matched by a positional gradient of guidance cues in the tectum (reviewed in 31).

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 (OR) expression invokes the existence of 1000 different sensory neurons, each expressing a unique combination of regulatory factors that governs the choice of a different OR gene. This deterministic model predicts that all OR 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 in other labs, provide evidence for a mechanism of receptor choice that is stochastic (36,37). 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 endoge-

A. Choice of functional receptor leads to feedback stabilization:



B. Choice of non-functional receptor leads to switching:

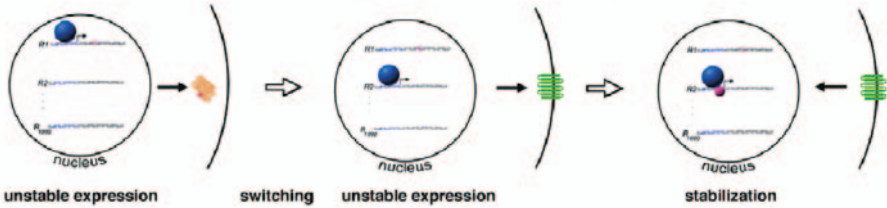


Figure 5. A Feedback Model Assuring the Stable Expression of a Functional Receptor. (A) The transcriptional machinery represented by a blue sphere expresses only one of 1000 odorant receptor genes (in this instance, R2). R2 encodes a functional receptor that elicits a feedback signal that leads to the stabilization of receptor choice (symbolized by a red sphere). (B) If the transcriptional machinery chooses the non-functional 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.

nous 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 Reed and Sakano labs devised genetic strategies that permit the analysis of the stability of receptor choice (38,39,40). 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 high frequencies to express alternate receptors such that a given neuron stably transcribes only a single receptor gene. These observations suggest a mechanism of OR 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 (Fig. 5).

The mouse genome contains 340 OR 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 transcriptional opportunity is provided 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 expression of a functional odorant receptor suppresses switching to other OR genes is reminiscent of one mechanism of allelic exclusion in T and B lymphocytes.

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), trypanosomes (42) and lymphocytes (43) with a mechanism to stochastically 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 OR 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 a 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 (Fig. 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

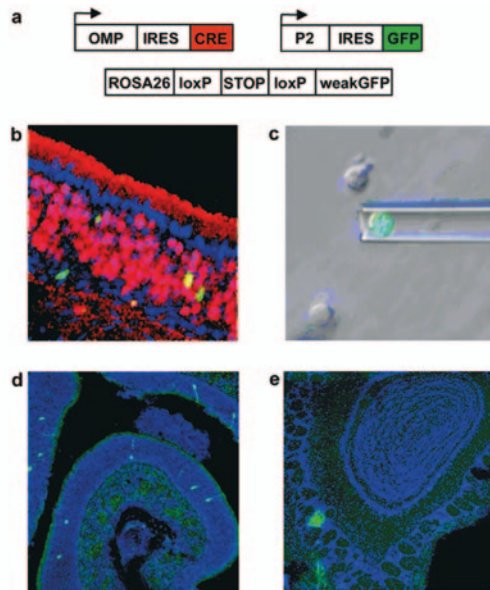


Figure 6. Cloning a Mouse from Olfactory Sensory Neurons Expressing the P2 Odorant Receptor. (a) A genetic strategy to label P2-expressing sensory neurons with GFP as well as to mark olfactory sensory neurons by virtue of a unique deletion in DNA. (b) The olfactory epithelium of a mouse with the genetic modifications described above. A single nucleus expressing the P2 odorant receptor gene was picked and introduced into an enucleated oocyte. The epithelium was stained with antibody to Cre recombinase (red) to mark sensory neurons and GFP (green) to identify P2-expressing cells. (c) A green neuron expressing P2-IRES-GFP was picked from dissociated olfactory epithelium of donor animals. (d) The olfactory epithelium from a mouse cloned from a nucleus expressing the P2 receptor shows the normal distribution of P2-expressing cells. Axons from these neurons converge on a single glomerulus in the olfactory bulb (e). All nuclei are stained with TOTO-3 blue. The observation that mice cloned from a nucleus expressing the P2 receptor gene do not preferentially express this gene in the sensory epithelium suggests that DNA recombination events do not accompany receptor gene choice. Adapted with permission from 44. Reprinted from *Nature* 428, 44-49, 2004, Eggen *et al.*, with permission from *Nature*.

generation of mice cloned from a single sensory neuron from the nose.

We would predict that if DNA recombination accompanies 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 (Fig. 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 olfactory neuron can re-enter the cell cycle and be reprogrammed to a state of totipotency after nuclear transfer. The stochastic choice of a single OR 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 OR gene.

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 (46,47). 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 (48,49,50) (Fig. 7).

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 (51). Jing Wang and Allan Wong in my lab developed an isolated *Drosophila* brain preparation that is

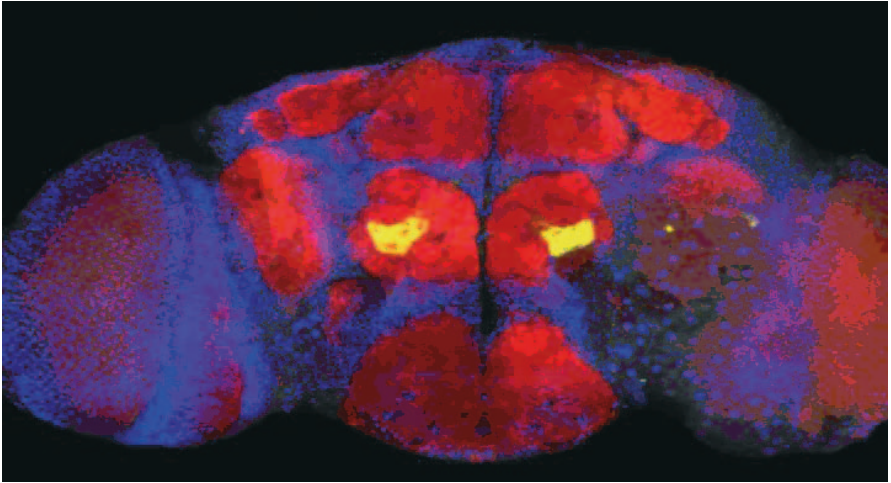


Figure 7. An Olfactory Sensory Map in the Fly Antennal Lobe. Neurons expressing the odorant receptor, OR47b, also express the transgene, synaptobrevin GFP, revealing convergence on a single spatially invariant glomerulus that is bilaterally symmetric in the antennal lobe.

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 (52). In the presence of calcium, calmodulin interacts with the M13 fragment eliciting 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 (53).

This imaging technique has allowed us to measure the responsivity of 23 glomeruli to 16 different odors (51). 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 (Fig. 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

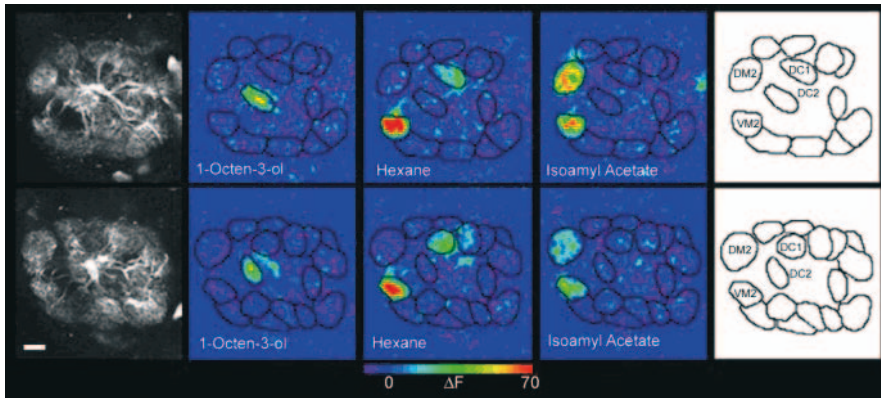


Figure 8. Different Odors Elicit Different Patterns of Glomerular Activation that are Conserved Among Different Organisms. Two different flies (upper and lower panels) bearing the GH146-Gal4 and UAS-G-CaMP transgenes were exposed to three odors. Glomerular responses reveal different patterns of activity for the different odors that are conserved in different animals. The panels to the left show the pre-stimulation images that reveal glomerular structure and the panels to the right identify the specific glomeruli schematically. Reprinted from *Cell*, Vol 112, 2003, pp 271-282, Wang *et al.*, with permission from Elsevier.

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 prior 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 (54,55), 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. Imaging experiments in vertebrates similarly reveal a functional representation of the anatomic map (56,57,58).

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 CO₂. Exposure of flies to CO₂ alone also elicits an avoidance behavior at levels of CO₂ as low as 0.1% (Fig. 9).

We therefore performed imaging experiments with the calcium-sensitive fluorescent indicator G-CaMP and two-photon microscopy to ask whether we

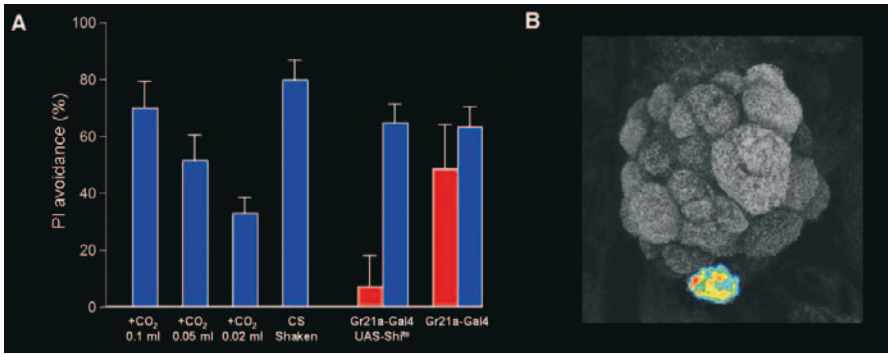


Figure 9. CO₂ Activates a Single Glomerulus and Elicits Avoidance Behavior. (A) Avoidance of air from stressed flies (CS) as well as of increasing concentrations of CO₂. Inhibition of synaptic transmission in GR21A neurons that project to the V glomerulus using *shi^{ts}* blocks CO₂ avoidance. Red and 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 V glomerulus.

could discern a pattern of glomerular activity in response to DSO and CO₂. 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 V glomerulus, whereas CO₂ activates only the V glomerulus. Activation of the V glomerulus was detected at CO₂ levels as low as 0.05% and this glomerulus was not activated by any of 26 other odorants tested (Fig. 9).

We demonstrated that axonal projections to V 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. CO₂, as well as DSO activated GR21A sensory termini in the V glomeruli. We next asked whether the GR21A sensory neurons are necessary for the avoidance response to CO₂. Inhibition of synaptic transmission in the GR21A sensory neurons that innervate the V glomerulus, using a temperature-sensitive *shibire* gene, *shi^{ts}* (60), blocks the avoidance response to CO₂ (Fig. 9). Inhibition of synaptic release in the vast majority of other olfactory sensory neurons or in projection neurons other than those that innervate the V glomerulus, 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.

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 with our eyes and our brain determine 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 (61,62). In this model, a given odor might activate a small number of glomeruli and a large ensemble of projection neurons (PNs) such that different odors elicit different temporal patterns of activity in the same PN. 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 (63). A similar experimental approach has been used to determine the lineage relationship of individual PNs and to examine their pattern of axonal projections (64,65). We observe that most PNs 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 (Fig. 10). PNs from different glomeruli exhibit patterns of axonal projections that are distinct, but often interdigitated (Fig. 11). Our data reveal a striking invariance in the spatial patterns of axon arbors of PNs that innervate a given glomerulus, a precision of connectivity that assures the specificity of information transfer.

The precision of projections of PNs 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

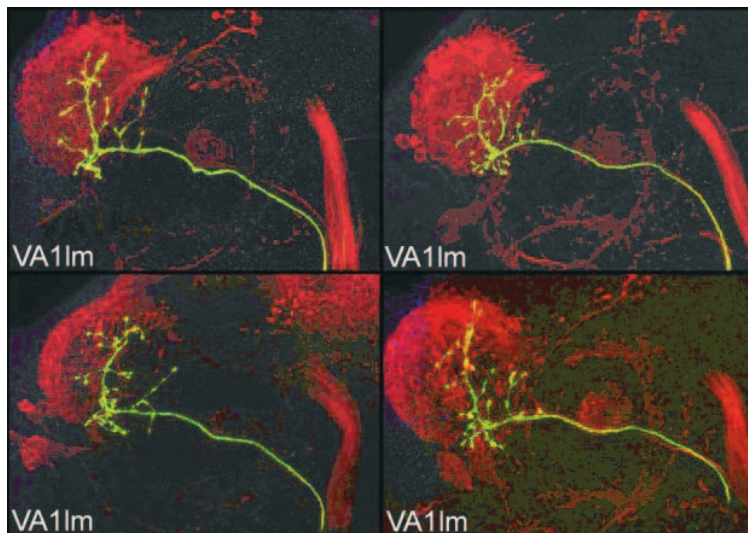


Figure 10. Projection Neurons that Innervate to the Same Glomerulus have Similar Axonal Projection Patterns. Individual projection neurons that connect to the VA1 LM glomeruli are visualized in the protocerebrum in different flies. These images reveal a striking constancy in the projection pattern among PNs that project to a given glomerulus. These observations reveal an invariant topographic map in the protocerebrum that differs in character from the map in the antennal lobe (with permission from 63). Reprinted from *Cell*, Vol 109, 2002, pp 229-241, Wong *et al.*, with permission from Elsevier.

diffuse and extensive, often extending the entire dimension of the brain hemisphere (Fig. 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 PNs from multiple distinct glomeruli, a necessary step in decoding spatial patterns to allow the discrimination of odor and behavioral responses.

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

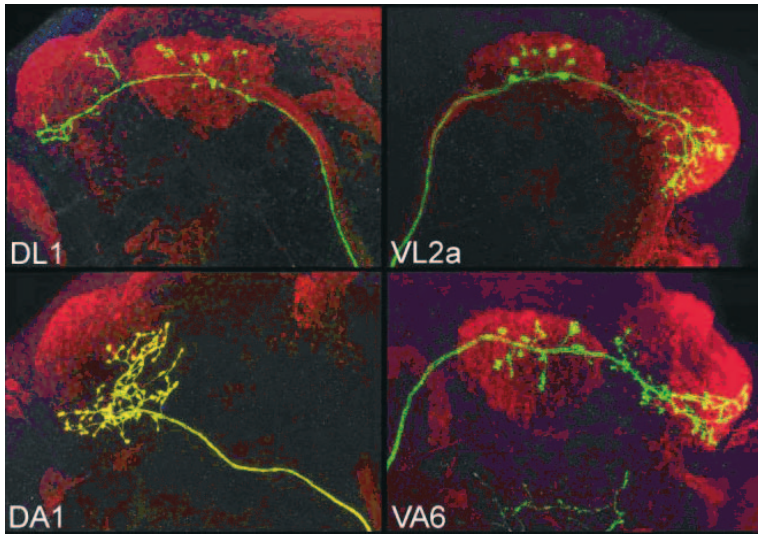


Figure 11. Axonal Patterns from Projection Neurons that Innervate to Different Glomeruli are Distinct. Axonal projections from single PNs can be visualized as they branch in the mushroom body and ultimately arborize in the protocerebrum. Projections neurons that connect to different glomeruli exhibit different patterns of axonal projections. The axon arbors in the protocerebrum are dispersed unlike the insular segregated arbors in the glomerulus, affording the possibility for integration in higher olfactory centers (with permission from 63). Reprinted from *Cell*, Vol 109, 2002, pp 229-241, Wong *et al.*, with permission from Elsevier.

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. Who in the brain is looking at the olfactory image? Who reads the map? 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*” (66).

“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.”

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This lecture encompasses the efforts in my laboratory over the past thirteen 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. 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 thirty 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.

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