

# Topographic Organization of Sensory Projections to the Olfactory Bulb

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## Summary

**The detection of odorant receptor mRNAs within the axon terminals of sensory neurons has permitted us to ask whether neurons expressing a given receptor project their axons to common glomeruli within the olfactory bulb. In situ hybridization with five different receptor probes demonstrates that axons from neurons expressing a given receptor converge on one, or at most, a few glomeruli within the olfactory bulb. Moreover, the position of specific glomeruli is bilaterally symmetric and is constant in different individuals within a species. These data support a model in which exposure to a given odorant may result in the stimulation of a spatially restricted set of glomeruli, such that the individual odorants would be associated with specific topographic patterns of activity within the olfactory bulb.**

## Introduction

In vertebrate sensory systems, peripheral neurons receive information from the environment and transmit this information to the brain, where it is processed to provide an internal representation of the external world. Mammals possess an olfactory system of enormous discriminatory power. Humans, for example, are thought to be capable of distinguishing among thousands of discrete odors. This diversity of odor recognition is coupled with remarkable specificity, such that subtle alterations in the molecular structure of an odor can often lead to profound changes in perceived odor quality.

The initial step in olfactory discrimination requires the interaction of odorous ligands with a family of seven transmembrane domain receptors on olfactory sensory neurons. The repertoire of mammalian olfactory receptors is extremely large and consists of about 1000 different genes (Buck and Axel, 1991; Levy et al., 1991; Parmentier et al., 1992; Ben-Arie et al., 1994). Discrimination among odorants requires that the brain determine which of the numerous receptors have been activated. In situ hybridization with olfactory receptor genes suggests that each cell expresses only one or a small number of receptor genes, such that individual olfactory neurons are functionally distinct (Ngai et al., 1993a; Ressler et al., 1993). The problem

of distinguishing which receptor has been activated can therefore be reduced to a problem of distinguishing which neurons have been activated.

How does the brain determine which of the functionally distinct sensory neurons have been activated? In other sensory systems, defined spatial patterns of sensory neurons or their projections are used to indicate the quality of the stimulus. In the somatosensory system, for example, the different somatic sensory submodalities (touch, proprioception, nociception, and thermoreception) result from the activation of distinct sensory cells that project to specific regions of the brain via topographically segregated pathways (Mountcastle, 1957; Perl et al., 1962; Dykes et al., 1982; Berkley, 1985). The olfactory system, therefore, may also use spatial segregation of sensory input to encode the identity of the odorant stimulus.

What features of the vertebrate olfactory apparatus might form the anatomic basis for a spatial map of olfactory information? Odorant stimuli are received from the environment by receptors on olfactory sensory neurons in the olfactory epithelium (Figure 1). Each olfactory neuron projects a single unbranched axon. As the collection of axons emerge from the olfactory mucosa, they fasciculate to form the olfactory nerve. The axons of the olfactory neurons synapse with dendrites of the mitral and tufted cells in the olfactory bulb, the first relay station for olfactory signaling in the brain. The mitral and tufted cells of the olfactory bulb in turn project axons to higher cortical centers via the olfactory tract. Thus, the anatomy of the olfactory system affords the opportunity for spatial segregation of afferent sensory input at all levels from the peripheral epithelium to the olfactory cortex (reviewed by Shepherd, 1991; Ressler et al., 1994).

We have used receptor genes as molecular probes to map the position of individual sensory neurons in the epithelium as well as their projections to the olfactory bulb. In situ hybridization with specific receptor probes in fish demonstrates that neurons expressing individual receptors are randomly distributed in the epithelium (Ngai et al., 1993a). In mammals, neurons expressing a specific receptor segregate within one of four broad but circumscribed zones within the epithelium (Ressler et al., 1993; Vassar et al., 1993). However, within a given zone, neurons expressing a specific receptor appear to be randomly distributed. Despite the restriction of specific receptors to one of a small number of broad domains, the most important feature of this sort of organization is the random distribution of receptor expression within a given domain.

These observations suggest that if spatial segregation is employed to encode odor quality, neurons expressing a given receptor, although randomly distributed throughout the epithelium, must project their axons to one or a subset of spatially defined glomeruli in the olfactory bulb. Such a model is consistent with anatomic considerations indicating that the number of glomeruli, 1000–3000 in mammals (Meisami, 1979; Royet et al., 1988) and 80 in fish (Baier and Korsching, 1994), approximates the number of

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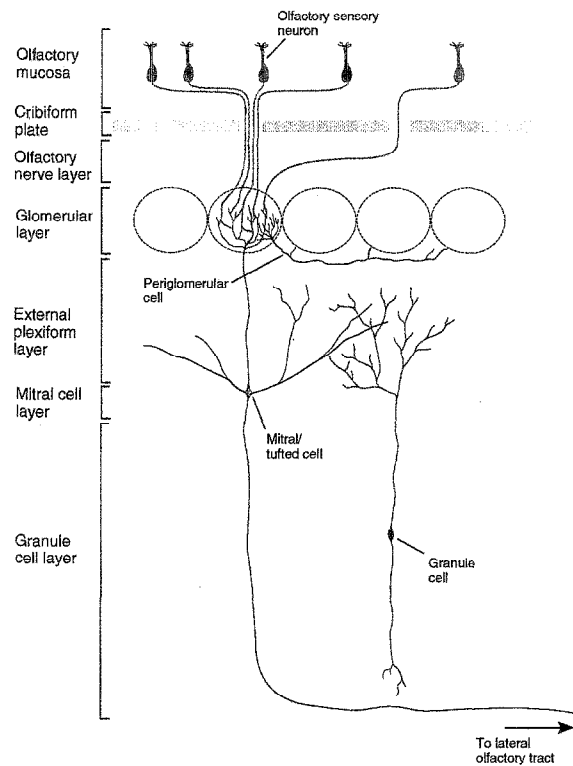


Figure 1. Cellular Organization of the Olfactory Bulb

Sensory neurons in the olfactory epithelium project axons to spherical regions of neuropil in the olfactory bulb called glomeruli. Each glomerulus represents a discrete unit of synaptic contact comprising the terminals of approximately 3000 sensory axons and the dendrites of mitral, tufted, and periglomerular cells. The principal output neurons of the bulb are the mitral and tufted cells, which project axons to the cortex via the olfactory tract. Granule and periglomerular cells are local inhibitory interneurons. (Adapted from Kandel et al., 1991.)

receptor genes in each organism (Buck and Axel, 1991; Ngai et al., 1993b). This model is in accord with previous experiments demonstrating that different odorants elicit spatially defined patterns of glomerular activity in the olfactory bulb. Optical recordings (Kauer et al., 1987), metabolic labeling (Stewart et al., 1979; Lancet et al., 1982), and electrophysiological studies (Imamura et al., 1992; Katoh et al., 1993) demonstrate that individual glomeruli are differentially responsive to distinct odors. These observations suggest that neurons responsive to a given odorant project to spatially defined glomeruli within the olfactory bulb.

In this study, we provide physical evidence that neurons expressing a given receptor project their axons to one or a small number of discrete glomeruli within the olfactory bulb. We have exploited the observation that receptor mRNA can be detected in the terminals of sensory axons in the olfactory bulb to map the projections of specific sensory neurons. We observe that neurons expressing a given receptor project their axons to one or a small number of topographically fixed glomeruli. The positions of specific glomeruli are bilaterally symmetric and are conserved in the brains of all animals within a species. These data sup-

port a model of olfactory coding in which the discrimination of odor quality would result from the detection of specific spatial patterns of glomerular activity within the olfactory bulb.

## Results

### The Establishment of a Topographic Map in the Olfactory Bulb

Experiments were designed to ask whether neurons expressing a given receptor converge upon a discrete subset of glomeruli within the olfactory bulb. Sensory neurons expressing a given receptor are randomly distributed within zones of the olfactory epithelium. To define a spatial map of the projections of the olfactory neurons within the bulb, it is necessary to identify markers that distinguish the axons of neurons expressing different receptors. The specific receptors or their respective mRNAs would provide obvious markers if they were expressed in axons. mRNA in neurons is largely localized to the cell body; occasionally, specific RNAs are observed in dendrites, but mRNA is rarely found in axonal projections (Jirikowski et al., 1990; Brunet et al., 1991; Mohr et al., 1991; Landry et al., 1992; reviewed by Steward and Banker, 1992). The mRNA encoding specific odorant receptors is abundant within the neuronal cell bodies in the sensory epithelium. Estimates from *in situ* hybridization analyses suggest that an olfactory sensory neuron expresses about 1000 copies of the mRNA for a single, specific receptor (data not shown). Each glomerulus in the bulb results from the convergence of axonal projections from about 3000 neurons (Meisami, 1979, 1989). If a receptor mRNA molecule is infrequently found at the axon terminal and neurons expressing a given receptor indeed synapse on a single glomerulus, this convergence should permit the detection of specific receptor mRNAs in individual glomeruli by *in situ* hybridization.

Control experiments were performed using the mRNA encoding the olfactory marker protein (OMP), which is expressed in all olfactory sensory neurons (Farbman and Margolis, 1980) at a level ten times higher than that of receptor mRNA (R. V. and R. A., unpublished data). Previous studies have detected OMP mRNA in the olfactory bulb by RNA blot hybridization (Rogers et al., 1987; Ehrlich et al., 1990). We therefore performed *in situ* hybridization experiments to ask whether OMP mRNA was detectable within axon terminals in the glomeruli of the olfactory bulb. Sensory axons from the olfactory epithelium enter the olfactory nerve layer of the bulb and synapse with the dendrites of the mitral and tufted cells to form discrete spherical glomeruli. The axons of the mitral and tufted cells form the major output tract of the bulb that projects to the olfactory cortex (Figures 1, 2A, and 2B).

Coronal sections through the olfactory bulb were annealed with a <sup>33</sup>P-labeled OMP probe and exposed to emulsion for 1 day. Strong hybridization signals are apparent throughout the olfactory nerve layer and in the vast majority of the glomeruli (Figure 2C). Hybridization is not observed in the mitral cell layer, nor in the granule cell layer. This pattern contrasts with *in situ* hybridization with a

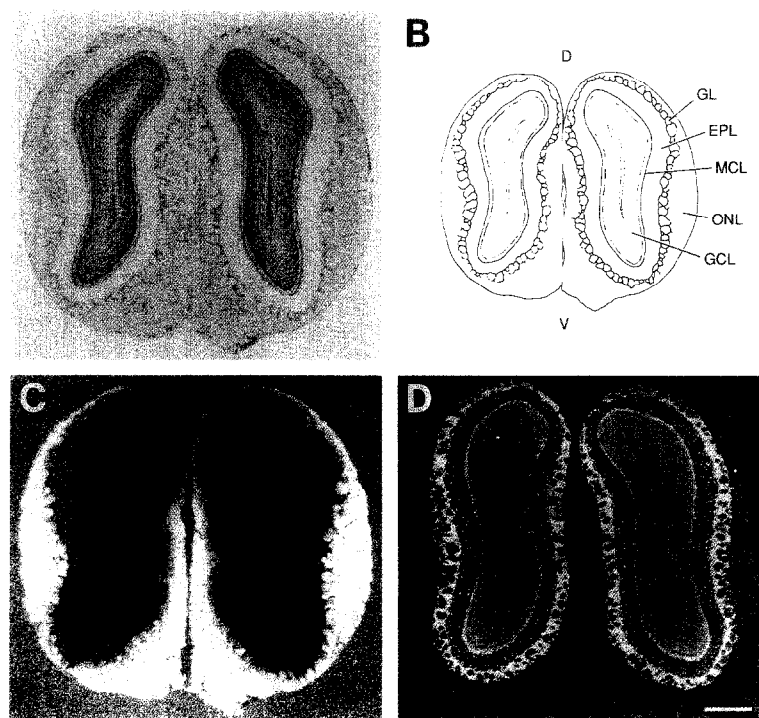


Figure 2. Anatomy of the Rat Olfactory Bulb

(A) Coronal section of rat olfactory bulb following in situ hybridization with a <sup>33</sup>P-labeled OMP antisense RNA probe and exposure to emulsion for 1 day. In this bright-field micrograph, silver grains appear light gray and are observed over the olfactory nerve layer and glomeruli. Neuronal cell bodies are stained blue. (B) Drawing of the section in (A), identifying the different layers of the olfactory bulb (GL, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; ONL, olfactory nerve layer; GCL, granule cell layer; D, dorsal; V, ventral).

(C) Dark-field micrograph of olfactory bulb section in (A). Strong OMP probe hybridization is apparent in the olfactory nerve layer and the glomeruli, demonstrating the presence of OMP mRNA in olfactory sensory axons and axon terminals. Signal is not observed over the cell bodies of olfactory bulb neurons.

(D) Coronal section of rat olfactory bulb annealed with a <sup>33</sup>P-labeled GluR1 antisense RNA probe, exposed to emulsion for one day, and photographed in dark field. Dense silver grain clusters appear over the cell bodies of mitral, periglomerular, and granule cells, while no hybridization is observed in the olfactory nerve layer or in the neuropil of glomeruli. Scale bar in (D) equals 760  $\mu$ m in (A), (C), and (D).

probe detecting the glutamate receptor subunit GluR1, which identifies the bulbar neurons including the periglomerular, mitral, and granule cells (Figure 2D; Hollmann et al., 1989; Keinänen et al., 1990; Nakanishi et al., 1990). These data suggest that the OMP mRNA detected is not derived from bulbar neurons, but rather results from RNA present within the axon terminals of olfactory sensory neurons.

The observation that OMP mRNA could be detected in sensory axon terminals prompted us to ask whether similar experiments could be used to detect receptor mRNA within individual glomeruli after significantly longer exposures. A <sup>33</sup>P-labeled RNA probe for the rat F12 receptor genes was used in situ hybridization experiments on coronal sections of the bulb (Figure 3). The F12 receptor probe detects five genes in the genome (Buck and Axel, 1991). In situ hybridization with the F12 probe on serial sections through the entire olfactory bulb reveals hybridization signals over only five discrete glomeruli. The five sections that exhibit the positive glomeruli are shown in Figures 3A–3E. Importantly, each of these five glomeruli is bilaterally symmetric; positive glomeruli occupy the same relative position in the right and the left bulb. These results provided initial evidence that sensory neurons expressing a given receptor project to one or a small number of glomeruli that are spatially defined within the olfactory bulb.

We have established a more complete topographic map by performing in situ hybridization with probes to four additional receptor subfamilies (Figure 4). Hybridization with a probe for the single copy receptor 17 identifies only two glomeruli. One glomerulus resides in the ventrolateral region of the bulb (Figure 4C), whereas the second is more

medially situated (data not shown). Moreover, these glomeruli are bilaterally symmetric. In situ hybridization with the F5 probe, which anneals with a subfamily of 11 receptor genes, identifies about seven positive glomeruli (Figures 4A and 4D). The F6 subfamily includes three genes, and F6 probes identify three glomeruli within the right and left olfactory bulbs (Figures 4B and 4E). The J7 probe hybridizes to two receptor genes and identifies two glomeruli (Figure 4F). Representative examples of a subset of glomeruli hybridizing with each probe are shown in Figures 4A–4F. The vast majority of positive glomeruli are bilaterally symmetric. However, the two symmetric glomeruli are not always observed in the same section, but may be displaced a few sections apart due to skewing of the bulb during embedding or sectioning procedures. In most instances, axons converge on an isolated glomerulus; occasionally, we observe that neurons expressing a given receptor converge on a small cluster of contiguous glomeruli.

It is not possible to estimate accurately the percentage of axons from neurons expressing a given receptor that project to an individual glomerulus. At one extreme, it is possible that all of the axons from a given class of sensory neuron project to only a single glomerulus. Alternatively, only a subpopulation of axons may converge on a single glomerulus, with the remainder broadly distributed throughout the olfactory bulb. The convergent axons would be readily detected by in situ hybridization, whereas the distributive projections would be below the level of detection in our assays. This notwithstanding, our data with five different receptor probes, which hybridize to 22 genes and identify only 19 of the 3000 glomeruli, strongly suggest that

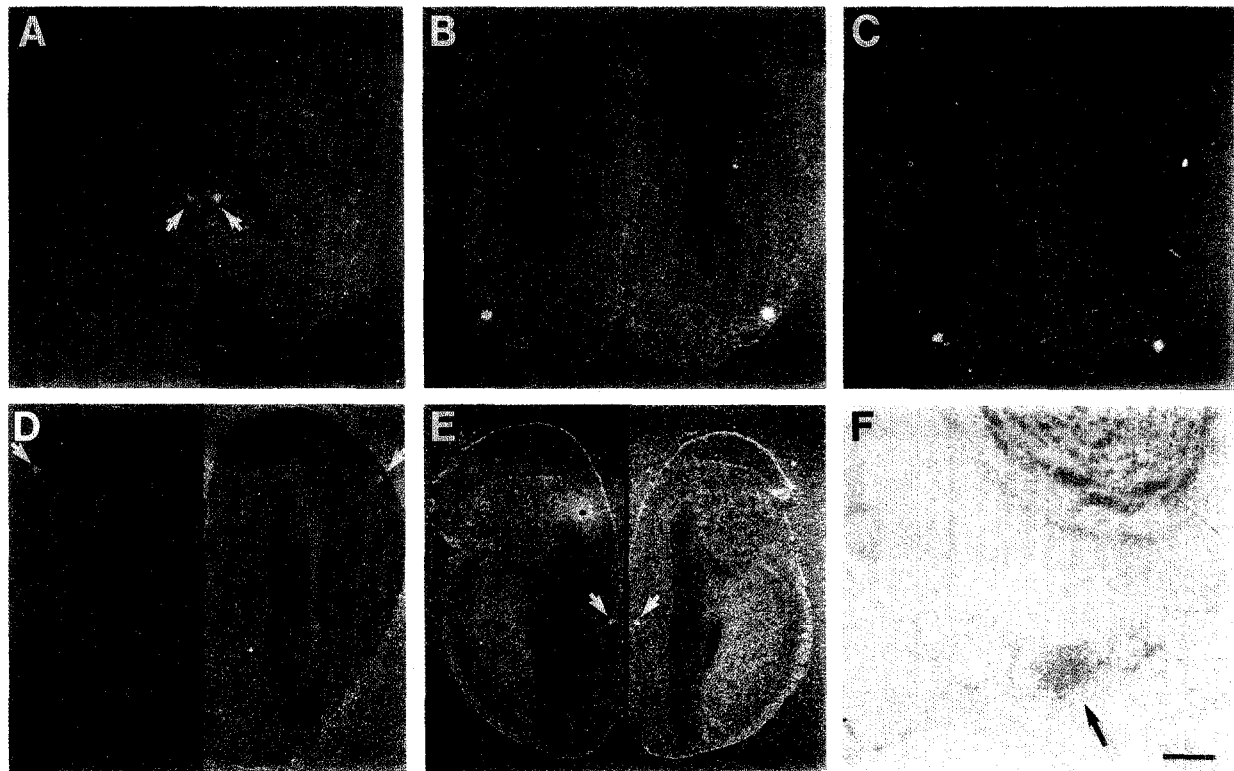


Figure 3. Localization of Odorant Receptor F12 RNA to Discrete Glomeruli within the Olfactory Bulb

Coronal sections of rat olfactory bulb were annealed with a  $^{33}\text{P}$ -labeled F12 receptor antisense probe, washed under high stringency conditions, and exposed to emulsion for 4 weeks.

(A–E) Dark-field micrographs of sections that demonstrate the five pairs of discrete glomeruli hybridizing with the F12 receptor probe. Sections are ordered in an anterior-to-posterior fashion. Arrows indicate the positions of weakly positive glomeruli. Note that positive glomeruli are bilaterally symmetric. Occasionally, glomeruli did not occur within the same section, a result most likely due to misalignment of the bulb during embedding procedures. In these cases, frames including each glomerulus of a pair were spliced together as in (A), (D), and (E). The F12 receptor probe recognizes five genes in the rat genome.

(F) High magnification bright-field micrograph of the positive glomerulus on the right half of the section in (C). The cell bodies of periglomerular cells (stained blue) indicate the boundaries of individual glomeruli. Note that silver grains are observed over a single glomerulus (black arrow), whereas neighboring glomeruli are negative. Scale bar in (F) equals 760  $\mu\text{m}$  in (A)–(E) and 150  $\mu\text{m}$  in (F).

a significant subpopulation of axons from sensory neurons expressing a given receptor converge on one or a small number of glomeruli within the olfactory bulb.

#### Hybridization Detects mRNA in Sensory Axons Rather than Bulbar Neurons

We have performed several experiments that indicate that hybridization to discrete glomeruli is the consequence of mRNA present within the axons of sensory neurons, rather than mRNA expressed in the cell bodies or processes of olfactory bulb neurons. First, hybridization with specific receptor probes is restricted to the glomeruli. Hybridization is never observed over the cell bodies of any of the olfactory bulb neurons. Moreover, we have performed control experiments with the glutamate receptor subunit GluR1 (Hollmann et al., 1989; Nakanishi et al., 1990). GluR1 is expressed by neurons within the olfactory bulb, but is not expressed by the presynaptic sensory cells (Keinänen et al., 1990). Hybridization with GluR1 is apparent over the mitral cell bodies and in the periglomerular cells

of the bulb that outline the glomeruli (see Figure 2D). This pattern contrasts with the restricted glomerular labeling observed upon *in situ* hybridization with odorant receptor probes. Receptor mRNA is abundant in the cell bodies of the sensory neurons, but no hybridization is apparent within the cell bodies of mitral cells. These data suggest that the receptor hybridization observed in glomeruli results from receptor mRNA present within the axons of sensory neurons rather than from receptor mRNA within the dendrites of a specific subpopulation of mitral cells.

Further evidence that the hybridization to discrete glomeruli reflects the presence of receptor mRNA in the axons of sensory neurons derives from the frequent detection of receptor mRNA in axon fascicles entering specific glomeruli. Receptor probes such as F5, which exhibit strong hybridization signals, reveal labeling not only over the glomeruli, but also in the fasciculated sensory axons entering the glomeruli (Figure 5). Serial sections reveal labeling over axon bundles in the olfactory nerve layer that then enter specific F5 glomeruli. These observations provide strong evidence that the mRNA detected within

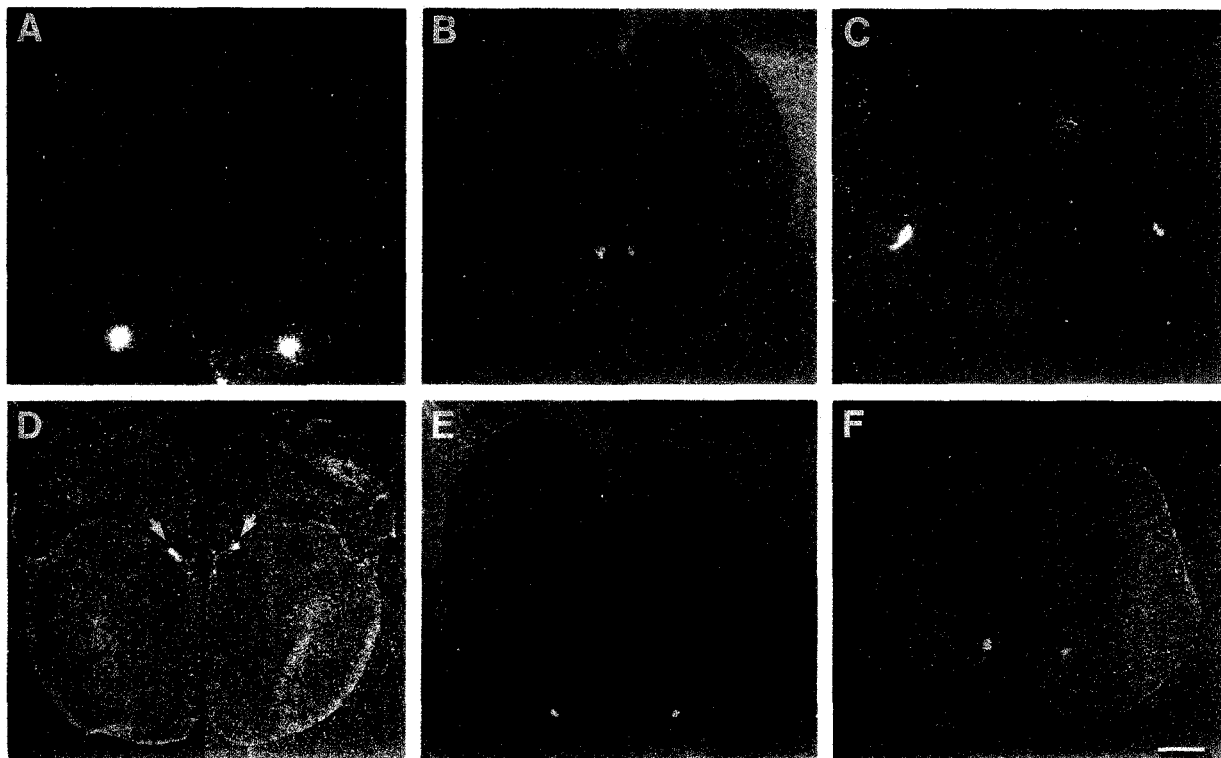


Figure 4. Localization of Additional Receptor RNAs to Specific Glomeruli

Dark-field micrographs showing coronal sections of rat olfactory bulbs following in situ hybridization with  $^{32}\text{P}$ - or  $^{33}\text{P}$ -labeled antisense RNA probes for receptor subfamilies F5 (A and D), F6 (B and E), I7 (C), and J7 (F). Representative examples of a subset of positive, bilaterally symmetric glomeruli are shown for each probe. Arrows indicate the positions of weakly positive glomeruli. The number of glomeruli identified and the number of genes recognized by a given probe, respectively, are noted for each probe: F5, 7 and 11; F6, 3 and 3; I7, 2 and 1; J7, 2 and 2. Scale bar in (F) equals 760  $\mu\text{m}$  for (A)–(F).

individual glomeruli reflects the presence of receptor mRNA in sensory axon terminals.

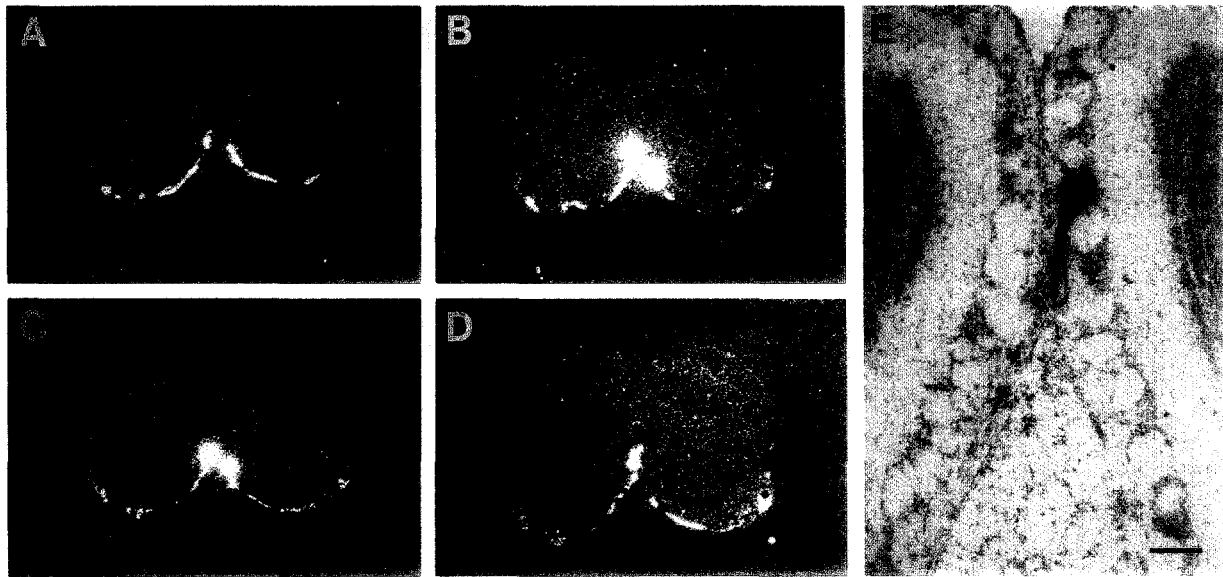
Finally, we have demonstrated that the receptor hybridization to glomeruli disappears after sensory axons are destroyed (Figure 6). When the sensory epithelium is exposed to the detergent Triton X-100, olfactory neurons die and the sensory axons degenerate within 1–2 weeks (Margolis and Grillo, 1978; Nadi et al., 1981; Verhaagen et al., 1990). If the hybridization to individual glomeruli results from the presence of mRNA within sensory axon terminals, we predict that deafferentation would result in the loss of glomerular hybridization signals. If, however, the hybridization signals derive from mRNA present within the dendrites of a specific subpopulation of mitral cells, we would expect that deafferentation should not alter the hybridization signal.

Chemical treatment produces a range of deafferentation varying from animal to animal. We therefore monitored the extent of axonal destruction in individual animals by examining the thickness of the olfactory nerve layer and the persistence of OMP mRNA in axon terminals. To provide an internal control for receptor hybridization, we chose an animal in which degeneration appeared to be severe on the right side and relatively mild on the left (Figures 6A and 6B). This degeneration correlated with de-

creased signal for OMP mRNA in the right olfactory epithelium compared with the left (data not shown). Hybridization with the OMP probe is significantly diminished on the right side of the bulb, consistent with the degeneration of sensory axons (Figure 6A). Degeneration in this animal was most severe on the lateral aspect of the right olfactory bulb. Hybridization with the F12 receptor probe reveals no positive glomeruli in the right olfactory bulb, whereas positive glomeruli were apparent in sections throughout the left bulb (Figure 6B). A similar result was obtained with the F6 receptor probe hybridized to alternate sections of the same olfactory bulb (data not shown).

As a control, we demonstrated that bulbar neurons remain intact despite the degeneration of the sensory axons. The patterns of hybridization for GluR1 mRNA, which identify the bulbar neurons, appear normal in an animal that received multiple chemical treatments and sustained severe deafferentation (Figure 6C).

These experiments provide strong evidence that the hybridization to individual glomeruli is the consequence of receptor mRNA present within the axon terminals of sensory neurons. Thus, the pattern of hybridization with receptor probes in the olfactory bulb is likely to reflect accurately the sites of convergence of axons from neurons expressing a specific odorant receptor.

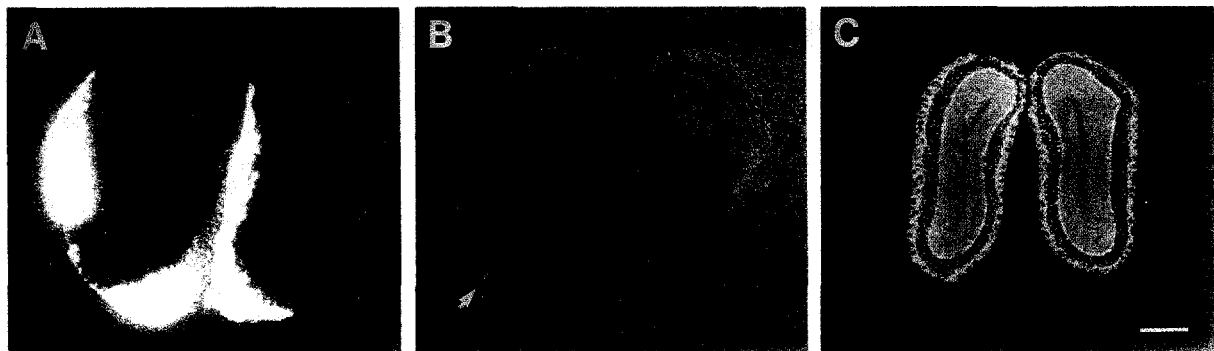


**Figure 5. Localization of Odorant Receptor RNA to Sensory Axons within the Olfactory Bulb**  
(A–D) Dark-field micrographs showing serial coronal sections of a rat olfactory bulb following in situ hybridization with  $^{32}\text{P}$ -labeled receptor F5 antisense RNA probe. Sections are ordered in anterior-to-posterior fashion. Intense hybridization is observed over bilaterally symmetric glomeruli (B and C). Labeling is also apparent over sensory axon fascicles in the olfactory nerve layer that enter the positive glomeruli (A–D). (E) High magnification bright-field micrograph of an olfactory bulb section from a different animal hybridized with the F5 probe. Silver grains are observed over a sensory axon fascicle entering a single glomerulus. Scale bar in (E) equals 760  $\mu\text{m}$  in (A)–(D) and 150  $\mu\text{m}$  in (E).

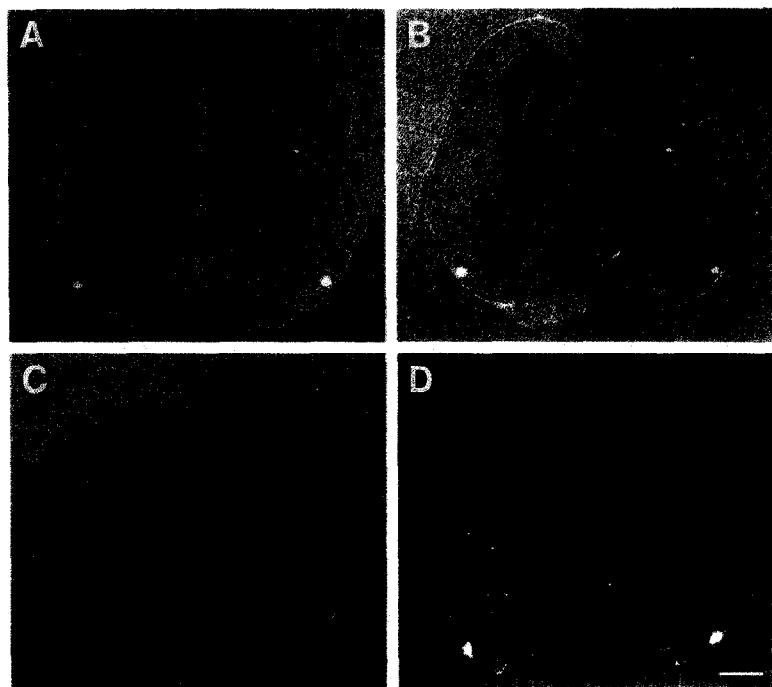
#### The Positions of Positive Glomeruli Are Relatively Constant in All Individuals within a Species

We next asked whether the convergent sites of projections from sensory neurons expressing a given receptor are topographically fixed, or whether the positions of these sites differ in different individuals. Each of the five receptor probes was therefore annealed to sections from at least three individuals, and the relative positions of the positive glomeruli were determined. In one particularly clear example, we identified the position of one of the five F12 glomer-

uli in four different animals (Figure 7). This F12 glomerulus is bilaterally symmetric and maintains the same relative position in the olfactory bulbs of all four animals. A more complete analysis of the relative position of 19 glomeruli confirms the constancy of glomerular position in different animals. It is difficult in these experiments to determine precisely the position of a specific glomerulus in different bulbs owing to the inherent variation in the size and shape of the olfactory bulb in different individuals. Nonetheless, most glomeruli can be identified in the same relative posi-



**Figure 6. In Situ Hybridization of Olfactory Bulb Sections Following Sensory Axon Degeneration**  
The nasal cavities of adult rats were exposed to Triton X-100 causing preferential deafferentation of the right olfactory bulb (see Experimental Procedures). Adjacent bulb sections from a treated animal were hybridized with either OMP (A) or receptor F12 (B)  $^{32}\text{P}$ -labeled antisense RNA probes and micrographed in dark field. In a separate experiment, olfactory bulb sections from a treated animal were hybridized with a  $^{32}\text{P}$ -labeled GluR1 antisense RNA probe (C). Sensory axon degeneration was most severe on the lateral aspect of the right bulb as measured by the thickness of the olfactory nerve layer (compare right and left bulbs in [A]). The intensity of OMP labeling is substantially decreased on the lateral aspect of the right bulb (A), correlating with sensory axon degeneration. Hybridization of adjacent bulb sections with the F12 receptor probe (B) reveals no positive glomeruli in the denervated right bulb, whereas positive glomeruli are apparent in the left bulb (arrow in [B]). In contrast, the pattern of GluR1 mRNA expression in the olfactory bulb is unchanged following deafferentation (C). Scale bar in (C) equals 760  $\mu\text{m}$  for (A)–(C).



**Figure 7. The Position of Individual Glomeruli Is Constant in Different Animals**

Dark-field micrographs of olfactory bulb sections from four individuals following in situ hybridization with the F12 receptor probe. The four panels show the position of one of the five F12-positive glomeruli in four different individuals. Note that the F12 glomerulus is bilaterally symmetric and maintains the same relative position in all four olfactory bulbs. Scale bar in (D) equals 760  $\mu\text{m}$  in (A)–(D).

tion in all individuals analyzed. These observations demonstrate that neurons expressing a given receptor project to topographically distinct glomeruli. Moreover, the positions of the specific glomeruli are not random; rather, they remain relatively constant in all individuals in a species.

## Discussion

### A Topographic Map Encoding Odor Quality

The initial event in the perception of odors involves the interaction of odorous ligands with receptors on sensory neurons. Discrimination among odors therefore requires a mechanism by which the brain can discern which of the numerous receptors have been activated. Individual olfactory sensory neurons are likely to express only one or a small number of receptor genes (Ngai et al., 1993a; Ressler et al., 1993). The problem of distinguishing which receptors have been activated therefore reduces to a problem of distinguishing which neurons have been activated. A topographic map defining the positions of specific neurons in the olfactory epithelium, or the positions of their axons in the olfactory bulb, may be employed to determine which of the neurons have been activated. In this manner, the olfactory system may use spatial segregation of sensory input to encode the quality of an olfactory stimulus.

Previous studies have examined the spatial distribution of neurons expressing specific odorant receptors in the sensory epithelium (Nef et al., 1992; Strotmann et al., 1992; Ngai et al., 1993a; Raming et al., 1993; Ressler et al., 1993; Vassar et al., 1993). In mammals, neurons expressing a specific receptor are distributed within one of four broad but circumscribed zones within the epithelium. Within a given zone, however, neurons expressing a specific receptor are not spatially segregated, but rather are

randomly dispersed within the epithelium (Ressler et al., 1993; Vassar et al., 1993). We have therefore asked whether neurons expressing a given receptor project their axons to common glomeruli within the olfactory bulb. The observation that individual neurons express about 1000 receptor mRNA molecules suggested an experimental approach to map axonal projections based upon the presence of mRNA at the axon terminal. A single glomerulus, about 200  $\mu\text{m}$  in diameter, represents the site of convergence of about 3000 sensory axons. If the neurons that project to a single glomerulus each express the same receptor mRNA and a small number of mRNA molecules are present at the axon terminal, then this convergence of axons might permit the identification of individual glomeruli by in situ hybridization with specific receptor probes. In this study, we demonstrate that the neurons expressing a given receptor project to one, or at most, a few glomerular targets among the thousands of glomeruli within the olfactory bulb. Our data with five different receptor probes, which hybridize to 22 genes and identify only 19 of the 3000 glomeruli, strongly suggest that a significant subpopulation of axons from sensory neurons expressing a given receptor converge on one or a small number of glomeruli within the olfactory bulb. Moreover, these projections define specific glomeruli that maintain a fixed position in the brains of all animals within a species.

Preliminary analysis indicates that the axons of neurons from one topographic zone in the epithelium tend to converge on glomeruli that are localized to a discrete region of the olfactory bulb. For example, neurons expressing the F6, J7, and F5 receptor families are expressed in the same epithelial zone and project to glomeruli that segregate within a ventromedial strip of the bulb (Figure 8, right). These observations are consistent with retrograde tracing

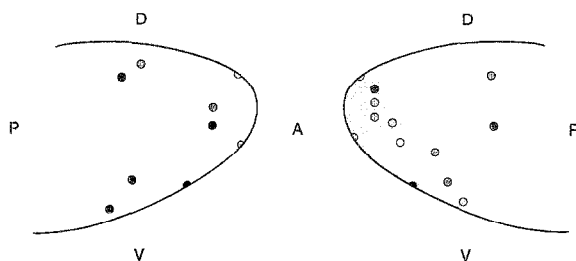


Figure 8. Summary of Specific Glomerular Positions within the Olfactory Bulb

Schematic representations of the lateral (left) and medial (right) surfaces of the right olfactory bulb. The relative positions of 19 glomeruli identified with five different odorant receptor probes are summarized. Glomeruli labeled with specific probes are color coded as follows: F5, green; F12, red; F6, blue; I7, black; J7, yellow. The majority of F5, F6, and J7 glomeruli segregate within an anterior-posterior strip on the ventromedial surface of the olfactory bulb (right; shaded gray). (A, anterior; P, posterior; D, dorsal; V, ventral.)

experiments demonstrating that neurons within defined regions of the epithelium project to broad but circumscribed regions of the olfactory bulb (Astic and Saucier, 1986; Saucier and Astic, 1986; Astic et al., 1987; Schoenfeld et al., 1994). Thus, the zonal organization of receptor expression in the epithelium may be preserved in the projection of sensory axons to the bulb. Compartmentalization of the epithelium and the bulb into anatomically and functionally discrete units of lesser complexity may reduce the problems inherent in regulating the expression of specific receptors and may facilitate the segregation of axons within the olfactory bulb.

Our data are consistent with previous studies suggesting that glomeruli represent functional units, such that specific odors elicit spatially defined patterns of glomerular activity in the olfactory bulb. For example, after exposure to a single odorant, enhanced 2-deoxyglucose uptake is observed over a restricted group of glomeruli. Moreover, this pattern of 2-deoxyglucose labeling of specific glomeruli is distinct for different odors (Stewart et al., 1979; Jourdan et al., 1980; Lancet et al., 1982; Royet et al., 1987). Optical recordings of the olfactory bulb using voltage-sensitive dyes similarly reveal diffuse yet circumscribed patterns of activity in response to single odors (Kauer et al., 1987). These patterns are distinct but overlapping for different odors.

Electrophysiologic studies provide independent support for a model of olfactory discrimination in which individual glomeruli are differentially responsive to different odors. Extracellular recording of spike responses from single mitral cells of a glomerulus indicate that individual glomeruli are "tuned" to discrete molecular structures (Mori et al., 1992; Imamura et al., 1992; Katoh et al., 1993). The individual mitral cells of a given glomerulus might all be maximally responsive to *ortho*-xylene, whereas the mitral cells of a topographically distinct glomerulus respond most strongly to *para*-xylene (Katoh et al., 1993).

These experimental approaches demonstrate the activation of specific glomeruli with specific odors, but cannot relate the activation of specific sensory neurons with spe-

cific patterns of glomerular activation. Our data provide direct anatomic evidence that neurons expressing a given receptor, and therefore responsive to a given odorant, project with precision to one or a small number of glomeruli within the olfactory bulb. Since the positions of the individual glomeruli are topographically defined, the olfactory bulb provides a two-dimensional map that identifies which of the numerous receptors have been activated within the sensory epithelium. One important implication of such a model is that exposure to a given odorant would result in the stimulation of a spatially restricted set of glomeruli, such that individual odorants would be associated with specific topographic patterns of activity within the olfactory bulb. The quality of an olfactory stimulus would therefore be encoded by the specific combination of glomeruli activated by a given odorant.

### Implications for Sensory Processing

How does the olfactory cortex decode this two-dimensional map within the bulb to allow the discrimination among a diverse array of odors? A given neuron is likely to express only one or a small number of receptors from the repertoire of 1000 genes (Ngai et al., 1993a; Ressler et al., 1993). Since the organism can detect far greater than 1000 discrete odors, this implies that a given odorant will interact with multiple receptors, and a single receptor will interact with multiple odorous ligands. In this manner, an odorant, even one that consists of a single molecular species, will interact with a unique combination of receptors, which in turn results in the activation of specific combinations of glomeruli. The maintenance of a fixed map of glomeruli in the bulb therefore provides a mechanism to relay the information resulting from the activation of receptors in the periphery to the brain.

How is such a combinatorial code of glomerular activation interpreted by the cortex? In one model, the topographic map in the olfactory bulb could project directly to olfactory cortex, providing a more central representation of the glomerular map. Cortical loci activated by a given odorant might then converge on higher centers to facilitate odor recognition. Alternatively, it is possible that each of the multiple glomeruli activated by a given odor would project fibers that converge on a single locus in the olfactory cortex. Each locus in the olfactory cortex would reflect the convergence of output fibers from a unique combination of glomeruli, such that the activation of a cortical locus would encode a given odor quality. In this model, each odor would result in the activation of a unique combination of glomeruli, and each combination would be encoded in a specific locus in the cortex. This model is consistent with anterograde tracing experiments indicating that mitral cells from a single glomerulus project to multiple loci in the olfactory cortex (Schwob and Price, 1978; Ojima et al., 1984; Buonviso et al., 1991). Moreover, retrograde tracing studies demonstrate that a cortical locus receives input from multiple glomeruli (Haberly and Price, 1977; Scott et al., 1980; Luskin and Price, 1982).

In either model, the ability of an odorant to activate a combination of glomeruli allows for the discrimination of a diverse array of odors far exceeding the number of recep-



tors and their associated glomeruli. Despite the diversity afforded by combinatorial activation of glomeruli, the organism is likely to be capable of discriminating only an extremely small subset of potential odors. As in other sensory systems, the olfactory system only perceives a meager image of the sensory information in the environment. This restricted array of recognizable odors will be a function of the repertoire of receptors expressed by the organism as well as the nature of the glomerular combinations that are encoded in the cortex. Those odors that are perceived from the universe of potential odorants presumably reflect selection for that subset of odorants that are biologically important for only the species. In this manner, individuals within a species will maintain a repertoire of receptor genes and a combination of cortical connections that permit the detection of those odors important for its survival and reproduction.

This model of olfactory perception shares several basic features with perception in other sensory systems. The brain analyzes a visual image by interpreting the individual components of the image: form, location, movement, color (Livingstone and Hubel, 1987; Zeki and Shipp, 1988). The unity of an image is accomplished by several parallel processing pathways, such that the image is initially dissected into tractable components and then reconstructed in higher visual centers of the cortex. A similar logic may be employed to discriminate olfactory stimuli. Olfactory processing initially requires that the odorant molecule is analyzed by "dissecting" its structural features, such that each of its structural elements will activate a unique subset of receptors, which in turn results in the activation of a unique subset of glomeruli. The odorous stimulus is then reconstructed by the olfactory cortex, which determines which of the numerous glomeruli have been activated.

### The Development of a Topographic Map in the Olfactory Bulb

The establishment of a topographic map of sensory projections might initially involve the expression of receptors by sensory cells. The choice of a given receptor would then be linked to the expression of guidance molecules, such that neurons expressing a given receptor project axons to a single glomerulus. Alternatively, it is possible that immature neurons, not yet expressing receptor, randomly project axons to any one of multiple glomeruli within the olfactory bulb. Contact with an individual glomerulus may then elicit a retrograde signal that directs the expression of specific receptor genes. In this manner, receptor gene expression would be regulated by the ultimate position of its axon terminal within the olfactory bulb. This model is unlikely, since we observe receptor expression in sensory neurons early in embryogenesis (rat embryonic day 14) prior to the birth of mitral cells and the presence of glomeruli (C. Dulac and R. A., unpublished data). Moreover, analysis of receptor gene expression in individual neurons suggests that the choice of receptor expressed by a neuron is not regulated, but rather is stochastic (Chess et al., 1994). Thus, it is likely that neurons stochastically express one receptor gene prior to synapse formation. Neurons ex-

pressing a given receptor will then form synapses with one or a small number of topographically fixed glomeruli.

How is this precise topographic map established? One attractive model combines guidance mechanisms during development with activity-dependent refinement to achieve the precision of connections between the sensory epithelium and olfactory bulb. It is possible, for example, that axons of neurons expressing a subpopulation of the receptor repertoire are restricted in their projections to broad but defined regions of the olfactory bulb. Neuronal growth cones may, for example, be guided to their appropriate target by a gradient of recognition molecules. This guidance process could establish a coarse topographic map. Activity-dependent mechanisms, as a consequence of the synchronous firing of neurons responsive to a given odorant, could refine the pattern of synaptic connections within this restricted region of the bulb to establish a relatively precise topographic map. Such a model draws heavily on observations in the visual system indicating that retinal axons first use activity-independent mechanisms to form a coarse retinotopic map. Activity-dependent processes progressively refine this coarse map to provide a level of precision required to perceive the visual image (reviewed by Constantine-Paton et al., 1990; Goodman and Shatz, 1993). Alternatively, mechanisms involving multiple guidance cues that provide each glomerulus with a unique identity, coupled with multiple receptors on the different sensory neurons, may provide the precision of bulbar connections.

The nature of the putative guidance cues in the target, as well as the recognition molecules on the sensory axons, remains elusive. One particularly attractive possibility is that the odorant receptors themselves may play a role in patterning the projections of olfactory sensory axons. The presence of receptor mRNA in sensory axons may result in the functional expression of receptors on axonal projections. These presynaptic receptors may be activated by guidance cues expressed by the postsynaptic mitral cells. In this manner, the odorant receptors may function in the dendrite in the recognition of odors and may exhibit a distinct function in the axon in the establishment of a topographic map that encodes odor quality in the olfactory bulb.

### Experimental Procedures

#### In Situ Hybridization

In situ hybridization was performed essentially as described (Schaeren-Wiemers and Gerfin-Moser, 1993), except that  $^{32}\text{P}$ - or  $^{33}\text{P}$ -labeled RNA probes were used. Probes were synthesized in a 10  $\mu\text{l}$  reaction with either 500  $\mu\text{Ci}$  of [ $^{32}\text{P}$ ]CTP or 330  $\mu\text{Ci}$  of [ $^{33}\text{P}$ ]UTP (3000 Ci/mmol and 1000 Ci/mmol, respectively, Amersham Life Sciences) using T3 or T7 RNA polymerases (Melton et al., 1984). Clones encoding full-length cDNAs (constructed in pBluescript II SK(+); Stratagene) were used as templates to synthesize probes (ranging from 1.0–2.0 kb in size) from the following odorant receptor sequences: F5, F6, F12, and I7. The sequence encompassing TM4 through TM7 (approximately 0.7 kb) of receptor J7 was isolated by the polymerase chain reaction (L. Buck and R. A., unpublished data). Sequences corresponding to the BamHI–Asp718 fragment of OMP cDNA (Rogers et al., 1987) were used to synthesize a 1 kb 3' OMP probe. Finally, a full-length GluR1 cDNA clone (Hollmann et al., 1989; Nakanishi et al., 1990) was used to synthesize a 1.4 kb probe.

Fresh-frozen olfactory bulbs of adult female Sprague-Dawley rats (11–13 weeks old) were oriented for coronal sections and cut to 15–30  $\mu\text{m}$  thickness. Serial sections of entire bulbs were allowed to dry and then were fixed for 10 min in 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature. After several rinses in PBS, sections were incubated in 0.25% acetic anhydride, 0.1 M triethanolamine (pH 8), washed again in PBS, and prehybridized for 2–4 hr at room temperature (Schaeren-Wiemers and Gerfin-Moser, 1993). Probes were diluted to a concentration of  $1 \times 10^8$ – $2 \times 10^8$  cpm/ml in hybridization buffer (Wilkinson et al., 1987a, 1987b), and 100  $\mu\text{l}$  was applied to each slide. Following a 14–20 hr incubation at 72°C, sections were washed in  $0.2 \times \text{SSC}$  for 1 hr at 72°C, treated with 2  $\mu\text{g/ml}$  RNase A for 30 min at 37°C, and washed again in  $0.2 \times \text{SSC}$  at 72°C. After dehydration, slides were dipped in undiluted NTB-3 emulsion (Kodak) and allowed to expose at 4°C. Slides hybridized with OMP and GluR1 probes were developed after 1 day, whereas slides hybridized with odorant receptor probes were exposed to the emulsion for 4 weeks. After development, sections were stained with toluidine blue O, dehydrated, and mounted in Accumount 280 (Baxter). Sections were photographed using a Nikon Labophot-2 microscope with 2 $\times$  and 10 $\times$  objectives. Dark-field illumination was with a vertical Darklite attachment (MicroVideo Instruments).

### Peripheral Deafferentation

Chemical deafferentation of rat olfactory bulb was adapted from procedures described for the mouse (Margolis and Grillo, 1978; Nadi et al., 1981; Verhaagen et al., 1990; F. L. Margolis, personal communication). Female Sprague-Dawley rats (11–12 week old) were lightly anesthetized with an intraperitoneal injection of ketamine (40 mg/kg). Approximately 0.5 ml of a 0.7% Triton X-100, 0.9% sodium chloride solution was injected into the right nostril of each animal using a blunted 23-gauge needle. Animals were sacrificed 2 weeks after this procedure, and the bulbs prepared for sectioning as described above.

In other experiments, we applied 1 ml of 1% Triton X-100, 0.9% sodium chloride, allowed the animals to recover for 2 days, and repeated the procedure for a total of four or five treatments. Animals were sacrificed after a recovery period of 2 weeks following the last treatment.

In pilot experiments, we added a blue dye to the Triton X-100 solution and examined the extent of staining in the left and right nasal cavities 10 min after treatment. We found extensive staining of olfactory epithelium on both right and left sides in some animals and preferential accumulation on one side in others. This variability was apparent in the extent of sensory axon degeneration seen in the experimental animals. Some animals had uniformly denervated olfactory bulbs, whereas others had more extreme axonal degeneration on one side only. This appeared to be independent of the number of treatments, although deafferentation was usually more complete in animals receiving multiple treatments.

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