An Olfactory Sensory Map in the Fly Brain

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Summary

We have isolated the "complete" repertoire of genes encoding the odorant receptors in *Drosophila* and employ these genes to provide a molecular description of the organization of the peripheral olfactory system. The repertoire of *Drosophila* odorant receptors is encoded by 57 genes. Individual sensory neurons are likely to express only a single receptor gene. Neurons expressing a given gene project axons to one or two spatially invariant glomeruli in the antennal lobe. The insect brain therefore retains a two-dimensional map of receptor activation such that the quality of an odor may be encoded by different spatial patterns of activity in the antennal lobe.

Introduction

In humans, smell is often viewed as an aesthetic sense, a sense capable of eliciting enduring thoughts and memories. For most animals, however, smell is a primal sense that affords organisms the ability to detect food, predators, and mates. Insects provide an attractive model system to understand the logic of olfactory perception because they exhibit complex behaviors controlled by an olfactory sensory system that is significantly simpler than that of vertebrates (Siddiqi, 1987; Hölldobler, 1995; Carlson, 1996; Menzel and Müller, 1996). Different subgroups of *Drosophila* have evolved different behavioral responses to the same odor to accommodate the unique ecological niche that they occupy. *Drosophila sechellia*, for example, is a monophagous dipteran attracted to *n*-caproic acid, abundant in the shrub *Morinda citrifolia*, its sole food source (Higa and Fuyama, 1993). In contrast, *Drosophila melanogaster* is repelled by *n*-caproic acid but is attracted to odors emanating from a variety of fermenting fruits (Cobb, 1999). Olfactory cues are also used by male moths to locate mates. The male hawkmoth (*Manduca sexta*) has the ability to detect vanishingly low concentrations of female pheromones and initiates flight behaviors in pursuit of females over long distances (reviewed in Hildebrand, 1995). Finally, social insects make extensive use of chemical cues in communication. Ants, for example, recognize nest odors to identify and ultimately kill conspecific intruders from neighboring nests (Isingrini et al., 1985; Hölldobler and

Wilson, 1990). The genetic analysis of olfactory-mediated behaviors in insects may therefore provide a facile system to understand the mechanistic link between the perception of odors and behavior.

The perception of odors requires the recognition of a diverse repertoire of odorous molecules in the periphery and neural mechanisms to allow the discrimination of odors more centrally. The initial step in the detection of odors involves the binding of an odorous ligand to G protein–coupled receptors that reside on the dendrites of olfactory sensory neurons. The genes encoding odorant receptors have been identified in worms, flies, and vertebrates. In the nematode, *C. elegans*, as well as many mammalian species, the family of odorant receptor genes is large and is likely to encode up to 1000 different seven transmembrane domain proteins (Buck and Axel, 1991; Levy et al., 1991; Ben-Arie et al., 1994; Troemel et al., 1995; Sengupta et al., 1996; Robertson, 1998). In the fruit fly, *Drosophila melanogaster*, as well as in vertebrate species such as birds and fish, the repertoire of receptor genes is smaller and is likely to encode from 50 to 100 receptor proteins (Ngai et al., 1993; Leibovici et al., 1996; Nef et al., 1996; Clyne et al., 1999b; Gao and Chess, 1999; Vosshall et al., 1999; Rubin et al., 2000). Thus, the recognition of the vast array of odors in the environment is accomplished by a large family of odorant receptor molecules.

Although the size of the receptor gene family is similar in nematodes and mice, the logic of olfactory perception differs in the two organisms. The discrimination of olfactory information requires neural mechanisms capable of distinguishing which of the numerous receptors have been activated by a given odorant. In *C. elegans*, a family of 1000 receptor genes is expressed in only 16 pairs of sensory cells, and each neuron expresses multiple odorant receptors (Bargmann and Horvitz, 1991; Colbert and Bargmann, 1995; Troemel et al., 1995, 1999). Activation of any one of the multiple receptors expressed in one cell will lead to chemoattraction, whereas activation of a receptor in a different cell results in chemorepulsion (Troemel et al., 1997). Thus, the behavioral response to a specific sensory input is a property of the neuron that is activated and not a function of the chemosensory receptor itself. This organization allows for the recognition of a diverse array of odorous ligands but diminishes the organism's discriminatory power.

A different logic is employed to discriminate odors by the mammalian olfactory system. In mice, each of the two million olfactory receptor neurons expresses only one of a thousand odorant receptor genes (Ressler et al., 1993; Vassar et al., 1993; Chess et al., 1994; Malnic et al., 1999). Neurons expressing a given receptor project axons with precision to two of 1800 discrete synaptic structures, the glomeruli, within the olfactory bulb (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Wang et al., 1998). The pattern of projections is spatially invariant, providing a two-dimensional representation of receptor activation in the brain. The existence of a physical map that segregates the projections of neurons expressing a given receptor (and therefore responsive to given odors) is in accord with both electro-

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physiological and imaging studies that demonstrate that different odors elicit defined patterns of spatial activity within the olfactory bulb (Stewart et al., 1979; Lancet et al., 1982; Kauer et al., 1987; Imamura et al., 1992; Mori et al., 1992; Katoh et al., 1993; Friedrich and Korsching, 1997; Rubin and Katz, 1999).

How is olfactory information represented in the insect brain? Does the logic of olfactory discrimination in the fruit fly, *Drosophila*, more closely resemble that of the invertebrate *C. elegans* or the more complex olfactory system of vertebrates? The anatomy of the insect olfactory system is reminiscent of vertebrates. Olfactory recognition in the fruit fly *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 43 glomeruli within the antennal lobe of the brain (Stocker, 1994; Laissue et al., 1999). The glomeruli are innervated by dendrites of the projection neurons, the insect equivalent of the mitral cells in the vertebrate olfactory bulb. These antennal lobe neurons in turn project to the mushroom body and lateral horn of the protocerebrum (reviewed in Stocker, 1994; Ito et al., 1998). 2-deoxyglucose mapping in the fruit fly (Rodrigues and Buchner, 1984; Rodrigues, 1988), functional tracing of pheromone-responsive neurons in the male moth (Hansson et al., 1992), and calcium imaging in the honeybee (Joerges et al., 1997; Galizia et al., 1999; Sachse et al., 1999) demonstrate that different odorants elicit defined patterns of glomerular activity, suggesting that in insects, as in vertebrates, a topographic map of odor quality is represented in the antennal lobe. However, in the absence of the genes encoding the receptor molecules, it has not been possible to define a physical basis for this spatial map.

In this study we have identified the "complete" family of *Drosophila* odorant receptors (*DOR*s) and employ these genes to visualize the projections of individual neurons to the fly brain. We identify 57 *DOR* genes within the *Drosophila* genome. Individual receptors are expressed in from 2 to 50 olfactory neurons in a conserved pattern that defines a topographic map on the surface of the antenna. Individual neurons are likely to express only one receptor gene. Neurons expressing a given receptor gene project with precision to spatially invariant glomeruli within the antennal lobe. In the fly, as in mammals, a topographic map of receptor activation in the peripheral sense organs is represented in the brain. These spatial patterns may then be decoded in higher sensory centers in the brain to translate stimulus features into meaningful neural information. These data suggest a logic of odor discrimination that has been maintained over the 500 million years of evolution separating insects from mammals, perhaps reflecting an efficient solution to the complex problem of olfactory sensory perception.

Results

In previous studies, difference cloning, along with analysis of genomic sequences, revealed a family of putative odorant receptors in *Drosophila melanogaster*. Nineteen candidate *Drosophila* odorant receptors (*DOR*) genes were initially identified in searches of databases representing 20% of the genome (Clyne et al., 1999b; Gao and Chess, 1999; Vosshall et al., 1999). Analysis of the recently completed euchromatic genome sequence of

Drosophila using BLAST (Altschul et al., 1990) and hidden Markov model (Eddy, 1998) searches with existing members of the *DOR* gene family indicate that a total of 57 *DOR* genes are present in the genome (Adams et al., 2000; Rubin et al., 2000). We tentatively define this family of 57 genes as the "complete" complement of *DOR* genes recognizing that 60 Mb of heterochromatic DNA remain to be analyzed.

Each of the 57 genes encodes a putative seven transmembrane domain protein of $\sim$380 amino acids. Analysis of these sequences does not reveal nonsense or frameshift mutations that characterize pseudogenes within the family of odorant receptors prevalent in both nematode and vertebrate genomes. Verification of these gene predictions, however, awaits the isolation and sequences of *DOR* cDNAs. The family as a whole is extremely divergent and exhibits from 17% to 26% amino acid identity. However, each of the genes shares short common motifs in fixed positions that define these sequences as highly divergent members of a gene family. Analysis of the sequence of all 57 receptors reveals the existence of discrete subfamilies whose members exhibit significantly higher sequence identity, ranging from 40% to 60%. One subfamily is aligned in Figure 1, and the remaining genes sequences are available at the URL provided below (Experimental Procedures).

The *DOR* genes are widely dispersed in the genome and most exist as single genes that distribute on each of the *Drosophila* chromosomes. There are two clusters of three-linked genes and eight examples of two-linked receptors. The approximate chromosomal position of each of the *DOR* genes was determined relative to sequence-tagged sites generated by the Berkeley *Drosophila* Genome Project (Berkeley *Drosophila* Genome Project, unpublished data) (Table 1). Thus, the 57 *DOR* genes identified within the euchromatic *Drosophila* genome may constitute the complete family of odorant receptor genes.

A Topographic Map of *DOR* Gene Expression in Olfactory Sensory Organs

In situ hybridization with the *DOR* genes isolated previously revealed that these genes were expressed in spatially defined subpopulations of olfactory sensory neurons (Clyne et al., 1999b; Gao and Chess, 1999; Vosshall et al., 1999). We have obtained a more complete profile of receptor expression by performing in situ hybridization with digoxigenin-labeled RNA antisense probes to each of the 57 *DOR* genes. The expression of 32 of the *DOR* genes is restricted to the antenna, seven are expressed solely in the maxillary palp, and one is expressed in both olfactory organs. We did not detect expression of 17 *DOR* genes in embryonic, larval, or adult olfactory organs nor in other regions of the adult head (Figure 2; Table 1; data not shown).

As described previously with a smaller subset of *DOR* genes, each receptor is expressed in a spatially restricted subpopulation of neurons. The number of cells that express a given receptor gene, as well as the spatial pattern of expression, is conserved between individuals and is bilaterally symmetric in the two antennae. *Or49b*, for example, is expressed in two cells at the lateral edge of the antenna at the midpoint of the proximodistal axis. At the other extreme, *Or47b* is expressed in $\sim$50 antennal neurons that reside in the lateral distal edge of the antenna. These patterns were conserved in over 30 individual flies examined for each gene.
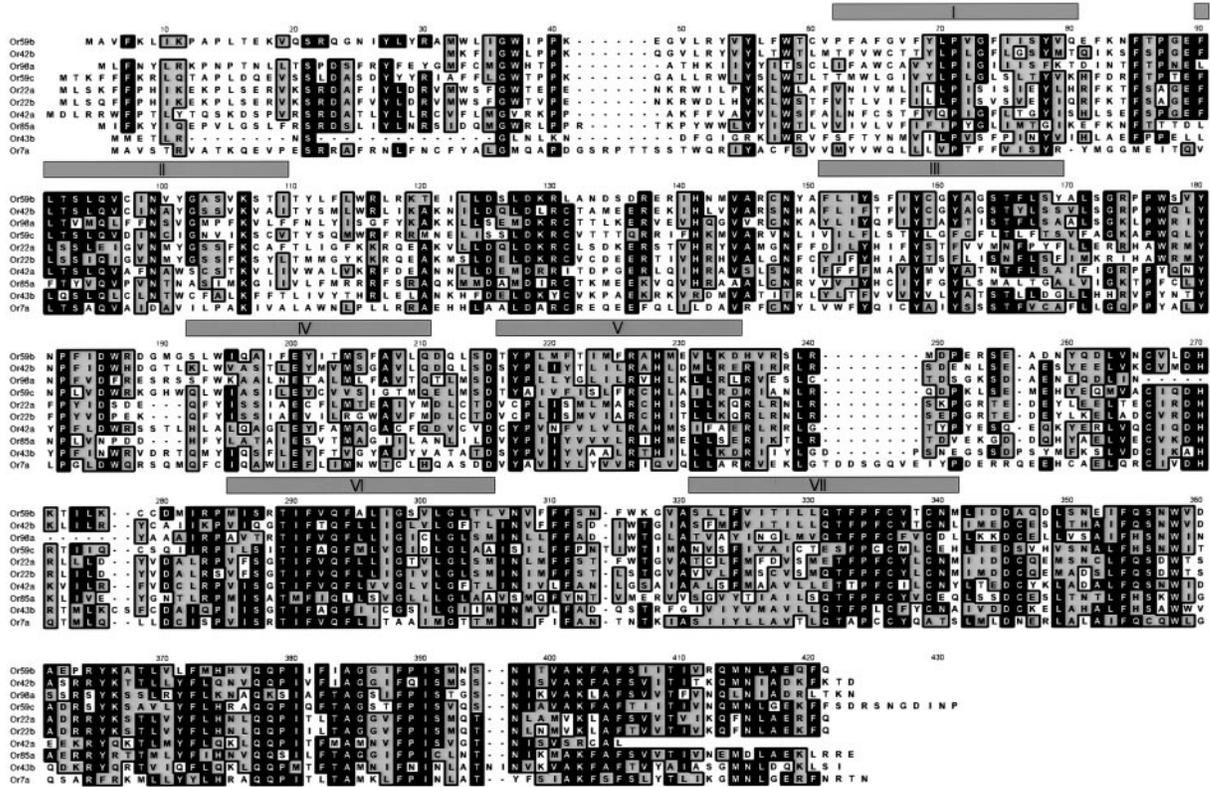
Figure 1. Sequence Alignment of the *Or22a* Subfamily

Proteins predicted by GENSCAN analysis or from cDNA sequences (Vosshall et al., 1999) were aligned using the ClustalW algorithm (MacVector; Oxford Scientific). Identical amino acids are indicated with black shading, while similar amino acids are shaded in light gray. The positions of the seven putative transmembrane domains are indicated by horizontal bars above the protein sequences.

In situ hybridization, coupled with immunocytochemistry with pan neuronal markers, demonstrates that this family of receptor genes is expressed in sensory neurons rather than support cells or glia within the antenna and the maxillary palp (Vosshall et al., 1999; data not shown). Expression of this gene family is only observed in cells within the antenna and maxillary palp. No hybridization was observed in neurons of the brain, nor was

Table 1. Expression Patterns of the "Complete" Repertoire of *Drosophila* Odorant Receptors

Antenna		Maxillary Palp	Antenna and Palp	Not Detected
Or47a (47F1)	*Or67c* (67D2)	*Or71a* (71B1)	*Or83b* (83A6)	*Or49a* (49A5)
Or47b (47F6)	*Or43b* (43F5)	*Or46a* (46E7-8)		*Or22c* (22C1)
Or7a (7D14)	*Or69b* (69E8-F1)	*Or1a* (1A8)		*Or46b* (46E7-8)
Or85a (85A3)	*Or69a* (69E8-F1)	*Or33c* (33B10)		*Or74a* (74A6)
Or85f (85D15)	*Or67a* (67B2)	*Or85e* (85B2)		*Or63a* (63B1)
Or19a (19B3-19C)	*Or43a* (43A1)	*Or85d* (85A11)		*Or83a* (83A6)
Or13a (13F16-18)	*Or35a* (35D1)	*Or59c* (59E1)		*Or59a* (59E1)
Or22a (22A5)	*Or10a* (10B15)			*Or24a* (24E4)
Or56a (56E1)	*Or9a* (9E1)			*Or30a* (30A3)
Or82a (82A3-4)	*Or88a* (88B1)			*Or45a* (45C5)
Or2a (2E1)	*Or49b* (49D1)			*Or85c* (85A9)
Or23a (23A3)	*Or98a* (98B5)			*Or45b* (45F1)
Or65a (65A7-11)	*Or85b* (85A9)			*Or94a* (94D9)
Or22b (22A5)	*Or83c* (83D5)			*Or94b* (94D9)
Or33b (33B10)	*Or42b* (42A2)			*Or92a* (92E8)
Or33a (33B10)	*Or59b* (59E1)			*Or42a* (42A2)
				Or98b (98D4)

The chromosomal positions of the 57 *DOR* genes are indicated in parentheses following the name of each receptor. Receptor names reflect a recent standardization of nomenclature, which uses the abbreviation *Or* for odorant receptors in *Drosophila* (*Drosophila* Odorant Receptor Nomenclature Committee, 2000 [this issue of *Cell*]). The map positions were determined relative to sequence tagged sites (Berkeley *Drosophila* Genome Project, unpublished data) or inferred from genome sequence by Celera Genomics.

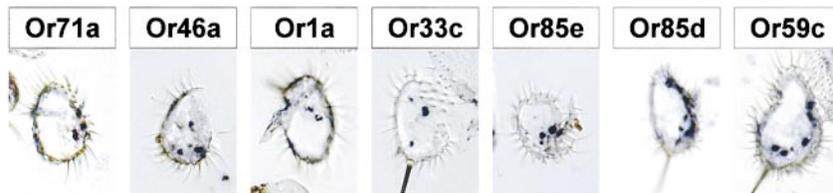## Antennal DOR Genes
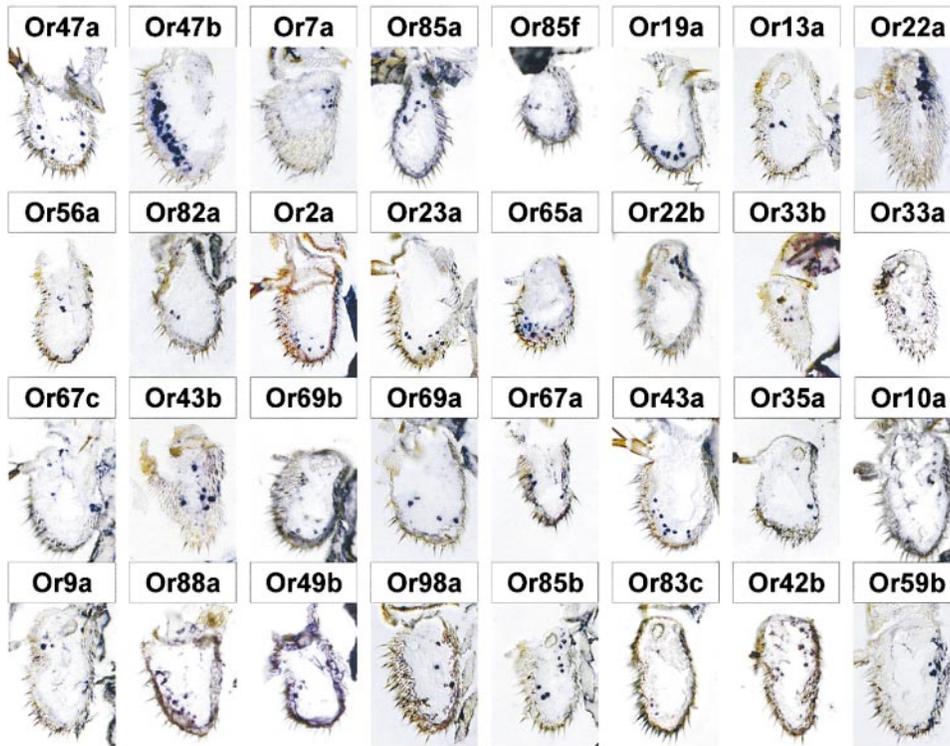
Maxillary Palp DOR Genes

Figure 2. Expression of 39 *DOR* Genes in Spatially Restricted Regions of the Antenna and Maxillary Palp

Frozen frontal sections from adult fly head were annealed with antisense digoxigenin RNA probes, and hybridization was visualized with an alkaline phosphatase-conjugated antibody. Each gene is expressed in a small subset of neurons in the antenna or the maxillary palp. The number and position of hybridizing cells is conserved in over 30 individual flies examined for each probe. The number of cells expressing each gene in the sections presented here is a fraction of the total number of positive cells in the whole antenna or maxillary palp. The antennal sections are mediated with dorsal up and medial right.

hybridization observed elsewhere in the adult fly (including the taste cells of the proboscis) or in any tissue at any stage during embryonic or larval development. Thus, it seems likely that this receptor family is dedicated to the perception of volatile olfactory stimuli by adult sensory neurons in the antenna and maxillary palp.

Individual Neurons Are Likely to Express Only a Single Receptor Gene

The diversity of receptor expression in individual sensory neurons will have important implications for the logic of odor discrimination. In *C. elegans*, individual neurons may express up to 20 different receptor genes (Troemel et al., 1995, 1999), whereas in mammals, olfactory sensory neurons transcribe only a single member of the gene family (Ressler et al., 1993; Vassar et al., 1993; Chess et al., 1994; Malnic et al., 1999). In previous studies, we have performed two-color in situ hybridization experiments with receptor probes to demonstrate that individual sensory neurons in *Drosophila* express different complements of receptors (Vosshall et al., 1999). At the extreme, these experiments are consistent with a model in which individual neurons express only a single receptor gene. The availability of the "complete" repertoire of receptor genes has allowed us to examine this question of diversity of receptor expression in greater detail.

This problem is best addressed in the maxillary palp, which contains about 120 sensory neurons and expresses only seven members of the *DOR* gene family. Each of the seven *DOR* genes expressed in the palp identifies about 20 neurons, consistent with a model in which individual sensory neurons are functionally distinct. We performed two-color in situ hybridization experiments in which the neurons expressing *Or85e* were
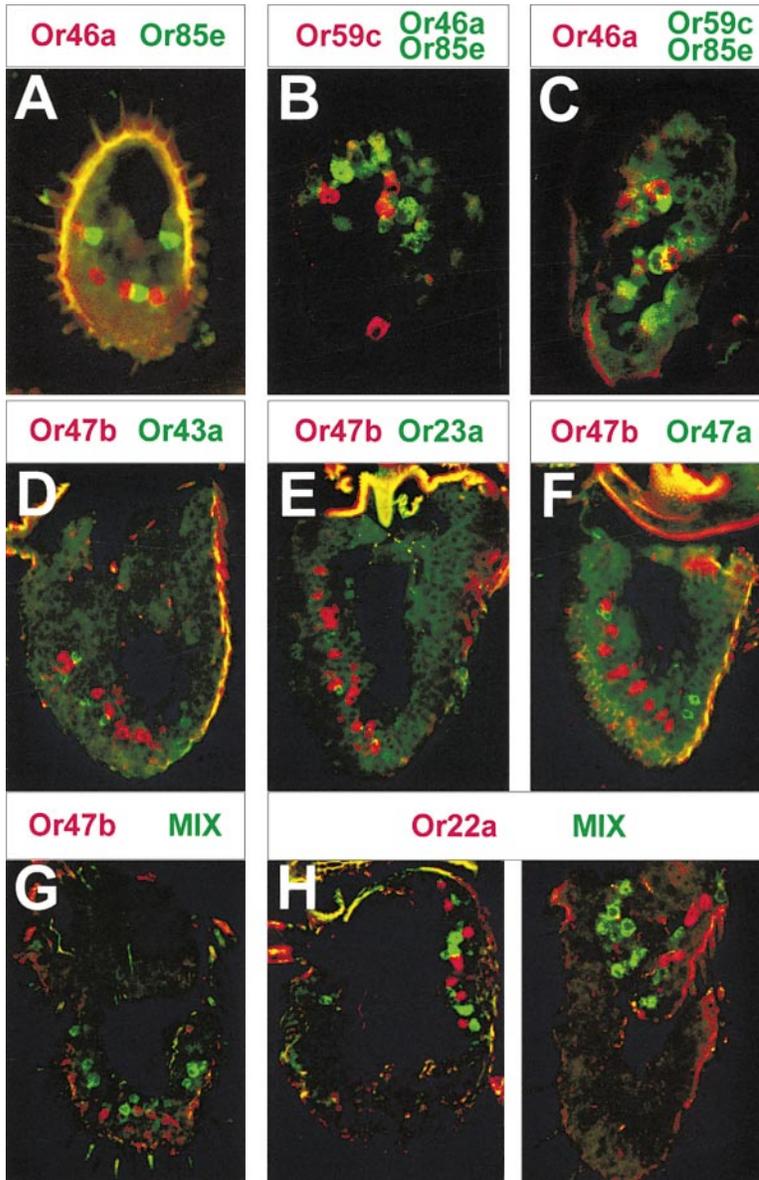
Figure 3. *DOR* Genes are Expressed in Distinct Subsets of Olfactory Neurons

(A–C) Two-color RNA in situ hybridization reveals that *Or85e*, *Or46a*, and *Or59c* are expressed in nonoverlapping subsets of maxillary palp neurons. Probe combinations are as follows: (A) *Or85e* (fluorescein-RNA, green) and *Or46a* (digoxigenin-RNA, red); (B) *Or59c* (fluorescein-RNA, red) and a mixture of *Or46a* and *Or85e* (digoxigenin-RNA, green); (C) *Or46a* (fluorescein-RNA, red) and a mixture of *Or85e* and *Or59c* (digoxigenin-RNA, green). All probes were annealed to frontal sections of adult head and visualized with antibodies that distinguish fluorescein- and digoxigenin-labeled RNA probes.

(D–G) *Or47b* and either single or mixed probes demonstrates that *Or47b* is expressed in subsets of neurons distinct from eight additional *DOR* genes also expressed in the lateral distal domain. Probes are as follows: (D) *Or47b* (digoxigenin-RNA, red) and *Or43a* (fluorescein-RNA, green); (E) *Or47b* (digoxigenin-RNA, red) and *Or23a* (fluorescein-RNA, green) (F) *Or47b* (digoxigenin-RNA, red) and *Or47a* (fluorescein-RNA, green); (G) *Or47b* (fluorescein-RNA, red) and a mixture of *Or67a*, *Or88a*, *Or49b*, *Or98a*, and *Or83c* (digoxigenin-RNA, green).

(H) Cells expressing *Or22a* are distinct from those expressing five other *DOR* genes present in the proximal medial region of the antenna. Sections were annealed with a probe against *Or22a* (fluorescein-RNA, red) plus a mixture of *Or7a*, *Or56a*, *Or85b*, *Or42b*, and *Or59b* (digoxigenin-RNA, green).

identified with antisense RNA probes labeled with fluorescein. Neurons expressing *Or46a* in the maxillary palp were detected with RNA probes labeled with digoxigenin, and expression was visualized with fluorescent antibodies that distinguish the two probes (Figure 3A). These two-color in situ hybridization experiments and additional experiments with mixed probes (Figures 3B and 3C) reveal that *Or85e*, *Or46a*, and *Or59c* are expressed in nonoverlapping subsets of cells.

Demonstrating the expression of a single receptor gene in individual sensory neurons within the antenna is more difficult since this olfactory organ expresses 32 members of the *DOR* gene family in ~1000 sensory cells. The lateral distal domain of the antenna contains about 300 neurons, 50 of which express *Or47b*. This same domain expresses 15 other receptors, including *Or47a*, *Or23a*, *Or67a*, *Or43a*, *Or88a*, *Or49b*, *Or98a*, and *Or83c*. In pairwise experiments, antennal sections were annealed with an *Or47b* antisense RNA probe and either individual or mixed probes for these eight additional receptors. RNA probes were labeled with either fluorescein or digoxigenin and visualized with antibodies that

distinguish the two types of probes. *Or47b* is expressed in a subpopulation distinct from that expressing any of these eight receptors (Figures 3D–3G). A similar experiment was performed to examine *DOR* gene expression in the medial proximal region of the antenna. *Or22a* probes were labeled with fluorescein and a mix of probes complementary to five additional genes expressed in this domain (*Or7a*, *Or56a*, *Or85b*, *Or42b*, and *Or59b*) was labeled with digoxigenin. Despite the interspersion of *Or22a* cells with cells expressing other *DOR* genes in the domain, there is no overlap in the expression of *Or22a* with any of these five *DOR* genes (Figure 3H). Taken together, these data provide strong support for a model reminiscent of the mammalian olfactory system, in which individual sensory neurons express only a single receptor.

This conclusion must be tempered by the previous description of an odorant receptor gene, *Or83b*, that is expressed in all olfactory sensory neurons in both the antenna and maxillary palp (previously known as *A45*; Vosshall et al., 1999). If *Or83b* does indeed recognize odorous ligands, then our data would indicate that all
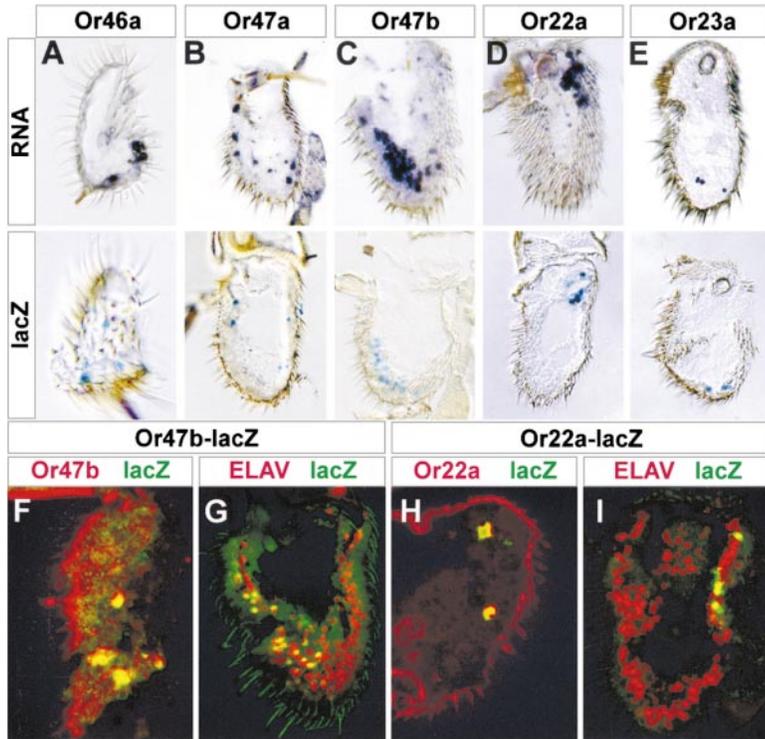
Figure 4. *DOR* Promoter Elements Recapitulate the Expression of Endogenous *DOR* Genes

(A–E) The spatial domains of expression of five *DOR* transgenes reflect the expression of the endogenous genes. Flies carrying *DOR-Gal4* transgenes were crossed to strains carrying UAS-*lacZ* responder transgenes and localization of LacZ was visualized by β-galactosidase activity staining. For each *DOR-Gal4* transgene, *Or46a* (A), *Or47a* (B), *Or47b* (C), *Or22a* (D), and *Or23a* (E), the expression of the endogenous gene is presented in the top panel and LacZ expression in the lower panel. RNA expression in the top panels is visualized as described in Figure 2.

(G and I) *DOR-Gal4* transgenes drive expression in sensory neurons in the antenna. Frontal antennal sections from *Or47b-Gal4*:UAS-*lacZ* (G) and *Or22a-Gal4*:UAS-*lacZ* (I) animals were incubated with antibodies that recognize LacZ (green) and the pan neuronal antibody Elav-9F8A9 (red). All cells expressing LacZ are also positive for Elav, confirming their identity as sensory neurons.

(F and H) *DOR-Gal4* transgenes promote expression of LacZ in cells that express the endogenous receptor. Sections from *Or47b-Gal4*:UAS-*lacZ* (F) and *Or22a-Gal4*:UAS-*lacZ* (H) were annealed with digoxigenin-labeled antisense RNA probes (red) and antibody against LacZ (green).

sensory neurons express two receptors rather than one, *Or83b*, and one additional gene from the family of *DOR* genes (see Discussion).

A Spatial Segregation of Like Axons in the Antennal Lobe

These experiments suggest that there are 39 distinct neuronal cell types within the *Drosophila* olfactory organs. In the mammalian olfactory system, neurons expressing the same receptor project their axons to two spatially invariant glomeruli (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Wang et al., 1998). We therefore determined whether cells expressing a given receptor in *Drosophila* converge upon spatially defined loci in the antennal lobe, the first relay station for olfactory information in the fly brain. Axons from the antenna and maxillary palp synapse with the dendrites of projection neurons in the antennal lobe. There are 43 morphologically distinct synaptic structures or glomeruli in the antennal lobe that are invariant in position and size in individual flies. The number of antennal lobe glomeruli dedicated to olfactory input (41) (Stocker, 1994; Laissue et al., 1999) approximates the number of different sensory neurons identified in the two olfactory organs (39), suggesting that olfactory neurons expressing a given receptor may converge on a single glomerulus.

We have therefore performed genetic experiments that permit us to visualize the axonal projections from neurons expressing a given receptor. Transgenic flies were generated in which *DOR* gene promoters direct the expression of the yeast transcriptional activator, *Gal4* (Brand and Perrimon, 1993). These flies were then crossed with stocks bearing a transgene in which the *Gal4*-responsive promoter, UAS, drives the expression of either β-galactosidase (LacZ) or a C-terminal fusion

of green fluorescent protein (GFP) to neuronal synaptobrevin (*nsyb*-GFP) (Estes et al., 2000). The expression of LacZ in specific subpopulations of sensory neurons allows the visualization of cell bodies, whereas the expression of *nsyb*-GFP, which selectively labels synaptic vesicles in nerve terminals, allows the visualization of terminal axonal projections (Ito et al., 1998; Estes et al., 2000).

Two to eight kilobases of DNA immediately upstream of the putative translation start from five *DOR* genes were fused to the coding sequence of *Gal4*. To demonstrate that these transgenes recapitulate the expression of the endogenous gene, we crossed the *DOR-Gal4* strains with UAS-*lacZ* responders. The progeny of these crosses express LacZ in spatially defined subsets of cells in the antenna or maxillary palp that mirror the pattern of expression of the endogenous receptor (Figures 4A–4E). To confirm that these cells are sensory neurons, we demonstrated that cells expressing LacZ are also labeled with an antibody that recognizes the neuron-specific RNA binding protein Elav (Robinow and White, 1988) (Figures 4G and 4I). As a further test for the fidelity of expression of the promoter fusions, we demonstrated that all cells expressing LacZ from the transgenes also express the corresponding endogenous odorant receptor RNA. In these experiments, RNA in situ hybridization was used to detect endogenous receptor RNA and immunofluorescence localized the expression of LacZ protein. Each of five *DOR* promoter fusions recapitulates the pattern of expression of the endogenous receptor gene (Figure 4F and 4H; data not shown).

We then used the *DOR-Gal4* transgenes to drive the expression of UAS-*nsyb*-GFP to visualize the projections of different populations of sensory neurons.
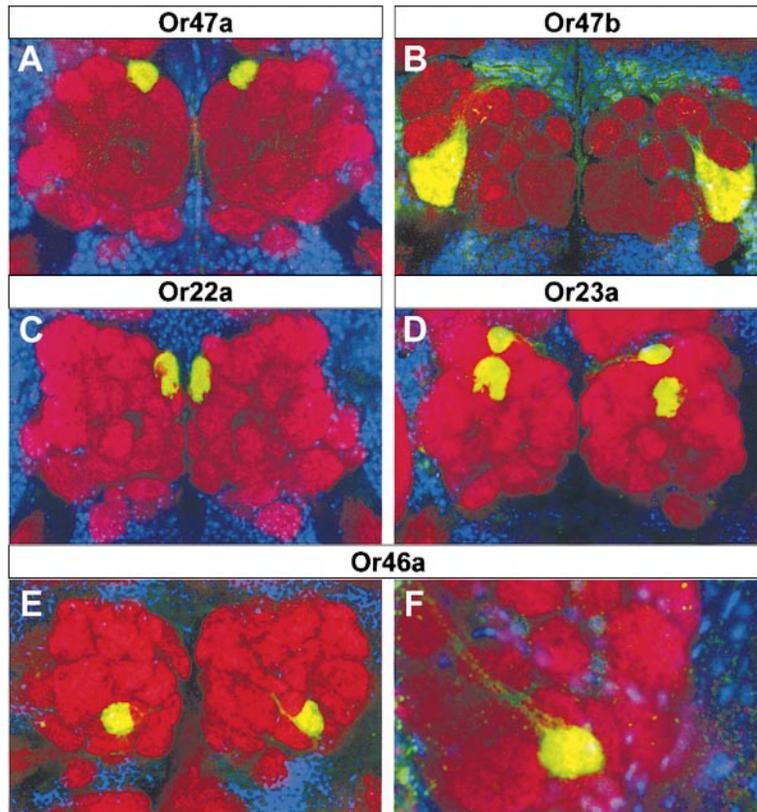
Figure 5. Neurons Expressing a Given *DOR* Gene Converge upon One or Two Spatially Invariant Antennal Lobe Glomeruli

(A–D) Cells expressing *nsyb*-GFP under the control of the four antennal *DOR* gene promoters (*Or47a*, *Or47b*, *Or22a*, and *Or23a*) project to distinct glomeruli. In all panels, *DOR-Gal4*:UAS-*nsyb*-GFP brain whole-mount preparations were stained with anti-GFP in green, the monoclonal antibody nc82 recognizing neuropil in red, and the nuclear stain TOTO-3 in blue. All preparations are fly brain whole mounts viewed frontally with dorsal up. (E–F) Neurons expressing *Or46a* converge upon a single ventral glomerulus located at an anterior position in the antennal lobe. (E) shows a low power view of the bilaterally symmetric *Or46a* glomeruli. (F) shows a higher power view of an antennal lobe highlighting the *Or46a*-expressing axon fascicles as they converge upon the *Or46a* glomerulus. The reagents used to stain these preparations are identical to those in Figures 5A–5D.

Flies carrying five different *DOR-Gal4* transgenes were crossed with animals bearing a UAS-*nsyb*-GFP transgene, and the brains of the adult progeny were then examined for localization of GFP. Immunofluorescence was performed on whole-mount brain preparations with antibody directed against GFP and with the monoclonal antibody, nc82, which labels neuropil in the fly brain and identifies the individual glomeruli in the antennal lobe (Laissue et al., 1999). Four *DOR-Gal4* promoter fusions (*Or47a*, *Or47b*, *Or22a*, and *Or23a*) are expressed in subpopulations of antennal neurons. When these flies are crossed with flies carrying the UAS-*nsyb*-GFP transgene, GFP labeling was seen in spatially invariant subsets of glomeruli within the antennal lobe (Figures 5A–5D). *Or47a*, for example, is expressed in 20 lateral distal neurons and their axonal projections converge on a single bilaterally symmetric glomerulus located at the dorsal medial limit of the antennal lobe (Figure 5A). *Or47b* is expressed in 50 lateral distal neurons and these cells project axons that converge on a single large glomerulus that lies at the ventral lateral edge of the antennal lobe (Figure 5B). Neurons expressing *Or22a* converge upon one dorsal glomerulus (Figure 5C). We have also visualized the projections of one of the seven different subpopulations of sensory neurons from the maxillary palp. Sensory neurons expressing the palp receptor, *Or46a*, project to a single glomerulus that resides ventrally in the antennal lobe (Figures 5E and 5F).

These four subpopulations of olfactory sensory neurons therefore project axons to single spatially invariant, bilaterally symmetric glomerulus within the antennal lobe. Cells expressing *Or23a*, however, send processes to two glomeruli (Figure 5D). *Or23a* fibers enter the brain and initially converge upon a small dorsal glomerulus.

Axons are seen extending more ventrally into the anterior of the antennal lobe where they converge upon a larger glomerulus. These two *Or23a* glomeruli are bilaterally symmetric and positionally invariant in multiple independent transgenic lines. It is not possible at present to determine whether individual fibers branch to form synapses within the two glomeruli nor if *Or23a*-expressing neurons sort such that individual subpopulations project to either one of the two glomeruli.

The topographic map of all five populations of olfactory sensory projections in the antennal lobe is unrelated to spatial domains of receptor expression in the antenna (Figure 7). For instance, while the 20 neurons that express *Or22a* in a proximal medial domain of the antenna converge upon a dorsal medial glomerulus, the *Or47a* glomerulus is situated directly adjacent to the *Or22a* glomerulus but receives input from cells in the lateral distal domain of the antenna. These precise patterns of glomerular convergence were observed in at least 20 individuals derived from multiple independent transgenic lines obtained for each of the four constructs. At a low frequency (1/100 flies), we find that *Or47a*-expressing olfactory neurons project to two bilaterally symmetric medial glomeruli in addition to the OR47a glomerulus. The basis for this variation in axon targeting is unknown.

In a control experiment, we demonstrated that the expression of *nsyb*-GFP does not perturb the normal pattern of axonal projections in the *Drosophila* visual system. In the adult visual system of the fly, the crystalline array of photoreceptors and the spatially ordered projection of axons to the optic lobe provides a sensitive readout for perturbations in the development of a sensory map (Simon et al., 1991; Martin et al., 1995). We
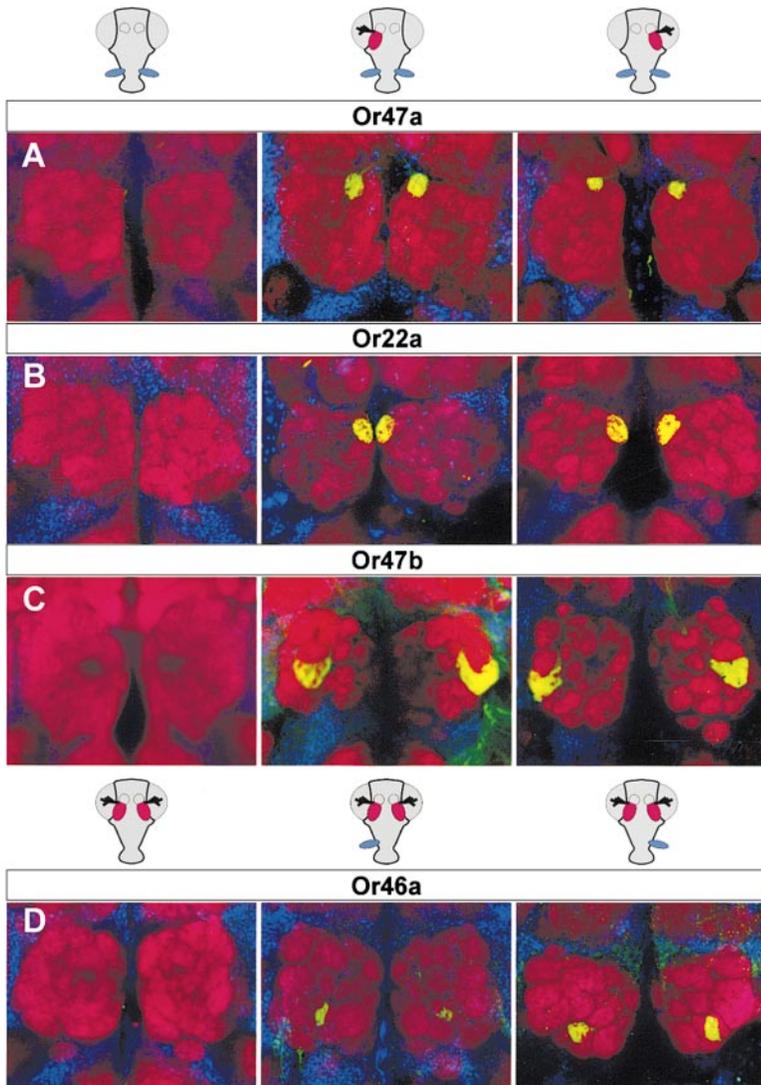
Figure 6. Olfactory Neurons Expressing a Given *DOR* Gene Synapse with Both Contralateral and Ipsilateral Glomeruli

Animals carrying *Or47a-Gal4*:UAS-*nsyb*-GFP (A), *Or47b-Gal4*:UAS-*nsyb*-GFP (B), or *Or22a-Gal4*:UAS-*nsyb*-GFP (C) transgenes were subjected to surgical deafferentation. Either both antennae (left panel), or only the left antenna (center panel) or right antenna (right panel) was removed and animals examined 15 days after surgery for glomerular convergence as described in Figure 5. Schematic drawings of fly heads above each panel indicate which organ has been removed. Bilateral deafferentation resulted in a complete loss of *nsyb*-GFP staining in the brains of all three strains (left panel). Animals lacking left antennae (center panel) or right antennae (right panel) nevertheless show labeling in positionally appropriate glomeruli in both antennal lobes. Therefore, sensory neurons from each side innervate both contralateral and ipsilateral antennal lobes. Similar analysis of the *Or46a* glomerulus in *Or46a-Gal4*:UAS-*nsyb*-GFP animals (D) using either bilateral or unilateral deafferentation of the maxillary palp produced the same result.

crossed UAS-*nsyb*-GFP flies to flies carrying the *GMR-Gal4* transgene that is strongly expressed in the visual system (Hay et al., 1994). We find no effect of *nsyb*-GFP expression on the external morphology of the eye, and the axonal projections of the photoreceptor neurons is wild type (data not shown). Thus, the expression of *nsyb*-GFP alters neither neuronal morphology nor circuitry and can be used to trace the projections of neurons in the fly brain. These control experiments provide additional support for a model in which individual sensory neurons expressing a given receptor gene project to one or a small number of spatially invariant glomeruli within the antennal lobe to generate an olfactory sensory map.

Antennal Neurons Project Both Ipsi- and Contralaterally

Anatomical tracing experiments in *Drosophila* have defined two subtypes of olfactory neurons in the antenna: those that project solely to the ipsilateral antennal lobe and others that branch and project to bilaterally symmetric glomeruli (Stocker et al., 1983, 1990). We have traced the axonal projections from neurons expressing a given

receptor in a single antenna or maxillary palp to both the left and right antennal lobe. Removal of both the antenna and maxillary palp from one side of the fly results in effective deafferentation. In control experiments, we surgically removed either both antennae or both maxillary palps from four different *DOR-Gal4* lines carrying a UAS-*nsyb*-GFP transgene. At 15 days postsurgery, no evidence of GFP labeling was evident in the antennal lobes (Figures 6A–6D, left panels). Thus, surgical removal of olfactory organs results in effective deafferentation.

When either the left or right sensory organs were removed singly, we find that the projections from the remaining antenna or maxillary palp converge upon the correct glomerulus in both the left and right antennal lobes (Figures 6A–6D, center and right panels, respectively). The position and size of the glomerulus is unaffected by unilateral deafferentation, but the density of *nsyb*-GFP-labeled fibers was decreased by about half. These data indicate that each of the four distinct subsets of olfactory neurons extend axons that innervate both the ipsilateral and contralateral antennal lobes equally. It is not possible from these studies to discern if individual
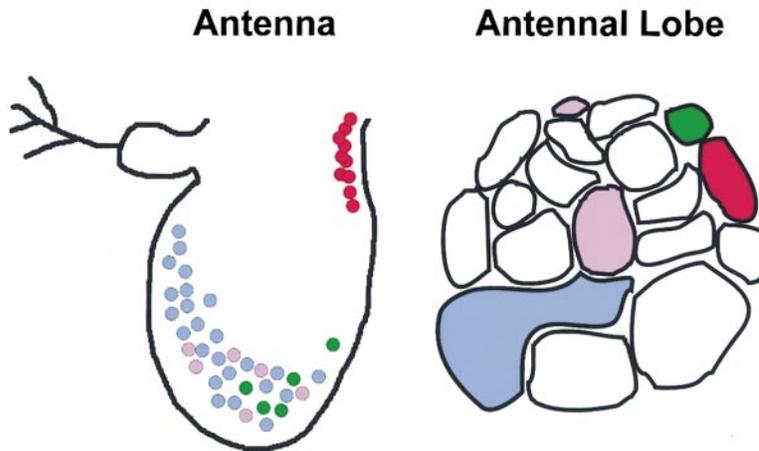
Antenna   Antennal Lobe

Figure 7. Spatial Maps in Peripheral and Central Olfactory Tissues in *Drosophila*

Schematic drawings of *DOR* gene expression in the antenna (left) and corresponding glomerular convergence in the antennal lobe (right). The relative position and number of cells expressing four *DOR* genes is indicated by the following color code: *Or22a* (red), *Or47b* (light blue), *Or47a* (green), and *Or23a* (lavender). In the right panel, a schematic drawing of an antennal lobe showing the relative dorsal (top) and medial (right) positions of glomeruli receiving projections from these four populations of neurons. Note that there is no relationship between the topographic position of cells in the antenna and the position of the glomerular target in the antennal lobe. While neurons expressing *Or22a* reside in a dorsal medial domain of the antenna and converge upon a dorsal medial glomerulus (red), the adjacent glomerulus (green) receives input from lateral distal neurons in the antenna expressing *Or47a*. There is, however, a relationship between the number of cells expressing a given receptor in the periphery and the size of the glomerulus. This is especially apparent for *Or47b*, expressed in ∼50 neurons and converging upon a very large ventral lateral glomerulus (light blue). Glomerular color codes match those in the antennal schematic: *Or22a* (red), *Or47b* (light blue), *Or47a* (green), and *Or23a* (lavender).

axons branch or if distinct populations of axons independently sort to the left and right antennal lobes. However, previous work by others demonstrated that afferent axons split into ipsilateral and contralateral branches (Stocker et al., 1983, 1990). Thus, neurons expressing a given receptor project axons both ipsi- and contralaterally to one or two spatially invariant glomeruli creating a topographic map of receptor activation in the antennal lobe.

Discussion

Most animals have evolved a mechanism to recognize olfactory 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. In this study, we address the way in which olfactory information is represented in the insect brain. Insects provide an attractive model system for the study of olfactory perception because they exhibit sophisticated olfactory driven behaviors that are controlled by an olfactory system that is anatomically and genetically simpler than vertebrates.

The Size of the Gene Family
An initial understanding of the logic of olfactory discrimination in *Drosophila* requires a knowledge of the number of odorant receptors expressed by the organism, the pattern of receptor expression in sensory neurons, and the way in which olfactory sensory neurons are wired into the brain. We have identified 57 *DOR* genes in the recent version (Version 1.0) of the *Drosophila* euchromatic genome (Adams et al., 2000; Rubin et al., 2000). Assuming that a disproportionately large number of *DOR* genes do not reside within the heterochromatic genome, the *DOR* gene family is small by comparison with *C. elegans* and mammals and more closely approximates the size of the receptor gene families in birds and fish. The individual receptor gene families not only differ vastly in size across species but are also often highly divergent with little or no sequence homology. Thus, a common function, olfactory perception, recruits different gene families of widely differing sizes and sequence to accommodate the unique requirements of individual species (Vosshall et al., 1999).

The diversity of odors recognized by a species is likely to be a function of the number of different odorant receptors it expresses. Thus, the repertoire of odors detected by the fruit fly may be small and may reflect the diminished importance of olfaction in insect species with a highly developed visual system. *Drosophila*, with rotting fruit as its sole source of food, may have evolved a narrow *DOR* gene repertoire that adequately accommodates its ecological niche. However, the true range of odors detectable by fruit flies in their natural environment has not been examined, nor has there been an analysis of subtle aspects of odor discrimination.

It should be noted that even a relatively small repertoire of receptors and an equivalent number of glomeruli could allow the discrimination of a vast array of odorants that numerically far exceed the size of the receptor repertoire. Imaging studies in both invertebrates and vertebrates reveal that a given odor, even if composed of a single molecular species, activates multiple glomeruli (Kauer et al., 1987; Joerges et al., 1997; Friedrich and Korsching, 1997; Galizia et al., 1999; Rubin and Katz, 1999; Sachse et al., 1999). This spatial pattern of glomerular activation may constitute a combinatorial code that would be read in higher sensory centers. If a given odor activates ten receptors and therefore ten glomeruli, then a repertoire of 40 receptors could generate over 10^8 different spatial patterns. Moreover, the relatively small number of odorant receptor genes in *Drosophila* may not be representative of other insects. The honeybee, *Apis mellifera*, has about 160 glomeruli and by inference 160 different odorant receptors, suggesting that in this insect, olfaction may be a more central means of acquiring sensory information from the environment (Menzel and Müller, 1996). Whatever the fruit fly's sensory capabilities, its olfactory sensory system is endowed with a relatively small number of receptor genes.

Of the 57 *DOR* genes in the *Drosophila* genome, 39 are expressed in subpopulations of sensory neurons, and one gene (*Or83b*) is expressed in all olfactory sensory neurons. We did not detect expression of 17 genes in either embryonic, larval, or adult olfactory organs or

in any other regions of the adult head. Despite the inability to detect expression of these 17 genes by in situ hybridization, analysis of their sequence does not reveal nonsense or frame shift mutations that characterize pseudogenes within families of odorant receptors in other species. If these sequences indeed represent pseudogenes, then the annotation generating an intact coding sequence may be incorrect. Alternatively, these 17 genes may be expressed either in olfactory organs or elsewhere at levels below that which can be detected by our in situ hybridization techniques (5–10 mRNAs per cell). This level of expression is far below estimates of receptor mRNA content in olfactory neurons in any species examined. However, other studies have employed RT–PCR to demonstrate the expression within olfactory sensory organs of several of the 17 *DOR* genes we fail to detect by in situ hybridization (Clyne et al., 1999b). Experiments in which the putative promoter regions from these genes are used to drive the expression of *Gal4* transgenes will provide more definitive information concerning these 17 genes.

Sensory Neurons Express One *DOR* Gene and *Or83b*

The diversity of receptor expression in individual sensory neurons has important implications for odor discrimination. In *C. elegans* it is estimated that chemosensory cells express up to 20 receptor genes, whereas in mammals neurons express only a single receptor. Discrimination requires that the brain determine which receptors have been activated by an odorant. If a sensory neuron expresses only one receptor, then this problem reduces to the simpler requirement that the brain discern which neurons have been activated by a given odorant.

In the fly, individual neurons express the *Or83b* along with only one of the remaining *DOR* genes. This is perhaps easiest to demonstrate in the maxillary palp, which expresses seven *DOR* genes and *Or83b*. The seven *DOR* genes expressed in the maxillary palp together hybridize with about 120 cells. This value is close to the total number of neurons in the palp, suggesting that we can identify every neuron in the palp with seven genes from the *DOR* gene family. Moreover, two-color in situ hybridization with either pairwise combinations or mixes of these genes as probes consistently reveal that a sensory neuron expresses a single receptor gene. Our data suggesting that neurons express only a single receptor are difficult to reconcile with electrophysiological recordings that have been interpreted to indicate that individual palp neurons express multiple receptors (Clyne et al., 1999a).

What is the role of *Or83b*, the only *DOR* gene family member expressed in all olfactory sensory neurons? *Or83b* might encode a receptor capable of binding odorous ligands, thus affording each cell a common and a unique specificity. A model we favor argues that *Or83b* does not serve as an odorant receptor but performs a function in sensory neurons independent of ligand binding. For example, recent studies reveal that the metabotropic GABA$_B$ receptor is composed of a heterodimeric pair of seven transmembrane domain proteins. Surface expression and functional ligand binding of the GABA$_B$R1 subunit is obtained only upon coexpression of a second GABA$_B$ receptor, GABA$_B$R2 (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998). Thus,

Or83b might perform a function essential for chemosensory signaling independent of odor binding, thereby retaining the one neuron–one receptor rule.

A Spatial Map in the Antenna

In situ hybridization with 39 of the 57 *DOR* genes reveals that each receptor is expressed in a spatially restricted subpopulation of cells in the antenna and palp. Both the pattern of receptor expression and the number of neurons expressing a given receptor is conserved in different individuals. Some genes are expressed in as few as two spatially defined cells in the antenna, whereas other genes are expressed in as many as 50 neurons. These experiments with the "complete" set of receptor genes define a neural epithelium in which each of 1000 cells in the antenna is marked by the expression of one of 32 unique genes. We presume that this spatial patterning is a consequence of axes of positional information that ultimately dictate the expression of specific genes. At present, we cannot determine the precision of the pattern of specific receptor expression we observe. Nonetheless, the fine control of neural identity reflected by receptor expression is likely to be distinct from the more coarse patterning that generates the three morphologically distinct sensilla (trichoid, basiconic, and coeloconic) that populate the olfactory sensory organs (Ray and Rodrigues, 1995; Reddy et al., 1997; Goulding et al., 2000).

If, as we suggest, graded positional information governs a spatial map of receptor expression in the periphery, why do we observe an interdigitating pattern in which two cells expressing a given receptor are interspersed by a cell expressing a different receptor? It is possible that precise patterning to the level of individual cells indeed occurs early in development and neural migration results in local disruptions in the peripheral map. Alternatively, positional information may dictate a set of defined spatial domains within which the expression of not one but a subset of receptors is permitted. This latter suggestion is reminiscent of a model of receptor expression in mammals in which a given receptor gene is expressed within one of four broad but circumscribed zones within the olfactory epithelium of the nose. Within a zone, however, neurons expressing a given receptor appear randomly dispersed (Ressler et al., 1993; Vassar et al., 1993). Such a mechanism would also be consistent with the patterns of expression of receptor genes in the fly antenna. More precise reconstructions of the spatial patterns of expression of multiple different receptors will be required to distinguish these alternatives.

One distinction between receptor expression in mice and flies is that in mice neurons express a single receptor gene from either the maternal or paternal allele, but never from both alleles (Chess et al., 1994). Moreover, in mice neurons that express an odorant receptor transgene do not express the same gene from its endogenous locus (Qasba and Reed, 1998; Shykind, Rohani, and R. A., unpublished data). In contrast, in *Drosophila* we demonstrate that sensory neurons that express a transgene driven by a *DOR* promoter always express the appropriate endogenous *DOR* gene. In the fly, therefore, two *DOR* promoters can be activated in the same cell, arguing that monoallelic expression is not occurring in *Drosophila* olfactory neurons.

A Sensory Map in the *Drosophila* Brain

How does the brain determine which neurons have been activated by an odorant? Genetic experiments with the family of *DOR* genes have allowed the visualization of a topographic map of sensory projections in the antennal lobe, the first relay of olfactory information in the fly brain. Neurons expressing a given receptor project their axons to either one or two topographically invariant glomeruli that generate an internal representation of receptor activation in the brain. Different odors will therefore activate different combinations of glomeruli such that the quality of an odor may initially be represented by different spatial patterns of activity within the antennal lobe.

The results of these genetic experiments are in accord with anatomic tracing experiments that demonstrate that individual sensory neurons project to only one of 43 morphologically distinct glomeruli (Stocker et al., 1983, 1990). Moreover, the number of anatomically defined glomeruli is remarkably close to the number of *DOR* genes expressed in the olfactory sensory organs. Our data are also consistent with 2-deoxyglucose mapping in the fruit fly (Rodrigues and Buchner, 1984; Rodrigues, 1988), functional tracing of pheromone-responsive neurons in the male moth (Hansson et al., 1992), and calcium imaging in the honeybee (Joerges et al., 1997; Galizia et al., 1999; Sachse et al., 1999) that demonstrate that different odorants elicit distinct patterns of glomerular activity. Our genetic tracing experiments demonstrating the convergence of like axons provide a physical basis for a functional map of odor quality. This spatial map may then be decoded in higher sensory centers within the brain to translate stimulus features into meaningful neural information.

The organization of the peripheral olfactory sensory system in the fruit fly is therefore remarkably similar to that in vertebrates. In mammals, olfactory neurons express only one of a thousand odorant receptor genes (Ressler et al., 1993; Vassar et al., 1993; Chess et al., 1994; Malnic et al., 1999). Neurons expressing a given receptor project with precision to two of 1800 glomeruli in the mouse olfactory bulb, the anatomical and functional equivalent of the antennal lobe (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Wang et al., 1998). In vertebrates, as in *Drosophila*, physiological studies reveal that different odors elicit different patterns of glomerular activity within the bulb (Stewart et al., 1979; Lancet et al., 1982; Kauer et al., 1987; Imamura et al., 1992; Mori et al., 1992; Katoh et al., 1993; Friedrich and Korsching, 1997; Rubin and Katz, 1999). Thus, the organization and functional logic of the complex process of olfactory sensory perception has been maintained over five hundred million years of evolution despite dramatic differences in other aspects of neural function. We suggest that this evolutionary conservation reflects the maintenance of an efficient solution to the complex problem of recognition and discrimination of a vast repertoire of odors in the environment.

Experimental Procedures

Experimental Animals

Flies were raised on standard cornmeal-agar-molasses medium at 25°C. Oregon R (*Drosophila melanogaster*) and *yw* strains were used for in situ hybridization experiments. Transgenic constructs were injected as mixtures of two constructs into *yw* embryos using a final concentration of 1 mg/ml DNA construct DNA and 0.225 mg/ml wings clipped turbo helper DNA. Single male transformants were outcrossed to autosomal balancer stocks and resulting progeny analyzed by polymerase chain reaction (PCR) to determine their genotype. At least five independent transgenic lines were generated for each construct. UAS-*nsyb*-GFP (UNG12) flies were a gift of Dr. Mani Ramaswami. Peripheral deafferentation was performed by manually removing left, right, or both antennae or maxillary palps from anesthetized young adult flies with forceps. Animals were allowed to recover in fresh vials and aged for 15 days at 25°C before projection patterns were analyzed as described below.

Isolation of New *DOR* Genes

Additional members of the *DOR* gene family were isolated by BLAST (Altschul et al., 1990) and hidden Markov model (Eddy, 1998) searches of *Drosophila* genome databases with existing members of the *DOR* gene family. The predicted protein sequences of all 57 *DOR* genes are available at this URL: http://cpmcnet.columbia.edu/dept/neurobeh/axel/dorg.html

DOR genes were isolated by PCR from antennal/maxillary palp cDNA or genomic DNA using primers that correspond to the extent of the predicted coding region. PCR products were subcloned into pGEM-T (Promega), and their ends sequenced using ABI chemistry and instrumentation.

Generation of *DOR* Promoter Element Transgenes

DOR gene regulatory sequences were obtained with long-range PCR using the Expand High Fidelity PCR System (Roche) and genomic DNA as a template. Sequences immediately adjacent to predicted ATG initiation codon and a variable distance upstream (*Or23a-Gal4*, 7.818 Kb; *Or22a-Gal4*, 7.717 Kb; *Or47a-Gal4*, 8.239 Kb; *Or47b-Gal4*, 7.467 Kb; *Or46a-VP22-Gal4*, 1.875 Kb) were isolated and subcloned into a CaSpeR-AUG-*Gal4* vector with rare-cutting enzyme sites in the polylinker (L. B. Vosshall, unpublished data). *Or46a-VP22-Gal4* consisted of a fusion of the HSV-1 structural protein VP22 (Elliott and O'Hare, 1997) to *Gal4*. The activity of this fusion protein was indistinguishable from that of unmodified *Gal4* and did not show the intercellular protein transfer properties of unmodified VP22 (Elliott and O'Hare, 1997).

In Situ Hybridization and Immunocytochemistry

Antisense digoxigenin riboprobes for all 57 *DOR* genes were generated using standard methods. RNA in situ hybridization visualized by alkaline phosphatase and immunofluorescence was performed as previously described (Vosshall et al., 1999; see also http://cpmcnet.columbia.edu/dept/neurobeh/axel/personal/insitu.html). Alkaline phosphatase experiments were documented with a Nikon SPOT-RT digital microscope system equipped with differential interference contrast. Fluorescent RNA in situ hybridization was performed as described (Vosshall et al., 1999) using digoxigenin- and fluorescein-labeled RNA probes, except that donkey anti-mouse coupled to CY3 (Jackson ImmunoResearch) and donkey anti-sheep coupled to Alexa Fluor 488 (Molecular Probes) were employed as secondary antibodies. For mixed in situ hybridization/immunofluorescence, sections were first annealed to DIG labeled antisense RNA probes, and then incubated with sheep anti-DIG (Roche) and rabbit anti-β-galactosidase (Cappel/ICN) antibodies and visualized with donkey anti-sheep Alexa Fluor 488 (Molecular Probes) and donkey anti-rabbit CY3 (Jackson ImmunoResearch) secondary antibodies. Images were analyzed with a Biorad 1024 confocal microscope.

Double immunofluorescence to colocalize LacZ and the neuronal marker Elav used a rabbit anti-β-galactosidase antibody (Cappel/ICN) and the mouse monoclonal Elav-9F8A9 and fluorescent secondary antibodies described above. The Elav-9F8A9 monoclonal antibody developed by G. M. Rubin was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242.

Dissection and antibody staining of adult brain whole mounts was performed exactly as described in Laissue et al. (1999), using the nc82 antibody (kindly provided by Professor Reini Stocker), which was visualized with a 1:100 dilution of goat anti-mouse IgG coupled to CY3 (Jackson ImmunoResearch). Expression of *nsyb*-GFP was

visualized with a 1:1000 dilution of anti-GFP antibody (Molecular Probes) and a 1:100 dilution of goat anti-mouse secondary antibody coupled to Alexa Fluor 488 (Molecular Probes). Nuclei were counterstained with a 1:1000 dilution of TOTO-3 (Molecular Probes). Brains were mounted in Vectashield (Vector Labs) using small cover slips as spacers and analyzed with a Biorad 1024 confocal microscope.

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Note Added in Proof

Since the submission of this paper, we have found two additional odorant receptors in *Drosophila* genome databases, *Or65b* and *Or65c*, tightly linked to *Or65a* at chromosomal position 65A7-11. Both are expressed in subsets of antennal neurons, bringing the total number of expressed *DOR* genes to 41, with 34 genes expressed in the antenna and seven expressed in the maxillary palp. The nomenclature of this gene family has recently been unified by a scientific consortium in consultation with FlyBase and is used throughout this paper. A complete description of the *DOR* nomenclature, including a comprehensive listing of previous names, map positions, and annotation information, appears in *Drosophila* Odorant Receptor Nomenclature Committee (2000).