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Odor Coding in the *Drosophila* Antenna

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Summary

Odor coding in the *Drosophila* antenna is examined by a functional analysis of individual olfactory receptor neurons (ORNs) in vivo. Sixteen distinct classes of ORNs, each with a unique response spectrum to a panel of 47 diverse odors, are identified by extracellular recordings. ORNs exhibit multiple modes of response dynamics: an individual neuron can show either excitatory or inhibitory responses, and can exhibit different modes of termination kinetics, when stimulated with different odors. The 16 ORN classes are combined in stereotyped configurations within seven functional types of basiconic sensilla. One sensillum type contains four ORNs and the others contain two neurons, combined according to a strict pairing rule. We provide a functional map of ORNs, showing that each ORN class is restricted to a particular spatial domain on the antennal surface.

Introduction

Animals have evolved olfactory systems of remarkable sensitivity and discriminatory power to process chemical information from their environments (Buck, 1996; Hildebrand and Shepherd, 1997). Olfactory receptor neurons (ORNs) encode an immense variety of odors. The identity of an olfactory stimulus, its intensity, and its temporal profile are all somehow represented in the activities of a diverse population of these neurons. An understanding of peripheral coding is essential to an understanding of how information is processed in the CNS, eventually providing instructions that guide the organism to resources in its environment.

Some insight into coding mechanisms can be gained by describing the anatomy—cellular or molecular—of the olfactory system. For example, detailed analysis of odor receptor gene expression and ORN projection patterns has led to new understanding of olfactory system organization and has been invaluable in formulating models of information processing (Buck, 1996; Mombaerts, 1999). A complementary approach is functional analysis of the system. Physiological measurement of the activity of ORNs allows a direct assessment of their responses to odors. Peripheral coding ultimately depends on the number of distinct functional classes of ORNs and their response spectra. The odor specificity, sensitivity, response dynamics, and spatial organization of ORNs within the sensory field can be determined to elucidate fundamental principles of odor coding.

We have carried out a functional analysis of odor coding in the *Drosophila* antenna, a system that is attractive for such a study in three respects. First, physiological recordings can be made from ORNs in vivo (Siddiqi, 1991; Clyne et al., 1997). Second, the fly's olfactory system is numerically simple; its ORNs can be classified into a limited number of distinct functional types (de Bruyne et al., 1999). Third, the results of physiological analysis can be integrated with the results of molecular, genetic, anatomical, and behavioral studies (Carlson, 1996; Vosshall, 2000) to construct an integrated model of odor coding.

Drosophila has two pairs of olfactory organs, the antennae and the maxillary palps (Figure 1A). Each antenna contains ~1200 ORNs (Stocker, 1994). As in other insects, these ORNs are compartmentalized in sensilla that protrude from the antennal cuticle, allowing easy access for electrophysiological recording (Boeckh, 1981). The sensilla are of three morphological types (Figure 1B): basiconic sensilla, trichoid sensilla, and coeloconic sensilla (Venkatesh and Singh, 1984). The most numerous are the basiconic sensilla ($n \approx 200$), which can in turn be divided into two subtypes distinguishable by light microscopy: large and small (Figure 1C). The antennal basiconic sensilla have previously been shown to be olfactory, and limited data have indicated that they are heterogeneous in terms of the odors to which they respond and in the distributions of response types (Siddiqi, 1991). However, there are virtually no published data concerning the responses of the individual ORNs that innervate these sensilla.

The axons of ORNs project directly to the antennal lobe, the insect equivalent of the vertebrate olfactory bulb, which in *Drosophila* contains 41 defined olfactory glomeruli (Laissue et al., 1999). A large family of genes likely to encode odor receptors has been identified and contains 60 defined members (Clyne et al., 1999b; Vosshall et al., 1999, 2000; see also Gao and Chess, 1999). Individual members are expressed in different distribution patterns in the antenna, and the axons of ORNs expressing different genes converge on distinct glomeruli of the antennal lobe (Vosshall et al., 2000; Gao et al., 2000), suggesting that ORNs with different odor specificities show different spatial distributions and project to distinct glomeruli.

Here we describe an electrophysiological analysis of odor coding in the fly antenna. We define 16 functional types of neurons and analyze their odor specificities with respect to a panel of 47 odors representing diverse chemical classes. ORNs are found to exhibit diversity not only in their odor response spectra but also in their response dynamics. We document both excitatory and inhibitory responses as well as different modes of response termination. We also show that these different ORN types are combined in stereotyped configurations within seven functional types of basiconic sensillum, and we provide a functional map of ORNs on the antennal surface. Their spatial distributions are reminiscent

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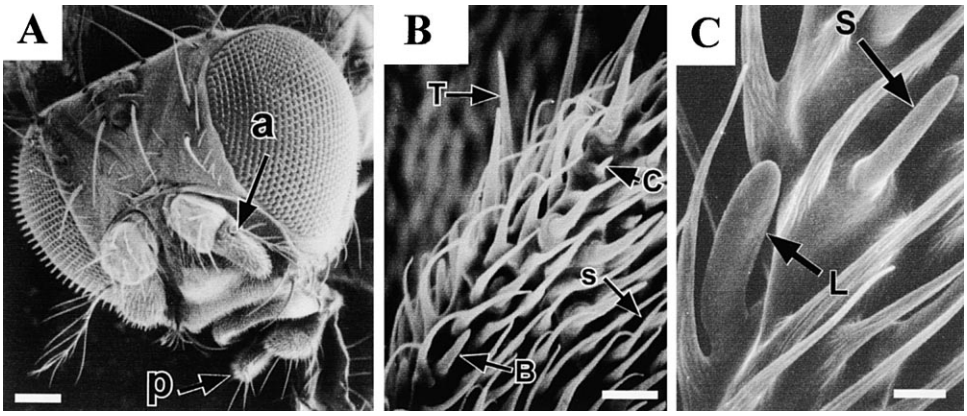

Figure 1. Olfactory Sensilla

(A) Olfactory sensilla on the third segment of the antenna (a) and the maxillary palp (p). Scale bar, 100 μm. From Riesgo-Escovar et al. (1995).
(B) The dorso-medial surface of the third antennal segment, showing the three morphological categories of olfactory sensilla: basiconic ("B"), trichoid ("T"), and coeloconic ("C"), as well as uninnervated hairs called spinules ("s"). Scale bar, 5 μm.
(C) The two varieties of basiconic sensilla: large (L) and small (S). Scale bar, 2 μm. (B) and (C) are from Riesgo-Escovar et al. (1997).

of receptor gene expression patterns. Most neurons appear tuned to a small subset of compounds, and dose-response analysis shows high sensitivity of several ORN types to particular odorants, which are likely to be high-affinity ligands for their respective receptors.

Results

Two or Four Distinguishable ORNs Occupy Each Antennal Basiconic Sensillum

Electrophysiological recordings from basiconic sensilla on the antenna consistently showed spontaneous action potentials (Figure 2A). Spikes from most recordings could be resolved into two populations based on their amplitudes (Figures 2A and 2B). The two populations of spikes in these sensilla indicate the presence of two neurons, as found in basiconic sensilla on the maxillary palp (de Bruyne et al., 1999). We will refer to the neuron with the larger spike amplitude as the A neuron and the one with the smaller spikes as the B neuron.

Neurons showed excitatory responses to odors, with different neurons excited by different odors. For instance, the A neuron in Figure 2C increases its firing frequency in response to ethyl acetate, while the B neuron, whose spikes are indicated by the dots, is unaffected. By contrast, the B neuron in the same sensillum is excited by hexanol (Figure 2D). In another sensillum (Figure 2E), the A neuron is excited by 1-octen-3-ol, whereas the B neuron is unaffected. In this sensillum, the B neuron is excited by ethyl butyrate (Figure 2F); the A neuron also shows a modest increase in firing frequency. Changes in spike shape or amplitude are in some cases observed during the course of an excitatory response, as seen at the beginning of the response in Figure 2C; such changes have previously been observed in recordings from the *Drosophila* maxillary palp by us, and in many other insect species by others (de Bruyne et al., 1999, and references therein), and do not generally interfere with the reliable attribution of spikes to the A or B neuron.

Recordings of spontaneous activity from some large basiconic sensilla revealed the presence of four neurons, whose spike amplitudes fell into four classes (Figures 2G and 2H). These physiological results confirm ultrastructural observations that while most basiconic sensilla house two neurons, many large basiconic sensilla contain four neurons (Stocker, 1994; Shanbhag et al., 1999). Strikingly, one of these neurons, the C neuron, responded strongly to CO_2 (Figure 2I).

Spike classification was more difficult during periods of high activity. When one neuron responded strongly to an odor, it was sometimes more difficult to distinguish the firing of a second neuron in the same sensillum. Expanding the time scale of such traces greatly facilitated spike classification (Figures 2J and 2K). Spikes were manually classified based on shape as well as amplitude. Often we could identify the firing of a second neuron by the presence of overlapping spikes, recognizable by their altered amplitude or irregular shape, representing the summed activity of two neurons (Figures 2J and 2K). In Figure 2J, the two immediately adjacent peaks above the dot represent the superposition of A and B spikes. The amplitude of the A spike (right) is reduced because it is out of phase with the B spike (left). The interval between the two spikes is too short to have arisen from the same neuron. The most difficult sensillum to analyze was ab1, the sensillum that contains four neurons (Figure 2I), of which an expanded trace is shown in Figure 2K. Among the train of C spikes are three additional spikes of other neurons; the one on the right overlaps a C spike to yield two closely adjacent peaks (arrowhead) followed by a trough, representing the superposition of a B spike with the C spike to its left. Our conclusions about the specificity of ab1 neurons were confirmed by testing with lower odor doses and by analysis of sensilla in which the response patterns were simplified by the ablation—physical or genetic—of subsets of neurons (not shown). We did not analyze recordings in which a low signal-to-noise ratio prevented reliable spike sorting.

We note that it is difficult to estimate quantitatively the reliability of our spike classification methods. More

sophisticated, quantitative means of spike sorting based on various algorithms and statistical techniques have been developed (Fee et al., 1996; Lewicki, 1998) and could be applied, particularly during periods of high activity, in an attempt to resolve spikes with optimal accuracy.

Sixteen ORN Classes Are Housed in Stereotyped Combinations in Seven Functional Types of Basiconic Sensilla

We chose a set of 47 odorants to characterize the responses of neurons in antennal basiconic sensilla. The odorants were chosen from a variety of chemical classes and include compounds that contain branched chains, double bonds, two functional groups, and other structural features. Most odorants were chosen because of evidence that they play a role in the chemical ecology of *Drosophila* or other flies. In addition to testing a large number of neurons with the entire set of 47 odorants, we identified a subset of 12 diagnostic odorants that were particularly useful in identifying and distinguishing among neuronal classes; these 12 odorants were used in most subsequent experiments.

Extensive recordings from large basiconic sensilla revealed that they fall into three distinct types based on the odor response spectra of their ORNs. We refer to these three types as antennal basiconic (ab) types ab1, ab2, and ab3, as distinct from pb sensilla (basiconic sensilla on the maxillary palp [de Bruyne et al., 1999]). Each ab type contains a stereotyped combination of neurons. The response spectra of the ORNs within ab1, ab2, and ab3 sensilla to the 12 diagnostic odors are shown in Figure 3.

The ab1 sensillum is unique in that it houses four neurons, as described above. The A neuron within this sensillum type, referred to as ab1A, responds most strongly to ethyl acetate. This neuron also responds to several other odorants, notably ethyl butyrate, but we note that it is unusual in responding strongly to the diluent control stimulus, paraffin oil. It is likely that for this particular neuron, much of the response observed to other odors represents a response to an element of the delivery system (see Experimental Procedures). The ab1B, C, and D neurons respond with a high degree of specificity to 2,3-butanedione, CO_2, and methyl salicylate, respectively; they do not respond to the diluent control stimulus. The other two types of large basiconic sensilla, ab2 and ab3, contain only two neurons. ab2 sensilla contain an A neuron that responds strongly to ethyl acetate, with a somewhat weaker response to 2,3-butanedione, and a B neuron that responds moderately to ethyl butyrate and hexanol. ab3 contains an A neuron that is excited by ethyl butyrate and pentyl acetate and a B neuron that responds to heptanone and hexanol. We note further that the ORNs in the three types of large basiconic sensilla exhibit different levels of spontaneous activity (Figure 3, lower right corner of each graph). The spontaneous activities of the neurons in an individual sensillum, taken as a group, allowed us to predict reliably which of the three types the sensillum belonged to before odor spectra were analyzed. These levels ranged from 1 $\pm$ 1 spike/s in the case of the ab2B neuron to 15 $\pm$ 4 spikes/s in the case of ab1C.

This functional classification of eight neuronal types in large basiconic sensilla was confirmed through a cluster analysis (not shown), similar to that used in analyzing the ORNs of the maxillary palp (de Bruyne et al., 1999). The eight ORNs described here are distinct from the six of the maxillary palp. Furthermore, of the 145 large basiconic sensilla from which we recorded, all could be classified as either ab1, ab2, or ab3; we found no evidence for an additional type. The only exceptional recordings were from a small number of sensilla in which one of the neurons was apparently missing.

In addition to recording from large basiconic sensilla, we recorded from 128 small basiconic sensilla on the anterior and posterior surfaces of the third antennal segment. We defined an additional four sensillum types, called ab4, ab5, ab6, and ab7, based on odor response profiles (Figure 3). Each of the four sensillum types contains two neurons distinct from each other and from all other previously defined neuronal types, including those on the maxillary palp. Thus, we have defined an additional eight ORN classes housed within the small basiconic sensilla. Moreover, the neurons in the small basiconic sensilla, like those in the large basiconic sensilla, are housed in characteristic combinations; they observe a strict pairing rule.

The ab4 sensillum houses a neuron, ab4A, that responds strongly to E2-hexenal, a "green leaf volatile"; other insects have ORNs that appear to be narrowly tuned to this molecule (Hansson et al., 1999). Not only does this odor elicit a high frequency of firing from the ab4A neuron, but the neuron continues to fire above spontaneous levels for more than a minute following a 0.55 s pulse of E2-hexenal at our standard test concentration (see below). ab5A responds strongly to geranyl acetate, a monoterpene ester, whereas ab5B responds strongly to pentyl acetate and heptanone. ab6A shows a strong response to a number of the selected subset of 12 odors, but 1-octen-3-ol was distinguished by evoking a long-lasting excitation (shown below in Figure 6L) such as that for ab4A. The ab7A neuron shows moderate responses to several odors. None of the 12 odors strongly excited ab4B, ab6B, or ab7B. However, ab6B is excited by 4-methylphenol (shown below in Table 1) and inhibited by ethyl acetate and pentyl acetate, and ab7B is mildly excited by ethyl butyrate. We note that in addition to the differences in odor response spectrum, the neurons in the small basiconic sensilla exhibit differences in their spontaneous rate of firing, ranging from 2 $\pm$ 1 spikes/s to 14 $\pm$ 3 spikes/s (Figure 3).

Although all of the large basiconic sensilla from which we recorded could be clearly identified as ab1, 2, or 3, some of the small basiconic sensilla (23 of 128) did not fall into a well-defined category. The neurons in these sensilla exhibited spontaneous action potentials, and most yielded weak excitatory responses to at least some odors, but none responded strongly to any of the 47 odors in the full panel.

Odor Coding across Antennal ORNs

The foregoing analysis has identified a set of 16 physiologically distinct classes of ORN available to the fly for encoding olfactory information. How is information about odor quality and quantity encoded across this

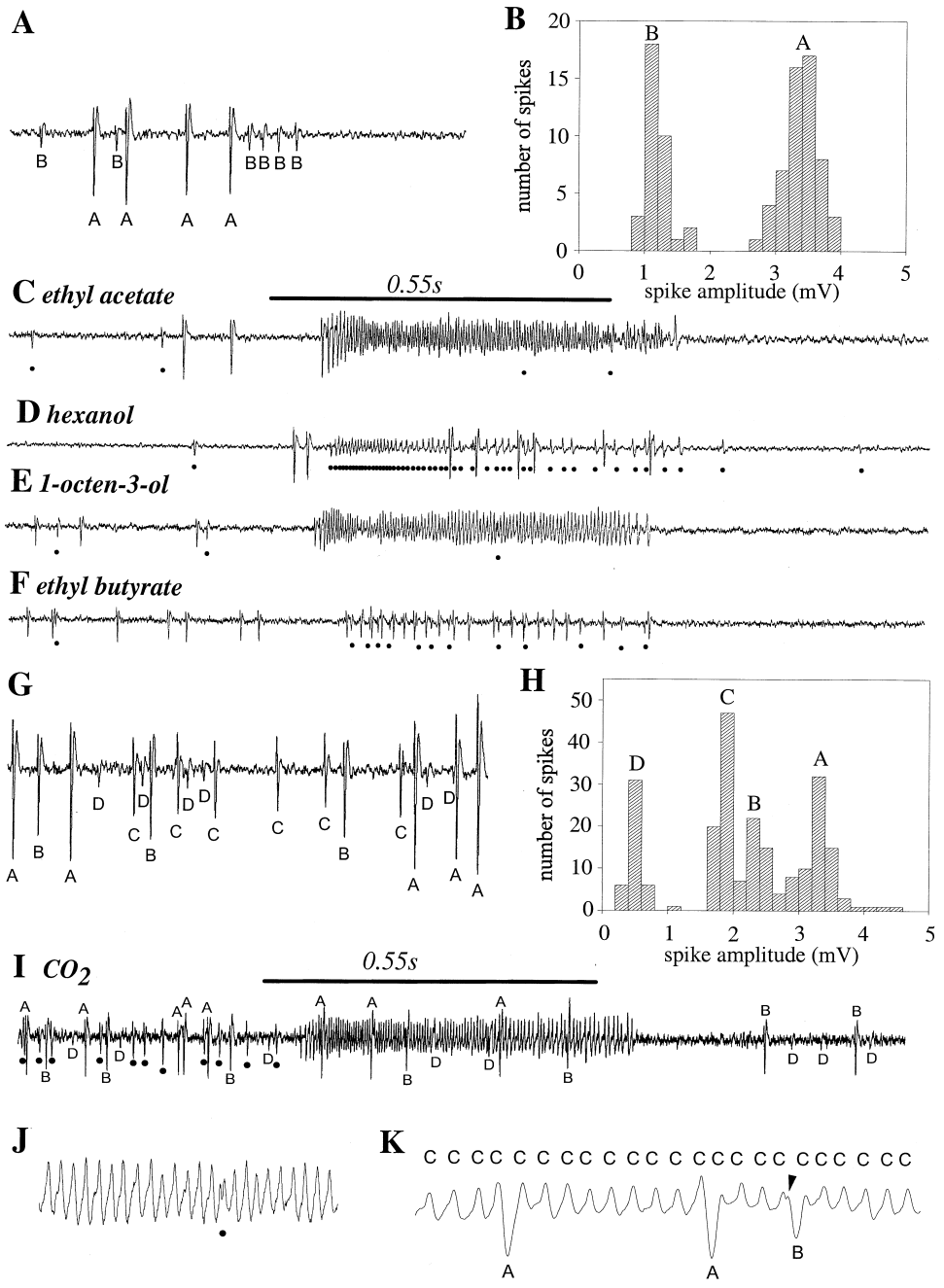

Figure 2. Extracellular Recordings from Basiconic Sensilla

(A) Spontaneous activity (1 s) of a basiconic sensillum later classified as ab3. Individual action potentials (spikes) are labeled A or B according to their amplitude.

(B) The bimodal distribution of spike amplitudes, as measured from peak to trough, in the recording shown in (A). Data shown represent 90 spikes from 6 s of the recording. "A" and "B" indicate subpopulations of spikes attributed to neurons A and B.

(C and D) Two 1500 ms traces of recordings from one sensillum, later classified as ab2, demonstrating different responses of the two neurons to different odors. Large action potentials, from the A neuron, increase in frequency in response to ethyl acetate (C). Dots indicate smaller action potentials from the B neuron, which is not excited by ethyl acetate but which responds to hexanol (D). The small spikes in the response to ethyl acetate can be distinguished more easily when the time scale is expanded. For odor stimulation (0.55 s, horizontal bar), air was expelled from a syringe over filter paper containing 20 μl of odorant, diluted 10^{-2} in paraffin oil. The concentration of the odor reaching the preparation is unknown.

(E and F) In another sensillum, subsequently classified as ab7, the A neuron is excited by 1-octen-3-ol, whereas the B neuron (dots) is unaffected. In this sensillum, the B neuron is moderately excited by ethyl butyrate; the A neuron also shows a modest increase in firing frequency.

(G) Spontaneous activity (1 s) from a large basiconic sensillum with four neurons, subsequently classified as ab1.

(H) Spike amplitude distribution, quadrimodal, of 231 spikes from 6 s of the recording in (G).

array of ORNs? Table 1 shows relative response magnitudes of these units to the 47 chemically diverse odors.

Most ORNs respond only to a limited subset of the tested stimuli. Specifically, 15% of the 752 entries (47 odors × 16 ORN classes) in Table 1 produced a response. Although we do not know how many molecules of each odorant reach the antenna in our paradigm, the odorant solutions used in this analysis were at a 10^{-2} dilution. Since higher concentrations of these odorants seem unlikely to be encountered often in nature, we suspect that the ORNs respond to an even smaller fraction of these compounds in the fly's natural environment. Moreover, the figure of 15% is not artificially low due to the choice of a large number of odors that the organism is unlikely to have encountered during the course of its evolution. Rather, many of the odors were selected on the basis of their roles in the chemical ecology of *Drosophila* or other flies, and 37 of the 47 odors elicit a response from at least one neuronal class. In fact, among the ten odors that did not elicit a response from the neurons characterized here are *cis*-vaccenyl acetate and 4-methylcyclohexanol, both of which evoke electroantennogram responses (Hing and Carlson, 1996; Borst, 1984), perhaps via antennal neurons innervating trichoid or coeloconic sensilla.

The response spectra exhibit a great deal of diversity. Most neurons respond most strongly to one or two odors of the test set, and the "best" odor is different for each neuronal class, with few exceptions. There are no cases in which two neuronal classes both exhibit the strongest category of odor response, designated "++++," to the same odor. For most odors, no more than 2 of the 16 neuronal classes respond; notably, 10 odors elicit a response from only one neuronal class.

The specificity of individual neurons ranges between two extremes. At one extreme lie five neurons that respond to one or none of the test stimuli. These neurons include ab1C, which responds strongly to CO_2, and to no other stimuli. CO_2 is an important cue for host-seeking insects such as mosquitos and tsetse flies, and it may also serve as a cue to *Drosophila* in locating fermenting fruits. ab4B responds to none of the tested odors, and ab7B responds to only one, weakly. These two neurons seem likely to be tuned to molecules not contained within our panel. At the other extreme of specificity lies ab6A, which is highly promiscuous. It responds to nearly half of the tested stimuli, many of which show little if any structural similarity. Interestingly, ab7A shows a spectral pattern highly related to that of ab6A; the two patterns are, however, distinct in that some odors (e.g., E2 hexenyl acetate) excite ab6A more strongly than ab7A while for other odors (e.g., iso-amyl acetate), the converse is true.

A particularly interesting spectrum is observed for ab5B. This neuron yields a strong response (designated "++++" or "+++" in Table 1) to only two odors, pentyl acetate and 3-(methylthio)-1-propanol. These molecules appear to have few if any structural features in common, and it is difficult to discern a common feature that distinguishes these two odors structurally from the others in the set.

In considering how this ensemble of ORNs encodes olfactory information, it is important to appreciate that dramatic differences are observed in the signal strengths elicited among neurons by a particular olfactory stimulus. For example, both ab1A and ab2A respond strongly to ethyl acetate, a product of ripening fruits that is indicated as the strongest stimulus for both neurons in Table 1. However, dose-response curves show that the response of ab1A is markedly stronger than that of ab2A (Figure 4A). A 10^{-4} dilution of ethyl acetate elicits 180 spikes/s from ab1A, but only 25 spikes/s from ab2A. It seems likely that a weak ethyl acetate stimulus is encoded exclusively by ab1A, whereas a strong stimulus is encoded by signals from both neurons.

Major differences in the sensitivities of neurons to the test odors that stimulate them most strongly are also observed for ab5B, ab6A, and ab4A (Figure 4B). ab5B shows a sigmoid response curve, with a half-maximal response at a dilution of approximately 10^{-4} pentyl acetate; ab6A shows a response half that of the maximum in this experiment at approximately 10^{-5} 1-octen-3-ol; ab4A shows a half-maximal response at approximately 10^{-6} E2-hexenal. Why these differences? One possibility is that the receptors in ab5B and ab6A have evolved to bind odors not included in our panel that are structurally related to, but distinct from, pentyl acetate and 1-octen-3-ol. It is striking that the greatest response observed in this study is to E2-hexenal, a critical olfactory cue for other insects in detecting leafy plants and also present in fruits. Perhaps ab4A has evolved to signal the presence of this compound.

Not only have some ORNs almost certainly evolved to encode odors other than those used here, but the olfactory system has evolved to encode odors in complex mixtures. Animals in their natural environments are rarely presented with pure odors, which raises the question of whether the responses we characterize in this study are in fact similar to those elicited by natural stimuli. To address this question, we measured responses to a complex natural food source, banana, and found that the response of ab2A was comparable to that observed with a 10^{-2} dilution of ethyl acetate (Figures 4C–4E). We do not know whether the response of ab2A to the banana stimulus is due to the presence of ethyl acetate in the banana; the results simply show that the response to a natural complex stimulus appears similar to those evoked by pure odors.

Multiple Modes of Response in an Individual Neuron

Most of the responses we have documented are excitatory, despite a few inhibitory responses of ab6B (Figure 3). In our study of basiconic sensilla on the maxillary palp (de Bruyne et al., 1999), we described a neuron that

(I) The C neuron, whose spikes prior to the burst of action potentials are indicated by dots, responds to CO_2.
(J) Expansion (200 ms) of the trace in (E); the dot corresponds to the dot shown during the spike train in (E).
(K) 75 ms trace from an ab1 sensillum exposed to CO_2 (see text).

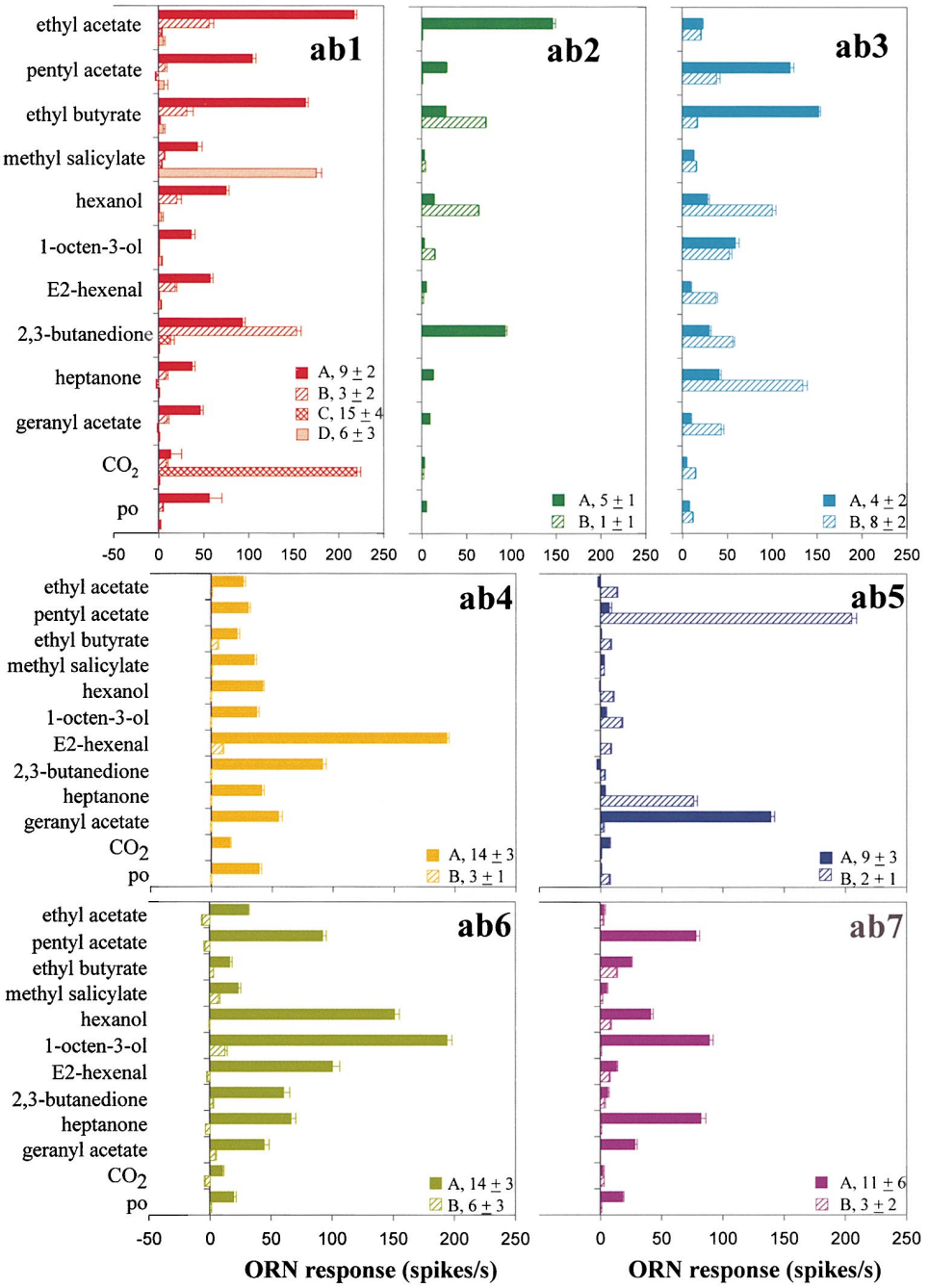

Figure 3. Sixteen Classes of ORNs Are Found in Characteristic Combinations in Seven Functional Types of Basiconic Sensilla

Response profiles of ORNs are shown for the three types of large basiconic sensilla (ab1, 2, and 3) and the four types of small basiconic sensilla (ab4, 5, 6, and 7) using a set of 11 diagnostic stimuli and the solvent control, paraffin oil (po). The indicated response is measured as the increase (or decrease) in spikes/s compared to the spontaneous firing frequency. Spontaneous frequencies of the neurons in each sensillum type are indicated in the lower right corner. Error bars are SEM. The number of ORNs was $10 \leq n \leq 15$ in all cases except that for ab1, $n = 6$.

was inhibited by several odors; however, that neuron showed a much higher spontaneous firing rate (32 ± 7 spikes/s) than any of the neurons documented here, and therefore inhibition—as measured by a reduction in firing rate below the spontaneous level—could be detected more sensitively. Are inhibitory responses more prevalent among antennal neurons than is suggested by Figure 3? To investigate this issue, we increased the sensitivity of our inhibition assay in two ways. We first increased the firing rate of a neuron by exciting it with one odor, and then tested other odors for their ability to decrease the firing rate of the excited neuron. This paradigm is likely to reflect the context of the ORNs in nature, as *Drosophila* reside in habitats rich

Table 1. Response Spectra of ORN Classes

	Large Basiconics								Small Basiconics							
	ab1				ab2		ab3		ab4		ab5		ab6		ab7	
	A	B	C	D	A	B	A	B	A	(B)	A	B	A	B	A	B
Ethyl acetate	++++	+	0	0	+++	0	0	0	0	0	0	0	0	0	0	0
iso-amyl acetate	+++	0	0	++	0	0	++	0	0	0	0	++	+	0	++	0
Pentyl acetate	++	0	0	0	+	0	+++	0	0	0	0	++++	++	0	++	0
E2-hexenyl acetate	++	0	0	0	0	0	+++	0	+++	0	0	+	++	0	+	0
Ethyl propionate	++++	+	0	0	++	+	++	0	0	0	0	0	0	0	0	0
Ethyl butyrate	+++	+	0	0	+	++	+++	0	0	0	0	0	0	0	0	+
ethyl 2-methylbutanoate	++	0	0	0	0	+	+++	0	0	0	0	0	0	0	0	0
cis-vaccenyl acetate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4-methylphenol	0	0	0	+	0	0	0	+	0	0	0	0	0	++	0	0
Benzaldehyde	0	0	0	++	0	0	0	0	+++	0	0	0	0	0	0	0
Phenylacetaldehyde	0	+	0	++	0	0	0	0	0	0	0	0	0	0	+	0
Acetophenone	0	0	0	+++	0	0	0	0	0	0	0	0	0	0	+	0
4-isopropylbenzaldehyde	0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	0
Eugenol methyl ether	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Methylsalicylate	0	0	0	++++	0	0	0	0	0	0	0	0	0	0	0	0
Butanol	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0
Iso-amyl alcohol	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0
E2-hexenol	0	0	0	0	0	++	0	+	+++	0	0	0	+++	0	++	0
Hexanol	0	0	0	0	0	++	+	++	0	0	0	0	+++	0	+	0
4-methylcyclohexanol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3-octanol	0	0	0	0	0	0	++	+++	0	0	0	+	+++	0	++	0
1-octen-3-ol	0	0	0	0	0	0	++	++	0	0	0	0	++++	0	++	0
E2-hexenal	0	0	0	0	0	0	0	+	++++	0	0	0	++	0	0	0
E2-octenal	0	0	0	0	0	0	+	0	++	0	0	0	++	0	0	0
Nonanal	0	0	0	0	0	0	0	0	++	0	0	0	0	0	0	0
Acetone	0	0	0	0	++	0	0	0	0	0	0	0	++	0	0	0
2,3-butanedione	++	+++	0	0	++	0	+	++	++	0	0	0	++	0	0	0
Cyclohexanone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heptanone	0	0	0	0	0	0	+	+++	0	0	0	++	+	0	++	0
(IR)-(+)-α-pinene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0
β-citronellol	0	0	0	0	0	0	0	0	0	0	0	0	++	0	0	0
Linalool	0	0	0	0	0	0	0	0	0	0	0	0	++	0	++	0
Cineole	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Geranyl acetate	0	0	0	0	0	0	0	+	0	0	+++	0	+	0	0	0
(R)-(+)-limonene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyrrolidine	0	0	0	0	0	0	0	+	0	0	0	0	++	0	0	0
2-isobutyl-3-methoxypyrazine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Indole	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Butyric acid	0	0	0	0	0	0	+	0	0	0	0	0	++	0	0	0
γ-valerolactone	0	+	0	+	0	+	+	0	0	0	0	0	++	0	0	0
Ammonia	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
Ethanolamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1,4-diaminobutane	++	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
Propanethiol	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
3-(methylthio)-1-propanol	0	0	0	0	0	0	0	0	0	0	0	+++	0	0	0	0
Dipropyldisulphide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carbon dioxide	0	0	++++	0	0	0	0	0	0	0	0	0	0	0	0	0

Specificity of response of each ORN class across a panel of 47 odors. Responses are indicated as excitatory if the firing frequency exceeds by 15 spikes/s that of the response to the diluent control. (15 spikes/s is approximately twice the mean standard deviation of the firing frequencies of the various neuronal types for the control stimuli). "+" indicates that the response exceeded that of the spontaneous frequency by $n < 45$ spikes/s; "++" by $45 \leq n < 113$ spikes/s; "+++" by $113 \leq n < 180$ spikes/s; "++++" by $180 \leq n$ spikes/s. These frequencies represent 20%, 50%, and 80% of 225 spikes/s, a typical maximal firing frequency for the ORNs in this study; 20% and 80% of the maximal firing frequency approximate the limits between which responses tend to be linear with respect to the logarithm of the dilution. Shaded entries designate the strongest responses for each neuronal type. All odorants were diluted 10^{-2} in paraffin oil except for those in italics, which were diluted 10^{-2} in water. Among the 752 entries in this table, all are based on recordings from at least two neurons, with most based on $3 \leq n \leq 6$ neurons.

in a wide diversity of odors, some of which are likely to keep certain ORNs firing at levels above the spontaneous frequency for extended periods of time. We also carried out a second experiment, designed to detect more sensitively the effects of odors on the spontaneous firing rate, i.e., the low rate of firing of a neuron exposed to charcoal-filtered air. To increase the sensitivity of detection, we lengthened the stimulus period to 2.2 s

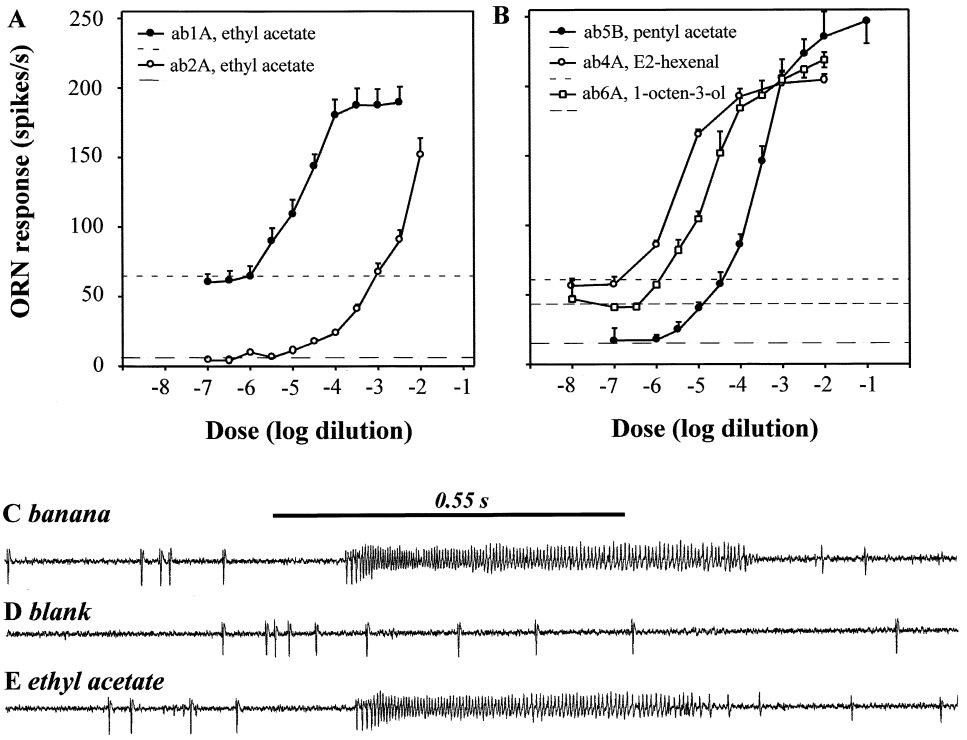

Figure 4. Responses to Odors at Different Doses and in a Natural Mixture

(A and B) Dose-response curves. Responses indicate increases over spontaneous frequency. Dashed lines indicate the mean response of the corresponding neuron to the paraffin oil diluent alone (see Experimental Procedures). (A) ab1A responds more strongly than ab2A to ethyl acetate. (B) Responses of three neurons to the test odors that stimulate them most strongly.

(C) Response of ab2A neuron to banana odor. A 5 ml syringe was used to withdraw 1 ml headspace from a 170 ml bottle containing ~40 g of overripe (brown) banana.

(D) Response to control stimulation with 1 ml headspace from an empty bottle.

(E) Response to 10^{-2} dilution of ethyl acetate.

(compared to the 0.55 s period used previously), which allows us to integrate the spike frequency over a longer period of time. For these experiments, we chose ab2A, whose large spike amplitude facilitates the analysis. As odors, we chose to analyze the terpenes, a class of compounds that are commonly found in high doses in plants but that had elicited excitatory responses from only four of the neurons defined here.

Inhibition of the ab2A neuron is shown in Figure 5. When the ORN was excited at a low level (15 spikes/s) by ethyl acetate, a pulse of linalool was found to decrease the firing rate to approximately 0 spikes/s (Figures 5A, 5B, and 5E). This inhibitory effect was specific, in that a number of other terpenes, such as citronellol, had no effect on the firing frequency (Figures 5C and 5E). Cineole reduced the firing frequency to an intermediate level (Figure 5E). As a control, a pulse of ethyl acetate was also administered and increased the firing frequency (Figure 5D). This ethyl acetate control shows that the neuron was still capable of excitation, even though it had adapted to the prolonged ethyl acetate stimulus.

In the second experiment, using a lengthened stimulation period, linalool was applied to ab2A while it was firing at its spontaneous level, and was again found to decrease the firing rate (Figure 5F). The effect again showed odor specificity (Figure 5F) very similar to that observed in the previous experiment (Figure 5E). These experiments together provide evidence that at least some antennal neurons in *Drosophila* exhibit two modes of response: excitation and inhibition.

Antennal neurons also show two modes of response at the end of a stimulus: the physiological response can terminate abruptly or it can be prolonged, continuing well beyond the end of the stimulus. An example of an abruptly terminating response is shown by ab3A, whose large spikes end shortly after the end of a 0.55 s ethyl butyrate stimulus (Figure 6A). By contrast, Figure 6B shows the response of ab5A to geranyl acetate, which continues long beyond the end of the odor stimulus.

An individual odor may elicit a quickly terminating response in some neurons, but a prolonged response in others. For example, both of the neurons in the ab3 sensillum are excited by heptanone, but after the end of odor stimulation, the ab3A neuron stops firing abruptly, while ab3B continues to fire for a prolonged period (Figure 6C). These results argue against the possibility that prolonged termination is a nonphysiological consequence of an odorant's chemical properties; rather, they show that the dynamics of response termination are a specific property of an individual neuron.

These differing response dynamics can be visualized by plotting the frequency of firing as a function of time. The abrupt termination in response of the ab3A neuron

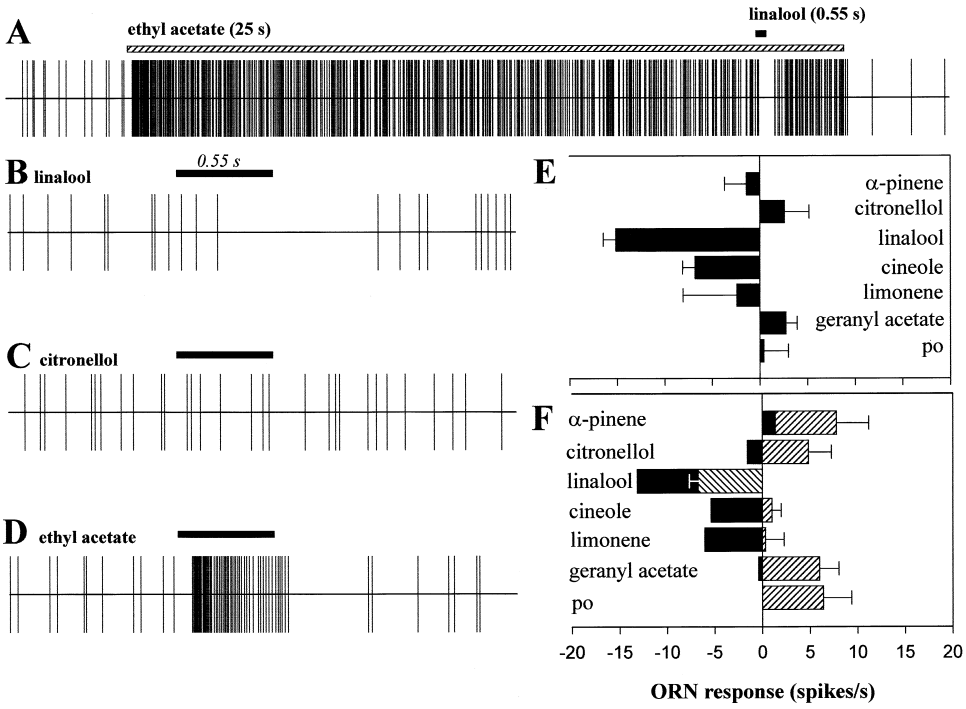

Figure 5. The ab2A Neuron Can Be Excited or Inhibited by Odor Stimulation

Action potentials of ab2A are represented by vertical lines of fixed height. (A) Inhibitory effect of linalool stimulation (0.55 s; black bar) on the A neuron during long stimulation (25 s; hatched bar) with ethyl acetate. (B–D) Odor-specific effects of linalool (B), which decreases the firing rate, citronellol (C), which has no effect, and ethyl acetate (D), which increases the firing rate when superimposed incrementally upon continuous ethyl acetate stimulation. The time scales are expanded compared to that in (A); the data in (B) are from panel (A). In each case, a 0.55 s pulse of the indicated odor is superimposed on the continuous 25 s stimulation with ethyl acetate. (E) Change in firing frequency following stimulation with the indicated odor during the course of continuous stimulation with ethyl acetate. Negative values indicate inhibition. Different odors, all terpenes, have different effects. po, paraffin oil diluent. Error bars indicate SEM; n = 6 recordings, each from a different ab2A neuron, for each odor. (F) Inhibition and excitation of ab2A neurons by the indicated odors in the absence of concurrent stimulation with ethyl acetate. Stimulation was for 2.2 s. The hatched bars show the changes in firing frequency; black bars indicate the changes in firing frequencies after subtraction of the change observed for the paraffin oil diluent alone in this experiment. Note the agreement between the black bars in panels (E) and (F). n = 8.

to ethyl butyrate, which was shown as a trace in Figure 6A, can also be seen in Figure 6D, which shows the spike frequency in 100 ms intervals during the course of the response, with the vertical line indicating the approximate end of odor stimulation. After the end of odor stimulation, the frequency abruptly declined from approximately 100 spikes/s to 0 spikes/s. The prolonged response of ab3B to heptanone, by contrast, is shown in Figure 6E. Both Figures 6D and 6E also illustrate that the firing frequencies are not constant during the course of stimulation; they decline between the initiation and cessation of stimulation.

An individual neuron may show an abruptly terminating response for some odors, but an extended response for others, as shown by comparison of the ab3B neuron's response to heptanone (Figure 6E) and 1-octen-3-ol (Figure 6F). These results suggest that the dynamics of neuronal response at the end of odor stimulation is a parameter of the odor-ORN interaction and could play a role in odor coding.

Consistent with a role in the coding of odor identity, the type of response dynamics shown by a neuron at the end of odor stimulation was independent of odor dose. For example, ab2A stopped firing abruptly after stimulation with a broad range of ethyl acetate concentrations (Figures 6G–6I), whereas ab6A continued to fire long after stimulation with a broad range of 1-octen-3-ol concentrations (Figures 6J–6L).

Another property of the response dynamics of some antennal neurons is also visible in Figure 6, a period of post-stimulus quiescence. The negative values for firing frequency observed after stimulus termination in, for example, Figure 6F indicate that the frequency after stimulation is lower than the spontaneous frequency observed before stimulation. We have observed this effect previously in maxillary palp neurons (de Bruyne et al., 1999).

A Functional Map of ORNs on the Antennal Surface

We have characterized 16 classes of ORNs on the antenna (Figure 7A). How are these classes distributed spatially? As useful coordinates in describing the distributions of these functional units, we refer to regions drawn on the basis of sensillar morphology. Specifically, the anterior and posterior surfaces of the third antennal segment are covered with olfactory sensilla, but the sensory field is heterogeneous in terms of sensillar morphology (Stocker, 1994) and can be divided accordingly into five regions (Figure 7B). Regions I and II contain

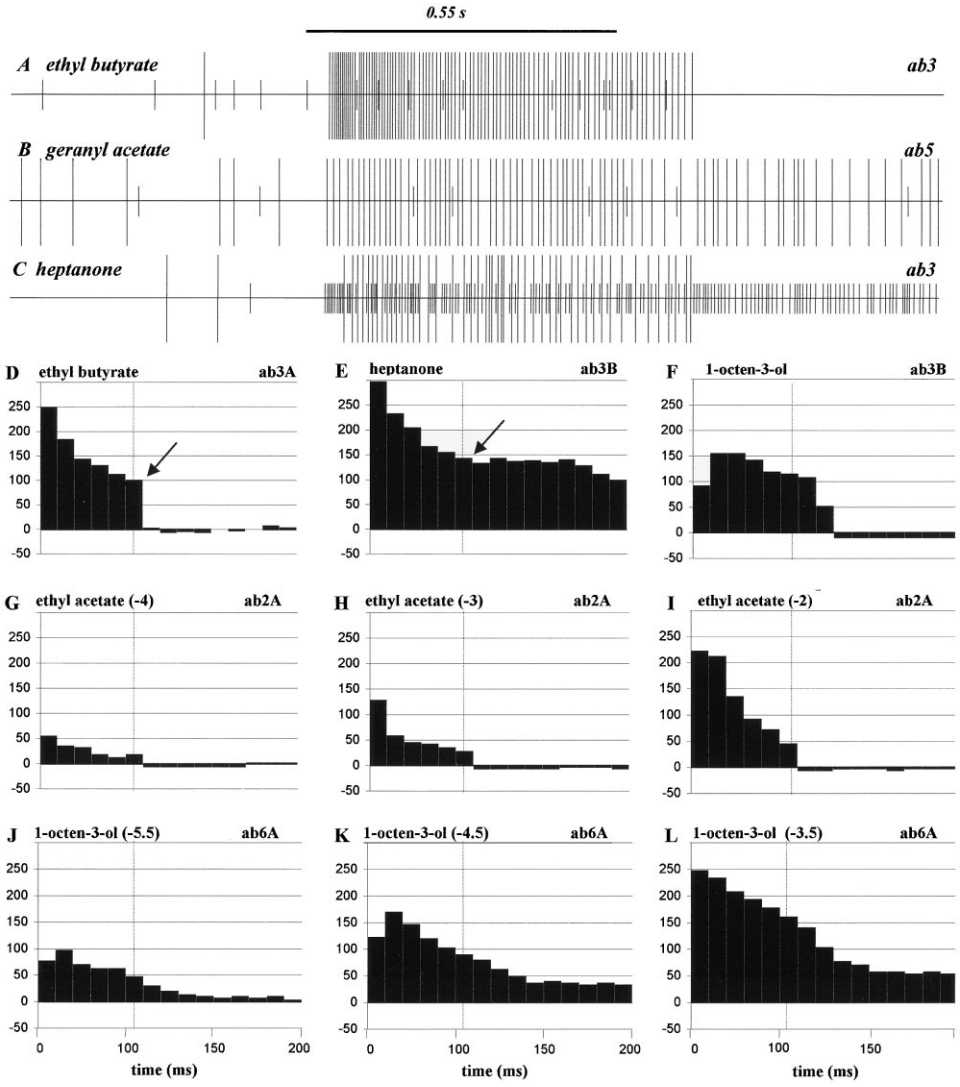

Figure 6. Alternate Modes of Response Termination

All odorants are diluted 10^{-2} unless otherwise indicated. In (A)–(C), tall lines indicate spikes of the A neuron; short lines indicate the B neuron. (A) Response of ab3A to ethyl butyrate. The A neuron shows an abrupt termination of firing shortly after the end of the stimulus. The bar indicates the period during which the odor release valve is open; odor continues to move through the delivery system for a short period after the valve has closed. (B) Prolonged response of ab5A (tall lines) to geranyl acetate. (C) Action potentials of ab3A (tall lines) terminate abruptly after the end of heptanone stimulus, whereas the B neuron shows a prolonged response.

(D–L) Mean spike frequency (spikes/s) is shown for successive 100 ms intervals. The beginning of the first 100 ms interval corresponds to the beginning of the train of action potentials. The vertical line represents the approximate end of odor stimulation; it is drawn 550 ms after the beginning of the train of action potentials, a period corresponding to the time during which the odor valve was open. The spontaneous frequency of firing, as determined during the 2 s period prior to stimulation, has been subtracted from all values; thus negative values observed in some cases after the end of stimulation represent post-stimulus quiescence. (D) Response of ab3A to ethyl butyrate shows sharp decline after end of stimulus (arrow). n = 6 recordings, each from a different neuron. (E) Response of ab3B to heptanone does not decline after end of stimulus (arrow). n = 6. (F) Response of ab3B to 1-octen-3-ol declines quickly after the stimulus, although not as abruptly as in (D). n = 6. (G–L) Termination kinetics at different doses of odors. Compare (G) to (J), (H) to (K), and (I) to (L); the number of spikes in the first bin is approximately equal for the two members of each pair. n = 3. Compare also (F) and (K), in which the same odor elicits different response dynamics from different neurons.

exclusively large basiconic sensilla. Region III is largely devoid of sensilla, except for a number of coeloconic sensilla. Region IV contains both small basiconic and coeloconic sensilla. Finally, region V contains a high density of trichoid sensilla, but also contains some small basiconic and coeloconic sensilla.

Each functional type of sensillum—and consequently each functional class of neuron—is restricted to a particular spatial domain of the antennal surface (Figure 7C). Although certain types are intermingled, each is compartmentalized by particular spatial boundaries. We note first that ab1, ab2, and ab3 are restricted to regions I and II, whereas the four remaining sensillar types are localized exclusively in regions IV and V (this segregation

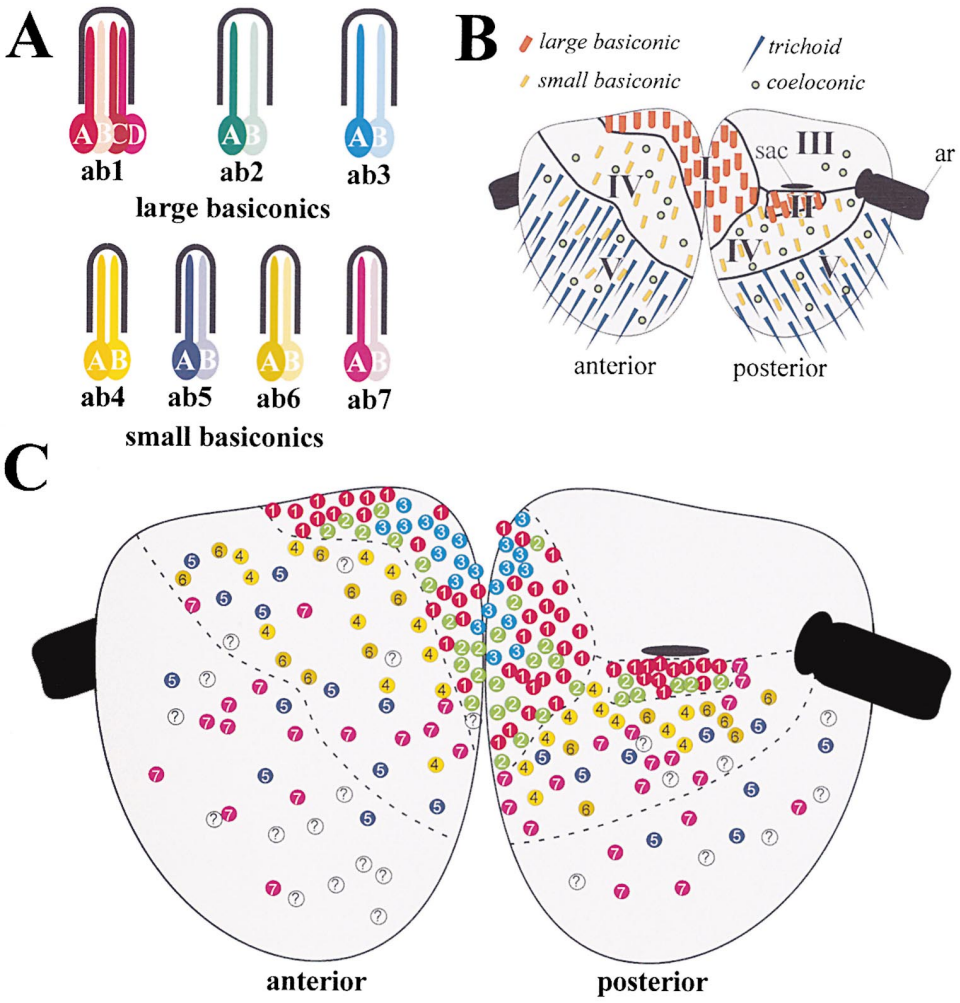

Figure 7. A Map of Neuronal Classes on the Antennal Surface

(A) The 16 functional classes of ORNs and their stereotyped organization within seven functional types of sensilla.

(B) Regions of the antenna as defined by morphological categories of sensilla (see Figure 1). The arista (ar) is a structure that extends from the antenna.

(C) The distribution of functional types of sensilla. Each circle represents a recording from one sensillum; question marks represent recordings that could not be classified. The distribution of recordings was intended to reflect the distribution of basiconic sensilla on the antenna, e.g., the high concentration of circles in region II reflects the high concentration of basiconic sensilla in that region. The dotted lines are those shown in (B), representing boundaries between morphological categories of sensilla. Dorsal is at top; medial is along the center of the diagram.

follows from the fact that ab1, ab2, and ab3 are the only large basiconic sensillar types, and from the definition of the regions). However, ab1, ab2, and ab3 do not show identical distributions. ab3 shows a more restricted distribution; it is limited to region I and, moreover, is restricted to the dorso-medial portion of region I. The distribution of ab1 overlaps closely with that of ab2. ab4 and ab6 are found in region IV but not region V, and their distributions appear similar. ab5 and ab7 are found in both regions IV and V; we are unable to distinguish between their distributions with the resolution afforded by this analysis.

Thus, four functional domains can be distinguished: those for (i) ab1 and ab2; (ii) ab3; (iii) ab4 and ab6; (iv) ab5 and ab7. Within a functional domain the distributions of functional types may not be identical (for example, we have found more ab1 sensilla than ab2 at the dorsal boundary of their domain), but a more detailed analysis will be required to establish these distinctions conclusively. We note finally that most of the unclassified basiconic sensilla are located in the ventro-lateral portion of the antenna, in region V.

How many neurons of each functional class are located on the antenna? The numbers of each sensillum type that we identified by physiological recording in this study are shown in Table 2, listed by region. Among the large basiconic sensilla, 50% were identified as ab1, with 30% as ab2 and 20% as ab3. Assuming that the distribution of the sensilla from which we recorded agrees reasonably well with the distribution of large basiconica on the antenna, and taking 90 as the number of large basiconica (Shanbhag et al., 1999), then our results suggest that there are on the order of 45 ab1A, B, C, and D neurons, 27 of the ab2 neurons, and 18 of

the ab3 neurons. We note that this estimate predicts that 50% of large basiconic sensilla would contain four neurons, which is in excellent agreement with the estimate of 52% from ultrastructural studies of males (Shanbhag et al., 1999). Similar calculations suggest that there are 13–28 of the neuronal types in small basiconic sensilla, again assuming that the sampling bias is not large.

Discussion

Odor coding in the *Drosophila* antenna has been examined directly in a functional analysis. A large number of antennal neurons have been characterized with respect to response spectrum, response dynamics, and spatial location within the sensory field. Here we define 16 functional classes of ORNs, each with a distinct response spectrum and each mapping to a restricted spatial domain on the antennal surface. We also document different modes of response dynamics, which may represent elements of an olfactory code.

Neuronal Organization of the Peripheral Olfactory System in *Drosophila*

The initial steps of odor coding occur in the olfactory organs of the fly. Each antenna contains ~1200 ORNs, and each maxillary palp contains ~120 ORNs (Stocker, 1994). Approximately half of the antennal ORNs innervate basiconic sensilla (Shanbhag et al.,1999) and are the subject of this study. Our physiological data indicate that each basiconic sensillum contains either two or four ORNs, consistent with anatomical data (Stocker, 1994; Shanbhag et al., 1999).

The physiological analysis shows that the ORNs in these sensilla are clustered in stereotyped combinations, as has been found in other insect olfactory sensilla (Boeckh, 1981). The consistent juxtaposition of neurons with particular response properties raises functional questions. Does the grouping of multiple neurons in the same local environment provide a cellular mechanism for increasing the fidelity of transmission of olfactory information? Inspection of Table 1 does not reveal an obvious relationship between the response spectra of neurons grouped in a common sensillum. However, it seems clear that the most potent ligands for many of these ORNs have yet to be identified, and the behavioral significance of the messages sent by each ORN type has not been defined. In species where the ecological significance of potent ligands has been identified, adjacent neurons sometimes transmit related information (Wojtasek et al., 1998; Grant et al., 1998), which affords a rich diversity of opportunities for modulation and processing of olfactory information.

The stereotyped combination of receptor neuron types within sensilla also raises developmental questions. The identity of one neuron in a sensillum can be reliably predicted from the identity of its neighbor(s), as if the choice of receptor genes made by an individual neuron is made in concert with, or is constrained by, the choice of its neighbor. We know little about the genetic and molecular mechanisms underlying receptor gene selection, except that the choices of some neurons appear to require the POU domain transcription factor gene *acj6* (Clyne et al., 1999a).

What relationship is there between the number of ORN classes and the number of glomeruli? The most informative data are available for the ORNs within large basiconic sensilla. We have defined eight classes of these ORNs and have no evidence for any additional classes. Approximately seven glomeruli are indicated by Stocker et al. (1983) as receiving input from the region of the antenna containing large basiconic sensilla. While there are some uncertainties in comparing the two studies, it is nonetheless striking that the number of ORN classes is similar to the number of identified glomeruli. This numerical similarity is consistent with the finding that neurons expressing a particular odorant receptor project to one or two glomeruli, both in vertebrates and in *Drosophila* (Mombaerts et al., 1996; Vosshall et al., 2000; Gao et al., 2000). Furthermore, the total number of ORN classes identified in this study, 16, when added to the 6 classes characterized in an extensive analysis of maxillary palp sensilla (de Bruyne et al., 1999) and the 7 classes for which we have so far found evidence in a preliminary characterization of trichoid and coeloconic sensilla on the antenna (Clyne et al., 1997), yields a total of 29, a number which approaches the number of olfactory glomeruli that have been identified in *Drosophila*, 41 (Laissue et al., 1999).

Table 2. Number of Each Sensillum Type Identified in Each Region

Large Basiconics				Small Basiconics					
Region	ab1	ab2	ab3	ab4	ab5	ab6	ab7	ab?	Totals
I	42	26	21	—	—	—	—	1	90
II	12	6	0	—	—	—	—	—	18
III	—	—	—	—	—	—	—	—	—
IV	—	—	—	22	13	15	20	6	76
V	—	—	—	0	8	0	14	16	38
Totals	54	32	21	22	21	15	34	23	222
Proportion	50%	30%	20%} 100%	19%	18%	13%	30%	20%} 100%	
Estimated #/antenna[a]	45	27	18	18	18	13	28	19	

The total numbers of recordings are smaller than those cited in the text because the text figures include sensilla whose locations were not defined precisely.

[a] The number of each functional type of sensillum per antenna was estimated by multiplying the indicated proportion by the total number of large or small basiconic sensilla as counted by Shanbhag et al. (1999) [for the number of small basiconic sensilla we used the sum of the numbers of SB, TB, and I sensilla, as defined by Shanbhag et al. (1999)].

An approximate numerical correspondence is also observed between the number of neurons per ORN class and the number of ORNs expressing an individual odor receptor gene. Specifically, we estimate that the number of neurons in a functional class ranges from 13 to 45 (Table 2). Our previous in situ hybridization experiments showed that among four individual odor receptor genes tested quantitatively, each was expressed in 16 $\pm$ 1 (n = 5) to 40 $\pm$ 1 (n = 6) cells per antenna (Clyne et al., 1999b), a figure that is in reasonable agreement with data for a number of other receptor genes (Vosshall et al., 1999, 2000). This numerical correspondence is consistent with the concept that neurons of a particular functional class, i.e., neurons with a particular odor response spectrum, express the same odor receptor gene(s).

We note that in situ electrophysiological studies of vertebrate olfactory epithelia (Sicard and Holley, 1984; Duchamp-Viret et al., 2000) have not allowed grouping of ORNs into discrete functional classes. It is possible that such classification has not been described because the number of classes is large and because the recordings are more difficult to obtain.

Mechanisms of Odor Coding

Three variables describe an olfactory stimulus: odor identity (analogous to hue), odor concentration (intensity), and time. We have described how each of these variables is represented in the action potential frequency of different receptor neurons in the *Drosophila* antenna. Different ORN classes show diverse odor response spectra, sensitivities, and dynamics. Correspondingly, a particular odor signal produces a different response among different ORN classes.

The ORN classes defined here exhibit a good deal of specificity, with only 15% of the odor stimulus-ORN combinations yielding a response (Table 1). In considering the degree of specificity, three issues arise. First, the apparent specificity is a function of the dose of odorants tested. We have used doses that we expect to be high in comparison to doses the organisms encounter in their natural environments; thus, we would expect ORNs to show an even greater degree of specificity if tested with doses encountered under natural conditions. Second, our estimate of specificity depends on the accuracy of spike classification, which, particularly in the case of the ab1 sensillum, can be difficult when a neuron responds strongly to an odor. This difficulty could lead to an underestimate of the response of another neuron in the same sensillum to the same odor, and thereby to an underestimate of tuning breadth. Finally, we have sampled a limited subset of volatile compounds; although we attempted to select these compounds judiciously, based on available knowledge of the chemical ecology of flies, certainly there are many odors important to the organism's survival or reproduction that were not tested. Just as the ab1A and ab4A neurons are extremely sensitive to ethyl acetate and E2-hexenal, respectively, other ORNs likely have ligands that elicit strong responses even at low concentrations and that represent ecologically important signals.

Although individual ORNs may have evolved to respond to particular ligands of ecological importance, "cross-stimulation" of ORNs by other ligands is likely to be critical in the coding of odor identity. Of the 16 ORN classes defined in this study, 11 responded to more than one of the test stimuli. Conversely, most (73%) of the test stimuli in Table 1 that elicited a response from any ORN elicited a response from multiple ORNs, and 23% elicited a response from five or more ORN classes. The response of individual ORNs to multiple odors, and the response of multiple ORNs to individual odors, is consistent with a number of other studies of vertebrate ORNs and some other insect ORNs (Buck, 1996; Hildebrand and Shepherd, 1997; Malnic et al., 1999).

An example of how odor identity could be encoded by integrating the responses of multiple ORNs is illustrated by the responses of ab1A and ab3A to ethyl acetate and ethyl propionate (Table 1). The ORN that is most sensitive to ethyl acetate, ab1A, is also highly sensitive to ethyl propionate. How then can the fly discriminate between a high dose of ethyl acetate and ethyl propionate? One possible answer is by integrating the response of ab1A with that of ab3A, which responds to ethyl propionate but not ethyl acetate.

Is the structure of the code arbitrary? In principle, if each ORN class represented a binary coding unit, the 16 classes described here could encode 2^{16}, or some 65,000, different odors. However, in interpreting the response matrix (Table 1), it is useful to consider that the ORN repertoire of *Drosophila* is not designed for maximal computational efficiency across the entirety of odor space, but rather has evolved to fit the organism's need for survival and reproduction. Esters and alcohols, which are commonly present in fermenting fruits, elicit responses from a relatively large number of neurons, which may thereby provide resolving power to aid in discrimination among these odors.

Coding of odor concentration is also likely to depend upon the multiplicity of responding ORNs. For example, ab1A is very sensitive to ethyl acetate, saturating at a 10^{-4} dilution (Figure 4). ab2A is less sensitive, showing a linear relationship that initiates at 10^{-4} and remains linear until 10^{-2}; thus the presence of two ethyl acetate neurons of differing sensitivities effectively expands the dynamic range of the response. Low doses of ethyl acetate are likely to be encoded by ab1A; at higher doses, not only is a stronger signal sent by ab1A to a glomerulus in the antennal lobe, but an additional signal is transmitted by ab2A, presumably to an additional glomerulus. We note that among the dose-response relationships we have characterized for ORNs of both the antenna and the maxillary palp (de Bruyne et al., 1999), the slopes are remarkably constant. Moreover, the linear phase of the curve in each case extends over at least a 100-fold range of odor dilutions.

Both excitation and inhibition of ORNs by odors have been documented (Figure 5). The ability of a neuron to exhibit two modes of response adds a degree of freedom that may be used in the coding of odor identity. Inhibition may provide a mechanism for enhancing signal recognition; for example, an odor may inhibit some ORNs while exciting others, which may enhance contrast in the glomeruli. In fact, we have shown that linalool can inhibit firing of the ab2A neuron (Figure 5), but can excite the ab4A, ab6A, and ab7A neurons (Table 1). Linalool is a terpene common among plants and excites ORNs from a variety of insect species; it has previously

been shown to inhibit pheromone-sensitive ORNs in a moth (Kaissling et al., 1989).

In addition to odor quality and quantity, the temporal profile of an odor stimulus also must be encoded by spike trains. The temporal profile of an odor is critical to flying insects that seek odor sources. Such insects encounter intermittent "odor pockets" while traversing an odor plume (Murlis et al., 1992), and the frequency of such encounters is an essential parameter in navigation (Mafre-Neto and Carde, 1994).

How is temporal information encoded? In addition to whether an individual ORN fires, and the frequency of firing, a third degree of freedom is the distribution of spikes in time. The spike frequency is not a simple reflection of the instantaneous odor concentration. For example, in some cases the spike train terminates abruptly after stimulus termination, and in other cases the spike train continues long after the odor stimulus has ceased; these radically different patterns can be observed for two neurons in the same sensillum, stimulated with the same odor (Figure 6C). We do not know the underlying molecular basis of these kinetic differences. One possibility is that they relate to differences in ligand-receptor binding affinities; another possibility is that they represent differences in the kinetics of receptor deactivation, with post-stimulus inhibition perhaps representing an extreme form of receptor deactivation. In any case, this third degree of freedom could provide a means of expanding the coding capacity of the system. Fast off-responses may convey information about rapid changes in odor concentration, such as those encountered by a flying insect. Slow off-responses may play another role in the fly's orientation behavior, perhaps by providing a "memory" of a recently encountered odor stimulus (Den Otter and Van der Goes van Naters, 1992). Thus, the two different response modes may simultaneously present a phasic and tonic representation of selected features of the odor environment.

The difference in temporal representation of odors by ORNs could also play a role in their identification; some odors could be encoded in part by the temporal dynamics that they elicit among different ORNs, as has been suggested in other organisms (Buck, 1996). It is clear from work in other insects that much olfactory processing and even the initiation of behavioral responses occur within less than 0.5 s (Vickers and Baker, 1996), so differences in the kinetics of termination would be most informative in the case of pulses shorter than 0.5 s. However, there is also evidence that odor identification depends at least in part on the synchronized oscillation of assemblies of neurons in the antennal lobes of insects (Stopfer et al., 1997), in a process that may take longer. It will be interesting to determine whether the differences we observe in the temporal dynamics of ORNs play a role in the temporal activity patterns of antennal lobe neurons.

The number of receptor genes expressed per ORN varies among species. *C. elegans* ORNs express multiple receptors (Troemel et al., 1995; Sengupta et al., 1996). By contrast, vertebrate ORNs are widely believed to express only one (Buck, 1996; Malnic et al., 1999) although there is evidence that some express more than one (Rawson et al., 2000). We have found evidence that the ab2A neuron is excited by some odors and inhibited by others. Excitation and inhibition have been found within the same ORN in a variety of insects, and there is evidence in the lobster that excitation and inhibition are mediated in the same ORN by different transduction pathways, via IP_3 in one case and cAMP in the other (Boekhoff et al., 1994; Hatt and Ache, 1994). A simple model for how two different odors elicit two different responses in the same cell is by acting through two different receptors. However, there is evidence consistent with a single receptor per ORN in *Drosophila* (Vosshall et al., 2000), and it is possible that two odors might act through a single receptor to produce either excitation or inhibition: for example, one odor might activate a receptor while the other might inhibit it. We note finally that a model in which some ORNs have two receptors would also provide an explanation for how the ab5B neuron responds to two odorants that are seemingly disparate in structure, pentyl acetate and 3-(methylthio)-1-propanol. However, other explanations consistent with a one-receptor model are also possible, e.g., in principle, one odorant may undergo hydrolysis to yield a product that is structurally similar to a second.

A Functional Map of the *Drosophila* Antenna

We have mapped the spatial distributions of the seven functional types of sensilla, and thus of the 16 functional classes of ORNs that they contain (Figure 7C). The distribution patterns define four spatial domains on the antennal surface. One domain is defined by the distribution of the ab3 sensilla, another by ab1 and ab2, a third by ab4 and ab6, and the fourth by ab5 and ab7. The four domains vary in area, and overlap partially with one another. Each domain is represented on both anterior and posterior faces of the antenna. It is possible that further analysis will establish fine distinctions between the distributions of the sensillar types that together define a particular domain.

While comparable functional maps of individual ORNs are not available in vertebrates, a recent calcium imaging study of the mouse olfactory epithelium has shown that ORNs are organized in a functional mosaic; ORNs responding to a given odor are widely distributed and interspersed with other ORN subtypes (Ma and Shepherd, 2000). Moreover, there is physiological evidence from field recordings, 2-deoxyglucose labeling studies, and voltage-sensitive dye recordings that the sensory field is not homogeneous (reviewed in Buck, 1996). The expression patterns of odor receptor genes, which are likely to reflect the distribution of functional classes of ORNs, has revealed a zonal organization in mouse and rat, with most receptor genes expressed in one of four defined zones (Buck, 1996). These zones, as defined through in situ hybridization experiments, appear comparable in some respects to the spatial domains that we have identified in functional studies. In situ hybridizations of receptor genes to the antenna indicates that subsets of genes are expressed in subregions of the antenna (Clyne et al., 1999b; Vosshall et al., 1999, 2000), at least one of which appears similar to one of the morphologically defined regions, region I.

The spatial organization of the antennal domains, as determined here by physiological recording, are of interest in a developmental context. The boundaries of these

domains are roughly perpendicular to the axis that extends from the dorso-medial portion of the antenna to the ventro-lateral portion. Thus, ab3 sensilla are the most dorso-medial, followed by ab1 and ab2, with ab4 and ab6 occupying an intermediate domain, and ab5 and ab7 located more ventro-laterally. This axis is parallel to the direction of many mitotic clones observed in developmental studies of the antenna (Postlethwait and Schneiderman, 1971) and to the orientation of individual sensilla. It is perpendicular to the boundaries between a number of sensillar types as defined anatomically (Shanbhag et al., 1999). Not only do large basiconic sensilla lie dorso-medial to the small basiconic sensilla, but at the dorso-medial extreme lies an ultrastructural subclass of large basiconic sensilla called LBI-1, which corresponds well in distribution and number to the ab3 sensilla. Another ultrastructural subclass of basiconic sensilla, TB-2, shows a distribution pattern similar to that of the ab4 and ab6 sensilla. We note also that the distribution of most of the sensilla that we could not classify is similar to that of "sensilla intermedia," a type of sensillum which, by electron microscopy, exhibits features intermediate between basiconic and trichoid sensilla. It is tempting to speculate that the different sensillar classes that we have defined functionally can also be distinguished anatomically, and that their distinct characteristics arise during development by differential expression of many genes, including receptor genes, along a morphogenetic axis.

Experimental Procedures

Single-Sensillum Recordings

Extracellular electrophysiological recordings were from Canton-S males at 20°C and 40%–60% relative humidity. A fly (2- to10-day-old) was wedged into the narrow end of a truncated plastic pipette tip and placed on a slide under a 1000× objective. The antenna was lifted and placed on a coverslip. The tip of a glass micropipette was used to hold the antenna in a stable position (see Clyne et al., 1997).

Action potentials were recorded by inserting a tungsten wire electrode in the base of a sensillum. The tip of the electrode was thereby brought in contact with the sensillum lymph surrounding the dendrites of the ORNs. The tungsten wire (0.1 mm diameter) was electrolytically sharpened (ca. 1 μm tip diameter) by repeated dipping in a 10% $NaNO_2$ solution while passing a 0.3–3 mA current through the solution. The reference electrode was inserted in the eye or at the base of the proboscis.

Signals were amplified 1000× (iso-dam, World Precision Instruments, Sarasota, FL) and fed into a computer via a 16-bit ADC to be analyzed offline with AUTOSPIKE software (Syntech, Hilversum, The Netherlands). AC signals (100–10,000 Hz) were recorded for 6 s, starting 2 s before stimulation, and action potentials were counted offline in a 0.5 s period before stimulation and for 0.5 s during stimulation. We noted a delay between the opening of the odor valve and the sharp onset of response to an odor stimulus. This delay was on the order of 75 ms and largely due to the travel time of the odor down the odor delivery tube (Clyne et al., 1997; de Bruyne et al., 1999). Accordingly, quantitation of response was begun 75 ms after opening the valve. Responses of individual neurons were calculated as the increase (or decrease) in action potential frequency (spikes/s) relative to the pre-stimulus frequency. In some experiments, action potentials were extracted by computer using the AUTOSPIKE algorithm, which distinguishes their peak-to-trough amplitudes from noise. Extraction was either offline from the primary data (Figures 6A–6C) or online to monitor the firing frequency of an individual ORN for up to 60 s (Figures 5A–5E).

Odor Stimulation

Odors were dissolved at a 10^{-2} dilution in paraffin oil or water and delivered via a syringe, using the system described in de Bruyne et al. (1999). Some ORNs showed relatively high responses to control stimuli consisting of diluent alone. Control experiments revealed that ab1A responded to an odor emanating from syringes. Limited data also suggest that aged paraffin oil elicits a greater response from ab1A; we thus used fresh solutions of odorants and paraffin oil control stimuli. A 0.55 s stimulus period was used in all recordings employing the "syringe puff" method except for Figure 5F, where odor stimulation was for 2.2 s. An interval of at least 60 s was allowed between odor stimuli, and we changed the order of presentation to minimize cross-adaptation effects. For dose-response relations, odorants were presented in increasing doses.

Odorants were from Aldrich (Milwaukee, WI) and were of the highest grade available (97%–99%), except that *cis*-vaccenyl acetate was from Sigma (St Louis, MO) and was 99% pure. We used (R)-(+)-α-pinene and (R)-(+)-limonene; β-citronellol, linalool, ethyl-2-methylbutanoate, and 1-octen-3-ol were racemic. 4-methylcyclohexanol was a mix of *cis* and *trans* isomers. The paraffin oil diluent was IR spectroscopy grade (Fluka, Buchs, Switzerland). Stimulation with gaseous carbon dioxide was by filling a 5 ml syringe with pure CO_2 (technical grade, Airgas, Cheshire, CT). When injected into the airstream with the syringe puff method, the concentration at the preparation was calculated to be ~5%.

For the prolonged stimulation experiment (Figure 5), we exposed ab2A to a prolonged ethyl acetate stimulus and allowed it to adapt, until it reached an approximately constant, low firing rate. We then superimposed a brief odor stimulus. To ensure that ethyl acetate concentration stayed relatively constant during an extended period, we established a dynamic equilibrium between ethyl acetate, diluted 10^{-2} in 200 μl of paraffin oil and applied to a filter paper on the inside wall of a 15 ml gas-wash flask, and a continuous air flow (2 ml/s). A solenoid valve then directed this odor-laden air via a needle either into the airstream toward the preparation or into a vacuum line to prevent odor buildup in the room. The equilibrium in this "flask flow" method was established during a period of at least 3 min before stimulation, and a single flask was not used for more than 20 min. The response of an ORN was recorded during stimulation for 30 s with the flask flow method; after 25 s of recording, a 0.55 s or 2.2 s stimulation with a second odor was delivered with the syringe puff method.

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