

Chemosensory Coding by Neurons in the Coeloconic Sensilla of the *Drosophila* Antenna

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Odor coding is based on the diverse sensitivities and response properties of olfactory receptor neurons (ORNs). In the *Drosophila* antenna, ORNs are housed in three major morphological types of sensilla. Although investigation of the *Drosophila* olfactory system has been expanding rapidly, the ORNs in one of these types, the coeloconic sensilla, have been essentially unexplored. We define four functional types of coeloconic sensilla through extracellular physiological recordings. Each type contains at least two neurons, with a total of at least seven distinct ORN classes that vary remarkably in their breadth of tuning. Analysis of 315 odorant–ORN combinations reveals how these neurons sample odor space via both excitation and inhibition. We identify a class of neurons that is narrowly tuned to small amines, and we find humidity detectors that define a cellular basis for hygroreception in *Drosophila*. The temporal dynamics of responses vary widely, enhancing the potential for complexity in the odor code. Molecular and genetic analysis shows that a broadly tuned ORN, antennal coeloconic 3B (ac3B), requires the odor receptor gene *Or35a* for its response *in vivo*. The activity of ac3B is not required for the response of the other ORN within that sensillum, ac3A. The functional analysis presented here, revealing a combination of highly specialized neurons and a broadly tuned ORN, along with the ancient origin of coeloconic sensilla, suggests that the specificities of these ORNs may reflect basic needs of an ancestral insect.

Key words: olfaction; *Drosophila*; antenna; coeloconic; physiology; receptor

Introduction

The first step in odor coding is the differential activation of olfactory receptor neurons (ORNs). Signals generated by ORNs undergo processing in the CNS, but the representation of an odor stimulus—its identity, intensity, and temporal structure—originates in the ORNs, whose diverse sensitivities and complex response properties provide the foundation of odor coding (Hildebrand and Shepherd, 1997).

The primary olfactory organ of *Drosophila* is the third antennal segment (Fig. 1A), whose surface is covered by sensilla ($n \sim 419$ in males and ~ 457 in females) (Shanbhag et al., 1999). These sensilla are innervated by up to four ORNs and fall into three major morphological types, the basiconic, the trichoid, and the coeloconic sensilla (Fig. 1B), as well as one minor type, the intermediate sensilla. The coeloconic sensilla ($n = 57$ in males and 54 in females) are the smallest of the types, 5 μm in height, and are architecturally distinct (Fig. 1C). Unlike the other types, they contain two walls, an outer and an inner wall, at the base of the

sensillum (Shanbhag et al., 1999). The inner wall encloses the ORN dendrites. The distal half of the sensillum appears longitudinally grooved. It is formed by ~ 8 closely apposed cuticular fingers, with grooves between the fingers that allow odors to pass through. Sensilla with this type of morphology have been conserved through hundreds of millions of years of insect evolution. They can be found in many and perhaps all insect orders (Steinbrecht, 1997), suggesting a critical function in chemosensory coding.

Although the basiconic sensilla of *Drosophila* have been extensively investigated (de Bruyne et al., 1999, 2001; Elmore et al., 2003), its coeloconic sensilla remain essentially unexamined. Preliminary investigation confirmed that at least some of its sensilla respond to odors (Clyne et al., 1997; Park et al., 2002); however, the number, specificities, and response properties of coeloconic ORN classes have not been defined, and their role in odor coding is unknown.

This paucity of information derives primarily from the technical difficulty of obtaining high-quality electrophysiological recordings from coeloconic sensilla. In addition to their small size, they are more susceptible to damage by the electrode and yield recordings with lower signal-to-noise ratios than the other types of sensilla. However, efforts to understand the integrated function of the *Drosophila* olfactory system (Hallem and Carlson, 2004) require an understanding of each of the three major subsets of olfactory input.

Here, we present a systematic investigation of this major type of olfactory sensillum and its contributions to odor coding. We define four functional types of coeloconic sensillum and seven classes of ORNs. The analysis identifies ORNs that represent ex-

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tremes in breadth of tuning, and recordings reveal diverse patterns of temporal dynamics. We identify humidity receptors and amine detectors. Genetic and molecular analysis reveals that *Or35a*, a member of the *Or* family of odor receptor genes, is necessary for the function of one ORN class, antennal coeloconic 3B (ac3B). Together, the data suggest a model in which the specificities of coeloconic ORNs reflect basic needs of an ancestral insect.

Materials and Methods

Drosophila stocks and transgenes. The *Or35a-GAL4* construct was created using primers to amplify a 5.0 kb DNA sequence immediately upstream of the translation initiation codon of *Or35a* and 1.2 kb of sequence downstream of the stop codon; the 5' end of the downstream region was 16 bp upstream of the *Or35a* stop codon. The transgenic construct was injected into *w¹¹¹⁸* flies. For cell-ablation experiments, the *Or35a-GAL4* line was crossed to *UAS-rpr* flies. *UAS-rpr/CyO* was a gift from B. Hay (California Institute of Technology, Pasadena, CA). The *Or35a* insertion line, PBac{WH}*Or35a*¹⁰²⁰⁵⁷ was from the *Drosophila* Stock Center (Bloomington, IN).

Electrophysiology. The preparation for extracellular single-unit recordings is essentially as described previously (Dobritsa et al., 2003). Canton-S flies aged <1 week were used for wild-type recordings. *Or35a-GAL4;UAS-rpr* flies were allowed to age for 7–10 d before recordings were taken, to allow time for cell death to occur. Electrical activity of the neurons was recorded extracellularly by placing an electrode filled with sensillum lymph ringer (Kaissling and Thorson, 1980) in the base of the sensillum. A reference electrode filled with the same ringer was placed in the eye. Signals were amplified using a patch-clamp amplifier (model 2400; A-M Systems, Carlsborg, WA) in voltage-clamp mode and fed into a computer via a 16-bit analog/digital converter (Digidata 1322A; Molecular Devices, Union City, CA). Impulses during the 0.5 s period before stimulation and the 0.5 s during stimulation were counted off-line using the AxoScope software (Molecular Devices). Responses of individual neurons were calculated as the increase (or decrease) in impulse rate (spikes per second) relative to the prestimulus rate. Each sensillum was tested with multiple odorants, and no more than three sensilla were analyzed per fly. The data shown are based on an analysis of 130 different sensilla. Neurons were often damaged during recording, as evidenced by the sudden initiation of high-frequency spike trains, presumably from rapid depolarization after membrane rupture. When a neuron in a sensillum was damaged, we discontinued recording from that sensillum.

Recordings were made by measuring currents in voltage-clamp mode (Redkozubov, 1995; Minor and Kaissling, 2003), which yielded a higher signal-to-noise ratio than we obtained when measuring voltages with a high-input impedance differential amplifier. To confirm that the measurement of currents did not confound comparisons with previous analyses based on measurement of voltages (Clyne et al., 1997; de Bruyne et al., 2001), we performed a direct comparison of odorant responses as determined by measurement of voltages versus currents: we tested the magnitudes and temporal dynamics of odor responses of the ab2A neuron (de Bruyne et al., 2001) and found no differences. Specifically, a 500 ms pulse of 2,3-butanedione produced 176 ± 13 spikes/s when measuring voltages and 188 ± 21 spikes/s when measuring currents ($n = 10$ in each case). A 500 ms pulse of ethyl acetate produced 262 ± 6 and 276 ± 13 spikes/s, respectively ($n = 10$). Moreover, a direct comparison of ethyl acetate responses at each 100 ms time point after odorant delivery up to 2 s after stimulus revealed no differences in the firing rate at any time ($n = 10$).

Odorant stimuli were prepared in Pasteur pipettes as described previously (Dobritsa et al., 2003). Odorants were from Sigma (St. Louis, MO) and were of the highest grade available (97–99%, except that phenylacetaldehyde was >90% pure). Alcohols are C1, and ketones are C2. Chem-

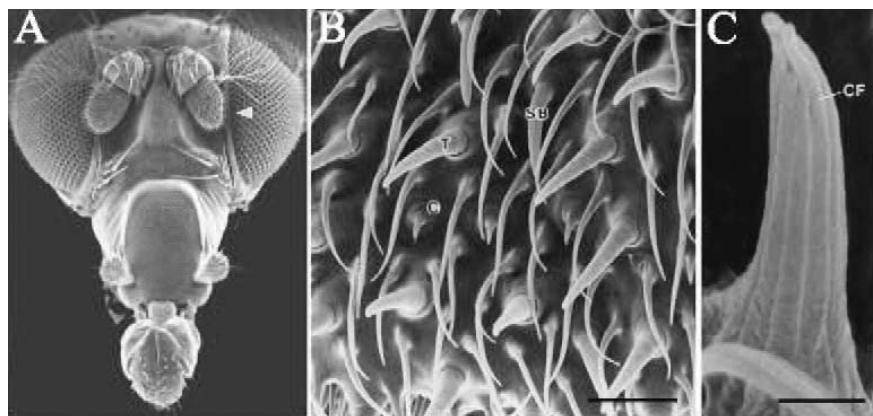


Figure 1. Coeloconic sensilla. **A**, Head of *Drosophila*. The arrowhead indicates the third antennal segment. **B**, Surface of the antenna, showing the coeloconic sensillum (C), small basiconic sensillum (SB), and trichoid sensillum (T). Scale bar, 10 μ m. **C**, Coeloconic sensillum. CF, Cuticular fingers. Scale bar, 0.5 μ m. **B** and **C** are from Shanbhag et al. (1999).

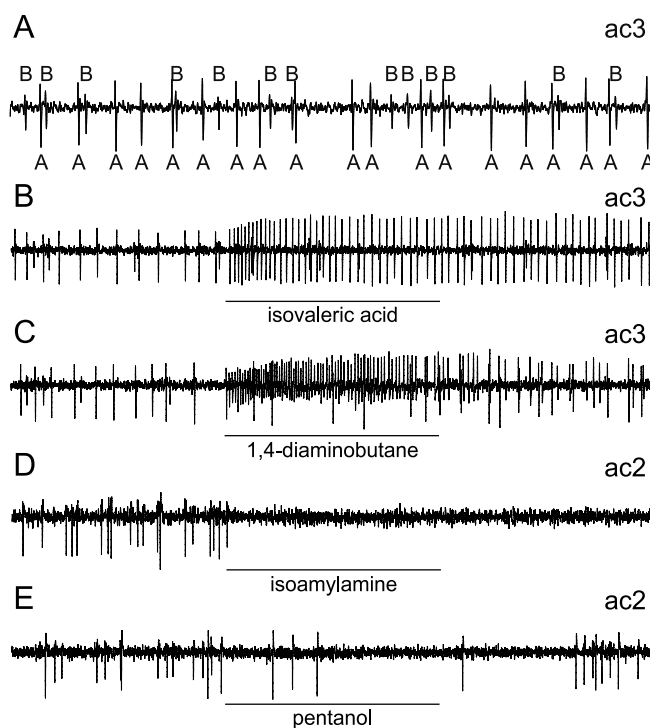


Figure 2. Recordings from the coeloconic sensilla. **A**, Spontaneous activity (750 ms) of two ORNs in a sensillum later classified as ac3. Spikes of large and small amplitude are designated A and B, respectively. **B**, Excitatory response of an A neuron, later classified as ac3A, to isovaleric acid. **C**, Excitatory response of a B neuron, later classified as ac3B, to 1,4-diaminobutane. **D**, Inhibitory response to isoamylamine. Both ORNs are inhibited by the odor. This sensillum was later classified as ac2. **E**, Inhibitory response of the B cell to pentanol. The A neuron is unaffected. The sensillum was later classified as ac2. Bars represent 0.5 s pulses of the indicated odors.

icals are racemic mixtures, unless otherwise stated. Propionic acid, butyric acid, ammonia, dimethylamine, 1,4-diaminobutane, and isoamylamine were diluted 10^{-2} in H_2O , unless otherwise noted. All other odorants were diluted 10^{-2} in paraffin oil (Fluka, Buchs, Switzerland). Stimuli were presented by placing the tip of the pipette through a hole in a glass tube carrying a humidified air stream (500 ml/min) directed at the fly and by administering a 0.5 s pulse of air (300 ml/min, giving a combined airflow of 800 ml/min during odor stimulation) through the pipette containing the odorant. All stimuli were used for a maximum of three presentations. The dilution used in most previous

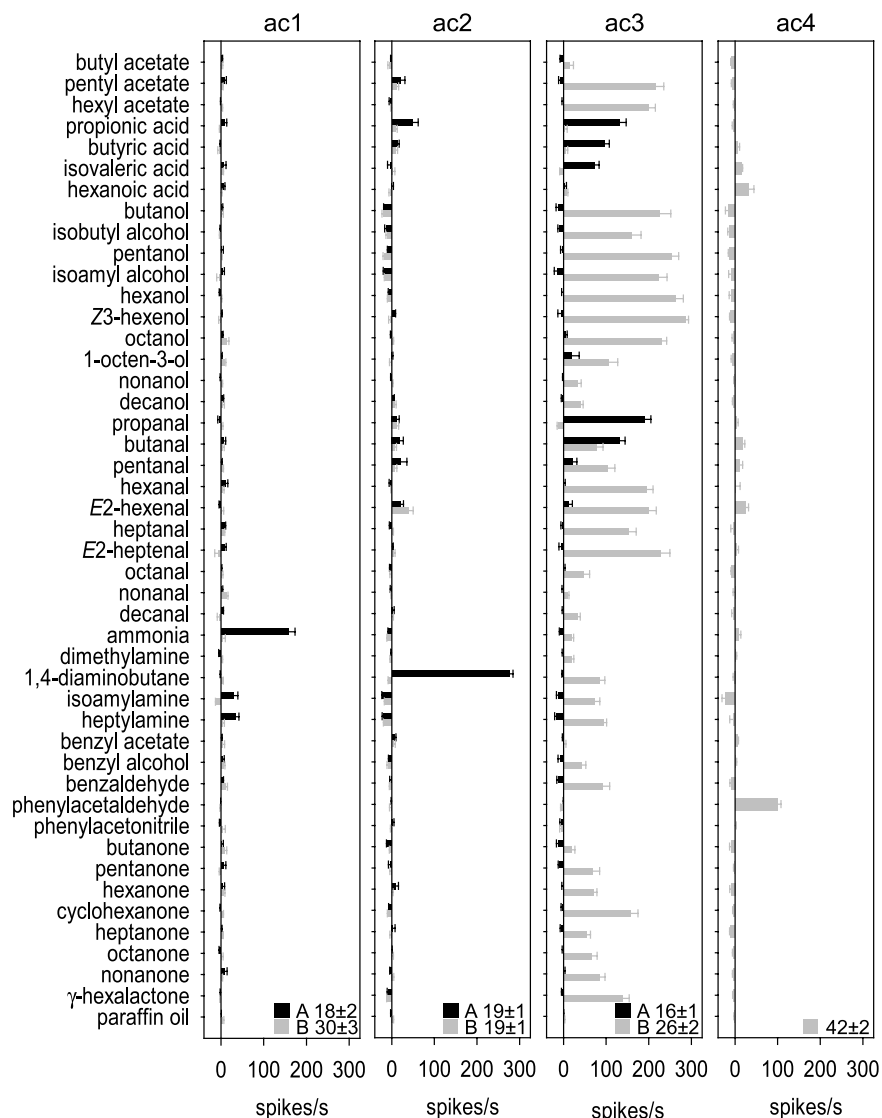


Figure 3. Responses of coeloconic ORNs to a panel of 45 odors. The spontaneous firing frequencies for each ORN are shown in the bottom right corner of each panel. We summed the activities of all ORNs in ac4, because we could not resolve the spikes with confidence. Recordings ($6 \leq n \leq 40$) were each from a different sensillum. Responses to the paraffin oil diluent ranged from 4.4 ± 2.2 spikes/s (ac2B) to -1.5 ± 1.2 spikes/s (ac2A). Error bars indicate SEMs.

characterizations of *Drosophila* ORNs and odor receptors is 10^{-2} (de Bruyne et al., 1999, 2001; Hallem et al., 2004; Goldman et al., 2005; Kreher et al., 2005). Although we refer to the dose in terms of the dilution of the odorant in solvent, i.e., 10^{-2} , we note that the 0.5 s pulse of air is further diluted in the humidified air stream, and we do not know how many molecules of odorant are carried from the solvent to the antenna or how this exposure compares with that of a fly in close contact with a fermenting fruit (Hallem et al., 2004).

For humidity recordings, the air stream was switched by a solenoid valve between 0 and $\sim 100\%$ humidity. The flow rate was kept at a constant 2 L/min. For 0% humidity air, we used an ultradry air source (Airgas, Cheshire, CT). To generate the $\sim 100\%$ humidity air, we bubbled the air through a container of water. A hygrometer (VWR International, West Chester, PA) was used to measure the relative humidities.

Results

Four functional types of coeloconic sensilla

Extracellular physiological recordings from coeloconic sensilla revealed the spontaneous activities of ORNs. In the case of many

sensilla, two ORNs could easily be classified by virtue of their distinguishable amplitudes: one ORN, termed the A cell, produced spikes of larger amplitude than the other ORN, termed the B cell (Fig. 2A).

Neurons in coeloconic sensilla showed excitatory responses to odors. For example, the A cell shown in Figure 2B produced a train of spikes when stimulated with the odor of isovaleric acid. Different neurons responded to different odors: for example the B cell in the same sensillum responded to the odor of 1,4-diaminobutane (Fig. 2C). Although the spike amplitudes shown in Figure 2 remained constant during the course of the recordings, the spike amplitudes in other recordings often changed during periods of intense activation; such changes have been observed previously in recordings of other ORNs from *Drosophila* (de Bruyne et al., 1999, 2001) and other insect species (Guillet and Bernard, 1972; Rumbo, 1989) and in general do not impair our ability to classify spikes.

In many cases, we observed inhibitory responses to odors: after odor stimulation, the frequency of firing fell below that of the spontaneous frequency. In some instances, both ORNs in a sensillum were inhibited such that little, if any, activity was observed after the odor stimulus (Fig. 2D). In other cases, only one of the ORNs showed strong inhibition (Fig. 2E, B cell).

We chose a set of 45 odors with which to characterize the ORNs of coeloconic sensilla. These odors were chosen to represent different chemical classes, such as acetate esters, organic acids, alcohols, aldehydes, amines, aromatics, and ketones. A number of these odors have been shown previously to play roles in the chemical ecology of *Drosophila* or other flies, and many are present in fruits (Stensmyr et al., 2003; TNO, 2004).

Extensive recordings, using these odors as stimuli, revealed four functionally distinct types of coeloconic sensilla, which we term ac1, ac2, ac3, and ac4. The magnitudes of the responses of the ORNs in these sensilla to the entire panel of odors are shown in Figure 3 and Table 1. The ac1 sensillum contains two ORNs. One responded strongly to ammonia, which is an important cue for a number of insects that seek human and animal hosts, including mosquitoes and ticks (Haggart and Davis, 1980; Braks et al., 2001). This ORN showed weaker responses to two other amines, isoamylamine and heptylamine, but none of the other tested compounds. The neighboring ORN showed no responses to any of the compounds. We will refer to the ammonia-sensitive cell as ac1A and to the nonresponsive cell as ac1B, although we note that ac1 is an example of a sensillum type in which the ORN classes are difficult to resolve (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). Difficulty in sorting spikes has also been reported for grooved, double-walled sensilla in *Anopheles* mosquitoes, al-

though spikes from trichoid sensilla of *Anopheles* could be classified (van den Broek and Den Otter, 2000).

The ac2 sensillum contains two ORNs, of which ac2A responds strongly to 1,4-diaminobutane. We observed only weak excitatory responses, and a few inhibitory responses, to other odors (Table 1, Fig. 3). The other ORN, ac2B, showed only one, weak excitatory response to odors of this set, and a few inhibitory responses.

Our analysis of the ac3 sensillum type defined two ORNs. ac3A is highly sensitive to propanal, and strong excitation was also observed in response to butanal and propionic acid, which are structurally related to propanal. ac3B differs from all other ORNs in this study in its breadth of tuning: it responds to a remarkably high fraction of the tested odors. Of the 45 odors tested, excitatory responses were observed to 36 (80%) (Table 1), and particularly strong responses to most alcohols were measured.

The ORNs in ac4 sensilla gave few excitatory responses, none of which exceeded 100 spikes/s. We were unable to sort the spikes with confidence in our recordings from ac4 and hence have presented the data in Figure 3 and Table 1 as the summation of all spikes from this sensillum elicited by each stimulus (for an example of an ac4 recording, see supplemental Fig. 1, available at www.jneurosci.org as supplemental material). However, it is clear that ac4 contains at least two and perhaps three ORNs, because we could often identify the firing of multiple neurons by the presence of summation events, i.e., overlapping spikes that are recognizable by their altered amplitudes or abnormal shapes; these superimposed spikes must arise from distinct neurons because the time interval between two such spikes is too short to have arisen from a single neuron.

Diverse patterns of temporal dynamics

The odor responses recorded from coeloconic ORNs revealed diverse patterns of temporal dynamics. In one pattern, the firing frequency rises quickly to a high level and then falls to a lower level, at which it continues firing for a sustained period of time (Fig. 4A). Although the graph in Figure 4A shows data only for the first 2.5 s, at the end of which the firing frequency was 28.7 ± 5.3 spikes/s above the spontaneous rate, the elevated firing rate persisted: at 10 s, the frequency was 30 ± 4.6 spike/s ($n = 6$; data not shown). A second pattern of temporal dynamics is also marked by a rapid rise, followed by a decline; however, in this case, the firing frequency decays to the spontaneous level by the end of the 2.5 s recording period (Fig. 4B). A

Table 1. Response spectra of ORN classes

Odorant	ac1		ac2		ac3		ac4
	A	B	A	B	A	B	Σ
Butyl acetate	0	0	0	0	0	+	0
Pentyl acetate	0	0	+	0	—	++++	0
Hexyl acetate	0	0	0	0	0	++++	0
<i>Propionic acid</i>	0	0	++	0	+++	0	0
<i>Butyric acid</i>	0	0	0	0	++	0	0
Isovaleric acid	0	0	0	0	++	0	0
Hexanoic acid	0	0	0	0	0	0	+
Butanol	0	0	—	—	—	++++	0
Isobutyl alcohol	0	0	—	—	—	+++	0
Pentanol	0	0	0	—	0	++++	0
Isoamyl alcohol	0	0	—	—	—	++++	0
Hexanol	0	0	0	0	0	++++	0
Z3-hexenol	0	0	0	0	0	++++	0
Octanol	0	0	0	0	0	++++	0
1-Octen-3-ol	0	0	0	0	+	++	0
Nonanol	0	0	0	0	0	+	0
Decanol	0	0	0	0	0	+	0
Propanal	0	0	0	0	++++	0	0
Butanal	0	0	+	0	+++	++	+
Pentanal	0	0	+	0	+	++	0
Hexanal	0	0	0	0	0	++++	0
<i>E2-hexenal</i>	0	0	+	+	0	++++	+
Heptanal	0	0	0	0	0	+++	0
<i>E2-heptenal</i>	0	0	0	0	0	++++	0
Octanal	0	0	0	0	0	++	0
Nonanal	0	0	0	0	0	0	0
Decanal	0	0	0	0	0	+	0
<i>Ammonia</i>	+++	0	0	0	0	+	0
<i>Dimethylamine</i>	0	0	0	0	0	+	0
<i>1,4-Diaminobutane</i>	0	0	++++	0	0	++	0
<i>Isoamylamine</i>	+	0	—	—	—	++	0
<i>Heptylamine</i>	+	0	—	—	—	++	0
Benzyl acetate	0	0	0	0	0	0	0
Benzyl alcohol	0	0	0	0	0	+	0
Benzaldehyde	0	0	0	0	—	++	0
Phenylacetaldehyde	0	0	0	0	0	0	++
Phenylacetone	0	0	0	0	0	0	0
Butanone	0	0	—	0	—	+	0
Pentanone	0	0	0	0	—	++	0
Hexanone	0	0	0	0	0	++	0
Cyclohexanone	0	0	0	0	0	+++	0
Heptanone	0	0	0	0	0	++	0
Octanone	0	0	0	0	0	++	0
Nonanone	0	0	0	0	0	++	0
γ-Hexalactone	0	0	0	0	0	+++	0
Paraffin oil	0	0	0	0	0	0	0

Responses are indicated as excitatory if the firing frequency exceeds by 15 spikes/s that of the spontaneous frequency. The response exceeded that of the spontaneous frequency as follows: +, by $15 \leq n < 45$ spikes/s; ++, by $45 \leq n < 113$ spikes/s; +++, by $113 \leq n < 180$ spikes/s; +++++, by $180 \leq n$ spikes/s (de Bruyne et al., 2001). The — symbol indicates inhibition to a level $\leq 50\%$ of the spontaneous firing frequency. Σ indicates that the responses represent the sum of the responses of all ORNs in the sensillum after odor stimulation, because the spikes of the ac4 ORNs could not be distinguished with confidence. All odorants were diluted 10^{-2} in paraffin oil, except for those in italics, which were diluted 10^{-2} in water (for explanation of dosages, see Materials and Methods). The 322 entries are each based on recordings from $6 \leq n \leq 40$ different sensilla from $n \geq 4$ different animals. A, A cell; B, B cell.

third pattern (Fig. 4C) is also characterized by a rapid rise in firing frequency but exhibits a subsequent period of poststimulus quiescence that begins almost immediately after the end of the stimulus. The inhibition is phasic, with the firing frequency returning to its basal level at 1.5 s in the graph. The final pattern, shown in Figure 4D, is one of inhibition, which begins immediately after

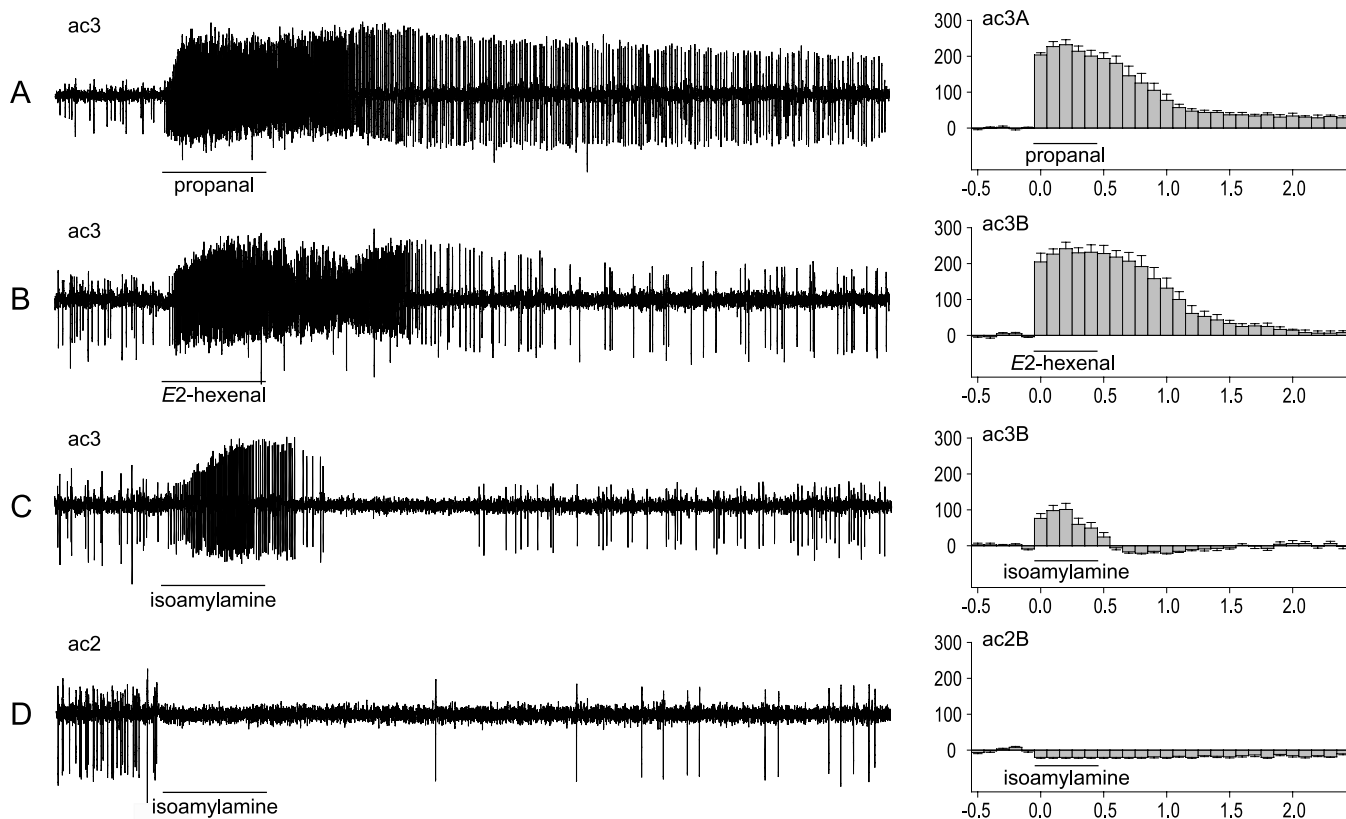


Figure 4. Differing temporal dynamics of coeloconic ORN responses. Bars represent 0.5 s pulses of the indicated odors. **A**, Response of ac3A to propanal. The amplitude of the A neuron increases after odor stimulation (see Results); the smaller spikes of the B cell can be seen in the second half of the trace. The vertical axis of the trace is reduced relative to the other traces in the figure. **B**, Response of ac3B to E2-hexenal. **C**, Response of ac3B to isoamylamine. The A cell is inhibited, as shown in Table 1. **D**, Inhibition of ac2B by isoamylamine. The A cell is also inhibited. In all graphs, $n = 6$. Error bars indicate SEMs.

the onset of the odor stimulus, with the inhibition lasting throughout the entire recording period. During the final 100 ms period shown in the graph, the firing rate is 10.3 ± 2.5 spikes/s below the spontaneous firing frequency; after 10 s, the rate was still 12 ± 3.0 spike/s ($n = 6$) below the spontaneous firing frequency.

Humidity sensors

Recordings from ac1 showed changes in spike frequency after changes in humidity. When the humidity was increased, the firing rate increased; when the humidity decreased, the firing rate decreased (Fig. 5A). Specifically, a change from dry air (0% RH) to moist air ($\sim 100\%$ RH) produced a rapid increase of ~ 50 spikes/s in the firing rate, and this increase was maintained until the humid air was again replaced by dry air, at which time the firing frequency fell abruptly (Fig. 5B, left two graphs). A similar response was observed in recordings from ac2 sensilla, although the change in firing frequency was smaller: ~ 20 spikes/s (Fig. 5B, right two graphs).

The changes in firing frequencies are sustained after a change in humidity. The graphs in Figure 5B show that, in ac1, the changes in firing frequency are sustained during a 5 s recording period, i.e., the firing frequency is relatively stable during the course of this period. In a separate experiment, we recorded activity during a 10 s period after a sustained change in humidity. We found that, in the case of ac1, the firing frequency was elevated by 47 ± 7 spikes/s after 5 s of increased humidity and by

41 ± 8 spikes/s after 10 s ($n = 6$ in both cases). The corresponding numbers for ac2 were 25 ± 5 and 28 ± 5 spikes/s, respectively. Likewise, the decreased frequencies at 5 s and those at 10 s were similar after a decrease in humidity (-33 ± 6 spikes/s at 5 s and -40 ± 8 spikes/s at 10 s for ac1; -23 ± 5 spikes/s at 5 s and -18 ± 5 spikes/s at 10 s for ac2; $n = 6$ in all cases).

We were initially motivated to test humidity because the ac1B and ac2B cells showed few, if any, excitatory responses to any of the diverse odorants we had tested (Table 1), and we therefore wished to test the hypothesis that they responded to humidity. The responses recorded from ac1 and ac2 after humidity changes are consistent with this hypothesis. However, we were unable to confirm with confidence that the humidity responses of ac1 and ac2 in fact derive from ac1B and ac2B cells: the spike amplitudes of A and B cells after changes in humidity were difficult to resolve. We cannot exclude the possibility of two humidity-responsive cells: one that responds to increases in humidity and one that responds to decreases, as has been found in the migratory locust (Altner et al., 1981) and in the cockroach (Tichy, 2003). Because experimentally induced changes in humidity can be accompanied by small changes in temperature, and because temperature-sensitive cells have been found in some cases to be combined with humidity-sensitive cells in coeloconic sensilla of other insects (Altner et al., 1977, 1981), we also tested ac1 and ac2 sensilla for responses to changes in temperature. Limited data revealed no responses to temperature changes in ac1 or ac2 or in ac3 or ac4.

We note that the presence of humidity had little, if any, effect

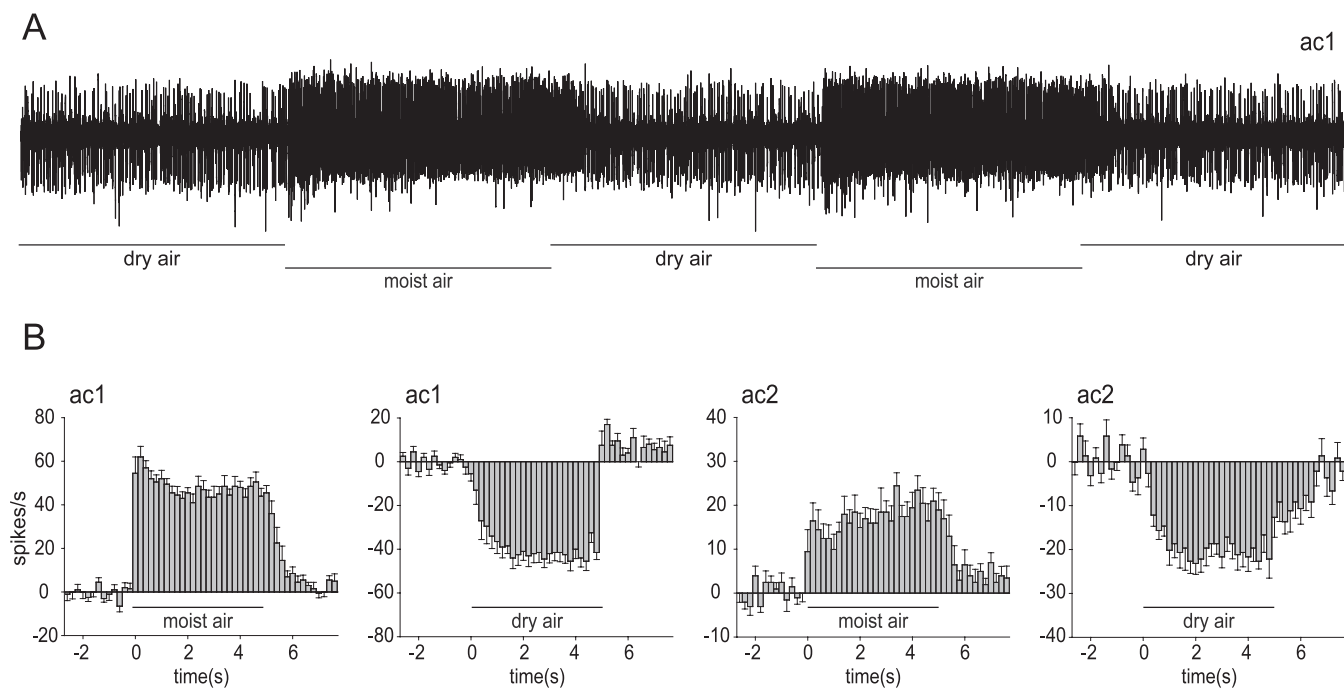


Figure 5. Responses to humidity. **A**, Recording from an ac1 sensillum. Bars represent 5 s pulses. **B**, Firing frequency (bin width, 200 ms) after changes in humidity. The two left panels show data from ac1 sensilla; the right panels represent ac2 sensilla. The vertical scales are different for the two sensillum types. In panels showing responses to “moist air,” the fly was exposed to dry air for 5 min before switching to humid air for 5 s, followed by a switch back to dry air. Likewise, in the panels showing responses to “dry air,” the fly was exposed for 5 min to humid air before switching to dry air for 5 s, followed by a switch back to humid air. In each graph, the number of spikes was counted during the 2.5 s period immediately before the change in humidity indicated by the bar. The average firing frequency during this 2.5 s prestimulus period was computed and subtracted from that of all bins in the graph. The spike counts represent all impulses recorded from each sensillum. $n = 10$ sensilla in each graph. Error bars indicate SEMs.

on the ammonia response of ac1A or the 1,4-diaminobutane response of ac2A, in limited testing. A 500 ms pulse of ammonia increased the firing frequency of ac1A by 159 ± 14 spikes/s ($n = 24$) in moist air and by 154 ± 6 spikes/s ($n = 10$) in dry air. Likewise, a 500 ms pulse of 1,4-diaminobutane increased the firing frequency of ac2A by 276 ± 8 spikes/s ($n = 35$) in moist air and 245 ± 10 spikes/s in dry air ($n = 10$).

Genetic and molecular manipulation of *Or35a* eliminates odor response of ac3B

Recently, the odor specificities of the Or family of antennal odor receptors were tested individually by expression of each receptor in an *in vivo* expression system (Hallem et al., 2004). Eleven of the tested receptors were found to confer odor response spectra that matched those of previously defined basiconic ORNs, and one, *Or35a*, conferred a response spectrum that corresponded to that of an undefined coeloconic ORN. This response spectrum appears similar to that defined here for ac3B, but we wished to determine directly the identity of the *Or35a*-expressing cell and to investigate the physiological effects of eliminating *Or35a* *in vivo*.

We obtained a stock containing a transposable element insertion 283 bp upstream of the *Or35a* translation start codon (Fig. 6A). The insertion lies in a region of noncoding DNA that is conserved between *D. melanogaster* and *D. yakuba*, suggesting that the region contains regulatory sequences essential for normal gene expression. Electrophysiological recordings from this insertion line revealed a population of coeloconic sensilla that gave responses characteristic of ac3A but not of ac3B (Fig. 6B, middle graph; compare with left graph). For example, the strong excitatory response to butanol recorded from ac3 sensilla in wild

type (Fig. 6C, top trace) is not observed in these *Or35a* mutant sensilla (Fig. 6C, middle trace). We examined the mutant sensilla in detail with 30 odors and found that all contained two ORNs: one that exhibited an odor response spectrum consistent with ac3A and one ORN that did not respond to any of 30 tested odors, most of which elicit strong or moderate responses from wild-type ac3B cells. Among ~30 coeloconic sensilla analyzed, we found none in which an ac3A cell, identified by virtue of its strong response to propionic acid, was paired with an odor-responsive cell. All ac3A cells in this analysis were paired with a cell that yielded spontaneous impulses, albeit at reduced frequency (6 ± 2 spikes/s compared with 26 ± 2 spikes/s in wild type for ac3B). Spikes in the insertion line did not show bursting, as was observed in cells suffering loss of the *Or22a* or *Or43b* genes (Dobritsa et al., 2003; Elmore et al., 2003).

We next generated an *Or35a-GAL4* construct and used it to drive synthesis of the reaper (*rpr*) cell death gene. Recordings from coeloconic sensilla of *Or35a-GAL4;UAS-rpr* revealed a population of sensilla that contain apparently normal ac3A cells but no other cells (Fig. 6B, right graph, C, bottom trace). We observed no spikes other than those attributable to ac3A.

The simplest interpretation of these results is that *Or35a* is the receptor that confers the odor response of ac3B and that the function of ac3A does not depend on the presence of the neighboring ac3B cell.

Discussion

The first step in the coding of an olfactory stimulus is the activation of ORNs in olfactory organs. In this study, we examined in detail the coding of olfactory information by ORNs in a major

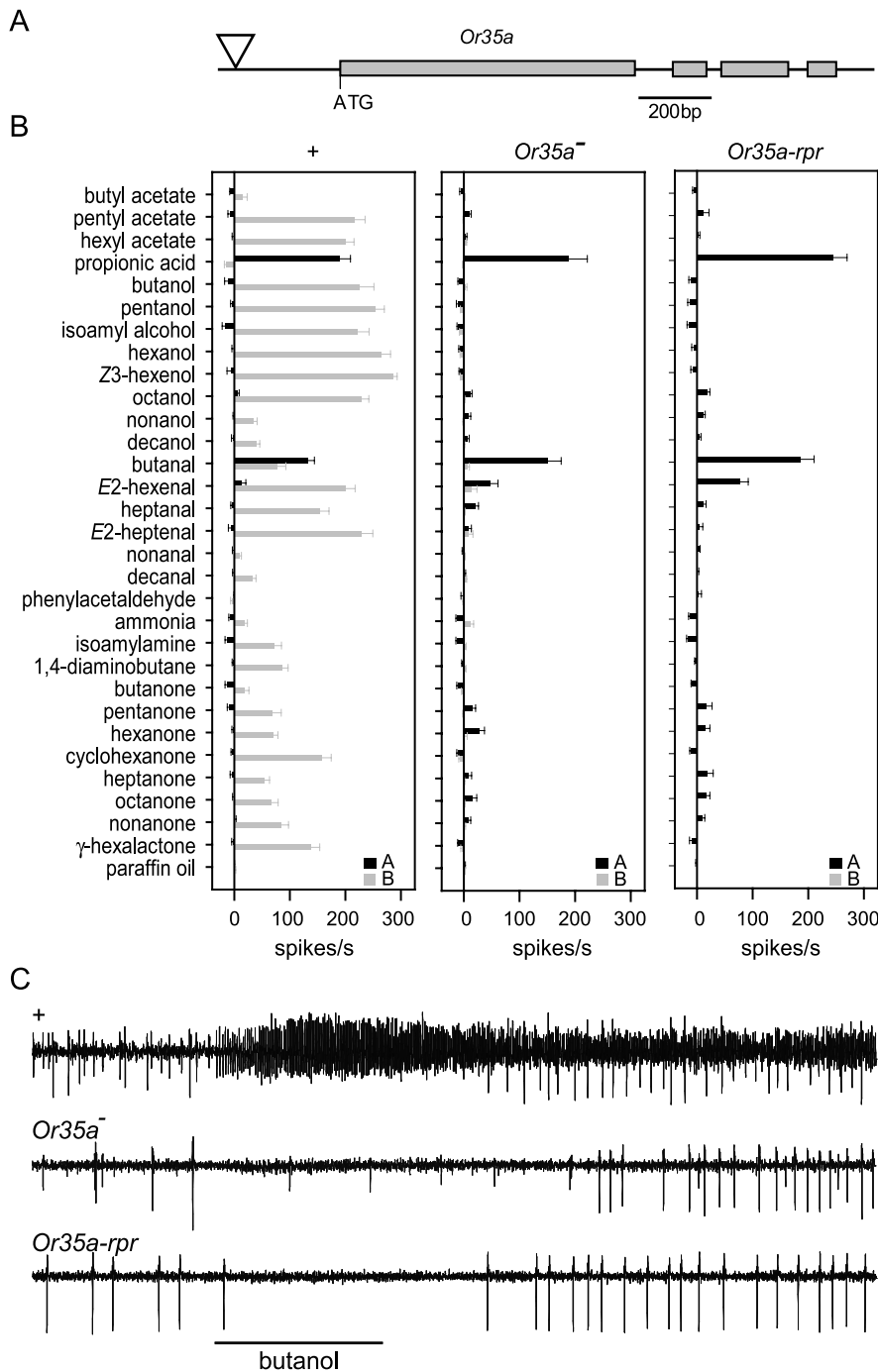


Figure 6. *Or35a* and the ac3 sensillum. **A**, The *Or35a* locus, showing insertion site (triangle) of a transposable element. **B**, Responses of wild type (left), an *Or35a* insertion mutant (center), and an *Or35a-GAL4;UAS-rpr* line (right). The *Or35a* insertion mutant was homozygous for the insertion, and *Or35a-GAL4;UAS-rpr* was homozygous for both the *Or35a-GAL4* driver and the *UAS-rpr* element. The data in the left panel are taken from Figure 3, except in the case of propionic acid, which was used in this experiment at a 10^{-1} dilution and for which $n = 27$. Middle panel, $n = 8$ for all odorants; right panel, $n = 11$ for all odorants. **C**, Traces of the different genotypes. The bar represents a 0.5 s pulse. Error bars indicate SEMs.

class of olfactory sensilla, the coeloconic sensilla, whose function in *Drosophila* has until now been essentially unexplored.

Physiological recordings identified four distinct functional types of coeloconic sensilla on the antennal surface. Each type contains at least two ORNs, and the data show that there are at least seven distinguishable classes of ORNs; we were unable to distinguish with confidence the activities of the different ORNs in

ac4 sensilla, but it is clear from the response to phenylacetaldehyde, for example, that at least one of the ORNs in ac4 is distinct from those in ac1, ac2, and ac3. We note that an anatomical study of coeloconic sensilla revealed two subtypes: one containing two neurons and the other containing three neurons (Shanbhag et al., 1999). It thus seems likely that at least one functional type, such as ac4, contains three ORNs.

We identified neurons in the *Drosophila* antenna that respond strongly to humidity. Hygroreception is critical in the insect world. Many insects, such as mosquitoes, lay eggs in water, and dry environments can lead to desiccation and death. *Drosophila* exhibits preferences when given a choice between environments of different humidities (Sayeed and Benzer, 1996); however, the molecular and genetic basis of hygroreception is not understood, and even its cellular basis has until now been unknown in *Drosophila*.

In defining a cellular basis of hygroreception in *Drosophila*, we provided a focus for a molecular and genetic analysis of the mechanism of transduction. We note that ablation experiments had provided evidence in a previous study that hygroreception localizes to the arista, a feathery projection extending from the third antennal segment (Sayeed and Benzer, 1996). However, this study also found that *spineless^{aristapedia}*, a homeotic mutant in which aristae are transformed into leg-like structures, had normal hygroreception. Our studies do not exclude the possibility of hygroreceptive cells associated with the arista or elsewhere; however, our identification of hygroreceptors in coeloconic sensilla is consistent with studies of other insects in which hygroreceptive cells were identified in morphologically similar sensilla (Altner et al., 1977; van den Broek and Den Otter, 2000).

The temporal dynamics of humidity responses, shown in the graphs of Figure 5, appear largely tonic. The responses to odors vary in their dynamics (Fig. 4). The diverse temporal dynamics shown by coeloconic ORNs may reflect another degree of freedom used by the antenna in odor coding: the temporal structure of olfactory information has been shown to be critical in odor coding in a variety of systems (Laurent et al., 2001).

We note with interest that, although the transduction mechanisms underlying hygroreception and olfaction may differ, they are likely to be housed in a common cell (i.e., in a multimodal neuron: isoamylamine inhibits all neuronal activity in ac2 sensilla) (Figs. 2D, 4D), including that of humidity-sensitive cells, suggesting that hygroreceptive cells contain a receptor for isoamylamine.

We also identified amine detectors. One class of ORN, ac1A, is highly sensitive to ammonia, and another, ac2A, responds strongly to 1,4-diaminobutane. A particularly important example of amine detection in insects is the attraction of mosquitoes to ammonia, a component of human sweat (Braks et al., 2001). Ammonia-sensitive cells have been detected in double-walled, grooved-peg sensilla on the antenna of *Anopheles* mosquitoes (van den Broek and Den Otter, 2000) and *Triatoma infestans*, a vector of trypanosomiasis, or Chagas disease (Taneja and Guerin, 1997). Identification of these cells in *Drosophila* lays a foundation for examining the molecular genetics of amine detection, which, in turn, could be useful in designing new means of pest control.

The response spectra of the coeloconic ORNs vary remarkably in their apparent breadth of tuning. At one extreme is ac3B, which reveals exceptionally broad tuning. ac3B is excited by 36 of 45 odors at the test concentrations; there is evidence that some coeloconic sensilla in the moth *Bombyx mori* yield responses to a variety of acids and alcohols (Pophof, 1997). ac1B, in contrast, is excited by none of these 45 odors; it may be the cell in ac1 that responds to humidity. ac1A responds strongly to ammonia and weakly to two other amines, but shows no responses to the other 42 tested odors. The profile of ac2A suggests that it may have evolved to signal the presence of 1,4-diaminobutane. No strong excitatory responses were detected among the neurons of ac4, other than a modest response to phenylacetaldehyde, and it seems likely that they detect the presence of biologically significant stimuli that are not included in our odor set. Conclusions about the breadth of ORN tuning are limited by the number of odors tested. Odor space is vast and discontinuous, and the sampling of odors here is necessarily limited. Nonetheless, the pattern that emerges from this analysis is one of a single broadly tuned ORN, ac3B, and others that are excited by only one or a small number of stimuli.

In light of the widely varying tuning breadths of these ORNs, it seems plausible that the coeloconic sensilla have evolved in large part to signal the presence of a small number of specific chemosensory stimuli, such as water vapor, ammonia, and 1,4-diaminobutane, also known as putrescine. The exceptionally broad spectrum of ac3B could have evolved as a more general sensor to signal the simultaneous presence of food sources. The coeloconic sensilla have an ancient origin (Steinbrecht, 1997), and perhaps their specificities reflect the most basic needs of an ancestral insect.

The specificities of these neurons are also of interest in light of the recent construction of a receptor-to-neuron map for the *Drosophila* antenna (Dobritsa et al., 2003; Hallem et al., 2004). *Or* genes were individually expressed in a mutant in which a particular ORN, ab3A, loses its odorant response because of a deletion that removes two odorant receptor genes, *Or22a* and *Or22b*. When particular *Or* genes were expressed in this mutant ORN, they conferred in many cases an odor response spectrum that matched that of a defined ORN. In this way, individual *Or* genes could be assigned to particular ORNs. Testing of virtually the entire repertoire of antennal *Or* genes mapped many receptor genes to basiconic ORNs, but only one, *Or35a*, to a coeloconic ORN. Because a number of receptors in that study conferred response profiles that did not match those of any defined ORNs, we further tested these unmapped receptors, using a panel of odorants from Table 1 that the present study has identified as diagnostic for particular coeloconic ORN classes. However, we were again unable to map any of these receptors to coeloconic ORNs (data not shown).

Why has only a single class of coeloconic ORN been found to

derive its odor response profile from an *Or* gene? One possible interpretation is that some coeloconic ORNs, for example those sensitive to humidity or ammonia, do not rely on receptors of the *Or* family to detect the molecules that activate them. We note that no *Or* gene was mapped to ab1C, a basiconic ORN that is a sensor of CO₂. A *Gr* (*Gustatory receptor*) gene (Clyne et al., 2000) has been mapped to this neuron (Suh et al., 2004), although at present there is no evidence that it is a CO₂ receptor. In the present study, we showed that *Or35a* is necessary for the response of ac3B. An important goal now is to determine which receptors are necessary for the responses of the other coeloconic neurons. The analysis of coeloconic ORNs presented here provides a foundation for investigating the molecular and genetic basis of the mechanisms by which coeloconic ORNs transduce chemosensory signals. It also provides a basis for investigating the roles of these neurons in olfactory-driven behaviors.

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