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Odorant-Selective Genes and Neurons Mediate Olfaction in C. elegans

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Summary

Olfaction is a versatile and sensitive mechanism for detecting volatile odorants. We show that the nematode C. elegans detects many volatile chemicals, which can be attractants, repellents, or attractants at low concentrations and repellents at high concentrations. Through laser ablation, we have identified chemosensory neurons that detect volatile odorants. Chemotaxis to volatile odorants requires different sensory neurons from chemotaxis to water-soluble attractants, indicating that C. elegans might have senses that correspond to smell and taste, respectively. Single neurons have complex sensory properties, since six distinguishable volatile odorants are sensed by only two types of sensory neurons. Chemotaxis to subsets of volatile odorants is disrupted by mutations in the *odr* genes, which might be involved in odorant sensation or signal transduction.

Introduction

Vertebrates can detect the presence of almost any volatile organic molecule, using the sense of olfaction, and can discriminate among similar molecules with high fidelity (Lancet, 1986; Reed, 1990). Olfactory neurons are able to recognize and encode this chemical information. Within the cilia of vertebrate and insect olfactory neurons, odorant binding is followed by the production of intracellular second messengers, including cAMP and phosphatidylinositides (Pace et al., 1985; Sklar et al., 1986; Boekhoff et al., 1990; Breer et al., 1990). These observations suggest that members of the seven membrane–spanning family of G protein–coupled receptors might be odorant receptors. Following this logic, Buck and Axel (1991) identified a candidate group of odorant receptor genes in the rat. These genes are expressed in the olfactory epithelium, and the sequences of their protein products are similar to those of G protein–coupled receptors. Hundreds or perhaps thousands of diverse sequences in this family are present in the rat. The structure and diversity of these molecules suggest that the interaction of odorants with subsets of these receptors is a key component of olfaction.

The existence of these genes opens many questions about the mechanism of odorant recognition and discrimination. How specific is the interaction of odorants and receptors? How many receptors are expressed on a single olfactory neuron? How is information about odorants transmitted to the brain to generate appropriate behaviors?

The small soil nematode Caenorhabditis elegans is an attractive organism in which to address these questions. C. elegans is accessible to genetic analysis and has a compact nervous system containing only 302 neurons in the adult hermaphrodite (Brenner, 1974; Wood et al., 1988). Each of these neurons can be identified in the live, transparent animal, and the neuronal morphologies and potential synapses have been described from electron micrographs of serial sections (White et al., 1986). The functions of individual neurons can be probed by killing neurons using a laser microbeam (Sulston and White, 1980; Avery and Horvitz, 1989).

C. elegans has long been known to chemotax to small water-soluble compounds like cAMP and NaCl, and neurons and genes required for these responses have been described (Ward, 1973; Dusenbery, 1974; Lewis and Hodgkin, 1977; Perkins et al., 1986; Bargmann and Horvitz, 1991a). Here, we show that C. elegans is also attracted to numerous volatile organic molecules. The chemosensory neurons required for chemotaxis to volatile attractants are distinct from those that sense salts. A screen for mutants that failed to chemotax to the volatile odorant benzaldehyde yielded *odr* (odorant response) mutants. Different *odr* mutants are defective in different, characteristic subsets of odorant responses.

Results

C. elegans Responds to Many Volatile Odorants

Chemotaxis of populations of C. elegans is measured by establishing a gradient of attractant from a point source and observing the accumulation of animals at the attractant source (Figure 1). A water-soluble attractant must diffuse through the agar substrate on the assay plate for several hours to form a gradient to which C. elegans will respond (Ward, 1973; data not shown). By contrast, if 1 μl of the volatile organic chemical isoamyl alcohol is spotted on the surface of the agar, C. elegans immediately orients its movement so that it accumulates at the isoamyl alcohol. Chemotaxis to isoamyl alcohol is fast and efficient for wild-type animals; the assay is complete within 1 hr (Figure 1).

The rapid response of C. elegans to isoamyl alcohol, a volatile organic molecule, suggests that the alcohol can diffuse through the air to be detected by the nematode. To test this possibility, isoamyl alcohol was spotted on the lid of the petri plate so that the attractant was not in direct contact with the agar on the plate. The animals in these assays accumulated immediately under the source of isoamyl alcohol, confirming that the alcohol was sensed even when it was not in direct contact with the agar. A dose response study of the response to isoamyl alcohol indicates that C. elegans chemotaxes equally well whether

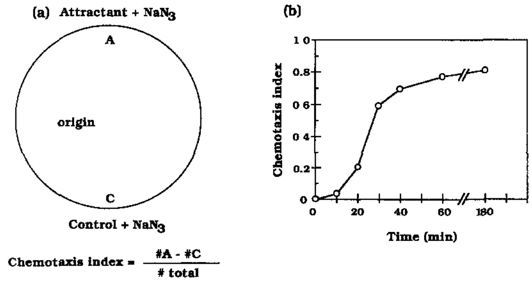

Figure 1. Chemotaxis Assays

(a) Population assays. A thin layer of agar in a petri dish was used as a substrate for chemotaxis, since C. elegans moves efficiently over the agar surface. Between 100 and 200 washed adult animals were placed near the center of a 10 cm assay plate with the attractant at one end of the plate and a control counterattractant at the opposite end of the plate. At various times, the number of animals at the attractant area and the control area was counted. A chemotaxis index was calculated based on the enrichment of animals at the attractant. The chemotaxis index can vary from +1.0 to −1.0. C. elegans adapts to an attractant after prolonged exposure, so the steady-state number of animals at an attractant will reflect both attraction and adaptation processes (Ward, 1973; data not shown). To minimize the effects of adaptation, sodium azide (an anesthetic) was used to capture animals at the attractant and control areas.

(b) Time course of chemotaxis to 1 μl of isoamyl alcohol (averages of 2 assays). The standard time point used to calculate the chemotaxis index in other figures was 60 min.

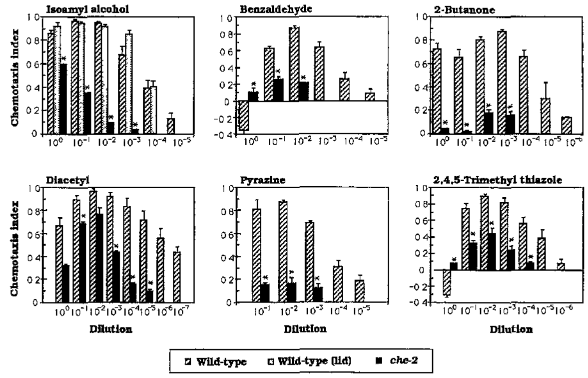

Figure 2. Dose Response Curves of Responses of Wild-Type Animals and *che-2(e1033)* Animals to Volatile Attractants

Wild-type animals, hatched bars; *che-2(e-1033)* animals, closed bars. One microliter of either undiluted attractant (10^0 dilution) or diluted attractant was present. Each data point represents at least three independent assays for N2 or *che-2*, with the SEM shown. In addition, the responses of wild-type animals to isoamyl alcohol suspended from the lid above the plate are shown (stippled bars). Asterisks, *che-2* values differing significantly from wild-type values at $p \leq 0.01$. Chemotaxis responses beyond the last dilution shown were not significantly above chance.

the attractant is placed on the lid above the petri plate or on the agar itself (Figure 2).

To identify other volatile attractants, 121 volatile chemicals were tested under the conditions diagramed in Figure 1. Among the attractants identified were numerous alcohols, ketones, esters, aldehydes, and aromatic compounds. Table 1 lists the attractants identified in these experiments. Fifty of the 121 compounds were strong attractants, 11 were variable or weak attractants, and 60 were not attractive to C. elegans.

Volatile Odorants Are Sensed at a Range of Concentrations

To characterize the responses to volatile attractants further, wild-type animals were tested at increasing dilutions of various attractants (Figure 2). 2-Butanone, diacetyl, isoamyl alcohol, and pyrazine were attractive through a broad range of concentrations. For example, a significant fraction of animals responded to 1 μl of undiluted diacetyl or to 1 μl of a 10^{-6} dilution of diacetyl. Benzaldehyde and 2,4,5-trimethylthiazole showed a more complex response. Both were attractive at low concentrations but somewhat repulsive when undiluted.

The responses to alcohols were examined further, since only a subset of alcohols appeared to be attractive (Table 1). A series of straight-chain alcohols from ethanol to 1-nonanol was tested at a range of concentrations (Figure 3). Ethanol and 1-propanol were only weakly attractive, while 1-butanol, 1-pentanol, and 1-hexanol were strongly attractive at low concentrations. However, 1-pentanol and 1-hexanol were not attractive at higher concentrations. 1-Heptanol, 1-octanol, and 1-nonanol were not attractive at any concentration and were repulsive at high concentrations.

Branched or secondary alcohols could also be attractive (Table 1). However, their attractiveness did not correspond strictly to the attractiveness of the n-alcohols. Thus, sec-butanol and t-butanol were less attractive than n-butanol, but 4-heptanol was attractive even though n-heptanol was not (data not shown).

Several general rules about attractive alcohols can be derived from these observations. First, a specific size and shape of alcohol seems to be attractive to C. elegans, with the optimum size being 4 to 6 carbons in a row followed by a hydroxyl group. As compounds vary either in size or in shape from this optimum, they become less attractive. A second, competing response to alcohols is also apparent. Alcohols with more than 5 carbons in a row are repulsive. For longer alcohols, either the attractiveness diminishes or the repulsion increases, or both, so that intermediate compounds like 1-hexanol exhibit both attractive and repulsive properties. The simplest explanation for these results is that two receptors for alcohols are found on C. elegans sensory neurons, one of which recognizes medium-sized alcohols and directs chemotaxis, and one of which recognizes longer-chain alcohols and directs repulsion.

Saturation Assays Define Categories of Volatile Odorants

Chemotaxis to volatile odorants could reflect either a nonspecific response to organic molecules or a specific chemical recognition of particular odorants. To distinguish between these possibilities, we attempted to saturate the responses to attractants by exposing C. elegans to high uniform concentrations of attractants. When 1 μl of benzal-

Table 1 Volatile Odorants Tested for Chemotaxis

Attractants	
Alcohols	1-Butanol, 1-pentanol, 1-hexanol, 2-pentanol, 2-hexanol, 3-hexanol, 4-heptanol, isobutanol, isoamyl alcohol, sec-butanol, β-mercaptoethanol, 4-penten-1-ol
Ketones	Acetone, 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, 3-pentanone, 3-octanone, diacetyl (2,3-butanedione)
Esters	Ethyl acetate, propyl acetate, n-butyl acetate, n-pentyl acetate, isoamyl acetate, ethyl propionate, ethyl butyrate, ethyl isobutyrate
Pyrazines	Pyrazine, 2-methyl pyrazine, 2,3-dimethyl pyrazine, 2,5-dimethyl pyrazine, 2,6-dimethyl pyrazine, 2-methoxypyrazine
Thiazoles	2-Ethoxythiazole, 2-isobutylthiazole, 2,4-dimethylthiazole, 2,4,5-trimethylthiazole
Aromatic compounds	Benzaldehyde, p-tolualdehyde, furfural, nitrobenzene, benzonitrile, methyl salicylate, aniline
Aldehydes	Valeraldehyde, capronaldehyde, heptaldehyde
Diethyl ether	
Weak attractants (attractive undiluted only)	
Citronellol, 2-phenylethanol, geraniol, methyl isobutyrate, benzene, cyclohexane, benzyl alcohol, benzylamine, pentylamine, heptylamine, 1-propanol	
Neutral or repellent compounds	
Alcohols	Ethanol, 2-heptanol, 3-heptanol, 2-octanol, 3-octanol, 1-nonanol, isopropanol, linalool, 1-heptanol,* 1-octanol,* 1-nonanol*
Ketones	2-Nonanone,* 3-nonanone,* 5-nonanone*
Esters	Ethyl valerate, ethyl hexanoate, propyl propionate, propyl butyrate, pentyl valerate, hexyl acetate, ethyl heptanoate,* butyl butyrate*
Pyrazines	2-Methoxyl-3-methylpyrazine, 2-methoxy-3-ethylpyrazine, 2-methoxy-3-isopropylpyrazine, 2-methoxy-3-isobutylpyrazine, 2-ethylpyrazine, 2-ethyl-3-methylpyrazine, 2-ethoxy-3-ethylpyrazine, 2-acetylpyrazine
Thiazoles	Thiazole, 4-methylthiazole, 2-acetylthiazole, 2,4,5-trimethylthiazole*
Aromatic compounds	Xylenes, chlorobenzene, toluene, benzyl benzoate, m,o-anisidine, m,p-anisaldehyde, trans-anethole, eugenol, 2-methylquinoxyline, 5-methylquinoxaline, 6-methylquinoxaline, vanillin, benzaldehyde*
Aldehydes	Butyraldehyde, caproylaldehyde, isobutyraldehyde
Amines	Diethylamine, triethylamine, butylamine, hexylamine, octylamine
Other	Limonene, +/−camphor, butyric acid, isovaleric acid, a-ionone +/−pinene, menthol

Repellents are listed with an asterisk. Some molecules (e g., benzaldehyde) are attractive at low concentrations and repellent at high concentrations. In general, a 10^{-2} dilution of odorant was tested; in some cases, when no response was observed, the odorant was also tested undiluted.

dehyde was evenly mixed into the 10 ml of agar contained in an assay plate, C. elegans did not chemotax to a point source of benzaldehyde (Figure 4). However, chemotaxis to the attractant diacetyl was normal on the same plates. If the agar included diacetyl but not benzaldehyde, the response to diacetyl was eliminated, but the response to benzaldehyde was normal. These results indicate that some specific, saturable molecule or process is required for chemotaxis to each of these compounds.

These saturation assays were used to define independent categories of volatile odorants. Seven classes of vola-

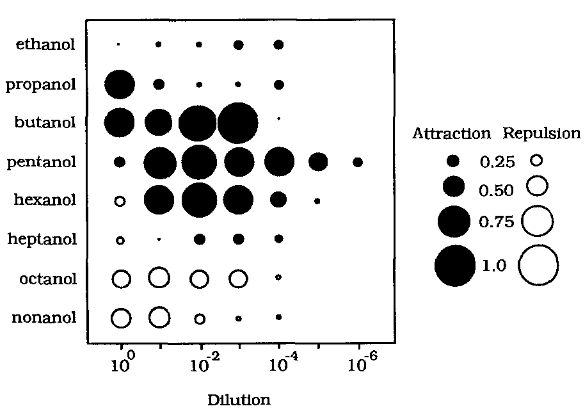

Figure 3. Responses of Wild-Type Animals to Straight-Chain Alcohols

Each alcohol was tested in populations of wild-type animals, as described in Figure 1. The diameter of the circle at each position represents the value of the chemotaxis index at that concentration of a given alcohol. For comparison, the sizes of the circles at chemotaxis indices of 0.25, 0.5, 0.75, and 1.0 are shown (the scale is linear). Closed circles indicate attraction; open circles indicate repulsion.

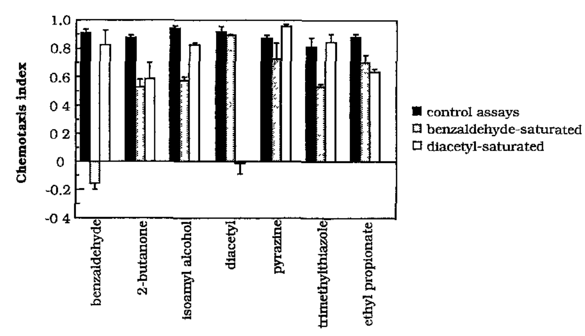

Figure 4. Saturation of Odorant Responses with High Concentrations of Benzaldehyde and Diacetyl

Wild-type animals were tested for their responses to each attractant on plates in which the 10 ml of agar contained 1 µl of either benzaldehyde or diacetyl. Attractant concentrations were 5×10^{-3} dilution (benzaldehyde), 10^{-3} dilution (butanone, diacetyl, and 2,4,5-trimethylthiazole), 10^{-2} dilution (pyrazine and ethyl propionate), and 10^{-1} dilution (isoamyl alcohol).

tile odorants were identified by testing some of the attractants listed in Table 1. Representative members of each class were benzaldehyde, 2-butanone, isoamyl alcohol, diacetyl, pyrazine, 2,4,5-trimethylthiazole, and ethyl propionate. Each of these odorants saturated its own response before eliminating responses to any of the other odorants. These odorants were used for further study of odorant responses and mutants, except for ethyl propionate, since responses to ethyl propionate were variable even in wild-type animals.

While at least these seven classes of odorants exist, some odorants were not discriminated from one another in such assays. Thus, high concentrations of isoamyl alcohol eliminated responses to other attractive alcohols, and high concentrations of benzaldehyde eliminated responses to nitrobenzene, benzonitrile, and furfural (data not shown). To a lesser extent, some cross-saturation was seen between members of different classes.

A Subset of *che* Mutations Affect Odorant Responses

A number of *che* (chemotaxis-defective) mutants have previously been identified based upon their effects on chemotaxis to water-soluble attractants such as NaCl (Dusenbery et al., 1975; Lewis and Hodgkin, 1977; Culotti and Russell, 1978; Albert et al., 1981; Perkins et al., 1986). These mutants were tested for chemotaxis to volatile odorants, including benzaldehyde, 2-butanone, and isoamyl alcohol.

Many of the *che* mutants were defective in chemotaxis to the volatile odorants, including *che-2*, *che-3*, *che-7*, *che-11*, *che-13*, *osm-1*, *osm-5*, *osm-6*, *daf-10*, *tax-2*, *tax-4*, *tax-6*, *unc-31*, *unc-86*, *mec-2*, and *lin-32* (data not shown; osm, osmotic avoidance defective; daf, dauer formation defective; tax, taxis defective; unc, uncoordinated; mec, mechanosensory defective; lin, cell lineage defective). However, other mutants that failed to chemotax to water-soluble attractants could still chemotax to volatile odorants. Among these mutants were *che-1*, *che-6*, *che-10*, *che-12*, *che-14*, *daf-6*, and *osm-3*.

One explanation for these results is suggested by the ultrastructural defects caused by some of the *che* mutants. The presumptive chemosensory organs of C. elegans include the amphids, the inner labial sensilla, and the phasmids (Ward et al., 1975; Ware et al., 1975). Each of these organs forms a pore through which the ciliated endings of some neurons are exposed to the environment. For example, 8 of the 12 neurons associated with each of the bilaterally symmetric amphids are exposed (Figure 6). Additional sensory organs present in the head are probably not chemosensory, since the neurons associated with these organs are embedded in the cuticle and are not exposed to the environment.

In *che-2*, *che-3*, *che-13*, *osm-1*, *osm-5*, and *osm-6* mutants, all ciliated sensory neurons in the head appear abnormal in electron micrographs (Lewis and Hodgkin, 1977; Perkins et al., 1986). Each of these genes is required for chemotaxis to volatile odorants, suggesting that ciliated sensory neurons are involved in those responses. By contrast, *che-10*, *che-12*, *daf-6*, and *osm-3* mutants, which still respond to volatile odorants, have more restricted ultrastructural defects (Albert et al., 1981; Perkins et al., 1986). All of these mutants have abnormalities in the exposed amphid neurons or in the structure of the amphid sensory opening. The exposed neurons of the amphid are important for chemotaxis to water-soluble attractants but apparently not for chemotaxis to volatile odorants.

The Ciliated AWC and AWA Neurons Sense Volatile Odorants

To identify neurons essential for chemotaxis to volatile odorants, we killed neurons in young animals using a laser microbeam, let them grow to adulthood, and tested the resulting animals for chemotaxis behaviors. The single-animal assays used to test operated animals were slightly different from the population chemotaxis assays described above (see Experimental Procedures) (Bargmann and Horvitz, 1991a). In brief, individual animals were placed on an assay plate on which a volatile odorant had been spotted and allowed to move freely for 1 hr. After 1 hr, the animal was removed and its pattern of movement, visible as indentations within the agar on the assay plate, was observed. Wild-type animals in the presence of a strong attractant tracked directly to the attractant. Some animals remained at the attractant for the entire hour, while others left and returned several times during the assay. The results from multiple operated animals were combined to calculate the fraction of animals that responded to a given attractant.

Two cell types of the amphid chemosensory organ, the AWA and AWC neurons, were required for chemotaxis to specific subsets of volatile attractants. Animals in which the AWC neurons were killed were severely defective in their responses to the volatile odorants benzaldehyde, butanone, and isoamyl alcohol (Figure 5). However, these animals responded normally to other volatile odorants and to water-soluble attractants such as Cl^- ions. Animals in which the AWA neurons were killed had defective responses to the volatile odorants diacetyl and pyrazine but normal responses to other volatile odorants and Cl^- ions (Figure 5).

Some attractants appeared to be sensed by both AWA and AWC. Killing AWA and AWC neurons together in the same animal reduced the responses to isoamyl alcohol and 2,4,5-trimethylthiazole more than killing either cell alone (Figure 5). Chemotaxis to Cl^- remained intact in these animals. Thus, AWA and AWC neurons are required for responses to distinct, but partly overlapping, subsets of volatile odorants.

The AWA and AWC sensory neurons are associated with the amphid sensory organ but are not directly exposed to the environment (Figure 6; Ward et al., 1975; Ware et al., 1975). The ciliated endings of AWA, AWC, and a third neuron type, AWB, are encased in a pocket that is continuous with the amphid opening; the endings of a fourth neuron type, AFD, are still further from the amphid opening. The branched ciliated endings of AWA, AWB, AWC, and AFD have more elaborate structures than those of the exposed amphid neurons that sense water-soluble attractants and repellents (Figure 6).

The exposed amphid neurons ASE, ADF, ASG, ASI,

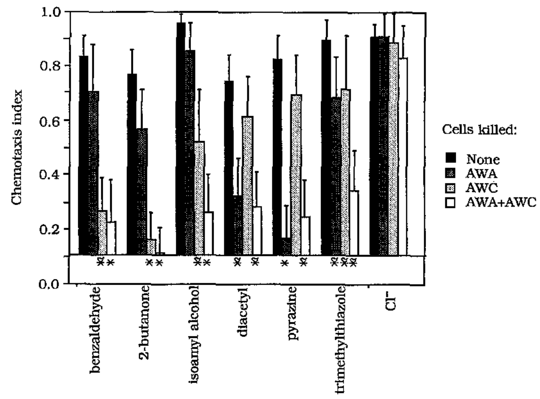

Figure 5. Chemotaxis Responses of Laser-Operated Animals

The baseline chemotaxis index is shown as 0.11; this value is the false-positive rate of the assay (see Experimental Procedures). Closed bars indicate unoperated animals; darkly stippled bars, animals in which both AWA neurons were killed; lightly stippled bars, animals in which both AWC neurons were killed; open bars, animals in which all AWA and AWC neurons were killed. Values less than those of unoperated animals at $p < 0.01$ are marked with asterisks. Values significantly less than those of unoperated animals but significantly greater than negative control values are marked with tildes (~). Error bars show 95% confidence intervals. Attractant concentrations were 5×10^{-3} dilution (benzaldehyde), 10^{-3} dilution (butanone, diacetyl, and 2,4,5-trimethylthiazole), 10^{-2} dilution (pyrazine), and 10^{-1} dilution (isoamyl alcohol). Each data point represents 21–46 operated animals and 57–81 controls.

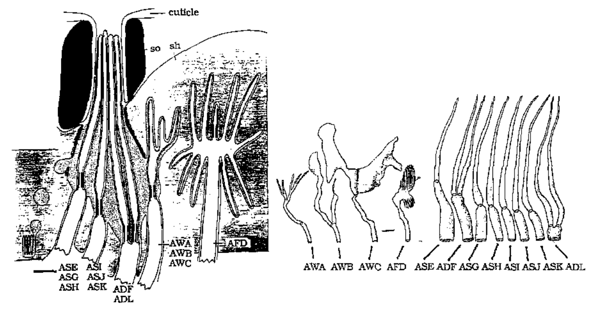

Figure 6. Structure of the Amphid and of the 12 Amphid Sensory Neurons

Modified from Ward et al. (1975) and Perkins et al. (1986).

(a) Schematic structure of the amphid opening. The endings of AWA, AWB, AWC, and AFD are encased within the amphid sheath cell (sh). The endings of the eight other neurons are exposed to the environment through the amphid socket cell (so). Scale bar, 1 μm. The cell bodies of the amphid neurons are located about 150 μm posterior to the amphid opening.

(b) Structures of the ciliated endings of the 12 amphid neurons. Scale bar, 1 μm.

and ASK account for all or nearly all chemotaxis to several water-soluble attractants (Bargmann and Horvitz, 1991a). When these neurons were killed with a laser, animals responded normally to benzaldehyde, diacetyl, and trimethylthiazole (chemotaxis indices of 0.77–1.0, n = 4–13 animals). Similarly, the exposed inner labial IL2 neurons are not required for chemotaxis to volatile odorants, since animals in which the IL2 neurons were killed could still chemotax to benzaldehyde, butanone, or isoamyl alcohol as well as control animals (chemotaxis indices of 0.89–1.0, n = 9 animals). In addition, *osm-3(p802)* mutants had defects in all exposed amphid or phasmid neurons but normal responses to all volatile odorants (data not shown). These results indicate that exposed neurons are not necessary for responses to volatile odorants.

After the AWA and AWC neurons were killed, most responses to volatile odorants were severely defective, but some residual responses to 2,4,5-trimethylthiazole, diacetyl, and pyrazine remained. Killing the AWB or AFD neurons together with the AWA and AWC neurons did not eliminate the responses. We have not been able to identify the neurons responsible for these residual responses.

The *odr* Mutants Are Defective in Responses to Volatile Odorants

To identify genes that might function specifically in the responses to volatile odorants, we isolated new mutants that failed to chemotax to the volatile odorant benzaldehyde at a 1:100 dilution (see Experimental Procedures). Eleven mutant strains that were defective in chemotaxis to benzaldehyde but normal in their responses to NaCl were isolated in our screens. These mutants defined six genes that are described below. In addition, we isolated a similar number of mutants that affected all chemotaxis responses; these mutants were not characterized further. To distinguish genes that affect all chemotaxis responses (*che* genes) from genes that affect only volatile odorant responses, the latter are designated as *odr* (odorant response) genes. Mutants defective in each of the *odr* genes were characterized both genetically and behaviorally (Figure 7). Serial section electron micrographs of the anterior sensory structures of these mutants were analyzed. Of the genes described below, only *odr-3* mutants had significant defects in the structures of amphid sensory neurons.

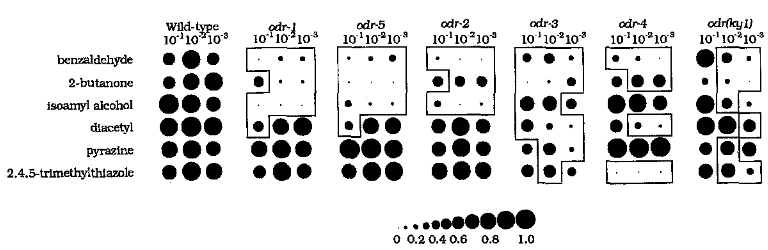

Figure 7. Chemotaxis Responses of Wild-Type and Mutant Animals

At least two independent assays of each strain were conducted at three different concentrations of each attractant. The size of the circle represents the chemotaxis index at that concentration. Boxes surround values different from wild-type at $p \leq 0.01$. The alleles for which data are shown are *odr-1(n1936)*, *odr-2(n2145)*, *odr-3(n2150)*, *odr-4(n2144)*, and *odr-5(ky9)*.

odr-1 *and* odr-5 *Mutations Eliminate the Function of the AWC Neurons*

A gene we have designated *odr-1* is defined by three mutant alleles, *n1930*, *n1933*, and *n1936* (see Experimental Procedures). *odr-1* mutants were defective in their responses to isoamyl alcohol, benzaldehyde, and 2-butanone (Figure 7). Their responses to diacetyl, pyrazine, and 2,4,5-trimethylthiazole were mostly normal. Responses to water-soluble attractants were also mostly normal, although variable defects in the responses to sodium acetate and cAMP were observed (data not shown). The defects caused by all three alleles of the gene were similar.

A fourth mutant strain, *odr-5(ky9)*, showed a spectrum of defects similar to those of *odr-1* mutants (Figure 7). The *odr-5* mutation complemented mutations in *odr-1* and mapped to a different interval. These data suggest that *odr-5* is a second gene that affects the same responses as does *odr-1*.

The behavioral defects of *odr-1* and *odr-5* mutants were similar to those observed when the AWC neurons were killed with a laser (Figure 5), so we examined the AWC neurons in these mutants. A nucleus that corresponds in position and appearance to the AWC nucleus was present in all *odr-1* and *odr-5* mutants, and superficially normal AWC cilia were present in electron micrographs of these mutants (data not shown).

odr-2 *Mutations Affect a Subset of Responses Mediated by the AWC Neurons*

The *odr-2* gene is defined by three mutations, *n1939*, *n2145*, and *n2148*. *odr-2* mutants were defective in their responses to benzaldehyde and isoamyl alcohol. They still responded to 2-butanone, although less well than wild-type animals (Figure 7). Their responses to diacetyl, pyrazine, 2,4,5-trimethylthiazole, and water-soluble attractants were normal. All three *odr-2* alleles caused similar behavioral defects.

All of the responses that were affected by *odr-2* mutations are mediated by the AWC neurons, but the cilia of the AWC neurons appeared normal in electron micrographs of *odr-2* mutants. The function of the AWC neurons was not eliminated completely by *odr-2* mutations, since mutants could still respond to 2-butanone. Either the *odr-2* gene is not essential for the 2-butanone response, or the three alleles are all leaky mutations with residual *odr-2* function. To explore the latter possibility, we examined the behavior of *odr-2* mutations in trans to genetic deficiencies that failed to complement *odr-2* mutations, presumably because they delete the *odr-2* gene (see Experimental Procedures). Weak mutations are often enhanced in severity when placed in trans to genetic deficiencies, but the effects of *odr-2* mutations were not enhanced by deficiencies. Animals with one copy of an *odr-2* mutation and one copy of the deficiency could still chemotax to 2-butanone as well as homozygous *odr-2* animals could (data not shown), as would be expected if the *odr-2* mutations are strong or null mutations.

odr-3 *Mutations Affect Responses Mediated by AWA and AWC Neurons*

Two mutations defined the *odr-3* gene. Responses to all of the volatile odorants were partly defective in *odr-3(n2150)* mutants, although the isoamyl alcohol response was only slightly affected (Figure 7). A residual response to most attractants was present in the mutants. *odr-3(n2046)* appeared to be a weaker mutation in the same gene as *odr-3(n2150)*. *odr-3(n2046)* had much less severe effects than *odr-3(n2150)* and failed to complement *odr-3(n2150)* for chemotaxis to benzaldehyde.

odr-3 mutants also failed to avoid high osmotic strength, and they were defective in chemotaxis to lysine but not to other water-soluble attractants (data not shown). Therefore, *odr-3* might affect functions of four kinds of sensory neurons: the AWA neurons, the AWC neurons, the ASH neurons (osmotic strength avoidance; Bargmann et al., 1990; J. H. Thomas and H. R. H., unpublished data), and perhaps the ASK amphid neurons (lysine response; Bargmann and Horvitz, 1991a).

The AWC cilia in *odr-3(n2150)* mutants were smaller than those in wild-type animals (Figure 8). This structural abnormality in the AWC cilia may contribute to the behavioral defects in these mutants. The ASH and ASK neurons were superficially normal in *odr-3* mutants; unfortunately, the AWA cilia could not be reliably followed in our sections of *odr-3* or wild-type animals.

odr-4 *Mutants Are Defective in Subsets of AWA and AWC Functions*

A single mutation in the *odr-4* gene was isolated. *odr-4(n2144)* animals failed to respond to benzaldehyde, diacetyl, and 2,4,5-trimethylthiazole but responded normally to isoamyl alcohol and pyrazine (Figure 7). The defects caused by the *odr-4* mutation did not correspond to the functions of any single sensory neuron, since some but not all functions of both AWA and AWC were affected by *odr-4*. The cilia of the AWC neurons were normal in *odr-4* mutants.

The genetic deficiency *nDf16* failed to complement *odr-4(n2144)*. When the *odr-4* mutation was placed in trans to *nDf16*, no additional behavioral defects were observed (data not shown). By the same arguments used above in discussing *odr-2*, this result argues that the *odr-4(n2144)* mutation affects only a specific subset of odorant responses.

odr(ky1) *Decreases the Sensitivity of Most Odorant Responses*

The mutation *odr(ky1)* has not been given a gene name, since its map position is not refined enough to demonstrate that it is not an allele of a previously described gene.

odr(ky1) mutants showed defective chemotaxis to all volatile attractants. In most cases, *odr(ky1)* decreased the sensitivity of the odorant responses (Figure 7): odorants were recognized at high concentrations but not at low concentrations. *odr(ky1)* animals could chemotax to water-soluble attractants (data not shown), but those assays were less quantitative than assays of chemotaxis to volatile odorants, so slight changes in sensitivity might have been overlooked.

Discussion

Sensation of Volatile Odorants Is Analogous to Olfaction

C. elegans lives in the soil at the air–water interface; it is likely to encounter both water-soluble and volatile chemi-

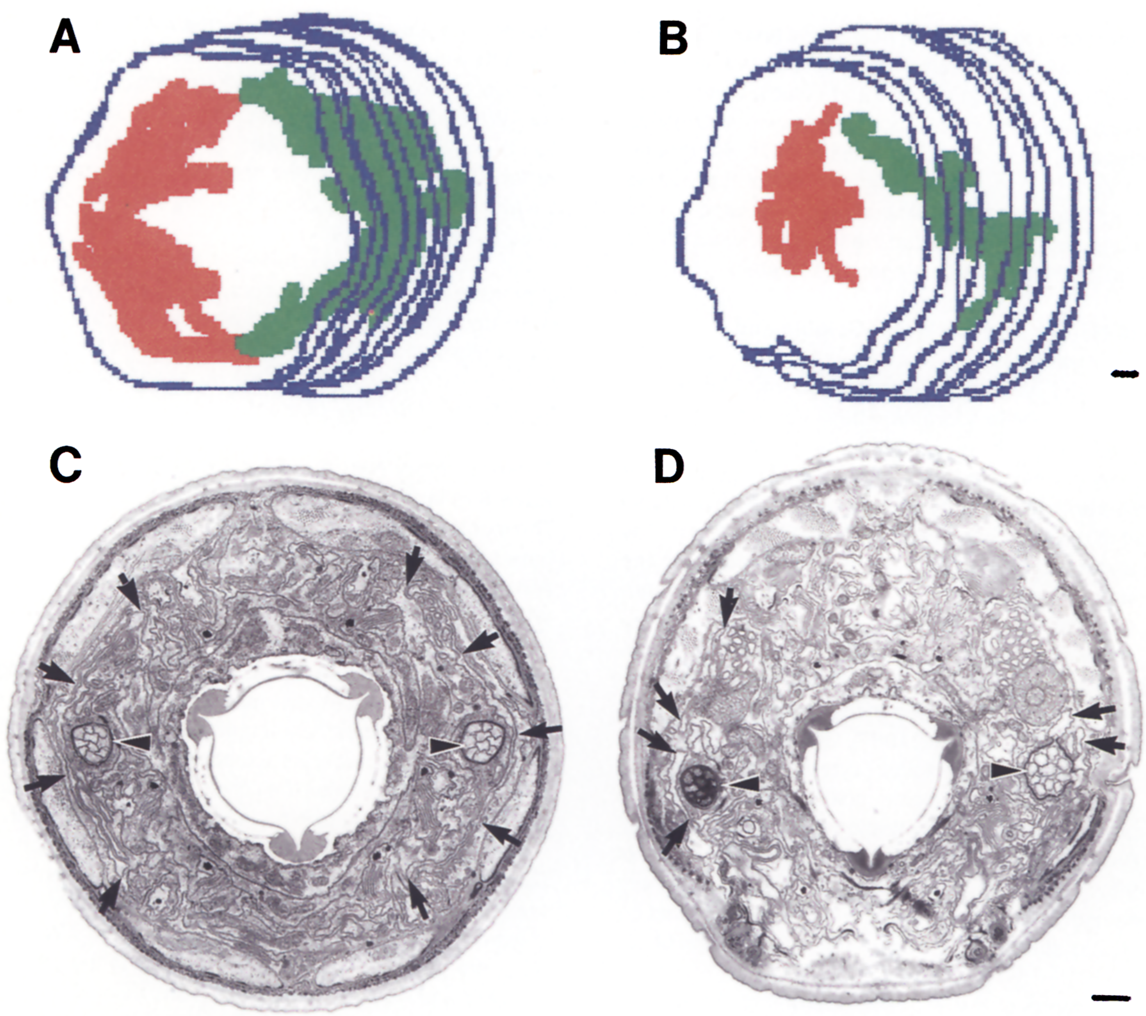

Figure 8. AWC Cilia in Wild-Type and *odr-3* Animals

(A and B) Reconstructions of the cilia of the AWC neurons in wild-type (A) and *odr-3(n2150)* (B) animals from serial section electron micrographs. The region shown begins approximately 1 μm from the anterior tip of the nose and includes the entire cilium of each AWC neuron up to the basal body. The left AWC neuron is shown in green, the right AWC neuron in red, and the outside of the animal's cuticle in blue. Dorsal is up, anterior is to the left; the reconstructions have been tilted by 45° so that both AWC cilia are visible.

(C and D) Representative sections of wild-type (C) and *odr-3(n2150)* (D) animals approximately 5 μm from the tip of the nose. The AWC cilia are continuous in the wild-type animal but fragmented and reduced in size in the *odr-3* mutant (arrows). The exposed cilia of the amphid channel are normal in *odr-3* (arrowheads). Scale bar, 1 μm.

cals that can influence its behavior. Among the water-soluble chemicals detected by C. elegans are a number of attractants, several repellents, and a pheromone that regulates nematode development (Wood et al., 1988; Bargmann and Horvitz, 1991a). Here, we demonstrate that C. elegans also responds to many different volatile odorants.

In the wild, C. elegans probably eats bacteria, including Pseudomonas, associated with decomposing material in the soil (Andrew and Nicholas, 1976). Pseudomonas and other naturally occuring microbes produce small alcohols, ketones, diacetyl, and esters as metabolic byproducts (Zechman and Labows, 1985; Dainty et al., 1985). These compounds could act as natural chemoattractants for C. elegans.

Sensation of chemicals is generally divided into taste and smell, but the nature of these different forms of sensation is not easily defined (Finger and Silver, 1987). Despite this ambiguity in definition, the chemosensation of volatile odorants exhibited by C. elegans appears to be analogous to the sense of olfaction. First, the chemicals sensed by AWA and AWC are volatile organic molecules that are recognized by the olfactory system in land animals and can travel through the air to be sensed (although volatile odorants must also penetrate an aqueous mucosal layer to reach the olfactory neurons in both vertebrates and invertebrates). Second, olfaction is generally more sensitive than taste, since it is used to sense molecules at a great distance. C. elegans can detect 2-butanone and diacetyl at concentrations comparable to the typical detection thresholds of vertebrate olfaction (Finger and Silver, 1987). Third, like vertebrate olfaction, the sensation of volatile odorants by C. elegans is versatile. Vertebrate taste buds detect only salts, sugars, acid, and a few other molecules, while vertebrates can smell almost any volatile molecule. Similarly, of hundreds of water-soluble compounds that were tested for chemotaxis, only a few were attractive to C. elegans (Ward, 1973; Dusenbery, 1974; Bargmann and Horvitz, 1991a). However, about half of the volatile molecules that we tested were attractive or repulsive to C. elegans, and it is likely that we have identified only a subset of odorants that C. elegans can detect.

Distinct Neurons Sense Water-Soluble and Volatile Attractants

The AWA and AWC neurons were essential for normal responses to volatile odorants. These neurons appear to be chemosensory, based on their anatomy and proximity to sensory openings in the amphid cuticle (Figure 6), but their differentiated sensory endings are distinct from those of most of the chemosensory neurons in the amphid. The AWA and AWC endings are not directly exposed to the environment, and they have complex, flattened cilia. Interestingly, complex cilia are characteristic of olfactory neurons in other animals (Lancet, 1986). Olfactory cilia are also usually protected from the environment by the presence of olfactory mucosa secreted by support cells (Lancet, 1986; Finger and Silver, 1987). Olfactory mucosa contain odorant-binding proteins, cytochrome P450, and UDP–glucuronosyltransferase, molecules that have been implicated in odorant concentration and odorant inactivation (Pevsner et al., 1985; Gyorgi et al., 1988; Lazard et al., 1990, 1991). Similarly, AWA and AWC endings are ensheathed by a secreted matrix material produced by the amphid sheath cell (Ward et al., 1975; Ware et al., 1975).

The functions of the exposed neurons of the amphid have been studied using laser killing experiments (Bargmann et al., 1990; Bargmann and Horvitz, 1991a, 1991b). Some of these neurons are required for chemotaxis, some for avoidance of chemical repellents, and some for entry into and exit from the dauer stage of development. All of these responses are primarily regulated by small water-soluble molecules, which might be accessible only to exposed neuron endings. Volatile odorants might be able to diffuse through the amphid matrix or through the cuticle of the nematode to reach the less accessible AWA and AWC neurons.

Single Olfactory Neurons Recognize Several Classes of Odorants

By saturating one odorant response and looking for effects on other odorant responses, we divided the volatile attractants into seven classes that can be discriminated by the animal. Similar experiments previously defined classes of water-soluble attractants (Ward, 1973). The existence of these classes suggests that some specific component exists for each class of attractants. This component might be a neuron, a high affinity odorant receptor molecule, one odorant recognition site on a multifunctional receptor, or another molecule in a signal transduction pathway.

Responses to six odorants of six different classes were measured after laser killing of sensory neurons. Three odorants were sensed primarily by the AWC neurons, two odorants were sensed primarily by the AWA neurons, and one odorant could be sensed by either AWA or AWC. Thus, the AWA and AWC neurons each directly or indirectly recognize multiple odorant classes. Single-unit recording of vertebrate olfactory neurons has also demonstrated that a single neuron can respond to many different odorants (Sicard and Holley, 1984).

Our results can be explained if each sensory neuron expresses several different odorant receptors that discriminate among different odorants. Alternatively, it is possible that sensory neurons other than AWA and AWC contribute to odorant discrimination but are not essential for chemotaxis to any individual odorant.

Chemosensory Neurons That Drive Chemotaxis Have Similar Predicted Connectivities

The morphological synapses of each neuron in C. elegans have been examined in electron micrographs of serial sections (White et al., 1976; Albertson and Thomson, 1976; White et al., 1986). The synaptic connections of the AWC neurons are extremely similar to those of the ASE neurons, although their sensory endings differ in morphology. These neuron classes are required for chemotaxis responses to dissimilar attractants: volatile odorants for AWC and water-soluble attractants for ASE. Their sensory endings might determine their chemical specificities, while their connections determine their effects on behavior.

Laser killing experiments have not revealed any individual interneuron type that is essential for normal chemotaxis (data not shown). However, the three sensory neuron classes most important in chemotaxis, AWA, AWC, and ASE, are particularly strongly connected to the interneurons AIY and AIZ. Both genetic data and laser killing implicate the AIZ interneurons in at least some chemotaxis behaviors (Chalfie et al., 1981; Finney and Ruvkun, 1990; C. I. B., unpublished data). Further analysis of the interneurons and motor neurons is necessary to elucidate the neuronal circuit that generates these behaviors.

Chemotaxis-Defective Mutants Define Distinct Steps in Sensory Processing

Genes with Broad Effects on Chemotaxis

A number of C. elegans mutants are defective in chemotaxis to all attractants (the *che-2* class in Table 2). Some of these mutants have structural defects in many ciliated sensory neurons and corresponding defects in chemotaxis to water-soluble and volatile attractants, avoidance of chemical repellents, dauer development and recovery, and male mating (*che-2*, *che-3*, *che-13*, *daf-10*, *osm-1*, *osm-5*, and *osm-6*; Dusenbery et al., 1975; Lewis and Hodgkin, 1977; Culotti and Russell, 1978; Albert et al., 1981; Perkins et al., 1986). A second group of mutants has defects in chemotaxis responses but appears to have structurally normal sensory neurons (*che-7*, *tax-2*, *tax-4*, *tax-6*, *unc-86*, *lin-32*, and *mec-2*; Perkins et al., 1986; this work). These genes might act in the subset of neurons that mediate chemotaxis behaviors or in the interneurons and motor neurons that regulate movement.

Like the mutants in this group, most olfactory mutants

Table 2. Summary of Wild-Type and Mutant Odorant Responses

	Benzaldehyde	2-Butanone	Isoamyl Alcohol	Thiazole	Pyrazine	Diacetyl	Na^+, Cl^-, Biotin, cAMP
Wild type	+	+	+	+	+	+	+
odr-1, *odr-5*	–	–	–	+	+	+	+
odr-2	–	+	–	+	+	+	+
odr-3	–	–	+	+/–	+/–	–	+
odr-4	–	+	+	–	+	–	+
odr(ky1)	–	–	+/–	+/–	+	+/–	+
che-1 class	+	+	+	+	+	+	–
che-2 class	–	–	–	–	–	–	–

Odorant concentrations considered here are the same as in Figure 6. At these concentrations, the first three odorants are detected primarily by the AWC neurons, diacetyl and pyrazine by the AWA neurons, and trimethylthiazole by both AWC and AWA neurons. The water-soluble attractants are detected primarily by ASE. The *che-1* and *che-2* classes of mutants are discussed in the text.

of the fruit fly Drosophila melanogaster are defective in responses to many odorants (Siddiqi, 1987). In addition, Drosophila olfactory mutants including *olfD/sbl*, *rdgB/ota1*, *pentagon*, and *acj2* also affect cells outside the olfactory system (Helfand and Carlson, 1989; McKenna et al., 1989; Woodard et al., 1989; Lilly and Carlson, 1990; Woodard et al., 1992). This pleiotropy might reflect the use of common signal transduction mechanisms in many cell types.

Genes That Affect Subsets of Chemosensory Neurons

Several genes appear to affect one or a few chemosensory cell types. Mutations in *che-1* and *che-6* eliminate the responses to water-soluble attractants but not volatile odorants (*che-1* class in Table 2). These genes are likely to affect ASE or its cellular targets but not AWA or AWC. *odr-1* and *odr-5* mutations affect responses to volatile odorants recognized by AWC but not responses mediated by ASE or AWA. These genes might be required either in the AWC neurons or in cells that interact with AWC to influence its development or its sensory function.

Two Drosophila genes similarly appear to be essential for particular chemosensory cell types. *lozenge* mutants have defects in the sensilla basiconica, one of the three kinds of olfactory sensilla in the antenna of the adult fly, and defects in eye development (Stocker and Gendre, 1988). *Pox-n* mutants lack peripheral larval chemoreceptors (Dambly-Chaudière et al., 1992). The *Pox-n* gene encodes a putative paired-box transcription factor that might specify chemoreceptor cell fate (Nottebohm et al., 1992).

Genes That Affect Odorant Sensation

Genes involved in the recognition of specific odorants should affect restricted subsets of olfactory responses. *odr-2* and *odr-4* appear to be such genes. All three *odr-2* mutations have a less severe effect on AWC than *odr-1* mutations, deleting some AWC functions while sparing others (Table 2). *odr-2* might function in chemoreception or sensory transduction in the AWC but not in the AWA neurons. Since chemotaxis to ketones depends on AWC but is still present in the *odr-2* animals, AWC might express a second sensory pathway that is independent of the *odr-2* function affected in these mutants.

odr-4 mutants show a more complex pattern of defects, including a subset of the functions of the AWC sensory neurons and a subset of the functions of the AWA sensory neurons. One model for *odr-4* action is suggested by biochemical studies of odorant recognition in vertebrates. Some odorants increase cAMP production in vertebrate sensory neurons, while others stimulate phosphatidylinositol turnover (Sklar et al., 1986; Boekhoff et al., 1990; Breer et al., 1990). If multiple signal transduction mechanisms operate within single C. elegans neurons, *odr-4* might be required in one but not the other second messenger pathway.

odr-3 and *odr(ky1)* also affect responses mediated by both the AWA and AWC neurons, but these genes are less selective in their effects than *odr-4*. Both *odr-3* and *odr(ky1)* affect most odorant responses. It is possible that both genes are generally involved in signal transduction, odorant concentration, or odorant clearing. *odr-3* mutants have defective AWC ciliated sensory endings as visualized in electron micrographs. These structural abnormalities in *odr-3* mutants might lead the AWC neurons to function less effectively. Alternatively, sensory deficits caused by *odr-3* could lead to morphological defects in the AWC cilia, just as mutations in some phototransduction components can lead to degeneration of the vertebrate or insect retina (Bowes et al., 1990; Dryja et al., 1990; Zuker, 1992).

Anosmic (smell-defective) humans usually have a decreased sensitivity of detection of particular odorants rather than a complete inability to sense such odorants (Amoore, 1977). Similarly, many of the *odr* mutants of C. elegans have greater defects at low odorant concentrations. This phenomenon could be explained if higher concentrations of odorants are sensed by several cell types or receptors in parallel, as is true for isoamyl alcohol and trimethylthiazole in C. elegans.

What might olfactory receptor molecules be in C. elegans? C. elegans might or might not use odorant detection mechanisms homologous to those in mammals. All *odr* mutations in C. elegans affect at least two categories of odorants as defined by the saturation experiments. If those categories correspond to single olfactory receptors, the known *odr* genes are unlikely to encode biochemical odorant receptors. Genes specific for a single odorant class might have been missed because we screened too few mutagenized animals, because redundant receptors recognize a single odorant category, or because the saturation categories do not correspond to single receptors.

Electrophysiological studies of vertebrate olfactory neurons indicate that single neurons can respond to many dissimilar odorants (Sicard and Holley, 1984). Similarly, we have found that C. elegans sensory neurons have broad specificities that do not correspond to the categories of odorants discriminated by the animal. The finer pattern of odorant discrimination might arise from information processing within broadly tuned sensory neurons. However, it is possible that more complex multicellular interactions contribute to odorant sensitivity and the integration of sensory information in C. elegans. Further genetic and behavioral analysis should reveal the nature of olfactory information coding within this simple nervous system.

Experimental Procedures

Strains

Wild-type worms were C. elegans variety Bristol, strain N2. Most mutations are described by Hodgkin et al. (1988) except for those in *tax* genes, which are described by Dusenbery et al. (1975). Mutant strains included CB1034 *che-1(e1034) fer-1(hc1ts) I*, PR672 *che-1(p672) I* (previously described as *tax-5*), PR674 *che-1(p674) I*, PR679 *che-1(p679) I*, PR680 *che-1(p680) I*, PR692 *che-1(p692) I*, PR696 *che-1(p696) I*, CB1033 *che-2(e1033) X*, CB1124 *che-3(e1124) I*, CB1073 *che-5(e1073) IV*, CB1126 *che-6(e1126) IV*, CB1128 *che-7(e1128)*, CB3329 *che-10(e1809) II*, CB3330 *che-11(e1810) V*, CB3332 *che-12(e1812) V*, CB3323 *che-13(e1805) I*, CB3687 *che-14(e1960) I*, PR808 *osm-1(p808) I*, PR802 *osm-3(p802) IV*, PR813 *osm-5(p813) X*, PR811 *osm-6(p811) V*, PR671 *tax-2(p671) I*, PR691 *tax-2(p691) I*, PR673 *tax-3(p673)*, PR678 *tax-4(p678)*, PR675 *tax-6(p675)*, CB1066 *mec-1(e1066) V*, CB75 *mec-2(e75) X*, CB398 *mec-8(e398) I*, CB1377 *daf-6(e1377) X*, and CB1387 *daf-10(e1387) IV*.

Generated during this work were strains MT4583 *odr-1(n1930) X*, MT4586 *odr-1(n1933) X*, MT4589 *odr-1(n1936) X*, MT4592 *odr-2(n1939) V*, MT5301 *odr-2(n2145) V*, MT5304 *odr-2(n2148) V*, MT5306 *odr-3(n2150) V*, MT4810 *odr(n2046) V*, MT5300 *odr-4(n2144ts)*, CX1 *odr(ky1) II*, and CX9 *odr-5(ky9) X*.

All nematodes were grown on petri plates with E. coli strain HB101 under uncrowded conditions (Brenner, 1974). Strains were grown at either 20°C or 25°C.

Chemotaxis Assays

Chemotaxis assays were based on the assays developed by Ward (1973) and Bargmann and Horvitz (1991a). Assay plates were 10 cm tissue culture dishes (Falcon) containing 10 ml of 1.6% BBL agar (Benton-Dickinson)–5 mM potassium phosphate (pH 6.0)–1 mM $CaCl_2$–1 mM $MgSO_4$. Two marks were made on the back of the plate at opposite sides of the plate, about 0.5 cm from the edge of the agar (see Figure 1). The diluted attractant (1 μl) was placed on the agar over one mark, and 1 μl of ethanol was placed over the other mark (attractants were diluted in ethanol). Animals to be tested were placed near the center of the plate, equidistant from the two marks. Attractants were obtained from Sigma Chemicals, Fluka Chemicals, or Pyrazine Specialties (Atlanta, Georgia). Pure pyrazine is a solid, so pyrazine dilutions are weight:volume rather than volume:volume as for other attractants.

Population Chemotaxis Assays

Well-fed adult animals were washed three times with S Basal (Brenner, 1974) and once with water to free them of bacteria, then placed near the center of a plate equidistant from the attractant and the counterattractant. At various times after the assay began, the numbers of animals at the attractant and the counterattractant areas were determined. The total number of animals in the assay was also determined. A specific chemotaxis index was calculated as

$$\frac{\text{Number of animals at attractant} - \text{Number of animals at counter-attractant}}{\text{Total number of animals in assay}}$$

The chemotaxis index could vary from 1.0 (perfect attraction) to −1.0 (perfect repulsion).

Although C. elegans will chemotax to many attractants, worms do not remain at the attractant source. Therefore, for most experiments the animals were anesthetized when they reached the attractant. Sodium azide (1 M; 1 μl) was placed at both the attractant source and the counterattractant source The azide anesthetized animals within about a 0.5 cm radius of the attractant. The rates at which animals accidentally wandered into the anesthetic region should be the same at the attractant and the counterattractant.

Control experiments with wild-type and mutant animals revealed that, after 60 min in assays with azide, the chemotaxis index reached a plateau and did not increase significantly thereafter (Figure 1). Therefore, in general, assays were counted only once after 60 min.

Single-Animal Chemotaxis Assays

Single-animal chemotaxis assays were similar to those described by Bargmann and Horvitz (1991a). Animals were placed on assay plates without bacteria for 1 hr before the first assay, then placed on assay plates equidistant from the attractant and the ethanol, slightly displaced from the center of the plate, about 5 cm from the peak of the gradient of attractant. Animals were permitted to move freely for 1 hr, after which they were removed and their tracks were observed as indentations on the agar. A single animal was tested in no more than three volatile assays in 1 day and three assays on a second day, then discarded.

Assays were scored as either positive or negative by counting the number of independent trips to the peak of the gradient (A) and subtracting the number of independent trips to the negative control region (C). If A − C was greater than zero, the assay was scored as positive; if A − C was less than or equal to zero, the assay was scored as negative. If the animal did not move at least 5 cm total in any direction during the assay, the assay was not counted.

Two hundred seventy assays were conducted on assay plates without any added attractant. In 30 out of 270 assays, an animal appeared to track to 1 of the 2 mock attractant areas on the plate. Thus, the apparent chemotaxis due to random movement was 11%. This value was used as a negative control level for all statistical comparisons.

Laser Killing of Neurons

Individual neurons were identified using Nomarski optics based on a combination of position and morphological cues, as previously described (Bargmann and Horvitz, 1991a). Cells were killed using a laser microbeam focused through the 100× Neofluor objective of a Zeiss Standard microscope, as described by Avery and Horvitz (1987, 1989). Adults were assayed within 48 hr of the L4–adult molt, which allowed at least 2.5 days following laser surgery for the ablated cells to lose function.

Isolation and Behavioral Characterization of *odr* Mutants

Wild-type animals were mutagenized with ethyl methanesulfonate as described by Brenner (1974) and allowed to self-fertilize for two generations. F2 animals were screened for defective chemotaxis in a modified version of the population chemotaxis assay. In most cases, animals were given a choice between 1 μl of a 1:100 dilution of benzaldehyde at one end of the plate and a weaker counterattractant at the other end. The counterattractant concentration was chosen so that over 90% of wild-type animals would prefer the benzaldehyde to the counterattractant in the choice. The counterattractant was either 1 μl of a 1:10,000 dilution of 2-butanone or a well containing 45 μl of 0.4 M sodium acetate. When sodium acetate was used as the counterattractant, it was allowed to diffuse to form a gradient for 12–20 hr before the assay.

F2 progeny of mutagenized animals were placed in the center of a plate with a choice of attractants. After 60–90 min, animals at or near the counterattractant were removed and placed in the center of a second plate with the same choice of attractants. Animals that preferred a counterattractant to benzaldehyde three times were isolated, and their broods were retested to determine whether they contained a heritable mutation. Mutants were backcrossed to wild-type worms at least twice before extensive characterization.

The *odr* mutants described in this work were derived from six independent mutagenesis experiments in which the progeny of approximately 32,000 mutagenized F1 animals were screened in total. Mutants carrying all alleles of the *odr* genes were tested at one or more concentration of each of the six odorants shown in Figure 7; mutants carrying the alleles not shown in Figure 7 gave results similar to those displayed. In addition, the mutants shown in Figure 7 were tested for chemotaxis to cAMP, biotin, lysine, and Na^+ and Cl^- and for avoidance of high osmotic strength. All were normal except *odr-3(n2150)* mutants, which were defective in their responses to the water-soluble attractant lysine and in avoidance of high osmotic strength.

Each *odr* mutant was characterized for chemosensory responses, mechanosensory responses, movement, and male and hermaphrodite fertility. No defects other than chemosensory abnormalities were observed (data not shown). The integrity of the exposed amphid neurons was examined by staining amphid neurons with the fluorescent lipophilic carbocyanine dye DiO C_{18} (Molecular Probes), which stains six pairs of amphid neurons and two pairs of phasmid neurons in C. elegans (Herman and Hedgecock, 1990). All of these neurons and their processes were normal in the *odr* mutants.

Mapping and Genetic Characterization of *odr* Mutants

All of the *odr* mutations described are recessive. Standard genetic mapping techniques depend on the ability to score a mutant phenotype in the presence of a second genetic marker with a visible phenotype; unfortunately, most genetic markers interfere with chemotaxis. Therefore, a strategy for mapping was devised based on dominant marker mutations. The strains used for mapping were MT4814 *unc-108(n501dm) dpy-5(e61) I*, MT4815 *sqt-1(sc1) II*, MT4816 *sqt-2(sc3) lin-31(n301) II*, MT4817 *unc-103(e1597sd) III*, MT1747 *dpy-13(e184sd) unc-8(n491sd) IV*, MT4818 *unc-70(n493sd) V*, and MT4819 *unc-58(e665dm) X*.

The general mapping strategy is outlined using *unc-103 III* as an example. After mating hypothetical mutation *odr-x* males to *unc-103/unc-103* hermaphrodites, all F1 cross-progeny were *unc-103/+* and uncoordinated. In the second generation one-quarter of the F2 progeny were coordinated; these coordinated F2s or their progeny were tested for chemotaxis. If *odr-x* was tightly linked to *unc-103*, all coordinated F2s were *odr-x/odr-x* and had the Odr mutant phenotype. If *odr-x* was unlinked, only one-quarter of the coordinated F2s were mutant for the *odr* gene, so most coordinated animals had normal chemotaxis.

Once a general chromosomal location for an *odr* gene had been assigned, subsequent mapping steps used recessive mapping strains and standard three-factor mapping crosses in which recombinants between two linked markers were scored for *odr* phenotypes (Brenner, 1974). Homozygous recombinant hermaphrodites were tested for complementation of the *odr* mutation by mating marked hermaphrodites with *odr* males and testing the cross-progeny for chemotaxis.

Some of these data are summarized below.

odr-1 X

n1936 was mapped near the gene *sem-1* by the following crosses: *n1936* × *unc-18(e81) dpy-6(e14)*, 7/7 Unc non-Dpy recombinants were Odr and 0/10 Dpy non-Unc recombinants were Odr. *n1936* × *lin-2(e1309) unc-9(e101)*, 9/9 Lin non-Unc were Odr. *n1936* × *unc-9(e101) lin-15(n765)*, 1/11 Unc non-Lin was Odr. *n1936* × *sem-1(n1382) unc-3(e151)*, 0/13 Sem non-Unc were Odr. *n1930* and *n1933* were both linked to *unc-58* in initial mapping experiments. *n1930* also mapped to the interval between *unc-9* and *lin-15* in three-factor crosses. *n1930*, *n1933*, and *n1936* all failed to complement one another.

odr-2 V

odr-2 mapped between the right breakpoint of the deficiency *nDf32* and the right breakpoint of the deficiency *sDf30* by the following crosses: *n1939*, *n2145*, and *n2148* were all mapped between *dpy-11(e224) unc-42(e270)* (*n1939*: 8/10 Unc non-Dpy were Odr, 1/10 Dpy non-Unc was Odr; *n2145*: 3/5 Unc non-Dpy were Odr, 0/4 Dpy non-Unc were Odr; *n2148*: 2/5 Unc non-Dpy were Odr, 2/6 Dpy non-Unc were Odr). Each mutation was also tested for complementation with *nDf32*, which they complemented, and *sDf30*, which they failed to complement. *n2145* also failed to complement *sDf20*. *n1939*, *n2145*, and *n2148* all failed to complement one another.

odr-3 V

n2150 was mapped to the interval between *sqt-3* and *unc-61* in the following crosses: *n2150* × *dpy-11(e224) unc-42(e270)*, 4/4 Dpy non-Unc recombinants were Odr, 0/10 Unc non-Dpy recombinants were Odr. *n2150* × *sqt-3(sc63ts) unc-61(e228)*, 1/6 Sqt non-Unc was Odr, 5/8 Unc non-Sqt were Odr. *n2046* failed to complement *n2150*. *n2046* was mapped to the right of *unc-42* in a cross of *n2046* with *dpy-11(e224) unc-42(e270)*: 0/10 Unc non-Dpy recombinants were Odr, 9/9 Dpy non-Unc recombinants were Odr.

odr-4 III

The following crosses placed *odr-4(n2144ts)* near the marker *sma-3*: *n2144* × *dpy-17(e164) unc-32(e189)*, 5/12 Unc non-Dpy recombinants were Odr, 4/6 Dpy non-Unc recombinants were Odr. *n2144* × *lon-1(e185) unc-36(e251)*, 6/10 Lon non-Unc were Odr, 3/5 Unc non-Lon were Odr. *n2144* × *sma-3(e491) unc-32(e189)*, 10/10 Unc non-Sma were Odr, 0/7 Sma non-Unc were Odr. *n2144* × *lon-1(e185) sma-3(e491)*, 0/9 Sma non-Lon were Odr. *n2144* failed to complement *nDf16*.

odr-5 X

ky9 was linked to *unc-58* in initial mapping experiments. The following crosses place *ky9* near the gene *vab-3*: *ky9* × *unc-9(e101) lin-15(n765)*, 0/4 Unc non-Lin recombinants were Odr. *ky9* × *lin-2(e1309) unc-9(e101)*, 0/2 Lin non-Unc were Odr, 3/3 Unc non-Lin were Odr. *ky9* × *vab-3(e648) lin-2(n1297)*, 12/12 Lin non-Vab were Odr. *ky9* × *dpy-6(e14) egl-15(n484)*, 3/9 Egl non-Dpy were Odr. *odr(ky9)/nDf19* heterozygotes were semisterile, so it was not possible to measure their chemotaxis.

odr(ky1) II

ky1 appeared to be loosely linked to *sqt-1* in initial mapping experiments. Additional mapping placed *ky1* in the *unc-52* region of chromosome II. *ky1* males were crossed with *unc-52(e1421)* hermaphrodites, and non-Unc F2s were picked. 6/6 F2 animals that did not have Unc progeny had all Odr progeny. *ky1* mapped right of *rol-l*: following a *ky1* × *rol-1(e91) unc-52(e444)* cross, two Rol non-Unc recombinants were Odr.

Electron Microscopy

Adult hermaphrodites were fixed in 0.8% glutaraldehyde–0.7% osmium tetroxide–0.1 M cacodylate buffer (pH 7.4) for 1 hr at 0°C in the dark, then fixed in 1% osmium tetroxide–0.1 M cacodylate buffer overnight at 4°C, embedded in agar, dehydrated, and embedded in a resin–araldite mixture. Sections (50–60 nm thin) were cut from the anterior tip of the animal for approximately 7 μm and stained with uranyl acetate and lead citrate for contrast. Pictures were taken at 80 kV with a Jeol JEM 1200 EX II. Intermittent sections were examined for each animal; the number of sections examined is listed in parentheses.

Wild type: three animals sectioned (55, 30, and 41 sections per animal); *odr-1(n1936)*: six animals sectioned (73, 69, 14, 50, 35, and 9); *odr-1(n1930)*: two animals sectioned (150 and 150), *odr-1(n1933)*: two animals sectioned (124 and 150); *odr-2(n2145)*: two animals sectioned (49 and 42); *odr-2(n1939)*: four animals sectioned (11, 10, 11, and 13); *odr-2(n2148)*: three animals sectioned (10, 13, and 10); *odr-3(n2150)*: six animals sectioned (49, 150, 100, 150, 56, and 19); *odr-4(n2144)*: three animals sectioned (150, 150, and 49); *odr-5(ky9)*: five animals sectioned (150, 12, 12, 10, and 7); *odr(ky1)*: three animals sectioned (10, 14, and 13).

All amphid sensory neurons were examined in each animal. Except for the AWC neurons in *odr-3* animals, all amphid neurons were superficially normal. Reconstructions were made from digitized images of 13–28 sections from each animal using the HVEM-3D IBM PC–based reconstruction system developed by Royer and Kinnamon (1991).

Statistical Analysis

Population Chemotaxis Assays

Mean values, SDs, and SEMs were calculated using standard methods for analyzing normal distributions (Rosner, 1986). Wild-type and mutant responses were compared using a two-tailed t test and the statistical program StatView 512+ (Brainpower, Inc., Calabasas, California).

Single-Animal Chemotaxis Assays

Ninety-five percent confidence limits for chemotaxis assays were calculated using the normal theory method for obtaining confidence intervals for binomial parameters and the following formula: 95% confidence range for chemotaxis index x = $x \pm 1.96\,[(x)(1-x)/n]^{1/2}$, where x is the chemotaxis index and n is the total number of assays from which x is derived.

In some cases in which the number of assays was small, this approximation was not valid, and confidence limits were obtained using tables (Rosner, 1986).

Mutant animals or laser-operated animals were compared with wild-type animals using χ^2 analysis (Rosner, 1986). Further discussion of statistical methods is provided by Bargmann and Horvitz (1991a).

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