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Reprogramming Chemotaxis Responses: Sensory Neurons Define Olfactory Preferences in C. elegans

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Summary

Different olfactory cues elicit distinct behaviors such as attraction, avoidance, feeding, or mating. In the nematode C. elegans, these cues are sensed by a small number of olfactory neurons, each of which expresses several different odorant receptors. The type of behavioral response elicited by an odorant could be specified by the olfactory receptor or by the olfactory neuron in which the receptor is activated. The attractive odorant diacetyl is detected by the receptor protein ODR-10, which is normally expressed in the AWA olfactory neurons. The repulsive odorant 2-nonanone is detected by the AWB olfactory neurons. Transgenic animals that express ODR-10 in AWB rather than AWA avoid diacetyl, while maintaining qualitatively normal responses to other attractive and repulsive odorants. Animals that express ODR-10 simultaneously in AWA and AWB have a defective response to diacetyl, possibly because of conflicting olfactory inputs. Thus, an animal's preference for an odor is defined by the sensory neurons that express a given odorant receptor molecule.

Introduction

Different olfactory cues can elicit distinct behavioral responses in an animal, allowing it to respond appropriately to its environment. To accomplish this task, olfactory neurons detect these cues, discriminate among them, and relay this information to the rest of the nervous system. For many animals, olfactory stimuli from food, predators, and pheromones induce stereotyped behaviors, suggesting these responses are specified by an innate genetic or developmental program. How such a program generates distinct responses for different stimuli remains to be understood fully.

The molecular and neural pathways that define specific behaviors can be identified and manipulated in the nematode C. elegans. C. elegans depends largely on its olfactory or chemosensory sense to acquire information about its environment. It can detect hundreds of water-soluble and volatile molecules, which can evoke attraction, repulsion, feeding, egg-laying, mating, and developmental changes in the animal (Bargmann and Mori, 1997). A given molecule is associated with a characteristic response in animals raised under standard conditions: for example, many short-chain ketone, alcohol, and aldehyde odorants are attractive to essentially all animals, while other ketones and alcohols are repulsive (Bargmann et al., 1993).

C. elegans detects chemicals using a small number of chemosensory neurons whose morphology and synaptic connections are known through reconstruction of the entire nervous system from serial electron micrographs (White et al., 1986). Functions for many of these neurons have been determined by examining the behaviors of animals in which specific neurons are killed with a laser microbeam (Bargmann and Mori, 1997). Eleven pairs of chemosensory neurons are found within the bilaterally symmetric amphid chemosensory organs, including three pairs of neurons with branched, extended sensory cilia called AWA, AWB, and AWC (Ward et al., 1975; Ware et al., 1975). The AWA and AWC neurons mediate responses to attractive volatile odorants (Bargmann et al., 1993). A single olfactory neuron can mediate responses to several compounds; for example, the AWA neurons detect both diacetyl and pyrazine, which can be distinguished by the animal in behavioral assays. These results imply that single olfactory neurons express multiple receptors, each of which binds different odorants.

Odorant receptors in C. elegans and other animals are G protein–coupled seven transmembrane proteins. The *odr-10* gene encodes a receptor in this class that likely detects the volatile attractant diacetyl (Sengupta et al., 1996). *odr-10* mutants fail to chemotax to low concentrations of diacetyl, but have normal responses to all other odorants tested, including pyrazine. An ODR-10::GFP fusion protein localizes to the cilia of the AWA neuron, where diacetyl detection is thought to occur. Experiments with *odr-7*, a mutant defective for AWA responses, provide further evidence for the diacetyl specificity of ODR-10. The *odr-7* gene encodes a transcription factor required for the expression of ODR-10 and possibly other AWA receptors. Expression of *odr-10* under an *odr-7*-independent promoter is sufficient to restore the diacetyl response but not the pyrazine response of *odr-7* mutants. These genetic arguments that ODR-10 is a diacetyl receptor have been confirmed by biochemical experiments demonstrating that expression of ODR-10 in human 293 kidney cells confers diacetyl sensitivity on those cells (Y. Zhang et al., unpublished data).

odr-10 is a member of a large family of candidate chemosensory receptor genes. At least 200 *odr-10*-like seven transmembrane-domain (*str*) genes are present in the 80% of the C. elegans genome that has been sequenced (Sulston et al., 1992). These genes encode putative G protein–coupled receptors, many of which are expressed in chemosensory neurons (E. R. T. and C. I. B., unpublished data). There are at least five other families of seven transmembrane-domain receptors that are also predominantly expressed in chemosensory neurons (*sra*, *srb*, *srd*, *sre*, and *srg* genes, over 100

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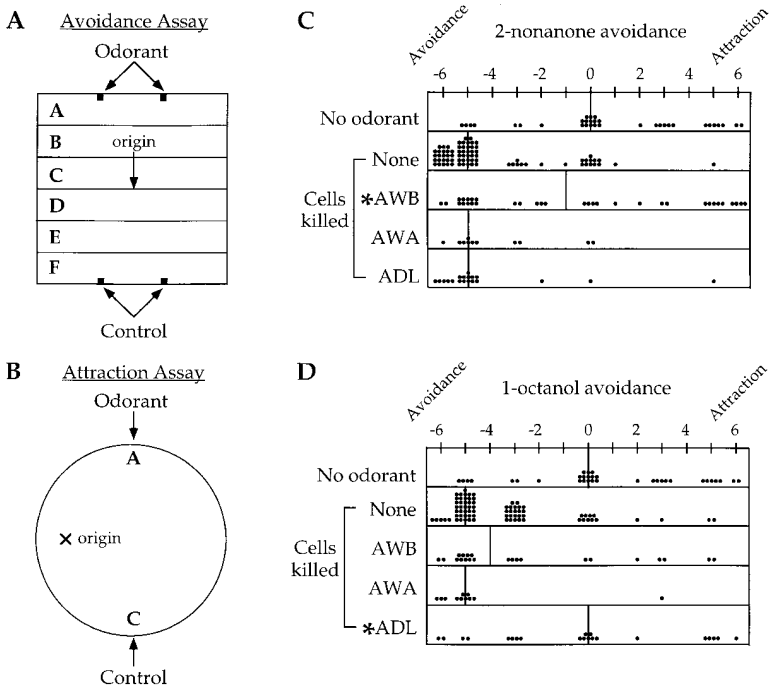

Figure 1. The AWB Olfactory Neurons Mediate Repulsion from 2-Nonanone

(A) Avoidance assays. For single animal assays, animals are placed in the center of the plate and observed to determine which sectors they enter during 1 hr (see Experimental Procedures). For population assays, azide is included to anaesthetize animals that enter sections A and F and animals are scored based on their final position after 1 hr. The scoring method for these square plate assays can detect either attraction (which generates a positive number) or repulsion (which generates a negative number). (B) Conventional chemotaxis assays are conducted on a round plate, which is less effective at scoring repulsion. (C) Avoidance of 2-nonanone (1:10 dilution) by intact and laser-operated animals in single animal assays. (D) Avoidance of 1-octanol (undiluted) by intact and laser-operated animals in single animal assays. For (C) and (D), each dot represents one assay, and the vertical line indicates the median response. Asterisks denote responses different from wild-type at $p < 0.01$. Intact animals were also scored in the absence of repellent.

members in total; Troemel et al., 1995, and our unpublished data). Although only ODR-10 has been shown to detect odorants, C. elegans may have a total of as many as 200–400 chemosensory receptor genes. Importantly, a single neuron can express multiple receptor genes, including receptors from different gene families. The expression and regulation of several different odorant receptor genes may allow each individual neuron to sense and discriminate among several odorants.

How is olfactory information encoded by the nervous system? In principle, all olfactory neurons might be equivalent, while olfactory receptors could be of different types. For example, attractant receptors could be different from repellent receptors, so that the response directed by a neuron depends on the type of receptor that is activated. In this model, olfactory information would be encoded by an odorant receptor and its effects on signal transduction within a neuron, and a neuron expressing receptors of both types could mediate both positive and negative responses. This sort of organization might be used in the lobster, where single olfactory neurons are depolarized by some odorants and hyperpolarized by others, suggesting that the cells express qualitatively distinct receptors (Michel et al., 1991). In an alternative model, each olfactory neuron might be dedicated to a characteristic response, so that any receptor it expresses drives the same behavior. In C. elegans, individual olfactory neurons have been shown to detect either attractants or repellents, but not both, suggesting that there are differences between these cell types (Bargmann and Mori, 1997). However, the interpretation of these cell ablation experiments is limited because they included only a small fraction of odorants; moreover, such loss-of-function experiments can only provide correlations. To determine whether the receptor or the neuron determines olfactory preference, we misexpressed *odr-10* in a cell that detects repellents rather than attractants. Our results indicate that ODR-10 generates a behavior that is specified by the sensory neuron in which it is expressed. Interestingly, the ODR-10 protein can couple to different signaling pathways in different sensory neurons.

Results

The AWB Sensory Neurons Detect the Repellent 2-Nonanone

The AWB neurons have branched, flattened cilia that are enclosed within a sheath cell, like the odorant-sensing AWA and AWC neurons (Ward et al., 1975; Ware et al., 1975). This morphology suggests that AWB neurons might recognize volatile molecules, like AWA and AWC, rather than water-soluble molecules, which are recognized by sensory neurons with more exposed single or double cilia. To analyze the function of the AWB neurons, we developed a new assay to measure long-range avoidance of volatile repellents (Figure 1A). The avoidance assay is similar to volatile attraction assays, but is conducted on a square plate with a repellent along one edge, while attraction assays are conducted on round plates with a point source of attractant (Figure 1B). Wild-type animals exhibit robust avoidance of repellents such as 1-octanol and 2-nonanone in the avoidance assay (Figures 1C, 1D, and 2C).

The contribution of the AWB neurons to avoidance was investigated by behavioral testing of animals in which the AWB neurons had been killed with a laser (Bargmann and Avery, 1995). AWB-ablated animals exhibited diminished avoidance of the volatile repellent 2-nonanone, but they had only minor defects in avoidance of 1-octanol (Figures 1C and 1D). Other forms of volatile avoidance rely on other neurons: killing the ADL neurons impaired long-range avoidance of 1-octanol (Figure 1D), and killing the ASH or ADL neurons impaired

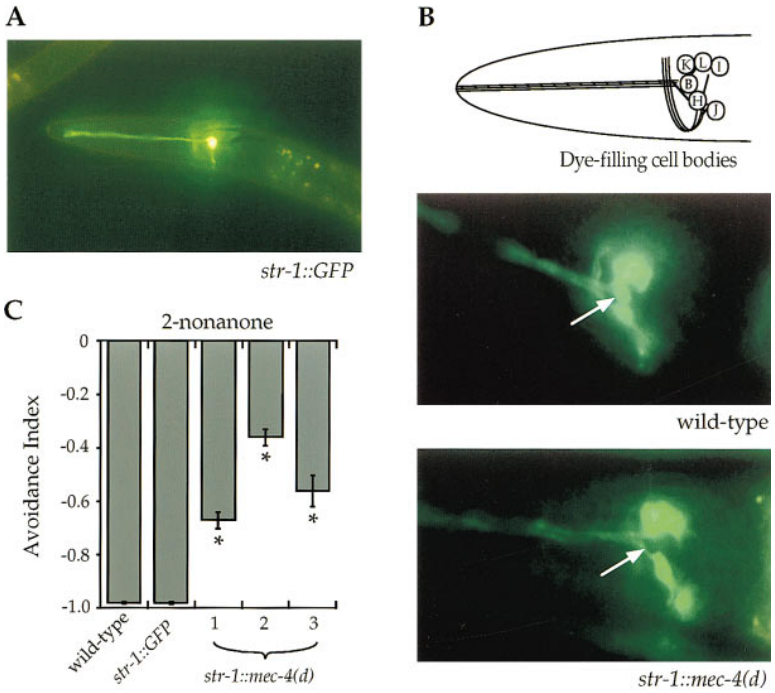

Figure 2. Regions Upstream of the *odr-10*-like Gene *str-1* Drive Expression in AWB

(A) Expression of the *str-1::GFP* reporter construct in AWB visualized by fluorescence microscopy. (B) Dye-filling with DiO of wild-type and *str-1::mec-4(d)* animals. K = ASK, L = ADL, I = ASI, B = AWB, H = ASH, J = ASJ. Arrows indicate the AWB cell body in the wild-type animal, and the expected position of the AWB cell body in an *str-1::mec-4(d)* animal. Anterior is at left and dorsal is up in (A) and (B). (C) Transgenic animals bearing the *str-1::mec-4(d)* transgene are defective in repulsion from 2-nonanone. Three transgenic *str-1::mec-4(d)* strains were compared, one with an integrated transgene (1), and two with extragenic arrays of the transgene (2 and 3). The percentage of animals that lacked DiO filling was 14% (1), 70% (2), 22% (3); in all strains, many of the remaining animals had only a single dye-filling AWB neuron. Asterisks denote responses that differ from wild-type at $p < 0.01$. Error bars equal the standard error of the mean (SEM). Each data point represents the average of at least eight independent assays.

short-range avoidance of 1-octanol (Troemel et al., 1995). Killing the ADL or AWA neurons did not affect 2-nonanone avoidance in this assay.

Since the AWB neurons appear to detect repulsive odorants, they should express odorant receptors and other signal transduction molecules. Indeed, in a survey of expression patterns of the *odr-10*-like *str* genes, we found that an *str-1::GFP* fusion gene was expressed at high levels in the two AWB neurons (Figure 2A). Expression from the *str-1* promoter in the AWB olfactory neurons suggests that *str-1* might encode a receptor for an AWB odorant. In addition, like the AWC olfactory neurons, the AWB neurons express the cyclic nucleotide–gated channel encoded by the *tax-2* and *tax-4* genes, which is predicted by genetic studies to transduce signals sent by G protein–coupled receptors such as the *str* genes (Coburn and Bargmann, 1996; Komatsu et al., 1996).

As noted above, avoidance of volatile repellents is distributed among several classes of neurons, and the contribution of each neuron appears to vary depending on the exact avoidance assay. Instead of performing the long-term tracking of single animals that is used to characterize laser-operated animals, we wished to examine the AWB contribution to avoidance in populations of animals. A population avoidance assay (Figure 1 and Experimental Procedures) was used to compare the behavior of wild-type animals and animals with compromised AWB function. In the latter animals, the AWB-specific *str-1* promoter was used to express the toxic gain-of-function degenerin gene *mec-4(d)* (*str-1::mec-4(d)* animals) (Driscoll and Chalfie, 1991; Maricq et al., 1995). AWB expression of *mec-4(d)* did not kill the AWB neurons efficiently, since their cell bodies could still be detected in the transgenic animals. However, the transgene resulted in a defect in the ability of the AWB sensory cilia to take up the lipophilic dye DiO (Herman and Hedgecock, 1990) (Figure 2B); in different transgenic strains, between 14% and 70% of animals lacked AWB dye-filling ($n > 35$ animals per strain). By contrast, no wild-type animals were defective in AWB dye-filling ($n = 70$). All other DiO-stained neurons retained normal dye-filling in *str-1::mec-4(d)* animals, confirming the cell type–specific effects of the transgene (Figure 2B).

In the population avoidance assay, which requires higher concentrations of 2-nonanone than single-animal assays to elicit robust avoidance, the transgenic *str-1::mec-4(d)* animals displayed diminished avoidance of 2-nonanone (Figure 2C). The *str-1::mec-4(d)* animals avoided 1-octanol normally and approached numerous volatile attractants normally (data not shown), indicating that AWB function is less important for these responses. The strength of the 2-nonanone defect in different strains correlated with the severity of the DiO-filling defect in the AWB neurons, but some nonanone avoidance persisted in all strains. It is unclear whether the residual 2-nonanone avoidance is due to residual AWB function or to the contribution of other sensory neurons to avoidance; because the population assay requires higher 2-nonanone concentrations than the single animal assay, it might involve more neurons. Regardless of whether other neurons sense 2-nonanone, these results provide evidence that the AWB neurons detect this repellent.

ODR-10 Expression in AWB Drives an Avoidance Response

odr-10 is normally expressed in the AWA olfactory neurons, but the expression of the *odr-10*-related gene *str-1* in AWB suggested that AWB might also support *odr-10* signaling. To determine whether ODR-10 could be expressed efficiently in AWB and localized to the AWB cilia, we used the *str-1* promoter to drive expression of

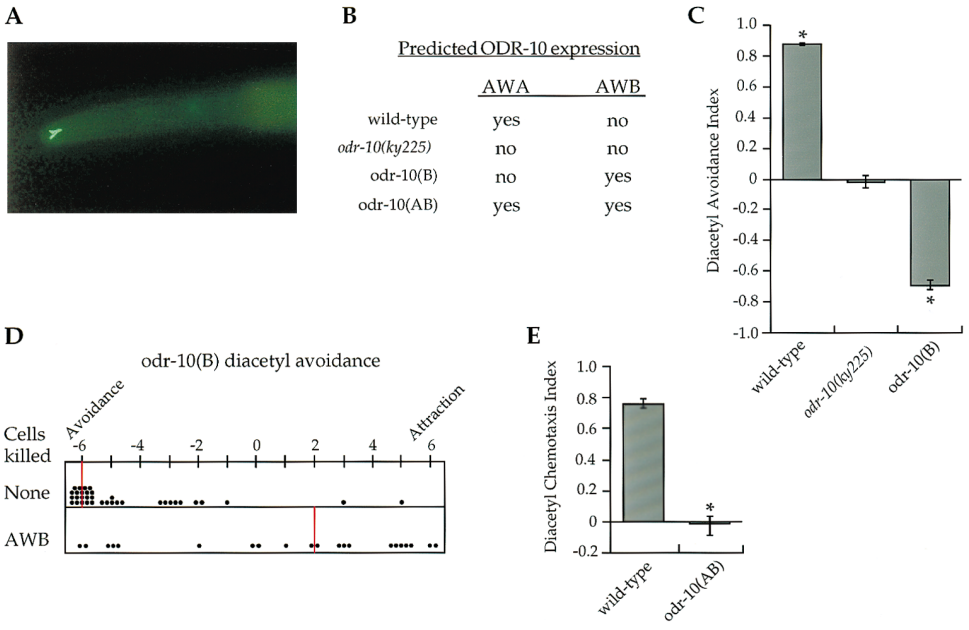

Figure 3. ODR-10 Expression in AWB Directs Repulsion from Diacetyl in an *odr-10* Null Background and Interferes with Attraction in a Wild-Type Background

(A) *str-1::odr-10::GFP* transgene expression in the AWB cilia. Identification of the AWB cilia was based on their characteristic bilobed structure, together with weak staining observed in the AWB cell body. Anterior is at left and dorsal is up. (B) Predicted ODR-10 expression in strains used for analysis. Expression in AWA indicates that animals are wild-type for *odr-10*, while expression in AWB indicates that the strain carries the *str-1::odr-10* transgene. (C) Diacetyl chemotaxis responses of wild-type, *odr-10(ky225)*, and odr-10(B) animals. Error bars equal the SEM. Asterisks denote responses that differ from *odr-10(ky225)* at $p < 0.01$. (D) Diacetyl responses of odr-10(B) intact and AWB ablated animals in single-animal avoidance assays. Data display is the same as in Figures 1C and 1D; differences are significant at $p < 0.01$. (E) Diacetyl chemotaxis responses of wild-type and odr-10(AB) animals. Asterisks denote responses that differ from wild-type at $p < 0.01$. Population assays in (C) were performed on square plates, which can measure either attraction or avoidance. Population assays in (E) were standard round-plate chemotaxis assays. Error bars equal the SEM. Each data point represents the average of at least 13 independent assays.

an *odr-10* cDNA fused to GFP at its carboxyl terminus. Animals with this transgene showed GFP expression predominantly in the AWB cilia (Figure 3A), confirming the expected cellular and subcellular distribution of the ODR-10 fusion protein.

To ask whether *odr-10* would function in AWB, we expressed an unmodified *odr-10* cDNA under control of the *str-1* promoter fragment. This transgene was introduced into *odr-10(ky225)* null mutants, to create a strain called odr-10(B) that expresses ODR-10 in AWB but not AWA (Figure 3B). *odr-10(ky225)* mutants are neither attracted nor repelled by low concentrations of diacetyl, and the *str-1::odr-10* transgene restored a diacetyl response to these nonresponsive animals (Figure 3C). Unlike wild-type animals, however, the odr-10(B) animals were repelled by diacetyl, indicating that misexpression of ODR-10 altered the behavior elicited by the odorant. Similar results were observed in eight independently derived odr-10(B) strains (data not shown; see Experimental Procedures).

Although *str-1::odr-10::GFP* and *str-1::GFP* fusions were expressed only in AWB, it was possible that a low level of *odr-10* expression in some other neuron was responsible for the diacetyl avoidance of the odr-10(B) strain. To ensure that repulsion from diacetyl in odr-10(B) animals was dependent on AWB, the AWB neurons were killed by laser ablation in the odr-10(B) strain, and the operated animals were tested in single-animal avoidance assays (Figure 3D). odr-10(B) animals in which AWB was killed no longer avoided diacetyl, indicating that diacetyl repulsion in this strain is probably due to ODR-10 signaling in AWB. After AWB killing, the response of these animals to diacetyl was not significantly different from the response of *odr-10(ky225)* animals (data not shown, $p > 0.5$).

Simultaneous Expression of *odr-10* in AWA and AWB Blocks Diacetyl Responses

Since wild-type animals expressing *odr-10* in AWA were attracted to diacetyl, and animals expressing *odr-10* only in AWB were repelled by diacetyl, the expression of the *str-1::odr-10* transgene in a wild-type strain might generate conflicting behavioral signals. The *str-1::odr-10* transgene was introduced into wild-type animals to generate the strain odr-10(AB), for ODR-10 in AWA and AWB (Figure 3B). These animals failed to respond to diacetyl (Figure 3E), suggesting that conflicting attractive and repulsive signals from AWA and AWB might be summed to generate a neutral response. Similar results were observed in eight independently derived transgenic strains that expressed ODR-10 in both AWA and AWB (data not shown). The severity of the diacetyl defect varied in magnitude among these strains, perhaps

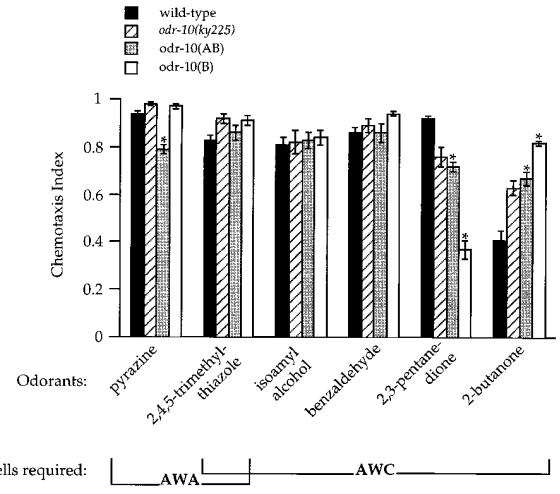

Figure 4. Odorant Responses of odr-10(AB) and odr-10(B) Strains

Responses of wild-type animals are indicated by closed bars, responses of *odr-10(ky225)* animals are indicated by hatched bars, responses of odr-10(AB) animals are indicated by gray bars, and responses of odr-10(B) animals are indicated by open bars. The cells required for responses to odorants are indicated below the odorant. Asterisks denote responses that differ from controls at $p < 0.01$. The control for odr-10(AB) is wild-type, and the control for odr-10(B) is *odr-10(ky225)*. Assays were standard population chemotaxis assays on round plates. Error bars indicate the SEM. Each data point represents the average of at least six independent assays. Dilutions of the odorants were as follows (in ethanol): 10 mg/ml pyrazine, 1:200 benzaldehyde, 1:100 isoamyl alcohol, 1:1000 2-butanone, 1:1000 2,3-pentanedione, 1:1000 2,4,5-trimethylthiazole.

because of differences in ODR-10 expression levels from different transgenes.

As a control for nonspecific promoter effects from *str-1*, the diacetyl responses of *str-1::mec-4(d)* and *str-1::GFP* strains in an *odr-10(+)* background were examined. Neither of these transgenes affected diacetyl responses, indicating that the promoter alone does not cause chemotaxis defects (data not shown). As a control for copy number of *odr-10*, we examined strains containing transgenic arrays of the wild-type *odr-10* gene under its endogenous promoter in both *odr-10(+)* and *odr-10(ky225)* backgrounds. These strains had normal attractive responses to diacetyl, indicating that high-copy expression of ODR-10 does not disrupt diacetyl responses or cause diacetyl repulsion (data not shown, and J. Chou and C. I. B., unpublished data).

To ask whether the effects of ODR-10 misexpression were specific to diacetyl, we examined the responses of the odr-10(B) and odr-10(AB) strains to pyrazine, which is sensed by AWA, and to 2,4,5-trimethylthiazole, which is sensed by AWA and AWC. Responses to these odorants were normal (Figure 4), except for a slight defect in the response of the odr-10(AB) strain to pyrazine, which was not present in other strains bearing the same transgene (data not shown). These results indicate that misexpression of *odr-10* did not interfere with all AWA functions.

odr-10(B) and odr-10(AB) animals responded normally to some volatile attractants sensed by AWC (Figure 4), such as isoamyl alcohol and benzaldehyde. However, the chemotaxis of the odr-10(B) strain to 2,3-pentanedione was diminished. 2,3-pentanedione is structurally similar to diacetyl (2,3-butanedione), but it is detected predominantly by the AWC neurons via an *odr-10*-independent pathway (Sengupta et al., 1996; P. Sengupta and C. I. B., unpublished data). The effect of the *str-1::odr-10* transgene suggests that ODR-10 may detect 2,3-pentanedione to some extent, even though this response is normally dominated by another receptor. The attractant 2-butanone is also sensed by AWC and is structurally similar to diacetyl; 2-butanone responses were somewhat enhanced in odr-10(B) and odr-10(AB) strains.

ODR-10 Requires Different Signaling Components in AWA and AWB

To define the signaling pathways used by ODR-10 in AWB, we examined avoidance responses in mutants defective in potential olfactory transduction molecules. *odr-3* encodes a putative G protein alpha subunit expressed in AWA, AWB, AWC, and other chemosensory neurons, and *odr-3* mutants are defective in chemotaxis to volatile attractants such as diacetyl (K. Roayaie, J. G. Crump, and C. I. B., unpublished data; Bargmann et al., 1993). *tax-2* and *tax-4* encode a putative cyclic nucleotide–gated channel that is expressed in AWB, AWC, and other neurons. TAX-2 and TAX-4 are hypothesized to be the transduction channel activated by olfactory receptors in AWC, but they are not required for AWA-mediated responses, including attraction to diacetyl, and do not appear to be expressed in AWA (Coburn and Bargmann, 1996; Komatsu et al., 1996). A candidate transduction channel for AWA is OSM-9, a novel protein with similarity to G protein–regulated channels that is required for AWA function and is expressed in AWA, AWC, and other sensory neurons, but does not appear to be expressed in AWB (Colbert et al., 1997). Candidate null alleles are available for each of these genes.

The effects of *odr-3*, *tax-2*, and *osm-9* mutations were analyzed in the odr-10(B) strain, whose diacetyl avoidance should be entirely dependent on AWB. odr-10(B); *odr-3* strains were defective in avoidance of diacetyl, suggesting that *odr-3* is required for ODR-10 signaling in AWB as it is in AWA (Figure 5A). However, odr-10(B); *osm-9* strains avoided diacetyl as effectively as odr-10(B) strains, demonstrating that OSM-9 is not required for ODR-10 signaling in AWB. odr-10(B); *tax-2* mutants did not avoid diacetyl, suggesting that ODR-10 signaling in AWB occurs through the TAX-2/TAX-4 channel (Figure 5A). Thus, the genetic requirements for diacetyl avoidance are different from those for AWA-mediated diacetyl chemotaxis, which depends on *odr-3* and *osm-9* but not *tax-2*.

odr-3, *tax-2*, and *osm-9* mutants were also tested using population avoidance assays for their avoidance of 2-nonanone, the normal AWB ligand (Figure 5B). *odr-3* and *tax-2* mutants displayed a partial impairment in 2-nonanone avoidance, but *osm-9* mutants were indistinguishable from wild-type animals (Figure 5B). The 2-nonanone defects could be caused by a lack of *odr-3*

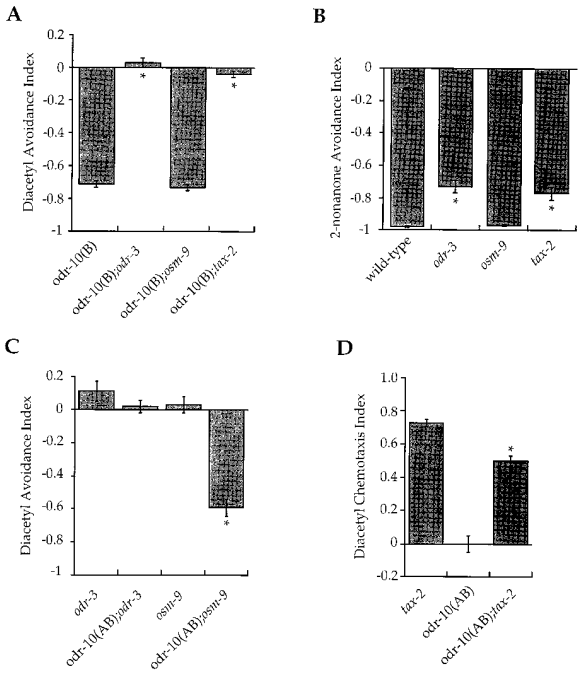

Figure 5. Diacetyl Avoidance Uses Some, but Not All, of the Signaling Components Required for Diacetyl Attraction

(A) Diacetyl responses of odr-10(B) animals in wild-type and mutant genetic backgrounds. (B) 2-nonanone responses of wild-type and mutant animals. (C) Diacetyl chemotaxis responses of odr-10(AB) animals in *odr-3* and *osm-9* mutant genetic backgrounds. (D) Diacetyl chemotaxis of odr-10(AB) animals in wild-type and *tax-2* mutant backgrounds. Asterisks denote responses that differ from controls at $p < 0.01$. For statistical comparisons, controls in (A) were odr-10(B) animals, controls in (B) were wild-type animals, and matched controls for odr-10(AB) transgenic animals in (C) and (D) were *odr-3*, *osm-9*, or *tax-2* mutants without the transgene. Population assays in (A), (B), and (C) were performed on square plates, which can measure either attraction or avoidance. Population assays in (D) were standard round-plate chemotaxis assays. Error bars equal the SEM. Each data point represents the average of at least 10 independent assays.

and *tax-2* function in the AWB neurons. Although these results are qualitatively similar to those observed with diacetyl avoidance, a strong second pathway for 2-nonanone avoidance persisted in each of these mutants. This second pathway could be due to other signaling molecules in AWB, since the *odr-3* and *tax-2* defects appeared to be milder than the defects in the *str-1::mec-4(d)* strain. If this were true, it would suggest that AWB has two signaling pathways, one used by ODR-10 to sense diacetyl that requires ODR-3 and TAX-2, and the other used to sense 2-nonanone that only partially requires ODR-3 and TAX-2. It also remains possible that other cells sense 2-nonanone at the high concentration used in the population assay.

Analysis of odr-10(AB) in *odr-3*, *tax-2* and *osm-9* mutant backgrounds also supported the conclusion that diacetyl avoidance and diacetyl attraction use different genetic pathways (Figures 5C and 5D). odr-10(AB); *odr-3* mutants had no diacetyl response, as expected if ODR-3 is required for both attraction to diacetyl in AWA and repulsion from diacetyl in AWB. odr-10(AB); *osm-9* mutants avoided diacetyl, consistent with the observation that *osm-9* is required for diacetyl attraction, but not diacetyl repulsion. Finally, diacetyl attraction was partially restored in an odr-10(AB); *tax-2* strain, consistent with a requirement for *tax-2* in diacetyl repulsion, but not diacetyl attraction. In summary, ODR-10 signaling in AWB requires the G protein ODR-3, as it does in AWA, but ODR-10 in AWB requires the TAX-2/TAX-4 channel, instead of the OSM-9 channel required in AWA.

Discussion

C. elegans Olfactory Neurons Have Preferred Behavioral Outputs

Individual olfactory neurons in C. elegans can detect several odorants, but there are no cases where one neuron detects both attractive and repulsive stimuli. One explanation for this bias is that receptor proteins intrinsically signal attraction or avoidance, and an individual neuron preferentially expresses receptors of only one class. An alternative explanation is that the receptors are versatile, and an odorant is attractive or repulsive based on the neuron in which its receptor is expressed and activated. Our results show that the diacetyl receptor ODR-10 can mediate distinct responses when expressed in different neurons, supporting the second model.

The AWB olfactory neurons mediate a long-range avoidance response to 2-nonanone; expression of ODR-10 in AWB generates a similar response to diacetyl, despite the fact that ODR-10 generates an attractive response in AWA. These results indicate that the olfactory neuron plays a major role in encoding information about chemotaxis responses. The mechanism by which AWA and AWB neurons direct different responses is unknown, but is likely to reside in the connections that they make with target neurons. AWA and AWB neurons share many of their presumed synaptic targets (White et al., 1986), but perhaps the precise combination of targets is crucial. Alternatively, AWA and AWB could differ in whether they are depolarized or hyperpolarized by odorant, or by the transmitter they release and its effect on target neurons.

These experiments have only been conducted with one receptor (ODR-10) and one olfactory cell type, the AWB neuron. They do not rule out the possibility that neurons, including AWA and AWB, are able to direct both an attractive and an avoidance response. It is possible that receptors encode information about olfactory preference in a cell type–specific manner: while ODR-10 directs attraction in AWA and repulsion in AWB, there may exist another class of receptors that can direct repulsion in AWA and attraction in AWB. Individual chemosensory neurons have been shown to be capable of directing more than one response: the ADF and ASI neurons direct chemotaxis responses to water-soluble attractants as well as developmental responses to the dauer pheromone, which controls progression through alternative larval stages (Bargmann and Horvitz, 1991a, 1991b). These neurons might use two classes of receptors to couple to chemotaxis responses or pheromone

responses. Distinct responses to sensory stimuli may also be generated by the ASH sensory neurons, which mediate avoidance of mechanical and chemical stimuli. *glr-1* codes for a glutamate receptor that is expressed in postsynaptic target cells of ASH, and *glr-1* mutants are defective for avoidance of mechanical, but not chemical stimuli (Kaplan and Horvitz, 1993; Hart et al., 1995; Maricq et al., 1995). The ASH neurons might use mechanoreceptors to couple to the *glr-1*-dependent synaptic pathway, while using chemoreceptors to couple to the *glr-1*-independent pathway.

Odorant Specificity and Signaling

Specificity of ODR-10

Previous studies demonstrated that ODR-10 was necessary for chemotaxis to diacetyl in wild-type animals and sufficient for chemotaxis to diacetyl in a mutant that lacks AWA responses (Sengupta et al., 1996). Expression of ODR-10 in AWB alters responses to diacetyl, but it also alters responses to two other odorants that are not strongly affected by loss of *odr-10* function. Both of these odorants, 2,3-pentanedione and 2-butanone, are structurally similar to diacetyl (2,3-butanedione).

Responses to the attractive odorant 2,3-pentanedione are reduced when ODR-10 is expressed in AWB, although they are less defective than diacetyl responses in the same odr-10(AB) strain. The reduced response might mean that ODR-10 can interact directly with 2,3-pentanedione, as it does with diacetyl, so that conflicting attraction and avoidance signals are generated in response to the odorant. In wild-type animals, 2,3-pentanedione is sensed mainly by the AWC neurons using an *odr-10*-independent receptor (Sengupta et al., 1996; P. Sengupta and C. I. B., unpublished data). ODR-10 may be part of a weaker pathway for sensing 2,3-pentanedione, and this response may be easier to detect in the AWB than the AWA neurons. Responses to the attractive odorant 2-butanone are paradoxically enhanced by *odr-10* expression in AWB, suggesting that there might be an antagonistic interaction between 2-butanone and ODR-10. It is also possible that ODR-10 does not bind 2,3-pentanedione or 2-butanone directly, but rather that complex interactions between sensory neurons can subtly alter the behavioral responses to some odorants.

The AWA, AWB, and AWC olfactory neurons, which are characterized by the complex morphology of their sensory cilia, are all utilized for directed movement in gradients of volatile odorants. However, the signaling molecules within these cells differ, and ODR-10 appears to be able to use different signaling pathways in different cell types. ODR-10 signaling in either AWA or AWB requires ODR-3, a putative G protein alpha subunit (K. Roayaie et al., unpublished data). Normal ODR-10 signaling in AWA requires OSM-9, a protein distantly related to the TRP phototransduction channel of Drosophila (Colbert et al., 1997), but not the cyclic nucleotide–gated channel proteins TAX-2 and TAX-4. By contrast, ODR-10 signaling in AWB requires TAX-2 but not OSM-9.

Interpretation of Olfactory Information by the Nervous System

Like mammals, C. elegans recognizes and discriminates among an enormous diversity of odorants using G protein–coupled receptors. The number of candidate chemosensory receptor genes is comparable between mammals and nematodes: perhaps 1000 genes in rodents and 200–400 genes in C. elegans. However, while the detection potential of the systems may be comparable, the smaller C. elegans nervous system has a more limited behavioral repertoire.

Our results suggest a model for the organization of olfactory information by the C. elegans nervous system. Individual C. elegans neurons express a spectrum of receptors that detect a characteristic set of odorants; each neuron also directs certain behavioral responses, which are presumably defined by the synapses it forms with other neurons. During normal development, we suggest that receptors that detect similar kinds of information are coexpressed in the same sensory neuron. For example, many structurally distinct odorants are produced by bacteria that serve as food for C. elegans (Zechman et al., 1986), and these odorants could have very different receptors, but all of those receptors might be expressed by the AWA and AWC neurons. This organization could allow the nematode to coordinate responses to its environment efficiently; for example, it could regulate responses from AWA and AWC depending on the availability of food (e.g., Colbert and Bargmann, 1997). The specificity of individual receptors can allow a high level of olfactory discrimination, and even specific olfactory adaptation, in which the attractiveness of an odorant can be altered without affecting responses to other odorants sensed by the same cell (Colbert and Bargmann, 1995). At the same time, the small nervous system constrains possible mechanisms of plasticity. Odorant-specific changes can be made by modifying signal transduction components, but modifying synapses will probably alter many odorant responses at once.

Our experiments indicate that if olfactory receptor expression is altered, the odorant detected by the receptor is interpreted in the context of the heterologous sensory neuron. In vertebrate olfactory neurons, altering the expression of olfactory receptors can lead to altered targeting of olfactory axons (Mombaerts et al., 1996). If this were true in C. elegans, altering the expression of receptors might change the behavioral responses generated by olfactory neurons. However, the AWA olfactory receptor ODR-10 can be expressed in AWB without altering the AWB behavioral output. Furthermore, ODR-10::GFP protein has only been observed in the AWA cilia, and not in the axons where target selection occurs (Sengupta et al., 1996). These results suggest that olfactory receptors are not major determinants of sensory axon targeting in C. elegans, though they are complicated by the fact that one neuron expresses many receptors, and ODR-10 was added to a complete endogenous repertoire of AWB receptors rather than replacing them.

Wild-type animals, odr-10(B) animals, and odr-10(AB) animals raised under identical conditions have different

responses to diacetyl, indicating that the intrinsic response to an odorant is defined not by experience but by the cells that express its receptor. A similar bias may underlie olfactory preferences in more complex animals. Many mammals display innate preferences for particular food sources and innate responses to pheromones the first time they are encountered (Halpern, 1987). Such responses can be directed by the vomeronasal olfactory organ, which evokes species-specific behavioral responses, or by the main olfactory organ. A developmental template for these intrinsic responses may be provided by the convergence of specific mammalian olfactory neurons onto invariant targets in the olfactory bulb (Mombaerts et al., 1996). We speculate that the initial projections from the olfactory bulb and the accessory olfactory bulb to brain centers that control feeding, mating, and other strongly determined behaviors may be similar from individual to individual as well, so that critical odorant responses are prewired to a behavioral map. The modification of olfactory preferences by experience may be superimposed on this initial hard-wired map of the olfactory system.

Experimental Procedures

Plasmid Construction

The predicted gene C42D4.5 is 21% identical to *odr-10* at the amino acid level, based on a splicing pattern altered from the Genefinder prediction, and has been named *str-1*. An *str-1::GFP* fusion gene was prepared by using the polymerase chain reaction (PCR) to amplify 4 kb of sequence upstream of the predicted *str-1* start site and the first 4 amino acids of the protein. A PstI site and a BamHI site engineered into the PCR primers were used to insert the amplified product into the GFP vector pPD95.75 (A. Fire et al., personal communication). The *str-1::odr-10::GFP* fusion gene was generated by PCR using the same 4 kb upstream region without any of the *str-1* coding region. A PstI site and a BamHI site engineered into the PCR primers were used to insert the amplified product into a vector containing an *odr-10* cDNA fused to GFP at its carboxyl terminus (Sengupta et al., 1996). The *str-1::odr-10* construct was made by replacing the *GFP* coding region in *str-1::GFP* with a KpnI/ApaI fragment containing the *odr-10* cDNA and *unc-54* 3′UTR. The *str-1::mec-4(d)* construct was made by replacing the GFP coding region in *str-1::GFP* with a KpnI/ApaI fragment containing the *mec-4(d)* allele (Driscoll and Chalfie, 1991; Maricq et al., 1995).

Transgenic Strains

Germline transformation was carried out as described (Mello et al., 1991). The *lin-15* clone pJM23 (50 μg/ml) (Huang et al., 1994) and *str-1::odr-10* (50 μg/ml) were injected into two *odr-10* mutant strains, *odr-10(ky225) lin-15(n765ts)* and *odr-10(ky32) lin-15(n765ts)*, and into *lin-15(n765)* animals. Transgenic animals were identified by rescue of the *lin-15(n765ts)* multivulval phenotype at 20°C. Many independent lines were characterized in each genetic background; eight in *odr-10(ky225) lin-15(n765ts)*, six in *odr-10(ky32) lin-15(n765ts)*, and eight in *lin-15(n765ts)*. Similar results were obtained in both *odr-10* mutant backgrounds. Transgenes were integrated into *odr-10(ky225) lin-15(n765ts)* and *lin-15(n765ts)* by gamma irradiation with 6000R delivered at 330R/min to generate the odr-10(B) and odr-10(AB) strains, respectively. The *str-1::odr-10* transgene integrated into chromosome V in the odr-10(B) strain, and into the X chromosome in the odr-10(AB) strain. An *str-1::GFP* transgene and an *str-1::mec-4(d)* transgene were also injected into *lin-15* animals with pJM23 and integrated. Four independent extragenic *str-1::mec-4(d)* lines were identified that had AWB dye-filling defects; the *str-1::mec-4(d)* transgene in strain 2 was integrated into chromosome V to generate strain 1 (Figure 2C). The *str-1::GFP* transgene integrated into the X chromosome, and is expressed strongly in AWB, with occasional weak staining in the interneurons SIA and AIN. Cell identification was based on the characteristic morphology and position of GFP-positive cell nuclei viewed by simultaneous fluorescence and Nomarski differential interference microscopy.

Chemotaxis Assays

Nematodes were grown at 20°C on E. coli strain HB101 under well-fed and uncrowded conditions (Brenner, 1974). Repulsion assays were performed on square plates containing 10 ml of 1.6% agar, 5 mM potassium phosphate, 1 mM calcium chloride, and 1 mM magnesium sulfate. These plates are divided into six equal sectors labeled A–F. For population assays, one microliter each of odorant and 1 M sodium azide were added in two spots in sector A, and 1 microliter each of control diluent (water or ethanol) and 1 M sodium azide were added in two spots in sector F. Adult animals were washed twice with S-Basal (Brenner, 1974) and once with water, placed in the center of the assay plate (between sectors C and D) and counted after 1 hr.

An avoidance index was calculated using the formula:

$$\text{Avoidance Index} = \frac{(A + B) - (E + F)}{N}$$

where A, B, E, and F are the number of animals in plate sectors A, B, E, and F respectively and N is the total number of animals in all six sectors of the plate.

Laser kills were performed on L1 animals as previously described (Avery and Horvitz, 1987; Bargmann and Avery, 1995). For single animal assays, square assay plates were prepared as for population assays except that just prior to the assay the plates were allowed to air dry for 1 hr with the lid of the plate removed. In addition, no sodium azide was used on these plates. Two spots with 1 μl each of 1-octanol, 2-nonanone, or diacetyl spotted onto sector A were used in single worm avoidance assays. 1-octanol was used undiluted, 2-nonanone was diluted 1:10, and diacetyl was diluted 1:1000 for these assays; diluent alone was spotted onto sector F. Laser-operated and control animals were transferred either in S-Basal or in halocarbon oil to the center of the plate and removed after 1 hr. No more than three repulsion assays were performed on any operated animal in one day. The order of repellent presentation was varied in an effort to minimize the possible effects of odorant interactions. All AWB ablations in the wild-type background were scored blind.

Upon completion of the assay, the path taken by the animal during the course of an assay was recorded by tracing the track on the agar. The nominal scores 3, 2, 1, −1, −2, −3 were assigned to sectors A–F, respectively, and an animal was given a score based on the sum of scores of the sectors in which it had traveled. For example, a score of −6 means the animal migrated into sectors D, E, and F but not A, B, or C while a score of 0 could represent an animal that crawled in all sectors, or in B, C, D, and E, or in C and D. These data sets were compared statistically using a Mann-Whitney rank sum test.

After the repulsion assays, laser kills were confirmed by behavioral tests (AWA) and by direct examination after DiO filling of the amphid neurons (AWB, ADL, ASH, ASI, ASK, ASJ). Only those animals with the appropriate neurons killed were included in the data sets for ablations in the wild-type background.

Population chemotaxis assays for attraction to volatile odorants were performed on round plates as described (Bargmann et al., 1993). Odorant dilutions were 1:1000 (diacetyl, 2,3-pentanedione, 2-butanone, 2,4,5-trimethylthiazole), 1:100 (isoamyl alcohol), 1:200 (benzaldehyde), and 10 mg/ml (pyrazine). These concentrations were chosen to maximize the response to each odorant while ensuring that the response is due to a single type of olfactory neuron, either AWA or AWC (except for 2,4,5-trimethylthiazole, which is sensed by both AWA and AWC). Population assay data for both attraction and repulsion assays were compared using the two-tailed t test.

Strain Construction

odr-10(B) and odr-10(AB) strains containing other mutations were constructed using single-animal PCR to confirm the presence of the *odr-10(ky225)* allele and the *str-1::odr-10* transgene, and the following behavioral assays to confirm the presence of the other

mutations: osmotic repulsion assays for *osm-9* and *odr-3* and benzaldehyde chemotaxis for *tax-2*.

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