Chemosensory Neurons with Overlapping Functions
Direct Chemotaxis to Multiple Chemicals in C. elegans

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Summary

The functions of the 11 classes of exposed chemosensory neurons of C. elegans were tested by killing cells with a laser microbeam. One pair of neurons, the ASE neurons, is uniquely important for chemotaxis: killing the ASE neurons greatly reduced chemotaxis to cAMP, biotin, Cl−, and Na+. Additional chemosensory function is distributed among several other cell types. Thus, 3 pairs of chemosensory neurons (ADF, ASG, and ASI) contribute to a residual response to cAMP, biotin, Cl−, and Na+. After ASE is killed, Chemotaxis to lysine similarly depends on the partly redundant functions of 4 pairs of chemosensory neurons (ASE, ASG, ASI, and ASK). The combined activity of several neuron types that act in parallel might increase the fidelity of chemotaxis.

Introduction

Chemosensation provides an animal with important information about its environment: attractive and repulsive molecules are recognized by organisms from bacteria to vertebrates (Adler, 1975; Van Houten et al., 1981; Van Haastert et al., 1982; Finger and Silver, 1987). In multicellular animals, specialized cells in the nervous system respond to chemical stimuli. It is known that different categories of molecules are recognized by different kinds of sensory neurons: for example, in vertebrates volatile molecules are recognized by olfactory neurons, and salts and sugars by taste bud neurons. However, the mechanisms by which these neurons process and integrate many diverse chemical stimuli are not well understood.

The nematode Caenorhabditis elegans is amenable to detailed study of nervous system structure and function. The C. elegans nervous system, consisting of 302 neurons in the adult hermaphrodite, has been analyzed using electron micrographs of serial sections (White et al., 1976, 1986; Albertson and Thomson, 1976). This work has led to a description of the number, process morphologies, and positions of all of the neurons, as well as identification of each of the morphologically identifiable synapses and gap junctions between neurons and between neurons and muscles. In principle, this "wiring diagram" should provide the basis for much of the behavior of this animal.

The structural description of a neuronal circuit permits analysis of the functional relationships between particular neurons and particular behaviors (Selverston and Miller, 1980; Chiel et al., 1988; Nusbaum and Marder, 1989). In C. elegans, this relationship can be elucidated by killing identified cell types with a laser microbeam (Sulston and White, 1980) and observing the effects on behavior (Chalfie and Sulston, 1981; Chalfie et al., 1985; Avery and Horvitz, 1987, 1989). Since C. elegans has few neurons and since most of the neurons can be unambiguously identified in living animals by morphology and position, it is possible to target particular cells for destruction with a high degree of accuracy. Laser damage to the nucleus of a neuron in young animals results in a loss of the function of that neuron (Chalfie et al., 1985; Avery and Horvitz, 1987, 1989). Thus, hypotheses concerning neuronal function can be tested by killing a cell and subsequently examining the animal for behavioral abnormalities. Using a combination of laser ablation experiments and genetic data, strong evidence for the involvement of mechanosensory neurons in touch sensation (Chalfie and Sulston, 1981; Chalfie et al., 1985) and for the involvement of particular motor neurons in movement (Chalfie et al., 1985; J. White, personal communication; S. McIntire and K. Horvitz, unpublished data), egg-laying (Trent et al., 1983; Desai and Horvitz, 1989), and feeding (Avery and Horvitz, 1987, 1989) has been gathered.

Among the behaviors that have been described for C. elegans, many are regulated by the presence of bacteria, its food. Bacteria cause increased feeding and egg-laying and decreased movement of the animal (Horvitz et al., 1982; Avery and Horvitz, 1990). In addition, the animal can find food in its environment. C. elegans can chemotax toward the peak of a gradient of a number of small molecules, including positively charged ions such as Na+ and K+, negatively charged ions such as Cl− and SO42−, basic pH, and several small organic molecules, including cAMP, cGMP, lysine, cysteine, and histidine (Ward, 1973; Dusenbery, 1974). In addition, using these or other cues, animals can locate bacteria and males can locate hermaphrodites (J. Sulston, R. Horvitz, and E. Jorgensen, unpublished data). Chemotaxis represents a complex response of the animal to its environment: the animal must sense continuously changing external conditions and convert sensory information into movement in a particular direction. In this work, we identify the C. elegans chemosensory neurons required for normal responses to a number of chemical attractants.

Results

Assays and Scoring of Chemotaxis Responses
Chemotaxis was measured using a modified version of an assay developed by Ward (1973; see also Experi-
Figure 1. Chemotaxis Assay and Scoring
A chemotaxis assay of a single wild-type animal toward Cl\textsuperscript{-} is shown at top, and the interpretation of the animal's behavior beneath. The animal was placed near the center of the plate at the origin. The labeled plug (at left) was the source of a gradient of Cl\textsuperscript{-}. A control area (unlabeled, at right) is indicated. After an hour, the animal was removed and the tracks the animal made on the surface of the agar plate were photographed in a contact print of the plate. These tracks are visible to the naked eye. According to the interpretation of this assay, the animal travelled to the peak of the gradient immediately after being placed on the plate (track I), left the peak of the gradient, and returned twice (tracks 2 and 3, which might have occurred in either order). The animal's pattern of approaching and leaving the attractant several times may reflect sensory adaptation during the assay (Ward, 1973). To score the assay, the number of trips to the Cl\textsuperscript{-} (A) was counted, and the number of trips to the control area (B) was subtracted from (A). If A - B is greater than zero, the animal is scored as responding to Cl\textsuperscript{-}. Here, A - B = 3, so this animal is scored as responding to Cl\textsuperscript{-}.

A chemotaxis assay of a single wild-type animal toward Cl\textsuperscript{-} is shown at top, and the interpretation of the animal's behavior beneath. The animal was placed near the center of the plate at the origin. The labeled plug (at left) was the source of a gradient of Cl\textsuperscript{-}. A control area (unlabeled, at right) is indicated. After an hour, the animal was removed and the tracks the animal made on the surface of the agar plate were photographed in a contact print of the plate. These tracks are visible to the naked eye. According to the interpretation of this assay, the animal travelled to the peak of the gradient immediately after being placed on the plate (track I), left the peak of the gradient, and returned twice (tracks 2 and 3, which might have occurred in either order). The animal's pattern of approaching and leaving the attractant several times may reflect sensory adaptation during the assay (Ward, 1973). To score the assay, the number of trips to the Cl\textsuperscript{-} (A) was counted, and the number of trips to the control area (B) was subtracted from (A). If A - B is greater than zero, the animal is scored as responding to Cl\textsuperscript{-}. Here, A - B = 3, so this animal is scored as responding to Cl\textsuperscript{-}.

mental Procedures). Briefly, a gradient of an attractant is established in a 10-cm petri plate containing agar by placing an agar plug soaked in attractant in a well near one edge of the plate and allowing the attractant to diffuse for 12-24 hr. A single animal is placed near the center of the plate, about 5 cm from the peak of the gradient. After an hour, the animal is removed and its tracks on the agar are examined.

A wild-type animal responds to an attractant in over 90% of chemotaxis assays. Typically, the animal will move to the peak of the gradient and wander away and back several times in an hour (Figure 1). Occasionally an animal will move to the peak of the gradient and remain there for the duration of the assay. A simple scoring method that counted each assay as either positive or negative was devised. Assays were scored by counting the number of times an animal that moved well arrived at the peak of the gradient and subtracting the number of times the animal arrived at a control plug at the opposite side of the plate. If the difference was greater than zero, the assay was scored as positive; if it was less than or equal to zero, the assay was scored as negative.

Table 1 lists the responses of wild-type animals to six different attractants. cAMP, Cl\textsuperscript{-}, and Na\textsuperscript{+} are each representative members of classes of attractants known to be distinguished from one another by C. elegans (Ward, 1973). Lysine is a weak attractant identified also by Ward (1973). Biotin and serotonin are strong attractants that we have identified (Table 1A; also unpublished data). Between 75% and 93% of the animals responded to each of these attractants.

Wild-type animals were observed also on similar plates without added attractants. Twenty-three percent of the animals gave an apparent response to the mock attractant in these control assays (see Experimental Procedures). Thus, there is a false-positive response rate of 23% in the assay as it is scored. More complex scoring methods that took into account total movement or graded responses were no more effective than the one we used in distinguishing the responses of wild-type animals in the presence or absence of attractants (data not shown).

Anatomical analysis of mutant animals defective in chemotaxis has indicated that exposed ciliated neurons are required for chemosensation (Lewis and Hodgkin, 1977; Perkins et al., 1986). To determine what component of the chemotaxis measured in our assay reflects the function of exposed ciliated neurons, we examined the mutant che-2(e1033). che-2 animals are chemotaxis-defective (hence the name che) and are also abnormal in other behaviors regulated by sensory stimuli (Lewis and Hodgkin, 1977). che-2 animals have structural abnormalities in most of the ciliated neurons of the head, including all of the neurons with endings exposed to the environment that are thought to be chemosensory (Lewis and Hodgkin, 1977). The severe structural defects in che-2 animals should lead to a profound defect in sensory neuron function.

The results of chemotaxis assays with che-2 animals are shown in Table 1A and Figure 3A. che-2 animals are defective in their ability to chemotax to all attractants tested when compared with wild-type animals (12% - 32% of animals responded to each attractant, p < 0.001 in all cases). An independent estimation of the level of chemotaxis for che-2 animals was made by scoring a number of assays in reverse, i.e., as if the control plug had been the attractant (see Experimental Procedures). In this case, the same chemotaxis index was
Twenty-six neurons in *C. elegans* have been proposed. The processes of these neurons run to the tip of the ASK, and ADL), and 6 cells of 1 type of inner labial cells. In general, we will refer, for example, to the left and right ADF cells collectively as the ADF cell type (IL2). In general, we will refer, for example, to the left and right ADF cells collectively as the ADF cell type. Thus, the chemosensory cells of the head can be considered to be of 9 types: 2 cells each of 8 types of amphidial neurons (ASE, ADF, ASG, ASH, ASI, ASJ, IC, IJ; Figure 3A). Chemotaxis to lysine and serotonin was assessed by killing particular cell types using a laser microbeam (see Experimental Procedures). Based on the combined responses of che-2 animals and of wild-type animals in the absence of attractant, a negative control value representing zero chemotaxis in the assay was defined (see Experimental Procedures). This value corresponds to an apparent response of 20% in the chemotaxis assays.

We were concerned that laser surgery itself might cause a slight decrease in chemotaxis, since surgery involves anesthetization, tissue damage, and trauma to the animal. There is a subtle but statistically significant decrease in the overall chemotaxis efficiency of animals that have undergone any laser surgery (Table 1M; also unpublished data). Therefore, we compared all operated animals with both intact wild-type animals and a control set of ablations in which cells apparently uninvolved in chemotaxis were killed (for each set of experiments, the statistical analysis is described in detail in Experimental Procedures).

**Functional Amphid Neurons Are Required for Chemotaxis**

Twenty-six neurons in *C. elegans* have been proposed to function as chemosensory cells based on the observation that they have ciliated endings that are exposed to the environment through specialized structures in the cuticle (Ward et al., 1975; Ware et al., 1975; White et al., 1986). The cell bodies of 22 of these cells are located in ganglia at the anterior end of the animal. The processes of these neurons run to the tip of the animal's nose, where they are presented to the environment by the amphid and inner labial sensilla. The other 4 cells have sensory endings in the phasmid sensilla at the posterior end of the animal. Figure 2 illustrates the positions of the cell bodies located on the left side of the animal (all cells belong to bilaterally symmetrical pairs) and the locations of the chemosensory process bundles.

There are 2 amphids, 1 each on the right and left sides of the animal, and each amphid contains 1 of a left-right pair of each type of neuron (Ward et al., 1975; Ware et al., 1975; White et al., 1986). The inner labial sensilla have a 6-fold symmetry, with the cells and sensilla radially arrayed around the animal's mouth. Thus, the chemosensory cells of the head can be considered to be of 9 types: 2 cells each of 8 types of amphidial neurons (ASE, ADF, ASG, ASH, ASI, ASJ, IC, IJ; Figure 3A). Chemotaxis to lysine and serotonin was assessed by killing particular cell types using a laser microbeam (see Experimental Procedures). Based on the combined responses of che-2 animals and of wild-type animals in the absence of attractant, a negative control value representing zero chemotaxis in the assay was defined (see Experimental Procedures). This value corresponds to an apparent response of 20% in the chemotaxis assays.

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**The ASE Neurons Are Important for Chemotaxis to Several Attractants**

To facilitate analysis of the amphid cell types, subsets of the amphid cells were killed in single animals, typically about 3 types of cells per animal. Pilot experiments included at least 5 animals in which each type of cell was killed. Despite the large number of cells killed in these animals, it was difficult to assess the role of each specific cell type in chemotaxis. This result indicates that some of these cells are required for normal responses. However, the large number of cells killed in these animals could potentially lead to nonspecific defects in behavior; this concern is addressed by experiments presented below.

By contrast, the inner labial IL2 cells are not essential for at least some chemotactic responses (overall response = 89%; Table 1B, 1I), and the inner labial sensilla were not studied further.

Animals in which 1 or more of the other (non-ASE) 7 exposed amphid cell types were killed were also tested for chemotaxis. None of these cells was essen-
### Table 1. Chemotaxis of Wild-Type, Mutant, and Operated Animals

#### Individual Assays

<table>
<thead>
<tr>
<th>Cells killed</th>
<th>cAMP 95% n</th>
<th>Biotin 95% n</th>
<th>Cl 95% n</th>
<th>Na+ 95% n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Controls</td>
<td>0.92 .85-96 106 0.93 .86-97 96 0.91</td>
<td>0.52-96 127 0.87 78-93 92 0.75</td>
<td>0.02-58 0.86</td>
<td>0.76-93 88 0.80</td>
</tr>
<tr>
<td>B. Amphid Cells or IL2 cells killed</td>
<td>0.40 .12-74 10 0.11 0.01-48 0.40-127 0.11</td>
<td>-0.12-74 0.01 0.01-48 0.40-127 0.11</td>
<td>-0.01-48 0.00</td>
<td>-0.05 0.14</td>
</tr>
<tr>
<td>C. ASE neurons killed</td>
<td>0.67 - 3 ND</td>
<td>0.92-62-99 12 1.0</td>
<td>-7 ND</td>
<td>0.85</td>
</tr>
<tr>
<td>D. Other amphid cells killed</td>
<td>0.84 .62-98 22 0.94</td>
<td>.71-99 18 0.92</td>
<td>0.76-98 30 0.70</td>
<td>0.49-86 27 0.47</td>
</tr>
<tr>
<td>E. Combinations of amphid cells killed</td>
<td>0.10 .01-43 10 ND</td>
<td>0.00</td>
<td>0.00-31 10 ND</td>
<td>0.00</td>
</tr>
<tr>
<td>F. Controls</td>
<td>0.39 .50-99 11 1.0</td>
<td>0.73-95 9 0.83</td>
<td>0.67-93 6 0.71</td>
<td>0.60-93 6 0.71</td>
</tr>
<tr>
<td>G. Controls</td>
<td>0.50-99 11 1.0</td>
<td>0.73-95 9 0.83</td>
<td>0.67-93 6 0.71</td>
<td>0.60-93 6 0.71</td>
</tr>
</tbody>
</table>

#### Calculated Values for Combined Assays

<table>
<thead>
<tr>
<th>Cells killed</th>
<th>cAMP 95% n</th>
<th>Biotin 95% n</th>
<th>Cl 95% n</th>
<th>Na+ 95% n</th>
<th>Lysine 95% n</th>
<th>Serotonin 95% n</th>
<th>All 95% n</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. Controls</td>
<td>1.00 .14-23 204 0.20-80</td>
<td>0.14-23 204 0.20-80</td>
<td>0.14-23 204 0.20-80</td>
<td>0.14-23 204 0.20-80</td>
<td>0.14-23 204 0.20-80</td>
<td>0.14-23 204 0.20-80</td>
<td>0.14-23 204 0.20-80</td>
</tr>
<tr>
<td>I. Amphid or IL2 cells killed</td>
<td>0.75-90 9 0.84</td>
<td>0.86-93 6 0.71</td>
<td>0.75-90 9 0.84</td>
<td>0.86-93 6 0.71</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 1 continued

<table>
<thead>
<tr>
<th>Cells killed</th>
<th>cAMP 95% n</th>
<th>Biotin 95% n</th>
<th>Cl 95% n</th>
<th>Na+ 95% n</th>
<th>Lysine 95% n</th>
<th>Serotonin 95% n</th>
<th>All 95% n</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. ASE neurons killed</td>
<td>0.45</td>
<td>0.38-51 214 0.41</td>
<td>0.38-51 214 0.41</td>
<td>0.38-51 214 0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

K. Other amphid cells killed | 0.80 | 0.75-90 9 0.84 | 0.75-90 9 0.84 | 0.75-90 9 0.84 |

### Table 1 continued

<table>
<thead>
<tr>
<th>Cells killed</th>
<th>cAMP 95% n</th>
<th>Biotin 95% n</th>
<th>Cl 95% n</th>
<th>Na+ 95% n</th>
<th>Lysine 95% n</th>
<th>Serotonin 95% n</th>
<th>All 95% n</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. Combinations of amphid cells killed</td>
<td>0.80</td>
<td>0.75-90 9 0.84</td>
<td>0.75-90 9 0.84</td>
<td>0.75-90 9 0.84</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### F. "Nonessential" controls

<table>
<thead>
<tr>
<th>Cells killed</th>
<th>cAMP 95% n</th>
<th>Biotin 95% n</th>
<th>Cl 95% n</th>
<th>Na+ 95% n</th>
<th>Lysine 95% n</th>
<th>Serotonin 95% n</th>
<th>All 95% n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various cells</td>
<td>0.30</td>
<td>0.19-40 7 0.24</td>
<td>0.19-40 7 0.24</td>
<td>0.19-40 7 0.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### M. "Nonessential" controls

<table>
<thead>
<tr>
<th>Cells killed</th>
<th>cAMP 95% n</th>
<th>Biotin 95% n</th>
<th>Cl 95% n</th>
<th>Na+ 95% n</th>
<th>Lysine 95% n</th>
<th>Serotonin 95% n</th>
<th>All 95% n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various cells</td>
<td>0.81</td>
<td>0.77-86 293 0.86</td>
<td>0.77-86 293 0.86</td>
<td>0.77-86 293 0.86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CJ;emosensory Neurons in C. elegans

Four Partially Redundant Cell Types Direct Chemotaxis to Many Attractants

Although animals in which the ASE neurons were killed were defective in chemotaxis compared with normal animals, the data indicate that the ASE neurons sense all of the attractants that act on C. elegans in chemotaxis. The ASE neurons are necessary for chemotaxis to all attractants, and the ASE neurons are sufficient, at least in three of the six assays tested, to conduct chemotaxis to all attractants tested (p < 0.01; see Experimental Procedures).

Nonspecific damage caused by laser surgery might kill cells other than those targeted for ablation. To rule out such a possibility, all exposed amphid and phasmid neurons except ASE (including all amphid and phasmid neurons including ASE) were killed in animals in which all amphid and phasmid neurons were killed together. These animals were indistinguishable from animals in which all amphid and phasmid cells, including ASE, were killed (overall response = 15%, p < 0.01; see Experimental Procedures). This experiment demonstrates that of the amphid and phasmid neurons, the ASE neurons are sufficient as well as necessary for chemotaxis. Although the numbers are too small to define the importance of the ASE neurons in chemotaxis, these data indicate that the ASE neurons are sufficient for chemotaxis to all attractants tested (p < 0.01; see Experimental Procedures).
Neuron 734

Figure 2. Positions of the Chemosensory Neurons of C. elegans
(Top) Approximate positions of the nuclei and sensory processes of putative chemosensory neurons. The animal is diagrammed lying on its side, with the dorsal side up and anterior to the left. The sensory processes of the amphid and inner labial neurons are exposed to the environment at the anterior tip of the animal, near the mouth opening (Ward et al., 1975; Ware et al., 1975). The phasmid neuron processes are exposed through posterior sensilla. Only neurons on the left side are shown; a bilaterally symmetrical set of neurons is present on the right side.
(Bottom) Positions of the nuclei of the neurons in the head region of a newly hatched L1, reproduced from Sulston et al. (1983). Again, the left side of the animal is shown, with the dorsal side up and anterior to the left. Most but not all of the neurons have bilaterally symmetrical counterparts on the right side. Putative chemosensory neurons are black. For more details about the anatomy of the head, see Sulston et al. (1983) and White et al. (1986).

wild-type animals, these animals still responded to all attractants at a level higher than the negative control value of 20% (p < 0.01 in all cases; Figure 3A; Table 1C). However, the killing of no other single type of cell gave a defect as great as that observed after killing ASE. Therefore, more than 1 type of cell must direct chemotaxis. To identify the additional cells responsible for this residual response, we killed ASE together with other types of cells in single animals. We reasoned that the additional cells might lie in the amphid, since killing all amphid neurons completely eliminated the chemotaxis responses.

Animals with the ASE cells and additional cells killed were compared with unoperated animals, with animals with only the ASE cells killed, and with the negative control value for the assay. No single amphid neuron accounted for the residual response after the ASE neurons were killed (Table 1; also data not shown). However, killing the ASE neurons together with the amphid neurons ADF, ASC, and ASI resulted in animals completely defective in chemotaxis toward biotin, Cl−, and possibly CAMP (Table 1E, 1L; Figure 3A).

All combinations of 3 of the 4 cell types ASE, ADF, ASC, and ASI were killed in many individual animals (Table 1E, 1L; Figure 3B). Only animals in which all 4 cell types were killed had as great a combined defect in chemotaxis to the attractants CAMP, biotin, Cl−, and Na+ as che-2 animals (p < 0.01).

The ability of operated animals to chemotax toward lysine and serotonin was not highly correlated with their ability to chemotax toward the other chemicals (Figure 3C). This difference can be seen most readily in animals in which ASE, ADF, ASC, and ASI have been killed (Figures 3A–3C). The responses to serotonin and lysine were the weakest of those measured in intact animals, but a significant component of these responses was still present after these 4 chemosensory cell types were killed. The responses to amino acids, which ordinarily are the least robust responses, became the most robust chemotaxis responses following laser surgery. These observations indicate that the defects in chemotaxis to CAMP, biotin, Cl−, and Na+ are not likely to be a result of general unhealthiness caused by sensory cell death.

The basis for the residual response to lysine was
Figure 3. Effects of Laser Killing of Amphidial Neurons on Chemotaxis

This figure summarizes data presented in Table 1. Error bars indicate the estimated 95% confidence limits (see Experimental Procedures). 

A) Chemotaxis of wild-type and che-2 animals and of wild-type animals in which ASE was killed, or ASE, ADF, ASG, and ASI were killed.

B) Chemotaxis of animals with combinations of cells ASE, ADF, ASG, and ASI killed toward CAMP, biotin, Cl-, and Na+. The chemotaxis index given is calculated by combining the results of assays to all of these attractants (Table 1) to give an average response for the group.

C) Chemotaxis of animals with combinations of cells ASE, ASG, ASI, and ASK killed toward lysine and serotonin.

D) Chemotaxis of animals with combinations of cells ASE, ASG, ASI, and ASK killed toward lysine. Since ADF does not contribute to the lysine response (Table 1), the numbers for animals with or without ADF have been combined in this figure for entries E-G-I-K-, E-G-I-, E-G-K-, G-I-K-. For example, the animals labeled G-I-K- include 20 animals, 11 of which had only ASG, ASI, and ASK killed and 9 of which had ASE, ASI, ASK, and ADF killed.

sought in a similar set of experiments. When the sensory cells ASE, ASG, ASI, and ASK were killed, the response to lysine was abolished (Figure 3D). All 4 of these cell types had to be killed to delete the response (Figure 3D). The responses to cAMP, biotin, Cl-, and Na+ in these animals were about the same as those in animals in which only ASE had been killed (49% versus 41%; Table 1J, 1L). The cells responsible for chemotaxis to serotonin have not been identified.

Redundant Chemosensory Cells Increase the Efficiency of Chemotaxis

The results of the above experiments indicate that some redundancy exists among the cells that sense a particular attractant. Nonetheless, in the responses to cAMP, biotin, Cl-, and Na+, the ASE cells appeared to be more important than the other cell types. There are several possible explanations for this observation. For example, the ASE cells and the minor cells might
have different thresholds for the attractants, in which case a different cell might appear to be most important depending on the concentration of attractant that was used. Alternatively, the minor cells might respond to the same concentration of attractant as do the ASE cells, but be less efficient at initiating directed movement.

We explored these possibilities by testing animals that had particular cells killed for their responses to different concentrations of attractants. In chemotaxis experiments with populations of intact wild-type animals, we found that NaCl was attractive when concentrations at the peak of a gradient varied from 25 mM to 800 mM and that attractive responses decreased when the concentrations were lower or higher (data not shown). Unoperated animals, animals in which the ASE neurons were killed, and animals in which ADF, ASG, and ASI (the minor cell types involved in chemotaxis to Na+ and Cl-) were killed were tested for their responses to each of six concentrations of NaCl (Figure 4).

Animals in which the ASE neurons were killed were significantly defective in their responses to all concentrations of NaCl over 25 mM. These data indicate that the ASE neurons direct chemotaxis responses throughout this range of NaCl concentrations. However, at all NaCl concentrations, a residual response was present after the ASE neurons were killed, indicating that some minor cells could direct an inefficient response to the attractant. The animals in which ADF, ASG, and ASI were killed were also defective compared with unoperated animals, suggesting that the minor neurons contribute to the normal chemotaxis response. The importance of the minor cells was most marked at 0.00 mM NaCl (p < 0.001), but was also apparent at lower concentrations of NaCl. The minor cells might be somewhat more effective in directing a response either to high concentrations of NaCl or to a steeper NaCl gradient.

Figure 4. Responses of Operated Animals to Different Concentrations of NaCl

Either unoperated wild-type animals, animals in which the ASE neurons were killed (ASE-), or animals in which the ADF, ASG, and ASI neurons were killed (A-F-G-I) were tested for their responses to NaCl. The concentration of NaCl at the peak of the gradient ranged from 25 mM to 800 mM. Chemotaxis indices at each concentration are derived from 80 or more unoperated animals, 50 or more animals in which ASE was killed and 30 or more animals in which ADF, ASG, and ASI were killed. Error bars indicate the upper limit of the 95% confidence limits for each number (the similar lower limits are omitted for clarity). The 20% value represents complete elimination of chemotaxis (see text).

Taken together, these data indicate that the strong response of wild-type animals to NaCl could result from a summation of the less efficient responses of several chemosensory neuron types.

Discussion

The ASE Neurons Are Required for Normal Chemotaxis

The ASE neurons are essential for normal chemotaxis to a number of attractants with different chemical structures. The simplest hypothesis explaining the observed requirement for the ASE neurons is that they directly sense CAMP, biotin, Cl-, Na+, and lysine and that sensory information from the ASE neurons is used to orient the animal's movement.

Several other explanations could account for the effect of killing the ASE neurons. The deaths of the ASE neurons might cause an animal to become generally deregulated in movement, or ASE might be necessary to maintain either the viability or the activity of the true chemosensory neurons (for example by a trophic interaction). These alternative explanations would predict that the deaths of some other cells, the true chemosensory neurons, would also lead to defective chemotaxis. However, no other class of exposed sensory neuron was necessary for chemotaxis to cAMP, biotin, Cl-, or Na+ (Table 1). Indeed, even after all amphid and phasmid neurons except ASE were killed together, animals responded to these attractants. Therefore, if ASE is not the true chemosensory neuron, chemosensory function must be highly delocalized among the amphid, phasmid, and inner labial sensilla, or found among cell types that are not exposed to the environment.

Our control experiments verify that no cells in the vicinity of ASE were essential for chemotaxis and that this procedure for laser surgery is able to kill individual cell types without damaging adjacent cells. Similar
specificity was demonstrated using laser surgery to kill cells in the pharyngeal nervous system of C. elegans (Avery and Horvitz, 1987, 1989).

**Additional Sensory Neurons Have a Minor Role in Chemotaxis**

The deaths of the ASE neurons reduced but did not eliminate the abilities of animals to chemotax to each of the attractants, and no single cell type accounted for the residual responses after ASE was killed. Thus, the ability to direct chemotaxis is distributed among multiple chemosensory cell types. We have identified groups of cells that contribute to five of the six responses that were examined.

The residual ability of ASE-killed animals to chemotax required ASC, ASI, and either ADF or ASK, depending on the attractant tested. For the responses to cAMP, biotin, Cl−, and Na+, at least 2 and probably all 3 of the ADF, ASG, and ASI cell types are involved in the residual chemotaxis following the deaths of the ASE neurons. However, the number of animals scored with respect to each attractant was not sufficient to determine whether all 4 cell types are equally involved in the sensation of all four attractants. Similarly, to eliminate the lysine response, 4 cell types (ASE, ASG, ASI, and ASK) had to be killed together. Since different cells are required for different responses, these chemotaxis defects are most likely explained by specific sensory defects rather than general nervous system abnormalities.

One simple model that explains these data is that the ASE, ASG, and ASI neurons sense cAMP, biotin, Cl−, Na+, and lysine; the ASK neurons sense only lysine; and the ADF neurons sense only the other attractants. However, more complex possibilities are also consistent with our data. Since we examined relatively few animals with multiple neurons killed, potentially interesting regulatory or inhibitory interactions among the neurons could have been obscured by statistical insignificance. Minor chemosensory neurons could have been overlooked if they contribute to a normal response but are unable to direct a response after ASE, ADF, ASG, ASI, and ASK are killed. Alternatively, only a subset of the identified cells might normally contribute to chemotaxis: 1 or more of the minor cells might participate in chemotaxis only after regulation, i.e., after it has assumed the function of a killed cell.

Only a subset of the C. elegans candidate chemosensory neurons defined by anatomy (Ward et al., 1975; Ware et al., 1975) were implicated in chemotaxis in these experiments. The other putative chemosensory neurons could be playing minor or regulatory roles in responses to compounds we assayed, sensing chemotractants that we did not assay, or performing other chemosensory functions.

The complete cell lineage of C. elegans is known ( Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston et al., 1983). Although many chemosensory neurons arise from a particular branch of the lineage, the ASE, ADF, ASG, ASI, and ASK cells are no closer to one another by lineage than they are to other cell types of the amphid. The anatomical connectivities within the nervous system are also known (White et al., 1986). The synaptic targets of ASE, ADF, ASG, ASI, and ASK all include several interneurons of the head, among which are at least 2 of the interconnected neurons AIA, AIB, AIE, AIZ, and RIA. Some or all of these

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**Figure 5. Assigned Functions for Chemosensory Cells of the Amphid**

Functions for the 8 exposed cell types of the amphid, as derived from these and other studies of chemotaxis (this manuscript), dauer formation (Bargmann and Horvitz, 1991), and avoidance of chemical repellents (including high osmotic strength and garlic; Bargmann et al., 1990; J. Thomas and R. Horvitz, unpublished data).
interneurons might receive information from the chemosensory cells for further sensory integration and processing.

Chemosensory Neurons Might Recognize and Distinguish Multiple Attractants

These experiments demonstrate multiple functions for single cells and overlapping functions among groups of cells in the C. elegans chemosensory nervous system. Figure 5 lists the 0 exposed amphid cell types and their apparent functions, as derived from these and similar experiments (Bargmann and Horvitz, 1991; Bargmann et al., 1990; J. Thomas and R. Horvitz, unpublished data). The identification of functions for these neuron types provides a framework for further behavioral and genetic analysis of these questions in C. elegans.

A single cell type, ASE, is involved in the sensation of several unrelated chemicals, including some that can be distinguished by the animal. For example, under conditions in which the response to Na+ is saturated, C. elegans can still chemotax to Cl- (Ward, 1973). In bacteria, similar saturation experiments indicate that a single bacterial cell can sense and distinguish several attractants by expressing and regulating different receptors at the bacterial membrane (Adler, 1975; Goy et al., 1977; Koshland, 1988). One simple way in which multiple attractants could be sensed by the ASE neurons in C. elegans would utilize an analogous mechanism: different attractants might be sensed by different receptors on the ASE sensory endings, with the receptors independently regulated to distinguish among them. Alternatively, C. elegans might distinguish among attractants by comparing the inputs from several different chemosensory neurons.

The properties of these sensory neurons in C. elegans are analogous to those in other animals. Individual olfactory neurons of vertebrates and the fruit fly Drosophila melanogaster respond to many distinct odorants (Sicard and Holley, 1984; Siddiqi, 1987). Single vertebrate taste bud neurons also respond to chemically and psychophysically distinguishable molecules (Scott and Chang, 1984). Thus, chemosensory neurons can have multiple reactivities even in animals in which the total number of neurons is much larger than in C. elegans.

Two very different responses to chemical stimuli are apparently initiated by the sensory neurons ADF, ASC, and ASI. Under conditions of crowding and starvation, C. elegans arrests its development in a specialized larval stage called the dauer larva (Cassada and Russell, 1975). Dauer larva formation is regulated by competing chemical stimuli from bacteria and a pheromone (Golden and Riddle, 1984). ADF, ASC, and ASI have a critical developmental role in the initiation of dauer larva formation (Bargmann and Horvitz, 1991), so they might be sensing food or pheromone signals (or both) to regulate development as well as sensing chemoattractants during chemotaxis. Similarly, the mechanosensory neurons of C. elegans apparently initiate several distinct responses to touch (Chalfie et al., 1985).

Multiple Chemosensory Neurons Contribute to the Fidelity of Chemotaxis

The responses of animals to different concentrations of NaCl suggest that parallel activity of the ASE neurons and the ADF, ASC, and ASI neurons might increase the efficiency of this response. Similarly, the left and right ASE neurons are not fully redundant in function, despite their similar morphologies and synaptic connections (White et al., 1986). Unlike insects that locate chemical stimuli by comparing their left and right antennae, C. elegans probably does not orient itself by comparing the left and right ASE neurons (Ward, 1973). However, the combined activity of this pair of neurons leads to increased fidelity of chemotaxis.

The C. elegans sensory neurons involved in chemotaxis (this work), dauer formation (Bargmann and Horvitz, 1991), mechanosensory responses (Chalfie et al., 1981; Way and Chalfie, 1989), and avoidance of chemical repellents (Bargmann et al., 1990; J. Thomas and R. Horvitz, unpublished data) have been identified. In each case, a hierarchy of sensory neurons exists in which different cell types respond with varying thresholds or efficiencies to related stimuli: major cell types (like ASE in chemotaxis) are assisted by additional cell types with superficially similar functions. Distribution of function among multiple neurons and parallel processing are widely observed in sensory systems (Burrrows, 1987; Heiligenberg, 1988; Lockery and Kristan, 1990). Our data suggest that these strategies are also used in the simple C. elegans nervous system and that the apparent redundancy and functional overlap among chemosensory neurons might serve to increase the robustness and the versatility of the behavioral response.

Experimental Procedures

Strains

Nematodes were grown on E. coli strain HB101 at 20°C (Brenner, 1974). All animals were raised with plentiful food in uncrowded conditions. Wild-type animals were C. elegans variety Bristol, strain N2. Mutant strains che-2 (e1033) X (Lewis and Hodgkin, 1977) and lin 17 (n677) J (Sternberg and Horvitz, 1988) were also used.

Chemotaxis Assays

Chemotaxis assays were based on the assay developed by Ward (1973). Assay plates were 10-cm tissue culture dishes (Corning) containing 10 ml of 1.6% BBL-agar (Becton-Dickinson) or 2% Difco-agar, 5 mM potassium phosphate (pH 6.0), 1 mM CaCl2, 1 mM MgSO4, 10 mM sodium phosphate (pH 7.2), except for assay plates for Na+ chemotaxis, in which the sodium phosphate was omitted. An agar plug 6 mm in diameter (volume of 36 μl) was removed from near one edge of the plate using a cork borer and replaced with a similar plug soaked in attractant. The attractant was equilibrated in the plate and form a gradient for 12–24 hr. The plug at the peak of the gradient was often removed at the beginning of the assay. A similar plug was removed from the plate at a point diametrically opposite the peak of the gradient; this plug was considered the negative control area (Figure 11). Assay plates were air-dried for 1 hr before the
tracks on the agar. The initial attractant concentration at the peak of the gradient was 0.2 M cAMP-NH₂ or biotin-NH₂, 0.4 M NH₄Cl, or sodium acetate; 0.5 M lysine acetate, or 0.026 M serotonin-creatinine sulfate complex (all attractants were obtained from Sigma Chemical Co.). These concentrations were chosen to maximize the responses to each attractant. The highest concentrations of CAMP, biotin, lysine, or serotonin that are freely soluble in water were used, and the highest concentrations of NH₄Cl or sodium acetate that did not induce an avoidance of high osmotic strength were used. Attractants were adjusted to neutral pH using ethylamine, sodium acetate, or sodium hydroxide and acetations are relatively unattractive to C. elegans (Ward, 1973).

Animals were placed briefly on fresh agar plates to free them of bacteria and then transferred to assay plates on a platinum wire using 2% methylcellulose (1500 centipoise; Sigma Chemical Co.). This viscous and unattractive compound was used to transfer the animals without injuring them, since nematodes adhere to it but can be easily released from it onto the agar. An animal was placed on the assay plate equidistant from the two plugs, 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 recorded. Occasional animals crawled off the edge of the agar surface and onto the plastic surface of the assay plate. These animals were gently removed and replaced at their starting position.

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 agar plug (B). If A > B was greater than zero, the assay was scored as positive; if A - B 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. An animal was usually subjected to 6 assays in 1 day, in the following order: CAMP, biotin, lysine, Cl⁻, Na⁺, serotonin. (There was no clear effect of order on efficiency of chemotaxis, although animals often moved more vigorously in the first two assays.) Some animals were tested only with a subset of these attractants; their responses were similar to those tested with all attractants. Occasionally a single animal was tested several times with the same attractant. If an animal was tested more than once, it was given a single final score for the attractant that was the average of the assays run; thus, if 1 assay to CAMP was positive and 1 negative, the animal was scored as 0.5 and considered to have been tested 1 assay.

Our scoring method is biased in favor of a positive outcome: an animal moving randomly will encounter the experimental plug more often than the control plug in some tests and hence be scored as positive at some frequency. This background frequency was measured for wild-type animals by placing the animals on plates without added attractant. An animal arrived at one of the two neutral plugs (mock attractants) in the agar in 46% of the assays (102/221 assays). Thus the apparent chemotaxis to a single plug as a result of this random movement was one-half of that, or 23%.

The identities of the 8 amphid chemosensory cells visualized by Nomarski microscopy in young animals were assigned by several criteria. First, larvae were soaked in the fluorescent dye fluorescein isothiocyanate (FITC), which stains 6 of the 8 putative amphid chemosensory cells (ADF, ASH, ASI, ASJ, ASK, and ADL; Hedegencock et al., 1985). The positions of the dye-filling cell bodies were related to the positions of particular nuclei visible by Nomarski microscopy (Figure 2). A series of criteria were defined based upon which of the nuclei of the dye-filling cells could be unambiguously identified in most animals viewed using Nomarski optics. These criteria included but were not limited to the following: the 3 cells ASK, ADL, and ASI are the 3 largest nuclei in the dorsal-medial region; ADF has a smaller nucleus than ASK and lies adjacent to ASK, slightly ventral to and laterally located from ASK; 7 cells, including ASH and ASJ, define a Y-shaped lateral group in which ASH and ASJ are 2 of the largest cells and always occupy stereotypic positions within the Y. The positions of ASE and ASG, which do not fill with FITC, were derived from those of the FITC-filled cells as indicated by Nomarski and electron-microscopic data provided by Sulston et al. (1983) and White et al. (1986). ASE has a large nucleus and is adjacent to ASH in the Y-shaped group of cells. ASG was assigned to be a small nucleus that occupies a position between ADL and ASI, slightly dorsal and usually slightly anterior to ASE. All of the amphid chemosensory cells in an animal were identified prior to ablation of any cell; if twisting of the animal or interference from the pharynx caused any of the assignments to be ambiguous, the animal was discarded.

The accuracy of the cell assignments made as described above was confirmed by FITC filling after laser ablation of candidate nuclei for all of the cells that stain with FITC. These results support the assignments that were made on the basis of position. ASG is a small nucleus that does not fill with HCL, and there are several other nuclei near this cell that are distinguished from ASG only by position, most notably AWA and AWB. If those positions are not invariant, it is possible that AWA or AWB was killed instead of ASG. To address this question, a single putative ASG cell was killed in each of 2 animals and the animals were analyzed by electron microscopy (D. Jacobson, C. Bargmann, and R. Horvitz, unpublished data). Animals were fixed in 2% paraformaldehyde, 2% glutaraldehyde in 0.1 M HEPES (pH 7.4) for 1 hr at 20°C, osmicated (1% OsO₄ in 0.1 M HEPES), and embedded in agarose, and the anterior 5-10 μm were sectioned. Thin sections were viewed on a JFC1600 EMII transmission electron microscope. The identities of the amphid neurons were assigned as by Ward et al. (1975) and Ware et al. (1975). In both animals, a single dendritic profile corresponding in position to the laser-killed ASG neuron sensory process was abnormally electron-dense and shrunken to 10%–20% of its normal size. All other amphid neuron processes were apparently normal.

The inner labial IL2 neurons were identified using the monoclonal antibody MAb22 (E. Hedegencock and H. Bhatt, personal communication; Okamura and Thomson, 1985). This antibody recognizes several cell types, including the IL2 neurons. Wild-type animals were doubly stained with MAb22 and the nucleic acid stain diamidinophenolindole (DAPI), using the protocol of Desai et al. (1988). The positions of the nuclei of the stained with MAb22 relative to all nuclei stained with DAPI were observed. Candidate IL2 nuclei were then identified in live L1 larvae using Nomarski optics and killed using a laser microbeam. After the animals grew to adulthood, they were tested for chemotaxis and then fixed and stained with MAb22. One IL2 cell stained in 6 animals generated in this way, i.e., the IL2 neurons were identified correctly in 35 of 36 cases (6 cells each in 6 animals).

Laser Ablation of Neurons

Cells were killed using a laser microbeam as described by Avery and Horvitz (1987, 1989). Animals in the first larval stage were
mounted on slides on 5% agar pads containing 3–5 mM NaN₃ as anesthet. Several different kinds of damage were considered to indicate that a cell had been killed: a change in nuclear appear-
tance to the flattened disc shape characteristic of programmed
cell death (Sulston and Horvitz, 1977); disappearance or striking altera-
tion in the appearance of the nucleus and appearance of a distinct region assumed to correspond to the cell body; or the appearance of scars that transected the nucleus. We observed that other nuclei could change position following the death of a cell, so the cell being killed was watched after surgery until sufficient damage was observed to consider the cell dead (5–60 min).

Animals were recovered from the slide in M9 buffer (Brenner, 1974) and placed on a bacterial lawn. Chemotaxis assays were conducted on operated animals after they reached adulthood, usually 3 days after surgery. Following the chemotaxis assays, surviving animals were soaked in FITC and visualized to confirm the death of FITC-filling cells (Hedgecock et al., 1985; Perkins et al., 1985). The criteria for nuclear death listed above usually led to the disappearance of the corresponding FITC-filling cell.

To confirm the specificity of the effect of killing the ASE neurons, all cells adjacent to ASE were killed and the animals were tested for chemotaxis. The cells that were killed adjacent to the ASE cell types are ASC, ASJ, ASH, AVB, AWA, and RIB (Figure 2; also unpublished data). The first 3 are amphid cells, the deaths of which have little effect on chemotaxis compared with the deaths of the ASE cells (Table 2). AVB regulates forward movement (Chaffie et al., 1985), but is nonessential for chemotaxis; when AVB was killed in 8 animals, their chemotaxis responses were completely normal: 8/8 responded to CAMP, 7/7 toбиотин, 6/7 to Cl⁻, and 3/4 to Na⁺. Similarly, killing RIB did not lead to a chemotaxis defect (6/6 animals responded to CAT and биотин, 4/6 responded to Cl⁻ and Na⁺), nor did killing AWA lead to a defect (2/2 animals responded to all attractants). Thus no other cell near ASE is required for chemotaxis.

When the ADF, ASC, ASI, and ASJ neurons are all killed in the young animals, many of the animals become dauer larvae and fail to develop to adulthood (Bargmann and Horvitz, 1991). For the experiments described here, only those animals that became dauer larvae were included in our analyses. The cells that were killed adjacent to the ADF, ASC, ASI, and ASJ cells were ASC, ASJ, ASH, AVB, AWA, and RIB (Figure 2; also unpublished data). The first 3 are amphid cells, the deaths of which have little effect on chemotaxis compared with the deaths of the ASE cells (Table 2). AVB regulates forward movement (Chaffie et al., 1985), but is nonessential for chemotaxis; when AVB was killed in 8 animals, their chemotaxis responses were completely normal: 8/8 responded to CAMP, 7/7 to биотин, 6/7 to Cl⁻, and 3/4 to Na⁺. Similarly, killing RIB did not lead to a chemotaxis defect (6/6 animals responded to CAT and биотин, 4/6 responded to Cl⁻ and Na⁺), nor did killing AWA lead to a defect (2/2 animals responded to all attractants). Thus no other cell near ASE is required for chemotaxis.

When the ADF, ASC, ASI, and ASJ neurons are all killed in the late L1 or early L2 stages; dauer larva form only if their development is initiated in the first larval stage (Swanson and Riddle, 1981). When the ADF and ASI neurons are killed and the ASJ neurons are not killed, many animals become dauer larvae transiently and then recover (Bargmann and Horvitz, 1991). Passing through the dauer stage did not significantly increase or decrease chemotaxis of the resulting adults (data not shown).

Statistical Analysis
Confidence limits of 95% for chemotaxis assays were calculated using the normal theory method for obtaining confidence intervals for binomial parameters and the formula

\[ 95\% \text{ confidence range} \quad x = x \pm 1.96 \sqrt{\frac{x(1-x)}{n}} \]

where \( n \) 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 exact confidence limits were obtained using tables (Rosner, 1986).

Most pairwise combinations between animals were compared with controls using chi square analysis (Rosner, 1986). In all cases, the results for each attractant were considered independently. To identify weaker general effects on chemotaxis, we also combined results from all six attractants and results from CAMP, биотин, Cl⁻, and Na⁺ for the different sets of animals. These comparisons are described individually below.

Owing to each other in their responses to each attractant using chi square analysis (\( p < 0.001 \) in all cases). The responses of che-2 animals were indistinguishable from the responses of wild-type animals in the absence of attractants, and these values were combined to define the negative control value (see Chemotaxis Assays).

Effect of Laser Ablation on Responses
In the course of these and other experiments, many animals have been generated with many combinations of cells killed. Generally, the responses of these animals are slightly weaker than those of wild-type animals, regardless of which cells are killed (Table 1D, IF, IK, IM; also unpublished data). We defined a set of control animals in which we killed only cells for which we had been unable to demonstrate any significant effect on chemotaxis either alone or in combination with other cells. One or more of the following cell types were killed in these animals: RIH, AVB, RIB, AIY, AIA, AFD, AWA, AWB, AWC, RIJ, ALA, BAG, GT₁, WT, RIF, and SMB. Between 40 and 60 animals in this set were tested for their responses to each attractant. When the data for all assays with these animals were compared with those with wild-type animals, the responses were found to be significantly reduced (\( p < 0.01 \)). The responses to serotonin of these animals were also reduced (\( p < 0.01 \)), but no other individual response was significantly lower than the corresponding response of unoperated animals.

All other chemotaxis responses have been compared with this set of responses as a control for effects of laser surgery and with the responses of unoperated animals. However, if any of the cells killed in the control set has a weak or cryptic effect on chemotaxis, the control level determined in these experiments might be inappropriately low.

Laser Ablation
Animals in which ASE was killed were chemotaxis-defective compared with intact wild-type animals (\( p < 0.01 \) for all attractants). However, when these animals were compared with animals having the control ablations, the defects caused by killing ASE were significant only for the attractants CAMP, биотин, Cl⁻, and Na⁺ (\( p < 0.01 \) in each case) and not for лизин and серотонин. Animals in which ASE was killed still had a significant residual response to each attractant (\( p < 0.01 \) in all cases) when compared with the negative control values for the assay.

The data from animals in which only ASE was killed were analyzed to determine whether these animals were truly intermediate in their responses, or whether they were composed of a mixture of normal (like intact animals) and fully defective (like che-2 animals). This comparison was done by examining animals that were tested for their responses to all four attractants CAMP, биотин, Cl⁻, and Na⁺. These animals were given a score of 0 to 4 according to the number of assays to which they gave a positive response (most intact wild-type animals were scored as 3 or 4; most che-2 animals as 0 or 1). The distribution of scores for ASE-killed animals was then tested to see whether it could be composed of a mixture of intact wild-type and che-2 animals. A computer program written by Dr. Rolf E. Bargmann (personal communication) for this purpose generated the best fit for the data for a mixture of wild-type and che-2 animals and then scored this fit by chi square analysis. ASE-killed animals were not well represented by a mixture of wild-type and che-2 animals (\( p < 0.01 \)).

Killing either the left or right ASE neuron decreased the combined responses to CAMP, биотин, Cl⁻, and Na⁺ compared with unoperated animals (\( p < 0.001 \) for either cell) or laser-operated controls (\( p < 0.05 \) for ASE left, \( p < 0.001 \) for ASE right, \( p < 0.001 \) for the 2 combined).

Other Amphid Cell Kils
Each of the 7 exposed amphid cells ADF, ASG, ASH, ASI, ASJ, ASK, and ADL was killed in at least 10 animals in which ASE was not killed. Many animals of this group had several different cell types killed. For example, 7 animals had both ASJ and ADL killed; these animals were composed of a mixture of normal (like intact animals) and fully defective (like che-2 animals) and then scored this fit by chi square analysis. ASE-killed animals were not well represented by a mixture of wild-type and che-2 animals (\( p < 0.01 \)).

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of animals following the deaths of ASH, ASI, ASK, or ADL were indistinguishable from unoperated animals or laser controls. The responses of animals in which ADF, ASG, or ASI were killed were significantly decreased compared with those of unoperated animals (p < 0.05 for ADF and ASG, p < 0.01 for ASI) but not significantly decreased compared with those of the laser controls. The responses of animals in which any of these cells was killed were much stronger than those of animals in which ASE was killed (p < 0.001 in all cases) or the negative control values (p < 0.001 in all cases). When the responses of these animals to CAMP, biotin, Cl-, and Na+ were combined, only ASI killed differed significantly from unoperated animals (p < 0.05) and no animals differed from the laser controls.

The responses to individual attractants were also examined. The following results were significant when compared with unoperated wild-type animals for the Na+ response, ADF (p < 0.05), ASH (p < 0.05), ASI (p < 0.05), and the lysine response, ALT (p < 0.05). However, when compared with the laser controls, only the decreased response to Na+ of animals in which the ASI cells were killed was significant (p < 0.05).

**Kills of All Amphid and Phasmid Neurons Except ASE**

The 5 animals with all amphid and phasmid neurons killed were compared with 5 animals with all amphid and phasmid neurons except ASE killed. No single response was significantly different between the two groups of operated animals (Table 1G). However, when the responses of these animals to all attractants were combined, the animals with ASE alive were clearly more efficient at chemotaxis than those in which ASE was killed (p < 0.001; Table 1H).

To estimate the minimum number of attractants to which ASE responded, we calculated the values for chemotaxis of these animals to different subsets of the attractants. In each case, animals with all amphid and phasmid cells killed were compared with animals with all except ASE killed. First, we combined the values for each group of three of the six attractants for example, all assays except the Cl- assay) and compared the two types of operated animals. In each case, the two groups of animals were different (p < 0.01 in all cases). This result indicated that no single attractant accounted for the difference between the two groups of animals and therefore that responses to at least two attractants were improved in the presence of ASE.

We then combined the values for each group of four of the six attractants (for example, all assays except the Cl- assay and the Na+ assay). Again, the animals were different in all cases (p < 0.01). This result indicates that the responses to at least three attractants were improved in the presence of ASE. Similar calculations grouping three attractants showed that at least four responses were probably improved in the presence of ASE (p < 0.05).

**Kills of ASE Together with Other Amphid Neurons**

Animals with multiple cell kills were compared with intact wild-type animals, laser controls, negative controls, and animals in which only ASE was killed. When results with all six attractants or with the four attractants CAMP, biotin, Cl-, and Na+ were combined, each combination of cells in which ASE was 1 of the cells killed was detected compared with all other unoperated wild-type animals or laser controls (p < 0.001 in all cases).

Most of these combinations of kills resulted in animals with a residual chemotaxis response compared with the negative control values; only animals in which ASE, ADF, ASG, ASI, and ASK were all killed and animals in which ASE, ASI, and ASK were killed were indistinguishable from the negative control levels (p < 0.01 for all other combinations of cells). Since killing ASE together with ADF, ASG, and ASI resulted in animals that did still respond to the attractants (p < 0.01), we attribute the results of the ASE, ASI, and ASK kills to the small numbers of animals tested and assume that some other cells must also sense the attractants.

When the responses from the four attractants CAMP, biotin, Cl-, and Na+ were combined, animals in which ASE, ADF, ASG, and ASI were killed were indistinguishable from the negative control value and significantly defective compared with animals in which only ASE was killed (p < 0.01). Animals in which any subset of 3 of those cells had been killed were significantly more effective at chemotaxis than the negative control values (p < 0.01 in all cases) and more effective than animals in which all 4 cells were killed (p < 0.01, except for kills of ASE, ASG, and ASI, for which p < 0.05; in the latter case, if the data from animals in which ASE, ASG, ASI, and ASK were killed are included, p < 0.01).

The responses to lysine were analyzed in a similar way. Killing ASE, ASG, ASI, and ASK resulted in animals whose response to lysine was as poor as that of the negative controls, and the effect of killing ASE, ASG, ASI, and ASK was more severe than the effect of killing any 3 of those cell types (p < 0.01).

Except for the responses listed above, no significant differences were seen between animals in which ASE and other neurons were killed and animals in which only the ASE neurons were killed.

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**References**


