ture but no behavioral abnormality [Z. Rezai and C. H. Yoon, *Dev. Biol.* **29**, 17 (1972); D. Goldowitz and R. J. Mullen, *J. Neurosci.* **2**, 1474 (1982); K. Herrup and E. Trenkner, *Neuroscience* **23**, 871 (1987)].
23. N. Inouye and S. Oda, *J. Comp. Neurol.* **190**, 357 (1980).
24. Granule cell densities were measured from three separate areas from midline sections of two mutant, one wild-type, and one heterozygous 129/Sv mice by counting cells in a 25,000-μm^2 sample. A mean of 156 ± 22 cells per 25,000 μm^2 was recorded for the six mutant samples and 147 ± 28 for the combined heterozygous and wild-type samples. We have observed no evidence for differences between wild-type and heterozygous mice and therefore have pooled data here. The data for midline length and cell number of the Purkinje layer used to calculate Purkinje cell density were obtained from the same sections with the addition of one outbred mutant and one wild-type 129/Sv mouse. The sample size in all cases was too small for statistical analysis and reflects the small number of mice currently available on the 129/Sv inbred background. All effects described, both in overall size and cerebellar patterning, have also been observed in an equivalent number of outbred mice examined.
25. R. L. Sidman, J. B. Angevine, E. T. Taber-Pierce, *Atlas of the Mouse Brain and Spinal Cord* (Harvard Univ. Press, Cambridge, MA, 1971).
26. J. Altman and W. J. Anderson, *J. Comp. Neurol.* **146**, 355 (1972); K. W. T. Caddy and T. J. Biscoe, *Philos. Trans. R. Soc. London Ser. B.* **287**, 167 (1979).
27. P. E. Neumann, G. G. Mueller, R. L. Sidman, *Brain Res.* **524**, 85 (1990).
28. K. R. Thomas and M. R. Capecchi, *Nature* **346**, 847 (1990).
29. A. P. McMahon and A. Bradley, *Cell* **62**, 1073 (1990).
30. D. G. Wilkinson, J. A. Bailes, A. P. McMahon, *ibid.*, **50**, 79 (1987).
31. S. DiNardo, E. Sher, J. Heemskerk-Jongens, J. A. Kassis, P. H. O'Farrell, *Nature* **332**, 604 (1988); A. Martinez-Arias, N. E. Baker, P. W. Ingham, *Development* **103**, 157 (1988).
32. The *en*-conserved domains are based on sequences of the human, mouse, and chicken *En-1* and *En-2* genes (C. Logan, S. Noble-Topham, M. Hanks, A. Joyner, unpublished results) and the *Xenopus En-2* gene (A. Hemmati-Brivanlou, J. de la Torre, C. Holt, R. Harland, *Development*, in press) and *Drosophila en* and *inv* (*6*, 7). Outside of the conserved domains, only the human and mouse *En-1* or *En-2* genes show homology.
33. Brains were dissected from 2-month-old *En-2^{hd}*/*En2^{hd}*, *En-2^{hd}*/+, and +/+ mice on the 129/Sv background, which had been perfused with 4% paraformaldehyde. The brains were bisected sagittally, fixed overnight, dehydrated, cleared, and embedded in paraffin wax. Serial sagittal sections were cut at a thickness of 10 μm, mounted on subbed slides, and stained with cresyl violet.
34. We thank the various members of our laboratories for helpful discussions and support; D. Nallainathan, M. Kownacka, and C. Ung for technical assistance; H. R. Horvitz for his input on the topic of redundancies; R. Kemler for the gift of the D3 ES cell line, and J. Nadeau for the gift of 129/Sv mice. Supported by grants from the National Cancer Institute (NCI) and Medical Research Council (MRC) of Canada to A.L.J. and J.R.; from the NIH to A.L.J., J.R. (HD25334), and K.H. (NS18381 and NS20591); and from March of Dimes (1-1175) to K.H. A.L.J. is an MRC Scholar, C.A.D. is a recipient of an MRC studentship, and J.R. is a Terry Fox Cancer Research Scientist of the NCI of Canada.

4 September 1990; accepted 29 November 1990

Control of Larval Development by Chemosensory Neurons in *Caenorhabditis elegans*

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Larval development of the nematode *Caenorhabditis elegans* is controlled by the activities of four classes of chemosensory neurons. The choice between normal development and development into a specialized larval form called a dauer larva is regulated by competing environmental stimuli: food and a dauer pheromone. When the neuron classes ADF, ASG, ASI, and ASJ are killed, animals develop as dauer larvae regardless of environmental conditions. These neurons might sense food or dauer pheromone, or both, to initiate the specialized differentiation of many cell types that occurs during dauer formation. Entry into and exit from the dauer stage are primarily controlled by different chemosensory neurons. The analysis of mutants defective in dauer formation indicates that the chemosensory neurons are active in the absence of sensory inputs and that dauer pheromone inhibits the ability of these neurons to generate a signal necessary for normal development.

THE NEMATODE *CAENORHABDITIS ELEGANS* enters one of two different life cycles after a developmental decision controlled by its nervous system. In the presence of ample food, an animal develops to adulthood through four larval stages in about 3 days (*1*). Under conditions of crowding and starvation, the development of young animals is arrested, and they differentiate into specialized larval forms called dauer larvae after the second larval molt (*2*). This alternative larval form is resistant to harsh environmental conditions, does not feed, and can survive without eating for several months. If food becomes available and the density of nematodes decreases, the dauer larva recovers to form a fertile adult.

The decision to become a dauer larva is made soon after hatching and is mediated through a competition between two chemical signals: food (usually bacteria) and a pheromone that reflects nematode density. High concentrations of pheromone and scarcity of food cause animals to enter the dauer stage (*3, 4*). This decision can be reversed at any time before or after dauer formation if food becomes available and pheromone concentrations decrease (*3, 5*).

In mutant nematodes with abnormal chemosensory neurons, dauer formation is not regulated correctly (*6, 7*). This finding suggests that the environmental stimuli that initiate dauer formation are interpreted by the nervous system. Many different cell types are subsequently altered during dauer formation, including neurons as well as hypodermal epidermal and gonadal cells that are not innervated (*8*).

The functions of particular *C. elegans* neurons can be elucidated by killing of identified cell types with a laser microbeam (*9, 10*). The nervous system of an adult hermaphrodite contains only 302 neurons, and the morphologies and synaptic specializations of all of the neurons have been reconstructed from electron micrographs of serial sections (*11*).

The amphid or phasmid sensory neurons have been implicated in the regulation of dauer formation (*6*). The cell bodies of the amphid neurons are located in ganglia in the head of the animal, and their processes run to the tip of the animal's nose, where they are exposed to the environment (*11, 12*). Each of the two amphids, one left and one right, contains eight exposed chemosensory cells: ASE, ADF, ASG, ASH, ASI, ASJ, ASK, and ADL. Two pairs of bilaterally symmetric phasmid neurons, PHA and PHB, are located in the tail.

We killed amphid and phasmid cells with a laser microbeam to assess their roles in dauer formation (*13*). When all 16 exposed amphid cells were killed in single animals, three of five animals became dauer larvae (Table 1)(*14*); when these cells and the phasmid cells were all killed, two of two animals became dauer larvae (Table 1). The animals that became dauer larvae after laser ablation were not exposed to a significant level of dauer pheromone at any point. Control animals raised under identical conditions did not become dauer larvae (Table 1).

Subsets of the chemosensory cells were killed so that the cells involved in dauer larva formation could be identified. When the amphid cells ADF, ASG, ASI, and ASJ were all killed in single animals, 30 of 37 animals became dauer larvae (Table 1). The killing of other amphid cells or phasmid cells in addition to these four cell types did not

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change the percentage of animals that became dauer larvae, so these four cell types account for the effect seen when all cells of the amphids are killed.

Thus, a developmental process normally regulated by chemosensory inputs is not regulated after the deaths of some chemosensory neurons. This result suggests that these sensory cells are involved in the sensation of dauer pheromone or food, the two chemical signals that determine whether dauer larvae will form and whether dauer larvae recover (*3, 5, 15*).

The killing of combinations of three of the four cell types ADF, ASG, ASI, and ASJ revealed functional differences among these cells. The deaths of ADF and ASI were crucial to the formation of dauer larvae. If either ADF or ASI neurons were alive, the animals developed to adulthood exactly as did mock-ablated controls.

ASJ had a different effect on dauer formation. When the other three cell types were killed but ASJ was left intact, the animals passed through a dauer stage transiently (Table 1). Unlike similar dauer larvae that formed after ADF, ASG, ASI, and ASJ were killed, the animals in which ADF, ASG, and ASI were killed recovered from the dauer stage after less than 1 day and developed to adulthood. Thus, ASJ is unable to prevent dauer formation after ADF, ASG, and ASI are killed, but it can reverse dauer formation caused by the deaths of these other cells.

The apparent role of the ASG cell type in dauer formation was variable. After laser ablation of ADF, ASI, and ASJ, 28 of 47 animals became dauer larvae (Table 1). When ASG was killed in addition to those three cell types, a greater fraction of the animals (30 of 37; $\chi^2 = 4.5$; $P < 0.05$) became dauer larvae. Thus, ASG might have a minor role in dauer formation compared to ADF and ASI.

Animals in which the four cell types ADF, ASG, ASI, and ASJ were killed formed dauer larvae that did not recover when exposed to food, so the cells mediating recovery seemed likely to be among those four. The transient dauer formation of animals in which the ADF, ASG, and ASI neurons were killed implicated the ASJ neurons in recovery.

To test the role of chemosensory cells in dauer recovery, we killed chemosensory cells in dauer larvae that had formed under conditions of crowding and starvation. After the addition of food, most animals quickly recovered from the dauer stage to form fertile adults. However, after the ASJ cells were killed, recovery from the dauer stage was inefficient (Table 2); many animals were unable to recover, and those that could did so more slowly than unoperated controls. The low residual ability to recover after ASJ death probably requires ADF, ASG, or ASI (Table 1), but the precise contribution of each of these cell types has not been determined.

The above experiments implicate ADF, ASI, and ASJ and suggest a role for ASG in the regulation of dauer development. However, it remains possible that other chemosensory cell types could also participate in this process. For example, antagonistic groups of cells might promote and prevent dauer formation. In this case, killing a particular additional cell type that prevented dauer formation as well as ADF, ASI, and ASJ might allow animals to grow to adulthood.

To determine the properties of individual chemosensory cell types, we examined the function of each chemosensory cell type in the absence of other chemosensory neurons. When all of the cells of the amphid and phasmid were killed in two animals (Table 1), both became dauer larvae, supporting the notion that the ground state of development in the absence of chemosensory cell function is dauer formation. All amphid and phasmid cells except a single amphid cell

Table 1. Dauer larva formation after killing of sensory cells. Cells were killed within 3 hours of hatching (*10, 13*).

Chemosensory cells killed*	Total no. of animals	No. of dauers	No. of recovered animals†	
			1 day	1 week
ASE +ADF+ASG+ASH+ASI+ASJ+ASK+ADL	5	3	0/3	ND
ADF+ASG+ASI +ASJ	37	30	0/30	0/17
ADF+ASG+ASI	18	16	16/16	
ADF+ASG +ASJ	8	0		
ADF +ASI +ASJ	47	28	4/28	6/18
ASG+ASI +ASJ	7	0		
ADF +ASI	12	5	5/5	
ADF+ASG+ASI +ASJ + some of ASE, ASH, ASK, or ADL	27	19	0/19	ND
All cells	2	2	0/2	0/2
All cells except ASE	3	2	0/2	0/2
All cells except ADF	2	0		
All cells except ASG	2	1	0/1	0/1
All cells except ASH	3	2	0/2	0/2
All cells except ASI	2	0		
All cells except ASJ	2	2	2/2	
All cells except ASK	2	2	0/2	0/2
All cells except ADL	2	2	0/2	0/2
None	>100	0		

*"All cells" includes the exposed amphid cells ASE, ADF, ASG, ASH, ASI, ASJ, ASK, and ADL; the nonexposed amphid cells AWA, AWB, AWC, and ADF; and the phasmid cells PHA and PHB. †The data are expressed as the total number of animals that had recovered after 1 day or 1 week/the total number of surviving animals. ND, not determined.

Table 2. Dauer larva recovery after killing of sensory cells. Dauer larvae were isolated from populations of starved animals with the use of 2% SDS, operated on, and incubated overnight without food before they were fed with bacteria. These animals were observed each day thereafter to determine recovery. All animals survived until the end of the observation period.

Cells killed	No. of recovered animals*			
	1 day	3 days	7 days	14 days
None	23/31	28/31	29/31	29/31
ASJ	1/15	2/15	2/15	3/15
ADF	4/10	4/10	7/10	8/10
ADF + ASG + ASI	3/4	4/4	4/4	4/4
ADF + ASJ	0/11	0/11	2/11	5/11

*The data expressed as the number of animals that had recovered after 1, 3, 7, or 14 days/the total number of surviving animals.

Table 3. Effects of killing chemosensory cells in dauer-defective mutants. The cells ADF, ASG, ASI, and ASJ (often in combination with the cell ADL) were killed in recently hatched animals of each dauer-defective mutant strain (*13, 14, 17*).

Strain	Total no. of animals	No. of dauers
N2 (wild type)	35	28
daf-3(e1376)	6	0
daf-5(e1386)	7	0
daf-6(e1377)	10*	1*
daf-10(e1387)	7	3
daf-12(m20)	9	0
daf-16(m26)	12	4
daf-17(m27)	10	0
daf-18(e1375)	17	0
daf-20(m25)	10	0
daf-22(m130)	3	3
che-2(e1033)	2	2

*These data are based on ten *daf-6* animals that had been maintained continuously at 20°C for several generations. It is known that *daf-6* is not fully penetrant for the dauer-defective phenotype (*6, 16*). In another experiment, four of five *daf-6* animals with parents that had been frozen at −80°C and thawed became dauer larvae after laser surgery. It seems likely that the freezing process affected the expression of the *daf-6* mutant phenotype for at least one generation.

type were killed in several animals (Table 1). When the one surviving cell type was ADF or ASI, the animals grew to adulthood. When only ASJ survived, two of two animals formed dauer larvae transiently and then recovered to adulthood. No other exposed amphid cell type efficiently promoted development to adulthood. These results support the hypothesis that either the ADF or ASI cells are necessary and sufficient to prevent entry into the dauer stage and ASJ is critical for exit from the dauer stage.

Dauer-defective mutations prevent dauer formation under adverse conditions, and dauer-constitutive mutations cause dauer larvae to form inappropriately under good conditions (*16*). Animals that have had their sensory cells killed have phenotypes similar to those of severe dauer-constitutive mutants. To determine whether the deaths of the sensory cells might bypass the lesion in dauer-defective mutants, we killed the sensory cells ADF, ASG, ASI, and ASJ in dauer-defective mutants (*17*).

When ADF, ASG, ASI, and ASJ were killed in the dauer-defective mutants *daf-10, daf-16, daf-22,* or *che-2,* dauer larvae formed (Table 3). By contrast, animals bearing mutations in *daf-3, daf-5, daf-6, daf-12, daf-17, daf-18,* or *daf-20* did not become dauer larvae efficiently after the deaths of the sensory cells. The defect in the latter set of mutants that makes them unable to form dauer larvae under starvation conditions also makes them unable to form dauer larvae if they lack functional sensory cells. These mutations bypass the normal requirement for chemosensory cell function in the prevention of dauer formation.

These results predict the sites of action of different genes in dauer formation. Dauer-defective mutations that are bypassed by killing the sensory cells might define genes that act within or upstream of the sensory cells to interpret environmental signals. By this argument, *che-2, daf-10,* and *daf-16* seem likely to have effects in the chemosensory cells (*18*). Because *daf-22* mutations prevent the production of dauer pheromone (*19*) they should act upstream of the chemosensory cells; indeed, *daf-22* animals still formed dauer larvae when the chemosensory cells were killed. By contrast, the genes *daf-3, daf-5, daf-6, daf-12, daf-17, daf-18,* and *daf-20* are unlikely to act only in the chemosensory cells because their mutant phenotype is still manifested when the chemosensory cells are dead (*18*). These genes could affect cells that respond to signals from the chemosensory cells, or they might cause other cells to produce the chemosensory cell signal inappropriately.

The killing of all amphid sensory cells leads to dauer formation (Fig. 1A). Yet many mutations that lead to defects in the amphids prevent dauer formation (Fig. 1B)(*6, 7, 20*). The defects in these mutants (truncated sensory cilia or occluded amphid openings) probably cause the chemosensory cells to be insensitive to exogenous signals. Thus, the absence of sensory cell function leads to an effect (dauer formation under good conditions) opposite to that caused by the absence of external stimuli (no dauer formation under harsh conditions).

These two observations can be reconciled by the assumption that external stimuli inhibit, rather than stimulate, the chemosensory neurons (Fig. 1C). This mechanism would be analogous to the inhibition (hyperpolarization) of vertebrate photoreceptor cells by light (*21*). The chemosensory cells ADF, ASI, and possibly ASG regulate the decision between dauer formation and development to adulthood by providing a signal that stimulates normal development (or inhibits dauer formation). The proposed signal could be a neurotransmitter, a hormone, or electrical activation of the neurons through gap junctions. If the cells are killed, the signal cannot be made, and dauer larvae form by default. If the cells are separated from the environment, the signal is produced constitutively so that the animals develop to adulthood.

The external stimulus that inhibits these neurons is likely to be dauer pheromone. Some of the same amphid cells might also act as the food sensors in dauer formation (Fig. 1D). The ADF, ASG, and ASI cells are chemosensory neurons that recognize several (possibly food-related) molecules to direct chemotaxis (*22*). The integration of food and pheromone inputs could take place within these sensory cells.

By contrast, for dauer recovery it appears that the ASJ neurons must be activated by external stimuli (presumably food) to initiate dauer recovery (Fig. 1E). Unlike the neurons that regulate dauer formation, the ASJ neurons might not trigger normal development in the absence of environmental stimuli.

Specific signals from the nervous system regulate the development of neuronal and nonneuronal cells in many vertebrates and invertebrates. Innervation by particular neurons is a requirement for specification of the differentiation and survival of muscular and neuronal targets in vertebrates (*23*) and also of nonneuronal cells associated with sensory organs (*24*). In addition, the development of cells that are not closely associated with the nervous system can be altered by neural activity (*25, 26, 27*).

The neurons that control dauer formation in *C. elegans* are likely to provide a link between environmental signals and developmental regulation. Nematode and trematode parasites colonize different organisms

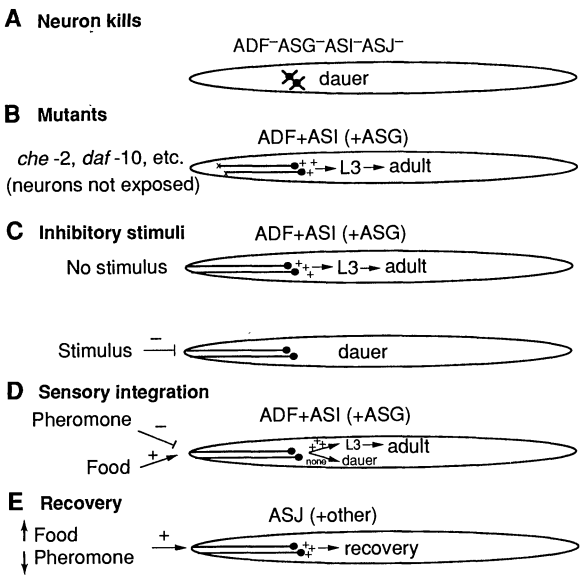

Fig. 1. A model for chemosensory cell function in dauer larva formation. Each diagram schematically shows a nematode and the nuclei and sensory processes of the chemosensory neurons involved in dauer formation. Only two neurons are shown in each diagram, regardless of the actual number of neurons implicated. The small + signs represent the signal released by the chemosensory neurons. This signal might be a neurotransmitter, a peptide, a hormone, or electrical activity. (**A**) Laser kills of the chemosensory cells ADF, ASI, ASG, and ASJ lead to dauer formation. (**B**) In mutants in which the sensory endings are abnormal and all amphid chemosensory cells receive no stimuli, dauer formation is prevented. Because the defect in these animals is bypassed by killing of the same chemosensory cells, the chemosensory cells must be active in the absence of external stimuli.(**C**). The activity of the sensory neurons ADF, ASI, and probably ASG is required for the animal to become an L3 larva and subsequently an adult. This activity is inhibited by environmental stimuli (probably dauer pheromone). Inhibition is represented by the ——| symbol. (**D**) To integrate the opposing signals of food and dauer pheromone, the ADF, ASI, and ASG neurons might simultaneously evaluate both signals and, depending on the ratio, direct development to either the L3 or the dauer stage. Stimulation is represented by the arrow. Alternatively, the food signal may be evaluated by different (unknown) neurons. (**E**) Recovery from the dauer stage is dependent on the activity of ASJ, and, to a lesser extent, other chemosensory neurons. High concentrations of food and low concentrations of pheromone may stimulate the resting ASJ neurons to permit recovery to the adult stage.

at different stages of their life cycles, and initiate developmental changes on encountering each host (*28*). In addition, a variety of marine invertebrates undergo metamorphosis after encountering a particular habitat (*29*). Perhaps mechanisms like those used by *C. elegans* for neuronal control of dauer formation and recovery are used by these other organisms to evaluate environmental signals and regulate their development.

REFERENCES AND NOTES

1. S. Brenner, *Genetics* 77, 71 (1974).
2. R. C. Cassada and R. L. Russell, *Dev. Biol.* **46**, 326 (1975).
3. J. W. Golden and D. L. Riddle, *ibid.* **102**, 368 (1984).
4. ———, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 819 (1984).
5. ———, *J. Chem. Ecol.* **10**, 1265 (1984).
6. P. S. Albert, S. J. Brown, D. L. Riddle, *J. Comp. Neurol.* **198**, 435 (1981).
7. L. A. Perkins, E. M. Hedgecock, J. N. Thomson, J. G. Culotti, *Dev. Biol.* **117**, 456 (1986).
8. D. L. Riddle, in *The Nematode* Caenorhabditis elegans, W. B. Wood, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988), pp. 393–412.
9. J. E. Sulston and J. G. White, *Dev. Biol.* **78**, 577 (1980); M. Chalfie and J. Sulston, *ibid.* **82**, 358 (1981); M. Chalfie *et al.*, *J. Neurosci.* **5**, 956 (1985).
10. L. Avery and H. R. Horvitz, *Cell* **51**, 1071 (1987); *Neuron* **3**, 473 (1989).
11. J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Philos. Trans. R. Soc. London Ser. B* **275**, 327 (1976); D. G. Albertson and J. N. Thomson, *ibid.*, p. 299; J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *ibid.* **314**, 1 (1986).
12. S. Ward, N. Thomson, J. G. White, S. Brenner, *J. Comp. Neurol.* **160**, 313 (1975); R. W. Ware *et al.*, *ibid.* **162**, 71 (1975).
13. Nematodes were grown under standard conditions (*1*). The eight amphid chemosensory cells were visualized by Nomarski microscopy in young animals and identified by several criteria including the abilities of the cells to fill with fluorescein isothiocyanate [E. M. Hedgecock, J. G. Culotti, J. N. Thomson, L. A. Perkins, *Dev. Biol.* **111**, 158 (1985); (*7*)] and their positions [J. E. Sulston, E. Schierenberg, J. G. White, J. N. Thomson, *Dev. Biol.* **100**, 64 (1983); (*11*)]. They were killed with a laser microbeam system (*10*). Detailed descriptions of cell identification and verification will be presented elsewhere (*22*). All operations were performed within 3 hours of hatching, because dauer larva formation can be initiated only within the first larval stage [M. M. Swanson and D. L. Riddle, *Dev. Biol.* **84**, 27 (1981)].

    For experiments assessing dauer recovery, we isolated dauer larvae by treating starved animals with 2% SDS (*2*). Laser operations on the dauer larvae were carried out as described (*9, 10*). After the operations, animals were allowed to recover in 200 μl of S Basal solution (*1*) in a 96-well microtiter dish, with one animal per well. *Escherichia coli* HB101 was added to the microtiter well 12 to 24 hours after surgery. Mock-operated dauer larvae were isolated with SDS and were mounted on a slide with NaN_3 for the same amount of time as the operated animals.
14. Dauer larvae were identified by their dark color, absence of pharyngeal pumping, and appearance of dauer-specific cuticular ridges (alae) visualized by means of Nomarski microscopy (*2, 8*). Additionally, dauer larvae are resistant to SDS treatment, so many candidate animals were tested for survival after treatment with 2% SDS for >10 min.
15. J. W. Golden and D. L. Riddle, *Science* **218**, 578 (1982).
16. D. L. Riddle, M. M. Swanson, P. S. Albert, *Nature* **290**, 668 (1981).
17. Mutant strains included *che-2 (e1033) X* (*20*), *daf-3 (e1376) X, daf-5 (e1386) II, daf-6 (e1377) X, daf-10 (e1387) IV, daf-12 (m20) X, daf-16 (m26) I, daf-17 (m27) I, daf-18 (e1375) IV, daf-20 (m25) X, daf-22 (m130) II* (*16*). Some strains were provided by the *C. elegans* genetic stock center.
18. These laser experiments identify the last point at which a dauer-defective mutant has a highly penetrant defect in dauer formation. Therefore, any mutant defective so that the animal is independent of chemosensory cell function could also have a defect in the chemosensory cells that is masked by the subsequent defect. The *daf-6* gene is probably one such gene, because it affects chemosensory sensilla (*6*) but is functionally independent of chemosensory cell function. In addition, *daf-16* might cause a partial defect at a later step in dauer formation and a strong defect in chemosensory cell function. Thus, several *daf-16* animals became dauer-like larvae after chemosensory cell kills, but did not differentiate fully into dauer larvae (C. I. Bargmann and H. R. Horvitz, unpublished data).
19. J. W. Golden and D. L. Riddle, *Mol. Gen. Genet.* **198**, 534 (1985).
20. J. A. Lewis and J. A. Hodgkin, *J. Comp. Neurol.* **172**, 489 (1977).
21. W. L. Hubbell and M. D. Bownds, *Annu. Rev. Neurosci.* **2**, 17 (1979).
22. C. I. Bargmann and H. R. Horvitz, in preparation.
23. A. J. Buller, J. C. Eccles, R. M. Eccles, *J. Physiol. (London)* **150**, 417 (1960); W. M. Cowan *et al.*, *Science* **225**, 1258 (1984).
24. A. A. Zalewski, *Exp. Neurol.* **24**, 285 (1969).
25. DeF. Mellon and P. J. Stephens, *Nature* **272**, 246 (1978).
26. J. P. Brockes, *Science* **225**, 1280 (1984).
27. J. W. Truman, *J. Comp. Neurol.* **216**, 445 (1983); K.-i. Kimura and J. W. Truman, *J. Neurosci.* **10**, 403 (1990).
28. P. J. Whitfield, in *Modern Parasitology*, F. E. G. Cox, Ed. (Blackwell, Oxford, United Kingdom, 1982), pp. 34–83; L. H. Chappell, *ibid.*, pp. 116–147; A. Maggenti, *General Nematology* (Springer-Verlag, New York, 1981).
29. D. J. Crisp, in *Chemoreception in Marine Organisms*, P. Grant and A. M. Mackie, Eds. (Academic Press, New York, 1974), pp. 177–265.

30. We thank J. Thomas and L. Avery for discussions and unpublished data and L. Avery, J. Brockes, M. Chalfie, M. Finney, E. Jorgensen, and J. Kaplan for comments on this manuscript. Supported by Public Health Service research grant GM24663. H.R.H. is an investigator of the Howard Hughes Medical Institute. C.I.B. is a fellow of the Helen Hay Whitney Foundation.

4 September 1990; accepted 27 November 1990

Failure to Elicit Neuronal Macroscopic Mechanosensitive Currents Anticipated by Single-Channel Studies

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Mechanosensitive channels can be observed in most cell types during single-channel recording and have been implicated in many cellular processes. Potassium-selective single-channel currents, both stretch-activated and stretch-inactivated, can be observed in growth cones and cell bodies of *Lymnaea stagnalis* neurons. Equivalent macroscopic mechanosensitive currents could not, however, be elicited while applying various mechanical stimuli. This discrepancy suggests that single-channel mechanosensitivity is an artifact of patch recording.

MECHANOSENSITIVE (MS) ION channels have been observed with single-channel recording in more than 30 cell types (*1, 2*), including neurons and their growth cones. For dynamic, tension-generating (*3*) structures, it is easy to conceive of a physiological role for these MS channels, yet direct evidence of a mechanical function for MS channels in growth cones (*4, 5*) or elsewhere is lacking. Mechanotransducing sites in specialized receptor cells remain inaccessible to patch electrodes (*1, 2*). Although the many reports of MS channels suggest that electromechanical irritability is present in most membranes, the cellular-level or macroscopic MS currents (I_{MS}) corresponding to these single-channel events have not been measured.

Neurons of the mollusk *Lymnaea stagnalis* are especially suitable for studying macroscopic I_{MS}. Molluscan growth cones, dynamic structures by definition, have MS channels (*4, 5*) and respond during outgrowth to mechanical stimuli (*6*). Distinguishing nonspecific I_{MS} carried in channels from nonspecific (nonchannel) leak is problematic, but *Lymnaea* MS channels are K^+-selective, so I_{MS} would reverse at potentials different from those for nonspecific leak (*7*). One can minimize space clamp problems and cytoplasmic disruptions by using isolated growth cones and perforated patch recording (*8*).

Tension in the membrane of stretched cells is neither measurable nor homogeneous. Nevertheless, procedures that, taken to their extreme, rupture membranes must increase tension. Figure 1 illustrates the tension-inducing procedures we used with *Lymnaea* neurons. In the following experiments, bath solution (MedK, Table 1) was spritzed (pressure-ejected, Fig. 1A) onto voltage-clamped isolated growth cones. After we verified fluid flow with visible dye on several growth cones, we routinely substan-

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