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C. elegans Responds to Chemical Repellents by Integrating Sensory Inputs from the Head and the Tail

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Summary

The phasmids are bilateral sensory organs located in the tail of *Caenorhabditis elegans* and other nematodes. The similar structures of the phasmids and the amphid chemosensory organs in the head have long suggested a chemosensory function for the phasmids [1]. However, the PHA and PHB phasmid neurons are not required for chemotaxis [2, 3] or for dauer formation [4], and no direct proof of a chemosensory function of the phasmids has been obtained. *C. elegans* avoids toxic chemicals by reversing its movement, and this behavior is mediated by sensory neurons of the amphid, particularly, the ASH neurons [5, 6]. Here we show that the PHA and PHB phasmid neurons function as chemosensory cells that negatively modulate reversals to repellents. The antagonistic activity of head and tail sensory neurons is integrated to generate appropriate escape behaviors: detection of a repellent by head neurons mediates reversals, which are suppressed by antagonistic inputs from tail neurons. Our results suggest that *C. elegans* senses repellents by defining a head-to-tail spatial map of the chemical environment.

Results and Discussion

We performed laser ablations to ask if phasmid neurons play a role in avoidance. The assay used to analyze the operated animals is the drop test (Figures 1A–1E, Experimental Procedures, and the Supplementary Material for the movie available with this article online). In this assay, a small drop of repellent is delivered near the tail of an animal while it moves forward. Once in contact with the tail, the drop surrounds the entire animal by capillary action (Figures 1A and 1B). If the substance is sensed as a repellent, the animal stops moving forward and starts moving backward (Figures 1C–1E). Wild-type animals avoid SDS when it is used as the repellent in the drop test. Animals in which the ASH neurons (left and right) were ablated showed reduced response to SDS (Figure 1F). Animals in which the ASH and the ASK neurons had both been ablated showed a response to SDS that was significantly lower than that of animals in which only the ASH neurons had been ablated (Figure 1F). These results demonstrate that both ASH and ASK neurons have a role in SDS avoidance.

The ablation of PHA and PHB did not result in an avoidance defect, so we ablated these phasmid neurons in combination with amphid sensory neurons. Interestingly, animals in which ASH, PHA, and PHB were killed showed a significantly stronger avoidance response to SDS than animals in which ASH alone was killed (Figure 1F). A similar enhancement of avoidance was observed when PHA and PHB were killed together with ASH and ASK (Figure 1F). One explanation for these results is that the phasmid sensory neurons act as negative modulators of the backing response driven by the amphid neurons.

Further support for this hypothesis was obtained using *tax-4* mutants. TAX-4 is a subunit of a cyclic nucleotide gated channel that is expressed in numerous chemosensory neurons and in thermosensory neurons [7]. *tax-4* animals show a strongly reduced avoidance response to SDS in the drop test (Figure 1G). However, ablation of PHA and PHB in *tax-4* animals restored SDS avoidance to nearly wild-type levels (Figure 1G). These results indicate that PHA and PHB antagonize the function of neurons that mediate the SDS avoidance response.

There are two possible mechanisms by which PHA and PHB could inhibit SDS avoidance. In one model, PHA and PHB would have a chemosensory function and would inhibit reversals when they sensed SDS with their exposed cilia. Alternatively, PHA and PHB could act as general negative modulators of the avoidance response that do not sense SDS directly. Three results support the hypothesis that PHA and PHB are indeed chemosensors.

In a first test for a chemosensory function of the phasmid neurons, we killed the hypodermal T cell in *tax-4* mutants. The T cell is the precursor of the phasmid socket cell, which forms the sensory channel through the cuticle to the outside world. When the T cell is killed, the PHA and PHB neurons are still present, but they cannot receive chemical stimuli from the environment, because the amphid channel is not formed and their sensory cilia do not reach the outside world. SDS avoidance by *tax-4* mutants was significantly enhanced by killing the T cell. Avoidance in the drop test rose to a level indistinguishable from that of PHA/PHB-killed *tax-4* animals (Figure 1G).

In a second approach, we designed an assay, the dry drop test, where stimulation of the phasmids was prevented by avoiding the capillary effect of the drop test (Figures 2A–2E, Experimental Procedures, and the Supplementary Material for the movie). In this assay, the drop is delivered on the agar a few millimeters ahead of an animal that is moving forward, so that it encounters the substance after the drop has been absorbed into the agar (Figures 2A–2C). If the substance is a repellent, the animal reverses immediately upon encountering it with its head (Figures 2D and 2E). In the dry

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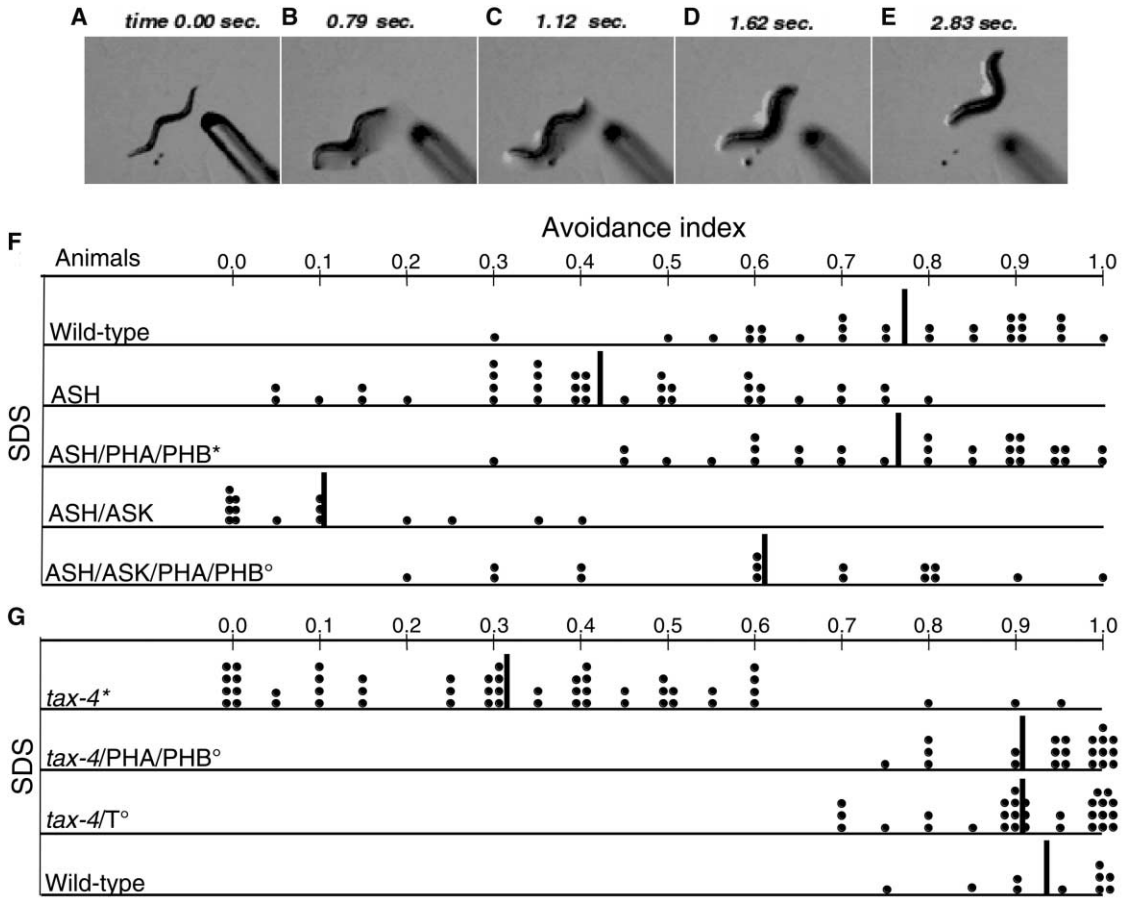

Figure 1. Phasmid Neurons Act as Chemosensory Neurons and Modulate Avoidance to SDS

(A–E) The drop test (see also the Supplementary Material for the movie). (A) The animal is moving forward on the agar plate, with its head at the bottom left. A glass micropipette filled with repellent is visible near the agar surface. Time 0.00 s. (B) The drop is delivered on the agar near the tail and reaches the anterior extremity of the animal by capillary action. Time 0.79 s. (C) The animal starts the avoidance reflex by moving backward. Time 1.12 s. (D and E) The backward movement continues. Time 1.62 s and 2.83 s.

(F and G) Scatter diagrams plotting the avoidance index of single operated animals. Individual assays were scored as positive or negative, and each black dot represents the fraction of positive responses in ten or more assays. Some animals were tested more than once; each row represents at least ten operated animals. Black vertical bars indicate the mean. (F) Ablations in a wild-type genetic background. Animals with ASH ablated are compared to animals with ASH/PHA/PHB ablated; animals with ASH/ASK ablated are compared to animals with ASH/ASK/PHA/PHB ablated. * indicates statistically significant difference with the ASH ablated animals; ° indicates statistically significant difference with the ASH/ASK ablated animals. (G) Ablations in a *tax-4 (p678)* genetic background. *tax-4* mutant animals were compared with *tax-4* animals in which either PHA and PHB neurons were killed or the T hypodermal cell (T) was killed. T is the precursor of the phasmid socket cell. Some wild-type animals were tested in the same session as positive controls. * indicates statistically significant difference with the wild-type strain; ° indicates statistically significant difference with the *tax-4* strain.

drop test, unlike the drop test, the repellent is presented to the head and not to the tail. The dry drop test performed with SDS induced robust avoidance behavior, and the concentrations required were comparable to those used in the drop test. *tax-4* animals responded like the wild-type to SDS in the dry drop test, although their response was reduced when using the drop test (Figure 2F). The comparison of this result with the ablations of the phasmid neurons or of the T cell suggests that an asymmetric presentation of the repellent overrides the antagonistic input from phasmids. In our interpretation, the different response of *tax-4* animals to SDS in the two assays is due to the fact that in the dry drop test the phasmids are excluded from direct contact with the repellent and cannot antagonize the avoidance response, which is thus significantly stronger.

In a third approach, we separated stimulation of anterior and posterior sensory neurons. To this end, wild-type animals were confronted with two separate dry drops of SDS. The first was delivered as in the normal dry drop test, just anterior to the animal nose; the second drop of SDS was deposited just posterior to the animal tail. With this strategy, the animal was forced to encounter the same repellent, first, anteriorly with the head and, then, during its backward movement, posteriorly with the tail. We compared the duration of the backward response in the regular dry drop test (only anterior stimulation) with the duration of the response when both drops were delivered. The duration of the backward response was reduced from an average of 3 s, when only the anterior stimulus was applied, to an average of 2 s, when both stimuli were delivered (Figures 3A and

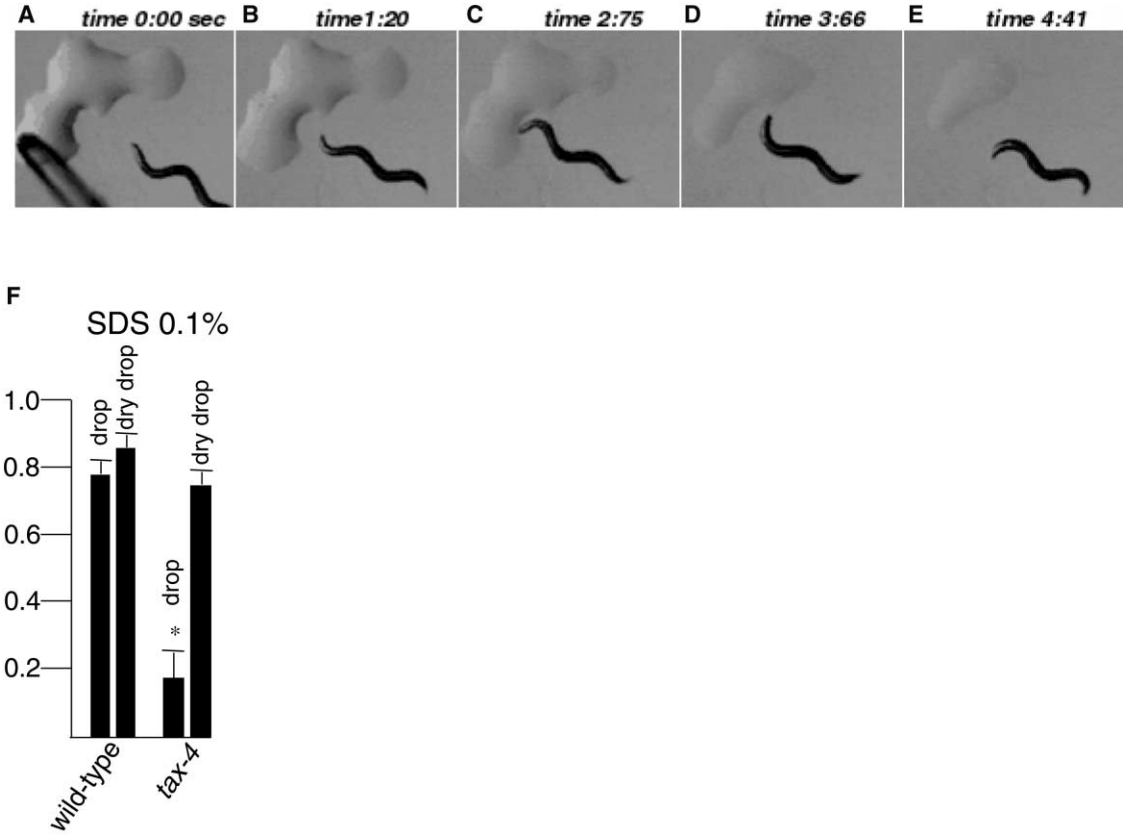

Figure 2. The Dry Drop Test

(A–E) Dry drop test (see also the Supplementary Material for the movie). (A) The animal is moving forward on the agar plate, with its head at top left, and the drop has been delivered on the agar surface ~500 μm ahead. The glass micropipette filled with the repellent is visible near the agar surface. Time 0.00 s. (B) The drop is being absorbed by the agar. Time 1.20 s. (C) The animal's head encounters the substance, but since the liquid has already dried out, there is no capillary action. Time 2.75 s. (D) Beginning of the avoidance reflex and backward movement; Time 3.66 s. (E) Backward movement continues. Time 4.41 s.

(F) Avoidance responses of *tax-4* mutant strain to 0.1% SDS. The *tax-4* mutant response to SDS was comparable to wild-type in the dry drop test but was reduced in the drop test. Each data point was obtained on populations of ≥10 animals tested with a minimum of ten drops each. The bars are the SEMs. * indicates statistically significant difference with the wild-type strain, $p < 0.01$.

3B). When the second drop near the tail did not contain the repellent (SDS) but only buffer (M13), the duration of the avoidance response remained unchanged (Figure 3C), indicating that SDS, not the delivery of the drop, inhibits the avoidance response. In addition, when using SDS we often observed a rapid forward acceleration of the animal just after its tail had come in contact with the repellent (data not shown).

These results suggest that PHA and PHB neurons act as chemosensors and demonstrate that the decision to initiate reversals incorporates information from head and tail sensilla. Inputs from the anterior amphid neurons ASH and ASK may act antagonistically to inputs from posterior phasmid neurons on a network of interneurons that can drive forward or backward movement. The backward response appears to predominate when anterior and posterior sensors are stimulated simultaneously. The organization of avoidance is thus reminiscent of the response to light touch, where anterior and posterior stimuli are also antagonistic and where backward movement triggered by anterior stimuli predominates over posterior stimuli [8]. Further similarity between chemical avoidance and mechanical response is observed in the pattern of connections of sensory neurons to interneurons. The posterior mechanosensor PLM and the posterior chemosensor PHB both synapse primarily onto AVA, AVD, and PVC interneurons (PHA synapses primarily onto PHB) [9]. These three interneurons together with AVB constitute the main network coordinating forward and backward movements [10, 11] (Figure 4).

Animals can map the chemical environment in which they live following two main strategies. In one strategy, chemosensory organs are clustered together, and animals discern the location of chemicals by moving through the environment, integrating signals using either temporal processing or proprioceptive input. A temporal processing strategy underlies chemotaxis in bacteria as well as chemotaxis to water-soluble attractants in *C. elegans* [12, 13]. In a temporal strategy, the response is time consuming by definition. In a second strategy, animals can have sense organs in different body regions so that activation of sensory neurons directly reflects the spatial distribution of chemical stimuli. Although anatomical considerations have suggested that such che-

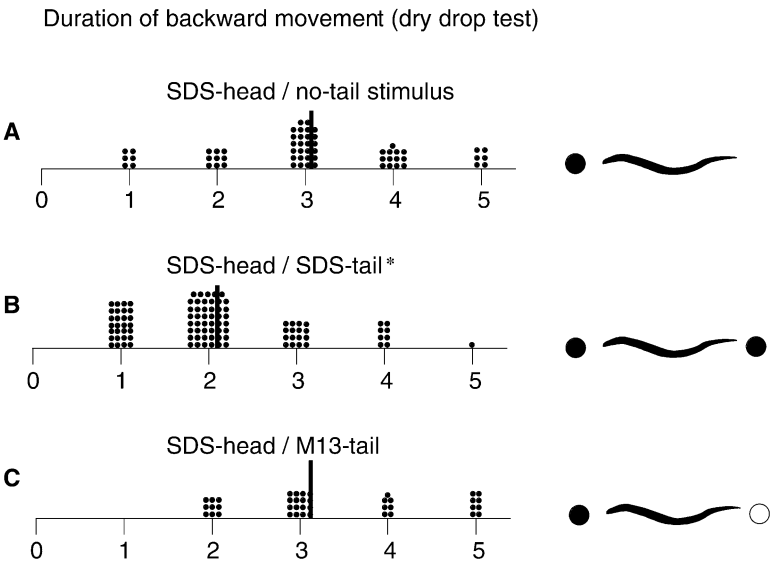

Figure 3. Dry Drop Stimuli Applied to Head and Tail Separately

Scatter diagram of the duration of the avoidance backward response. Each dot represents the duration of backward movement by a single animal in seconds; vertical bar shows the mean. (A) Duration of the response induced by dry drop at anterior only with 0.1% SDS; (B) duration of the response when the anterior SDS dry drop stimulus is followed by a posteriorly applied SDS dry drop; (C) duration of the response when the anterior SDS dry drop stimulus is followed by a posteriorly applied M13 buffer dry drop. * indicates statistically significant difference with the head-only stimulus, $p < 0.01$.

mosensory maps might exist, this model has not been directly demonstrated. A head-to-tail sensory map of the environment can be very rapid, but it is limited in spatial resolution by the number and location of the sensory organs. We suggest that rapid avoidance of toxic molecules or conditions by *C. elegans* utilizes a simple head-tail chemical sensory map, whereas chemotaxis to food sources and mating partners uses a more flexible temporal strategy that can locate signals in any direction.

Our results demonstrate a specific chemosensory function of the phasmids in *C. elegans* and suggest potential functions for similar chemosensory organs along the body and tail of other Nematoda, Annelida, and Arthropoda. The presence of the phasmids is used by evolutionists to divide the phylum of Nematoda in Phasmida (Secernentea), which have this sensillum, and Aphasmida (Adenoforea), where this sensory organ is absent [1]. Most of the Aphasmida are aquatic free-living species, whereas most of the Phasmida are terrestrial. It is possible that the antero-posterior spatial separation of two chemosensory organs is not as useful in aqueous media because of the high turbulence of molecules moving through water. Terrestrial species might make a better use of a head-tail chemical map to receive and integrate stimuli coming from different directions.

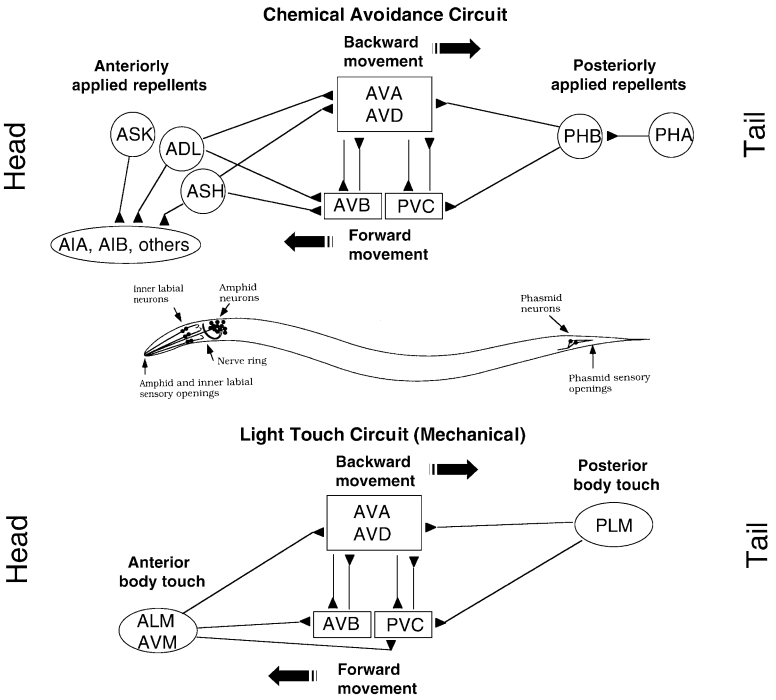

Figure 4. Model for Chemical Avoidance Circuit

For comparison, the light-touch mechanical circuit is shown (adapted from [15]). Chemical avoidance sensory neurons are circles, mechanical sensory neurons are ovals, and interneurons are boxes. ASH, ASK, and ADL sense chemical repellents (ASH also senses nose touch, a mechanical stimulus). In chemical avoidance, the amphid sensory neurons ASH and ADL directly contact the command movement interneurons AVB, AVA, and AVD, and all three avoidance sensory neurons contact the amphid interneurons AIA and AIB. The phasmid sensory neuron PHA contacts the PHB sensory neuron, which synapses onto AVA, AVD, and PVC interneurons. In the light touch circuit, the PLM sensory neuron, which triggers forward movement to tail touch, contacts AVA, AVD, and PVC interneurons. The AVM and ALM neurons, which trigger backward movement to light anterior touch, contact AVB, AVD, and PVC interneurons. In both circuits the backward response predominates when stimuli are simultaneously applied anteriorly and posteriorly. Numerous other connections of these sensory neurons and interneurons are not shown.

Experimental Procedures

Drop Test

A drop of a solution containing the repellent or a drop of buffer is delivered on the agar near the tail of a moving animal. The drop touches the tail, surrounds the animal, and reaches the anterior amphid sensory organs by capillary action. Drops are delivered using 10 μl glass capillaries (Blaubrand intraMARK) pulled by hand on a flame to reduce the diameter of the tip. The capillary is mounted in a holder with rubber tubing and operated by mouth or by 5 ml syringe. We calculated the average drop size to be ~5 nanoliters. The animal usually starts a backward motion within 1 s of the delivery of the drop. The response to each drop is scored as positive if the animal reacts within 4 s. The avoidance index (a.i.) is the number of positive responses divided by the total number of trials.

Drop tests were conducted on unseeded NGM plates using adult animals. All repellents were dissolved in Tris, 30 mM; NaCl, 100 mM; KCl, 10 mM (M13 buffer). SDS repellent was added to a final concentration of 0.1%.

A number of control experiments confirmed that the drop test specifically measures chemical avoidance responses. First, wild-type animals challenged in the drop test with neutral substances or with attractants showed avoidance indexes of 0–0.15. No animal ever showed an avoidance index higher than 0.3. Second, several chemosensory-defective mutants tested in the drop test with 1 M glycerol (high osmotic strength) exhibited strongly reduced avoidance responses [*osm-1(p808)*, a.i. = 0.15; *osm-3(p802)*, a.i. = 0.16; *osm-5 (p813)*, a.i = 0.16; *osm-6 (p811)*, a.i. = 0.2; *osm-9 (n1601)*, a.i. = 0.14; *osm-10 (n1602)*, a.i. = 0.19; *che-3(e1124)*, a.i. = 0.13; *daf-10 (e1387)*, a.i. = 0.13]. These results indicate that the drop test overlaps with the osmotic avoidance assays that were used to isolate and characterize these mutants [14]. Third, mechanosensory mutants challenged with SDS in the drop test showed an avoidance index comparable to that of wild-type animals [*mec-4* (*e1611*), a.i. = 0.88; n > 10, >100 trials; *mec-10* (*e1515*) a.i. = 0.9; n > 10, >100 trials], indicating that mechanical stimulation is not essential for the drop test avoidance response.

Dry Drop Test

The dry drop test resembles the drop test except that the animal encounters the repellent after the drop has been absorbed into the agar. This assay should provide spatial resolution between anterior and posterior sensory neurons. The drop is delivered ~0.5–1 mm anterior to the animal (head stimulus). In Figure 3 (separate head and tail stimuli), a second drop was applied ~0.3–0.5 mm posterior to the animal. Attractants and neutral stimuli did not induce obvious forward acceleration or backward movement in the dry drop test. *mec-4* (*e1611*) and *mec-10 (e1515)* mutants avoided SDS in the dry drop test (a.i. = 0.95; n > 10; >100 trials; a.i. = 0.9, n > 10; >100 trials), indicating that mechanosensation is not essential for the dry drop test. *osm-10 (n1602)* animals, which fail to avoid high osmotic strength, avoid instead SDS (a.i. = 0.95; n > 10; >100 trials), proving that the avoidance response we observe is not due to a change in osmolarity of the medium.

Supplementary Material

Supplementary Material including movies can be found at http://images.cellpress.com/supmat/supmatin.htm.

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