View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Neuron, Vol. 26, 619–631, June, 2000, Copyright ©2000 by Cell Press

C. elegans Locomotory Rate Is Modulated by the Environment through a Dopaminergic Pathway and by Experience through a Serotonergic Pathway

Elizabeth R. Sawin,† Rajesh Ranganathan, and H. Robert Horvitz*
Howard Hughes Medical Institute
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Summary

***Caenorhabditis elegans* modulates its locomotory rate in response to its food, bacteria, in two ways. First, well-fed wild-type animals move more slowly in the presence of bacteria than in the absence of bacteria. This basal slowing response is mediated by a dopamine-containing neural circuit that senses a mechanical attribute of bacteria and may be an adaptive mechanism that increases the amount of time animals spend in the presence of food. Second, food-deprived wild-type animals, when transferred to bacteria, display a dramatically enhanced slowing response that ensures that the animals do not leave their newly encountered source of food. This experience-dependent response is mediated by serotonergic neurotransmission and is potentiated by fluoxetine (Prozac). The basal and enhanced slowing responses are distinct and separable neuromodulatory components of a genetically tractable paradigm of behavioral plasticity.**

Introduction

An important function of the nervous system is to allow animals to respond flexibly to changing environmental stimuli. Such behavioral plasticity is manifested in numerous ways. In associative learning, associations between stimuli are recorded by the nervous system, and the stored information is used to alter behavioral outputs (Kupfermann and Kandel, 1995). In forms of nonassociative learning, habituation and dishabituation alter the strength of reflexive responses based on an animal's past experience of repetitive or noxious stimuli, respectively, and sensitization enhances the response to a baseline stimulus after exposure to a novel or stronger stimulus. Another form of behavioral plasticity, also referred to as an alteration in behavioral state, or behavioral arousal, occurs when an animal's responsiveness to specific stimuli is altered as a result of a change in its internal state, a change that is often influenced by the animal's environment or past experience (Flicker et al., 1981). Understanding the mechanisms that produce such flexibility in behavioral output has been a major challenge in neurobiology.

Behavioral plasticity often involves the alteration of the properties of neurons and synapses. These alterations can be caused by the actions of neuromodulators, such as dopamine and serotonin. In both invertebrate and vertebrate nervous systems, dopamine plays a critical role as a neuromodulator by altering the intrinsic properties of neurons within circuits, both presynaptically (Cameron and Williams, 1993; Harris-Warrick et al., 1995) and postsynaptically (Barnes et al., 1994; Maguire and Werblin, 1994; Pereda et al., 1994). Genetic analyses of dopaminergic neurotransmission in *Drosophila melanogaster* (Tempel et al., 1984; Neckameyer, 1998) and mice (Drago et al., 1998, and references cited therein) have implicated dopamine in locomotory and spatial learning behaviors. Furthermore, the disruption of dopaminergic systems in human brains is involved in disorders such as Parkinson's disease (Marsden, 1992) and has been suggested to be involved in schizophrenia (Hietala and Syvalahti, 1996, and references cited therein), and dopaminergic neurons contribute to the circuitry involved in human motivation, reward, and drug addiction (Koob, 1992; Self and Nestler, 1995).

Serotonin is also an important neuromodulator in many animals. In the sensitization of defensive reflexes in the leech (Sahley, 1995, and references cited therein) and in *Aplysia californica* (Hawkins et al., 1993, and references cited therein), the application of the sensitizing stimulus releases serotonin and enhances the strength of reflex pathways. Serotonin mediates changes in behavioral state associated with food deprivation in the leech (Groome et al., 1993; Brodfuehrer et al., 1995) and social dominance in the lobster (Kravitz, 1988). Serotonergic neurotransmission is implicated in numerous human processes and diseases (Osborne and Hamon, 1988; Takada and Curzon, 1995; Lucki, 1998, and references cited therein). Fluoxetine (Prozac) and other selective serotonin reuptake inhibitors used in the clinic to treat many of these human disorders are thought to work by primarily affecting serotonergic neurotransmission (Wong and Bymaster, 1995).

The simple anatomy, genetics, and behavior of the nematode *Caenorhabditis elegans* make this animal attractive for analyses of behavioral plasticity (Brenner, 1974; Wood et al., 1988). The chemotaxis response of *C. elegans* to volatile attractants adapts to the presence of high levels of certain attractants, while animals maintain the ability to respond to other attractants (Colbert and Bargmann, 1995). *C. elegans* is capable of nonassociative learning in the form of habituation when repeated mechanical stimulation is applied, dishabituation when the noxious stimulus of electric shock is administered, and sensitization when stronger mechanical stimulation is administered prior to the baseline stimulus (Rankin et al., 1990; Wicks and Rankin, 1997). Several hours of starvation affect *C. elegans* thermotaxis (Hedgecock and Russell, 1975; Mori, 1999) and olfaction (Colbert and Bargmann, 1997).

Several dopaminergic (Sulston et al., 1975) and serotonergic (Horvitz et al., 1982; Desai et al., 1988; Loer and Kenyon, 1993; E. R. S. and H. R. H., unpublished data) neurons have been identified in *C. elegans*. Locomotion and egg laying in *C. elegans* involve dopaminergic signaling (Schafer and Kenyon, 1995; Weinshenker et al., 1995), and serotonin affects several *C. elegans* behaviors, including locomotion, egg laying, and pharyngeal pumping (Horvitz et al., 1982; Trent et al., 1983; Avery

*To whom correspondence should be addressed (e-mail: horvitz@mit.edu).
† Present address: Sustainability Institute, Hartland, Vermont 05048

and Horvitz, 1990; Schafer and Kenyon, 1995). Furthermore, the selective serotonin reuptake inhibitor fluoxetine (Prozac) affects *C. elegans* egg laying (C. Johnson, personal communication; Weinshenker et al., 1995), nose contraction (Choy and Thomas, 1999), and locomotory rate (Nurrish et al., 1999).

We have identified and analyzed a new paradigm for *C. elegans* behavioral plasticity. This paradigm provides a model for how behavioral state is controlled and how it affects the response to a specific stimulus. We observed that well-fed *C. elegans* hermaphrodites, when washed free of bacteria and later reintroduced to bacteria, moved more slowly than when transferred to an environment without bacteria. Furthermore, this slowing response to bacteria was enhanced if the animals were not well fed but rather had been deprived of food for only 30 min. We note that in earlier experiments that have studied the effect of food withdrawal on *C. elegans* behavior, a brief 30 min absence from bacteria did not effect a behavioral change (Colbert and Bargmann, 1997; Mori, 1999), suggesting that our paradigm may involve a distinct mechanism of plasticity. We found that the responses of well-fed and food-deprived animals represent two distinct and separable modulatory behaviors and that dopamine and serotonin function in a nonoverlapping manner to mediate the behaviors of well-fed and food-deprived animals, respectively.

Results

Locomotory Rate Decreases in the Presence of Bacteria

We quantified the locomotory rate of wild-type *C. elegans* hermaphrodites moving on an agar surface of a petri plate by counting the number of bends in the anterior body region during a 20 s interval. Animals that had been continuously cultured on the bacterium *Escherichia coli* (well-fed) were washed free of bacteria and then transferred to assay plates containing or lacking a bacterial lawn. Well-fed animals that were transferred to assay plates containing bacteria moved more slowly upon reentering the bacterial lawn than well-fed animals that were transferred to assay plates lacking bacteria ($p < 0.0001$, Student's t test) (Figure 1A, closed bars). We refer to this response of well-fed animals after their reentry into the bacterial lawn as the "basal slowing response."

Food-Deprived Animals Show More Pronounced Slowing in Response to Bacteria

The strength of an animal's slowing response to bacteria depended on the animal's recent experience, in particular on whether the animal had been removed from bacteria 30 min before the behavioral assay (food deprived). We compared the locomotory rate of well-fed animals to that of food-deprived animals on assay plates containing or lacking a bacteria lawn. We found that food-deprived animals showed a more pronounced slowing response to the presence of bacteria than did well-fed animals ($p < 0.0001$, Student's t test) (Figure 1A, shaded bars). We refer to this response of food-deprived animals after their reentry into the bacterial lawn as the "enhanced slowing response."

The enhanced slowing response was a specific modulatory response rather than physiological exhaustion resulting from food deprivation, since food-deprived animals in the absence of bacteria moved as rapidly as well-fed animals in the absence of bacteria (Figure 1A). These results indicate that *C. elegans* can use information about its prior exposure to bacteria to alter its behavior when it reencounters bacteria.

The Basal Slowing Response Requires Dopamine

To explore the mechanisms underlying the basal and enhanced slowing responses, we examined several *C. elegans* mutant strains with defects in neurotransmission. We first examined the behavior of animals bearing mutations in one or more of three genes known to affect biogenic amine synthesis: *cat-2, cat-4,* and *bas-1. cat-2(e1112)* (catecholamine-defective) and *cat-4(e1141)* mutants do not show the formaldehyde-induced fluorescence (FIF) indicative of the neurotransmitter dopamine (Sulston et al., 1975). *cat-4* mutants also are reduced in serotonin expression (Desai et al., 1988; Weinshenker et al., 1995), whereas *cat-2* mutants have normal levels of serotonin (G. Garriga, personal communication; E. R. S. and H. R. H., unpublished data). *cat-2* encodes a tyrosine hydroxylase, an enzyme required for the biosynthesis of dopamine (Lints and Emmons, 1999). *cat-4* encodes a GTP cyclohydrolase I (C. Loer, personal communication), an enzyme required for the synthesis of a cofactor needed for dopamine and serotonin biosynthesis (Kapatos et al., 1999). *bas-1(ad446)* (biogenic amine synthesis–defective) mutants have reduced serotonin levels (Loer and Kenyon, 1993) and also lack the FIF indicative of dopamine (E. R. S. and H. R. H., unpublished data). *bas-1* encodes an aromatic amino acid decarboxylase, an enzyme needed for the biosynthesis of serotonin and dopamine (C. Loer, personal communication). Thus, *cat-4* and *bas-1* mutants appear to be deficient in both dopamine and serotonin, while *cat-2* mutants are deficient in only dopamine.

We found that animals with mutations in any of these three genes were completely defective in the basal slowing response (Figures 1B–1D, closed bars). For example, in the presence of bacteria, the locomotory rate of well-fed *cat-2* mutants (Figure 1B, closed bars) on bacteria was significantly faster ($p < 0.0001$, Student's t test) than that of well-fed wild-type animals that had been assayed in parallel. We generated a *bas-1(ad446); cat-4(e1141)* strain and found that the double mutant was indistinguishable from the single mutants with respect to the defect in the basal slowing response (Figure 1E, closed bars). All of these mutants moved at the same rate as wild-type hermaphrodites in the absence of bacteria (Figures 1A–1E). Hence, the lack of the basal slowing response of these mutants was not a result of a general enhancement of locomotory rate or physical vigor but rather was likely to be a consequence of their common defect in dopamine expression. Moreover, the existence of mutants defective in the basal slowing response argues that the slowing of locomotory rate of well-fed animals on a bacterial lawn is a specific behavioral response to the presence of bacteria rather than a physical inability to move faster on a bacterial lawn.

The Enhanced Slowing Response Requires Serotonin

In addition to a defect in the basal slowing response, animals with mutations in *bas-1* or *cat-4,* but not *cat-2,* were also defective in the enhanced slowing response (compare Figures 1B–1D, shaded bars, with Figure 1A, shaded bars). That *cat-2* mutants displayed a normal

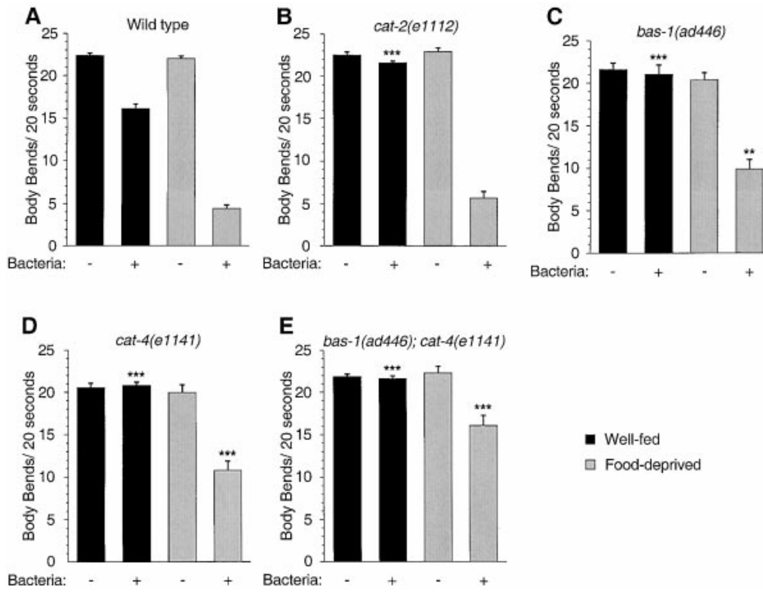

Figure 1. Modulation of Locomotory Rate of Wild-Type Animals and Dopamine- and/or Serotonin-Deficient Mutants

Well-fed and food-deprived animals were transferred to assay plates with or without a bacterial lawn, and 5 min later, the locomotory rate of each animal was recorded. Food-deprived animals were transferred to plates without bacteria 30 min prior to the transfer to locomotory assay plates (see Experimental Procedures for details). In this and all subsequent figures, unless otherwise stated, each trial involved testing at least five animals in each of the conditions; a given animal was tested in only one of the conditions.

(A) The wild-type basal (closed bars) and enhanced (shaded bars) slowing responses. Eleven trials with wild-type animals. See Results for p values.

(B–E) Mutants defective in the basal and/or enhanced slowing responses. The p values were calculated by comparing the combined data for the mutants from all of the separate trials with the combined data for the wild-type animals assayed in parallel in each condition of each separate trial. Wild-type data from these parallel trials are not shown, since in no case were these data significantly different from the data in (A).

(B) Ten trials with *cat-2(e1112)* mutants.

(C) Four trials with *bas-1(ad446)* mutants.

(D) Five trials with *cat-4(e1141)* mutants.

(E) Six trials with *bas-1(ad446); cat-4(e1141)* mutants.

Error bars represent the SEM; double asterisk: $p = 0.0002$, triple asterisk: $p < 0.0001$, Student's t test.

enhanced slowing response suggested that dopamine was not needed for this behavior and that the loss of serotonin biosynthesis in the *bas-1* and *cat-4* mutants was responsible for the defect in the enhanced slowing response. These results also suggested that this experience-dependent modulatory behavior was not just a stronger form of the basal slowing response. In support of this hypothesis, other treatments that affected the enhanced slowing response did not affect the basal slowing response (see below).

bas-1(ad446); cat-4(e1141) mutants were more defective in their enhanced slowing response (Figure 1E, shaded bars) than was either of the single mutants (Figures 1C and 1D, shaded bars). A plausible explanation for the increased defect is that the double mutants are more defective in serotonin biosynthesis than is either single mutant. It is unlikely, however, that the double mutants completely lack serotonin, since they do not have the developmental defects seen in animals with a large deletion in the *tph-1* gene, which encodes a tryptophan hydroxylase, an enzyme essential for serotonin biosynthesis (Sze et al., 2000). Alternatively, it is conceivable that *bas-1* and *cat-4* are both involved in the biosynthesis of a signaling molecule other than dopamine or serotonin and that the increased loss of that molecule led to the increased behavioral defect in the double mutant. We consider this second possibility unlikely, since we could restore wild-type basal and enhanced slowing responses to the double mutant by preincubating the animals in dopamine or serotonin, respectively (see below).

Exogenous Dopamine Restores the Basal Slowing Response of *cat-2* and *bas-1; cat-4* Mutants

We tested the ability of exogenous dopamine to restore the basal slowing response to *cat-2(e1112)* and *bas-1(ad446); cat -4(e1141)* mutants. Prior to the behavioral assay, we incubated wild-type, *cat-2(e1112),* and *bas-1(ad446); cat-4(e1141)* animals on bacteria-containing plates with 2 mM dopamine and then examined the basal slowing response of these animals. Preincubation on plates containing dopamine caused *cat-2* and *bas-1; cat-4* mutants to exhibit a nearly normal basal slowing response (Figure 2). For example, control *cat-2* mutants pretreated without dopamine had a significantly higher locomotory rate (Figure 2A, asterisks) than did control wild-type animals pretreated without dopamine, whereas *cat-2* mutants pretreated with dopamine did not have a significantly different locomotory rate from wild-type animals pretreated with dopamine (denoted by an "R" for rescue; see Experimental Procedures for rescue criteria). *C. elegans* locomotion can be inhibited by high concentrations of dopamine (Schafer and Kenyon, 1995). Therefore, the concentration of dopamine used in this experiment was titrated so that it did not affect the locomotory rate of well-fed wild-type animals either in the absence or presence of bacteria (Figure 2), thereby allowing us to conclude that the rescue by dopamine in this assay was not the result of a general dopamine-induced locomotory slowing that bypassed the modulatory response to the stimulus of bacteria. Also, preincubation on dopamine-containing plates did not decrease the locomotory rate of either mutant class in the absence of bacteria (Figures 2A and 2B); thus, it is unlikely that the slowing of the dopamine-treated *cat-2* and *bas-1; cat-4* mutants in response to bacteria resulted from a hypersensitivity of the mutants to dopamine. Pretreatment with exogenous serotonin did not rescue the defect in the basal slowing response of well-fed *cat-2* or *bas-1; cat-4* mutants (Figure 2), suggesting that the effect of dopamine was specific and that exogenous serotonin cannot bypass the need for dopamine in the basal slowing response.

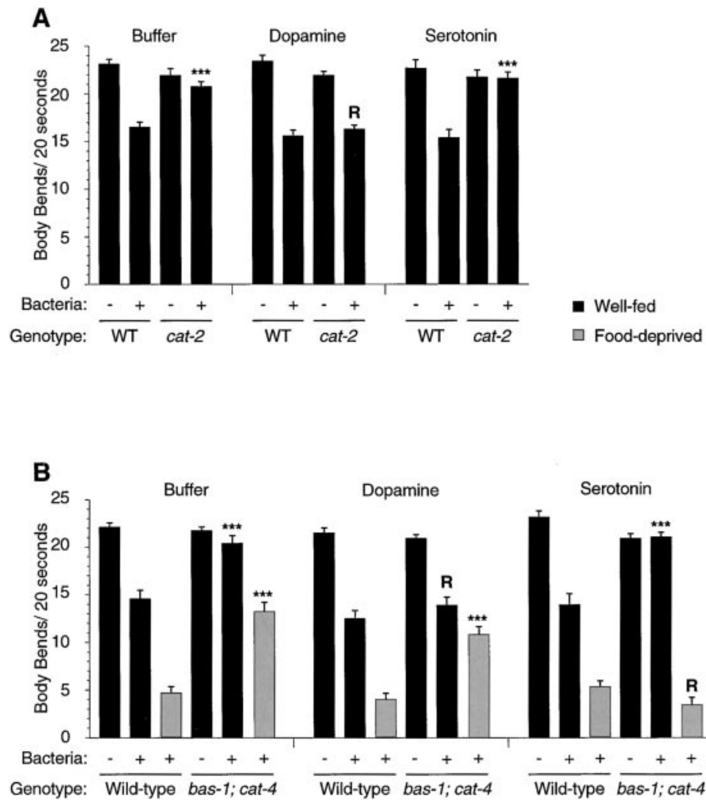

Figure 2. Dopamine Pretreatment Rescues the Defect in the Basal Slowing Response of Dopamine-Deficient Mutants, and Serotonin Pretreatment Rescues the Defect in the Enhanced Slowing Response of Serotonin-Deficient Mutants

Following preincubation on bacteria-containing plates with no neurotransmitter (Buffer), or with dopamine or serotonin, animals were transferred directly to assay plates, and locomotory rate was measured (closed bars, [A and B]), or to food deprivation plates with buffer, dopamine, or serotonin for 30 min and then to assay plates, and locomotory rate was measured (shaded bars, [B]). Abbreviation: R, rescued (see Experimental Procedures for details of rescue criteria).

(A) Rescue of the defect in the basal slowing response of *cat-2* mutants (four trials). Abbreviation: WT, wild type.

(B) Rescue of the defect in the basal and enhanced slowing responses of *bas-1; cat-4* mutants (four trials).

Error bars represent the SEM; triple asterisk: $p < 0.0001$, Student's t test.

The CEP, ADE, and PDE Dopaminergic Neurons Sense Mechanosensory Stimuli Required for the Basal Slowing Response

The *C. elegans* hermaphrodite has eight dopaminergic neurons: the four CEPs, the two ADEs, and the two PDEs (Sulston et al., 1975). Ultrastructurally, the eight dopaminergic neurons have ciliated endings embedded in the cuticle, indicating likely mechanosensory functions (Ward, 1973; Perkins et al., 1986; White et al., 1986). The CEP sensory processes extend to the tip of the nose, the ADE sensory endings are located laterally in the anterior third of the body, and the PDE sensory endings are located laterally in the posterior body region.

To test if these dopaminergic sensory neurons were required for the basal slowing response, we ablated combinations of dopaminergic neurons in young larvae and examined the locomotory behavior of the resulting adults. Among the single cell-type ablations, only the ablation of the four CEPs resulted in a detectable defect in the basal slowing response (Figure 3A), and this defect was only modest. Among the double cell-type ablations, animals lacking the four CEPs and the two PDEs moved slightly faster than did mock ablated controls (Figure 3B). Animals lacking the four CEPs and the two ADEs did not show a defect in the basal slowing response; perhaps a larger number of experiments are necessary to obtain a statistically significant difference; alternatively, the lack of a defect in these animals could have a biological basis. Ablating all three classes of dopaminergic neurons resulted in animals that were completely defective in the basal slowing response (Figure 3B). This result implied that these three classes of dopaminergic neurons function redundantly to mediate the basal slowing response.

If these dopaminergic neurons were directly involved in sensing the presence of bacteria, only those neurons with sensory endings in contact with bacteria might be required for the basal slowing response. Since *C. elegans* moves through bacteria lying on either its left or its right side (White et al., 1986), we were able to test this hypothesis by ablating only the dopaminergic neurons having sensory endings on one side of the animal's body. Since the sensory endings of the CEP neurons are located in the tip of the animal's nose, the entire circumference of which is in contact with bacteria, we ablated all four CEP neurons. We found that the ADE and PDE neurons were required only on the side of the animal's body in contact with bacteria (Figure 3C). When animals lay on the unoperated side, they responded normally to bacteria, but when these same animals lay on the opposite, operated side, they were defective in the basal slowing response.

To explore the nature of the stimulus detected by the dopaminergic neurons, we tested if substances other than bacteria could elicit the basal slowing response. We found that wild-type hermaphrodites slowed their rate of locomotion as they crawled through a three-dimensional matrix of sterile Sephadex G-200 beads (Figure 4A). G-200 Sephadex beads (20–50 μm) are much larger than bacteria are and cannot be ingested by *C. elegans*. Therefore, any stimulus presented by these beads had to be sensed on the exterior of the animal. We found that *cat-2(e1112)* dopamine-deficient mutants did not exhibit the basal slowing response to the Sephadex beads (Figure 4A). Similarly, animals in which all of the dopaminergic neurons had been ablated by laser ablation also did not exhibit the basal slowing response to Sephadex beads (Figure 4B).

These data argue that the slowing response to a nonbacterial stimulus was also mediated by dopaminergic

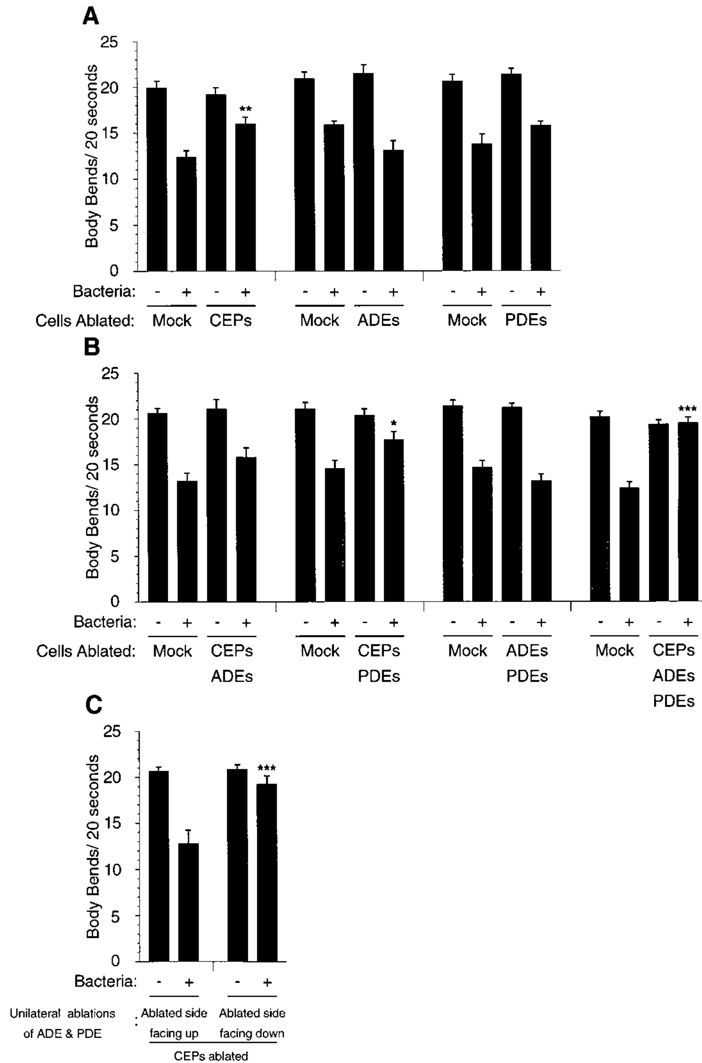

Figure 3. Dopaminergic Neurons Function Redundantly to Sense Mechanosensory Stimuli

(A and B) Basal slowing response of animals with dopaminergic neurons ablated. The dopaminergic neurons indicated were ablated in larvae 2 days before the behavioral experiment. In parallel, an equivalent number of mock ablated animals were also tested. A given animal was tested first in the presence of bacteria and then in the absence of bacteria (see Experimental Procedures for details).

(A) Twenty-three CEP-ablated, 14 ADE-ablated, and 13 PDE-ablated animals were assayed.

(B) Fifteen CEP-ablated, ADE-ablated; 21 CEP-ablated, PDE-ablated; 20 ADE-ablated, PDE-ablated; and 17 CEP-ablated, ADE-ablated, PDE-ablated animals were assayed.

(C) Only dopaminergic neurons in contact with bacteria were required for the basal slowing response. The locomotory rates of five animals in which CEPDL/R, CEPVL/R, ADEL, and PDEL had been ablated and six animals in which CEPDL/R, CEPVL/R, ADER, and PDER had been ablated were recorded in the presence and absence of bacteria, with animals lying first on one side and then on the other (the order in which the sides were tested was random; see Experimental Procedures for details).

Error bars represent the SEM; asterisk: $p = 0.01$, double asterisk: $p = 0.0009$, triple asterisk: $p < 0.0001$, Student's t test.

neurotransmission. Since we added no bacteria in these experiments (and, thus, there were presumably no chemosensory cues associated with bacteria), these results, taken together with the fact that the dopaminergic neurons are likely to be mechanosensory, suggested that dopaminergic neurotransmission is required to respond to a mechanical attribute of bacteria to elicit the neuromodulatory basal slowing response.

Exogenous Serotonin Restores the Enhanced Slowing Response of *bas-1; cat-4* Mutants

As discussed above, the enhanced slowing response appears to be independent of the dopamine-mediated basal slowing response and requires serotonin. We tested the ability of 2 mM exogenous serotonin to restore the enhanced slowing response to *bas-1; cat-4* mutants. Preincubation on serotonin-containing plates caused *bas-1; cat-4* mutants to exhibit a normal enhanced slowing response (Figure 2B). The concentration of serotonin used in this experiment was titrated so that it did not affect the locomotory rate of wild-type animals (Figure 2) or of *bas-1; cat-4* mutants when well fed (Figure 2B), or of food-deprived *bas-1; cat-4* mutants in the absence of bacteria (data not shown), thereby allowing us to conclude that the slowing caused by serotonin in this assay is a restoration of the enhanced slowing response and not the result of a general serotonin-induced locomotory slowing that bypassed the modulatory response to the stimulus of bacteria. Exogenous dopamine did not rescue the defect in the enhanced slowing response of *bas-1; cat-4* mutants (Figure 2B) but did rescue the basal slowing response of *bas-1; cat-4* mutants (Figure 2B), indicating that the serotonin rescue of the defect in the enhanced slowing response is specific. Thus, endogenous dopamine was not required for the enhanced slowing response (*cat-2* mutants; Figure 1B, shaded bars), and exogenous dopamine could not bypass the need for serotonin in the enhanced slowing response.

The Serotonergic NSMs Are Required for a Full Enhanced Slowing Response

A number of serotonin-immunoreactive neurons have been reported to be present in the *C. elegans* hermaphrodite; the most reproducibly staining neurons are the bilaterally symmetric pairs of NSMs (Horvitz et al., 1982), HSNs (Desai et al., 1988), ADFs, PHBs, AIMs, and the single I5 and RIH neurons (E. R. S. and H. R. H., unpub-

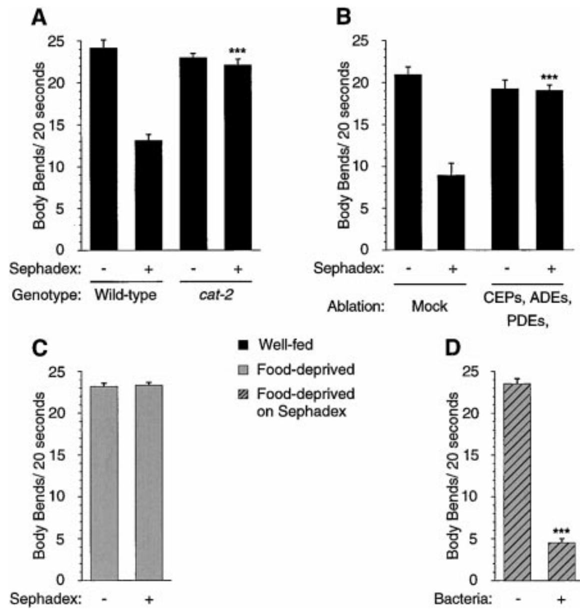

Figure 4. The Basal Slowing Response Involves a Mechanosensory Stimulus, While the Enhanced Slowing Response Does Not

(A) Response of wild-type and *cat-2* animals to Sephadex beads; 35 wild-type and 35 *cat-2* animals were assayed in three trials.

(B) Animals with dopaminergic neurons ablated do not respond to Sephadex beads. Five animals in which the CEPs, ADEs, and PDEs had been ablated and five mock ablated animals were tested in each of the two conditions. A given animal was tested first in the presence of Sephadex and then in the absence of Sephadex (see Experimental Procedures for details).

(C) Food-deprived wild-type animals do not slow in response to Sephadex beads; 20 animals were tested in each condition in four trials.

(D) Animals food deprived in the presence of Sephadex beads exhibit a normal enhanced slowing response; 20 animals were tested in each condition in four trials.

Error bars represent the SEM; triple asterisk: $p < 0.0001$, Student's t test.

lished data). Expression of a green fluorescent protein (GFP) reporter of the *tph-1* gene is also observed in the NSMs, HSNs, ADFs, and AIMs, and in the RIH (Sze et al., 2000).

To test if these serotonergic sensory neurons were required for the enhanced slowing response, we ablated combinations of serotonergic neurons in young larvae and examined the locomotory behavior of the resulting adults. After ablating the NSMs using laser microsurgery, we found that well-fed animals lacking the NSMs ($n = 45$) exhibited a normal basal slowing response (a change from 21.9 ± 0.4 body bends/20 s [error represents the SEM] on plates with no bacteria to 14.8 ± 0.5 body bends on plates with bacteria compared with mock ablated controls [$n = 45$], which changed from 21.3 ± 0.5 body bends to 14.6 ± 0.6 body bends in the respective conditions). However, on plates with bacteria, food-deprived animals lacking the NSMs ($n = 45$) moved slightly but significantly faster (7.3 ± 0.6 body bends) than did mock ablated controls (5.1 ± 0.4 body bends, $n = 45$, $p = 0.002$, Student's t test), suggesting that the NSMs contribute to the enhanced slowing response. That the ablation of the NSMs did not cause a defect in the enhanced slowing response as strong as the one seen in *bas-1; cat-4* mutants (Figure 1E, shaded bars) suggested that other cells, possibly including other serotonergic neurons, also contribute to the response.

We performed several double, triple, quadruple, quintuple, sextuple, and septuple ablations (all ablations included both members of the bilaterally symmetric classes), all of which included the ablation of the NSMs. In none of these multiple cell-type ablation experiments did we see a statistically significant defect in the enhanced slowing response beyond the defect seen in the NSM-ablated animals (data not shown).

It is possible that the NSMs are the only serotonergic neurons involved in the enhanced slowing response. For example, the NSMs might have continued to function even though their nuclei had been ablated; such residual function has been observed for the M4 neuron after its ablation in older animals (Avery and Horvitz, 1987). However, we consider this possibility unlikely, since we have found that the ablation of the NSMs leads to a complete loss of the potentiation of the enhanced slowing response by fluoxetine (see below). It is also conceivable that the NSMs provide both positive and negative inputs for the enhanced slowing response and that the ablation of the cell removes both classes of inputs, leading to a weaker behavioral defect than would be seen if only one or the other class of input was individually perturbed. The VC4 and VC5 neurons may also contain serotonin, based on extremely weak immunoreactivity to anti-serotonin antibodies (G. Garriga, personal communication). We have observed such weak immunoreactivity in these neurons only very rarely (E. R. S. and H. R. H., unpublished data). Since tryptophan hydroxylase GFP reporter expression is not observed in the VC4 and VC5 neurons (Sze et al., 2000), it is possible that the variable and low level of serotonin immunoreactivity seen in these neurons is the result of these neurons taking up serotonin released by other serotonergic neurons (e.g., the HSNs, which are nearby). Nevertheless, it remains conceivable that the VC4 and VC5 neurons or other serotonergic cells not visualized by available anti-serotonin antibodies might function in the enhanced slowing response.

The Enhanced Slowing Response Is Not Triggered by Mechanosensory Stimuli that Trigger the Basal Slowing Response

We found that food-deprived wild-type hermaphrodites did not slow their locomotion rate as they crawled through a three-dimensional matrix of sterile Sephadex G-200 beads (Figure 4C). These data argue that in contrast to the basal slowing response, which is likely to be triggered by a mechanosensory stimulus to the surface of the animal, the enhanced slowing response appears not to be. Moreover, animals attained the food-deprived state even in the presence of Sephadex beads, since animals that were food deprived in a three-dimensional matrix of Sephadex beads and then reintroduced to bacteria still exhibited the normal enhanced slowing response (Figure 4D). These observations demonstrate that the physical stimulus provided by Sephadex beads neither mimics the cues that food-deprived animals normally receive when reintroduced to bacteria nor prevents animals from attaining the food-deprived state.

Serotonin Antagonists Blocked the Enhanced Slowing Response

To test further the serotonin dependence of the enhanced slowing response, we preincubated wild-type

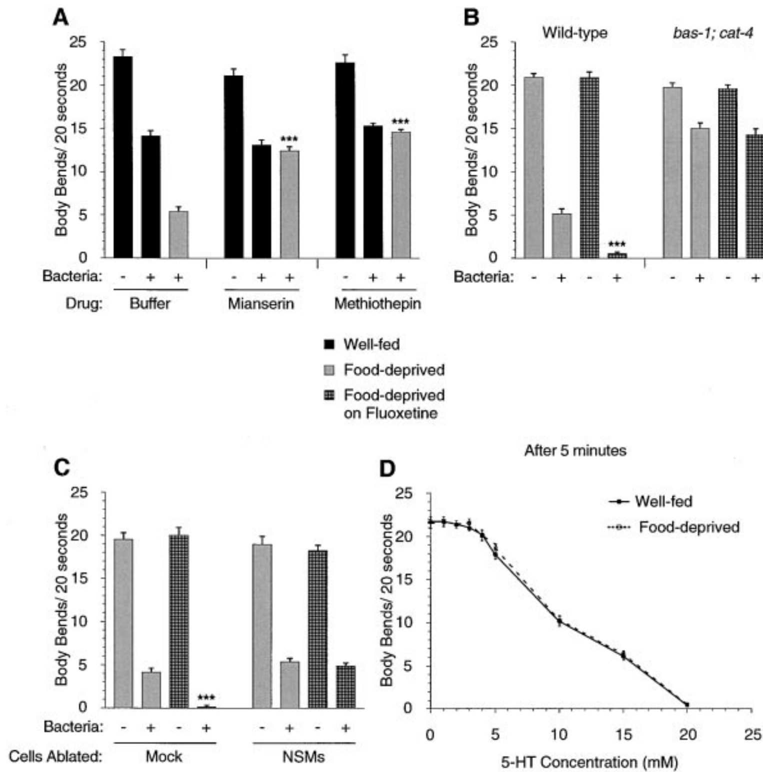

Figure 5. The Enhanced Slowing Response Involves Serotonin, Is Potentiated by Fluoxetine, and Is Not a Result of Serotonin Hypersensitivity

(A) Serotonin antagonists disrupted the enhanced slowing response. Mianserin hydrochloride was at 20 μM, and methiothepin mesylate was at 44 μM (five trials).

(B) The potentiation of the enhanced slowing response by fluoxetine requires endogenous serotonin; 20 wild-type and 20 *bas-1; cat-4* animals were tested in each condition in four trials.

(C) The potentiation of the enhanced slowing response by fluoxetine acts through the NSMs. Locomotory rates of seven mock ablated and eight NSM-ablated animals were assayed in each of the four conditions (see Experimental Procedures for order of conditions tested). The effect of the NSM ablations on the enhanced slowing response in the absence of fluoxetine was statistically significant ($p = 0.044$). The p values denoted by the asterisks in Figures 5B and 5C were calculated by comparing the fluoxetine-treated animals with untreated animals of the same genotype or ablation state.

(D) Dose–response of serotonin sensitivity; 20 animals were tested at each concentration over 20 trials (1 animal per condition per trial). Well-fed or food-deprived animals were transferred to plates (no bacteria) with the indicated concentration of serotonin, and locomotory rate after 5 min was plotted.

Error bars represent the SEM; triple asterisk: $p < 0.0001$, Student's t test.

animals on petri plates with two serotonin receptor antagonists, mianserin hydrochloride (Glennon, 1987) and methiothepin mesylate (Mylecharane, 1989), and tested these animals for the basal and enhanced slowing responses. While the specificities of these drugs on *C. elegans* behavior have not been established, we found that both blocked the inhibition of locomotion induced by treatment with high concentrations of exogenous serotonin (data not shown), suggesting that these drugs interfere with serotonergic neurotransmission in *C. elegans.* Neither mianserin (20 μM) nor methiothepin (44 μM) had any effect on the basal slowing response (Figure 5A, closed bars). However, food-deprived animals pretreated with either drug were defective in the enhanced slowing response (Figure 5A, shaded bars), providing further support for the role of serotonin in this modulatory behavior.

Fluoxetine Potentiates the Enhanced Slowing Response

We examined the effects of low concentrations of the selective serotonin reuptake inhibitor fluoxetine (Prozac) on the locomotory rate of food-deprived animals. On plates without bacteria, there was no difference between the locomotory rates of animals that been food deprived in the presence or absence of fluoxetine (Figure 5B). Since fluoxetine is a serotonin reuptake blocker that most likely potentiates the concentration of serotonin at synapses, this observation suggested that in food-deprived animals, in the absence of bacteria, no serotonin is released, and hence no potentiation by fluoxetine occurs. By contrast, on plates with bacteria, wild-type animals that had been food deprived in the presence of fluoxetine moved much more slowly than did wild-type animals that had been food deprived in the absence of fluoxetine (Figure 5B). This observation suggests that fluoxetine amplifies the signaling process triggered when bacteria are encountered by food-deprived animals. We propose that bacteria trigger the release of serotonin, and fluoxetine amplifies the effect of the released serotonin by blocking its reuptake. Furthermore, we postulate that fluoxetine affects the levels of only serotonin to potentiate the enhanced slowing response, since on plates containing bacteria, *bas-1; cat-4* mutants (which lack serotonin and dopamine) that had been food deprived in the presence of fluoxetine had the same locomotory rate as did *bas-1; cat-4* mutants that had been food deprived in the absence of fluoxetine (Figure 5B), while *cat-2* mutants (which lack dopamine but not serotonin) were no different from wild-type animals in this experiment (data not shown).

The NSM-ablated animals were resistant to the effects of fluoxetine. On plates containing bacteria, NSM-ablated animals that had been food deprived in the presence of fluoxetine did not move any more slowly than did NSM-ablated animals food deprived in the absence of fluoxetine (Figure 5C). These data suggest that the potentiation of the enhanced slowing response by fluoxetine depends on the NSMs and that it was serotonin released by the NSMs that was specifically potentiated by fluoxetine. This result also indicates that if there is another, as yet unidentified, source of serotonin that modulates this behavior, the serotonin signaling from that source is not potentiated by the fluoxetine treatments used in these experiments.

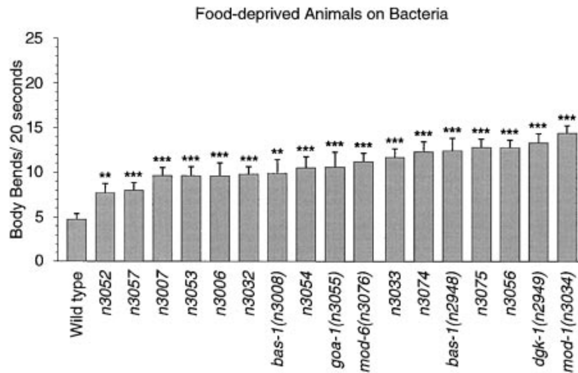

Figure 6. Mutants Defective in the Enhanced Slowing Response

At least 20 animals were tested for each genotype in at least four trials. The p values were calculated by comparing the combined data for the mutants from all of the separate trials with the combined data for the wild-type animals assayed in parallel in each condition of each separate trial. Wild-type data from these parallel trials are not shown, since in no case were these data significantly different from the representative data for the wild type shown in the first column. Error bars represent the SEM; double asterisk: $0.001 < p < 0.05$, triple asterisk: $p < 0.0001$, Student's t test.

Food Deprivation Does Not Lead to Serotonin Hypersensitivity

To determine whether the treatment of food deprivation altered the sensitivity of the animals to serotonin, we compared the responsiveness of well-fed and food-deprived animals to exogenous serotonin. High concentrations of exogenous serotonin decrease the locomotory rate of well-fed *C. elegans* (Horvitz et al., 1982). We found that food-deprived worms were not hypersensitive to exogenous serotonin, since the locomotory rate of well-fed and food-deprived animals was inhibited by serotonin to the same extent over a range of concentrations (Figure 5D). This assay for serotonin sensitivity was done in the absence of food, since the presence of food would have led to the enhanced slowing response by food-deprived animals. The threshold concentration of serotonin required to affect well-fed and food-deprived animals was also very similar (Figure 5D). In addition, the well-fed and food-deprived animals did not show any significant difference in the kinetics of the response to any of the concentrations used in Figure 5D (data not shown). These results suggest that the increased responsiveness of food-deprived animals to bacteria is not a result of hypersensitivity to a food-induced serotonin signal.

Isolation of Modulation-Defective Mutants

We performed a genetic screen for mutants with defects in the enhanced slowing response. From a screen of ~16,000 haploid genomes, we isolated 17 mutant strains that when food deprived and reintroduced to bacteria exhibit an abnormally fast locomotory rate (Figure 6). Our complementation studies indicated that 4 of these strains contain mutations in the known genes, *bas-1*, *goa-1* (G protein O, alpha subunit), and *dgk-1* (diacylglycerol kinase). *goa-1* and *dgk-1* have both been implicated by previous studies to be components of a serotonin signaling pathway (Mendel et al., 1995; Segalat et al., 1995; Nurrish et al., 1999). That we isolated alleles of these three genes in this genetic screen provides further evidence for the need for serotonin signaling in the enhanced slowing response.

All 17 mutant strains display the same locomotory rate as do wild type on plates without bacteria, whether they were well fed or food deprived (data not shown). *bas-1(n2948)* and *bas-1(n3008)* mutants also showed a strong defect in the basal slowing response (data not shown), as expected given our characterization of the *bas-1(ad446)* mutant (Figure 1C). Of the remaining 15 strains, 3—*n3007*, *n3033*, and *goa-1(n3055)*—also have weak defects in the basal slowing response (data not shown). We assayed *goa-1(n363)* null mutants (Segalat et al., 1995) and found that they have a more severe defect in both the basal and enhanced slowing responses than does our new *goa-1(n3055)* mutant (data not shown). This defect in the basal slowing response of the *goa-1* mutants suggests that the G protein encoded by *goa-1* might be involved not only in a serotonergic signaling pathway but also in a dopaminergic pathway.

Thus far, we have genetically mapped two of the mutations (*n3034* and *n3076*) to small regions on chromosomes V and I, respectively (data not shown). These genetic positions are distinct from the location of all genes implicated to date in the basal and enhanced slowing responses (data not shown). These mutations define two new genes, *mod-1* and *mod-6* (modulation of locomotion defective).

Discussion

Food is a powerful stimulus that affects many *C. elegans* behaviors, including pharyngeal pumping (Avery and Horvitz, 1990), defecation (Liu and Thomas, 1994), and egg laying (Horvitz et al., 1982; Trent et al., 1983; Weinshenker et al., 1995). A prolonged withdrawal from food can affect *C. elegans* behavior (Colbert and Bargmann, 1997; Mori, 1999). We report that a brief 30 min period of food deprivation, a period insufficient to affect the other behaviors that have been studied (Colbert and Bargmann, 1997; Mori, 1999; E. R. S. et al., unpublished data), elicits a robust change in behavior, namely the response to the presentation of the stimulus of food. In other words, 30 min of food deprivation can cause *C. elegans* to exist in an alternative behavioral state that allows it to respond appropriately to changes in the availability of food by modulating its locomotory rate. A dopamine-dependent process acts to reduce the locomotory rate of well-fed animals in response to a mechanosensory stimulus from bacteria. A serotonin-dependent process causes food-deprived animals to respond with an enhanced slowing of locomotory rate upon being transferred to bacteria. Both of these modulatory behaviors seem likely to be of adaptive significance (see below).

The ability of *C. elegans* to respond with differing intensities to the same environmental stimulus, depending on the animal's state, is similar to examples of behavioral plasticity seen in many animals. In the leech, a prolonged period without feeding results in an increase in the frequency of swimming and biting behaviors (O'Gara et al., 1991). Food-deprived leeches also exhibit other behavioral responses that are distinct from the responses of satiated leeches (Groome et al., 1993; Brodfuehrer et al., 1995). Food-induced arousal in *Aplysia* leads to an increase in the speed and strength

of biting responses (Rosen et al., 1989). Simliarly, a mammal's internal state can modulate the animal's responsiveness to various environmental stimuli (Kupfermann and Schwartz, 1995).

We have shown that our paradigm for behavioral plasticity in *C. elegans* involves dopamine and serotonin and is affected by fluoxetine. We propose that this experience-dependent modulatory behavior of *C. elegans* provides a simple model for the control of behavioral state and the related phenomena of motivation and mood.

The Basal Slowing Response Involves a Mechanosensory Stimulus Mediated by Dopamine

Recently, using the assay for locomotory rate we developed (Sawin, 1996) and used in the studies described in this paper, Duerr et al. (1999) observed that *cat-1* mutants, which are defective in loading presynaptic vesicles with monoamines, do not show the basal slowing response. This observation is consistent with our finding that dopamine-deficient mutants are defective in the basal slowing response. The apparent redundant function of the different dopaminergic neurons in the basal slowing response established by our laser ablation experiments is similar to redundancies seen in other sensory systems in *C. elegans*. For example, three classes of mechanosensory neurons function together to sense touch to the nose (Kaplan and Horvitz, 1993), and four partially redundant cell types are required for direct chemotaxis to many attractants (Bargmann and Horvitz, 1991).

Since the dopaminergic neurons involved in the basal slowing response appear to sense a mechanical attribute of both bacteria and the Sephadex matrix (perhaps surface tension or pressure exerted on the cuticle), we suggest that in the basal slowing response, the dopaminergic neurons transduce a mechanosensory stimulus through interneurons and motor neurons to effect a slowing of locomotory rate (Figure 7A).

C. elegans is capable of discerning several attractive chemical cues in its search for food (Colbert and Bargmann, 1997). Why then might *C. elegans* sense bacteria mechanically? Perhaps while the chemosensory system is used to find new distant bacterial food sources, the mechanosensory system we have identified allows the animal to remain in the vicinity of the food source once that source has been reached.

The Enhanced Slowing Response Is Modulated by Serotonin and Is Distinct from the Basal Slowing Response

The process that allows food-deprived *C. elegans* to respond more strongly when transferred to bacteria is distinct from the process that well-fed *C. elegans* uses to respond to bacteria. The results of our rescue, ablation, and antagonist experiments strongly indicate that the experience-dependent enhanced slowing response is not simply a stronger form of the basal slowing response but rather is completely distinct. We therefore expect that complete loss of the enhanced slowing response would result in food-deprived animals having the same locomotory rate on plates with bacteria as they do on plates without bacteria. For this reason, we conclude that none of the mutants tested or the treatments performed completely abolished the enhanced slowing response.

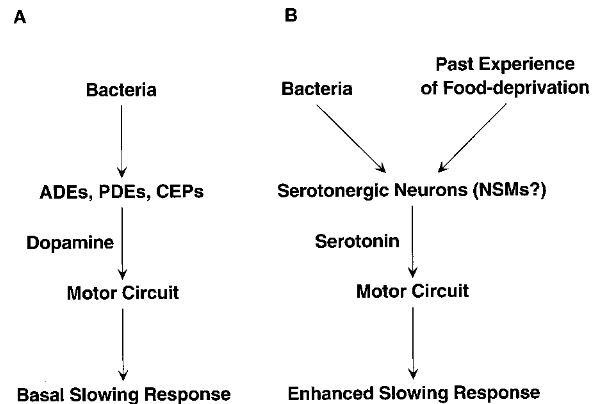

Figure 7. Models for the Basal and Enhanced Slowing Responses

(A) In well-fed animals, the three classes of dopaminergic neurons (CEPs, ADEs, and PDEs) function redundantly to sense a mechanosensory stimulus from bacteria and then modulate the motor circuit to effect the basal slowing response.

(B) In food-deprived animals, serotonergic neurons sense the current level of food in the pharynx and integrate this information with information about the past experience of food deprivation. The serotonergic neurons then release an increased amount of serotonin, which inhibits the motor circuit to a greater extent than in the basal slowing response to effect the enhanced slowing response (see Discussion for details).

If, as discussed above, serotonergic cells other than the NSMs are required for the enhanced slowing response, then the complete lack of potentiation of the enhanced slowing response by fluoxetine in NSM-ablated animals suggests that fluoxetine acts only on the NSMs and not on any of the other serotonergic cells involved. Given that the unique ultrastructure of the NSMs suggests that they might be neurosecretory (Albertson and Thomson, 1975), it is possible that fluoxetine acts on only neurosecretory cells in *C. elegans*. Alternatively, a nonserotonergic modulatory pathway insensitive to fluoxetine may act in parallel to the serotonergic pathway to transduce the signal(s) required for the enhanced slowing response.

The Food Deprivation Signal Is Likely to Act Upstream of the Serotonin-Responsive Circuit

We sought to determine whether information about food deprivation was stored in cells pre- or postsynaptic to the serotonergic synapses in the animal. If serotonin were released as an indicator of the presence of bacteria, then food deprivation could result in diminished levels of serotonin release and perhaps a consequent sensitization of serotonin receptors by pathways well established for G protein–coupled receptors (Lefkowitz, 1993). If so, when animals are exposed to bacteria after food deprivation, release of serotonin would resume, and because serotonin receptors had become sensitized, the same amount of released serotonin would have a greater behavioral effect. We do not believe that such a mechanism is responsible for the enhanced slowing response, since food-deprived and well-fed animals had similar sensitivities to exogenous serotonin. Rather, these findings suggest that the enhanced slowing response is regulated by a mechanism that does not depend either on altering the number of serotonin receptors on the cells that directly respond to serotonin or

on enhancing the sensitivity of the serotonin receptors or of any of the downstream components of the relevant signaling pathway(s).

One alternate model is that serotonin is released as an indicator of the absence of bacteria, i.e., during the 30 min period of food deprivation. Without such serotonin release, the enhanced slowing response could not occur. However, we observed that food-deprived animals did not move more slowly than well-fed animals did on plates without bacteria and that the locomotory rate of food-deprived animals on plates without bacteria was unaffected by the fluoxetine treatment during food deprivation. Given these observations, such a model would necessitate proposing that the reintroduction to food provides a permissive signal that allows the increased levels of serotonin in the synaptic cleft to effect a greater slowing of locomotory rate.

Instead, we favor a simpler model. We propose that the effect of food deprivation is to increase the amount of serotonin released when food is reencountered. Such an increase in serotonin release could be achieved by several possible molecular mechanisms, including an increase in the number of presynaptic vesicles that fuse to release serotonin or a faster fusion and exocytosis process of the normal number of presynaptic vesicles.

It is not obvious how the food-deprived state of an animal might lead to an increase of serotonin release from serotonergic neurons when bacteria are encountered. The information about food deprivation could be stored in the serotonergic neurons themselves, through several possible molecular mechanisms, including transcriptional, translational, or posttranslation changes of specific genes and proteins. One possible manifestation of such changes could be a depolarization in the resting membrane potential of the serotonergic neurons, such that the neurons are primed to release serotonin when the next bacterial stimulus is encountered. Alternatively, the food deprivation state could be stored anywhere in the nervous system upstream of or parallel to the serotonergic cells. Another possibility is that some nonneuronal physiological state, such as the extent to which the pharynx or the intestine is filled, is changed by food deprivation and that such a change is detected and transduced by the nervous system. The serotonergic NSMs are plausible candidates to sense the presence of food, since these neurons have apparent mechanosensory endings (Albertson and Thomson, 1975) that could sense either bacteria in the pharynx or pharyngeal movements that occur during feeding.

In a simple model for the serotonergic regulation of the enhanced slowing response, serotonergic neurons (perhaps only the NSMs) might both detect the presence of food and store information about prior food deprivation; these two inputs would then be integrated within these cells to regulate neural activity (Figure 7B). Alternatively, a serotonergic circuit, which may or may not include the NSMs, might mediate either the stimulus of bacteria or the food deprivation signal, while another circuit responds to the other input, and the two inputs are integrated downstream of the serotonergic neurons. As discussed above, another modulatory pathway may act in parallel to the serotonergic pathway, and such redundancy could account for our failure to identify any pharmacological treatment, laser ablation protocol, or mutation that completely abolished the enhanced slowing response. Such branched pathways are conceivable but are not the simplest interpretation of the data.

Adaptive Significance of the Basal and Enhanced Slowing Responses

Both the basal and the enhanced slowing responses could well be important for the survival of *C. elegans* in the wild. *C. elegans* eats bacteria, which in turn feed on decaying organic material in the soil, and the *C. elegans* population increases until the food source is exhausted, at which point the population disperses (Nicholas, 1984). As discussed above, the basal slowing response could provide a mechanism to increase the likelihood that an animal will remain in the presence of a proximal bacterial food source. The enhanced slowing response would increase the certainty that an animal that had briefly wandered from or exhausted its food supply would, upon locating a fresh food supply, remain in the vicinity of that food source. In other words, the basal slowing response ensures that a well-fed animal will remain in the presence of food and continue feeding, while the enhanced slowing response ensures that an animal away from a food source for 30 min, for which food is likely to be even more important, stops when it encounters a new food source. Perhaps in its natural habitat, a food-deprived animal is unwilling to forage further afield in search of a better or larger food source, or some alternative joy, since it risks going too far from a recently discovered and vital food source, whereas a well-fed animal may be willing to chance an exploration of its environment.

Our finding that *C. elegans* can modulate the rate of an ongoing motor program based on environmental conditions and its recent experience highlights the behavioral flexibility that is possible even with a nervous system comprising only 302 neurons of 118 distinct types (White et al., 1986). We have readily isolated a collection of mutations with defects in the enhanced slowing response and identified new genes involved in this paradigm of behavioral plasticity. We believe that the further dissection of this modulatory behavior at the level of defined cells, neurotransmitters, and genes will lead to an understanding of the complex mechanisms an animal uses to alter its behavioral output based on its prior experience.

Experimental Procedures

Strains and Strain Constructions

Nematodes were grown in noncrowded conditions at 20°C as described by Brenner (1974), except that *E. coli* strain HB101 instead of OP50 was used as the food source, since thin bacterial lawns of uniform thickness were more reliably generated with overnight growth of HB101 (data not shown); such uniformity was critical for reproducible results in the locomotory rate assays. Wild-type animals were *C. elegans* strain N2. Mutant strains used were CB1112 *cat-2(e1112)* II, CB1141 *cat-4(e1141)* V (Sulston et al., 1975), MT7988 *bas-1(ad446)* III (Loer and Kenyon, 1993), MT8943 *bas-1(ad446)* III; *cat-4(e1141)* V, MT363 *goa-1(n363)* I, MT7990 *bas-1(n2948)* III, MT7991 *dgk-1(n2949)* X, MT8201 *mod(n3006)*, MT8203 *mod(n3007)*, MT8202 *bas-1(n3008)*, MT8539 *mod(n3032)*, MT8630 *mod(n3033)*, MT8541 *mod-1(n3034)* V, MT8623 *mod(n3052)*, MT8624 *mod(n3053)*, MT8625 *mod(n3054)*, MT8626 *goa-1(n3055)* I, MT8627 *mod(n3056)*, MT8628 *mod(n3057)*, MT8689 *mod(n3074)*, MT8690 *mod(n3075)*, and MT8720 *mod-6(n3076)* I.

Locomotory Rate Assays

Assay plates were prepared by spreading the *E. coli* strain HB101 in a ring with an inner diameter of ~1 cm and an outer diameter of ~3.5 cm on NGM agar (Brenner, 1974) in 5 cm petri plates. Assay plates were always freshly spread with bacteria, incubated overnight at 37°C, and allowed to cool to room temperature before use. Plates

for measuring locomotory rate in the absence of bacteria were also incubated at 37°C. Only synchronized young adult hermaphrodites (16 hr after the late L4 larval stage) were tested. In all cases, plates were coded so that the experimenter was blind to the genotype or the laser ablation state of the animal and to the neurotransmitter content of the pretreatment plate.

For well-fed animals, locomotory rate was measured by removing 5 animals from plates with ample bacteria, washing the animals twice in S basal buffer (Brenner, 1974), and transferring them to an assay plate in a drop of buffer using a capillary pipette. If the assay plate contained a ring-shaped bacterial lawn, the animals were transferred to the clear zone at the center of the ring. The drop of buffer used to transfer the animals was absorbed with a Kimwipe. Five minutes after transfer, the number of body bends in 20 s intervals was sequentially recorded for each of the 5 animals on the assay plate. This slowing response of well-fed animals was not an artifact of the wash step, since animals transferred directly from the culture plates to assay plates also exhibited the basal slowing response (data not shown). Since food-deprived animals (see below) were washed, we washed all of the animals to enable direct comparisons.

For food-deprived animals, 5–15 animals were washed free of bacteria in S basal buffer (two washes) and then transferred to 5 cm NGM agar plates that had no bacteria but had a ring of high osmolarity fructose on the outer edge. The drop of buffer used to transfer the animals was absorbed with a Kimwipe. The animals were incubated on these plates for 30 min at room temperature. *C. elegans* avoids high osmolarity (Culotti and Russell, 1978), and this avoidance reflex prevented the animals from swimming off the agar and dying on the plastic edge of the petri plate. The high osmolarity ring was created by adding ~100 μl of a 4 M fructose solution to the outer circumference of the agar surface and waiting ~5–10 min for this solution to dry into the plate. To make the high osmolarity ring visible, we added a small amount of bromophenol blue to the fructose solution. The presence of the high osmolarity ring or the bromophenol blue had no effect on the behavior of animals in the locomotory rate assays (data not shown). At the end of 30 min of food deprivation, five worms were transferred in a drop of S basal buffer to assay plates, and locomotory rate was measured as described above for well-fed animals.

Locomotory rate measurements for mock and laser-ablated animals were done in a similar fashion, except that only 1 animal was transferred to each assay plate. Its locomotory rate was tested in the presence of bacteria, and it was allowed to recover for 2–3 hr on plates containing bacteria and then transferred, as described above, to food deprivation plates. After 5 min on the food deprivation plates, the locomotory rate of these well-fed animals in the absence of food was recorded; these locomotory rates were not different from locomotory rates on regular assay plates without bacteria that did not have the high osmolarity ring (data not shown). After 30 min, the food-deprived animals were transferred to assay plates containing ring-shaped bacterial lawns, and the locomotory rate was again recorded. For the ablation and serotonin antagonist experiments (Figures 5B and 5C), locomotory rate data for food-deprived animals on plates without bacteria were not collected, since we have never observed any difference between the behaviors of well-fed and food-deprived animals on plates without bacteria. For the unilateral ablations, there was no systematic bias to the order in which the sides were assayed, since the plates were coded prior to the locomotory assay, and the experimenter did not know when assaying a given side whether or not the neurons had been ablated on that side.

Sephadex G-200 beads (Sigma Chemicals) were resuspended in S basal buffer at 30 mg/ml and autoclaved for 45 min. Approximately 200 μl of this mixture was transferred to an agar plate. Excess buffer that pooled at the edge of the Sephadex was removed with a Kimwipe. The rate of locomotion was measured 2 min after transferring the animals to the Sephadex matrix. The food deprivations on Sephadex were performed by washing the animals twice in S basal buffer and transferring them to a 5 cm plate whose surface was completely covered by a layer of Sephadex beads. Thirty minutes later, animals were transferred to assay plates.

Statistical analysis was performed using the unpaired Student's t test of the Statview program.

Laser Ablations

Neurons were ablated during the second larval stage using a laser microbeam, as previously described (Avery and Horvitz, 1987, 1989; Bargmann and Horvitz, 1991). Behavioral assays of young adult animals were performed 2 days later. Mock ablated animals were transferred to agar pads and anesthetized in parallel to the animals that underwent laser ablation.

Successful identification and ablation of the dopaminergic neurons were confirmed using the FIF technique of Sulston et al. (1975), which generates fluorescence in the cell bodies of dopaminergic neurons, with modifications adapted from Jagdale and Gordon (1994). The eight dopaminergic cell bodies were routinely visible in animals of the first, second, and third larval stages.

To confirm that the behavioral phenotype we observed in our presumptive CEP-ablated, ADE-ablated, PDE-ablated animals was specifically caused by ablation of those neurons and not by accidental damage to neighboring cells, we ablated the nearest neighbors of the dopaminergic neurons in 2 animals. We ablated all cells adjacent to dopaminergic neurons (RID, ALA, URX, FLP, ADA, AQR, PVD, PVM, and SDQL) ($n = 2$), except for the sheath cells of the dopaminergic neurons and cells that are postsynaptic to dopaminergic neurons. These animals showed a normal basal slowing response (data not shown). Similarly, for the NSM ablation experiments, we ablated I2 ($n = 3$) and MC ($n = 4$) and saw no effect on the enhanced slowing response (data not shown).

Neurotransmitter and Drug Pretreatment

Solutions of serotonin creatinine sulfate complex (50 mM, Sigma) or dopamine hydrochloride (RBI) were prepared fresh in M9 buffer (Brenner, 1974), and 400 μl of these solutions or of M9 buffer was added to each 5 cm plate containing ~10 ml of agar and a bacterial lawn to obtain an equilibrium concentration of ~2 mM of each neurotransmitter. The plates were allowed to dry at room temperature with their lids removed for 1 hr, and then 40–50 animals of each genotype that had been picked as L4 animals 16–20 hr earlier were transferred to each plate. The animals were incubated on these plates for either 2 hr (*bas-1; cat-4* rescue experiments) or 6–7 hr (*cat-2* rescue experiments) at 20°C and then assayed according to our standard locomotory rate assay, with the modification that the food deprivation plates contained the appropriate neurotransmitter. The assay plates did not contain the neurotransmitters. For both *cat-2* and *bas-1; cat-4* mutants, pretreatment with dopamine resulted in the appearance of FIF indicative of dopamine in the cell bodies of eight neurons in the positions of the dopaminergic neurons of the wild type (data not shown). For these drug experiments, pretreatment was considered to rescue the mutant phenotype if the data from mutants were not statistically different ($p > 0.05$) from the data from wild-type assayed in parallel under the same conditions; $p = 0.49$ for rescue by dopamine of the basal slowing response of *cat-2* mutants, $p = 0.28$ for rescue by dopamine of the basal slowing response of *bas-1; cat-4* mutants, $p = 0.1$ for rescue by serotonin of the enhanced slowing response of *bas-1; cat-4* mutants.

Plates containing methiothepin mesylate (RBI) and mianserin hydrochloride (RBI) were prepared in a manner similar to the serotonin- and dopamine-containing plates. Animals were incubated for 30 min on either plates with bacteria and the antagonist or on plates without bacteria but containing antagonist and then assayed according to our standard locomotory rate assay. The assay plates did not contain the antagonists.

Fresh NGM agar plates containing either no fluoxetine or 75 μg/ml fluoxetine (Sigma) were prepared the day before the assays and stored overnight in the dark. The fluoxetine stock (300 μg/ml) was dissolved in water and added after the agar solution had cooled to just above gelling temperature. Animals were incubated for 30 min on plates without bacteria and with or without fluoxetine and then tested on assay plates (without fluoxetine). For testing the response of NSM-ablated animals, the locomotory rates of the mock ablated and NSM-ablated animals were assayed, 1 animal at a time, first on plates with bacteria after food deprivation in the absence of fluoxetine, then on plates with bacteria after food deprivation in the presence of fluoxetine. Then all of the animals were tested as a group, first on plates without bacteria after food deprivation in the absence of fluoxetine, then on plates without bacteria after food

deprivation in the presence of fluoxetine. The animals were allowed to recover on plates with bacteria for 1–2 hr between each of the four conditions.

To examine the serotonin response of food-deprived versus well-fed animals, we prepared fresh NGM agar plates with the appropriate amounts of serotonin. The serotonin stock was dissolved in water and added after the agar solution had cooled to just above gelling temperature. After incubating the plates overnight at room temperature, we placed a single food-deprived or well-fed animal on a plate, and the locomotory rate of the animal was recorded at the start of every minute for 5 min.

Mutant Screen

N2 hermaphrodites (L4) were mutagenized with EMS (Brenner, 1974), and their F2 progeny were food deprived for 30 min and then transferred to the center of large petri plates spread with *E. coli* strain HB101 in the form of a ring-shaped lawn. After 5–10 min, the assay plates were examined for worms that had both reached the outer edge of the bacterial lawn and were moving rapidly. Such animals were picked to individual plates, and their progeny were retested. Prior to detailed behavioral analysis, each strain was outcrossed at least twice.

Acknowledgments

The authors thank Mark Alkema, Brad Hersh, Peter Reddien, and Hillel Schwartz for helpful comments concerning the manuscript. This research was supported by United States Public Heath Service research grant GM24663. E. R. S. was supported by predoctoral fellowships from the National Science Foundation and the W. M. Keck Foundation. R. R. was supported by a predoctoral fellowship from the Howard Hughes Medical Institute. H. R. H. is an Investigator of the Howard Hughes Medical Institute.

Received February 14, 2000; revised May 15, 2000.

References

Albertson, D.G., and Thomson, J.N. (1975). The pharynx of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. *275*, 299–325.

Avery, L., and Horvitz, H.R. (1987). A cell that dies during wild-type C. elegans development can function as a neuron in a *ced-3* mutant. Cell *51*, 1071–1078.

Avery, L., and Horvitz, H.R. (1989). Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of C. elegans. Neuron *3*, 473–485.

Avery, L., and Horvitz, H.R. (1990). Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. J. Exp. Zool. *253*, 263–270.

Bargmann, C.I., and Horvitz, H.R. (1991). Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in C. elegans. Neuron *7*, 729–742.

Barnes, S., Syed, N.I., Bulloch, A.G., and Lukowiak, K. (1994). Modulation of ionic currents by dopamine in an interneurone of the respiratory central pattern generator of *Lymnaea stagnalis*. J. Exp. Biol. *189*, 37–54.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics *77*, 71–94.

Brodfuehrer, P.D., Debski, E.A., O'Gara, B.A., and Friesen, W.O. (1995). Neuronal control of leech swimming. J. Neurobiol. *27*, 403–418.

Cameron, D.L., and Williams, J.T. (1993). Dopamine D1 receptors facilitate transmitter release. Nature *366*, 344–347.

Choy, R.K., and Thomas, J.H. (1999). Fluoxetine-resistant mutants in *C. elegans* define a novel family of transmembrane proteins. Mol. Cell *4*, 143–152.

Colbert, H.A., and Bargmann, C.I. (1995). Odorant-specific adaptation pathways generate olfactory plasticity in C. elegans. Neuron *14*, 803–812.

Colbert, H.A., and Bargmann, C.I. (1997). Environmental signals modulate olfactory acuity, discrimination, and memory in *Caenorhabditis elegans*. Learn. Mem. *4*, 179–191.

Culotti, J.G., and Russell, R.L. (1978). Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. Genetics *90*, 243–256.

Desai, C., Garriga, G., McIntire, S.L., and Horvitz, H.R. (1988). A genetic pathway for the development of the *Caenorhabditis elegans* HSN motor neurons. Nature *336*, 638–646.

Drago, J., Padungchaichot, P., Accili, D., and Fuchs, S. (1998). Dopamine receptors and dopamine transporter in brain function and addictive behaviors: insights from targeted mouse mutants. Dev. Neurosci. *20*, 188–203.

Duerr, J.S., Frisby, D.L., Gaskin, J., Duke, A., Asermely, K., Huddleston, D., Eiden, L.E., and Rand, J.B. (1999). The *cat-1* gene of *Caenorhabditis elegans* encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. J. Neurosci. *19*, 72–84.

Flicker, C., McCarley, R.W., and Hobson, J.A. (1981). Aminergic neurons: state control and plasticity in three model systems. Cell Mol. Neurobiol. *1*, 123–166.

Glennon, R.A. (1987). Central serotonin receptors as targets for drug research. J. Med. Chem. *30*, 1–12.

Groome, J.R., Clark, M., and Lent, C.M. (1993). The behavioural state of satiation in the leech is regulated by body distension and mimicked by serotonin depletion. J. Exp. Biol. *182*, 265–270.

Harris-Warrick, R.M., Coniglio, L.M., Barazangi, N., Guckenheimer, J., and Gueron, S. (1995). Dopamine modulation of transient potassium current evokes phase shifts in a central pattern generator network. J. Neurosci. *15*, 342–358.

Hawkins, R.D., Kandel, E.R., and Siegelbaum, S.A. (1993). Learning to modulate transmitter release: themes and variations in synaptic plasticity. Annu. Rev. Neurosci. *16*, 625–665.

Hedgecock, E.M., and Russell, R.L. (1975). Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *72*, 4061–4065.

Hietala, J., and Syvalahti, E. (1996). Dopamine in schizophrenia. Ann. Med. *28*, 557–561.

Horvitz, H.R., Chalfie, M., Trent, C., Sulston, J.E., and Evans, P.D. (1982). Serotonin and octopamine in the nematode *Caenorhabditis elegans*. Science *216*, 1012–1014.

Jagdale, G.P., and Gordon, R. (1994). Role of catecholamines in the reproduction of *Romanomermis culicivorx*. J. Nematol. *26*, 40–45.

Kapatos, G., Hirayama, K., Shimoji, M., and Milstien, S. (1999). GTP cyclohydrolase I feedback regulatory protein is expressed in serotonin neurons and regulates tetrahydrobiopterin biosynthesis. J. Neurochem. *72*, 669–675.

Kaplan, J.M., and Horvitz, H.R. (1993). A dual mechanosensory and chemosensory neuron in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *90*, 2227–2231.

Koob, C.F. (1992). Dopamine, addiction and reward. Semin. Neurosci. *4*, 139–148.

Kravitz, E.A. (1988). Hormonal control of behavior: amines and the biasing of behavioral output in lobsters. Science *241*, 1775–1781.

Kupfermann, I., and Kandel, E. (1995). Learning and memory. In Essentials of Neural Science and Behavior, E.R. Kandel et al., eds. (Stamford, CT: Appleton and Lange), pp. 651–666.

Kupfermann, I., and Schwartz, J. (1995). Motivation. In Essentials of Neural Science and Behavior, E.R. Kandel et al., eds. (Stamford, CT: Appleton and Lange), pp. 613–627.

Lefkowitz, R.J. (1993). G protein–coupled receptor kinases. Cell *74*, 409–412.

Lints, R., and Emmons, S.W. (1999). Patterning of dopaminergic neurotransmitter identity among *Caenorhabditis elegans* ray sensory neurons by a TGFβ family signaling pathway and a Hox gene. Development *126*, 5819–5831.

Liu, D.W., and Thomas, J.H. (1994). Regulation of a periodic motor program in *C. elegans*. J. Neurosci. *14*, 1953–1962.

Loer, C.M., and Kenyon, C.J. (1993). Serotonin-deficient mutants

and male mating behavior in the nematode *Caenorhabditis elegans*. J. Neurosci. *13*, 5407–5417.

Lucki, I. (1998). The spectrum of behaviors influenced by serotonin. Biol. Psychiatry *44*, 151–162.

Maguire, G., and Werblin, F. (1994). Dopamine enhances a glutamate-gated ionic current in OFF bipolar cells of the tiger salamander retina. J. Neurosci. *14*, 6094–6101.

Marsden, C.D. (1992). Dopamine and basal ganglia disorders in humans. Semin. Neurosci. *4*, 171–178.

Mendel, J.E., Korswagen, H.C., Liu, K.S., Hajdu-Cronin, Y.M., Simon, M.I., Plasterk, R.H., and Sternberg, P.W. (1995). Participation of the protein G_o in multiple aspects of behavior in *C. elegans*. Science *267*, 1652–1655.

Mori, I. (1999). Genetics of chemotaxis and thermotaxis in the nematode Caenorhabditis elegans. Annu. Rev. Genet. *33*, 399–422.

Mylecharane, E.J. (1989). The classification of 5-hydroxytryptamine receptors. Clin. Exp. Pharmacol. Physiol. *16*, 517–522.

Neckameyer, W.S. (1998). Dopamine and mushroom bodies in *Drosophila*: experience-dependent and -independent aspects of sexual behavior. Learn. Mem. *5*, 157–165.

Nicholas, W.L. (1984). The Biology of Free-Living Nematodes (New York: Clarendon Press).

Nurrish, S., Ségalat, L., and Kaplan, J.M. (1999). Serotonin inhibition of synaptic transmission: $G\alpha_o$ decreases the abundance of UNC-13 at release sites. Neuron *24*, 231–242.

O'Gara, B.A., Chae, H., Latham, L.B., and Friesen, W.O. (1991). Modification of leech behavior patterns by reserpine-induced amine depletion. J. Neurosci. *11*, 96–110.

Osborne, N.N., and Hamon, M. (1988). Neuronal Serotonin (New York: John Wiley and Sons).

Pereda, A.E., Nairn, A.C., Wolszon, L.R., and Faber, D.S. (1994). Postsynaptic modulation of synaptic efficacy at mixed synapses on the Mauthner cell. J. Neurosci. *14*, 3704–3712.

Perkins, L.A., Hedgecock, E.M., Thomson, J.N., and Culotti, J.G. (1986). Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. *117*, 456–487.

Rankin, C.H., Beck, C.D., and Chiba, C.M. (1990). *Caenorhabditis elegans*: a new model system for the study of learning and memory. Behav. Brain Res. *37*, 89–92.

Rosen, S.C., Weiss, K.R., Goldstein, R.S., and Kupfermann, I. (1989). The role of a modulatory neuron in feeding and satiation in Aplysia: effects of lesioning of the serotonergic metacerebral cells. J. Neurosci. *9*, 1562–1578.

Sahley, C.L. (1995). What we have learned from the study of learning in the leech. J. Neurobiol. *27*, 434–445.

Sawin, E.R. (1996). Genetic and cellular analysis of modulated behaviors in *Caenorhabditis elegans,* PhD thesis, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Schafer, W.R., and Kenyon, C.J. (1995). A calcium-channel homologue required for adaptation to dopamine and serotonin in *Caenorhabditis elegans.* Nature *375*, 73–78.

Segalat, L., Elkes, D.A., and Kaplan, J.M. (1995). Modulation of serotonin-controlled behaviors by G_o in *Caenorhabditis elegans*. Science *267*, 1648–1651.

Self, D.W., and Nestler, E.J. (1995). Molecular mechanisms of drug reinforcement and addiction. Annu. Rev. Neurosci. *18*, 463–495.

Sulston, J., Dew, M., and Brenner, S. (1975). Dopaminergic neurons in the nematode *Caenorhabditis elegans*. J. Comp. Neurol. *163*, 215–226.

Sze, J.Y., Victor, M., Loer, C., Shi, Y., and Ruvkun, G. (2000). Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. Nature *403*, 561–564.

Takada, A., and Curzon, G. (1995). Serotonin in the Central Nervous System and Periphery: Proceedings of the Symposium on Serotonin in the Central Nervous System and Periphery, April 1–2, 1995, Nagoya, Japan (New York: Elsevier).

Tempel, B.L., Livingstone, M.S., and Quinn, W.G. (1984). Mutations in the dopa decarboxylase gene affect learning in *Drosophila*. Proc. Natl. Acad. Sci. USA *81*, 3577–3581.

Trent, C., Tsung, N., and Horvitz, H.R. (1983). Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. Genetics *104*, 619–647.

Ward, S. (1973). Chemotaxis by the nematode *Caenorhabditis elegans*: identification of attractants and analysis of the response by use of mutants. Proc. Natl. Acad. Sci. USA *70*, 817–821.

Weinshenker, D., Garriga, G., and Thomas, J.H. (1995). Genetic and pharmacological analysis of neurotransmitters controlling egg laying in *C. elegans*. J. Neurosci. *15*, 6975–6985.

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. *314*, 1–340.

Wicks, S.R., and Rankin, C.H. (1997). Effects of tap withdrawal response habituation on other withdrawal behaviors: the localization of habituation in the nematode *Caenorhabditis elegans*. Behav. Neurosci. *111*, 342–353.

Wong, D.T., and Bymaster, F.P. (1995). Development of antidepressant drugs. Fluoxetine (Prozac) and other selective serotonin uptake inhibitors. Adv. Exp. Med. Biol. *363*, 77–95.

Wood, W.B., and the Community of *C. elegans* Researchers (1988). The Nematode *Caenorhabditis elegans* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).