# 27

# Neural Plasticity

**Erik M. Jorgensen**
Department of Biology
University of Utah
Salt Lake City, Utah 84112

**Catharine Rankin**
Psychology Department
University of British Columbia,
Vancouver, British Columbia V6T 1Z4



## I. INTRODUCTION: CONNECTIVITY AND PLASTICITY

Neural plasticity refers to functional changes in the nervous system and therefore encompasses a range of phenomena from changes at synapses observed on a microscopic scale to changes in behavior observed in the whole animal. These diverse phenomena are related since changes in synapses are believed to underlie changes in an animal's behavior (Greenough and Bailey 1988). Ideally, both the physical changes to the nervous system and the resultant behavioral changes could be identified and studied together to yield an integrated understanding of nervous system structure and behavior.

Nervous systems were once thought to be "hardwired" during development. In most vertebrate central nervous systems, cell proliferation occurs during embryogenesis and new neurons are not added to the mature

nervous system (Jacobson 1991). An unusual exception to this rule is found in some species of birds in which neurons are added to the brains of juveniles to accommodate song learning (Paton and Nottebohm 1984). Sensory systems such as the visual cortex form their functional connectivity during a limited "critical period" during development, and new connections in most systems are not made after this period (Hubel and Wiesel 1970). This stability was thought to be an essential requirement for reliable processing of sensory information. However, this general stability has been shown to have its exceptions. The nervous system of even the dimmest among us can learn new tasks and thereby reveals a degree of plasticity in our brains. Even the inability of the brain to produce new neurons has been recently challenged when it was demonstrated that mature brain cells can be induced to divide and proliferate in vitro (Reynolds and Weiss 1992; Morshead et al. 1994). Additionally, the functional connectivity of the visual cortex reorganizes after sensory lesions (Das and Gilbert 1995), illustrating a flexibility in a circuit once thought to be rigidly fixed.

Even a nervous system with a stable anatomical connectivity can exhibit profoundly variable output. Anatomical connectivity refers to the physical arrangement of synapses among cells of a circuit. Functional connectivity defines the effects of cells in a circuit upon one another (Getting 1989). A cell may form synapses to another cell, but these synapses have no effect on the postsynaptic cell. Thus, the synapses form part of the anatomical connectivity but not the functional connectivity. In some cases, these latent synapses can be activated to alter the output of the circuit after high-frequency electrophysiological stimulation (Charpier et al. 1995; Liao et al. 1995). In other cases, the functional connectivity may be quite dynamic; synaptic relationships between cells can change instantaneously when inputs into the circuit change (Dickinson 1989). For example, the mollusk *Tritonia* first responds to threat by reflexive withdrawal. Withdrawal is followed by rapid escape swimming, which is driven by alternating contractions of the dorsal and ventral muscles. The same group of interneurons mediate both behaviors; these cells inhibit one another when they participate in reflexive withdrawal, and they excite one another when they participate in escape swimming (Getting 1989).

Because the output of a nervous system is a function of the input into the circuit and the anatomical and functional connectivities, changes in the output can be caused by alterations to any one of these three properties. First, altering the input of a circuit can radically change the functional connectivity. These inputs can be either synaptic or humoral.

Second, the functional connectivity can be changed by altering the individual strengths of the existing synapses. Third, synapses can be added or eliminated to change the anatomical connectivity. Observations of these first two mechanisms require electrophysiological recordings from the relevant circuit, and although such techniques are being developed in *Caenorhabditis elegans* (Raizen and Avery 1994; Davis et al. 1995; Lockery and Hall 1995), they have not yet been applied to the analysis of dynamic neural circuits. For this reason, we are confined to observing changes in the physical connectivity of the nematode. Nevertheless, there are several examples of such synaptic remodeling during *C. elegans* development.

The *C. elegans* nervous system is thought to be even more rigid than other nervous systems. Unlike vertebrate nervous systems, the number of cells is invariant, and anatomical reconstructions have shown that neuronal connectivity is similar between individuals (White et al. 1986; Hall and Russell 1991). For these reasons, *C. elegans* once appeared to be a poor organism in which to study neural plasticity. However, recent data indicate that the *C. elegans* nervous system is also flexible. In this chapter, we review examples of neural plasticity during development and behavioral plasticity in adult animals, including examples of learning such as habituation and classical conditioning.

## II. SYNAPTIC PLASTICITY DURING DEVELOPMENT

During development in vertebrates, there is a dynamic interaction between a neuron and its target. In a process called activity-dependent development, the activity of the target can affect its innervation from the input neuron. Thus, the connectivity cannot be hardwired; i.e., it cannot be predetermined by the genome. Studies of the vertebrate visual system indicate that many of the initial synaptic contacts appear to be temporary or inappropriate. Use of the visual system strengthens appropriate synapses and eliminates inappropriate synapses (Constantine-Paton et al. 1990; Shatz 1990; Hockfield and Kalb 1993). Similarly, it has been hypothesized that in the adult animal, the strengths of synapses may be altered in an activity-dependent manner during learning (Bliss and Collingridge 1993; Hawkins et al. 1993). Thus, in both a developing and a mature nervous system, there must be signal transduction systems that link activity of the presynaptic and postsynaptic cells and cause changes in the strength of the connection. Moreover, the mechanisms used to alter an existing circuit in a mature animal may be the same mechanisms used early in development to establish the original connectivity in the embryo (Kandel and O'Dell 1992 and references therein).

### A. Neuromuscular Junction Formation

Dynamic interactions between neuron and target were first observed during the formation of vertebrate neuromuscular junctions. In early development, a muscle fiber is innervated by several motor neurons. There is then a competitive interaction among the motor neurons that eliminates all but one input and serves to sharpen the compartment boundaries between adjacent motor units (Lichtman 1995). The sorting-out process is activity-dependent; it seems that the more effective motor neuron is favored and the weaker motor neuron is disfavored by a retrograde signal from the muscle.

Observations of *C. elegans* neuromuscular junctions indicate that synaptic plasticity may occur in nematode development as well. First, rudimentary plasticity is a prerequisite to normal development and growth of the animal. The length of a *C. elegans* individual increases fivefold during development, and neuromuscular junctions are added to maintain synaptic density (Fig. 1). At the end of the L1 stage, additional muscles and motor neurons are added to the embryonic nervous system and must be incorporated into the existing nerve cords (White et al. 1978). During the L4 stage, sex-specific muscles and motor neurons are added to both hermaphrodites and males. It is likely that the addition of these components requires accommodations in the existing nervous system so that a well-ordered motor system is maintained. These observations indicate that the motor neurons and target muscles interact dynamically during the formation of neuromuscular junctions, but it is not yet clear whether this is an activity-dependent interaction.

Nematodes differ from most other animals in that a motor neuron does not leave the nerve fasicle to innervate its target; instead, the muscle extends a process, the muscle arm, to the nerve cord to form a neuromuscular junction at the edge of the nerve bundle. Analyses of *unc-6* and *unc-104* mutants indicate that the migrating muscle arm may respond to chemotropic cues to locate its synaptic partner. In the absence of function from the *C. elegans* netrin homolog UNC-6, axons that normally run in the dorsal nerve cord are displaced ventrally (Fig. 2) (Hedgecock et al. 1990). In this mutant, the dorsal muscles send their arms ventrally across the lateral epidermis to locate the misplaced axons. *unc-104* mutants lack the neuron-specific kinesin-like protein. Although these mutants possess a relatively normal axon morphology, the motor neurons accumulate vesicles in the ventrally located cell bodies instead of transporting them dorsally into the axons (Hall and Hedgecock 1991). Muscle arms in *unc-104* mutants project to the vesicle-rich cell bodies instead of the axons. Both phenotypes suggest that a chemotropic molecule, perhaps stored in

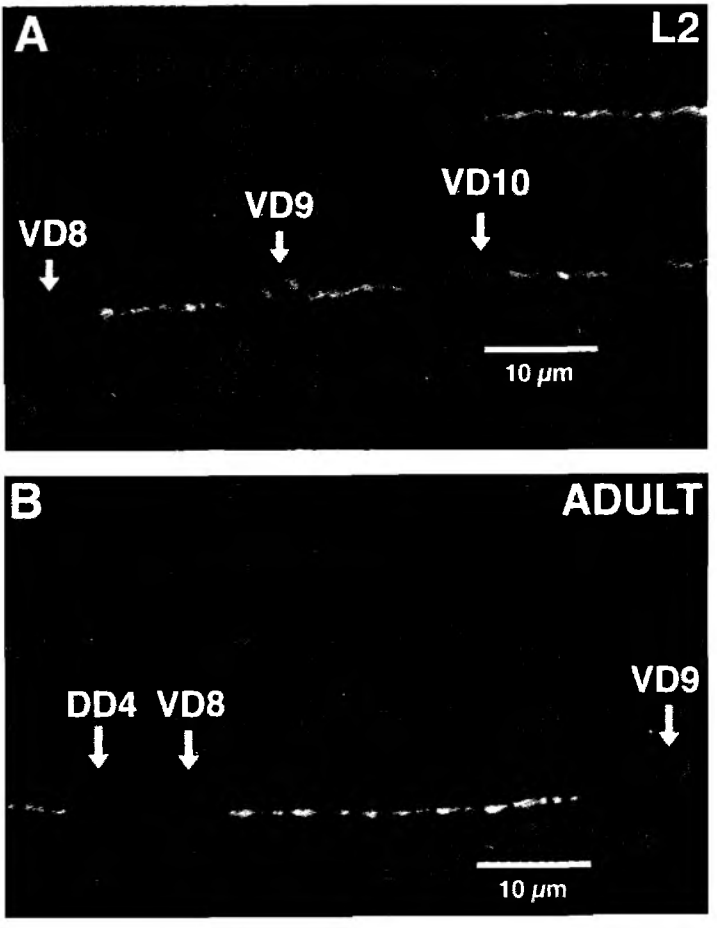


*Figure 1* Neuromuscular junctions are added to axons during development. Green fluorescent protein (GFP; Chalfie et al. 1994) was fused to the *C. elegans* homolog of the synaptic vesicle protein synaptobrevin and expressed in the D-type motor neurons (a gift from M. Nonet and Y. Jin). This fluorescent construct appears to label clusters of synaptic vesicles at neuromuscular junctions. Note the increase in synaptic sites between the motor neurons VD8 and VD9 in the adult (*B*) compared to the L2 larvae (*A*). (Confocal photomicrograph by K. Knobel and E. Jorgensen.)

vesicles, is released by axons and guides the muscle arms to the motor neurons. Alternatively, what we see in the adult mutant may be the result of promiscuous muscle arm projection to many targets during development and only the correct projection may persist, stabilized by adhesive interactions during neuromuscular formation. In either case, these mu-

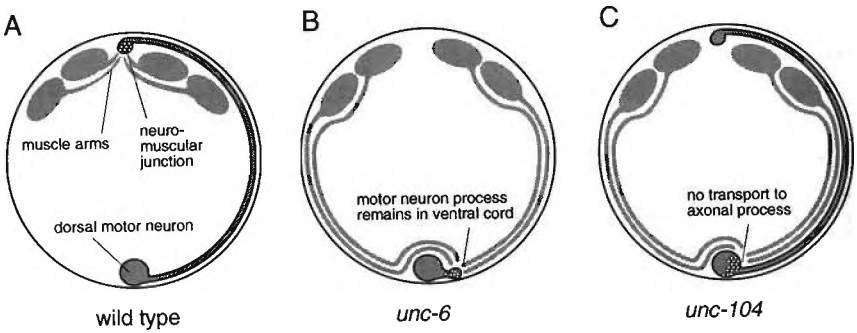


*Figure 2* Muscle arms can locate misplaced motor neurons. (*A*) In wild-type animals, processes from the muscles, called muscle arms, extend to the motor neuron axons in the dorsal and ventral cords. Only the dorsal muscles are shown. (*B*) In *unc-6* mutants, the motor neuron axons do not extend to the dorsal cord, and the muscle arms extend to the misplaced axons on the ventral side (Hedgecock et al. 1990). (*C*) In *unc-104* mutants, axonal outgrowth is normal but vesicles are not transported along the axonal processes (Hall and Hedgecock 1991). Muscle arms extend ventrally and form synapses to the vesicle-rich motor neuron cell bodies.

tants reveal that the muscle can respond flexibly to perturbations of the motor neurons.

Axonal outgrowth in *C. elegans* does not depend on the target muscle. During embryogenesis, when the ventral cord motor neurons are extending axons, the muscles have already differentiated; i.e., the muscle cells are in place, the contractile apparatus is assembled, and the muscles are spontaneously contracting (Durbin 1987; Hresko et al. 1994). However, in animals in which the muscle precursors have been killed, the DD motor neurons still extend processes into the dorsal cord (Plunkett et al. 1996).

The motor neuron may not be completely passive in the search for a synaptic partner. Reconstructions of adult worms demonstrate that motor neuron axons shift to the edge of the nerve cord to form neuromuscular junctions with the muscle arms (White et al. 1976). Although this displacement seems to be a very subtle migration on the part of the neuron, it may reflect a latent ability of the motor neuron to actively seek a synaptic partner. When a motor neuron's normal target has been killed, the motor neuron can extend a process to an ectopic muscle (Plunkett et al. 1996). Thus, both the muscle and the motor neuron can respond to perturbations in a plastic manner to form a neuromuscular junction.

Moreover, the regionalization of junctions in *C. elegans* suggests that motor neurons may compete for synaptic targets. The regions of body

muscle innervated by adjacent motor neurons of the same class do not overlap (White et al. 1976). The extent of the junctional domain is not limited by the length of the axon since the axon of any particular motor neuron extends across two adjacent domains of innervation. Such a distribution may be formed by competition between members of a class for targets.

The experiments described above indicate that bidirectional communication between muscle and neuron is important for neuromuscular junction formation in *C. elegans*. As stated above, the formation of synapses between the motor neuron and the muscle is activity-dependent in vertebrates. For example, when the acetylcholine agonist carbachol is added to vertebrate neuromuscular junctions, acetylcholine receptors are inappropriately activated, and synaptic sites are eliminated to compensate for the increase in muscle depolarization (Bloch 1986). Conversely, blocking neurotransmission with curare leads to an increase in synaptic sites (Dahm and Landmesser 1991). These experiments indicate that the muscle regulates its synaptic input in an activity-dependent manner. In contrast, synaptic density is not altered in *C. elegans* mutants that have altered muscle activation. For example, the lack of acetylcholine in *cha-1* mutants (J. Rand, pers. comm.) or GABA in *unc-25* mutants (E. Jorgensen et al., unpubl.) does not lead to an increase in the frequency of neuromuscular junctions. These mutants may up-regulate the activity of the motor neurons or the responsiveness of the muscles in other ways, but by ultrastructural criteria, there seems to be no increase in synaptic density. The number and position of synaptic sites must be controlled by other signals that may include cell surface receptors, components of the extracellular matrix, secreted growth factors or peptides, or signals that pass through gap junctions (Cash and Poo 1995).

### B. DD Rewiring

The most concrete example of synaptic plasticity occurs in the DD motor neurons of the ventral nerve cord during larval development. At the end of the L1 stage, additional body muscles and five additional classes of motor neurons are born. These neurons and muscles are incorporated into the existing motor circuit. During this time, the embryonic DD motor neurons change their synaptic partners (Fig. 3). During embryonic development, the GABAergic DD motor neurons innervate the ventral muscles and receive input from the DA and DB motor neurons on the dorsal side. At the end of the L1 stage, the DD motor neurons rewire their synaptic contacts so that they form neuromuscular junctions on the dorsal

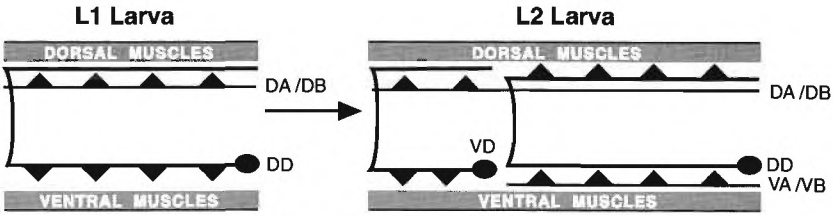


*Figure 3* Rewiring of the DD motor neurons during larval development. In the L1 larva, the DD neurons receive input from the DA and DB motor neurons and innervate the ventral muscles. These neuromuscular junctions are eliminated, and the DD neurons innervate the dorsal muscles in the L2 larva (White et al. 1978). Several classes of motor neurons are added during this period (VA, VB, VC, VD, and AS neurons). The connectivity of a new class of GABAergic motor neurons, the VDs, resembles the DD connectivity in the L1 larva.

side (White et al. 1978). Synaptic input from the dorsal motor neurons is lost and new inputs from the VA and VB motor neurons are developed. During this period, a new class of GABAergic motor neurons, the VD neurons, form neuromuscular junctions to the ventral muscles and receive input from the dorsal motor neurons. One possibile mechanism for this rewiring is that the generation of the VD contacts causes the DD motor neurons to rewire. However, in *unc-55* mutants, the VDs inappropriately form synaptic outputs onto the dorsal muscles, and the DD motor neurons rewire even in the absence of VD contacts to the ventral muscles (Walthall and Plunkett 1995). The cholinergic VA and VB neurons form new synapses to the DDs during this period. It is possible that these new inputs induce the changes in the DDs. However, in *lin-6* mutants, none of the postembryonic motor neurons are born, including the VA and VB neurons, and again the DD motor neurons redirect their neuromuscular junctions to the dorsal muscles (White et al. 1978). As a consequence, the DD neurons in *lin-6* mutants form normal outputs but have no inputs. Because the remodeling of the DD neurons does not require the presence of these other neurons, it is likely that the DD neurons are executing an intrinsic program initiated during the L1 to L2 transition.

### C. Remodeling of Dauer Larva Sensory Endings

A second developmental period during which synaptic remodeling occurs is during the formation of the dauer larvae. Dauer larvae behave in a manner obviously different from nondauer larvae (see Riddle, this volume). One way in which they differ is in their chemotactic behavior. Although capable of movement, dauer larvae do not chemotax to food

(Albert and Riddle 1983). Instead, sensation of food initiates recovery from the dauer stage. After resuming pharyngeal pumping, the dauer larva will then chemotax to food, which eventually results in the resumption of normal development. In addition, chemosensory responses change with the age of the dauer larvae. Dauer pheromone inhibits food-induced dauer recovery, but after 1 week spent in the dauer state, animals are much less sensitive to this inhibitory effect of dauer pheromone (Golden and Riddle 1984b). These changes in chemosensory responses may be caused by the remodeling of the sensory endings that occurs during dauer formation (Albert and Riddle 1983). In the amphid, the AWC and AFD cells elaborate larger sensory processes, whereas the ASG and ASI sensory endings are retracted somewhat in the amphidial pore. In addition, the inner labial sensory endings no longer protrude at the anterior tip of the nose. The amphidial sensory neurons are important for entry (ADF, ASG, ASI) and exit (ASJ) from the dauer stage (Bargmann and Horvitz 1991b). Why are the sensory endings remodeled in the dauer larvae? These modifications might be required for the altered chemotactic behavior of the dauer larva, or they may be specializations that allow recovery from the dauer stage. Finally, the modifications may simply protect the sensory endings from the harsh environmental conditions experienced for the duration of the dauer stage.

In addition to altered chemotaxis, dauer larvae exhibit other unique behaviors (Cassada and Russell 1975). They are sluggish and do not actively forage, but they will rapidly move away if prodded. They also will climb to the top of a vertical object such as a strand of fungus and wave their bodies in the air. In the soil, this behavior may be an attempt to become lodged on the body of a passing insect and thereby propagate dispersal. It is unlikely that the remodeling of the sensory neurons can be responsible for these changes in behavior. It is more likely that alterations of the connectivity in the central nervous system or changes in the humoral environment of the worm are responsible for these new locomotory responses. A reconstruction of dauer connectivity may yield insight into the circuits mediating these behavioral changes. Molecular genetic analysis of dauer behavioral mutants could be used to identify signaling molecules contributing to the behavioral change.

### D. Synaptic Plasticity in the Male

*C. elegans* males change their behavior when they mature from larvae into adults. L4 stage male larvae behave in a manner similar to hermaphrodites. For example, L4 males exhibit slow locomotion on food and avoid media preconditioned with hermaphrodites (E. Jorgensen, un-

publ.). Adult males are more active than L4 males on food, particularly in the absence of hermaphrodites, and will chemotax to hermaphrodite-conditioned media. In the presence of hermaphrodites or media preconditioned with hermaphrodites, males spontaneously back and coil, presumably in search of a mate. Once contact with an adult hermaphrodite has been made, the male proceeds through a series of steps to locate the vulva, insert the spicules, and transfer sperm (Liu and Sternberg 1995; Emmons and Sternberg, this volume). These behaviors in male adults but not in hermaphrodites or male larvae are probably caused by the extensive modifications to the nervous system that take place during the L4 molt. There are 87 neurons found in the adult male that are not found in the hermaphrodite (Sulston et al. 1980, 1988). It is also possible that the connectivity of the neurons common to both sexes is remodeled in the male. Although a complete reconstruction of the nervous system in an adult male has not been yet been attempted, such a reconstruction would directly address this question. Mutational analyses of male mating behavior may identify molecules that are involved in remodeling the male nervous system between the L4 and adult stages.

The behavioral changes seen in the adult male also require remodeling of existing muscles. Specifically, one of the enteric muscles that controls expulsion in the larval stages is reshaped to play a part in copulation. In the L4 male, GABA release from AVL and DVB causes contraction of the enteric muscles, including the anal depressor and sphincter muscles (for a review of the defecation cycle, see Avery and Thomas, this volume). The anal depressor in the L4 male is attached on one side to the dorsal hypoderm and on the other side to the roof of the rectum. During the L4 molt, the anal depressor contractile apparatus detaches from the dorsal hypoderm and attaches to the dorsal spicule protractor (Sulston et al. 1980). As a consequence, AVL and DVB are required for eversion of the spicules in the adult (E. Jorgensen, unpubl.). AVL and DVB function is also involved in enteric muscle function in the adult. In the L4 male, GABA release from AVL and DVB contracts the sphincter muscle, but in the adult male, GABA relaxes the sphincter muscle (Reiner and Thomas 1995). Thus, not only do the enteric muscles become reconfigured in the adult male, but there also must be alterations at the neuromuscular junctions that change the activity of the neurotransmitter.

## III. BEHAVIORAL PLASTICITY IN THE ADULT

Instead of studying the morphology of the nervous system, one can observe changes in the behavior of an animal. In fact, there are a number of

ways that an organism can express behavioral plasticity without a morphological change. Neuromodulators can toggle between stereotyped behavioral states. For example, the egg-laying hormone of *Aplysia* elicits a complex behavioral program by exciting and inhibiting specific neurons throughout the nervous system (Scheller et al. 1982). Alternatively, the strengths of existing synapses can be altered as a result of experience. We present evidence of such complex behavioral changes in worms ranging from examples of neuromodulators to paradigms for learning and memory.

Neuromodulators can instantaneously alter the range of synapses that are active in any particular group of neurons. The modulator can thereby bias a pattern generator toward one of several output patterns. Neuromodulators can act globally as a circulating hormone or they can act on a specific cell via synaptic connections (Dickinson 1989). In invertebrates, aminergic neurotransmitters have been demonstrated to act humorally to control behavioral states. In the lobster, a high ratio of serotonin to octopamine initiates dominant and aggressive behavior, whereas a low ratio initiates submissive behavior (Kravitz 1990). Alternatively, the neurotransmitters can act locally via synaptic contacts to strengthen or weaken a second connection in a circuit. For example, serotonergic or FMRFamidergic synapses from sensory neurons can, respectively, strengthen or weaken response of the sea slug *Aplysia* to mechanosensory stimuli (Byrne 1987; Montarolo et al. 1988). In the mollusk *Tritonia*, a central pattern generator produces rythmic output to the motor neurons that control swimming during escape; serotonergic input dynamically modulates synaptic strengths in this circuit during ongoing behavior (Katz et al. 1994).

### A. Aminergic Neuromodulators

The best evidence for neuromodulators in *C. elegans* comes from studies of aminergic neurotransmission. The most common aminergic neurotransmitters in invertebrates are dopamine, serotonin, histamine, and octopamine. Although a number of behaviors are affected by raising or lowering levels of aminergic neurotransmitters in worms, it is not known whether these neuromodulators are acting humorally as neurohormones or as classical neurotransmitters at discrete synapses. However, in some cases, the actions appear so global that a humoral role seems likely.

Dopamine release might signal the presence of food to a well-fed nematode. Worms alter a number of behaviors upon exiting a lawn of bacteria. They become hyperactive, stop pharyngeal pumping, and do not

activate the motor movements of the defecation cycle. Upon entering a bacterial lawn, wild-type worms slow, resume pumping, and resume motor movements of the defecation cycle. Some of these changes in behavior are probably mediated by dopamine transmission. Exogenous application of dopamine causes animals to become inactive, mimicking food abundance (Schafer and Kenyon 1995). Dopamine antagonists such as haloperidol cause animals to become hyperactive, mimicking food depletion. *cat-2(e1112)* mutants have low levels of dopamine (Sulston et al. 1975), and these animals are hyperactive in the presence of food in comparison to wild-type animals (B. Sawin, pers. comm.). However, they do not lack defecation cycles nor do they exhibit slow pharyngeal pumping, which are the other two behavioral changes that take place when a worm exits food. Laser killing of the dopamine neurons phenocopies the *cat-2* defect and thus confirms the role of the dopaminergic nervous system in the suppression of the hyperactive behavior (B. Sawin, pers. comm.). It is not clear whether this behavior is mediated indirectly by a humoral effect or by the direct release of dopamine from sensory neurons onto postsynaptic elements of interneurons. However, the dopamine neurons lack neurosecretory ultrastructure, so the effects of dopamine may be mediated at synapses (White et al. 1986).

Serotonin may signal the presence of food to a hungry worm. Worms that have been removed from food for 30 minutes are very sensitive to the presence of food; upon entering a bacterial lawn, they stop swimming, initiate rapid pharyngeal pumping, begin laying eggs, and suppress the contraction of the enteric muscles when they reinitiate the defecation cycle (B. Sawin, pers. comm.; E. Jorgensen, unpubl.). Application of exogenous serotonin can also induce these behaviors even in a well-fed worm. Bath application of serotonin stimulates pharyngeal pumping (Croll 1975b; Avery and Horvitz 1990), induces egg laying (Trent et al. 1983), causes sluggish locomotion, and inhibits enteric muscle contractions (Ségalat et al. 1995). In addition, hungry worms have altered chemotactic behaviors, and these alterations are abolished by the application of serotonin (C. Bargmann, pers. comm.). *cat-4* mutants lack serotonin and dopamine (Sulston et al. 1975; Desai et al. 1988; Weinshenker et al. 1995) and are defective for several of the behaviors that can be induced by serotonin application. These mutants pump slowly (Avery and Horvitz 1990) and are hyperactive (J. Kaplan; B. Sawin; both pers. comm.). Although these mutants are not egg-laying-defective, mutations in *cat-4* can enhance egg-laying defects in other mutants (Avery et al. 1993). Finally, *cat-2* mutants that express serotonin but not dopamine

still have enhanced sensitivity to food after starvation, suggesting that dopamine is not required for this behavior (B. Sawin, pers. comm.).

Serotonin probably acts humorally as well as synaptically to mediate behavior. It is most intensely expressed in two pharyngeal motor neurons, the NSMs, which appear secretory by morphology (Albertson and Thomson 1976; Horvitz et al. 1982). Killing the NSMs along with other serotonergic cells phenocopies at least some of the *cat-4* behavioral defects (B. Sawin, pers. comm.). Since NSM synapses are directed toward the nerve ring and do not directly synapse onto the cells that are likely to mediate these behaviors, the NSMs are probably acting at a distance. In other organisms, it is known that serotonin binds seven-pass transmembrane receptors that activate trimeric G-proteins. In *C. elegans*, serotonin acts via the $G_o$ GTPase. The $\alpha$-subunit of $G_o$ is encoded by the gene *goa-1* (Lochrie et al. 1991), and mutations in *goa-1* disrupt serotonin signaling in several behaviors, including locomotion and defecation (Mendel et al. 1995; Ségalat et al. 1995). Together, these results suggest a model in which a starved worm has very low levels of circulating serotonin; as a consequence, the animal is hyperactive. When it enters the bacterial lawn, serotonin is released humorally, and this induces a number of appropriate behaviors, including active pumping, restricted movement, and egg laying.

Octopamine antagonizes the effects of serotonin in lobsters (Kravitz 1990), but its functions in *C. elegans* are relatively unexplored. Octopamine is found in *C. elegans*, but the cells expressing octopamine have not been identified (Horvitz et al. 1982). In contrast to serotonin application, octopamine causes loopy or kinked locomotion and depresses egg laying and pharyngeal pumping. Phentolamine, an octopamine antagonist in invertebrates, stimulates egg laying. Thus, serotonin and octopamine appear to act antagonistically in *C. elegans* as they appear to do in other invertebrates, but the basic cell biology of octopamine neurotransmission has not yet been investigated.

### B. Peptidergic Neuromodulators

Neuropeptides can act as hormones, neuromodulators, or neurotransmitters (Krieger 1983). Although there is circumstantial evidence that neuropeptides can act independently and at a distance to modify the activity of many neurons, it is believed that most neuropeptides act in concert with a classical neurotransmitter to simply modify the output of the primary neurotransmitter on the postsynaptic cell (Cooper et al. 1991). This may make the study of neuropeptides by genetic methods rather difficult because mutants lacking a specific neuropeptide may have only

subtle changes in behavior unrecognizable in the rather crude behavioral screens that are presently practical.

The actions of modulatory peptides are only beginning to be explored in nematodes. Studies so far have concentrated on a single family of peptides, the FMRFamide-like peptides (FLPs). When *C. elegans* is stained with an antibody that recognizes all members of this family, about 10% of the neurons express FMRFamide immunoreactivity, including motor neurons and interneurons (Schinkmann and Li 1992). Some of these peptides are likely to originate from the gene, *flp-1*, that encodes multiple peptides ending with the amino acid sequence FLRF (Rosoff et al. 1992; see Rand and Nonet, this volume). One neuron class that expresses a FMRFamide-like peptide is the VC class of motor neurons. The VCs synapse to the ventral body muscles and the vulval muscles. Because egg laying requires the contraction of the vulval muscles, the effect of FLRFamide on egg laying was tested. Application of FLRFamide alone caused no change in egg laying, but it was capable of potentiating the induction of egg laying by serotonin. A genetic analysis of these peptides has been complicated by the discovery of at least four genes that could encode FMRFamide-like peptides (C. Li, pers. comm.).

An analysis of the role of peptides in nematodes has been more extensively carried out in the parasitic nematode *Ascaris*. Because of its large size, *Ascaris* is more amenable to electrophysiological analyses, and despite the disparity in size, the *Ascaris* and *C. elegans* nervous systems are remarkably similar (Stretton et al. 1985). In *Ascaris*, a large number of neuropeptides have been characterized by immunoreactivity (Stretton et al. 1991), and 12 peptides related to FMRFamide have been purified (AF1–12). Bioactivity of some of these peptides has been tested. AF1 abolishes the spontaneous oscillations of the ventral cord inhibitory motor neurons by reducing the input resistance of the membrane (Cowden et al. 1989). AF2 causes rhythmic contractions of the body muscle (Cowden and Stretton 1993), and AF4 induces continuous contraction of the body muscle (Cowden and Stretton 1995). Despite the extreme differences in lifestyle, the parasitic *Ascaris* and the free-living *C. elegans* nervous systems are remarkably similar in cell number, morphology, and neurotransmitter type. However, in *C. elegans*, less than 10% of the neurons are immunoreactive for FMRFamide-like peptides; in *Ascaris*, more than 60% of the neurons express FMRFamide-like immunoreactivity (Cowden et al. 1993). The differences in peptide distribution between these two species might be mechanisms to generate very different behaviors in nematode species that share an evolutionarily rigid nervous system (Stretton et al. 1991).

### C. Sensory Adaptation

A simple form of behavioral plasticity as a result of experience is sensory adaptation. Sensory adaptation is the decrease or fatigue of sensory neuron response following prolonged exposure to sensory input. In most cases, the sensory response recovers after the stimulus has been removed. In *C. elegans*, responses to soluble compounds (taste) and responses to volatile compounds (olfaction) show a decrease following prolonged exposure (Ward 1973; Dusenbery 1980b; Colbert and Bargmann 1995). Similarly, worms raised at a specific temperature will avoid other temperatures, but after 2 hours, they no longer avoid these novel temperatures (Hedgecock and Russell 1975). Olfactory adaptation is selective, i.e., an animal will not move toward an adapted odorant, but it will still move toward a novel odorant, even when the two odorants are sensed by the same olfactory neuron (see Bargmann and Mori, this volume). Mutational analysis has shown that the molecular mechanisms for adaptation differ for different odorants. Colbert and Bargmann (1995) characterized two mutants that show normal chemotactic responses to volatile compounds but fail to adapt to different subsets of odors mediated by the AWC neurons: *adp-1* mutants fail to adapt to benzaldehyde and butanone but adapt to isoamyl alcohol, and *osm-9* mutants do not fully adapt to isoamyl alcohol or butanone but adapt normally to benzaldehyde.

### D. Learning and Memory

Another way that behavior can change as a result of experience is through learning. Traditionally, theorists have divided learning into two categories: nonassociative and associative. Nonassociative learning occurs when an individual is exposed to a single type of stimulus and behavior is changed as a result of that exposure. Examples of nonassociative learning include habituation and sensitization. Like sensory adaptation, habituation is a simple decrement in response to a repeated stimulus, but it can be distinguished from sensory adaptation by a number of features (see below). Sensitization is an increase in response to a wide variety of stimuli following a noxious stimulus. Associative learning occurs when animals learn to link a stimulus or behavior with a second temporally associated stimulus. Associative learning includes classical conditioning and operant conditioning. The most prominent example of classical conditioning is Pavlov's experiments with dogs in which the animal learns to associate the ringing of a bell with food. In operant conditioning, an animal learns to associate one of its own behaviors with a stimulus. For example, in B.F. Skinner's classic operant conditioning experiments, a rat learns to press a lever for a reward of food.

Whereas learning is a change in behavior as a result of experience, memory is the ability to store and recall those changes to behavior. Research on both vertebrates and invertebrates has suggested that there may be a number of phases of memory (ranging from two in *Aplysia* to three in rats and birds to four in flies; for review, see DeZazzo and Tully 1995). Memory can last in these various phases from as short as seconds as is found in short-term memory or as long as hours to a lifetime as is found in long-term memory. The cellular and molecular mechanisms behind these phases of memory seem to be distinct. For example, long-term but not short-term memory can be disrupted by treatments such as electroconvulsive shock or inhibitors of protein synthesis (Davis and Squire 1984).

### *1. Habituation*

Perhaps the simplest and most ubiquitous form of learning is habituation, which is a decrease in a response to a given stimulus after repeated trials. Observations of worms that bumped into glass beads (Croll 1975a) or that had been touched with a fine hair (Chalfie et al. 1985) demonstrated that the backing response declined with repeated mechanosensory stimulation. However, to distinguish this decrement in response from sensory adaptation or fatigue, a number of features of habituation must be observed (Groves and Thompson 1970). For example, habituation occurs more slowly with more intense stimuli or with longer interstimulus intervals. Habituation can be built up with repeated training sessions. Habituation, sensory adaptation, and fatigue all diminish gradually with time, but the rate of recovery from habituation depends on the interstimulus intervals of training. Finally, only habituation can be rapidly abolished with the application of a novel or noxious stimulus in a phenomenon known as dishabituation.

One simple stimulus that can be used to study habituation in *C. elegans* is a controlled tap to the side of the petri dish (Rankin et al. 1990). Such a tap causes an animal that is motionless or moving forward to move backward. As taps are repeated, the average distance a worm moves backward decreases. Following habituation training, an electrical stimulus delivered to the agar on either side of the worm causes dishabituation, i.e., the shock restores the normal response to tap.

In other organisms such as *Aplysia* (Rankin and Carew 1987) and rat (Davis 1970), the speed and degree of response decrement are dependent on the interstimulus interval. Similarly, worms rapidly habituate to stimuli delivered at 10-second intervals but slowly and less completely to

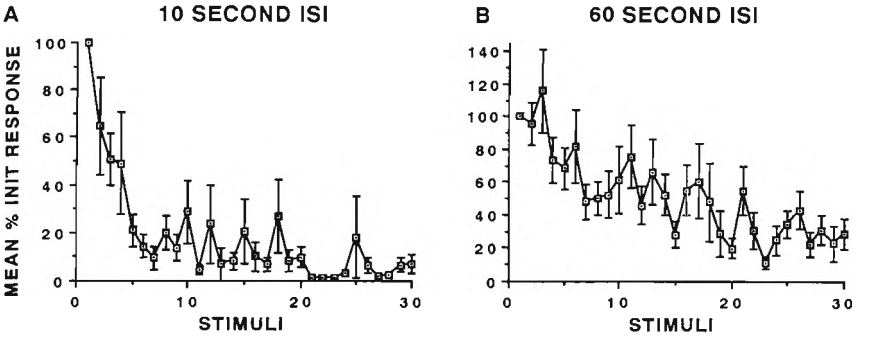


*Figure 4* Habituation in *C. elegans*. (*A*) Habituation of backing to trains of taps for 30 stimuli delivered at a 10-sec interstimulus interval (ISI). (*B*) Habituation of backing to trains of taps for 30 stimuli delivered at a 60-sec interstimulus interval. The shorter interstimulus interval (10 sec) produces greater and more rapid decrement than the longer interstimulus interval (60 sec). Habituation is expressed as the mean percent of the initial backing response (INIT). Bars indicate the standard error of the mean. (Modified, with permission, from Rankin and Broster 1992; copyright by the American Psychological Association.)

stimuli delivered at 60-second intervals (Fig. 4) (Rankin and Broster 1992). Spontaneous recovery from habituation is also dependent on the interstimulus interval. Worms recover more rapidly from habituation induced by short interstimulus intervals than they do from long interstimulus intervals (Rankin and Broster 1992). These data indicate that the different intervals are recorded in the nervous system at least 1 hour after the delivery of the last stimulus and can continue to influence behavior differentially. They also suggest that habituation to short and long stimulus intervals may recruit different cellular mechanisms.

Work with other organisms (e.g., *Aplysia*; Carew and Kandel 1973) has shown that with repeated habituation training sessions, two things happen: (1) a build up of habituation over blocks of trials and (2) the possibility of the formation of long-term memory. A paradigm used to study long-term habituation in *Aplysia* was modified and applied to *C. elegans* (Beck and Rankin 1995). In this paradigm, worms were given three blocks of 20 stimuli each and stimuli within blocks were delivered every 60 seconds, with an hour rest between blocks. To determine whether the training produced long-term habituation, these same worms were given a block of 20 stimuli at 60-second intervals on the second day. The results showed that there was a build up of habituation over the course of the three training blocks on the first day. In addition, the worms were capable of long-term memory as demonstrated by retaining memory of habituation training for at least 24 hours. This long-term memory was

disrupted by heat shock during the rest intervals (Beck and Rankin 1995). The current hypothesis is that heat shock disrupts cellular processes such as protein synthesis that are necessary for memory formation (Davis and Squire 1984).

Although habituation is a form of learning found in many organisms, surprisingly, little is known about the cellular processes underlying this form of learning. Studies in *Aplysia* (Bailey and Chen 1983) have suggested that there may be a decrease in the amount of neurotransmittor available for release in terminals that have undergone habituation training. Although studies in *C. elegans* have not yet progressed to the molecular level, *C. elegans* with its simple nervous system may offer new insights into the cellular mechanisms underlying habituation. Two interacting neural circuits are activated during response to head and tail touch (Chalfie et al. 1985; see Driscoll and Kaplan, this volume): Stimulation of the tail touch receptors activates the interneurons that direct the worm to move forward, and stimulation of the head touch receptors activate the interneurons that direct the animal to move backward. In response to tap, these neural circuits are activated simultaneously and the behavior results from an integration of two competing outputs (Wicks and Rankin 1995).

Given that the observed response to tap in intact animals is actually an integration of two competing responses, what is the response of each of the competing circuits alone to habituation training? The two circuits do not produce the same pattern of behavioral outputs in response to habituation training (Wicks and Rankin 1996). When the posterior touch neurons (PLM) are killed, the circuit that moves the animal backward can be viewed in isolation. In the operated animals, the reversals habituate more slowly and less completely than they do in the wild-type worms. Ablation of the anterior touch neurons ALM and AVM produce animals that respond to tap by accelerating forward. When such ablated animals are given habituation training, a different pattern of response was observed. With short interstimulus intervals (10 seconds), accelerations first increase in magnitude (sensitization) before habituating. With long interstimulus intervals (60 seconds), there is no evidence of sensitization. Again, the data suggest that habituation in unoperated animals is the result of a balance of two competing behaviors: reversals and accelerations. For example, with short interstimulus intervals, animals rapidly habituate within the first few stimuli. This rapid habituation may reflect the increased input of the sensitized accelerations that decrease the magnitude of the reversals. As habituation continues, reversals become infrequent and the animals often accelerate forward in

response to tap. Presumably, the rapid habituation of reversals and the slower habituation of accelerations are integrated as a net movement forward. These results suggest that there might not be a single mechanism underlying habituation, nor might all cells involved in a behavior respond in the same way to repeated stimulation. Instead, each cell type may have a unique response to repeated stimulation, and the behavior that is observed is the integrated output of all of the cell types.

In the future, genetic analyses of the mechanisms involved in the long- and short-term memory phases of habituation should lead to additional insights into the similarities and differences between memory processes in this simple nervous system and in more complex organisms such as *Drosophila*, *Aplysia*, and mammals.

### 2. *Sensitization*

A second form of nonassociative learning is sensitization (Groves and Thompson 1970), which refers to the increase in reflexive responses due to the application of a noxious stimulus. Sensitization is not a form of associative learning because the stimulus is not specifically paired with another stimulus. The stimulus merely raises the arousal level of the animal so that all reflex pathways are facilitated. Sensitization in *C. elegans* has been demonstrated in several ways, but it has not been investigated to the same extent as habituation. Sensitization was first shown by presenting worms with a single tap, then a stronger stimulus, in the form of trains of taps, and then looking at the response to a single tap again (Rankin et al. 1990). Worms showed larger responses to the single tap following the train of taps than they did to the initial single tap.

### 3. *Associative Learning*

In the simple nonassociative forms of learning, an animal alters its behavior to a single stimulus; in contrast, in associative learning, an animal learns to use a previously neutral stimulus to predict the presence or absence of a second more significant stimulus. *C. elegans* is capable of this more advanced form of learning as demonstrated by several different paradigms. Examples of associative learning by *C. elegans* come from classical conditioning paradigms in response to chemosensory stimuli (J.Y.M. Wen et al., in prep.). In this discriminative classical conditioning assay, one ion is associated with food and a second ion is associated with the absence of food; the conditioned animals will then selectively migrate to the ion paired with food. First, adult hermaphrodites are deprived of food for 5 hours, and then an ion, either sodium or chloride,

is presented to the animals with bacteria for the first hour and the other ion is presented without bacteria for a second hour. In the test phase, the animals are then given a choice between diffusive gradients of sodium and chloride for 1.5 hours. The results show that conditioned animals display significant preference for the ion paired with food and that the preference lasts up to 7 hours after training (Fig. 5). Switching the order of the conditioning stimuli did not affect the results. Presentation of the ion paired with no food before presentation of the ion paired with food resulted in identical degrees of learning.

Learning can be assayed in individual animals as well. In this paradigm, worms are conditioned in liquid medium in test tubes containing solutions of the ions and *Escherichia coli.* To test for learning, individuals are placed on test plates with a gradient of each of the ions, and the initial heading of the worm is assayed. Conditioned animals show initial headings similar to their final accumulation; thus, learning can be assayed within 30 seconds, rather than waiting the 1.5 hours required in the chemotaxis assay.

*C. elegans* can also learn aversive associations. In this type of experiment, an ion, such as sodium or chloride, is paired with a noxious stimulus, and thereafter the worms avoid the conditioned ion. For example, adult hermaphrodites can first be conditioned with an ion associated with an aversive stimulus such as garlic. Subsequently, when tested in a chemotaxis assay, these animals avoided the ion that had been paired with garlic (Fig. 5C). Aversive learning can also be tested in individuals as well as in populations. In this kind of experiment, worms are exposed to an attractive ion and the aversive stimulus, for example, copper ion. To test for learned aversion, conditioned worms are placed on a spot containing the paired or unpaired ion in the absence of copper, and the time required for the animal to leave the spot is measured.

The eventual goal is to employ genetic techniques in *C. elegans* to identify molecules essential for learning and memory. Having established that *C. elegans* shows discriminative classical conditioning in a variety of paradigms, van der Kooy and colleagues screened for mutants defective in associative learning (J.Y.M. Wen et al., in prep.). They isolated two lines of ethylmethanesulfonate (EMS)-induced learning-deficient mutants that show normal chemosensory responses but no evidence for classical conditioning in any of the discriminitive classical conditioning paradigms. These mutations define two loci, *lrn-1* and *lrn-2* (learn).

Another possible case of associative learning is a phenomenon first described by Hedgecock and Russell (1975) who demonstrated not only that *C. elegans* could detect thermal gradients and selectively migrate

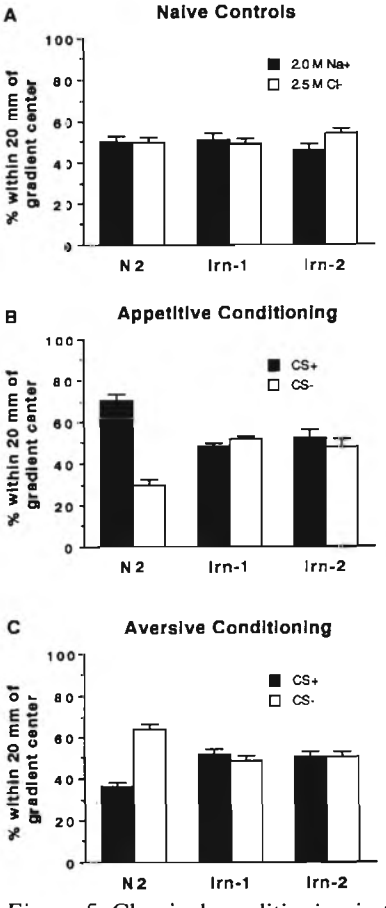


*Figure 5* Classical conditioning in the *C. elegans* wild type and in *lrn-1* and *lrn-2* mutants. (*A*) Both wild-type and mutant lines show the same response levels in an unconditioned (without training) discrimination test. (*B*) In appetitive conditioning, one ion is paired with *E. coli* (CS+) and the other ion without *E. coli* (CS–). Wild-type animals show conditioning by preferring the CS+; neither mutant strain shows a preference for the CS+. (*C*) In aversive conditioning, the CS+ ion is paired with garlic extract. Wild-type worms show conditioning by preferring the CS– ion; neither mutant strain avoids the CS+ ion. Bars indicate the standard error of the mean. (Modified from J.Y.M. Wen et al., in prep.)

along an isothermic contour, but also that such thermotaxis could be modified by experience. The temperature at which the animals were raised and fed determines the temperature to which they migrate. A brief starvation of 2 hours induces strong dispersion from the starvation temperature; a 4-hour period of starvation decreases these responses

(Hedgecock and Russell 1975; Mori and Ohshima 1995). Although it is possible that dispersion from the starvation temperature involves a learned association between a specific temperature and a lack of food, new data indicate that starvation may simply suppress thermotaxis (see Bargmann and Mori, this volume).

## IV. CONCLUSIONS

For such a simple organism, *C. elegans* shows a great deal of behavioral plasticity. The conclusion that one must draw is that behavioral plasticity is an important component in the everyday life of a worm. Once well-defined learning paradigms become established in *C. elegans*, genetic analysis in this organism may resolve several long-standing issues in our studies of learning and memory. First, what are the molecules that mediate neural plasticity? Genetic analysis of associative learning, combined with studies of the proteins involved in neurotransmission (see Rand and Nonet, this volume), will identify the molecular substrates of synaptic strengthening and weakening. Second, does long-term potentiation (LTP) equal learning? A long-standing debate in the field of learning and memory has been whether the mechanisms that underlie cellular models of learning such as LTP reflect the mechanisms used in the formation of memory as measured in behavioral assays. Genetic analysis can circumvent this dispute because the initial criteria for selecting a mutant will be a demonstrable defect in a learning task. Third, is plasticity in the adult related to plasticity in the embryo? Genetic studies may reveal that the mechanisms that establish connectivity in the embryo are the same as those that modify behavior in the adult.

Studies in *C. elegans* may eventually link the different levels at which plasticity is measured, so that we will have a mechanistic understanding of changes in behavior. Such experiments will demonstrate how changes in the behavior of an animal are caused by physiological or morphological changes in a neural circuit. In turn, the changes in the neural circuit can then be correlated with the activity of molecules in individual cells.

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