The Journal of Neuroscience, March 1995, 15(3): 2434–2444

Integration of Mechanosensory Stimuli in *Caenorhabditis elegans*

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The tap withdrawal reflex in *Caenorhabditis elegans* demonstrates various forms of nonassociative learning. A first step in determining the cellular mechanisms of this learning is to identify the neuronal circuitry that underlies this reflex. Studies by Chalfie et al. (1985) have defined the touch-circuit that mediates the response to a stimulus related to tap—a light touch. We used the touch circuit as a starting point in the identification of the tap withdrawal circuitry. Here we report the effects of lesions of identified neurons on the tap withdrawal reflex. Ablations of the sensory neurons and interneurons of the touch circuit produce effects on the tap withdrawal response that generally confirm and expand upon the roles of these cells in mechanosensory integration as proposed by Chalfie et al. (1985). However, no role for the LUA interneurons could be identified in the production of the tap withdrawal response. Furthermore, the effects of ablating some neurons outside the touch circuit suggest roles for two of these cells in the integration of the tap withdrawal response. Ablation of either the midline neuron DVA or the PVD neurons resulted in a decrease in both the frequency and magnitude of reversals that were elicited by tap. Additionally, the ablation of either cell decreased the magnitude of accelerations produced by animals in response to tap.

[Key words: mechanosensation, circuitry,* Caenorhabditis elegans*, habituation, laser ablation, tap withdrawal, touch]

A key step in the analysis of the neural mechanisms underlying learning and memory is the delineation of the neural circuitry that mediates the plastic response. Adult *Caenorhabditis elegans*, when subjected to a vibratory stimulus applied through the medium upon which they locomote, will swim backward. This response, termed the tap withdrawal reflex (Chiba and Rankin, 1990), shows habituation of both magnitude and frequency in response to repeated stimulation. The tap withdrawal reflex also shows sensitization, dishabituation, long-term (24 hr) retention of habituation training (Rankin et al., 1990), and context conditioning (Rankin, 1993).

Received Apr. 15, 1994; revised Oct. 11, 1994; accepted Oct. 12, 1994.

This work was supported by an NSERC scholarship to S.R.W. and NSERC and HSF operating grants to C.H.R. Some nematode strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). We thank M. Chalfie and J. Thomas for discussions. We also thank Chris Beck, Cathy Chiba, and Emma Wood for editorial comments, Terry Zulynik and Richard Faber for data acquisition, and two anonymous reviewers for insightful comments on an earlier version of the manuscript.

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In many systems (e.g., *Aplysia*, Castellucci et al., 1970; *Hermissenda*, Farley et al., 1983; *Limax*, Prior and Gelperin, 1977; *Drosophila*, Han et al., 1992), circuitry has been identified using anatomical mapping techniques. Several unique characteristics of the nematode *C. elegans* make the anatomical identification of the circuitry underlying a given reflex straightforward. First, the adult nematode possesses only 302 neurons, all of which have been completely described in terms of their location and synaptic connectivity (White et al., 1986; Hall and Russell, 1991; Achacoso and Yamamoto, 1992). Second, the developmental lineage of each of these cells has been traced from the zygote. Thus, a complete spatiotemporal map of the nervous system is available (Sulston and Horvitz, 1977; Sulston et al., 1983). Finally, the worm is amenable to single-cell laser microsurgery (Sulston and White, 1980). Using this technique, individual neurons can be destroyed with little or no damage to the remaining nervous system.

The neural circuitry that mediates the tap withdrawal reflex can be identified by ablating putative circuit cells and noting the effects of the ablation on the animal's withdrawal reflex (Wicks and Rankin, 1992). Once the role of a cell in the reflex has been established via laser ablation, other candidate cells can be identified on the basis of their connectivity. With a nervous system of 302 neurons, it should be possible to investigate the roles of all candidate neurons. Neural circuits underlying behaviors as diverse as pharyngeal pumping, chemotaxis, and mechanosensation have been identified using this technique (Chalfie et al., 1985; Avery and Horvitz, 1989; Bargmann et al., 1990; Kaplan and Horvitz, 1993). Perhaps the best-described example of circuit analysis in the worm is the delineation of the touch withdrawal circuit, which mediates head-touch-induced forward movement and tail-touch-induced backward movement (Chalfie and Sulston, 1981; Chalfie et al., 1985; Fig. 1). Since both touch and tap are mechanical stimuli, and since a pilot study involving genetic lesions of the touch circuit showed altered response to tap (Rankin and Chalfie, 1989), the touch withdrawal circuit served as a starting point in our analysis of the tap withdrawal circuitry.

In this study we have defined the roles of the interneurons and sensory neurons of the touch withdrawal circuit in the production of the tap withdrawal response. In addition, results from the ablation of a number of neurons outside the touch circuit suggest a possible role for some of these cells in the tap withdrawal reflex.

A preliminary report of these findings has been presented in abstract form (Wicks and Rankin, 1992).

Materials and Methods

Cell designations. All cell classes are described using the classification of White et al. (1986). Unless otherwise noted, all references to a par-

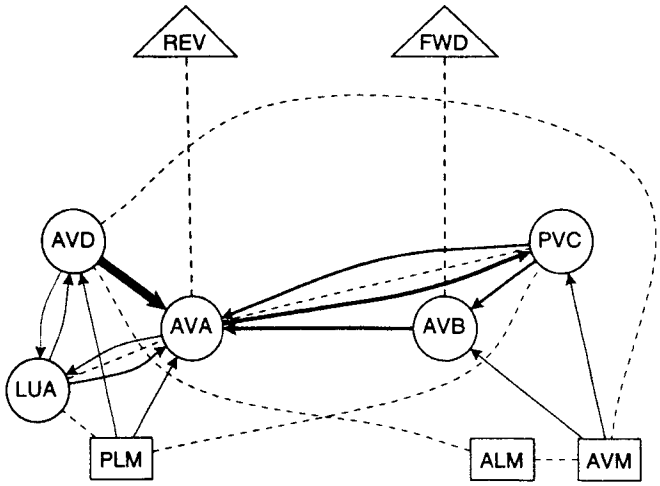

Figure 1. The touch withdrawal circuit. This circuit consists of five sensory neurons (*squares*), five pairs of interneurons (*circles*), and two motoneuron pools (not shown) and divides the animal into two somatosensory fields. Anterior touch input is largely transduced by ALM and AVM, whereas posterior touch is transduced by PLM. The LUA cells act as connectors between PLM and the interneuronal level in this circuit. The AVD and PVC classes act as head- and tail-touch modulators, whereas the AVB and AVA act as forward and backward movement driver interneurons, respectively. All cells are bilateral except AVM, which along with the AS motoneurons represent the only cells in this circuit that arise postembryonically. Chemical connections are indicated by *arrows*, with the number of synaptic contacts being proportional to the *arrow width* (for reference, the connection between AVD and AVA represents approximately 140 chemical connections). Gap junctions are indicated by *dashed lines*. Pools of motor neurons drive forward (*FWD*) and backward (*REV*) motion. Not all connections are shown. Adapted from Chalfie et al. (1985).

ticular cell class (e.g., ALM) refer to a pair of bilaterally symmetric cells. Reference to a group of animals with one or more names of particular cell classes followed by a negative sign (e.g., ALM$^-$) indicates that all members of the indicated classes were ablated in the group and that all other cell classes were left intact.

Subjects. A total of 590 hermaphroditic *C. elegans* Bristol (N2) were used. Animals were originally obtained from the Caenorhabditis Genetics Center and synchronously grown on Nematode Growth Medium agar seeded with *E. coli* (OP50) as described by Brenner (1974). For laser studies, highly synchronous animals were obtained by washing large numbers of eggs and adults in M9 buffer solution followed by washing the animals in an alkaline hypochlorite solution (as described in Wood, 1988, for the cleansing of infected colonies). The resulting solution was spun down in a tabletop centrifuge. The pellet was washed and resuspended in a drop of buffer and spread on an unseeded plate. After 2–3 hr, larval worms were collected for ablation.

In addition, 60 mutant animals [20 each of *cat-1* (e1111)X, *cat-2* (e1112)II, and the double mutant *cat-2*(e1112)II;*cat-1*(e1111)X] were analyzed. For these mutant studies, 4-d-old mutant animals (as well as 20 N2 control animals) were isolated from synchronous colony plates and transferred to testing plates. Each animal was allowed to recover from the transfer for at least 1 min prior to the application of a single tap stimulus.

Apparatus. Laser pulses were delivered by a VSL-377 nitrogen laser (Laser Science, Inc., Cambridge, MA). The beam was directed through a laser dye module (Laser Science, Inc., Cambridge, MA) containing a coumarin 440 dye (Laser Science, Inc., Cambridge, MA) that reemitted with a peak gain of 437 nm. Single-cell ablations were performed under a 100× oil immersion lens mounted on a Ziess Axioskop equipped with Nomarski (differential interference contrast) optics (Carl Zeiss Canada). The beam was directed down through the optics of the microscope with a semisilvered mirror and targeted into the plane of optical focus with a beam expander (Laser Science Inc., Cambridge, MA).

All behavioral testing was done by observing worms on petri plates filled with 10 ml of NGM agar, under a stereomicroscope (Wild M3Z, Wild Leitz Canada). All behavior was recorded by a video camera (Panasonic Digital 5100) attached to a VCR (Panasonic AG1960) and monitor (NEC). A time-date generator (Panasonic WJ-810) was used to superimpose a digital stopwatch and time-date display on the video record. Taps (force of 1–2 newtons) were delivered to the side of the plate as described previously (Rankin, 1991) with the following modifications. Each animal was raised on an individual plate seeded with *E. coli* and tested only once, 4 d after ablation. Handling was minimized by assessing an animal's behavior on the same plate upon which it was raised. Animals from the three mutant strains studied [*cat-1* (e1111)X, *cat-2* (e1112)II, and the double mutant *cat-2*(e1112)II;*cat-1*(e1111)X] as well as 20 control N2 animals were placed on testing plates (no food present) just prior to testing.

Scoring. In response to tap, animals either reversed (swam backward) through some distance or accelerated (swam forward more rapidly), depending on their complement of cells. Response magnitude was quantified by tracing the path of the response using stop-frame video analysis onto acetate sheets. The length of the traces were then digitized into machine readable form on a Macintosh computer using a bitpad and MACMEASURE software. Acceleration magnitude was assessed by subtracting the worm's velocity immediately prior to the tap from its velocity 1 sec after the tap. Velocity in these experiments was defined as the distance through which the worm moved in a 1 sec interval. Frequency measures were taken by counting the number of animals performing a given response type.

Analysis. Reversal magnitude data were analyzed by first expressing the length of all reversals that occurred in response to a single tap stimulus as a percentage of the individual worm's body length. This standardized measure was then compared across groups using a factorial ANOVA with Fisher's PLSD post hoc tests (STATVIEW, Abacus Concepts, Inc., Berkeley, CA). Any animals that did not demonstrate a reversal to a single tap were not included in the calculation of group means. If the animal paused in response to tap, its reversal magnitude was zero. Acceleration magnitudes were compared with a t test on the mean of the first eight responses during habituation (a decrement in responding not due to motor fatigue or sensory adaptation). All frequency data were expressed as the fraction of worms reversing and analyzed with the χ^2 statistic.

Procedure. Single-cell laser ablations (Sulston and White, 1980; Chalfie and Sulston, 1981; Avery and Horvitz, 1989) were conducted by mounting highly synchronous animals (about 10 at a time) in a small volume of sterile M9 buffer (<1 μl) on a wet agar pad containing 10 mM sodium azide (an anesthetic; Wood, 1988). Animals were covered with a 12 mm round glass coverslip sealed at the edges with Vaseline. Control animals were left under the microscope for approximately 45 min before being removed. Experimental animals were subjected to cellular ablations (bilateral where appropriate) before being recovered. The intensity of the laser beam was attenuated by interposing glass microscope slides between the laser and the microscope such that when the beam was focused in the plane of the coverslip it would just barely damage the glass coverslip (this intensity was ideal in that single laser pulses did little damage to a cell, but repeated pulses would destroy neurons). All damage was monitored visually. Any animals in which the damage was considered either incomplete or extraneuronal, as well as any animals in which the targeted cell was not clearly identifiable were destroyed. All cells were ablated in early L1, within 3 hr of hatching. All ablations were performed at the same stage in the development of the animal to control for the nonspecific effects of anesthesia, handling, and food density on the testing plates. As a consequence, portions of the nervous system were still developing at the time of ablation. Some cells, derived from postembryonic blast cells, were not yet present at the L1 larval stage. These cells included AVM (nonbilateral, ablated Q_R), PVM (nonbilateral, ablated Q_L), PHC/PVN/PLN (ablated T), and PVD/PDE (ablated V5). Thus, AVM (nonbilateral), for example, was ablated in its precursor form by destroying the Q_R blast cell prior to cell division. All animals were recovered from the microscope slide and placed on individual agar plates seeded with OP50 *E. coli* within 1 hr of initial anesthesia and placed in a 20°C incubator. Approximately 25% of the animals were remounted without anesthesia 2–3 hr later and checked to ensure that the target cell was destroyed. Although the target neuron(s) was destroyed in all of these animals, two worms were eliminated from the study due to initially undetected damage to adjacent cells.

Behavioral testing of ablation animals was done on the same plates on which the animals were isolated, shortly after the onset of egg laying at between 3 and 4 d posthatching. Reversals were assessed by mea-

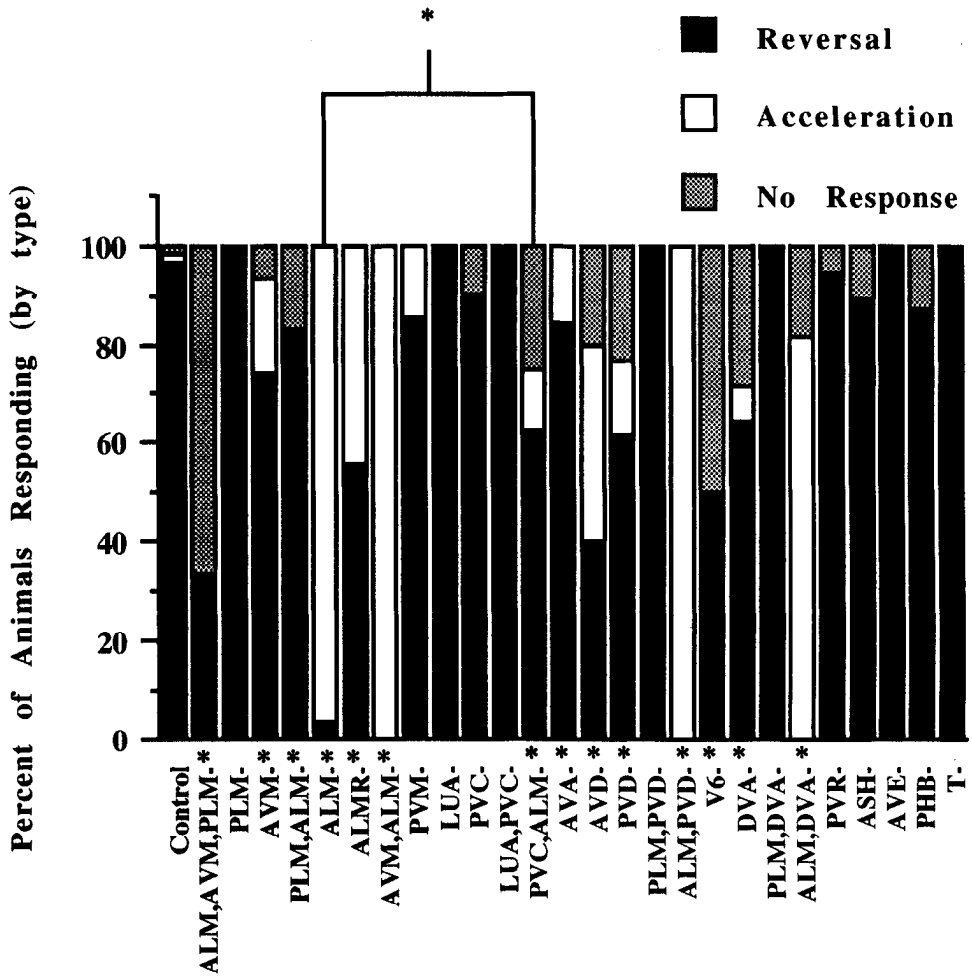

Figure 2. Changes in the frequency of response type as a consequence of ablation. This graph summarizes the effect of a variety of cellular ablations on the type of response elicited by a tap stimulus. Animals were scored either as not responding to a tap (*gray bars*), accelerating in response to tap (*white bars*), or reversing in response to tap (*black bars*). An *asterisk* beside the group name indicates a significant change in the percentage of reversals in response to a tap compared to control condition ("acceleration" and "no response" conditions were combined for this analysis). Note that PVD arises from the V5 lineage and that AVM arises from the Q_R lineage and that these two cells were removed by ablating the respective blast cell precursors.

suring the magnitude of each animal's response to a single tap. The measurement of accelerations involved measuring the magnitudes of responses during trials with repeated stimulation to test for habituation. These animals received tap stimuli at a 10 sec interstimulus interval (ISI). The mean of the first eight responses was calculated for each animal and used for comparisons.

Results

Experiment 1: effects of touch cell ablations

Chalfie and Sulston (1981) described five specialized microtubule sensory neurons that transduced the touch stimulus used in their experiments (a gentle touch applied with a hair to either the head or tail of the animal). These are divided into a bilateral pair of tail-touch cells (PLM), a bilateral pair of head-touch cells (ALM), and a single midline head-touch cell (AVM). A sixth midline microtubule cell (PVM) did not appear to play any significant role in the transduction of the touch stimulus (Chalfie and Sulston, 1981; Chalfie et al., 1985). These sensory neurons may transduce both the touch stimulus and the tap stimulus used in this analysis. The touch withdrawal circuit analysis may not, however, in itself be sufficient to describe the tap withdrawal reflex. The cells that mediate the touch withdrawal response are not necessarily the same as those that mediate the tap withdrawal response and the roles of cells common to both responses may be different. Although it has been noted that a tap stimulus does not elicit movement in touch insensitive mutants (Chalfie and Sulston, 1981; Chalfie and Au, 1989), touch and tap are distinct stimuli. The tap stimulus is diffuse and nondirected. It would presumably activate both the head- and tail-touch subcircuits simultaneously and therefore activate competing excitation and inhibition in the two subcircuits (Rankin, 1991; Wicks and Rankin, 1991). Touch, on the other hand is a relatively intense and highly directed stimulus. The ethological significance as well as the neural substrates of the two stimuli are likely related, but not identical. The tap withdrawal response in the intact animal is sensitive to neural rewiring during development (Chiba and Rankin, 1990) whereas the touch withdrawal behavior in the intact animal shows no developmental change, despite the observation that some of the cells in the touch circuit do arise postembryonically. Additionally, response to tap has been more amenable to quantitative analysis than response to touch, because the tap is a repeatable, mechanically delivered stimulus, making it possible to reliably evaluate the magnitude of the withdrawal reflex. The measurement of response magnitude has proven to be a more sensitive measure of the relative roles of cells in this circuit than the frequency measure.

The first step in our analysis was to determine whether the tap stimulus was being transduced by the touch cells. Ablation of all five touch cells resulted in animals that generally did not respond to tap; however, two animals did respond with very small reversals. The animals ($n = 6$) showed a significantly lower frequency ($\chi^2 = 32.91, p < 0.0001$; see Fig. 2; Table 1, Touch cells) of reversal response to tap, and the size of those reversals that occurred were significantly smaller than responses of control

Table 1. Summary of ablation results: touch cells and interneurons

Ablation	(*n*)	Compared to	Reversal frequency	Response magnitude	Notes
Touch cells					
ALM,AVM,PLM	(n = 4)	Con	Decrease	Decrease	Slightly sensitive to anterior mechanosensory input
PLM	(n = 16)	Con	Increase	Increase	Always reverses
PLML	(n = 7)	Con	Increase	No change	Cell makes gap junctions only
PLMR	(n = 13)	Con	Increase	Increase	Makes all chemical connections
ALM	(n = 19)	Con	Decrease	NA	Always accelerates
ALMR	(n = 9)	Con	Decrease	NA	Accelerates half the time
AVM (Q_R Blast)	(n = 18)	Con	Decrease	Decrease	Q_R blast cell ablated
ALM,AVM	(n = 13)	Con	Decrease	NA	Always accelerates
		ALM	No change	Increase	Larger accelerations than ALM$^-$
Interneurons					
LUA	(n = 16)	Con	No change	No change	
LUA,PVC	(n = 10)	PVC	No change	No change	
LUA,PLMR	(n = 14)	PLMR	No change	No change	
PVC	(n = 20)	Con	No change	No change	Always reverses
PVC,ALM	(n = 6)	ALM	Increase	NA	
AVD	(n = 4)	Con	Decrease	Decrease	
AVA	(n = 11)	Con	Decrease	NA	Animal is backward Unc
AVB	(n = 1)	Con	No change	NA	Animal is forward Unc

The number of animals in each ablation group used to quantify the response to a tap stimulus is shown, along with a summary of the results of those ablations for the sensory neurons (touch cells) and the interneurons of the touch circuit described by Chalfie et al. (1985). NA, not applicable; Con, control group; Unc, uncoordinated.

animals (F = 5.48, p = 0.005; Fig. 3*A*). These results suggest that the tap stimulus is largely transduced by the five cells that Chalfie et al. (1985) described. However, there does appear to be some residual anterior input in the absence of these five cells that is sometimes sufficient to produce a reversal response. An electron microscopic reconstruction of the anterior sensory anatomy suggested that there were several cells in the tip of the head that could be mechanosensory in nature (Ward et al., 1975). Chalfie and Sulston (1981) noted some residual touch sensitivity in the tip of the head after ablation of the head touch receptors (ALM and AVM). This observation was later confirmed and expanded upon by Kaplan and Horvitz (1993), who identified other mechanosensory receptors in the head by laser ablation that were distinct from the touch cells.

Animals in which the head-touch receptor ALM was bilaterally removed (n = 27) accelerated rather than reversed in response to the tap (Fig. 2). Thus, in the absence of the ALM cells, the posterior input to the tap withdrawal circuit predominated and the animals accelerated forward. The ablation of the third remaining head-touch receptor alone (AVM, n = 29) resulted in a significant reduction in the frequency of reversal to tap (χ^2 = 9.763, p = 0.002; Fig. 2); AVM$^-$ animals occasionally accelerated rather than reversed. When AVM was ablated in addition to ALM in the same animal (n = 28), these animals, like ALM$^-$ animals, always accelerated in response to tap (Fig. 2). Animals lacking the PLM cells (n = 35)—the only tail-touch receptors described by Chalfie et al. (1985)—always responded to a tap stimulus with a reversal.

An analysis of the response magnitude produced by animals lacking touch cells further clarified the roles of these cells. In the absence of the tail-touch cells (PLM), the reversals elicited by tap were significantly larger than control reversals (n = 16, F = 5.48, $p < 0.0001$; Fig. 3*A*). The ablation of AVM also had a large effect on reversal magnitude, but in the opposite direction; AVM$^-$ animals reversed a shorter distance than did controls (n = 23, F = 5.48, $p < 0.0001$; Fig. 3*A*). This effect may be due to the loss of gap junction input to ALM and AVD from AVM and/or the loss of putative inhibitory input (Chalfie et al., 1985) onto the AVB and PVC cells.

To further assess the role of AVM in the tap-withdrawal response, two additional ablations were performed. First, to determine whether AVM itself was capable of supporting a reversal response, the other two pairs of touch cells (PLM and ALM) were removed. Although animals in this group (PLM,ALM$^-$, n = 8) responded with reversals to a single tap with a frequency equivalent to controls (χ^2 = 0.135, p = NS), the magnitude of reversals produced was much smaller than control responses (F = 5.48, p = 0.0036; Fig. 3*A*). Second, as reported above, ALM and AVM,ALM ablations resulted in animals that tended to accelerate forward rather than reverse in response to tap. If AVM is actively inhibiting the posterior tap-response circuitry, the coablation of AVM and ALM in the same animal should produce larger accelerations than the ablation of ALM alone. However, the interpretation of the mean magnitude of acceleration to a single tap is complicated by a ceiling effect inherent in the measure used, as animals are already accelerating at near maximal levels to the tap stimulus. We therefore analyzed the magnitude of accelerations as the animals habituated, taking a mean acceleration of the first eight responses in a habituation protocol, reasoning that the measure of response magnitude in a decremented state may reveal group differences concealed by the ceiling effect. The mean magnitude of the first eight accelerations produced in response to tap stimuli delivered at a 10 sec interstimulus interval (ISI) was significantly smaller for ALM$^-$ animals (n = 19) than for AVM,ALM$^-$ animals (n = 13, F = 11.78, p = 0.0031; Fig. 3*B*). A comparison of the rates of ha-

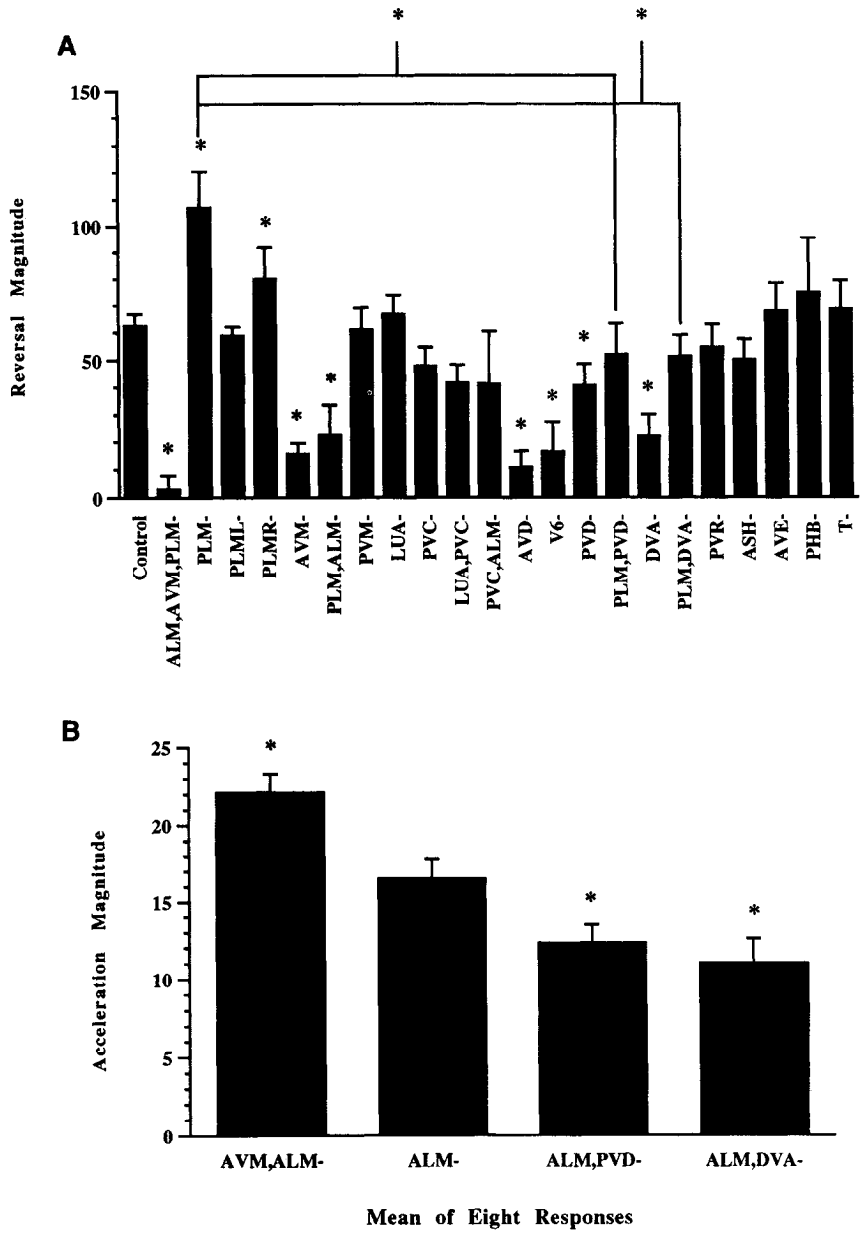

Figure 3. Changes in the tap withdrawal response magnitude as a consequence of ablation. The effects of cellular ablations on the magnitude of reversals (*A*) and accelerations (*B*) produced in response to a tap stimulus are shown standardized to the length of the worm. *A*, Significant differences in the reversal magnitude between an ablation group and the control group (*far left bar*) is indicated with an *asterisks* over that group. Two other comparisons were made, between PLM$^-$ and PLM,PVD$^-$ and between PLM$^-$ and PLM,DVA$^-$, and are indicated by the *bracketed lines*. Of the cells not in the touch withdrawal circuit, only two are implicated in response to tap on the basis of the data presented here: PVD and DVA. *B*, The accelerations were measured by taking the mean of eight stimuli (see Materials and Methods). The addition of an AVM ablation to ALM$^-$ animals resulted in larger accelerations than ALM$^-$ alone, whereas the addition of either a PVD or a DVA ablation to ALM$^-$ animals resulted in smaller accelerations than ALM$^-$ alone. Note that PVD arises from the V5 lineage and that AVM arises from the Q_R lineage and that these two cells were removed by ablating the respective blast cell precursors. Error bars indicate SEM.

bituation of these two groups yielded no significant differences; thus, the differences in mean magnitude were not the result of differences in the rate of habituation (using a within-subject ANOVA, $F = 0.821$, p = NS). The detailed effects of ablation of tap withdrawal circuit elements on the reflex habituation dynamics will presented in detail elsewhere (S. R. Wicks and C. H. Rankin, unpublished observations).

Ablation of the PVM cell ($n = 28$) had no effect on either the magnitude ($F = 5.48$, p = NS; Fig. 3*A*) or frequency ($\chi^2 = 3.121$, p = NS; Fig. 2) of the tap withdrawal response. No evidence for its involvement in mechanosensory transduction could be identified.

We also investigated whether we could demonstrate an effect of the ablation of a single PLM or single ALM cell. Chalfie and Sulston (1981) were unable to demonstrate an effect of removal of a single posterior sensory neuron (PLM) with a touch assay. The two PLM cells make asymmetric connections with the interneurons in the circuit and are not gap junctioned with each other. Both cells make gap junctions with other cells in the tap circuit; however, only the right PLM sensory neuron makes chemical connections with the interneurons in the tap circuit (White et al., 1986; Achacoso and Yamamoto, 1992). Ablation of PLML ($n = 7$) had no effect on the magnitude of reversals produced (one-tailed t test, $t = 0.337$, p = NS; Fig. 4). In contrast, the removal of PLMR ($n = 13$) resulted in animals that responded to tap with larger reversals than control animals (one-tailed t test, $t = -1.718$, $p = 0.046$; Fig. 4). This result is consistent with the hypothesis of Chalfie et al. (1985) that the chemical connections from the sensory neurons are functionally inhibitory.

When we ablated a single ALM cell ($n = 9$) (we only ablated ALMR; the connectivity data do not suggest a functional asymmetry of the anterior touch cells) we found that the animal's pattern of behavior was intermediate between the control pattern of reversals and the pattern of accelerations seen in bilateral ALM$^-$ animals. Of the nine ALMR$^-$ animals tested, five re-

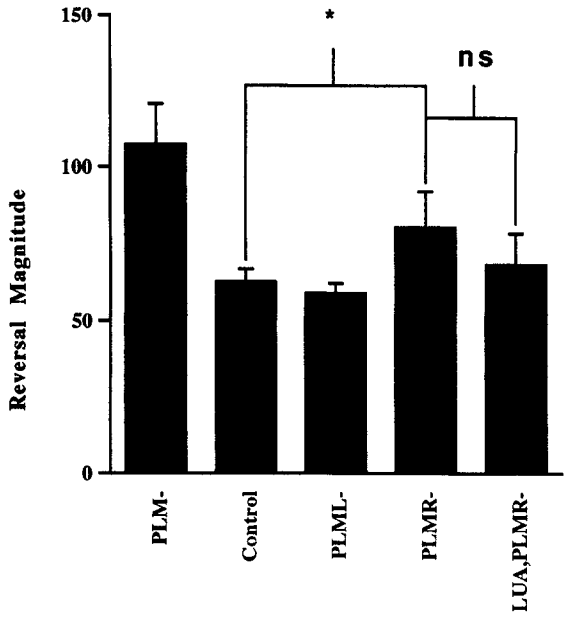

Figure 4. Effects of unilateral tail touch cell ablation on the tap withdrawal response magnitude. The ablation of PLML had no effect on the reversal response magnitude, whereas the ablation of PLMR resulted in a slight increase in reversal magnitude evoked by a tap stimulus. Removal of LUA in addition to PLMR, had no further effect on the reversal magnitude elicited by tap. Error bars indicate SEM.

sponded to a tap with a reversal and four animals accelerated ($\chi^2 = 56.23$, $p < 0.0001$). Thus, there appears to be a limited form of redundancy inherent in the bilaterality of the anterior touch cells; a single ALM cell is capable of supporting a reversal response to tap, but does not do so as effectively as the bilateral pair of cells.

These results suggest that directed sensory input to the tap withdrawal circuit can be divided into three components: a posterior component completely mediated by the posterior touch cell class PLM, an anterior component carried by the two anterior touch cell classes ALM and AVM, and a small anterior component mediated by as yet unidentified cells. In general, removal of either PLM or ALM/AVM biases the animal such that its response is dominated by the other input. These antagonistic subcircuits may compete to produce the animal's behavior. It is not the case that a lesion results necessarily in the reduction of a behavior (indeed, PLM ablations result in an increase in response magnitude), but rather that behavior is composed of a number of competing subcomponents and an alteration in the circuitry mediating the behavior alters which subcircuit is expressed in behavior at any one time.

Experiment 2: the effects of interneuronal ablations

Based on the analyses of Chalfie et al. (1985), the five pairs of interneurons described in the touch circuit can be placed into three classes. The first class of interneurons—consisting of the AVD interneurons in the head touch subcircuit and the PVC interneurons in the tail touch subcircuit—are not required for spontaneous locomotion. Rather, they are required for normal modulation of locomotion by head- and tail-touch, respectively. In the absence of PVC, animals are tail-touch insensitive; in the absence of AVD, animals are head-touch insensitive [although Chalfie et al. (1985) recognized that animals do regain some head touch sensitivity when the cell AVM makes functional synapses late in larval development]. The second class of interneurons—consisting of the AVA interneurons in the head touch subcircuit and the AVB interneurons in the tail touch subcircuit—make electrical connections with the motor neurons required for backward and forward motion, respectively (see Fig. 1). In the absence of either AVA or AVB, the animals are described as being backward- and forward-uncoordinated (Unc), respectively. That is, these two pairs of interneurons are required for normal spontaneous movement and can be described as driver cells. Thus, the symmetry in the circuit evident at the level of the touch cells also seems to be expressed at the level of the interneurons. The third class of interneurons, the LUA cells, are described as connector cells that act to couple the PLM sensory neurons to the interneuronal level.

We systematically laser-ablated these five pairs of interneurons in an attempt to define their roles in response to a tap stimulus. The results of this experiment are summarized in Table 1 (Interneurons). In general, animals missing the class of driver cells (i.e., AVA and AVB) were studied only in terms of a description of the form of their tap withdrawal response. As such animals were uncoordinated, any analysis of the magnitude of the withdrawal response would be difficult to interpret. A further limitation to these studies was that the two pairs of interneurons AVB and AVD are located adjacent to the pharynx deep in the lateral ganglia. Such a position makes it difficult to unambiguously identify and ablate these cells without damaging either the basement membrane of the pharynx or the adjacent neurons. Consequently, the number of animals in which these two cell pairs were confidently removed was limited (AVD, $n = 4$; AVB, $n = 1$).

Removal of the tail-touch modulators (PVC, $n = 34$) resulted in animals that consistently responded to a tap stimulus with reversals much like the PLM$^-$ animals described above. However, the reversals produced by PVC$^-$ animals were not significantly different than control responses ($F = 5.48$, $p = 0.066$; Fig. 3*A*). This ablation leaves the PLM cells and their chemical synaptic connections with the anterior mechanosensory circuit interneurons (AVD and AVA) intact. Chalfie et al. (1985) has suggested that these connections are functional and act to inhibit the anterior tap response circuitry much like the analogous connections from AVM discussed above act to inhibit the posterior tap response circuitry. Our results provide support for this hypothesis.

However, the observation that the ablation of PVC—a neuron that is anatomically central to the touch circuit—leaves a functionally intact tap withdrawal response is paradoxical. Although the ablation of PVC might be expected to result in larger reversals due to the loss of putative excitatory input from the tail touch sensory neurons (PLM), this ablation also disrupts complex recurrent connections in the circuit at the interneuronal level (see Fig. 5). These two effects might counteract each other and result in a superficially normal tap withdrawal response in PVC$^-$ animals. To further explore our observations, we ablated PVC in ALM$^-$ animals. If PVC truly had no role in the integration of the tap withdrawal reflex, then the PVC,ALM$^-$ animals should accelerate to tap, much like the ALM$^-$ animals do. The PVC,ALM$^-$ animals ($n = 8$), however, responded with significantly more reversals than did the ALM$^-$ animals ($\chi^2 = 16.3$, $p < 0.0001$; see Fig. 2). Thus, it appears that PVC does play a role in the integration of the tap withdrawal reflex.

Removal of the anterior AVB driver cells resulted in an animal that was forward-uncoordinated (Unc) as described by Chal-

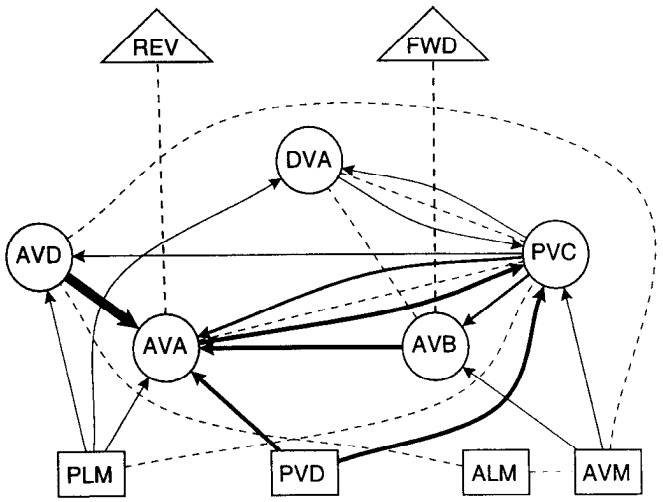

Figure 5. The simplified nematode tap withdrawal circuit. The hypothesized circuit that mediates the nematode tap withdrawal reflex consists of seven sensory neurons (*squares*), nine interneurons (*circles*), and two motoneuron pools (not shown) that produce forward and backward locomotion (*triangles*). All cells represent bilateral classes of cells except AVM and DVA that are single cells. Chemical connections are indicated by *arrows*, with the number of synaptic contacts being proportional to the *arrow width*. Gap junctions are indicated by *dashed lines*. This circuit has been simplified for ease of presentation in two ways. First, the bilateral symmetry of the circuit has been collapsed, and second, only connections with an average of greater than five synapses are shown.

fie et al., (1985), but appeared to respond to tap stimuli with normal reversals. Although this animal was capable of forward movement the form of this behavior was disturbed. In general, the animal was able to propagate a waveform down approximately one-half of its body length. The tail of the animal was dragged along passively behind the animal as it moved. In response to tap, the worm reversed normally, with full coordinated involvement of the entire body length. The reversals often terminated with a slight kinking of the posterior body. Chalfie suggested that this residual ability to move forward was due to the presence of a complex sensory-motor network for control of head movements.

Removal of the posterior driver cells AVA resulted in animals that were backward-Unc, as described by Chalfie et al. (1985). This phenotype is the backward analog to the AVB$^-$ phenotype described above. That is, AVA$^-$ animals were incapable of producing a normal reversal but despite this, still attempted to respond to tap stimuli with reversals, although at a slightly lower frequency than control animals ($n = 11$, $\chi^2 = 6.265$, $p = 0.0123$; Fig. 2). These reversals were abrupt, often resulting in the animal kinking up and freezing in response to tap.

Finally, ablation of the AVD cells ($n = 4$) resulted in animals that moved normally, but had a tendency to accelerate in response to a tap stimulus. Two animals produced very small reversals ($F = 5.48$, $p = 0.015$; Fig. 3*A*); however, this response type was less common than in wild-type animals ($\chi^2 = 19.608$, $p < 0.0001$; Fig. 2). This observation is consistent with the suggestion (Chalfie et al., 1985) that AVD acts as a functional connector between the head-touch cells (AVM and ALM) and the backward locomotion driver cell (AVA); the ablation of AVD attenuates the putative excitatory input to the motor neurons responsible for backward motion.

When Chalfie et al. (1985) ablated the LUA cells they were unable to demonstrate any change in the worm's touch sensitivity, although it was hypothesized that these cells might still play a significant role as connector cells between PLM and the touch withdrawal circuit interneurons by inhibiting the production of backward movement in response to tail touch. This hypothesis was well suited to testing using our quantitative assay. Removal of the LUA cells had no effect on either the reversal frequency ($n = 16$, $\chi^2 = 0.22$, $p =$ NS) or the reversal magnitude ($F = 5.48$, $p =$ NS) when compared to control animals (see Figs. 2, 3*A*). We also attempted to demonstrate a role for the LUA cells by ablating them in animals that lacked the PVC cells and thus much of the presumptive competing (forward movement inducing) input to the circuit. Again, however, no effect of LUA ablation was observed. The LUA,PVC$^-$ animals ($n = 10$) were indistinguishable from PVC$^-$ animals. Both groups of animals consistently responded with reversals (Fig. 2) of comparable magnitude ($F = 5.48$, $p =$ NS; Fig. 3*A*).

One further test of a role for LUA as a connector between the PLM cells and the interneurons in this circuit was suggested by the connectivity data. The PLM cells make asymmetric connections with the interneurons; PLML fails to make any direct chemical connections with AVD or AVA, whereas PLMR makes several synapses with each. The left PLM cell does, however, make indirect connections with AVA and AVD via the LUA cells. Thus, LUA may act as a connector only for the left tail touch cell. To test this possibility we compared PLMR$^-$ animals with LUA,PLMR$^-$ animals. These two groups each possess the left tail touch cell; however, the LUA,PLMR$^-$ animals lack the LUA cells and therefore also lack any direct or indirect chemical synapses between PLMR and the AVA and AVD interneurons. The magnitude of reversals elicited in LUA,PLMR$^-$ animals were not significantly different from the PLMR$^-$ group (*t* test, $t = 0.768$, $p =$ NS; Fig. 4). Thus, the LUA cells do not appear to play a significant role in integration of the tap stimulus.

Experiment 3: ablation of cells outside the touch circuit

Having delineated the roles of the touch circuit neurons in integrating the tap withdrawal response, we then attempted to define roles for any cells outside the touch withdrawal circuit that might also play a role in the response to tap. The results of these experiments are summarized in Table 2.

Hypotheses concerning which cells might play significant roles in this response were formed on the basis of two sets of observations. First, a survey of the connectivity data provided by White et al. (1986) yielded many candidates. Any cells that made significant monosynaptic connections with known cells in the touch withdrawal circuit could play a role in the production of the tap withdrawal response. Second, literature concerning the roles of neurons in other behaviors (i.e., chemotaxis, Bargmann et al., 1990, 1993; Bargmann and Horvitz, 1991; head touch, Kaplan and Horvitz, 1993) was used to refine the list of candidates.

The cells investigated were PVR, PVD/PDE, ASH, PHB, AVE, DVA, and the daughters of the T-blast lineage (PHC/PLN/PVN). The connections each of these cells make with identified cells of the touch withdrawal circuit are shown in Table 2. Several of these cells have been tested for roles in mechanosensation (Way and Chalfie, 1989; Kaplan and Horvitz, 1993). Others stain for a marker of mechanosensory function (Siddiqui et al., 1989). Others make conspicuous connections with the touch circuitry and motor neurons required for movement. The reasons that each of these cells was considered are briefly outlined below.

Table 2. Summary of ablation results: cells outside the touch circuit

Ablation	(*n*)	Compared to	Reversal frequency	Response magnitude	Synapses to	Synapses from or gap junctions with
AVE	(*n* = 7)	Con	No change	No change	AVA, AVB, PVC	AVA, AVB, ALM, PVC
PVD (V5 Blast)	(*n* = 31)	Con	Decrease	Decrease	AVA, PVC	—
V6 Blast	(*n* = 5)	Con	Decrease	Decrease		
PLM,PVD	(*n* = 12)	PLM	No change	Decrease		
ALM,PVD	(*n* = 9)	ALM	No change	Decrease		
ASH	(*n* = 18)	Con	No change	No change	AVA, AVB, AVD	—
PHB	(*n* = 10)	Con	No change	No change	AVA, AVD, PVC	—
PVM	(*n* = 28)	Con	No change	No change	AVM, PVC	—
PLN (T-blast)	(*n* = 6)	Con	No change	No change	—	—
PHC (T-blast)	(*n* = 6)	Con	No change	No change	PVC, AVB, AVA	PVC, PLM
PVN (T-blast)	(*n* = 6)	Con	No change	No change	PVC, AVB, AVA, AVD	AVB
FLP	(*n* = 1)	Con	No change	No change		
IL1,OLQ	(*n* = 1)	Con	No change	No change		
DVA	(*n* = 14)	Con	Decrease	Decrease	AVB, PAVC,AVA	PVC, PLM
PLM,DVA	(*n* = 9)	PLM	No change	Decrease		
ALM,DVA	(*n* = 11)	ALM	No change	Decrease		
PVR	(*n* = 19)	Con	No change	No change		PLM, ALM, AVM

The number of animals in each ablation group used to quantify the response to a tap stimulus is shown. The relevant connectivity of the nontouch circuit neurons is also presented. Con, control group.

The role of the PVD cells in the response to touch was assessed by Way and Chalfie (1989) with touch stimuli. It was thought that PVD may be a mechanoreceptor (Ward et al., 1975; E. Hedgecock, cited in Way and Chalfie, 1988). Way and Chalfie (1989) were able to show that PVD was required for sensitivity to "harsh touch." That is, in the absence of touch receptors, the animals would still react to a harsh touch stimulus (the worms were prodded with a platinum wire near their midbody region) by locomoting away from the stimulus (usually backward); removal of the PVD cell attenuated this response. The PVD cell also expresses *mec-3*—a gene that controls the character of the six touch cells (Way and Chalfie, 1988)—and this mechanosensory function of PVD was absent in mutant *mec-3* animals (Way and Chalfie, 1989).

The PVD cells arise in a postembryonic lineage as part of a pair of structures referred to as the postdeirids. Ablation of the V5 blast cells that give rise to these structures ensures that the PVD neurons are not formed. This procedure had a significant effect on the response to tap: the frequency of reversals in response to tap was significantly depressed as compared to control animals ($n = 39$, $\chi^2 = 9.372$, $p = 0.0022$; Fig. 2), and the magnitude of those reversals that were produced was also reduced ($n = 31$, $F = 5.48$, $p = 0.0143$; Fig. 3*A*).

One other pair of neurons (the PDE cells) was also ablated by this procedure and the effect of the ablation may have been mediated by the loss of these cells. However, the PDE cells make only sparse connections with the cells of the touch withdrawal circuit (PDE makes a total of 189 chemical synapses, only four of which are with members of the touch circuit), whereas the postsynaptic partners of the PVD cells are almost exclusively members of the touch withdrawal circuit (specifically, connections with PVC and AVA represent 110 of the 120 chemical synapses that PVD forms) (Achacoso and Yamamoto, 1992). Furthermore, the PDE cells are known to be dopaminergic and several mutants that have known defects in these neurons are available (Sulston et al., 1975). We tested three of these mutants [*cat-1*(e1111)X, which lacks dopamine in the processes; *cat-2*(e1112)II, which has greatly reduced or absent dopamine; and the double mutant] on the tap withdrawal assay. No differences were noted in the magnitude or frequency of responding of either the *cat-1*, *cat-2*, or the double *cat-2*;*cat-1* strains when compared to the N2 strain (data not shown). Thus, the effect of V5 blast cell ablation on the tap withdrawal reflex appears to be independent of the normal functioning of the chemical connections from the PDE cells and is tentatively assigned to the loss of the PVD neurons. It has also been reported that the ablation of the V6 blast cell (the posterior neighbor of V5) affected the lineage specification of V5 such that the postdeirids were not formed: the V5 daughters go on to assume a hypodermal fate (Kenyon et al., 1992). Ablation of the V6 blast cells in four animals had effects on the tap-withdrawal response that were consistent with this observation: animals reversed less often than controls ($\chi^2 = 15.05$, $p < 0.0001$; Fig. 2) and produced smaller reversals in response to tap than controls ($F = 5.48$, $p = 0.013$; Fig. 3*A*). Furthermore, we ablated PVD in animals that also had the tail touch receptors (PLM) ablated. These animals ($n = 12$) consistently reversed to tap, but the magnitude of the reversal produced by the PVD,PLM$^-$ animals was significantly smaller than those produced by PLM$^-$ animals ($F = 5.48$, $p < 0.0001$; Fig. 3*A*).

There are two possible explanations for the attenuation of the reversal magnitude produced by PVD ablations. Either PVD—like AVM—biases the circuit toward reversals, in which case ablation of PVD would be expected to reduce the magnitude of the resultant reversal, or PVD acts to set the degree of excitability within the circuit by providing information to both the anterior and posterior portions of the circuit about the degree of background mechanosensory input. The connectivity of PVD suggests a role more in line with the second possibility: PVD makes approximately an equal number of connections with both anterior (AVA) and posterior (PVC) circuitry used in the touch response. These two hypotheses concerning the role played by PVD produce different predictions about the effects of coablation of PVD and ALM. If the PVD cell biases the circuit toward

reversals, then the predicted effect of coablation of PVD and ALM in the same animal would be to increase the acceleration magnitude. However, if PVD provides some level of excitation to the circuitry, then the mean acceleration magnitude of PVD,ALM$^-$ animals should be attenuated.

The results of tests with PVD,ALM$^-$ animals provide support for the hypothesis that PVD acts as a sensory neuron that sets excitability of the reflex circuitry, modulating the level of the animal's responsiveness. The mean acceleration magnitude of eight stimuli presented at a 10 sec interstimulus interval of PVD,ALM$^-$ animals (n = 9) was significantly smaller than the mean magnitude of responses produced by ALM$^-$ (n = 19) animals (F = 11.78, p = 0.045; Fig. 3*B*).

Some anterior circuitry not described by Chalfie et al. (1985) clearly has a significant role in the animal's movement and response to mechanical stimuli. None of the ablations described so far completely eliminate the animal's response to anterior touch, nor will any ablation completely destroy the animal's ability to move forward. As mentioned earlier, AVB ablations will produce animals that are unable to recruit the posterior body muscles in forward motion but are still capable of some rudimentary forward motion. Thus, there must be some redundant function of the anterior sensorimotor circuitry. Candidates were identified and tested.

The AVE cells are one of the three major interneuron sets that make connections with the more anterior motor neurons of the ventral nerve cord. Ablations of these cells (n = 7) had no detectable effect on the form of the animal's spontaneous movement. Furthermore, this ablation produced no significant change in the worm's response to a tap stimulus either in terms of the reversal frequency (χ^2 = 0.113, p = NS; Fig. 2) or reversal magnitude (F = 5.48, p = NS; Fig. 3*A*). A possible interpretation of this result is that the function of AVE is entirely redundant with that of AVB in the intact animal, and that a role for AVE could only be demonstrated in animals that lack both AVE and AVB. We were unable to obtain animals in which both of these interneurons were unambiguously coablated.

A number of other cells were selected as possible candidate members of the tap withdrawal circuit on the basis of their involvement in other related behaviors. For example, several cells have been implicated in the foraging behavior and nose-touch sensitivity exhibited by the animal. Kaplan and Horvitz (1993) have studied the effects of various ablations on the animal's response to a light touch to the tip of its head. Among the cells that have been implicated in this behavior via laser ablation is ASH. Ablation of ASH (n = 19) had no effect on either the frequency (χ^2 = 3.100, p = NS; Fig. 2) or magnitude (F = 5.48, p = NS; Fig. 3*A*) of reversals.

A number of cells in the tail ganglia of the animal also make extensive connections with the cells so far discussed. Three of these (PHC, PVN, and PLN) arise from a single postembryonic lineage (that of the T-blast cell). The PHC cells have been reported to express touch-cell-like markers in a specific mutant strain (Mitani et al., 1993; Basson and Horvitz, cited in Mitani et al., 1993) and although this observation does not directly address the function of the PHC cells in wild type animals, it does suggest that PHC was worth investigating further. Ablation of the T-blast cell in early larval animals (2 hr posthatching, prior to the first T cell division) prevents all three of these cells from forming. This ablation (n = 6) had no significant effect on the tap withdrawal reflex (reversal frequency: χ^2 = 0.085, p = NS, Fig. 2; reversal magnitude: F = 5.48, p = NS, Fig. 3*A*). Similarly, ablation of the PHB cells (n = 10), a pair of lumbar ganglion cells that makes connections with PVC and AVA, also had no effect on the tap withdrawal response (reversal frequency: χ^2 = 2.902, p = NS, Fig. 2; reversal magnitude: F = 5.48, p = NS, Fig. 3*A*).

Another candidate cell in the tail ganglia is DVA. The single DVA cell has a process that synapses to both AVA and PVC (see Table 2, Fig. 4; the synaptic input from DVA to AVA is not represented on Fig. 4 because this set of connections contains less than five members), the same cells to which PVD is also primarily presynaptic. Ablation of this single cell (n = 14) reduced the frequency of reversal in response to a tap stimulus (χ^2 = 17.661, p < 0.0001). Furthermore, the magnitude of the reversal response was attenuated as a result of this ablation (F = 5.48, p = 0.0003). In addition to the connections with the interneurons noted above, DVA also makes chemical synapses with some of the motor neurons that mediate forward movement.

Two additional ablations were performed to further explore the finding that DVA appears to play a role in integrating the tap withdrawal response. First, we ablated DVA in addition to PLM to determine if the removal of DVA would attenuate the reversal magnitude of the large reversal produced by the PLM ablation. These animals (PLM,DVA$^-$, n = 9) consistently reversed to tap, but the mean reversal magnitude of the responses was significantly smaller than the mean magnitude of PLM$^-$ animals (F = 5.48, p = 0.0002; Fig. 3*A*). Second, we removed DVA in animals that also had ALM ablated. Thus, we were able to analyze the effects of the DVA ablation on the magnitude of accelerations produced in response to tap. The accelerations produced by ALM,DVA$^-$ animals (n = 11) were significantly smaller than the accelerations produced by ALM$^-$ animals (F = 11.78, p = 0.0057; Fig. 3*B*). This pattern of results, in which the magnitude of both reversals and accelerations was decreased, is similar to that obtained for the PVD ablation.

Finally, the cell PVR was ablated to determine if it had any role in producing a normal tap withdrawal response. The microtubule cells are a group of six cells [AVM, PVM, PLM(x2), ALM(x2)] that are genetically and biochemically distinct from other cells in the organism (Chalfie and Thomson, 1982; Savage et al., 1989; Chalfie, 1993). Consequently, markers exist that recognize these cells specifically. In particular, one microtubule antibody that uniquely stains the six cells mentioned above also stains the PVR cell (Siddiqui et al., 1989). Given that the PVR cell also make significant monosynaptic connections with the circuitry used in the touch response, it was tested for its role in the tap reflex. We performed postembryonic PVR ablations (n = 19) and found no effect of this ablation on either the reversal frequency (χ^2 = 0.756, p = NS) or reversal magnitude (F = 5.48, p = NS).

Discussion

The results presented here suggest that the tap withdrawal reflex is mediated by the five sensory neurons (ALMs, PLMs, and AVM), and eight interneurons (AVAs, AVBs, AVDs, and PVCs) that Chalfie et al., (1985) described, as well as three other neurons (PVDs and DVA). No role in mechanosensory integration for the LUA cells was demonstrated. Figure 5 shows the simplified anatomical connectivity of this circuitry. In general, the tap-withdrawal circuit can be roughly divided into circuitry designed to integrate anterior sensorimotor input and circuitry designed to integrate posterior sensorimotor input. These two subcircuits appear to functionally inhibit each other, and thus the

behavioral output is the result of a balance of the activities of these two subcircuits. Of all the nontouch circuit neurons that were tested, only PVD and DVA could be implicated in the tap withdrawal reflex on the basis of the data presented here. However, it is possible that other cells may play small roles that may only be detected by ablating large numbers of neurons at the same time. Further ablation studies may detect these effects, but given that ablation of these cells on their own has no effect on the tap withdrawal response, it is unlikely that any of these cells will prove to play a major role in mechanosensory integration.

The effects of ablation of the two novel neurons (PVD and DVA) suggest a role for these cells in mechanosensory integration. Both PVD and DVA synapse with both the anterior integration and posterior integration circuitry (see Table 2, Fig. 5). We suggest that a possible functional role for PVD may be to provide a level of excitation to the circuitry. This excitation or "tone" is either a reflection of background mechanosensory input, perhaps making the animal more responsive in a noisy world, or it is a reflection of activity in the *milieu interieur*, in which case PVD may be responding to paracrine or neurohumoral signals. PVD may play a sensory role akin to that of a stretch receptor (Hedgecock, cited in Way and Chalfie, 1988) or a background mechanosensory input detector. This possibility is supported by the observations that PVD has virtually no partners presynaptic to it and has previously been implicated in the integration of harsh touch (Way and Chalfie, 1989). The role played by DVA in the integration of mechanosensory information is similar; however, DVA is less likely than PVD to be a mechanoreceptor. The connectivity of DVA is consistent with that of an interneuron rather than that of a sensory neuron. It is a midline interneuron that receives input from putative tail chemosensory neurons (the PHA cells are morphologically similar to other chemosensory neurons, in that they have sensory endings in the phasmid sensilla through which they can take up a fluorescein dye; Hedgecock et al., 1985) and might therefore modulate the tap response according to the chemical environment. Thus, we have assigned DVA a role as an interneuron in the tap withdrawal reflex circuitry.

An underlying assumption of this work is that the neuroanatomical connections described by White et al. (1986) have some corresponding functionality. This assumption underlies the main criteria used to identify candidate cells for ablation. Although it is unlikely that a cell with sparse or absent anatomical links to those cells described by Chalfie et al. (1985) could have a significant role in mediating the response to tap, it is possible that the functional links do not necessarily correspond to the anatomical ones identified under an electron microscope. The designation of a synapse was made by White et al. (1986) on the basis of the presence of a presynaptic specialization visible in electron micrographs. All membranes adjacent to this specialization were designated as postsynaptic partners. Thus, many of the anatomical synapses may not be functional (e.g., some might lack the appropriate receptor phenotype). Also, this method of synapse identification is not sensitive to neurohumoral or paracrine effects. That is, any neuromodulator released into the neuropil might alter the circuit properties of the nematode nervous system and thus behavior. This is especially a concern given the small size of the nematode. Multiple reports of circuit switching as a result of bath application or endogenous release of neuromodulators have been made (for recent reviews, see Getting, 1989; Harris-Warrick et al., 1992) and an extensive battery of neuropeptides has been described in *Ascaris*, a related nematode (Stretton et al., 1992).

An additional assumption made in this work is that the effect on behavior produced by the ablation of a cell is due to the loss of that cell's function directly on that behavior. However, there are an additional number of caveats that need to be kept in mind when discussing the interpretation of ablation results. In general, any lesion may produce an effect if the lesioned area is permissive to a behavior, even if the lesioned area is not actually contributing directly to that behavior. This limitation of lesion studies also applies to the ablation of single neurons in a circuit. Also, when there are recurrent connections within a circuit, it can sometimes be difficult to interpret the consequences of the ablation of a neuron (although, as discussed below, a computational modeling approach can help make predictions). The effects of the ablation may be more accurately attributed to the alterations in complex interactions between circuit elements than to the loss of a specific role of the target cell. A further specific caveat of this work is that the ablations reported in this article were all done early in development, and it is possible that the nervous system of these animals have compensated for the loss of these connections. Although this is an important consideration, it is clear that if there is compensation, it is not complete for many of the ablations.

The results presented here support the relation between the anatomical connectivity and the functionality of synapses in the nematode. Specifically, because the tap withdrawal reflex can be quantified (unlike the response to touch, e.g.) it has been possible to detect subtle effects of cellular ablation. For example, the observation that both ALM$^-$ and AVM,ALM$^-$ animals invariably accelerate in response to tap but the AVM,ALM$^-$ animals accelerate more vigorously suggests that the connections between AVM and the circuitry used in the posterior touch response are functional and inhibitory in nature as hypothesized by Chalfie et al. (1985). At the very least it can be said that AVM has an effect on mechanosensory integration that is independent of ALM and thus provides more than just parallel processing of anterior mechanosensory information.

The relationship between circuitry and behavior in *C. elegans* is robust and bidirectional. Changes in the nervous system as a consequence of ablation produce predictable changes in the form of the behavior; observed changes in behavior may provide information about the nature of the underlying nervous system. This latter approach may be used to assign polarities (excitatory or inhibitory) to the chemical synapses studied in the tap withdrawal circuit. Hypotheses about what polarity configurations might best account for the behavioral observations reported here are difficult given the complexity of the circuitry involved. However, these hypotheses can be aided by the formulation of an appropriate computational model of the circuitry. Such a model could be used in conjunction with these studies to form specific predictions about the polarities of chemical synapses within the tap withdrawal circuit. Work demonstrating the feasibility of this approach has been carried out (Hutcheon et al., 1993; Wicks, Roehrig, and Rankin, unpublished observations). This might in turn facilitate the determination of neurotransmitters and neurotransmitter–receptor pairings in these cells—work that is in its infancy.

Now that the tap withdrawal circuitry has been identified, it is possible to start exploring the cellular and molecular basis of the simple forms of behavioral plasticity observed in the tap withdrawal reflex. It has been demonstrated that cellular ablation

of elements of the tap withdrawal circuit can modulate habituation dynamics in predictable ways (Wicks and Rankin, unpublished observations) and such observations may suggest possible sites of plasticity in the circuitry that then could be explored at a molecular/genetic level. Also, many of the cells in the tap withdrawal circuit are genetically characterizable; gene expression in these cells may be manipulated either directly (Way and Chalfie, 1989; Hamelin et al., 1992) or indirectly (Stringham and Candido, 1993). With the rapid sequencing of the *C. elegans* genome (Waterston et al., 1992) the stage is set to identify candidate learning genes in this system utilizing both classical and reverse genetic approaches. This, in combination with the type of study presented here, makes *C. elegans* a powerful tool for the understanding of the processes underlying learning. The strength of the nematode as a model neurobiological system is that it is not necessary to study a particular component of the system in isolation; with only 302 neurons to account for its entire behavioral repertoire, the possibility exists of describing the roles of every neuron in the organism in every behavior that the animal exhibits.

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