A DEVELOPMENTAL ANALYSIS OF THE REVERSAL RESPONSE IN THE NEMATODE *CAENORHABDITIS ELEGANS*

by

CATHERINE MEYE CHIBA

B.Sc., The University of British Columbia, 1986

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ARTS

in

THE FACULTY OF GRADUATE STUDIES

Department of Psychology

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

August 1989

Department of Psychology

The University of British Columbia
Vancouver, Canada

Date Oct. 10, 1989

Abstract

The nematode Caenorhabditis elegans exhibits a number of locomotory behaviours, among them a family of reversal responses which can occur either spontaneously, in the absence of obvious stimuli, or reflexively, in response to mechanical stimuli. These behaviours are thought to be mediated by a common neural circuit, the touch withdrawal circuit, whose genetics, neuranatomy, and development have been exceptionally well-characterized. Despite known changes in the functional neuroanatomy of this circuit over development, at least one behaviour mediated by this circuit, the touch withdrawal reflex, shows no apparent changes over development. This thesis examined the possibility that these changes may be reflected in two other reversal responses, spontaneous reversals and the tap reversal reflex.

In the experiments reported here, both spontaneous reversals and the tap reversal reflex were examined over six developmental stages: four larval stages (L1, L2, L3, and L4), and two adult stages (young adult and 4 day olds). In all experiments, animals were observed individually under a light microscope and their behaviour videotaped for later analysis.

Results indicated that both spontaneous reversals and the tap reversal reflex show developmental changes in C. elegans. Young adult animals showed an increase in the frequency of spontaneous reversals relative to the other

developmental stages; this increase was neither a function of increased activity or change in sensory threshold. Larval animals of all stages also showed significantly different patterns of responding to taps relative to adult stages, with L2 animals showing the most pronounced differences. Although animals of all stages reversed in response to touch, taps elicited not only reversals, but a previously undescribed response, accelerations. While adults consistently showed reversals in response to single taps, larval animals showed accelerations on approximately half the responses.

The tap reversal reflex was also found to be graded, showing a significant trend to increase in magnitude with increasing stimulus magnitude. Increasing stimulus magnitude also increased the probability of accelerations relative to single taps for all developmental stages, though larval animals still showed fewer reversals and more accelerations than adult animals.

There appeared to be two phases in the development of the reflex: one, the transition from newly hatched L1 animal to subsequent larval stages; the other, the transition from larval stage L4 to young adult, when the pattern of responding to taps changes from the larval to the adult pattern. These periods correlated with periods of known neuronal change in the neural circuit. It was hypothesized that the transition from larval to adult patterns of responding might be the result of the addition

of a specific sensory neuron, AVM, and its associated connections.

Table of Contents

List of Tables

List of Figures

Acknowledgements

I didn't do this alone.

Thanks to Ann Rose and her students for all their help: their assistance, advice, and essential supplies for the care and feeding of C. elegans (and their provision of C. elegans itself) made this work possible. Thanks especially, Ann, for introducing our lab to C. elegans in the first place: it's a wonderful animal.

Thanks also to Rod Wong, who was there at the beginning, and introduced a romantic to the study of animal behaviour. Thank you for your consideration, good will, and encouragement. I have cherished your point of view.

Finally, I would like to thank Catharine Rankin, who hung on to the end, and in doing so taught me a lesson in friendship, craftsmanship, and tenacity that I never thought I had to learn. And I thought I was stubborn.

This thesis is dedicated to John T.M. Kam: In the worst of it you were my solace, my sanctuary, my sanity. Thank you. You made the difference.

A fundamental issue in both psychology and biology is the question of how behaviour is produced. There is general agreement that the nervous system plays a key role in producing behaviour. One assumption that follows from this is that changes in the structure of nervous systems lead to changes in behaviour. The relationship, however, is not always clear cut.

One way to analyze the relationship between nervous systems and behaviour is to study behaviour as a nervous system develops. A developing animal does not start with a functioning nervous system: it assembles it, and the nature of behaviour during this assembly can give us insights into the relationships between nervous system structure and nervous system function.

The difficulty comes in determining the relationships between the two, as nervous systems and behaviour are not identical. Both are products of interactions and activities at several levels of organization, ranging from the level of molecules to the level of neurons; from the level of neurons and neural circuits to the level of entire organisms. Changes can take place at any of these levels, with no guarantee that changes at one level are necessarily reflected in changes at another level. Furthermore, the sheer complexity of interactions at any one level--be it molecular, cellular, anatomical, or behavioural, often precludes our ability to draw conclusions about the relationship between interactions

across levels.

Yet ultimately, to understand how behaviour arises out of the workings of a nervous system, we must be able to integrate information across levels. Our goal is to understand how changes at one level of organization may be reflected in another; to understand how these levels may interact. No single level is the sole producer of behaviour; all appear to be interdependent. The question is how to integrate the inputs and outputs of various levels.

One approach to the problem is to study a very simple animal such as the nematode Caenorhabditis elegans. In many respects, C. elegans is ideally suited for just such an integrative approach. Sidney Brenner, who selected and promoted this species as a model organism in the early 70's (Brenner, 1974; see also Brenner 1988) recognized the problem of complexity as a major hindrance to progress in our understanding of the processes of development and nervous system function. He saw in C. elegans the potential to become to the study of nervous systems and development what bacteria had been to molecular biology: a powerful model organism from which to extract first principles.

With only 302 neurons, consistent, precise, and highly determinate development, and a genome and lifestyle highly conducive to genetic dissection, C. elegans is one

of the most intensively studied and extensively described multicellular organisms in biology today. Measuring less than a millimeter long as a young adult, this tiny, transparent, free-living soil nematode is the model system of choice for the study of problems as diverse as developmental genetics, toxicology, and aging (e.g., see references in Zuckerman, 1980a; 1980b; also Wood, 1988).

Such intensive study has made C. elegans a tempting choice for the neurobiologist in search of a model system. In addition to a nearly completed physical map of the wild-type genome (Coulson, Sulston, Brenner, & Karn, 1986), there now exist complete developmental lineages for every cell of every type in the adult hermaphrodite (Sulston & Horvitz, 1977; Sulston, Schierenberg, White, & Thomson, 1983), as well as a complete neuroanatomical map of the entire adult nervous system, including locations of putative chemical and electrical synapses and anatomical wiring diagrams for several behaviours (White, Southgate, Thomson, & Brenner, 1986).

One family of behaviours in this animal has been especially well studied--behaviours related to locomotion. C. elegans exhibits a number of locomotory behaviours, one of which is a reversal response that can occur either spontaneously or reflexively (Croll, 1975). The animal normally swims forward on a solid substrate, propelling itself on its side with undulatory waves of muscular contraction that pass down the length of its body from

head to tail. When the animal reverses, the flow of these waves reverses direction, the contractions passing from tail to head resulting in the animal's backward motion. This behaviour can occur in response to direct mechanical stimulation, such as touch to the head, and is termed the head touch withdrawal reflex (Chalfie & Sulston, 1981). A similar response, the tail touch withdrawal reflex, results in the animal's forward motion (Chalfie & Sulston, 1981). Other stimuli, such a vibration passing through the substrate beneath the animal, can also elicit reflexive reversals in adult animals. We have called this latter response the tap reversal reflex, as it can be elicited by tapping the side of the animal's dish (Rankin & Chiba, 1988; Rankin, Beck, & Chiba, in press).

The tap reversal reflex has been the subject of studies of behavioural plasticity. C. elegans is capable of non-associative learning--habituation, dishabituation and sensitization--and is also capable of long-term memory (Rankin & Chiba, 1988; Rankin, Beck, & Chiba, in press). In addition, the tap reversal is inhibited by gentle touch to the tail, suggesting an interaction between competing responses (Rankin, unpublished data).

The touch withdrawal reflex has been extensively studied by Martin Chalfie and his colleagues (Chalfie & Sulston, 1981; Chalfie, Thomson, & Sulston, 1983; Chalfie, 1984; Chalfie, Sulston, White, Southgate, Thomson, &

Brenner, 1985; Walthall & Chalfie, 1988; Chalfie & Au, 1989). The functional anatomy of the neural circuit for this behaviour has been worked out in considerable detail using a combination of genetic lesions and laser microsurgery to determine the roles of individual cells.

Both the touch withdrawal reflex and the tap reversal reflex are thought to be mediated by the neural circuit elucidated by Chalfie et al (Chalfie, Sulston, White, Southgate, Thomson, & Brenner, 1985) Animals defective in critical elements of the touch withdrawal circuit and known to be touch insensitive also show abnormal responses to tap (Rankin & Chalfie, 1989).

In addition, Chalfie and his colleagues have detailed many features of the neuroanatomical development of this circuit (Chalfie & Sulston, 1981; Chalfie, Thomson, & Sulston, 1983; Chalfie, 1984; Walthall & Chalfie, 1988) One intriguing aspect of this response to touch is that it is present throughout post-embryonic development, despite the occurence of substantial changes in the circuit's neural anatomy (Chalfie & Sulston, 1981).

The reversal responses thus form a family of responses especially suitable for the study of the relationships between developmental changes in nervous systems and the behaviour they produce. They are particularly interesting given that at least one behaviour, the touch withdrawal reflex, appears not to reflect substantial neuroanatomical changes as they occur

over development.

The following experiments represent an examination of both the touch and the tap reversal response throughout development. The aims of these experiments were threefold: (1) to examine the relationship between two topographically similar behaviours--the touch withdrawal reflex and the tap reversal reflex; (2) to examine the possibility that the substantial changes in the underlying neural circuit may be reflected in tap reversal reflex; and (3) to lay the groundwork for an examination of the development of behavioural plasticity in C. elegans by a detailed characterization of the tap reversal reflex over development.

Before outlining the experiments I will first describe the relevant characteristics of the animal's development and neural anatomy.

BACKGROUND

The Life Cycle

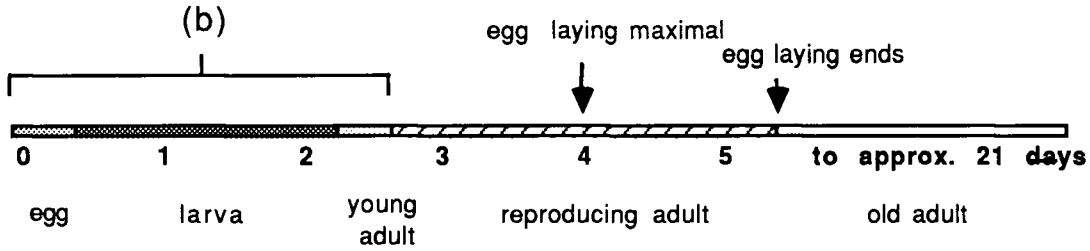
C. elegans develops rapidly, proceeding from egg to reproducing adult in approximately three and a half days at 20° C (see Figure 1). Eggs are laid approximately three hours after fertilization, and the developing animal passes through four larval molts before reaching its final adult form. Egg-laying begins several hours after the last molt and continues for about four days, after which the

Figure 1. The life cycle of C. elegans. From data presented in Byerly et al, 1975. Figure 1(a) shows the typical life cycle of Caenorhabditis elegans in days at 20° C. Eggs are laid approximately 3 hours after fertilization; 0 represents the time at which the eggs are laid. Animals spend approximately 2.5 days in larval development (including embryogenesis), and begin to lay eggs at approximately 3 to 3.5 days. Arrows indicate time of peak egg-laying and effective end of egg-laying.

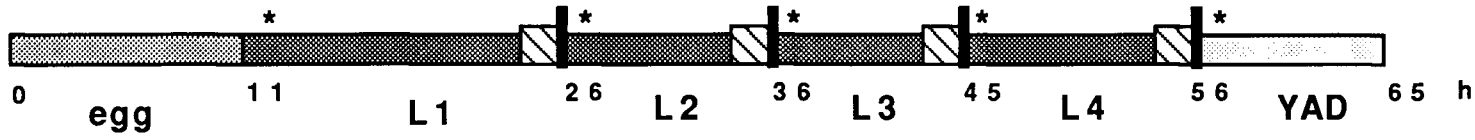
Figure 1(b) shows a more detailed view of early development. Times are given in hours. On average, eggs hatch approximately 11 hours after being laid. Dark rectangles mark stages of larval development from L1 through L4; the thick vertical black lines indicate approximate times of molting. Boxes with diagonal lines indicate approximate periods of lethargus. Young adulthood ends when the animal begins to lay eggs. Approximate periods of testing are marked with asterisks.

THE LIFE CYCLE

(a)



(b)



animal may continue to live to an estimated maximum of about 21 days.

As shown in Figure 1, each of the four larval stages lasts approximately ten to fifteen hours, the longest stage being larval stage 1, or L1. The animal grows continuously between molts, and the pattern and timing of cell division between individuals under similar conditions is highly consistent (see Sulston, 1988, for summary). The animal ends a given larval stage when it sheds its cuticle, about two hours after entering a period of inactivity.

The Behaviour

Immediately after hatching, the animal is able to locomote, swimming over or through semi-solid substrates such as agar with sinusoidal waves that propagate down the length of its body. It responds to gentle touch on both the head and the tail by swimming away from the stimulus, and, when swimming undisturbed, shows continous head oscillations which appear independent of the sinusoidal body waves. (See Croll, 1975, for a detailed analysis of locomotory behaviour in C. elegans.)

The animal also shows a similar reflex, the tap reversal reflex, which is elicited when a vibrational stimulus is transmitted to the animal via the underlying substrate. In studies of non-associative learning, this reflex is elicited by tapping the side of a plate containing the worm on agarose gel. Both resting and

forward-travelling animals stop and reverse, undulating backwards for some distance. The tap reversal reflex is the primary focus of the experiments reported here.

The Touch Circuit

The tap reversal reflex appears to be mediated by the same circuit that underlies the reversal response to gentle head touch. The functional neuroanatomy, genetic control, and development of this circuit has been described by Chalfie and colleagues (Chalfie & Sulston, 1981; Chalfie, Thomson, & Sulston, 1983; Chalfie, 1984; Chalfie, Sulston, White, Southgate, Thomson, & Brenner, 1985; Walthall & Chalfie, 1988; Chalfie & Au, 1989).

The basic elements of the touch circuit in C. elegans are 6 touch receptors, 5 pairs of interneurons, and 69 motor neurons. The touch receptors, also known as microtubule cells, are the sensory elements of the circuit. The six touch receptors of the adult animal can be divided into three groups based upon their position. Two of the cells, the left and right anterior lateral microtubule cells (abbreviated as ALML and ALMR, respectively: see White et al, 1986, for a more detailed explanation of abbreviations), are positioned laterally on either side of the anterior half of the animal, extending their processes anteriorly. Two other cells, the left and right posterior lateral microtubule cells (PLML and PLMR,

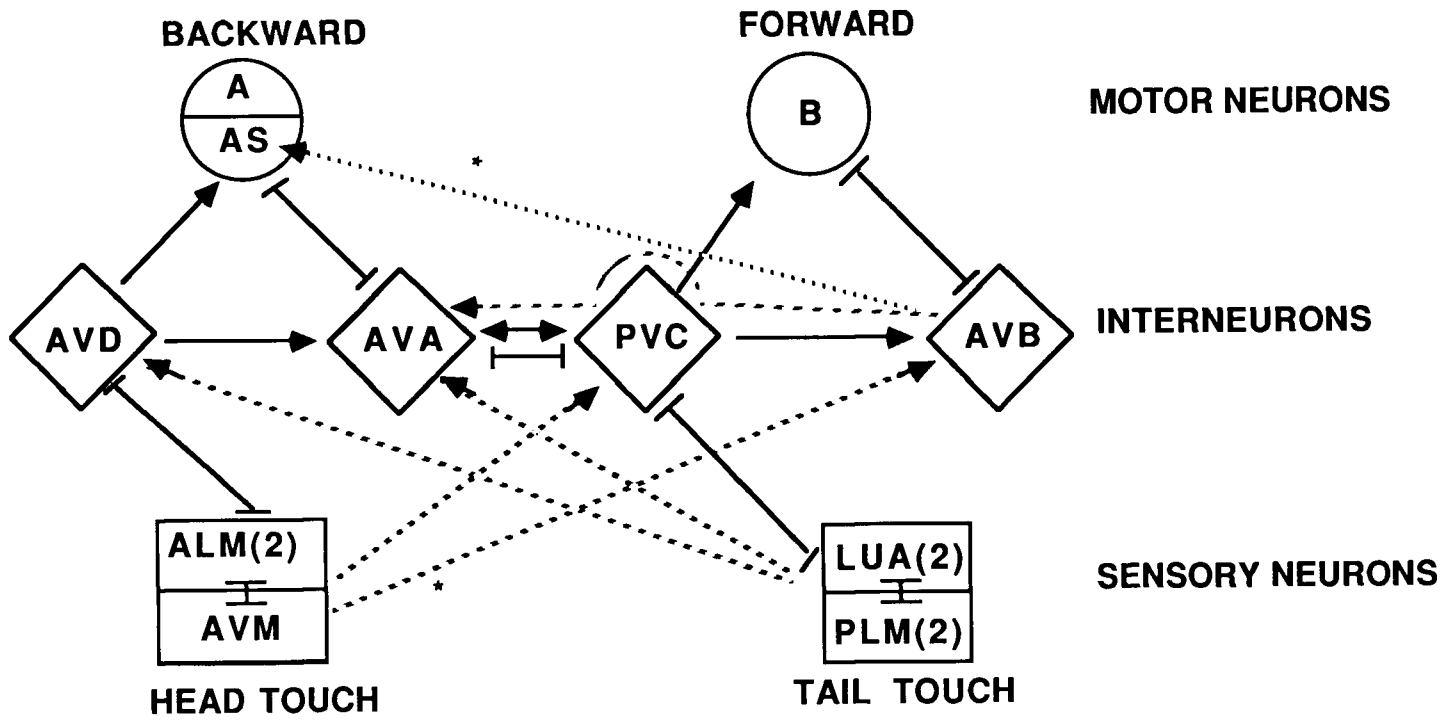
respectively) are positioned posteriorly, on either side of the animal's tail. These also extend their processes anteriorly, to the animal's longitudinal midline. The two other cells, the anterior and posterior ventral microtubule cells (AVM and PVM, respectively) are positioned medially and ventrally: AVM in the anterior half of the animal, extending its process anteriorly; and PVM in the posterior half of the animal, extending its process anteriorly into the anterior portion of the animal. Of these cells, five are known to contribute directly to the withdrawal responses seen to posterior and anterior touch. The function of the sixth, PVM, is not yet known, but may involve the mediation of other touch-related behaviours.

The touch circuit in C. elegans can be divided into two interconnected units: (1) the neural circuit for anterior touch (head touch) and (2) the neural circuit for posterior touch (tail touch). (See Figure 2). The ALM (R and L) cells and the AVM cells are the sensory receptors for anterior touch. These cells are coupled to each other by gap junctions and are thus electrically coupled, working as a unit. When stimulated, these cells cause the response to gentle touch via gap junctions to a pair of interneurons, the AVDs, which form excitatory chemical synapses onto the motor neurons responsible for backward movement, the A and AS cells. AVD also makes indirect connections to these motor neurons via a chemical synapse

Figure 2. The touch circuit of *C. elegans* (adult hermaphrodite). From information presented in Chalfie et al., 1985, and from Chalfie, personal communication to Catharine Rankin.

The touch cells are represented as rectangles, as are the touch cell connectors, LUA. Interneurons are represented as diamonds; motor neurons as circles. Chemical synapses are indicated with arrows (→) and gap junctions with bars (├─┤). Inhibitory and excitatory connections are also depicted: dotted lines indicate inhibitory connections, solid lines excitatory.

The AVB cells form chemical synapses with the AS cells but not the A cells; only AVM of the anterior touch receptors synapses with AVB. Both the latter connections (marked with *) arise postembryonically; the connection from AVM to AVB is completed approximately 40 hours after hatching (Chalfie & Sulston, 1981; Sulston & Horvitz, 1977). Gap junctions between identical motor neurons and interneurons are not shown.


BACKWARD
FORWARD
A
AS
B
MOTOR NEURONS
AVD
AVA
PVC
AVB
INTERNEURONS
ALM(2)
AVM
LUA(2)
PLM(2)
SENSORY NEURONS
HEAD TOUCH
TAIL TOUCH

to AVA, a large interneuron coupled by gap junctions to the A and AS motor neurons. The ALM and AVM cells are also thought to make inhibitory connections onto the posterior touch circuit (see Figure 2): one to PVC, the interneuron analogous to AVD in the posterior touch circuit, and one to via AVM to AVB, the interneuron analogous to AVA in the touch circuit.

The posterior touch circuit is structured similarly. The sensory receptors, the PLM cells, are electrically coupled via gap junctions to PVC, an interneuron making excitatory chemical synapses onto the B motor neurons. When stimulated by gentle touch to the posterior half of the animal, the PLMs activate the B motor neurons via PVC, eliciting forward movement. PVC, like its counterpart AVD, makes a second set of excitatory connections to the B motor neurons via AVB, which (like AVA) forms gap junctions with the B cells. The PLM cells are also electrically coupled to a pair of interneurons, the LUAs, which appear to act as extensions of the PLMs, allowing them both to make contact with the AVA and AVD cells of the anterior touch circuit (Chalfie et al, 1985). These chemical synapses, along with chemical synapses from the AVBs onto the AVA interneurons and the AS motor neurons, are thought to be inhibitory (see Figure 2).

Post-Embryonic Changes in the Touch Circuit

Many of the elements of the adult touch circuit arise post-embryonically. The newly hatched L1 animal, although

capable of locomoting and responding to gentle head touch (Chalfie & Sulston, 1981), has a nervous system substantially different from that of the adult.

The newly hatched L1 starts with a touch circuit in which only the four lateral touch receptors are present; AVM and PVM arise post-embryonically. The interneuron pairs AVD, AVA, PVC, and AVB are present, but the connections between AVBs and the ALMs are missing due to the absence of AVM. The AS motor neurons are absent as well, so that this connection between AVB and the circuit for anterior touch is also missing.

The newly hatched L1 animal has only 22 of the 76 ventral cord motor neurons it will have as an adult (Sulston, 1976; Sulston & Horvitz, 1977). At hatching, only three types of motor neurons, the dorsal A (DA), dorsal B (DB), and dorsal D (DD), are present; the adult complement consists of eight types, including the ventral counterparts of these neurons (the VA, VB, and VD motor neurons), the C neurons, and the AS neurons. In addition, one of these classes of motor neurons, the DD motor neurons, completely reverses its pattern of connectivity from larva to adult (White, Albertson, & Anness, 1978).

Changes begin in the larval nervous system three hours after hatching. At this time cell division is reinitiated. Within the next 10 hours, many of the cells that will comprise the adult nervous system are generated.

The touch receptors AVM and PVM arise during this time, as do the ventral A, ventral B, ventral D, and AS motor neurons. Although these new cells are born at this time, they are not necessarily functional. Some structural changes (such as the rewiring of the DD neurons) may be essentially complete by the onset of the second larval molt (eg., see White et al, 1978). However, others may not be complete until as late as the fourth larval molt; some neurons, such as the AVM neuron, may need to migrate and extend processes to their appropriate targets before becoming functional (see Walthall & Chalfie, 1988).

The occurrence of such large changes in the neural circuit would seem indicate that there should be correspondingly large changes in behaviour. However, this is apparently not the case: both the head and the tail touch withdrawal reflex are observed at all larval stages (Chalfie & Sulston, 1981).

The experiments reported here address the possibility that connectivity changes in the touch circuit might be reflected in other behaviours mediated by the circuit, such as spontaneous reversals and the tap reversal reflex. To date neither of these behaviours have been examined with respect to development. These experiments were therefore designed to test for developmental differences in spontaneous reversals and in the tap reversal reflex.

General Methods

Animals

C. elegans Bristol (strain N2) were maintained at room temperature (20 ± 2° C) on *E. coli* (strain Op50)-seeded NGM agar (as described by Brenner, 1974).

All animals were tested in the first five hours of each larval stage, and at various ages in the adult animal. Juvenile animals were thus tested in early L1, L2, L3, and L4; adult animals immediately after the final molt (at 55-60 hours; the "young adult" stage), and at 4 days, when most animals have reached their peak rate of egg-laying. The L1 animals were tested within the first three hours of hatching, as cell division resumes 3 hours after hatching (Sulston, 1988).

Establishment of Synchronous Colonies and Verification of Stages

In order to ensure that the animals tested were the same age, synchronous colonies were established by allowing several gravid adults to lay eggs on *E. coli*-seeded plates for 30 to 90 minutes. As the youngest animals are almost invisible in thick bacteria, colonies for L1 animals were established on plates dotted with bacteria just prior to the addition of adults.

The hours given in Figure 1 served as rough guides to the development of the animals. However, given that the temperatures under which these animals were kept were

subject to small fluctuations, other methods were used to verify stages. These included checking the animals for hatching or lethargus (defined by inactivity and lack of pharyngeal pumping in the majority of the animals within a colony) prior to the expected time of molting, as well as making visual checks for features characteristic of particular stages and times. Size consistency was also checked using a gradicule (a measurement device inserted into one of the microscope eyepieces).

Characteristics of Developmental Stages

L1 animals. Newly hatched L1 animals are approximately 200 µm long. Under the dissecting light microscope, they are completely transparent, with no variation in body colouring, and can be distinguished from older L1s by their torpedo-like shape. Slightly older L1s are more cylindrical.

L2 animals. L2 animals are approximately 370 µm long at the time of the first molt (Byerly et al, 1976). The head of the animal is transparent, and takes up approximately one-third of the animal's body length. The rest of the body is darkly coloured, with the future gonad appearing as a narrow white oval approximately one-third of the animal's body length from the tail.

L3 animals. After the second molt, L3 animals are approximately 480 µm long (Byerly et al, 1976). Newly molted L3 animals are clear (no dark body colouring) and faintly mottled along their lengths. As L3 animals become

older, the body posterior to the pharyngeal bulb becomes darkly coloured, although the head remains clear and light-coloured. The gonad is thin, flat, and slightly elongated in appearance, a short white line on one side of the animal.

L4 animals. Shortly prior to the third molt, L3 animals lose their dark colouring and become transparent again. After shedding their L3 cuticles, the 640 µm long (Byerly et al, 1976) L4s are transparent for a short time but regain their dark body colouring soon after. The light-coloured head appears to take up about one-fifth of the animal's body length, and the pharyngeal bulb is clearly visible. The gonad appears as a large white crescent with its flat end apposed to the location of the future vulva. In early L4s, the gonad appears as a tight, round semicircle against a grayish body; in older L4s, the gonad resembles a wide and flattened quarter-moon against a blackish body.

Young adult animals (YAD). After the final larval molt, young adult animals are approximately 850 µm long (Byerly et al, 1976). They have normal adult proportions, with the head (portion of the body anterior to and including the pharyngeal bulb) taking up approximately one-fifth of the body. The vulva has opened to the outside; however, no eggs are visible and the animal is still transparent.

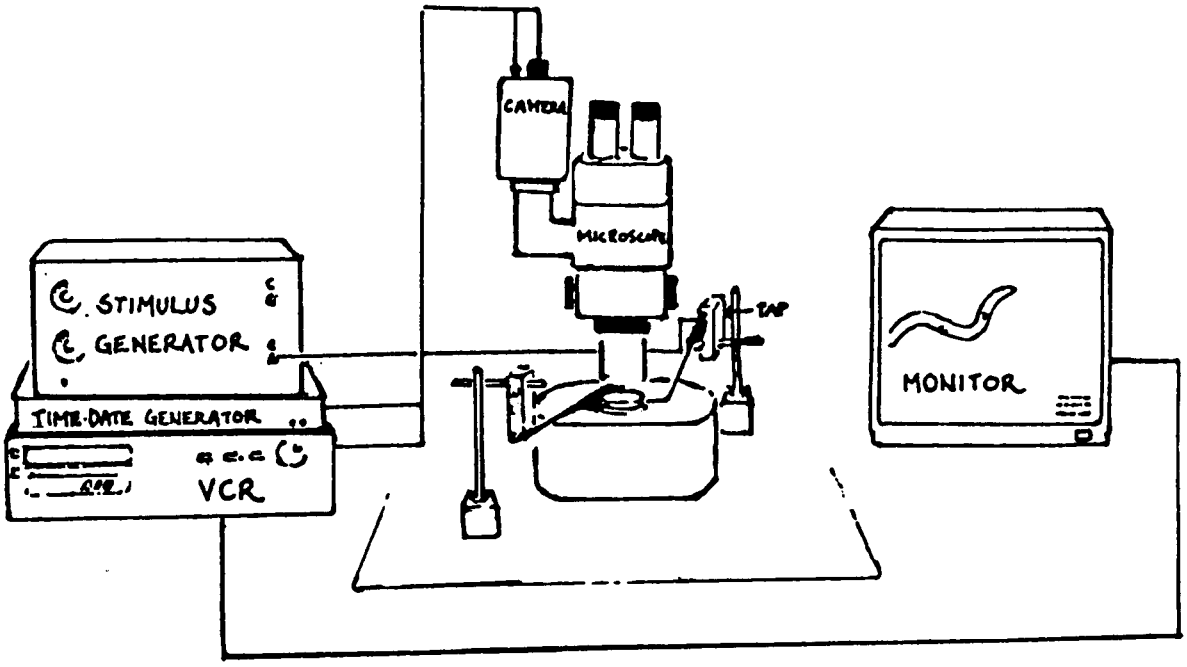
Adults. Four day old adults (4D) can be distinguished from young adults by their darker colouring and large size (1 mm and larger). Ripening eggs are visible in the animal's uterus; these eggs are grayish under the light microscope. Adults continue to grow throughout the rest of the life span, and their intestines appear to become mottled with dark, blackish pigment as they age.

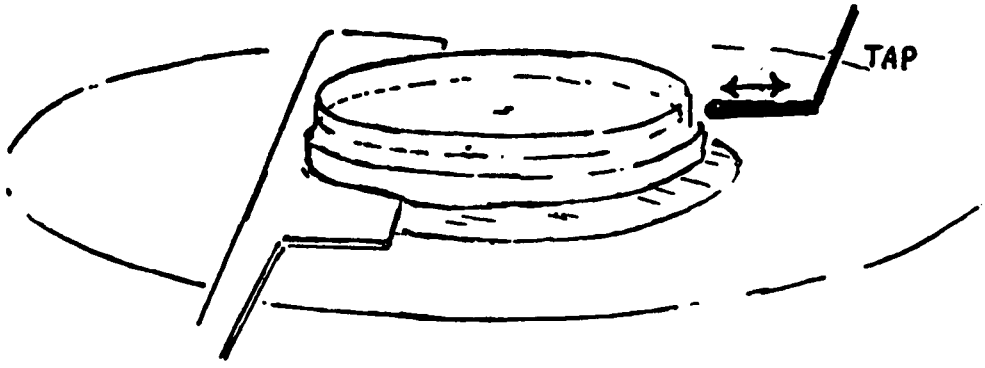
Males. Males arise spontaneously in populations approximately once every 1000 animals at 20° C (Hodgkin, Horvitz, & Brenner, 1979; Rose and Baillie, 1979). As only hermaphrodites were used in these experiments, any males found in the stock and subject populations were removed. This can be done visually after the L3 stage, due to the male's distinctive tail shape. Adult males are distinguishable by their spade-shaped tails. Animals tested at L3 or earlier, however, were maintained on their original test plates with bacteria until their sex could be firmly established.

Apparatus and General Technique

For all experiments, observations were made through a Wild M3Zoom stereomicroscope equipped with a phototube for videotaping (see Figure 3). All observations were made under bright field illumination and videotaped using a Panasonic D5000 low-light, high-resolution video camera. To facilitate scoring, the date, time, and time elapsed for individual animals were superimposed on the videotaped

Figure 3. Apparatus used for studies of behaviour in *C. elegans*. Top depicts microscope with video recording equipment and stimulus generator; bottom shows detail of plate holder and tapper. See General Methods for detailed description.


CAMERA
MICROSCOPE
TAP
STIMULUS
GENERATOR
TIME-DATE GENERATOR
VCR
MONITOR


TAP

record using a Panasonic WJ810 time-date generator.

Animals were removed from plates for testing with a fine hair (thicker hairs were used for larger animals) and transferred to a bacteria-free plate (a 5 cm diameter Petrie plate) containing 10 mL of NGM agar. These plates were then placed within a holder mounted in a Marzhauser MM33 micromanipulator on a magnetic base. The holder allowed the dish to vibrate freely when tapped, while still allowing smooth tracking of the animal during videotaping. In Experiments 2 and 3, taps were delivered using a mechanical tapper mounted in a separate micromanipulator. The number and rate of taps was controlled with a Grass S88 stimulator.

Scoring

In Experiment 1 videotaped records were scored for total number of spontaneous reversals and percent of time active. In Experiments 2 and 3 the records were scored for frequencies of specific response types and magnitudes of responses observed. The methods of scoring are described below.

Numbers of Spontaneous Reversals. When counting numbers of spontaneous reversals in Experiment 1, the animal was said to be reversing if backward-propagating (head to tail) waves were seen passing down the animal's body. This normally resulted in the animal's backward movement; however, some of the smaller animals (L1s in particular) were occasionally unsuccessful in moving

backward (sometimes the anterior part of the animal appeared to "bunch up" in front of the tail). Each continuous bout of backward-propagating waves was counted as one reversal.

Percent Time Active. In Experiment 1, the amount of time spent active in the observation period was scored to the nearest second using a stopwatch. Only those periods in which backward- or forward-propagating waves were passing down the animal's body were counted as "active" periods (momentary pauses caused by transitions from backward to forward movement were ignored). Those periods in which an animal remained stationary without undulation were scored as inactive periods, even if the animal moved its head occasionally: movements involving only that portion of the body immediately anterior to the pharynx were thus ignored if unaccompanied by waves of contraction involving the rest of the body. The number of seconds active was then converted to a percentage of the total number of seconds in the observation period.

Response Type. Responses to tap and touch in Experiments 2 and 3 fell into one of two major categories: (1) reversals (for definition, see Numbers of Spontaneous Reversals, above), and (2) accelerations, defined as increases in swimming speed relative to the average speed of the animal 2 seconds prior to the stimulus. Only responses occurring within 1 second of stimulus completion

were counted. In cases in which both types of responses occurred within a single second, the response was classified as whatever type occurred first. Responses that fell into neither of the reversal or acceleration categories but were clearly responses to the stimulus were classified as "other", as were responses in which no detectable change in behaviour occurred.

Magnitude. Magnitudes of responses in Experiments 2 and 3 were obtained by tracing the paths travelled by animals onto acetate sheets with the aid of stop-frame video analysis. As animals of various stages were filmed at different magnifications, the length of the video image of each animal's body was also traced, and all measurements expressed in terms of the animal's own body length. Thus the measure of response magnitude was distance travelled relative to body size (distance travelled / body length), allowing an examination of the effects of stage on responding rather than the effects of body size (an L1 animal traversing the actual distance covered by two adult body lengths travels a distance equivalent to 8 to 10 times its own body length).

Reversals were scored by tracing the path travelled by the head of the animal immediately following the end of the stimulus. A reversal ended when the animal either stopped or changed direction, resuming forward motion.

Accelerations were scored by tracing the path travelled by the tail of the animal immediately following

the stimulus. A measure of the pre-stimulus speed was obtained by tracing the path travelled by the animal's tail 2 seconds prior to the onset of the stimulus and averaging the distance travelled for the two seconds. Both records were marked in intervals of one half second to determine changes in speed.

An acceleration was judged to have occurred if the distance travelled in either interval in the second immediately following the stimulus exceeded the average distance travelled per interval in the 2 seconds prior to the stimulus. The beginning of the acceleration was defined as the interval in which this increase in speed occurred.

The end of the acceleration was defined as the interval after which the animal either stopped, changed direction, or decreased its speed for two consecutive intervals to a speed less than or equal to either (a) the average speed it was travelling in the 2 seconds prior to the stimulus or (b) half the speed of the first interval of the acceleration, whichever occurred first. The time elapsed between the beginning and end of the acceleration was also recorded for each acceleration.

Lengths of the paths travelled by animals in either accelerations or reversals were obtained by retracing the tracings on a digitizing pad (Summagraphics Bit Pad Plus), which transferred the image into a MacIntosh SE

microcomputer. The lengths of the images were then computed using MacMeasure, a program suitable for measuring the lengths of irregular curves.

Statistical Analyses

Two basic types of measurements were obtained in the experiments: ratio and categorical measurements. Numbers of spontaneous reversals, percents of time active, and response magnitudes were ratio measurements; frequencies of response type were categorical measurements.

Ratio Measurements. In order to test for age-related changes in these types of measurements, analyses of variance (ANOVAs) were used for the initial analyses of the data. The ANOVA allows one to decide whether differences observed between groups are large enough to be attributable to the factor (e.g., stage) being tested, rather being attributable to the kind of variation that occurs within all groups regardless of treatment. (for discussion, see Bailey, 1981, pp. 99-114; Glass & Hopkins, 1984, pp. 324-359). The test statistic, F, is a ratio of the amount of variation present attributable to the factor being tested to the amount of variation attributable to error. ANOVAs can be modified to suit the experimental design, the primary change being in composition of the error term.

As the ANOVA tests all groups simultaneously, a significant value for F indicates only that a difference between group means exists, not where a difference occurs.

For most analyses, any test reaching significance with a probability of type I error of .05 or less was followed with an application of the Newman-Keuls method for making multiple pairwise comparisons between means. An alternative method, trend analysis, was used in Experiment 3 to test response magitudes to stimuli of graded magnitude. This test, which generates F-statistics, is used in those situations in which there is a continuum underlying the independent variable, and one is interested in determining whether the variation present can be attributed to specific trend across levels of the independent variable. (For discussion of the Newman-Keuls, trend analysis, and basic rationale for choosing multiple comparisons, see Glass and Hopkins, 1984, pp. 368-392, and in particular pp. 374-376, 396-391).

Categorical Data. As counts of response types were categorical data, they could not be analyzed using an analysis of variance. Instead, contingency tables were constructed for the data analyzed and chi-squared tests of association (Glass & Hopkins, 1984, pp. 285-289) applied to proportions of reversals and accelerations observed with respect to the stages tested. This test is similar to the analysis of variance in that it tests the hypothesis that there exist no differences between groups in the proportion of individuals exhibiting a given attribute. No theoretical values for the proportions

expected are assumed; rather, expected frequencies are calculated based on the data actually obtained. This test works well even when the average expected frequency of the response in a cell is as low as 2 (see notes in Glass & Hopkins, 1984, p. 288).

As with the F-test for the ANOVA, a significant test statistic (χ^2) indicates only that a statistically significant difference exists between proportions observed in a contingency table, with no indication as to which proportions can actually be considered different. Significant ($p < .05$) chi-square values were thus followed with multiple pairwise comparisons between the proportions observed (see Glass & Hopkins, 1984, pp. 391-392). These tests compare the variance of the proportions tested in the contrast with the expected variance of the contrast, the ratio having a chi-squared distribution with degrees of freedom equal to one less than the number of groups in the data set. Confidence intervals for the proportions were also constructed, using confidence interval tables given for binomial proportions (pp. 280-281, Glass & Hopkins, 1984).

Experiment 1:
Spontaneous Reversals

C. elegans shows at least two types of reversal. One, discussed previously, is the reflex reversal: a reversal that occurs in response to mechanical

stimulation. Both the head touch withdrawal response and the tap reversal reflex are reflex reversals. The other, seen frequently in adult animals, is the spontaneous or uninduced reversal. Adult animals frequently reverse direction while swimming undisturbed on a bare agar surface, and although they may be responding to unseen factors in the agar or to minute vibrations not detectable by observers, the response is not caused by any obvious exogenous mechanical stimulation.

Evidence from another invertebrate species suggests that developmental changes may be reflected in the frequency of spontaneous reversals. Rankin et al (Rankin, Stopfer, Marcus, & Carew, 1987) found that juvenile Aplysia showed a high frequency of spontaneous contractions of the newly developed gill and siphon. The frequency of spontaneous contractions decreased as the animals matured. This suggests that as new elements in a circuit develop, there may be a concurrent high rate of spontaneous activity in those elements. If this reflects a general principle of neuronal development and its effect upon behaviour, then perhaps similar increases will occur in the occurrence of spontaneous activity in the touch circuit.

The first experiment was thus designed to examine the frequency of spontaneous reversals as they occurred over development.

Method

Subjects

Ten animals for each of the six developmental stages were used (60 animals in total).

Protocol

Single animals were transferred to a test plate 3 min prior to filming. The plate was then left on the lighted microscope stage for these 3 minutes, after which these animals were filmed for 10 minutes under the same illumination. For all experiments and all stages, the bright-field illumination was held at a constant level. Animals at each stage were filmed at magnifications allowing the best combination of resolution and ease of tracking. In order to equate the amount of tracking required between stages, magnifications giving approximately equivalent-sized video screen images were chosen.

Scoring and Statistical Analyses

Videotapes were scored for total number of spontaneous reversals (in ten minutes) and percent of time spent active.

One-factor between groups analyses of variance were used to analyse the data. Developmental stage was set as the between groups factor.

Results

Developmental stage had a significant effect on the mean number of spontaneous reversals observed during the 10-minute observation period; $F(5, 54) = 4.84$, $p < .005$.

Newman-Keuls posthoc multiple comparisons revealed that the young adult stage showed significantly larger numbers of reversals than any other age group ($p < .05$; see Figure 4).The number of spontaneous reversals was similar across all other stages tested.

Animals of all stages were active for most of the ten minute observation period. The percentage of time an animal spent active showed a significant effect of developmental stage; $F(5, 54) = 3.106$, $p < .02$. Multiple comparisons showed that the L1 animals spent significantly less time active than any other stage ($p < .05$; see Figure 5). Other developmental stages were not significantly different from each other.

Discussion

Spontaneous reversals occur at similar rates across most developmental stages tested. However, in the hours immediately following the last larval molt, young adult animals show a greatly elevated tendency to engage in spontaneous reversals. This increase was not the result of a increase in locomotor activity in general, as all stages with the exception of L1 were active for approximately the same percentage of time. Interestingly, it is at approximately this stage (about 40 hours post-hatch) that Chalfie and Sulston (1981) report that the AVM-ALM connections become functional.

Figure 4. Total number of spontaneous reversals in ten minutes, all developmental stages. Black bars indicate mean number of spontaneous reversals seen during a 10 minute observation period for each stage tested. Only the young adult stage (*) was significantly different from the other stages tested. Means are presented with standard errors (which in the case of the ANOVA, is $\sqrt{MS_e / n}$, the square root of [the averaged standard deviation / n]) ; see Glass & Hopkins, 1984, pp. 217, 233, 436, for discussion of standard errors).

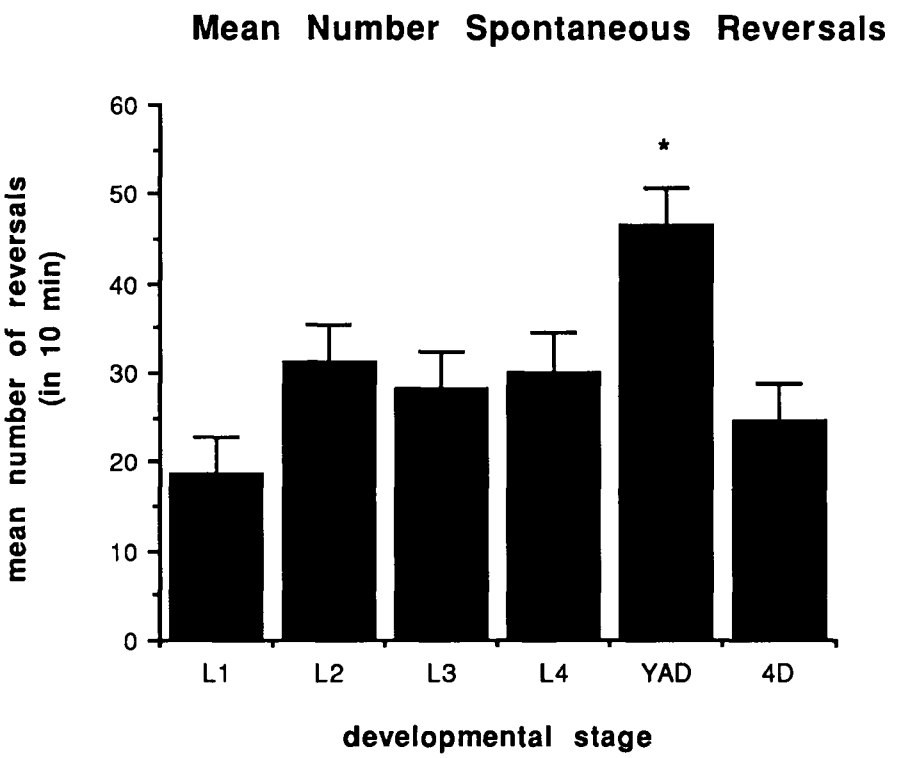
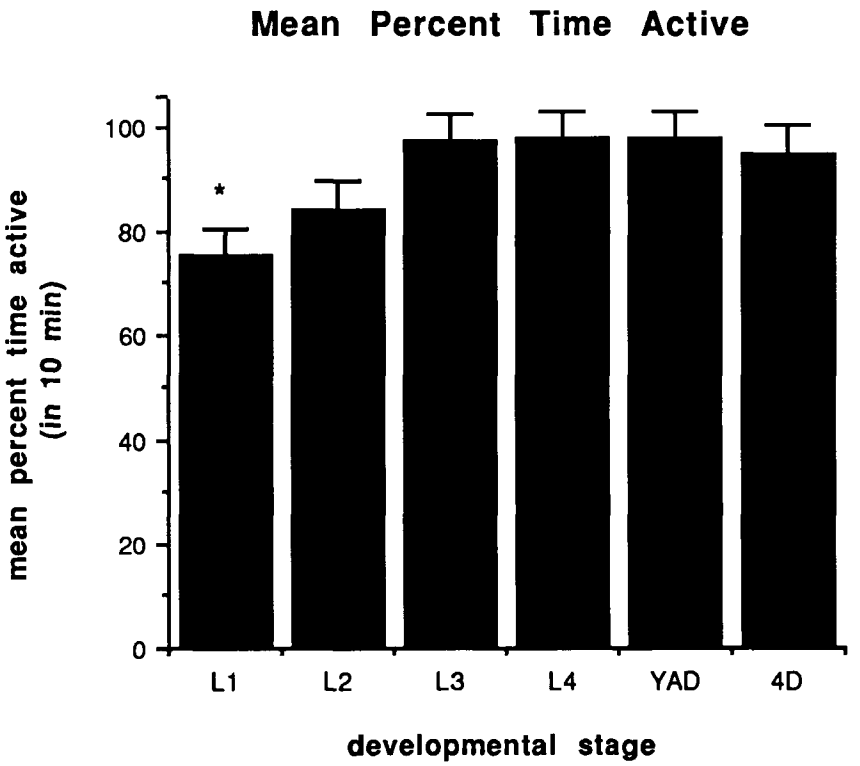

Mean Number Spontaneous Reversals
mean number of reversals
(in 10 min)
60
50
40
30
20
10
0
*
L1
L2
L3
L4
YAD
4D
developmental stage

Figure 5. Mean percent time active in 10 minute observation period, ± standard errors. L1 animals (*) spend significantly less time active than the other developmental stages tested, with the exception of L2.


Mean Percent Time Active
mean percent time active
(in 10 min)
100
80
60
40
20
0
*
L1
L2
L3
L4
YAD
4D
developmental stage

It is possible that the change in spontaneous reversal frequency is due to a neuroanatomical change manifesting itself in behaviour. However, it is difficult to say whether the observed change is the direct result of connectivity changes occuring in the circuit mediating reversals, or the result of another factor, such as a change in sensory threshold. One method of addressing this problem is to examine reflex behaviours in which the source of the stimulus for reversal is clearly identified and quantifiable.

Experiment 2:
Reflex Reversals to Touches and Taps

In the adult, both tap and head touch elicit the reversal reflex. Although we hypothesize that both stimuli are acting upon the touch withdrawal circuit, it is possible that the two stimuli are interacting with the circuit in slightly different ways. As the tap operates by sending vibrations through the agar, it may actually be stimulating touch receptors in both the head and the tail of the animal. The head touch stimulates the touch receptors in the head alone.

Thus, in the second experiment the responses elicited by head touches were compared to those elicited by taps across developmental stages. This experiment was useful both in determining whether younger animals respond to the tap at all, and in determining whether responses differed

as a function of developmental stage.

Method

The general methods and apparatus for this experiment are described under "General Methods". Detailed below are the relevant features of Experiment 2.

Subjects

Twenty animals were used for each of the six developmental stages (total: 120 animals). Half the animals in each stage were randomly assigned to a "touch first" group, while the other half were assigned to a "tap first" group. This was done to control for order effects.

Protocol

All animals were given two stimuli, 7-15 minutes apart, one a gentle touch to the head of the animal, delivered using a fine hair glued to the needle of a 1 cc syringe; the other a single tap, delivered to the side of the dish with a mechanical tapper.

Scoring and Statistical Analyses

Responses to touches and taps were scored for both response type and response magnitude. Frequencies of animals giving specific response types were analyzed using chi-squared tests of association to test for effects of developmental stage. Reversal and acceleration frequencies were analyzed separately. Magnitudes of responses (regardless of response type) were first analysed with a 2-factor repeated measures ANOVA with

developmental stage as the between groups factor and stimulus type as the within-groups factor, nested within stage (as each animal in a given developmental stage was tested on its responses to both the touch and the tap).

Results

In experiments with adults, taps almost always elicit reversals. In this experiment, larval animals sometimes reversed to the tap, and sometimes showed a previously undescribed response to tap, an acceleration.

When animals accelerate to a tap, they travel forward rather than backward, moving forward if they had been stationary prior to the stimulus, moving faster if they had been moving forward. Regardless of stage, the speed of the animal prior to the stimulus was on average 43.3% of the speed attained during acceleration.

Response Frequencies

Regardless of developmental stage, head touches always elicited reversals from animals (see Figure 6a). This was not the case for taps, which elicited both reversals and accelerations (see Figures 6a & 6b). Separate chi-squared tests of association were applied to the reversal and acceleration frequencies exhibited in response to taps.

I. Frequency of Reversals. The proportion of animals exhibiting reversals in response to tap differed significantly as a function of stage; $\chi^2(5) = 41.6$, $p < .0001$. These proportions and their associated confidence

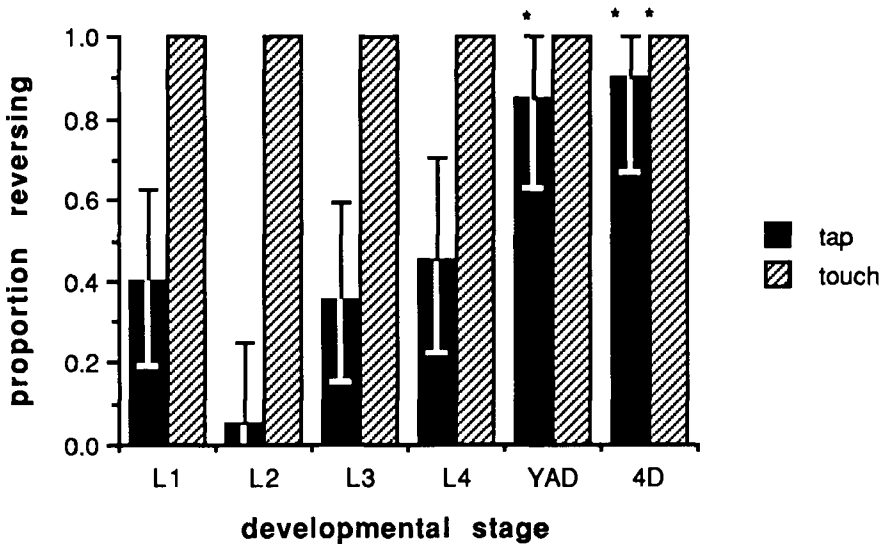
intervals are plotted in Figure 6a. Multiple pairwise comparisons of the proportions indicated that the 4 day old adults showed significantly higher proportions of reversals to taps than any of the larval stages. Young adults were significantly different from L2s and L3s, but not from either L1s or L4s, although both proportions were lower than that observed for young adults. Multiple comparisons revealed no significant difference between larval stages.

II. Frequencies of Acceleration. The proportions of animals exhibiting accelerations in response to tap are plotted with their associated confidence intervals in Figure 6b. A chi-squared test of association on the proportion of accelerations as a function of stage indicated a significant difference between stages; $\chi^2(5) = 42.44$, $p < .0001$. Multiple pairwise comparisons on these proportions indicated that all larval stages showed significantly more accelerations than either adult stage ($p < .05$ or smaller), although the hypothesis that L4 animals were different from young adults was not rejected.

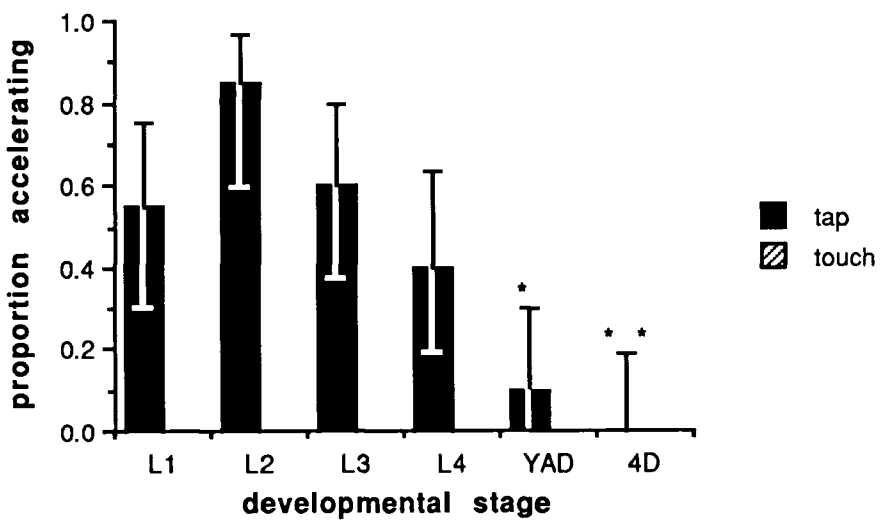
III. Other Responses. Only ten out of 120 animals showed responses that were not clearly defined as either accelerations or reversals. Of these, five showed no observable response to tap (1 animal out of the 20 in each of stages L1, L2, and YAD; 2 animals out of 20 in L4),

Figure 6. Proportions of animals reversing and accelerating to tap and touch stimuli. Each bar represents data for 20 animals; each animal was tested with both tap and touch. Figure 6(a) shows the proportions of animals reversing at each stage to tap (black bars) and to head touch (hatched bars). Figure 6(b) shows proportion of animals that accelerated to the same stimuli (black bars, responses to tap; non-existent hatched bars, responses to touch). All animals at all developmental stages reversed to head touch; none accelerated. Proportions of response types to tap are depicted with 95% confidence intervals. Both adult stages showed significantly higher proportions of reversals and lower proportions of accelerations than larval stages (YAD (*) significantly more reversals and fewer accelerations than L1, L2, and L3 stages; 4D (**) significantly more reversals and fewer accelerations than all larval stages.)

(a) Reversals to Tap and Touch



(b) Accelerations to Tap and Touch



4 paused or ceased moving for more than 1 second following the tap (1 animal in L4; 2 in 4D), and 1 exhibited a slight movement forward followed by a reversal that occurred just beyond the 1 second cut-off point (1 animal in L2). As very few of these responses fell into the "other" category, and as they appeared to be distributed evenly between groups, the frequencies of these responses were not analyzed.

Response Magnitudes

All magnitudes were expressed as distance travelled relative to the animals' own body lengths. There was a significant effect of stimulus type (tap versus touch) on the magnitudes of the responses observed; $F(1, 114) = 98.897$, $p < .0001$. Responses to touches showed significantly larger relative magnitudes than responses to taps regardless of stage (overall means and standard errors for touches and taps were 2.33 ± 0.13 and 1.04 ± 0.13 body lengths respectively). There were also significant age ($F(5,114) = 4.561$; $p < .0008$) and age by stimulus interaction ($F(1,114) = 4.417$; $p < .001$) effects on response magnitude, but these effects were manifested only in responses to touch. As the actual magnitude of touch was neither controlled nor quantifiable (touches were delivered by hand with a non-calibrated hair), this difference was not pursued. Additional one-factor analyses of variance performed on the reversals and accelerations to tap showed no significant effects of

stage upon magnitudes of either reversals or accelerations observed; $F(5,54) = 0.556$, $p > .7$ for reversals; $F(4, 45) = 1.299$, $p > .25$ for accelerations), nor were there any detectable differences in velocity or duration of acceleration for any of the stages tested ($F(4, 45) = 0.283$; $p > .85$ for velocity; $F(4, 45) = 0.698$, $p > .5$ for duration).

Discussion

As Chalfie and Sulston (1981) found, both larval and adult animals reversed to head touch at all ages tested. This was not the case, however, with responses to tap. Larval and adult animals differed significantly in their tendency to reverse to taps: young adults and 4 day old adults nearly always reversed to taps, whereas larval animals were almost as likely to accelerate as reverse.

The accelerations were an unexpected response. In adult animals, in studies of non-associative learning in *C. elegans*, only reversals had been recorded as responses to tap. Other responses appeared only infrequently, and some, such as pauses, typically occurred once the animal had been habituated. It is clear from this data, however, that accelerations are a significant class of responses to the tap, at least in larval animals.

It is also clear from the data that touches and taps produce different response patterns in *C. elegans*. Not only do they elicit different response types across

developmental stages, they also elicit different magnitudes of response regardless of developmental stage. Responses to touch elicited significantly larger magnitudes of response than did taps across all developmental stages.

In addition, response magnitude to touch showed an apparent effect of developmental stage. However, it is difficult to make statements about response magnitudes to touch as the magnitude of the touch was not controlled. The tap, however, was of uniform magnitude. Animals gave approximately equivalent responses to tap across developmental stages. The final experiment was thus designed to test the effects of tap stimuli of quantifiably different magnitudes across developmental stages.

Experiment 3: Graded Response

Adult *C. elegans* show a graded response to taps of increasing magnitude. Single taps elicit short reversals, while multiple taps elicit longer reversals (Rankin, personal communication). Experiment 3 was designed to examine the effects of differing stimulus magnitudes on response magnitude in each of the developmental stages. Large, intermediate, and small magnitude taps were used in order to determine whether the response is graded throughout development.

Method

Subjects

Sixty animals of each of the six developmental stages were used (total: 360 animals).

Protocol

Each animal received only a single stimulus. In each stage, animals were tested for their response to either (a) weak (single tap), (b) intermediate (train of three taps) or (c) strong (train of six taps) stimuli. A Grass S88 stimulus generator was used to generate the taps via a mechanical tapper: single taps were generated by a single 60 volt, 25 msec signal to an electromagnetic relay in the mechanical tapper; trains of three taps by a 60 V, 300 msec signal with a frequency of 8.5 pulses a second; and trains of six taps by a 60 V, 600 msec signal with a frequency of 8.5 pulses per second.

Scoring

Responses were videotaped and scored for frequency and magnitude as described in the General Methods.

Statistical Analyses

Frequency of Response. Separate sets of contingency tables were constructed for reversals and accelerations. Chi-squared tests of association were used to examine frequencies of response type, first as a function of age and second as a function of stimulus type. As both the effects of age and stimulus type were significant,

additional chi-squared tests of association were applied to responses at each stimulus magnitude to test for differences across developmental stages. Significant results on these tests were followed with multiple pairwise comparisons, as described previously.

Magnitude. Magnitudes of responses were tested initially with 2-factor analyses of variance, with between group factors of stage and stimulus magnitude. Significant results were followed with separate one-factor analyses of variance (where appropriate) and trends analyses.

Results

Response Frequencies

I. Frequencies of Reversals. The proportion of animals exhibiting reversals was affected by both stage and stimulus magnitude; $\chi^2(5) = 104.191$, $p < .0001$ for stage; $\chi^2(2) = 21.055$, $p < .0001$ for magnitude. Regardless of stimulus magnitude, adults tended to reverse: 40 out of 60 young adults and 49 out of 60 four day old adults reversed. Larval animals tended not to reverse: under the same conditions, only 19 out 60 L1s, 4 out of 60 L2s, 13 out of 60 L3s, and 16 out of 60 L4s reversed. However, when frequencies of reversals to the three different stimulus magnitudes were examined without respect to developmental stage, the data indicated that single taps produced the greatest number of reversals (67 out of a possible 120 reversals), while multiple taps elicited fewer reversals (38 out of 120 for 3 taps, 36 out

of 120 for 6 taps). Data were then analyzed separately to determine effects of developmental stage at each stimulus magnitude.

Single taps. The proportion of responses to single taps differed significantly across stages; $\chi^2(5) = 52.075$; $p < .0001$; see Figure 7a). Pairwise comparisons of the proportions indicated that larval animals (L1, L2, L3, and L4) were less likely to reverse to single tap than adults of either age. Larval stage L2 animals showed the lowest frequencies of reversals to tap of all larval stages (see Figure 7a); this difference did not reach significance.

Three taps. Fewer animals tended to reverse when given a train of three taps. However, developmental stage continued to have a significant effect upon the proportions of reversals observed; $\chi^2(5) = 34.044$; $p < .0001$. Although all larval stages (L1, L2, L3, and L4) showed fewer reversals to trains of 3 taps than adults, only the L1s and L2s were significantly different from the two adult stages (see Figure 7b).

Six taps. The train of six taps also produced a general trend for fewer reversals relative to those given for single taps, though this trend was not uniform (see Figure 7c). Developmental stage continued to exert a significant effect upon the proportions of reversals observed; $\chi^2(5) = 39.524$; $p < .0001$. Larval stages L2, L3, and L4, all showed significantly fewer reversals than

did 4 day old adults; L3 animals also show significantly fewer reversals than young adults. L1 animals did not differ significantly from either adult stage.

II. Frequencies of Acceleration. Not surprisingly, accelerations to the taps mirrored the trends seen for reversals. In general, larval animals accelerated more frequently than did adults, and multiple taps produced more accelerations overall than did single taps. Regardless of stimulus magnitude, the proportion of animals accelerating to taps differed significantly as a function of stage; $\chi^2(5) = 96.518$, $p < .0001$). Similarly, regardless of developmental stage, the proportion of animals accelerating differed significantly as a function of stimulus magnitude; $\chi^2(2) = 26.131$, $p < .0001$. Single taps produced the fewest accelerations (48 out of a possible 120 accelerations); multiple taps produced accelerations more frequently (79 out of a possible 120 and 84 out of a possible 120 for 3 and 6 taps, respectively).

Single taps. The proportion of accelerations to single taps differed significantly across stages: $\chi^2(5) = 46.25$; $p < .0001$; see Figure 8a). Pairwise comparisons indicated larval animals were significantly more likely to accelerate than 4 day old animals; all larval stages except L1 were also significantly different from young adults. The adult stages did not differ significantly from each other. Again, L2 animals showed the highest

frequencies of acceleration to single taps of all larval stages, but were significantly different only from L1s and adults.

Three taps. Regardless of developmental stage, animals given a train of three taps produced a higher proportion of animals accelerating relative to animals given a single tap (see Figure 8b; compare with Figure 8b). Developmental stage continued to have a significant effect upon the proportions of accelerations observed; $\chi^2(5) = 31.751$; $p < .0001$. All larval stages showed more accelerations to trains of 3 taps than did adults; the L1s, L2s, and L3s were significantly more likely to accelerate than 4 day old adults. The L2s also gave significantly more accelerations than young adult animals.

Six taps. The train of six taps tended to produce more accelerations than the single tap; however, this effect was not uniform (compare Figure 8a and 8c). Developmental stage continued to exert a significant effect upon the proportions of animals accelerating; $\chi^2(5) = 39.524$; $p < .0001$. Larval stages L2, L3, and L4 were significantly different from the 4 day olds. L1 animals did not differ significantly from either adult stage.

III. Other Responses. Other responses contributed only 8 out of the total 360 responses. Of these, 5 occurred in response to single tap, three in response to

Figure 7. Proportions of animals reversing to tap stimuli of varying magnitude. Twenty animals were tested for each combination of age and stimulus magnitude; proportions of animals accelerating in each of these groups are depicted in Figure 8. Figure 7(a) represents proportions of animals reversing to single tap; Figure 7(b), to 3 taps; Figure 7(c), to 6 taps. All proportions are presented with 95% confidence intervals.

REVERSALS

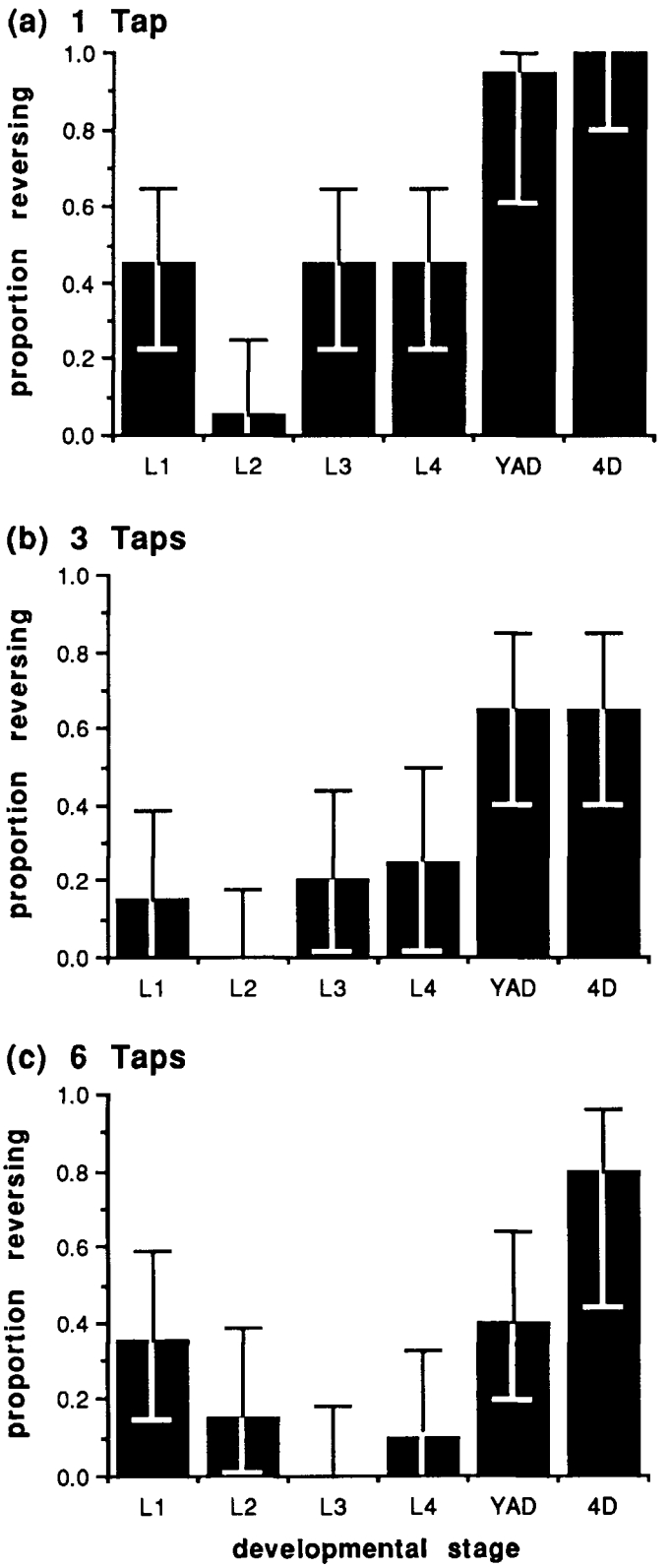
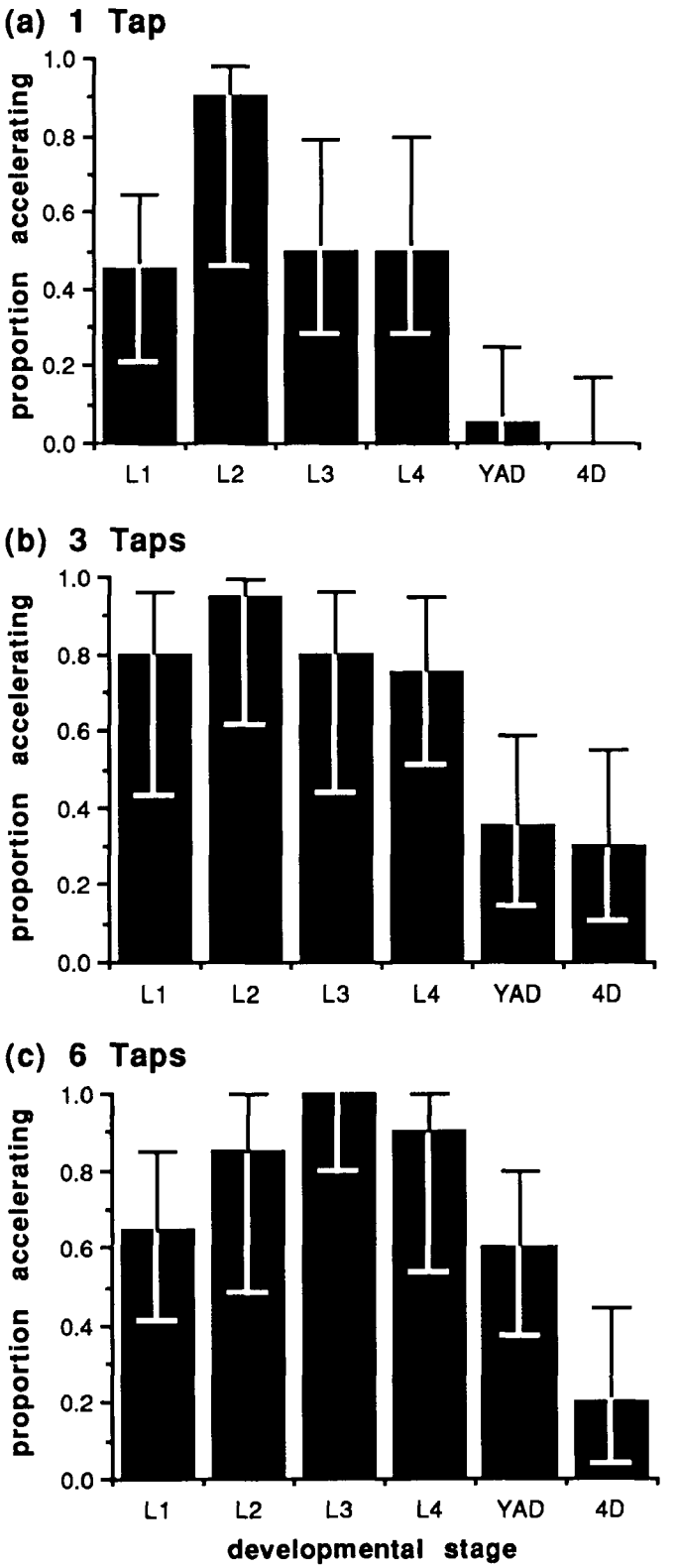

(a) 1 Tap
proportion reversing
1.0
0.8
0.6
0.4
0.2
0.0
L1
L2
L3
L4
YAD
4D
(b) 3 Taps
proportion reversing
1.0
0.8
0.6
0.4
0.2
0.0
L1
L2
L3
L4
YAD
4D
(c) 6 Taps
proportion reversing
1.0
0.8
0.6
0.4
0.2
0.0
L1
L2
L3
L4
YAD
4D
developmental stage

Figure 8. Proportions of animals accelerating to tap stimuli of varying magnitude. Twenty animals were tested for each combination of age and stimulus magnitude; proportions of animals reversing in each of these groups are depicted in Figure 7. Figure 8(a) represents proportions of animals reversing to single tap; Figure 8(b), to 3 taps; Figure 8(c), to 6 taps. All proportions are presented with 95% confidence intervals.

ACCELERATIONS



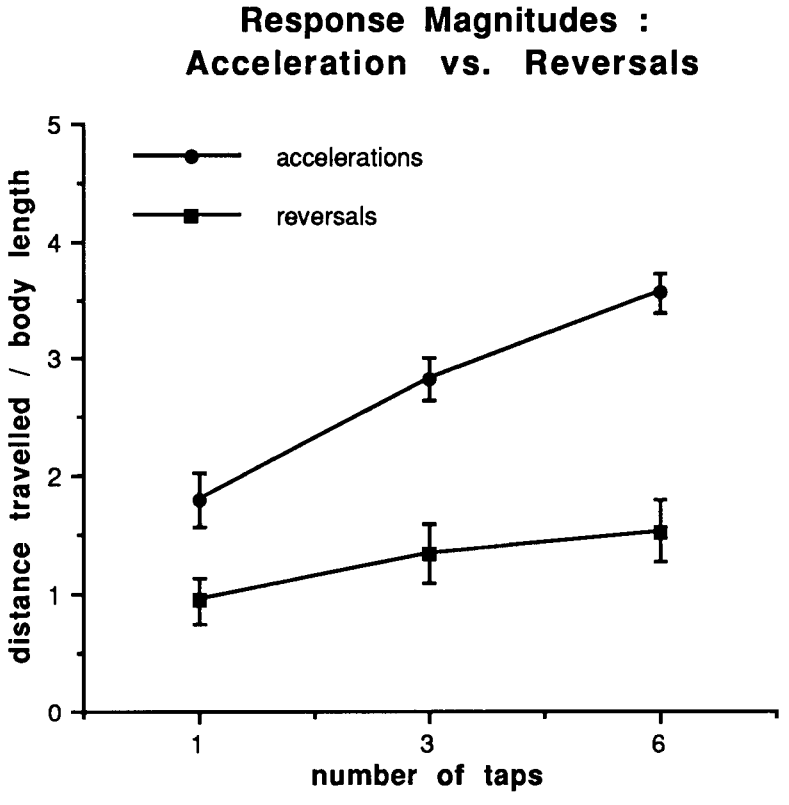
a train of 3 taps, and none in response to 6 taps.

Response Magnitude.

Because both stage and stimulus magnitude significantly affected the frequencies of reversals and accelerations, the next step was to determine whether accelerations and reversals differed in magnitude regardless of developmental stage. In order to assess the effects of stimulus type and response type on response, a two-factor analysis of variance was performed using response magnitude as a between subjects factor. This analysis revealed significant main effects of both stimulus magnitude and response type ($F(2,346) = 15.12$, $p < .0001$ for stimulus magnitude; $F(1, 346) = 68.271$, $p < .0001$ for response type) as well as a significant interaction effect ($F(2, 346) = 3.812$, $p < .05$) between the two variables. This interaction is plotted in Figure 9. Both accelerations and reversals show a tendency to increase as stimulus magnitudes increase; however, accelerations increase more markedly. In addition, average magnitudes of accelerations were uniformly greater than average magnitudes of reversals at all stimulus magnitudes (see Figure 9).

The analyses of frequencies of response types showed that, regardless of stimulus magnitude, the predominant response of larval animals to taps was an acceleration, the predominant response of adult animals a reversal.

Figure 9. Interactions between response type and stimulus magnitude on response magnitudes, collapsed across developmental stages. Distances travelled by animals (relative to body length) are greater when the responses are accelerations rather than reversals, and increase more steeply as a function of increasing stimulus magnitude when the responses are accelerations rather than reversals. Mean distances travelled are presented with standard errors.


Response Magnitudes :
Acceleration vs. Reversals
accelerations
reversals
distance travelled / body length
0
1
2
3
4
5
1
3
6
number of taps

Because the interaction between stimulus magnitude and response type significantly affected the magnitudes of responses seen, the question as to whether response magnitude differed as a function of stimulus magnitude was addressed with respect to accelerations in larval animals and with respect to reversals in adult animals.

Magnitude of accelerations in larval animals. When only larval (L1, L2, L3, and L4) animals were examined, both developmental stage and stimulus magnitude had significant effects upon magnitudes of acceleration; $F(3, 169) = 5.016$, $p < .005$ for developmental stage; $F(2,169) = 11.744$, $p < .0001$ for stimulus magnitude. Trend analysis of the mean magnitudes of acceleration for each of the three stimulus magnitudes showed that a positive linear ($F(1, 169) = 17.07$, $p < .001$) trend accounted for most of the variability in the data. Regardless of larval stage, mean magnitudes of accelerations tended to increase as stimulus magnitude increased from 1 to 3 to 6 taps. However, the difference between means at 3 and 6 taps was less than the difference between means at 1 and 3 taps (mean overall magnitudes [in body lengths] and standard errors for 1, 3, and 6 taps regardless of larval stage were 1.84 ± 0.27, 2.85 ± 0.23, and 3.43 ± 0.23 respectively).

As there were no significant interaction effects between stage and stimulus magnitude ($F(6, 169) = 1.141$; $p > .30$), separate one factor ANOVAs were used to examine

the effects of stimulus magnitude on acceleration size within each larval stage. Larval stages L1, L3, and L4 all showed significant effects of stimulus magnitude on acceleration magnitude (F (2, 35) = 3.328, $p < .05$ for L1; $F(2,43) = 6.396$, $p < .005$ for L3; $F(2, 40)$, $p < .002$ for L4). In each of these stages, trends analyses of the mean acceleration magnitudes showed significant linear trends ($F(1, 35) = 5.228$, $p < .05$ for L1; $F(1,43) = 11.379$, $p < .01$ for L3; $F(1, 41) = 14.497$, $p < .001$ for L4) Again, mean magnitudes of acceleration tended to increase as stimulus magnitude increased, but tended to increase less from 3 to 6 taps than from 1 to 3 taps (see Figure 10a). Although L2 animals also seemed to show an upward trend, an analysis of variance showed that the differences between means was not significant ($F(2, 51) = 0.468$, $p > .5$).

Acceleration magnitudes for adult stages were not analyzed due small and highly unequal n's (ranging from 0 to 12 for a given combination of stage and stimulus magnitude). These results are summarized for comparison in Table 1.

Magnitude of reversals in adult animals. There was a significant effect of both stage ($F(1, 83) = 24.025$, $p < .0001$) and stimulus magnitude ($F(2, 83) = 8.008$, $p < .001$). Trend analysis of the mean magnitudes of reversals to stimuli of increasing magnitude regardless of

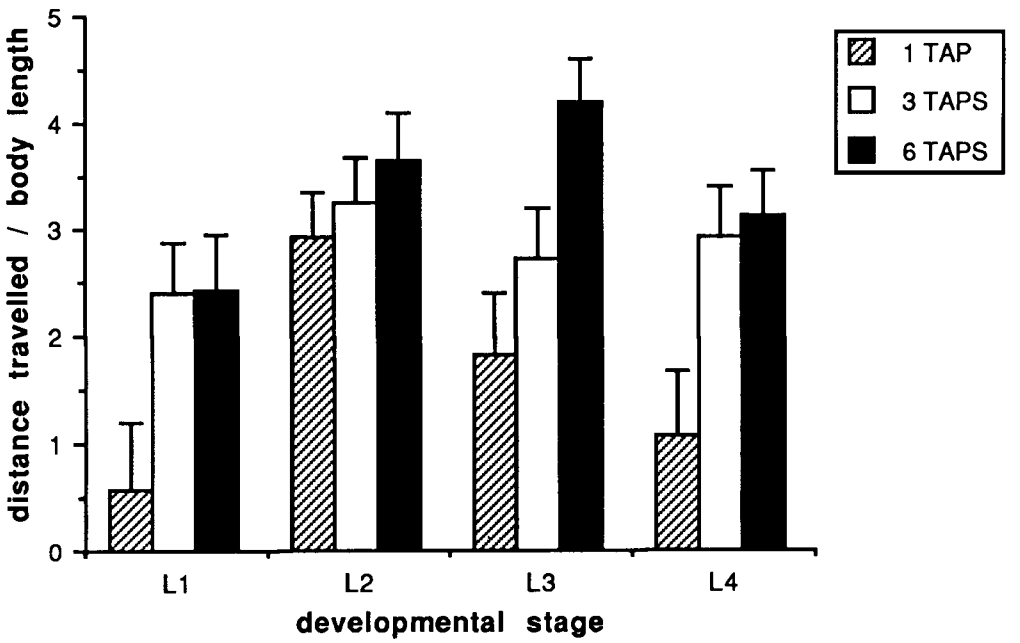
adult stage showed a significant linear ($F(1,83) = 16.072$, $p < .001$) trend. As with accelerations for larval animals, mean reversal magnitudes for the combined adult stages tended to increase with stimulus magnitude, but increased most when increasing from 1 to 3 taps (mean overall magnitudes and standard errors for reversals in adults were 1.01 ± 0.11, 1.573 ± 0.13, and 1.72 ± 0.14 body lengths for 1, 3, and 6 taps, respectively).

As there were no significant interaction effects between adult stage and stimulus magnitude ($F(2, 83) = 0.63$, $p > .5$), separate one factor ANOVAs were performed to examine the effects of stimulus magnitude in each adult stage. Reversal magnitudes of four day old adult animals showed a significant effect of stimulus magnitude ($F(2, 46) = 6.594$, $p < .003$); trends analysis showed that a significant linear ($F(1, 46) = 9.555$, $p < .01$) trend accounted for much of the variability in the data. Although young adult animals also appeared to show a tendency to give increasing magnitudes of reversal in response to increasing magnitudes of stimuli (see Figure 10b), the differences between means were not significant ($F(2,37) = 2.176$; $p > .10$). Mean reversal magnitudes for both adult stages are plotted in Figure 10b.

Mean reversal magnitudes for larval stages were also recorded, but not analyzed due to small and highly unequal n's (ranging from 0 to 9 for a given combination of stage and stimulus magnitude). These results are summarized in

Figure 10. Response magnitudes for stimuli of varying magnitude; larvae and adults. Hatched bars represent mean response magnitudes (relative to body length) to single tap; open bars, to three taps; black bars, to six taps. Means and standard errors are presented; see Tables 1 and 2 number of animals contributing to each mean. Figure 10(a) represents mean acceleration magnitudes for larvae; Figure 10(b) represents mean reversal magnitudes for adults.

(a) Acceleration Magnitudes: Larvae



(b) Reversal Magnitudes: Adults

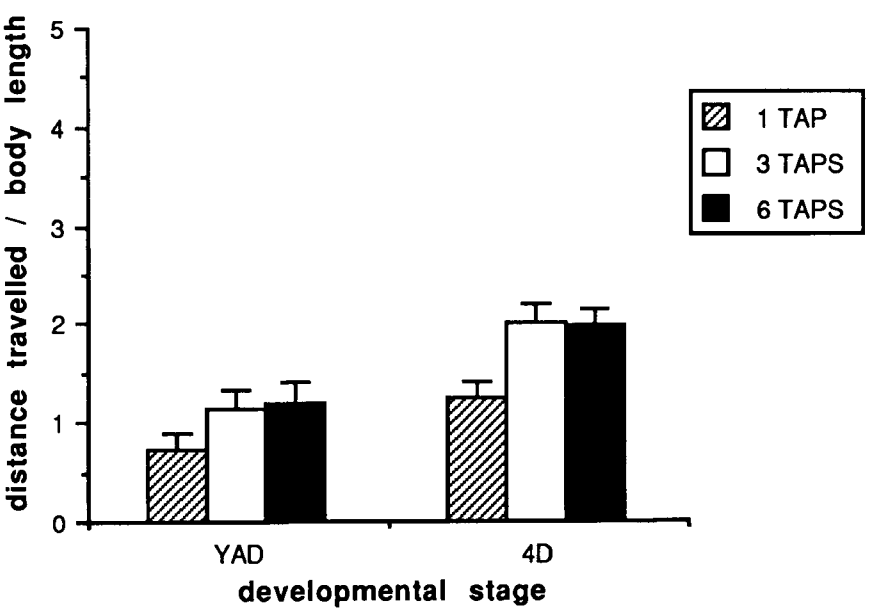


Table 1

Mean Acceleration Magnitudes To Tap Stimuli of Three Different Intensities: All Developmental Stages

	Stimulus Intensity		
Stage	1 Tap	3 Taps	6 Taps
Larvae			
L1	0.56 ± 0.62 (9)	2.41 ± 0.47 (16)	2.43 ± 0.52 (13)
L2	2.91 ± 0.44 (18)	3.25 ± 0.43 (19)	2.00 ± 0.34 (17)
L3	1.82 ± 0.59 (10)	2.73 ± 0.47 (16)	4.19 ± 0.42 (20)
L4	1.07 ± 0.59 (10)	2.92 ± 0.48 (15)	3.11 ± 0.44 (18)
Adults			
YAD*	0.13; no SE (1)	2.61 ± 0.41 (7)	3.88 ± 0.50 (8)
4D*	-- (0)	2.75 ± 0.90 (6)	4.80 ± 1.52 (4)

Note. Means shown with standard errors when applicable; number of accelerations contributing to mean in brackets (n). Total possible accelerations in each cell = 20. All magnitudes in body lengths.

*n's for these groups were too small and too unequal for application of the ANOVA. In these cases, standard errors (SE) were calculated as $\sqrt{s^2 / n}$. Otherwise, SE = $\sqrt{MS_e / n}$.

Table 2

Mean Reversal Magnitudes To Tap Stimuli of Three Different Intensities: All Developmental Stages

	Stimulus Intensity		
Stage	1 Tap	3 Taps	6 Taps
Larvae			
L1*	1.12 ± 0.19 (9)	1.14 ± 0.43 (3)	0.82 ± 0.24 (7)
L2*	1.01; no SE (1)	-- (0)	2.00 ± 0.34 (3)
L3*	0.68 ± 0.17 (9)	0.95 ± 0.24 (4)	-- (0)
L4*	0.81 ± 0.19 (9)	0.58 ± 0.27 (5)	1.00 ± 0.70 (2)
Adults			
YAD	0.74 ± 0.15 (19)	1.14 ± 0.19 (13)	1.19 ± 0.24 (8)
4D	1.26 ± 0.15 (20)	2.00 ± 0.19 (13)	1.98 ± 0.17 (16)

Note. Means shown with standard errors when applicable; number of reversals contributing to mean in brackets (n). Total possible reversals in each cell = 20. All magnitudes in body lengths.

*n's for these groups were too small and too unequal for application of the ANOVA. In these cases, standard errors (SE) were calculated as $\sqrt{s^2 / n}$. Otherwise, SE = $\sqrt{MS_e / n}$.

Table 2.

Other Features of Accelerations

Not surprisingly, when accelerations were examined for all developmental stages, there were significant effects of stimulus magnitude on the duration of accelerations, with duration increasing as magnitude increased ($F(5,194) = 4.11$, $p < .02$). Mean durations of acceleration regardless of developmental stage were 4.1 ± 0.59 s for 1 tap (n = 48), 5.7 ± 0.46 s for 3 taps (n = 79), and 7.2 s ± 0.45 s for 6 taps (n = 84). No significant effects of either stage or stimulus type were found for acceleration velocity; $F(5, 194) = 0.418$, $p > .80$ for stage; $F(2, 194) = 0.493$, $p > .60$ for stimulus magnitude.

Discussion

The analysis of graded response in this experiment showed three major trends: (1) larval animals accelerated more frequently to taps than adults at all stimulus magnitudes; (2) multiple taps elicited greater frequencies of accelerations than single taps, regardless of developmental stage; and (3) larger stimulus magnitudes tended to elicit responses of larger magnitudes. In addition, magnitudes of accelerations tended to be greater than magnitudes of reversals, and were more sensitive to changes in stimulus magnitude.

As the purpose of this experiment was to determine

whether response magnitude was graded as a function of stimulus magnitude, and as larval and adult animals differed significantly in their tendencies to either reverse or accelerate in response to taps regardless of stimulus magnitude, only the magnitudes of the predominant response types given by these groups were analyzed. Overall, both accelerations in larval animals and reversals in adults were graded in magnitude as a function of stimulus magnitude: magnitudes of both accelerations and reversals tended to increase as tap stimuli increased in magnitude from 1 to 3 to 6 taps, although the increases occurring between the two multiple taps than were smaller than those occurring between single and multiple taps. These trends were maintained for accelerations in larval stages L1, L3, and L4, and for reversals in the four day old adults; both groups of animals gave larger responses in response to larger stimuli, but seemed to differentiate less between the two largest stimuli. Only larval stage L2 and the young adult stage did not show a significant trend towards gradedness of response, although their group means were consistent with the trend to increase as a function as stimulus magnitude (see Tables 1 and 2).

It therefore appears that, at least with accelerations in larval animals and reversals in adults, the response magnitudes to tap stimuli increase as the taps increase in magnitude. The largest increases in

response magnitude occur as the stimuli increase from single to multiple taps; however, animals tend to differentiate less between the two multiple taps. This trend also appears to be reflected in the proportions of animals giving either reversals or accelerations for stimuli of increasing magnitude: regardless of developmental stage, more animals tended to accelerate (and not reverse) to multiple taps relative to single taps, but did not necessarily accelerate more (and reverse less) to a multiple tap consisting of 6 taps relative to a multiple tap consisting of 3 taps.

It should also be noted that the gradedness of the response was not reflected in differences in velocity attained by animals that accelerated. The sole differences between responses to differing stimulus magnitudes seemed to lie in the total time and distance that passed before the responses ended.

It is also interesting to note that the results obtained in Experiment 3 for responses to single tap replicated the pattern of results obtained for single tap in Experiment 2. As in Experiment 2, larval animals in Experiment 3 tended to give fewer reversals and more accelerations to single tap than either adult stage. More interestingly, larval stage L2 animals again showed the lowest proportion of reversals (and highest proportion of accelerations) to single taps of all developmental stages.

GENERAL DISCUSSION

Both spontaneous reversals and the tap reversal reflex show developmental changes in C. elegans. In Experiment 1, young adult animals showed an increase in the frequency of spontaneous reversals occurring relative to the other developmental stages. In Experiments 2 and 3, larval animals of all stages showed significantly different patterns of responding to taps than either of the adult stages. Although animals of all stages reversed in response to touch, taps elicited not only reversals, but a previously undescribed response, accelerations. Larval animals showed reversals on approximately half the responses to taps, while young adult and 4 day old adults consistently showed reversals in response to single taps. Increasing stimulus magnitude also increased the probability of accelerations relative to single taps; this was true for all developmental stages, though larval animals still showed fewer reversals and more accelerations than adult animals.

Spontaneous Reversals. Young adults showed a significantly elevated tendency to exhibit spontaneous reversals over a ten-minute observation period. This was not merely a function of increased activity, as other stages (except L1s) showed similar activity rates and failed to show elevated rates of spontaneous reversing. The elevation was also unlikely to be the result of a

difference in sensory threshold as young adults did not differ significantly from any other stage in the magnitude of response to either touches or taps.

Although other studies have also looked at the frequency of spontaneous reversals in C. elegans and related nematodes (e.g., see Croll, 1975; Pollock & Samoiloff, 1976), none have looked specifically at the young adult stage. In Panagrellus redidivus, a related nematode, Pollock and Samoiloff (1976) obtained data that seemed to indicate a general trend towards increasing frequencies of reversals per unit time as a function of age (data recomputed by Dusenberry, 1980); however, the ages of the adult animals studied were not specified.

The increase in frequencies of spontaneous reversals in young adult appears to be a transient phenomenon. Adult animals at 4 days of age do not differ from larval stages, indicating that if this increase is a function of some neuronal change, it is one that occurs only briefly, as an event rather than a long-term adjustment. One possibility is that the increase in spontaneous reversals is a result of the incorporation of new elements into the touch circuit. (e.g., see Rankin, Carew, 1987; see also Bekoff, 1981, for a discussion of spontaneous activity in developing motor systems).

Touches and Taps. As Chalfie & Sulston (1981) showed, the touch withdrawal reflex was present in C. elegans throughout development: all animals at all

developmental stages responded to head touch by reversing. It was not clear whether differences in response magnitude occurred over development, as the touches in these experiments were neither controlled nor quantifiable. This question could be addressed, however, by using touch stimuli that apply force of known magnitude (e.g., von Frey hairs).

The tap reversal reflex was also present throughout development. However, not all animals at all developmental stages reversed in response to tap: larval animals sometimes accelerated, moving forward if initially stationary, moving faster if already in motion. In response to single tap, larval animals were at least as likely to accelerate as reverse; young adult and four day old animals nearly always reversed. This difference between larval and adult stages was particularly pronounced at larval stage L2, which in both Experiments 2 and 3 showed the lowest probability of reversing to single tap relative to all other developmental stages tested. The pattern of results obtained for single tap in Experiment 2 was thus reproduced in Experiment 3.

Magnitudes of responses to single tap did not differ significantly between developmental stages, nor did the velocity or durations of the accelerations, suggesting that the single tap did not differ in magnitude across developmental stages. The single tap did, however, differ

in apparent intensity from touch: touches tended to elicit greater magnitudes of response than single taps.

Graded Response. Similar developmental changes occurred in the tap reversal reflex when assessed with tap stimuli of varying magnitude. Increasing the magnitude of tap stimuli from single to multiple (trains of 3 or 6) taps had two general effects: (a) a tendency to elicit greater magnitudes of response and (b) a tendency to elicit greater proportions of accelerations relative to reversals.

The tendency for animals to give greater magnitudes of response to tap stimuli of increasing magnitude indicated that the tap reversal reflex in *C. elegans* is graded as a function of stimulus magnitude: larger stimuli elicit larger responses. In general, larval animals tended to show significantly larger magnitude accelerations as stimulus magnitude increased, while adult animals showed significantly larger magnitude reversals as stimulus magnitude increased. Young adult and L2 animals were the only stages in which the trends were not pronounced enough to reach statistical significance. Interestingly, in those stages where statistically significant differences occurred, it appeared that the largest difference in response magnitude occurred as the tap stimuli increased from single to multiple taps; relatively little difference appeared to exist between responses to three versus six taps. This finding may

indicate that there is a ceiling effect for responses to stimuli greater than a magnitude of 3 taps; it may also indicate that 3 and 6 taps may have been too close in magnitude to be distinguishable by the animal. Additional tests using stimuli of more widely varying magnitude would help to clarify this issue.

The analysis of graded response was restricted to accelerations in larvae and reversals in adults for two reasons. First, accelerations in general were larger in magnitude and more sensitive to increases in stimulus magnitude than reversals, making it unwise to combine magnitudes of accelerations and reversals to give a single measure of response magnitude. Second, the proportion of larval animals exhibiting reversals and adult animals exhibiting accelerations were at some stimulus magnitudes so low that meaningful analyses were not possible. The responses that were obtained in each of these categories were not inconsistent with the trends for graded response (see Tables 1 and 2); however, larger numbers of responses would have to be obtained before a clear picture of these trends could be established.

The effect of increasing stimulus magnitudes on proportions of response types added another dimension to the results obtained in Experiment 2. The shift in proportions of accelerations relative to reversals occurred not only as a function of developmental stage,

but also as a function of stimulus magnitude. Results of Experiment 3 indicated that as the number of taps given in a stimulus increased from single to multiple taps, the proportion of animals accelerating in all stages increased--in adult stages as well as larval. Although adult animals still tended to reverse more frequently to taps than larval stages at any stimulus magnitude, an adult animal responding to multiple taps was almost as likely to accelerate as a larval animal responding to single tap. Again, the greatest difference seemed to occur between the patterns elicited by single taps relative to the patterns elicited by multiple taps; responses to three taps and responses to six taps showed roughly similar overall frequencies of both response types.

The dual effects of increasing magnitude of tap stimuli observed in Experiment 2 and the differences observed between touches and taps observed in Experiment 2 suggest a number of areas for further exploration. One is the nature of the stimuli used: what features of the tap relative to touch make it more likely to elicit accelerations, especially in larval animals? Another major area to explore is the nature of the effects of varying stimulus magnitudes on responses to tap, namely, what aspects of the stimuli are responsible for the increases seen in (a) response magnitude and (b) proportion of accelerations.

The relationship between touches and taps as stimuli was an issue first raised in Experiment 2. Touches not only always produced reversals at all developmental stages, they also appeared to be more intense stimuli, eliciting larger overall magnitudes of response regardless of developmental stage. Was this because the touches used were of greater physical magnitude than the single taps used? Or do touch stimuli always produce larger magnitudes of response relative to taps, even if both are of equivalent physical magnitudes? In order to address this issue, one needs to know the physical magnitudes of the forces applied by both the tap and the touch, and be able to find calibrate the stimuli so that one can give an animal both touches and taps of known and controllable magnitude. Such an analysis would provide information about the ways in which touch and tap stimuli interact with the neural circuits underlying the responses observed.

Another question that bears further exploration is the nature of the sensory signals that touches and taps represent. In the introduction to Experiment 3, one speculation put forth was that the taps may be stimulating receptors from both the head and the tail of the animal, whereas the head touch stimulates receptors only in the head. This is an attractive hypothesis given that the acceleration response appears to be similar to the tail

touch withdrawal response--that is, the animal moves forward--and might be mediated by this portion of the neural circuit. In addition, if it is supposed that touch stimulates the anterior head touch receptors directly, while tap stimulates both anterior and posterior responses, it might be that response magnitudes to touch are always larger than responses magnitudes to tap, given that they are likely to be less inhibited or inhibited later by competing responses mediated by the portion of the circuit mediating the opposing response.

One way to test this hypothesis is to examine the effects of stimuli over development in mutants that lack either the anterior or posterior touch receptors (but not both). Mutants missing the tail touch receptors (PLMs) throughout development should always respond to taps with reversals regardless of developmental stage. Mutants missing all of the anterior tail touch receptors (ALMs and AVM) throughout development should always show accelerations to tap, regardless of developmental stage. In addition, in normal animals, the use of a stimulus that stimulates both the head and tail of the animal simultaneously (perhaps a pair of hairs) should elicit patterns of responding over development similar to those elicited by tap.

Another issue raised by Experiments 2 and 3 is whether the increases in response magnitude and frequency of accelerations were produced by the same features of

stimulus magnitude. This issue is raised because the stimuli used to test graded response in Experiment 3 varied along two parameters: (1) the total amount of force striking the animal (the sum of the forces of the individual taps) and (2) the total number of taps. Examination of the results of Experiment 2 suggests that the two behavioural changes may have independent causes: the magnitude of the response to single tap did not change over development , while the number of accelerations elicited did.

One way to resolve this issue is compare stimuli that vary in magnitude along only one parameter. Thus, single taps of varying force could be used, with corrections (such as lower water content in the agar) made to damp out oscillations. If the shift in proportions of animals exhibiting accelerations and reversals is solely a function of the magnitudc of the force striking the animal in a given tap stimulus, then it should occur when only high magnitude single taps are used, perhaps showing a ceiling effect at highest magnitudes. Similarly, if the shift in proportions is a function of the qualitative nature of the stimulus--that is, the fact that the taps are multiple rather than single, or in rapid succession rather than singular--then varying these parameters should cause the shift in proportions seen, even if the total force of the stimuli were equivalent. These experiments

should also help to determine what aspects of the tap stimuli are responsible for the increases seen in response magnitude.

The results of Experiments 2 and 3 also raise a number of issues for the analysis of plasticity in the taps reversal reflex over development in this animal. The first of these is practical: as accelerations appeared to be greater in magnitude than reversals, and as larval animals tended to show both types of behaviours in response to tap, it may be more informative to use a simple measure of response occurrence (i.e., yes/no, did a response occur or not) rather than a measure of response magnitude in order to track the changes that may occur due to learning. Another alternative would be to use a strictly categorical method of classifying responses--into reversals, accelerations, pauses, and no responses, for instance. Studying the habituation of responses to touch may be another way of assessing whether the tendency to accelerate to tap is a sole function of stimulus magnitude: if it is the case that accelerations tend to occur to large stimuli and reversals to smaller stimuli, then we might see an increase in the probability of reversals during habituation, when the perceived intensity of the stimulus is thought to become progressively smaller.

The results of these experiments indicate that there are two key transition points in the development of the

tap reversal reflex. These are the transition represented from the newly hatched larval stage L1 to larval stage L2 and the transition from larval stage L4 to the young adult. Newly hatched L1s were the least active of all developmental stages, and the L2s consistently showed the lowest frequency of reversals and highest frequency of accelerations to single taps and trains of three taps in Experiments 2 and 3. Young adults not only showed increased frequencies of spontaneous reversals relative to any other stage, but were also the first stage at which animals started to show an adult-like pattern of responding, predominantly reversing rather than accelerating to tap.

Both of these periods of behavioural change appear to correlate with periods of neuronal change within the animal. The L1 to L2 stage is the period in which the juvenile (embryonic) nervous system begins the switch to the adult nervous system. New cells are born during this time, including many members of the touch circuit. AVM, the AS motor neurons, and the ventral A and ventral B motoneurons all arise during this time (Sulston & Horvitz, 1976, 1977; Chalfie et al, 1985; White et al, 1978). In addition, the existing dorsal D motorneurons, responsible for the coordination of contraction between muscle groups in the animal, lose their embryonic patterns of connections and become completely rewired (White et al,

1978). The young adult stage represents a second transition: at approximately 40 hours after hatching, the coupling between the AVM and ALM neurons becomes functional.

Figure 2 represents relationships between the neural circuit for touch sensitivity as worked out thus far by Martin Chalfie and colleagues (Chalfie et al, 1985; Chalfie, personal communication to C. Rankin). If the circuit depicted is correct, then the following hypothesis appears tenable: Adult animals reverse to the taps more frequently than larval animals because of a new inhibitory connection between AVB via AVM, and possibly because of the addition of asensory neuron, AVM.

If the relative number of inhibitory connections affects the probability that a response will inhibited, it becomes clearer why adults reverse more frequently to taps than do larval animals. In the adult, there are two routes of inhibition going from the anterior (head touch) circuit to the posterior (tail touch) circuit; in larval animals, there is only one. One goes from the ALMs to PVC, which should prevent forward movement by preventing PVC from exciting to the B motoneurons and AVB. The other goes from the AVM cell to AVB. The AVBs are the only interneurons in the posterior circuit whose inhibitory connections are not mirrored by connections coming from the anterior circuit. They are also the only neurons that inhibit the motor neurons producing the competing response

directly.

The critical difference between adult and larval animals may thus be the addition of AVM, and the consequent addition of an inhibitory connection to AVB. Because AVB makes two inhibitory connections upon the anterior touch circuit, the inhibition of this neuron reduces the inhibition of backward movement by the tail touch circuit while simultaneously inhibiting the probability of forward movement. Thus, while the posterior touch circuit has more routes to inhibit the workings of the anterior touch circuit, two of these routes are blocked by the addition of AVM. Removal of AVM (or more particularly, the AVM to AVB connection) via laser ablation or genetic lesion should result in adults that show the larval pattern of responding.

Conclusion

Both spontaneous reversals and reflexive reversals to tap show developmental changes in C. elegans. These changes, seen at the behavioural level, appear to correlate with specific changes the neuronal circuit thought to mediate the responses. These results suggest further investigations to explore the nature of the relationship between these behaviours and their neuronal correlates.

References

Bailey, N.T.J. (1981). Statistical methods in biology (2nd ed.). New York: John Wiley and Sons.

Bekoff, A. (1981). Embryonic development of the neural circuitry underlying motor coordination. In Studies in developmental neurobiology, W.M. Cowan, Ed., pp. 134-170. New York: Oxford University Press.

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

Brenner. S. (1988). Foreword to The Nematode Caenorhabditis elegans, W.M. Wood, Ed., pp.ix-xii. New York: Cold Spring Harbor.

Byerly, L., Cassada, R.C., & Russell, R.L. (1976). The life cycle of the nematode Caenorhabditis elegans: I. Wild type growth and reproduction. Developmental Biology, 51, 23-33.

Chalfie, M. (1984). Neuronal development in Caenorhabditis elegans. Trends in Neuroscience, 7, 197-202.

Chalfie, M. & Au, M. (1989). Genetic control of differentiation of the Caenorhabditis elegans touch receptor neurons. Science, 243, 1027-1033.

Chalfie, M., & Sulston, J. (1981) Developmental genetics of the mechanosensory neurons of Caenorhabditis elegans. Developmental Biology, 82, 358-370.

Chalfie, M., Sulston, J.E., White, J.G., Southgate, E., Thomson, J.N., & Brenner, S. (1985). The neural circuit for touch sensitivity in Caenorhabditis elegans. The Journal of Neuroscience, 5, 956-964.

Chalfie. M., Thomson, J.N., & Sulston, J.E. (1983). Induction of neuronal branching in Caenorhabditis elegans. Science, 221, 61-63.

Chalfie, M., & White, J. (1988). The nervous system. In The Nematode Caenorhabditis elegans, W.M. Wood, Ed., pp. 337-391. New York: Cold Spring Harbor.

Coulson, A., Sulston, J., Brenner. S., & Karn, J. (1986). Towards a physical map of the genome of the nematode Caenorhabditis elegans. Proceedings of the National Academy of Sciences, 83, 7821-7825.

Croll, N.A. (1975). Components and patterns in the

behaviour of the nematode Caenorhabditis elegans. Journal of Zoology (London), 176, 159-176.

Dusenberry, D.B. (1980). Behavior of free-living nematodes. In Nematodes as biological models: Vol. 1. Behavioral and developmental models, B.M. Zuckerman, Ed., pp. 127-158. New York: Academic Press.

Glass, G.V., & Hopkins, K.D. (1984). Statistical methods in education and psychology (2nd ed.). Englewood Cliffs: Prentice-Hall.

Hodgkin, J., Horvitz, H.R., & Brenner, S. (1979). Nondisjunction mutants of the nematode Caenorhabditis elegans. Genetics, 91, 67-94.

Pollock, C., & Samoiloff, M.R. (1976). The development of nematode behaviour: Stage-specific behaviour in Panagrellus redivivus. Canadian Journal of Zoology, 54, 674-679.

Rankin, C.H., Stopfer, M., Marcus, E.A., & Carew, T.J. (1987). Development of learning and memory in Aplysia. I. Functional assembly of gill and siphon withdrawal. Journal of Neuroscience, 7, 120-132.

Rankin, C.H., & Chalfie, M. (1989). Analysis of non-associative learning in C. elegans: I. Neural circuit mutations. Society for Neuroscience Abstracts, 15, in press.

Rankin, C.H., & Chiba, C.M. (1988). Short and long-term learning in the nematode C. elegans. Society for Neuroscience Abstracts, 14, 607.

Rankin, C.H., Beck, C.D.O., & Chiba, C.M. (1989). Caenorhabditis elegans: A new model system for the study of learning and memory. Behavioral Brain Research, in press.

Rose, A.M., & Baillie, D.L. (1979). The effect of temperature and parental age on recombination and nondisjunction in Caenorhabditis elegans. Genetics, 92, 409-418.

Sulston, J.E. (1976). Post-embryonic development in the ventral cord of Caenorhabditis elegans. Philosophical Transactions of the Royal Society of London, B. Biological Sciences, 275, 287-298.

Sulston, J.E. (1988). Cell lineage. In The Nematode Caenorhabditis elegans, W.M. Wood, Ed., pp. 123-155. New York: Cold Spring Harbor.

Sulston, J.E., & Horvitz, H.R. (1977). Post-embryonic cell lineages of the nematode Caenorhabditis elegans. Developmental Biology, 56, 110-156.

Sulston, J.E., Schierenberg, E., White, J.G., & Thomson, J.N. (1983). The embryonic cell lineage of the nematode Caenorhabditis elegans. Developmental Biology, 100, 64-119.

Walthall, W.W., & Chalfie, M. (1988). Cell-cell interactions in the guidance of late-developing neurons in Caenorhabditis elegans. Science, 239, 643-645.

Wood, W.M. (Ed.) (1988). The nematode Caenorhabditis elegans. New York: Cold Spring Harbor.

White, J.G., Albertson, D.G., & Anness, M.A.R. (1978). Connectivity changes in a class of motoneurone during the development of a nematode. Nature, 271, 764-766.

White, J.G., Southgate, E., Thomson, J.N., & Brenner, S. (1976). The structure of the nervous system of Caenorhabditis elegans. Philosophical Transactions of the Royal Society of London B. Biological Science, 314, 1-340.

Zuckerman, B.M. (Ed.) (1980a). Nematodes as biological models: Vol. 1. Behavioral and developmental models. New York: Academic Press.

Zuckerman, B.M. (Ed.) (1980b). Nematodes as biological models: Vol. 2. Aging and other model systems. New York: Academic Press.