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**THE TOUCH SENSITIVE BEHAVIOR OF *CAENORHABDITIS ELEGANS*:
A SIMULATION APPROACH USING NEURAL NETWORKS**

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THE TOUCH SENSITIVE BEHAVIOR OF *CAENORHABDITIS ELEGANS*: A SIMULATION APPROACH USING NEURAL NETWORKS

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Abstract

The paper describes a neural network model of the touch sensitivity circuit of the nematode *Caenorhabditis elegans*. Much of the worm's biology, genetics, neurobiology, and behavior is known (Wood 1988) and therefore *C. elegans* can be considered as a good experimental model for both neurobiology and psychology. We describe a serie of simulations in which neural networks are trained, using a genetic algorithm, to reproduce the habituation of the nematode's touch sensitive behavior. A lesion study of the network allows to make a direct comparison between the fine functioning of the model and the data collected by Chalfie *et al.* (1985) using the ablation method of single neurons. The model accords well to the known neurobiological data, and it suggests some hypotheses on the neural functioning of the circuit and of single neurons. It needs, however, further refinements and testing.

1. Introduction

Computational models of the nervous system may be classified into two classes: realistic and simplifying models (Sejnowski, Koch, and Churchland, 1988; Churchland and Sejnowski, 1992). Realistic models tend to simulate the nervous system by incorporating as many known details as possible of its structure and function. For example, the Hodgkin-Huxley model of the neuron (Hodgkin, 1976) simulates the biochemical functioning of single neurons and, more specifically, action potentials as the result of properties of membrane channels.

Simplifying brain models are computational models that attempt to simulate the brain at the level of the general principles that appear to regulate its structure and function but ignore many known details of the nervous system. Most work on neural networks belongs to this second class of brain models. Neural networks not only

ignore the fine details of individual neurons, cell membranes, neurotransmitters, ion channels, etc., but they generally do not have particular neural structures in mind and do not refer to the nervous system of particular species of animals. This general rule has exceptions, however, since in some cases neural network models have been used to study specific neural structures (e.g. the Cowan-Friedman model of eye-brain maps; cf. Cowan and Friedman, 1990) or specific behaviors/neural structures of specific animals (cf. the Lockery and Sejnowski model of the bending reflex in the leech (Lockery and Sejnowski (1992) or the Stork *et al.* (1992) model of the tailflip circuit in the crayfish).

The work reported in this paper belongs to this second subclass of neural network applications. Our purpose is to construct a neural network model of the touch sensitivity circuit in the nematode *Caenorhabditis elegans* (*C. elegans*). While low level properties of the nervous system of *C. elegans* are ignored, the model incorporates many known properties of its real neural circuit architecture and attempts to reproduce the nematode's withdrawal response to touch in the head or tail regions. Furthermore, the model is expected to simulate the habituation of this response, i.e., a reduced reaction with repeated presentation of the stimulus.

The paper is divided into two parts. Part A briefly discusses the potentialities of *C. elegans* for the study of the biological and neural factors in behavior. *C. elegans* is a relatively simple animal (less than 1000 cells in total of which about 300 are neurons) and, furthermore, it is a very well studied animal. In fact, due to systematic research efforts many details of its genes, development, neural circuitry, and behavior are well known (Wood, 1988). We give a detailed description of the worm's touch sensitive behavior and of the habituation of this response following repeated stimulation. Also, the neural circuit controlling this behavior is analytically described on the basis of the findings reported by Chalfie and his colleagues (Chalfie, Sulston, White, Southgate, Thompson & Brenner, 1985).

In part B we describe a series of simulations of the nematode's touch sensitive behavior. Neural networks that reproduce *C. elegans*' neural circuit architecture are evolved using Holland's Genetic Algorithm (Holland, 1975). The evolved networks show backward movement in response to stimuli localized in the worm's head region and forward movement in response to stimuli in the tail region. Moreover, the model is able to reproduce the habituation of touch sensitivity, i.e. a decreased response to repeated stimuli. Finally, we report the results

of some neuron lesion simulations in order to compare the role of single neurons in the artificial neural network with the known role of real neurons as determined with laser ablation experiments.

Part A.

***C. elegans* as a biological and behavioral model**

2. *C. elegans*

C. elegans is a small round worm with a simple structure and a simple genome. Since Sydney Brenner's 1965 proposal to use *C. elegans* as a biological model to arrive to a complete knowledge of the micro-biology (cellular development, genetic control, neural structure, etc.) of a simple living organism, the worm has been studied for more than 30 years. *C. elegans* has some properties that facilitate its biological study: it has a short life cycle, it can be easily cultivated, it generally reproduces by self-fertilization, it has a limited number of cells and a simple genome. Much is known about the worm's biology (Wood 1988). One adult hermaphrodite organism is constituted by 959 somatic nuclei (1031 in the adult male) with a largely invariant cellular development. Complete cell lineage is known. Embryonic development, larval development, and sex differentiation mechanisms are known. Many genetic studies, conducted using mutant phenotypes, have clarified the genetic mechanisms controlling development.

C. elegans is a good model for the study of the genetic and cellular aspects of behavior because of its relatively simple nervous system. Much work has been dedicated to the study of the genetic control of behavior using mutations that affect normal activity in a way that allows the identification of genetic determinants and physiological factors (Bargmann, 1993). Despite its small nervous system (only 302 neurons in adult hermaphrodites), the worm shows a variety of behaviors. Much behavior is a coordinate contraction/relaxation of muscles that results in a wave-like movement. Other behaviors are chemotaxis and osmotic avoidance, i.e. attraction to and repulsion from chemical substances, thermotaxis, i.e. migration to determinate temperatures, sex-related behavior, i.e. egg laying and male mating behavior, and mechanosensation such as touch sensitivity.

Some psychologists have reported experiments on learning and memory using *C. elegans* (Rankin, Beck & Chiba, 1990; Rankin & Broster, 1992). They present a series of experimental data demonstrating that the nematode shows some form of non-associative learning such as habituation, dishabituation, and sensitization for reflex responses to mechanical vibration stimuli. The authors propose *C. elegans* as a new model for the study of the detailed developmental, genetic, and physiological mechanisms involved in learning and memory.

3. Touch sensitive behavior

C. elegans shows mechanosensation, i.e. touch sensitivity, in different situations. It moves away from stroking with a fine hair or moves away from a strong metal touch, even if it does not respond to gentle touch stimuli. Moreover, the male uses touch sensitivity to locate the hermaphrodite's vulva, and all individuals stop pharyngeal pumping when touched, are affected by touch during their egg-laying activity, etc. Individuals that do not respond to hair stimulation, even if responding to strong metal stimulation, are considered touch insensitive.

Animals respond to touch when stimulated both anteriorly (in the head) and posteriorly (in the tail) but not in the vulval region. When an animal is touched in the head, it moves backward. When it is touched in the tail, it moves forward. Backward/forward movement requires coordination of muscle responses. After repeated stimulation animals show habituation and become refractory (Chalfie & Sulston, 1981). Another habituation phenomenon was observed in *C. elegans* by Ward (1973) for chemotactic behavior.

4. The touch sensitivity neural circuit

4.1 Some general observations about *C. elegans*' nervous system

C. elegans' nervous system is simple. An adult hermaphrodite has a nervous system of 302 cells grouped in 118 classes on the basis of their anatomy and connectivity pattern. Various classes of neurons share a simple general topology with only one process and few branches. Their simplicity notwithstanding, each neuron synapses onto about 18 other neuron classes. Neuron synapses are multifunctional, that is, they have effects inside different

functional circuits. Neuronal cell bodies are distributed in about 10 ganglia. Ganglia are not functionally specialized. A very large and important ganglion is the "Nerve Ring", located anteriorly around the pharynx. Neuronal processes, that run longitudinally along the worm's body, are grouped into four main bundles: ventral cord, dorsal cord, and two lateral cords.

The nervous system (location of neurons, direction and branching of neuronal processes, patterns of synapses, and neuromuscular junction) is similar in isogenic organisms, even if some differences in synaptic organization among distinct individuals can be observed. Plasticity phenomena are observed during neural development both for post-embryonic presence of new neurons and for changes in connectivity.

(The main references on *C. elegans*' nervous system are Chalfie & White 1988, for a complete description of the nervous system, and Chalfie 1984, for data on neural development.)

4.2 The neural circuit

The neural circuit for touch sensitivity has been described in detail, and much is known as well about its genetic control. (The most complete description is found in Chalfie *et al.*, 1985. Other studies are reported in Chalfie & Sulston 1981; Herman 1987). The neural network involved in touch sensitivity is constituted by 85 neurons: 6 sensory neurons, 10 interneurons, and 69 motor neurons. (Cf. figure 1). The organization of the network is left-right symmetrical, even if there are some slight differences between the left and the right side (cf. figure 2). Furthermore, anterior/posterior sensitivity corresponds to two separate sensory sub-circuits that are integrated by interneurons. Body cells and neurite processes are schematically represented in figure 3.

There is a relevant difference between the early developing touch sensitivity circuit, formed by few neurons, and the mature more complex neural circuit. During late development the neural network is subject to rearrangement of old circuits with regard both to connectivity and the appearance of new neurons.

The three main neuron classes of the touch sensitivity network are: sensory neurons, interneurons, and motor neurons. The function of single neurons in touch sensitivity behavior has been accurately investigated by means

of cell ablation by laser beams (see below) and the use of mutant animals lacking some of the neurons.

4.2.1 Sensory neurons

There are six sensory neurons belonging to the same neuron type: the microtubules cells (Chalfie & Thompson, 1979; 1982; Chalfie & Au, 1990). These neurons are characterized by the presence of large microtubules whose function is probably related to mechanosensory mechanisms. The six sensory neurons are: ALMR and ALML (the right and left Anterior Lateral Microtubule cell), AVM (Anterior Ventral Microtubule cell that is formed post-embryonically), PLMR and PLML (the right and left Posterior Lateral Microtubule cell) and PVM (the Posterior Ventral Microtubule that is formed post-embryonically).

The two ALM cells are essential for anterior touch sensitivity while AVM mediates a very weak touch response. AVM is a cell that arises post-embryonically and it increases the complexity of the neural network. PLM cells are essential for posterior touch sensitivity, while PVM does not mediate touch sensitivity.

Sensory neurons have chemical synapses and gap junctions onto interneurons. Furthermore, they synapse onto cells of other neural circuits such as the egg-laying system, the pharyngeal pumping circuit, and other neurons whose function is not known.

4.2.2 Interneurons

There are 10 interneurons in the touch sensitivity network. They serve to integrate signals from the anterior and posterior touch neurons. The 10 interneurons are: AVDR and AVDL (the right and left Anterior Ventral interneuron D), AVAR and AVAL (the right and left Anterior Ventral interneuron A), AVBR and AVBL (the right and left Anterior Ventral interneuron B), PVCR and PVCL (the right and left Posterior Ventral interneuron C), and the posterior LUAR and LUAL interneurons.

Interneurons have a complementary pattern of connectivity with respects to the sensory cells. That is, each interneuron receives chemical synapses from either anterior or posterior touch cells and has a link with neurons

of opposite side. Through analytical study using laser ablation of single cells, it has been possible to test the role of different interneurons in the neural control of the touch response (Chalfie *et al.*, 1985). These studies demonstrate that the two AVD cells are essential for motor response (backward movement) to anterior touch stimuli, while the two PVC cells are essential for response (forward movement) to posterior touch stimuli.

The two AVA and the two AVB cells are necessary for normal coordinated movement. AVA interneurons are required for normal backward movement and for termination of forward movement. The opposite role is played by AVB neurons. The main AVB pathway for control of A motor neurons (see below) passes through AVA interneurons.

The two LUA interneurons serve to link the two PLM sensory cells to AVA and AVD interneurons. In fact, only PLMR cell have a direct connection to AVA and AVD. They are considered to function as sensory neurons more than as interneurons (Chalfie *et al.*, 1985). In fact, all interneurons, except LUA, send connections to either A or B or to both A and B motor neurons.

4.2.3 Motor neurons

There are 69 motor neurons grouped in the following classes:

- DA 9 dorsal A motor neurons that innervate the dorsal muscle;
- VA 12 ventral A motor neurons (post-embryonic);
- AS 11 ventral AS motor neurons that innervate the dorsal muscle;
- DB 7 ventral B motor neurons;
- VB 11 dorsal B motor neurons (post-embryonic);
- DD 6 ventral D motor neurons;
- VD 13 dorsal D motor neurons (post-embryonic).

The A and B neurons receive input from interneurons and send signals to muscles through neuromuscular junctions. Instead, D neurons receive input from A and B motor neurons and send signals to the same muscles.

The function of A neurons is to contract muscle for backward movement while B neurons control muscle contraction for forward movements. D neurons inhibit contralateral muscles thereby contributing to the generation of a coordinated movement. As a consequence of post-embryonic formation of ventral motor neurons, DD cells are subject to changes in connectivity (White, Albertson & Anness, 1978).

4.3 Connectivity pattern

The connectivity pattern of the touch sensitivity circuit is well known (cf. figures 1). Identification of synapses and gap junctions has been possible using techniques of serial section electron micrography reconstruction. (A complete description of the connectivity pattern can be found in Chalfie *et al.*, 1985.)

Because of the difficulty in conducting electrophysiological studies in small *C. elegans*, some data have been gathered, for analogy, from the larger nematode *Ascaris lumbricoides*. This worm has a remarkable morphological similarity with *C. elegans* and, due to its larger size, it allows an ultrastructural analysis of neuron activity and connectivity. (Cf. Stretton, Fishpool, Southgate, Donmover, Walrond, Moses & Kass, 1978; Stretton, Davis, Angstadt, Donmoyer & Johnson, 1985; Walrond, Kass, Stretton, & Donmover, 1985; Walrond & Stretton, 1985a; 1985b).

Many data especially concerning the function of motoneurons have been collected. A table of correspondences between *C. elegans* motoneurons and *Ascaris* motoneurons exists, and between motoneuron function and the corresponding chemical transmitters (Walrond *et al.*, 1985a). In *Ascaris*, DE1, DE2, DE3, V1, and V2 excitation leads to muscle depolarization and contraction, causing worm movement. The neurotransmitter released by these neurons is acetylcholine. More analytically, excitation of DE1, DE3, and V1 causes backward movement; excitation of DE2 and V2 causes forward movement. DI and VI motoneurons play a contralateral inhibition function because their neurotransmitter is GABA, a hyperpolarizing substance. By analogy, in *C. elegans* DAS, DA, and VA motoneurons control backward movement, DB and VB cause forward movement, and DD and VD motoneurons play a contralateral inhibition function.

Part B.

Neural networks model of *C. elegans*' touch sensitivity behavior

5. Neural networks model for the touch sensitivity circuit

The goal of our research is to design and test a computational model of *C. elegans*' touch sensitivity neural circuit that reflects many of the neurobiological data observed in the nematode. Chalfie *et al.* (1985)'s description of the neural circuit for touch sensitivity (cf. Part A) is the main source of data for designing the architecture of a neural network that simulates the worm's mechanosensory behavior.

The neural network model is constituted by three layers of neurons: the sensory-input layer, the interneuron layer, and the motor-output layer (cf. figure 4). Because of the symmetrical right-left organization of the worm's neural circuit, the neural network model represents just one side of the circuit.

The sensory-input layer has four neurons. Two are anterior sensors that correspond to the anterior microtubules cells AVM and ALM. The other two neurons are the posterior microtubules cell PVM and PLM.

The interneuron layer is constituted by four of the interneurons observed in the worm. The neurons that are represented in the network are AVD, AVA, AVB, and PVC. The two LUA neurons are not included in the model because they play a role mostly in the circuit's symmetrical organization, a factor not considered in our model.

The motor-output layer consists of four neurons. This is the part of our model which is most distant from the actual circuit. The real motoneurons are 69. However, these motoneurons control the motor output for all the movement behavior of the nematode, not only its touch sensitivity behavior. In fact, motor neurons innervate muscles all over the worm's body, and they are organized in segments with repeated neural organization. To avoid modelling the complex organization of motor neurons, and because this is not the principal goal of the present research, our model uses just four motor neurons, each of them playing the collective function of a group of motoneurons. In fact, we know that A and B motoneurons respectively control forward and backward

movement while D neurons play a coordinating role, in that they determine a contralateral inhibition that creates a wave-like process for coordinated worm movement. (In simulations currently in progress the model includes a more detailed representation of the repeated pattern of organization of motoneurons.) The four motor neurons of the neural network model are: one A neuron, whose activation excites muscles causing forward movement; one B neuron, whose activation excites muscles causing backward movement; and two inhibitor neurons each sending inhibiting connections respectively to the A and B neurons and each receiving exciting connections respectively only from the B neuron and the A neuron. In fact, in the worm D motoneurons receive input only from motoneurons and not from interneurons.

The set of connections linking the neurons in the model reflects exactly the connection pattern observed in *C. elegans*. Since the type of each synapse, whether excitatory or inhibitory, is known, in some of our simulations the model will also reflect this property, that is, the connection sign (plus or minus) will be fixed and not subject to change during learning.

The logistic function is used for unit activation. Because the interneuron and motoneuron layers include recursive connections, in each spreading the activation function is applied after calculation of the net-input for all neurons. Therefore, the activation potential coming from each sending neuron results from the activation state of the neuron in the previous cycle. In this way we synchronize neuron activity without using action potentials produced at different times.

The output responses to touch, that is, the backward and forward movements, are determined by combining motoneuron outputs. Backward movement is calculated as the algebraic sum of the positive output of motoneuron A and of the negative output of the first motoneuron D. Forward movement is calculated as the algebraic sum of the positive output of motoneuron B and of the negative output of the second motoneuron D.

6. Simulation parameters

6.1 Genetic Algorithm parameters

To develop an appropriate set of weights for the neural network model we implemented a version of Holland's Genetic Algorithm (1975; cf. also Goldberg, 1989). The choice of the Genetic Algorithm (GA) is due to various reasons: (a) it is a well studied learning method for neural networks (cf., for example, Parisi, Cecconi & Nolfi, 1990; Belew, McInerney & Schraudolph, 1991); (b) in simulations of realistic neural models GA seems to be a better and more natural learning algorithm than other methods such as supervised back-propagation; (c) GA allows to observe various phenomena and their interactions, i. e. evolutionary learning, individual learning, adaptation events, etc...

6.1.1 Genetic coding

The information encoded in a genotype represents neuron biases (thresholds) and connection weights. Values are encoded as real numbers. In the initial generation all organisms are assigned a randomly generated genetic code, i.e. a random set of biases and connection weights. In some of the simulations the sign of the connection weights is fixed, i.e. it is chosen according to the type of synapse observed in real animals (excitatory or inhibitory) and it is not changed during evolution. In other simulations the sign is randomly selected and it is free to change during evolution.

6.1.2 Population size and selection strategy

In every generation there are 100 worms. After testing the 100 organisms are ordered according to fitness value. The 20 individuals with highest fitness value are selected for reproduction. Each of the selected worms generates 5 copies (offspring). Therefore, the next generation is constituted by 100 new worms (20x5). Reproduction is agamic, which is the most frequently reproduction modality observed in *C. elegans*. Each offspring inherits its parent's genotype, i.e. it has an identical copy of the parent's genetic string except for some mutations. Because the genetic string is a list of real numbers, the mutation operator acts by substituting a random number in randomly selected positions. The probability of a mutation varies from 0 to 20%.

6.1.3 Fitness criterion

In each generation all the 100 individuals are tested for their touch sensitive behavior. In order to test individual behavior a fitness criterion was defined according to the principles identifying touch sensitivity. A worm is touch sensitive if (a) when it is stimulated anteriorly (in the head) it moves backward away from stimulus; (b) when it is stimulated posteriorly (in the tail) it moves forward away from stimulus. Moreover, since *C. elegans* shows habituation in touch sensitivity response, the withdrawal response to repeated stimuli should decrease at each stimulus presentation.

The fitness criterion is the following:

- (1) Plus 200 fitness points (a) if motoneurons controlling backward movement determine worm movement and motoneurons controlling forward movement inhibit forward movement, and (b) if backward movement intensity is less than the intensity of the previous response (habituation). Both (a) and (b) criteria have to be satisfied except for the first stimulus where only criterion (a) is applied.
- (2) Plus 50 points if only one of the two (a) and (b) criteria is satisfied.
- (3) Minus 100 points if none of the above criteria are satisfied.

The sum of fitness points after 20 stimulations is an individual worm's fitness value, i.e. it describes the individual's performance in touch sensitive behavior. Each test consists of 20 cycles. For the first 10 cycles only anterior touch sensors are activated, that is, the ALM and AVM neurons receive 1 in input while the other input neurons receive 0. For the successive 10 cycles posterior touch sensors (PVM and ALM) are activated.

7. Results

The simulations are organized into two different groups.

In a first set of simulations we evolved neural networks with fixed synapse type. All the network's neurons have excitatory connections except for the two D motoneurons which have inhibitory connections. We run 10 replications of the simulation with different random seeds for initial genetic code generation. In 4 of the 10

replications the population of networks has reached a good performance level in touch sensitive behavior at around generations 60-70. (Cf. figure 5.) The organisms move away from stimulus (they move backward when stimulated anteriorly and forward when stimulated posteriorly) and their response habituates, that is, movement intensity decreases after repeated stimulation. In the other 6 simulations organisms evolve only a habituation response to either anterior or posterior stimulation only.

The histograms of figure 6 represent graphically the habituation response showing the strength of motor response after the first stimulus and after the tenth stimulus for both anterior and posterior stimulation. The data concern the means of motor response intensity of 20 different individuals (5 organisms from each of the 4 successful simulations). To test if the response decrease is statistically significant we have applied an analysis of variance with repeated measures (MANOVA), since we compare the response of the same animal at the beginning and at the end of the stimulation set. The data used in the statistical test concern the response intensity of the 20 selected best individuals. The results of the MANOVA test confirm that response intensity decreases significantly with $p < 0.0001$ and $F_{1/19} = 416.9$ for anterior stimulation, and $p < 0.001$ and $F_{1/19} = 215.9$ for posterior stimulation. These results fit well with data reported from experimental work on habituation of touch response in real *C. elegans* (Rankin *et al.*, 1990). Similarly good results are obtained in simulations where the stimulus set included 20 or 30 repeated stimuli on each side.

The second group of simulations differs from the previous simulations in that the connection signs were not fixed a priori for all neurons. The network architecture is the same but connections departing from interneurons can have a positive or a negative sign depending on random initial assignment and on evolutionary history. However, the sign of connections departing from the same interneuron is the same for all connections. This is because a single neuron can release just one neurotransmitter which can have either an excitatory or an inhibitory role.

In these simulations too populations with a good performance level in touch sensitive behavior tend to evolve, even if the solution is harder to find. In fact, in only 3 of the 20 different simulations an optimal behavior evolved. Moreover, analyzing the neural networks of the best individual, in 2 of the 3 successful simulations the evolved connection signs of four interneurons are all positive, while in the third one there are some inhibiting

connections. This is an interesting result because apparently the model is able to predict that the four interneurons of touch sensitivity circuit should be excitatory in real animals.

8. Discussion

The results of our simulations indicate that the proposed neural network model is a good computational model of *C. elegans'* touch sensitivity neural circuit and behavior. The computational model replicates the same behaviors observed in the real nematode. The neural network processes anterior/posterior input stimuli in such a way that a withdrawal reaction to the stimulus source is generated. Moreover, if the same stimulus is repeatedly presented to the network, the network gives a continuously decreasing response.

However, a computational model's ability to replicate the behavior of a real organism is only a first proof of the validity of the model. There must be agreement between the computational model and the real organism both in what the model/organism does and in how the model/organism does it. That is, a good computational model must reflect the same mechanisms and processes present in the real organism.

To study this aspect of the model we analyzed the way our neural networks process input signals and the role of the various neurons in producing the correct motor output. Chalfie's and colleagues (Chalfie *et al.*, 1985) have examined the role of single neurons in the touch sensitivity neural circuit by utilizing the method of laser ablation. Using this method (Sulston & White, 1980) it is possible to kill by a laser beam a single cell in the alive worm (or to kill the precursor of particular cells during development, determining indirectly the absence of target cells). This makes it possible to observe the effects on behavior of the absence of a particular cell and to infer the functional role of the cell.

In neural networks simulations one can use the method of unit lesion that is very similar to the laser ablation technique. By excluding some neurons from the functioning network it is possible to test the effects of the absence of these neurons on the network's behavior. We have used the lesion method to analyze the contribution of particular neurons to the functioning of evolved networks. To do this we have lesioned 20 simulated individuals (5 individuals from each of the 4 successful replications of the simulation with fixed synapse type)

and then we have tested their performance on the usual set of 20 stimuli (10 anterior stimuli followed by 10 posterior stimuli).

The results of these lesion tests are showed in the following table. The table also reports the effects of the cell ablation experiments observed by Chalfie *et al.* (1985) and by other authors. The results, when they are compared with the corresponding experimental data observed in real worms, suggest that very similar roles are played by units in the artificial neural networks and by the corresponding neurons in the nervous system of real animals, although there are some differences.

As in real animals, AVD and PVC artificial interneurons are essential for backward and forward movement, respectively. Lesion of AVA and AVB interneurons has the effect that motor reactions do not habituate, and this could be consistent with their role in real animals in controlling the coordination of movements. Our model does not allow us to observe clearly the coordination underlying wave-like movements, but the presence of disturbances in habituation behavior after lesion indicate an influence of AVA and AVB neurons in the regulation of global aspects of movement, rather than in the simple generation of movement (as in the case of AVD and PVC neurons).

The presence of some nonmatching data between the real neural circuit and artificial neural networks indicate that the model needs adjustment. Some differences can be explained as due to a different network organization caused by different evolutionary histories. In fact, in neural network simulations using genetic algorithms many factors, like the initial randomly assigned connection weights and other genetic parameters, can influence evolution. But other differences may have deeper and more interesting explanations. Neural organization is not only the result of inherited genetic instructions but also of the interaction between the genetic program and epigenetic events such as environmental experiences, developmental events, and so on. Therefore, it is possible that if we introduce in our model some epigenetic factors the final network organization can have a different shape. Because the present model simulates only genetic phenomena, evolved neural networks can have different a functional organization with respect to the neural circuit of the real animal.

That epigenetic events can change the final network organization is shown by the functioning of the four

sensory neurons. From lesion experiments it emerges that the role of the input neurons of the artificial neural network is different from the functional role of real sensory neurons. In real animals the ALM neuron is essential for anterior touch sensibility, while the AVM neuron plays only a secondary role. In fact, when ALM neurons are ablated the worm shows reduced anterior sensibility (Chalfie & Sulston, 1981). The same phenomenon is observed for posterior touch sensitivity, with the difference that PVM alone does not mediate any sensibility. In contrast with this, in our simulations the functional roles of the two anterior neurons are equivalent. In fact, AVM neurons by themselves alone mediate a good touch sensitivity, like ALM neurons. And we find the same results for PVM and PLM artificial neurons.

It is known (White *et al.*, 1978) that the touch sensitivity circuit is subject, during the worm's life cycle, to reorganization because of postembryonic formation of some sensory and motor neurons. Sensory neurons AVM and PVM arise postembryonically and the presence AVM causes circuitry changes in *C. elegans*. This could explain why lateral microtubule cells ALM and PLM play the main role in mediating touch stimuli, while AVM and PVM have a secondary role (or no role in the case of PVM neurons). And it could explain why in our simulations no functional distinction between AVM and ALM, and between PVM and PLM, emerges evolutionarily. Because no developmental events are introduced, both lateral and ventral microtubule sensory neurons exist at the same stimulation time and they tend to acquire an equivalent function evolutionarily.

To test this hypothesis we have run a new set of simulations which include the simulation of a developmental event. The life of each individual is divided into two periods: a embryonic period and a postembryonic period. During both periods individuals are tested with a set of 20 stimuli, 10 successive anterior stimuli and 10 posterior stimuli. The difference between the two life periods concerns the different neural network used to process touch stimuli. In the embryonic period, the network includes only two sensory units, the ALM neuron for anterior stimuli and the PLM neuron for the posterior ones. The rest of the network is the same as in the preceding simulation. The neural network of the postembryonic period has all four sensory neurons. All other genetic algorithm parameters are the same as in the preceding simulations. An individual's fitness is calculated as the sum of the fitnesses of the two periods.

The results indicate that maximum fitness is reached at around generation 150, i.e. later than in the simulations

without the two-stage development. The lesion test was executed as in the other simulations. The lesions indicate that all neurons except the sensory neurons play the same function that they have in the simulations without development. However, in the sensory layer of the neural network it is possible to observe a different functioning. The sensory neurons function in a way which is more similar to that of real worms, i. e. lateral ALM and PLM neurons play a prominent role in mediating touch response while ventral microtubule cells AVM and PVM have a minor or nonexistent sensory role. This result confirms the hypothesis that developmental events, even if limited to the modeling of a simplified two-stage development, may influence mature network functional organization, yielding, in our case, a better matching between artificial neural network and real *C. elegans*'s touch sensitivity circuit.

From the lesion experiments some indication on the possible role of single neurons in the habituation mechanism has emerged. The lesion table (table 1) shows that all 4 interneurons affect motor response habituation, even if it seems that it is mainly the AVA and AVB neurons, especially when lesioned together, that have a bad effect on habituation after repeated stimulation. Moreover, inhibitor D motoneurons seem to have a role in controlling the habituation process. To better understand how the 4 interneurons and the two D neurons cause response habituation we recorded the activation level of these neurons from the first stimulus to the tenth. A neuron whose activation changes during this time period is considered to be responsible for the habituation response.

The results of this analysis show that for the habituation of the backward response, of the 2 AVD and AVA interneurons that determine backward movement, only the AVA neuron changes its activation state and determines a decrease in the motor response by communicating these changes to the next neural layer, i.e. the motoneurons of the output layer. For forward movement in response to posterior stimuli both the AVB and PVC neurons determine habituation by changing their activation state stimulus after stimulus. Finally, in the output layer, the D motoneurons by increasing their activation state in response to each successive stimulus determine the reduced intensity of the final muscle response.

These data suggest a partially asymmetric neural mechanism responsible for motor response decrease after repeated stimulation. The analysis of our artificial neural networks suggests a subtle neural mechanism that

determines habituation in *C. elegans*' touch sensitivity circuit. But these results on habituation need to be confirmed by neurobiological experiments on real animals or, perhaps, by the development of a more realistic model since it is very hard to make electrophysiological analyses on small 1mm long *C. elegans*. A more detailed model that would allow us to extract more fine data on the neural mechanisms of habituation should probably incorporate lower level mechanisms than those at the neuron level. For example, it should support a system of short-term modification of synaptic strength. In fact, it is known (Groves & Thompson 1970) that most of the mechanisms involved in habituation operate at the synaptic level. A more realistic model that incorporates synaptic level details could allow us to study the neural mechanisms of various habituation phenomenon observed in *C. elegans* (Rankin & Broster, 1992).

9. Conclusions

The results presented in this paper constitute the first step toward the validation of a computational model of the real neural circuitry observed in *C. elegans*. The model accords well to some neurobiological data, even if it needs further refinements and further testing. It can be extended to include modelling of a more detailed motor apparatus, in order to simulate the organization in repeated segments along the worm's body, the left-right symmetrical organization of the circuit, the synaptic mechanisms of habituation, etc. A very important field that could be explored by designing and testing detailed neural network models of touch sensitivity circuit is neural development. In some of the simulations the neural circuit is subject to reorganization during worm development. But much is known about the genetic and environmental factors controlling circuit development that should be included in the simulations. For example there exists a specific literature on mutant phenotypes with abnormal touch sensitivity circuit, like the *mec* and *unc* phenotypes. The use of genetic algorithms on populations of neural networks together with a more complex genetic encoding system could help us explore this virgin field with interesting results.

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List of Captions

- Figure 1:** *C. elegans*'s neural circuit for touch sensitivity
- Figure 2:** Touch sensitivity neural circuit: left-right organization
- Figure 3:** Touch sensitivity neural circuit: body cells and neuritis processes localization. Only some of the 69 motor neurons are showed.
- Figure 4:** Architecture of neural networks used in simulations
- Figure 5:** Fitness across the 200 generations in a simulation with fixed predefined connection signs
- Figure 6:** Strenght of motor response after the first stimulus and after the tenth stimulus in anterior and posterior stimulation. The strenght values are the average of the motor response of 20 individuals.
- Table 1:** Results of the lesion test in simulated organisms versus laser ablation test in real worms. For each neuron, the data in first rows refer to anterior stimulation and the data in second rows are related to the observations after posterior stimuli. The numbers between brackets refers to number of organisms that show that behavior.

Table

Neuron	Lesions in Neural Networks Model	Laser ablations in <i>C. elegans</i>
AVM	No backward mov. (10); no habituation (10)	Minor loss of anterior touch sensitivity

ALM	No backward mov. (3); no habituation (15)	Low anterior sensitivity

PLM	---	No posterior sensitivity
	No forward mov. (11); no habituation (9)	
PVM	---	No effect: the neuron does not mediate any movement response
	No forward mov. (14); no habituation (6)	
AVD	No backward movement (17)	No backward movement
	Absent or very low forward habituation (20)	
AVA	Absent or very low backward habituation (20)	Uncoordinate backward mov.; animals do not stop forward mov.
	Absent or very low forward habituation (20)	
AVB	No backward mov. (9); no habituation (11)	Uncoordinate forward mov.; animals do not stop backward mov.
	No forward mov. (8); no habituation (12)	
PVC	Absent or very low backward habituation (20)	No forward movement
	No forward movement (20)	
AVA+	No backward mov. (4); no habituation (16)	No normal coordination
AVB	No forward mov. (5); no habituation (15)	
A	No backward movement (20)	No backward movement

B	---	No forward movement
	No forward movement (20)	
D	Unc. backward mov. and bad habituation (20)	Uncoordinate movement
	Unc. forward mov. and bad habituation (20)	

Table 1

Figures

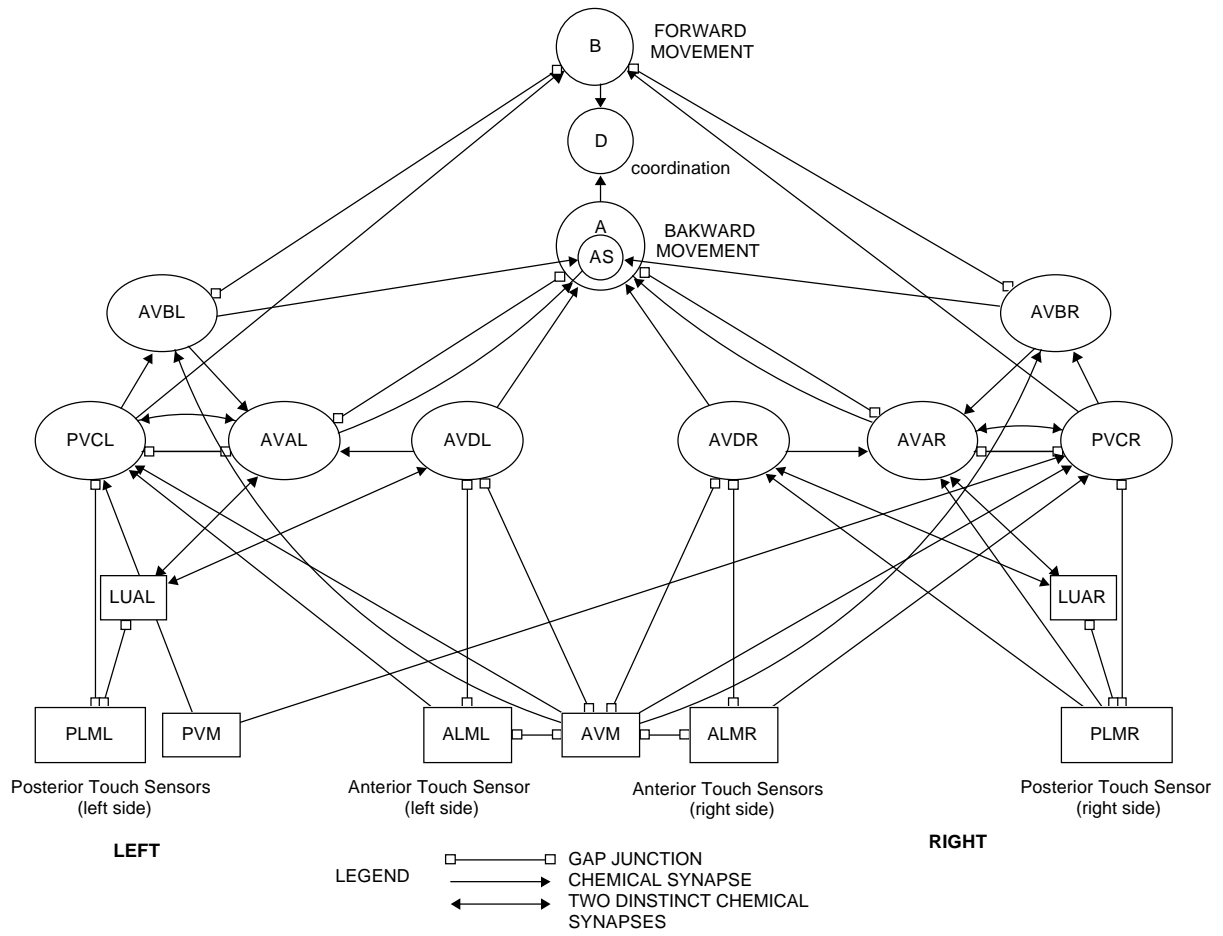


Figure 1

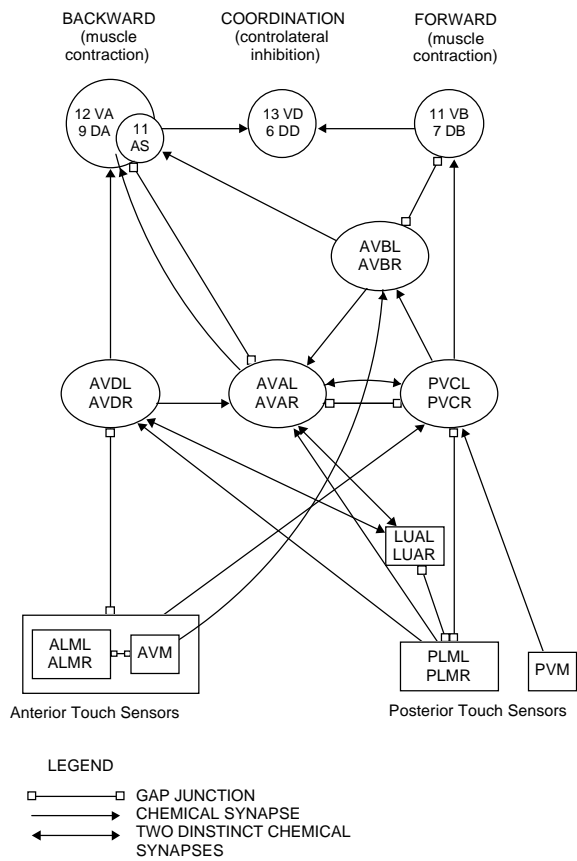


Figure 2

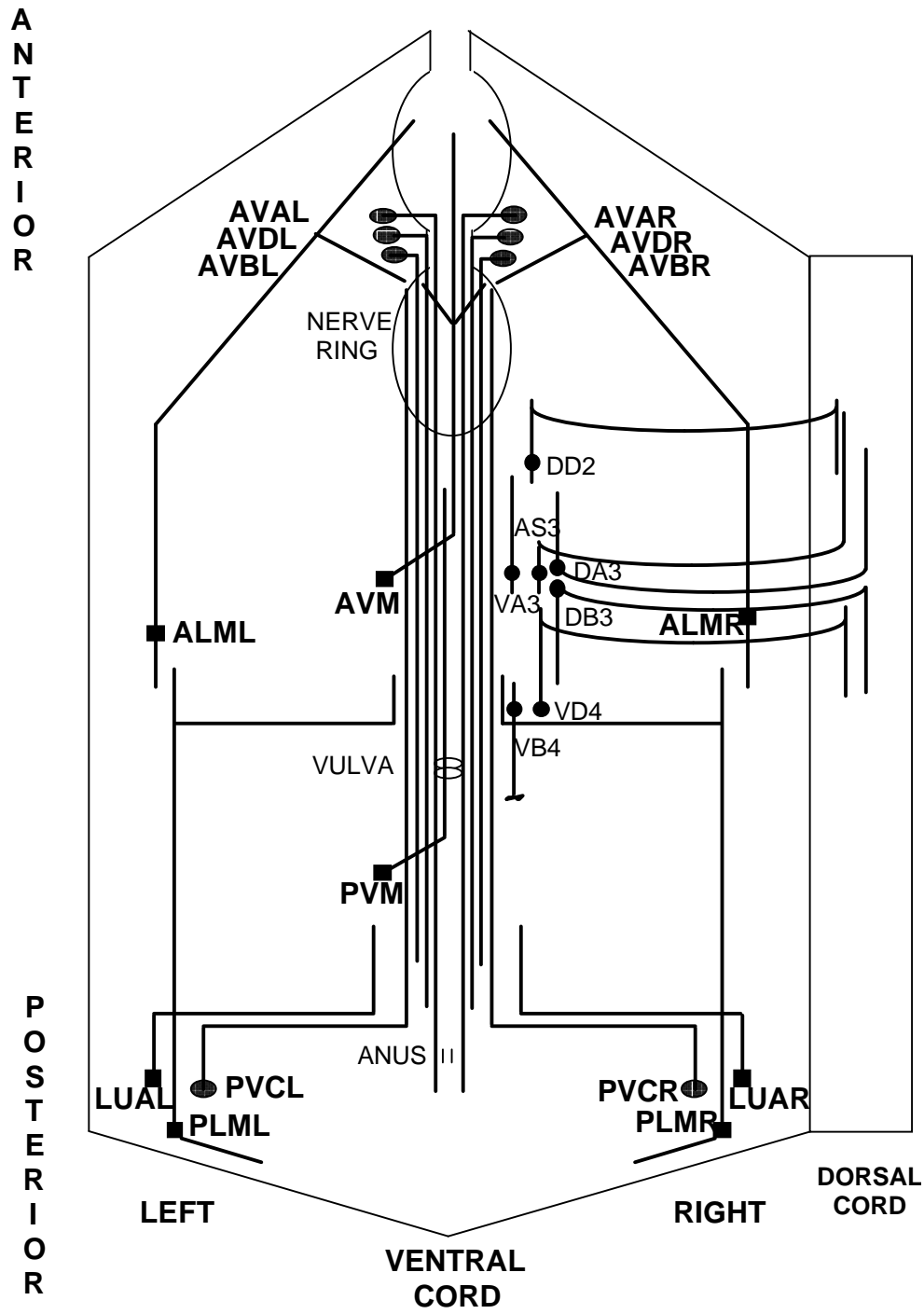


Figure 3

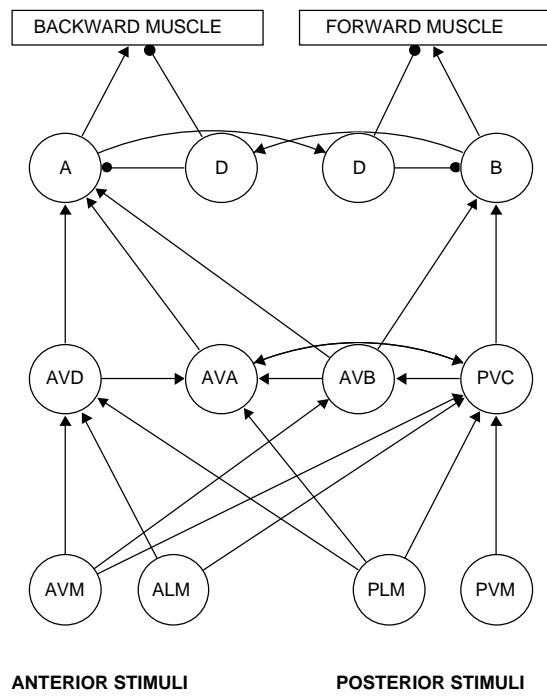


Figure 4

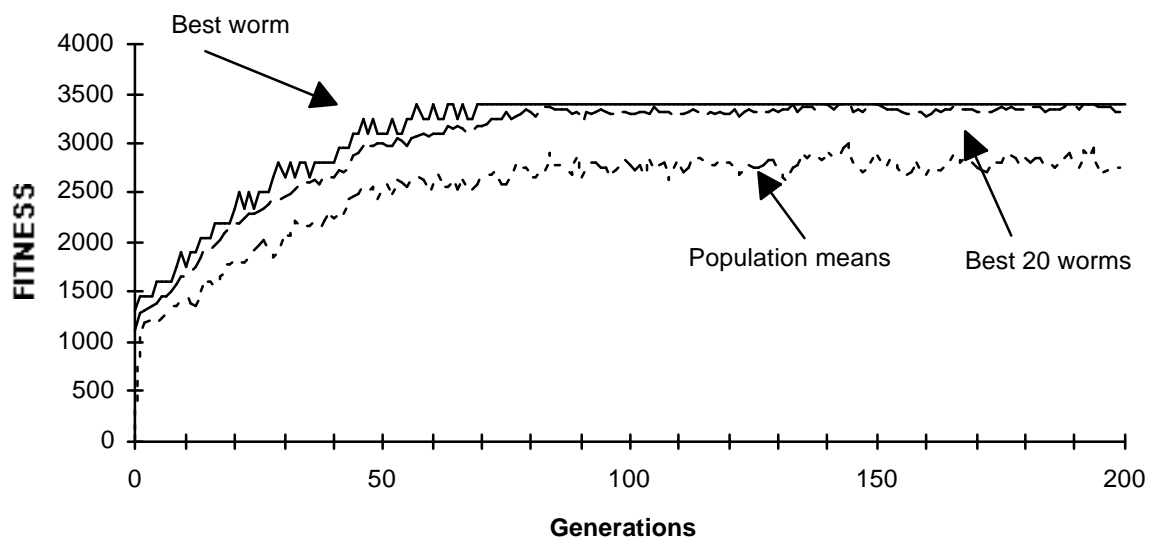


Figure 5

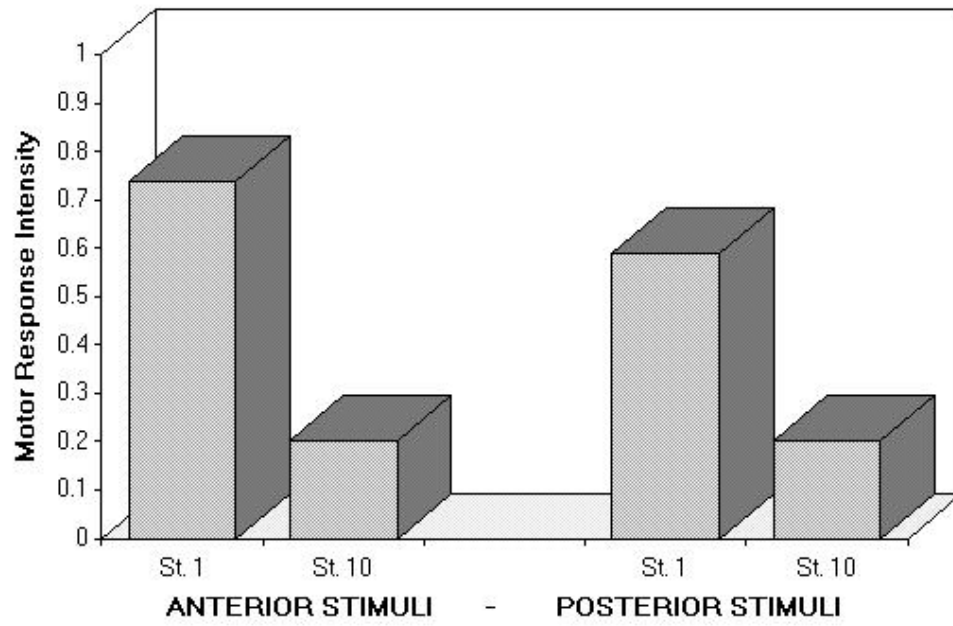


Figure 6