Modeling Locomotion and Its Neural Control in Nematodes

The complete "wiring diagram" (a list of all neurons with the location of all synaptic connections between them) is thus far known only for a single species of multicellular animals, the nematode Caenorhabditis elegans. We used this information to develop a detailed computer simulation of a significant part of its nervous system, which is responsible for the undulatory locomotion of the worm. We conjecture that the nervous system of small nematodes such as C. elegans might function without active membrane properties (e.g., action potentials), but that this is not possible for the larger nematodes, such as Ascaris lumbricoides. In order to identify the spatiotemporal muscle activation patterns generated by the nematode nervous system, we also studied the biomechanics of undulatory locomotion. We found that the absence of a rigid backbone makes available to the nematode a richer repertoire of activation patterns than those used by snakes, but that the simplicity of the nematode nervous system might restrict the exploitation of these possibilities.

Key Words: nematodes, locomotion, neural control, modeling locomotion, computational modeling

The primary goal of the neurosciences is to understand the functioning of the nervous systems of animals, including humans. We believe that experimental work in this field has to be complemented by theoretical studies and we describe in this comment a theoretical line of approach toward this goal. The rationale of this approach is to try to obtain as much anatomic and neurophysiologic information as possible about a living organism, and then to construct a physico-mathematical model of its nervous system. If well constructed, this model should be able to simulate...
correctly the behavior of the animal when stimuli are presented to it. If the
simulation yields the same answer to the stimuli as the living organism, a
great advance toward the understanding of the functioning of the organ-
ism has been made.

What makes nematodes (roundworms) favorite objects of
neurobiologic studies is, in the first place, the simplicity of their nervous
system. This was recognized in the last century and gave rise to exhaus-
tive light microscopic studies of the neuroanatomy of the larger nematode
species [1]. Attractive features were the small number of neurons (about
300), their relatively simple morphology, and the constancy of the mor-
phology of single cells when comparing different individuals. This made
the identification of individual neurons in different animals possible.
Goldschmidt [2,3] made a serious effort to reconstruct the whole nervous
system of the nematode Ascaris lumbricoides* by tracing all its neural
processes, but his ambitious goal was hampered by the limited resolution
of the light microscope. It took the advent of electron microscopy and a
decade of dedicated work before Goldschmidt’s goal was achieved by
other researchers, and the diagram of connections of the complete nerv-
ous system of a nematode was reconstructed entirely [4,5]. One of the im-
portant results was that reproducible identification of features is not
limited to the cellular level but that it continues to the ultrastructure: the
nervous systems of two Caenorhabditis elegans individuals are identical
even at the level of individual synapses. It turned out, however, that the
complete knowledge of the anatomy does not immediately entail func-
tional insight. Unfortunately, one of the most important experimental
tools for the understanding of the function of nervous systems, the elec-
trophysiologic recording of the neuronal activity with microelectrodes,
cannot be used (at least at present) in C. elegans due to the small size of its
neurons.

Another important property of nematodes comes to the rescue here,
namely, the fact that the nervous systems of different nematode species
are remarkably similar. Although the lengths of C. elegans and A.

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*"How helpful it would be for an understanding of the physiological processes if one could
obtain complete knowledge of the structure of the nervous system of any organism. . . . If at
all, this can only be achieved for a very simple organism. . . . I doubt that many such organ-
isms exist, in any case, I only know about a single one [Ascaris lumbricoides]" [2] (our
translation).
lumbricoides are vastly different (1 mm versus 300 mm), the number of neurons is almost the same in individuals of both species. Due to the large size of A. lumbricoides, it is impractical to obtain the same kind of detailed information about its ultrastructure as was possible for C. elegans, because the ultrastructural information is obtained by cutting the animal in slices of a thickness of less than 1 μm and examining each slice. On the other hand, it is the large size of A. lumbricoides and of its neurons that makes electrophysiologic experiments feasible in this animal. Therefore, one can combine information by doing complementary experiments with these two nematode species—ultrastructure analysis in C. elegans and electrophysiological study in A. lumbricoides. Furthermore, by using methods that can be used in both species, many homologous classes of neurons were identified, which indicates that, indeed, the agreement of the cell numbers is not accidental but reflects a functional similarity of the two nematode nervous systems. For instance, it was shown by immunohistologic techniques that neurons of homologous classes in C. elegans and A. lumbricoides contain the same neurotransmitter (McIntire, S.: Personal communication) [6].

Despite the simplicity of their nervous systems, nematodes are “real” animals that are born, age, and die, that have nerves, muscles, and reproductive organs, that show reflexes and more sophisticated behavioral schemes [7,8] as well as plasticity of their nervous system and, apparently, even ability to learn from experience [9]. Nematodes may, therefore, offer the possibility to study nontrivial properties of higher animals in a simple system. As John Hulston, one of the pioneers of modern nematode biology, expressed it, “In a sense, one organism like this [C. elegans] contains all of biology” (cited by Roberts [10]). One of the aims of our study was to get functional information from detailed anatomic information, by using the latter to develop a simulation of the function of the nervous system.

We note that the structure of the nervous system of C. elegans is better known than that of any other animal, as is its development and possibly its genetics. For an overview of these subjects we refer the reader to Wood [11]. Here we remark only that, concerning its development, C. elegans is the only multicellular animal whose complete cell lineage is known, from the fertilized zygote to the fully developed worm with its 959 nongonadal cells [12]. Concerning genetics, a nearly complete physical map of its genome has been established [13]. At present, the genome of C. elegans is being sequenced at a rate of more than 100 kb per year, and the total
genome is scheduled to have been sequenced by the year 2000 [14]. *C. elegans* will be the first multicellular animal for which this will be accomplished and it will serve as an important proving ground for the Human Genome Project. Knowledge in the domains of development and genetics is useful also for functional studies. For instance, knowledge of the cell lineage proved to be important for the laser ablation experiments mentioned later. In a laser ablation experiment performed on an adult individual, the aim is to kill a specific neuron. Sometimes one is not sure that the right neuron has been killed. If, instead of killing the neuron in the adult, the precursor of a neuron is killed in a developing animal, this ensures that the neuron in question will be absent in the adult. Likewise, genetic knowledge has been used for answering functional questions, such as by studying the anatomy and behavior of some of the numerous *unc* mutants with locomotory disturbances [15].

THE NEURAL CONTROL OF LOCOMOTION IN NEMATODES

We will call the totality of data about the neurons and their mutual connections by synapses the "wiring diagram" of the nervous system.

A wild-type adult hermaphrodite* *C. elegans* has 959 nongonadal cells, of which 302 are classified as neurons. These numbers are so much smaller than the corresponding numbers in higher animals that, since the wiring diagram of *C. elegans* is available, one might be tempted to model its whole nervous system. This might have been a viable approach if the details of the function of the synapses (such as whether they are excitatory or inhibitory and their temporal behavior) were known. However, since the wiring diagram contains only anatomic and not functional data, we have resisted this temptation and decided to start by modeling only a part of the nervous system. Only the existence and location of membranes and synapses are known, but not their properties. We thought that two parts of the *C. elegans* nervous system are particularly appropriate to be modeled, one being the neural net controlling the function of the pharynx and the other the set of neurons responsible for the control of locomotion.

*One in about a thousand worms is a male; the others are self-fertilizing hermaphrodites.
The main attraction of the pharynx nervous system is that this network of 20 neurons is almost independent from the other 282 neurons of *C. elegans* [5]. Two important reasons motivated us not to choose the pharynx nervous system for a modeling effort. The first is that the pharynx, which pumps food into the digestive tract, has been found to be a very complicated mechanical system [16]. The second reason is that the control of the pharynx might be effectuated to a large extent by mechanical, electrical, or other interactions between the pharynx muscle cells and the neurons may have mainly a modulatory function. In fact, it was shown that the pharynx continues to pump (albeit with a significant loss of efficiency) even after all pharyngeal neurons have been ablated by a laser microbeam [17]. For these reasons, we thought that the pharyngeal nervous system is not appropriate for a first modeling effort.

The other part of the *C. elegans* nervous system that seemed particularly suitable for being modeled is the circuitry that controls the undulatory locomotion of the animal. Locomotion is not only one of the most important activities of the animal, but it is also the easiest to observe. Therefore, it might seem surprising, but it is a fact, that there are important questions related to this activity that remain unanswered. Even the basic mechanism that controls the locomotion is unknown and this is precisely what we simulated. Similarly to the case of the pharyngeal nervous system, there is also a well-defined group of neurons that is known to be responsible for the control of the undulatory locomotion. This group seems less isolated from the other parts of the nervous system than the pharynx neurons, but there is a well-defined interface between this group and the rest of the nervous system. An important point in favor of detailed simulations of this part of the nervous system is the fact that the first laser ablation experiments in identified neurons of *C. elegans* have all been performed in neurons of the locomotory system. This is due to technical reasons, such as the easy access to these neurons, to the particularly simple morphology of these neurons, which have few, if any, branching points (Fig. 1), and to the fact that the motor neurons are arranged in repeating units, which facilitates analysis of their function.

Having outlined the reasons for the choice of our subject of investigation, in the remaining part of this section we discuss the structure of the nervous system of nematodes. The most important parts are: the nerve ring, a toroidal structure of neuropile that is wound around the pharynx and is connected to the sensory neurons of the head; several ganglia containing the bodies of the cells that contribute to the neuropile in the nerve
FIGURE 1 Example of a typical *C. elegans* motor neuron (DB3; notation used is that of White et al. [5]) showing the detail to which the structure of the nervous system is known. Chemical synapses are indicated by arrows, directed from the presynaptic to the postsynaptic neuron. NMJ: neuromuscular junctions. Gap junctions are represented by H-like symbols. The dorsal cord process (right) is drawn on a larger scale than the ventral cord process (left) and only the first part of the dorsal cord process is shown. Data from White et al. [5]. Analogous information is available for all 302 neurons of *C. elegans*.

ring; some more ganglia located in the caudal part of the body (again connected to sensory cells); six tracts of nerve fibers along the long axis of the body. By far the most prominent of these tracts is the ventral cord, which
also contains the cell bodies of the somatic motor neurons that control the somatic musculature. This musculature is used for the undulatory locomotion of the worm by making neuromuscular junctions with ventral muscle. Some of these neurons send processes (called commissures) halfway around the cylindrical body to another of the six tracts of nerve fibers, the dorsal cord, where they extend parallel to the body axis and make neuromuscular junctions with somatic muscles. Since these muscles are functionally divided into dorsal and ventral subfields, the worm has to move by throwing its body in dorsoventral waves. (In its "natural" laboratory habitat, the surface of an agar layer, the worm is lying on its side and the dorsoventral waves correspond to left-right movements.) The somatic motor neurons receive input from interneurons that, in turn, receive their major input in the nerve ring or the caudal ganglia. Only four pairs of interneurons are presynaptic to the somatic motor neurons and run along the whole ventral cord.

There is direct experimental evidence showing that these interneurons control the locomotion of *C. elegans*. Using a laser microbeam, cells can be killed selectively and the behavior of a treated worm can then be compared with that of untreated animals. Using this technique, it was found that undulatory locomotion is controlled by two circuits. If either the four interneurons AVBL, AVBR, PVCL, and PVCR or the motor neurons of the classes VB and DB are destroyed, worms can no longer move forward. Killing the interneurons AVAL, AVAR, AVDL, and AVDR or the motor neurons AS, DA, and VA produces worms that cannot move backward. (We will use the terminology of White *et al.* [5], which assigns a unique name to each of the *C. elegans* neurons. Suffice it to say here that each neuron name is composed of the name of the class to which the particular neuron belongs, which may be followed by either L (left) or R (right), or by a number. Class names consist of either two or three capital letters.)

**UNDULATORY LOCOMOTION**

*C. elegans* advances on a solid surface by undulatory movement of its body. This movement resembles the typical motion of a snake rather than that of an earthworm. Several characteristic features of this motion are of importance for the following analysis. First, the nematode *C. elegans* can move forward or backward. If its head or tail touches an obstacle, it usu-
ally reverses the direction of its motion. Second, when in motion or at rest, the shape of the nematode’s body is well represented by a portion of a sine curve, and the length of the body corresponds approximately to one complete wavelength of the sine curve. The third characteristic of the motion, as observed through a microscope, is the oscillatory right-left movement of the head, and often of the tail.

The mechanism of the undulatory locomotion of snakes was analyzed by the British zoologist J. Gray in the 1950s [18,19]. He came to the conclusion that if a snake is to produce a forward thrust with respect to its support, muscles of certain well-defined regions of the sinusoidally curved animal body have to contract periodically. In addition, the contracting and relaxing regions form a periodic sequence along the body, and these regions move together backward along the body at approximately the same speed as the animal moves forward. When the animal moves backward the regions of muscular contraction seem to move forward relative to the body. In the absence of lateral slipping, the regions of muscular contraction are stationary with respect to the support on which the animal moves.

Gray’s further analysis shows that for a resulting thrust it is necessary that the muscles in the exterior arc of the curved body contract in those regions in which the radius of curvature increases (that is, the curve straightens) in the direction of the motion and relaxes in the other regions. For an elementary, but only approximately correct, demonstration of this assertion, see Erdös and Niebur [20].

Since the sliding motion of *C. elegans* is similar to that of a snake, one may ask whether the just described muscular contraction pattern of snakes is also necessarily the one used by the worm.

An important structural difference between snakes and worms has to be taken into account when studying this question. Snakes have a skeletal backbone, whereas from the dynamic point of view, nematodes are more like rubber hoses filled with a viscous liquid. The presence of the skeleton plays an important role in Gray’s analysis: consider what happens when the muscles contract in a given segment on one side of the body, while they are relaxed on the other side of the same segment. Because of the presence of the incompressible backbone, the skin contracts on the side of the muscle contraction and expands on the other side. Since the skin is in frictional contact with the support on which the body moves, forces are exerted on the support by the body, and the resultant of these forces has a component parallel to the body axis and in the forward direction if the segment in question has a lesser curvature on its front end than on its rear end.
Our work demonstrated that the same muscular excitation pattern that will propel the snake will also provide a thrust to the *C. elegans*, because the body liquid, which is only slightly compressible, provides something like a “hydrostatic skeleton.” This concept has been introduced by Harris and Crofton [21]. On the other hand, our analysis showed that locomotion can be induced also by patterns other than the one in which only muscles on the exterior arc of those parts of the body contract where the curvature decreases.

To obtain a concrete idea of what is meant by these statements, we fore- stall later developments of this report and look at Figures 5 and 6. Here we see the computer simulation of two muscle contraction patterns. The first one, in Figure 5, corresponds to the criterion of Gray as previously enunciated. The dark areas represent those muscles that are momentarily contracted in the forward-moving nematode. In Figure 6, muscles on both the exterior and the interior arcs are contracted, albeit again in places, where the curvature decreases in the forward direction, that is, toward the right. This pattern does not correspond to Gray's criterion, because the muscles are contracted simultaneously on the inside and outside arcs of the body outline as projected on the plane of the support. Such a simultaneous contraction would result in a shortening of the segment, which would be prevented by the presence of the backbone. Therefore, such a pattern could not develop in an animal with a backbone.

A detailed mathematical analysis [22] shows that in the case of a “hydrostatic skeleton,” this type of contraction pattern also produces locomotion: it turns out that, with the same muscular strength, the pattern shown in Figure 6 produces a higher speed than the pattern shown in Figure 5.

It follows from the foregoing discussion that it is appropriate to speak of muscular waves propagating backward along the body of the nematode. Therefore, one of the important questions to be answered was how these muscular waves were generated. Although myogenic control plays an important role in nematodes, as was explained in the introduction using the example of the pharynx of *C. elegans*, we assume, in agreement with most workers in the field, that the spatiotemporally varying muscle contraction pattern has to be generated by an equally varying neural excitation pattern. The question then could be reformulated by asking how the relatively simple nematode nervous system produces this intricate excitation pattern.

In the next sections we will attempt to answer this question.
SIGNALING MODES IN THE NEMATODE NERVOUS SYSTEM

Textbook examples of "typical" neurons comprise a dendritic arbor in which synaptic inputs are summated electrotonically, a cell body (soma), and an axon along which action potentials are propagated in an all-or-nothing mode. Whether an action potential is generated or not in these "typical" neurons depends on the voltage at the "axon hillock," which is the junction between the soma and the axon, and this voltage is determined by the synaptic input.

It is becoming more and more obvious that this picture is incorrect for large classes of neurons in invertebrates and also in vertebrates [23]. Experimental evidence is accumulating that nematode neurons do not fall into the described category of "typical" neurons. As was mentioned previously, C. elegans neurons are too small to be impaled with electrodes and therefore no electrophysiologic data are available for this animal. In contrast, intracellular recordings of muscle cells as well as of neural cells are available for A. lumbricoides [24]. In the following, we will discuss briefly structure and function of the muscle cells before coming back to the nervous system.

The innervation of nematode muscle is somewhat unusual: muscle cells send noncontractile extensions to the areas where the nerve cells make neuromuscular junctions. The muscle cell extensions interdigitate extensively in the neuromuscular junction area and they are coupled by gap junctions, thus forming a functional syncytium. The reason for this arrangement is probably that it economizes nervous tissue, which makes it possible for an animal as large as A. lumbricoides, with more than 50,000 muscle cells, to be controlled by less than 300 neurons. Graded action potentials have been observed in A. lumbricoides muscle cells [25]. Their presence is easily explained as a consequence of the described morphology of these cells as follows: the synaptic potentials generated at the neuromuscular junctions have to travel along the muscle arms before arriving at the cell body and the contractile parts of the cell. Passively spreading electronic potentials would be too strongly attenuated, and therefore useless, due to the impedance mismatch between the thin muscle arms on the one hand and the large cell body and contractile cell parts on the other hand. Therefore, action potentials in the muscle cells are a functional necessity.
On the other hand, intracellular recordings in *A. lumbricoides* somatic motor neurons revealed no action potentials [26]. Instead, signals are transmitted electrotonically. The intracellular voltage ($V$) in a neural process can then be approximated by the following partial differential equation:

$$
\lambda^2 \frac{\partial^2 V}{\partial x^2} - \tau \frac{\partial V}{\partial t} = (V - V_R) + E(V - V_E) + I(V - V_I)
$$

(1)

(see Rall [27] and Jack *et al.* [28] for the assumptions made in deriving this equation). In this equation, $t$ is the time, $x$ is the spatial coordinate along the nerve fiber, and $\lambda$ and $\tau$ are the characteristic length and time, respectively, of the fiber. The terms on the right-hand side represent the competition between the various ion channels in the membrane, each of which tries to pull $V$, which is a function of $x$ and $t$, toward its specific reversal potential: the excitatory synapses toward $V_E$, the inhibitory synapses toward $V_I$, and the leakage channels toward the resting potential $V_R$. The amplitudes of the synaptic terms, $E > 0$ and $I > 0$, have been normalized with respect to the leakage conductance. Excitatory synapses depolarize the membrane, inhibitory synapses hyperpolarize it; it follows that $V_I < V_R < V_E$. In an interacting neural network, there is one equation as equation 1 for every neuron, and the equations are coupled by the synaptic terms $E$ and $I$, which thus depend on the voltages of the presynaptic neurons. See Niebur [22] and Niebur and Erdös [29] for a formal expression for the resulting system of equations.

$V(x,t)$ in equation 1 describes graded potentials that spread decrementally along the neural processes of *A. lumbricoides* motor neurons. (Small deviations from the behavior described in equation 1 [anode and cathode break potentials] have been observed, but in no case was it possible to evoke action potentials [30].) If the motor neurons are stimulated by application of a voltage pulse to an extracellular electrode in the ventral cord, it is possible to observe the propagation of the resulting decrementally spreading voltage maximum (called “excitation” in the following) along the commissure and in the dorsal musculature to which the neuron is presynaptic. By intracellular electric stimulation and recording in the commissure of inhibitory motor neurons, the velocity of the excitation spreading in the neuron was determined to be in the range 20 to 25 cm s$^{-1}$ [31]. In the muscle, the excitation was found to propagate with different velocities, dependent on whether the muscle receives direct neural input
or not: in that part of the dorsal muscle that receives synaptic input from the motor neurons, the propagation velocity of the excitation was found to be in the similar range of 24 to 30 cm s\(^{-1}\). In portions of the muscle located anteriorly or posteriorly to the output region of the stimulated neuron, the velocity of the excitation was found to be in the range of 11 to 15 cm s\(^{-1}\) [32].

These results are consistent with the hypothesis that the excitation travels with a velocity of more than 20 cm s\(^{-1}\) in the motor neurons and with less than 15 cm s\(^{-1}\) in the muscular syncytium [32]. After a series of related experiments, other workers had come to the opposite conclusion [33]. We therefore decided to simulate the propagation of excitations in neurons with the experimentally determined [26] electrical parameters of \textit{A. lumbricoides} motor neurons. The result is shown in Figure 2, together with measured data from Walrond and Stretton [32]. We found a velocity of about 22 cm s\(^{-1}\) [22].

Our results thus corroborate the conclusion of Walrond and Stretton [32] that the fast excitations \((v > 20 \text{ cm s}^{-1})\) are carried by the motor neurons and the slow excitations \((v < 15 \text{ cm s}^{-1})\) by the muscle. The dissenting conclusion of Weisblat and Russell [33] may be due to the somewhat different experimental paradigm.

Equation 1 describes the behavior of a neural process with a passive membrane (that is, the membrane impedance is voltage independent). Since action potentials (which would require additional terms in this equation) have never been observed in \textit{A. lumbricoides}, we asked ourselves whether the nematode neurons need this mode of operation at all. Does the nervous system of nematodes work with pure electrotonic interactions, not using any nondecrementally propagating action potentials? Experiments show that this is the case for \textit{A. lumbricoides motor} neurons, but until now it was not possible to do the corresponding experiments in \textit{A. lumbricoides interneurons} or in any \textit{C. elegans} neurons. Therefore, we have studied this question by computer simulations.

The results were that the synaptic input to the interneurons in the nerve ring generates sufficient depolarization to control all motor neurons in the ventral cord of \textit{C. elegans}, but that this is not the case in \textit{A. lumbricoides}, due to the larger length of this animal [22].

Before the systematic electrophysiologic studies of nematode neurons (mostly by the group of A. O. W. Stretton [24]) the discovered nonspiking neurons had been mostly sensory cells or central, local circuit interneurons [23]. The observation of nonspiking motor neurons in \textit{A. lumbricoides} removed a critical difference between \textit{C. elegans} and \textit{A. lumbricoides}.
FIGURE 2 Neural excitation spreading in real and simulated motor neurons of *Ascaris* and in *Ascaris* muscle tissue. Symbols: A simulated neuron of length 5 cm with $\lambda = 5$ mm and $\tau = 50$ ms (similar to values computed from the measured membrane capacity and resistance, see Davis and Stretton [26]) was excited in a region of length 0.5 cm at one of its ends between $t = 0$ and 10 ms (triangles) or 100 ms (diamonds). Plotted is the time when the maximum of the excitation appears as a function of the distance between stimulation site and recording site. Note that after different transients, both excitations spread with the same velocity (common slope). Lines: The solid line (slope: 0.04 s/cm) represents the measured [37] spreading of excitation in *Ascaris* dorsal muscle where the muscle is postsynaptic to the excited motor neuron; presumably the excitation travels in the neuron here. The dashed line (slope: 0.083 s/cm) represents the spreading of excitation in *Ascaris* dorsal muscle where the muscle is postsynaptic to the excited motor neuron; presumably the excitation travels in the neuron here. It is seen that the excitation in the neuron travels at about the same speed as in our simulation and much faster than in the muscle.

*lumbricoides* showed that motor neurons can also function by relying on graded (nonspiking) signals for information transfer. Therefore, on the basis of our simulation results, we suggest that the nervous system of *C. elegans*, and possibly that of other nematodes of similar size, may function purely electrotonically. The simulations also indicate that *A. lumbricoides*, and possibly other larger nematodes, need active mem-
branes, such as voltage-dependent ion channels of as yet unknown nature in the interneurons of the ventral cord.

MATHEMATICAL MODEL OF UNDULATORY LOCOMOTION

In the section "Undulatory Locomotion," we gave a qualitative explanation of the principles on which this type of locomotion of nematodes is based. In this section we enter into a quantitative analysis, which was necessary in order to carry out a computer simulation of the motion.

The program of this section is as follows: we first identify all forces acting on the nematode body. These forces are partly external and originate in the friction between the moving body and its support, and partly internal, coming from the fluid pressure, the elastic properties of the cuticle, and from the muscular contraction.

We then define certain muscle contraction patterns, which vary along the body and with time. These patterns are generated by the nervous system, as described in the previous sections. Finally, we describe how we formulated and solved the equations of motion of the body subject to the forces mentioned.

Since the nematode is a continuous elastic body, the number of its degrees of freedom in the sense used in mechanics is infinite. In order to reduce this number to a finite value, which can be dealt with by computer simulation techniques, we introduced a number of approximations. The validity of these approximations was carefully considered, but will not be discussed here.

The support of the body has been assumed to be a plane. The position of the body has been defined by the projection of its outline on this plane. The closed curve obtained by this projection was then divided into 19 segments by lines perpendicular to the body axis. In the straightened body, in the absence of externally applied forces, these lines intersect the body axis at equal distances, including the distance of the first line from the point of the head, and that of the last line from the end of the tail. The two cartesian plane coordinates of the corners of the segments thus obtained were designated by the vectors (indicated by bold letters throughout this article) $x_r(i)$ and $x_l(i)$, where $r$ and $l$ refer to the right and left sides of the body, and $i$ numbers the corners on the given side, beginning at the head. Note that $i = 1, \ldots, 20$. 

122
In its natural habitat, *C. elegans* lives in a water film that covers its support, such as the soil. In the laboratory, the worms are usually bred on an agar layer, where the animal produces a shallow groove. We can observe three types of locomotion of *C. elegans* under these circumstances: if the groove is deep, the body exerts forces against the walls of the groove, and the worm creeps along the groove produced by its anterior part without slipping. If the groove is shallow, the creeping is accompanied by simultaneous lateral movements, called slipping. Finally, if the water layer is sufficiently thick, the worm swims. In these comments we report only on our study of the pure creeping motion. Under these circumstances, the forces to be taken into account are those resulting from the interior pressure of the body, the elastic forces produced by the cuticle, the muscular forces, and the mechanical forces of the environment exerted on the body. The calculation shows that the forces of inertia are negligible, since the body's acceleration is very small: the mass of *C. elegans* is of the order of $10^{-9}$ kg, its acceleration of the order of $10^{-3}$ m s$^{-2}$. Therefore the inertial forces amount to $10^{-12}$ kg·m s$^{-2}$. On the other hand, measured friction forces are of the order of $10^{-5}$ kg·m s$^{-2}$.

The pressure forces on each segment are directed outward (perpendicularly to the segment under study), and their magnitude at time $t$ is calculated from:

$$F(t) = p(t)l(t)$$  \hspace{1cm} (2)

Here, $p(t)$ is the pressure at time $t$ (which is the same for all segments) and $l(t)$ is the length of the borderline of this particular segment, that is, the distance between its two limiting corners. Obviously, $l$ varies with time, as the shape of the moving body varies. We assume the liquid in the body to be incompressible; therefore the total body volume has to remain constant. However, it is by no means evident that having solved the equations of motion for all corner points $x_r(i)$ and $x_l(i)$ of the segments at successive times, these points will move in such a way that the volume of the body (which in the plane model corresponds to the area delimited by the polygonal contour) will remain constant. In fact, it is clear that the volumes of the individual segments will vary in time, since the shape of these segments constantly changes during the motion.

To make sure that the total volume does remain constant, we apply the same principle in the computation as used by nature, namely, if the vol-
ume increases, we decrease the pressure, and vice versa. This is done by computing the pressure from:

\[ p(t) = \left( \frac{p(0)V(0)}{V(t)} \right)^a \]  

(3)

In this equation, \( V(t) \) is the total body volume at time \( t \) (the time \( t = 0 \) is chosen arbitrarily), which is obtained from another formula that expresses the body volume in terms of the coordinates of all the corner points of the contour. The variable \( p \) is dimensionless. The constant \( a \) was chosen as 4, 6, or 8 and we observed that the results depended little on the choice. This self-regulating mechanism works fairly well, inasmuch as the relative changes in the volume are less than 1% during the whole simulation.

Next, we discuss the forces generated by the elastic cuticle. Observation shows that the body diameter stays practically constant, whereas the cuticle does stretch and contract in the longitudinal direction during the motion. Therefore, we assumed that the elastic constant, which is the factor of proportionality between the strain and the stress, is 1000 times larger in the transverse than in the longitudinal direction of the body. (The outcome of the calculations did not depend crucially on this somewhat arbitrarily chosen number.) In the transverse direction, we calculated the cuticle forces from a simple linear relationship between the distance of two contralateral points of the body, that is, between two points that are on opposite sides of the line dividing two segments.

For ipsilateral points, such a simple relationship would not correspond to reality. It is observed that the cuticle can easily be stretched or compressed in the longitudinal direction only within narrow limits. More substantial deformation is strongly resisted. Therefore a nonlinear stress-strain relationship (not described here) was applied to account for this effect.

Muscular forces were simulated similarly to the ipsilateral elastic cuticle forces. Somatic *C. elegans* muscles are all parallel to the local body axis and, therefore, they are situated in our model between neighboring corner points. Activation of a muscle generates an additional force between the two corner points to which it is attached. In the next section we will study patterns of activity that are suitable for the propulsion of the body.
The forces of the environment will be described next. These are most important, since without them the animal would not progress at all, just wriggle around despite all of its intricate neural and muscular machinery.

The forces of the environment on the body of the nematode are of a frictional nature. Since the worm moves in the groove it produces, the forces opposing movement perpendicular to the body axis are much stronger than those parallel to it. Therefore we assumed the following form of these forces, acting on each of the segment corner points

\[ F_j = c_L v_L(j) - c_N v_N(j) \]  

(4)

Here, \( c_L \) is the longitudinal friction coefficient, \( c_N \) is the transverse friction coefficient, \( v_L(j) \) and \( v_N(j) \) are the vectors of longitudinal and transverse velocity, and \( j \) is the index of the corner point. The words "longitudinal" and "transverse" always refer to the local direction of the body axis. It can be assumed that the transverse friction coefficient of an elongated body, like that of a nematode, is much larger than the longitudinal. This difference between \( c_N \) and \( c_L \) is still accentuated by the longitudinal ridges (alae) found along the body of the animal. In the computation, we used \( c_N/c_L = 10^4 \). The velocity of every point was calculated by the finite difference method. In this method, the velocity is equated to the ratio obtained by dividing the vector difference between two successive positions of a point of the body by the difference between the times at which the point finds itself in these two positions.

Before we enter into the topic of the formulation and solution of the equations of motion, we need to explain how we simulate the path-finding mechanism of the animal. As was remarked, the observation shows that the animal follows a more or less sinusoidal path, with occasional deviations and changes in direction of motion. It is also observed that when moving forward, its head turns to the left and to the right, mapping out the direction to be followed by the body.

These head movements are generated by a set of muscles that are innervated by neurons different from those in the somatic musculature. We did not simulate this part of the nervous system, but calculated at every instant of the simulation of the motion the direction in which the head has to turn in the next phase of the motion to assure a sinusoidal track. We skip the details of this calculation, which are described elsewhere [34].

The equations of motion are formulated in the following way: in uniform motion the sum of all forces acting on the body must vanish. There-
fore, the sum of all internal forces must equal the negative of the sum of external forces. The latter, which are frictional forces, give rise to the uniform motion, since the velocity of a given point in contact with a support will be equal to the frictional force acting on that point, divided by the coefficient of sliding friction. (On the basis of experimental evidence, we assume linear proportionality between the frictional force and velocity.)

Consequently, we have the following system of linear differential equations for the $2N$ ($N = 20$) positions of the corners of the segmented body contour:

$$\frac{d}{dt} x_L(j) = \frac{1}{c_L} F_L(j)$$

$$\frac{d}{dt} x_N(j) = \frac{1}{c_N} F_N(j)$$

Here the index $j$ runs over all corner points and $\frac{d}{dt} x_L(j)$ is that component of the velocity vector of the $j$-th corner point that is parallel to the local body axis. Likewise, $\frac{d}{dt} x_N(j)$ is the component of the vector that is orthogonal to this axis, and $F_L(j)$ and $F_N(j)$ are the corresponding components of the sum of all internal forces at this corner point. In other words, $(F_L(j) + F_N(j))$ is the sum of all forces on this point, which result from the interior pressure, the muscles, and the elastic cuticle and which are all time-varying.

The simulation of the movement of the nematode consists of solving these equations of motion numerically to obtain the successive positions of the body starting from an initial position. We do not wish to dwell on the method of calculation and mention only that the backward differentiation formula was applied, in conjunction with a modified Newton method. A Cray 2 computer was used.

Some instructive tests were done in the course of the simulation. The initial position being a sinusoidal state, we first assumed that the body muscles are not excited. In this case, after about 10 seconds simulated time, the body straightened out and did not move either forward or backward. Using different types of muscle excitation patterns, the creeping motion of the nematode was successfully simulated in the forward as well as in the backward direction. These excitation patterns are described in the next section. Figure 3 shows three successive phases of the motion of the simulated worm as it appears on the computer screen. When the neural circuitry responsible for forward motion is deactivated after 2 seconds
and the circuitry for backward motion is switched on instead, the direction of motion is reversed. This is illustrated in Figure 4 where the coordinates of the center of gravity of the body are plotted as functions of time. After about 2 seconds of forward motion, during which the $x$-coordinate increases at a fairly steady rate, the $x$-coordinate starts to decrease at the same rate. This indicates reversal of the direction of motion. During all this time, the $y$-coordinate of the center of gravity remains almost constant, which shows that the worm does not slip sideways.

**MUSCLE EXCITATION PATTERNS**

Propulsion by undulatory locomotion requires periodic actuation of different body muscles of a worm or a snake. We have described previously the work of J. Gray who elucidated muscle activation patterns that are suitable for the locomotion of a snake, that is, a body with a backbone skeleton. The locomotion of a nematode is more difficult to analyze, since local muscle contractions lead to global pressure variations (equation 3)
FIGURE 4 Position of the center of mass of the simulated nematode body as function of time. The solid line shows the x coordinate and the broken line the y coordinate. At $t = 0$, the worm starts moving in the $+x$ direction, at $t = 2$, we reverse the direction by exciting the interneurons responsible for backward movement. Note that the y coordinate stays essentially constant.

and therefore a local analysis, as performed by Gray in the case of the snake, is not applicable. We have resorted to computer simulation to find suitable activation patterns in this case.

As described in the previous section, we determined the forces that act on the body of a creeping worm (that is, hydrostatic pressure of the body fluid, elastic forces of the cuticle, muscle forces, and friction with the support) and solved the equations of motion of the body, equations 5 and 6. Different muscle activation patterns were then simulated by using different sets of muscle forces and we were able to study which activation patterns generate propulsive power for the worm. It turned out that this procedure provided not only insight in the working of the nervous system of nematodes, but that it is also applicable to the analysis of the forces and the control of machines and robots [35].

Our results show that several classes of substantially different activation patterns are suitable to impart propulsive power to a body of sinusoidal shape, which shows the robustness of undulatory locomotion. Gray proved that a locally controlled muscle activation scheme is suitable to propel a body with a backbone. This scheme is defined by the requirement that, for forward movement, a given muscle has to contract if the ra-
dius of curvature of the body part, which is located anteriorly to the muscle, is smaller than the radius of curvature posterior to the muscle. Our simulation shows that this pattern is also suitable to propel a body with a hydrostatic skeleton (Fig. 5).

A different class of muscle activation patterns was found by regarding the set of differential equations as a constrained optimization problem for the velocity of the center of mass of the worm’s body and by solving the system for the greatest velocity, either in forward or in backward direction. In contrast to the pattern that was characterized by muscle contraction on one side of the body (unilateral contraction), this “optimal” pattern is characterized by bilateral contractions (Fig. 6). Since a worm does not dispose of a digital computer to solve the optimization problem, we wondered whether similar patterns can be generated by other means. We found that this is the case and that activation patterns similar to the “optimal” one can be found by combining information about the lengths of different body parts (Fig. 7). These lengths differ from each other because in the course of motion they are stretched differently. It seems that the gen-
FIGURE 6 Simulation of nematode locomotion using "optimal" muscle control: a muscle is excited if its contraction contributes to locomotion in the +x direction.

FIGURE 7 Simulation of nematode locomotion using "quasi-optimal" muscle control (see text).
eration of such patterns (which we will refer to as "quasi-optimal") requires us to determine the sign of the product of the elongations of different body parts, which may be positive or negative (see Niebur [22] for details). In principle, neural systems may be able to perform this kind of computation, but we do not see how the simple nematode nervous system could do so. Therefore we conclude that such quasi-optimal patterns cannot occur in nematode locomotion.

The generation of the last class of muscle excitation patterns we studied is based on the assumption of stretch receptors being located about one fifth of the body length anteriorly to the muscle controlled by the receptor for backward movement, and the same distance posteriorly to the controlled muscle for forward motion. We showed [22] that this excitation pattern, called "stretch receptor" pattern (Fig. 8), can also provide a thrust that is sufficient to propel the worm's body in the desired direction.

It is interesting to speculate on the question of how the worm varies its velocity of motion. A nematode creeping over a surface can vary its velocity from zero (no motion) to some maximal value that is of the order of 0.1 cm s\(^{-1}\) for *C. elegans*. We found that for all muscle activation patterns, the velocity can be varied by changing the value of the muscular tension. We conjecture that the muscular tension is controlled by the amount of

![FIGURE 8 Simulation of nematode locomotion using stretch receptor muscle control (see text).](image-url)
neurotransmitter emitted at the neuromuscular junctions by the somatic motor neurons. On the other hand, the amount of neurotransmitter emitted is presumably controlled by the excitation status of the motor neurons, which, in turn, is varied by the neural input from the ventral cord interneuron. Since the latter receives all of its input either in the nerve ring or in the caudal ganglia (except for a few synapses from motor neurons in the ventral cord), we may suppose that the variation of the level of interneuron excitation by the synapses in the nerve ring or in the caudal ganglia leads to a variation of the speed of the moving worm.

DISCUSSION

Nematodes offer today an unprecedented possibility for the study of nervous systems. *C. elegans* is unique with respect to the simplicity of, and the available knowledge about, its nervous system. This knowledge can be combined with the wealth of data available about its development and its genetics. Furthermore, other nematodes (in particular *A. lumbricoides*) are sufficiently similar in structure to *C. elegans* that many experiments that are difficult to do with *C. elegans* can be performed with them, and the data can be used to characterize a “generic nematode.”

Most probably the computational mechanisms for the control of locomotion are implemented in vivo by hard-wired stretch receptors. “Hard-wired” means that the synaptic connections are determined genetically. Recent experiments show that the behavior of *C. elegans* is not completely programmed genetically and that its nervous system has at least some degree of plasticity [9], but for a stereotypic behavior like undulatory locomotion a hard-wired “program” seems to be the most efficient implementation.

It was recognized 15 years ago by R. L. Russell (personal communication), that the distal ends of the somatic motor neurons might have the function of stretch receptors. This hypothesis was based on several pieces of evidence. The processes are devoid of synapses, gap junctions, or other differentiations that can be discerned by electron microscopy. They are situated close to the cuticle and therefore ideally located for monitoring the shape of the body. Their length (about a fifth to a quarter of the body length) and their consistent position relative to the neuromuscular junction regions (in neurons used for backward motion, always anterior to
their neuromuscular junctions and in neurons used for forward motion, always posterior to their neuromuscular junctions) make it improbable that they are functionless appendages or developmental errors. The position of these processes was a puzzle, though: why are the sensors used for forward locomotion arranged posteriorly to the output region of their neurons, and why are the putative stretch receptors located so far away from the muscles they influence? These questions were answered by our analysis of the control of the forces involved in undulatory locomotion [34]. We found that the greatest curvature of a sinusoidally shaped body is located about a quarter of a body length away from the muscles that have to contract to generate motion-generating thrust. Furthermore, stretch receptors are generically excited when they are dilated rather than compressed (we thank Professor E. Florey for pointing this out to us), and during forward locomotion stretched regions are located posteriorly to the muscles that have to be excited. The opposite is true for backward locomotion. This explains the location of the putative stretch receptors in the body of *C. elegans*.

New experimental evidence in favor of the stretch receptor hypothesis has been provided by laser ablation experiments performed in the laboratory of R. L. Russell (personal communication). The experiments were done in newly hatched animals, which have a smaller number of excitatory motor neurons than adult worms (15 instead of 69), which makes it possible to kill all motor neurons of the ventral cord. It was found that the midbody, whose muscles get their only innervation from the somatic motor neurons, was always flaccid. One of the most important results of this study was the observation that movements of head and tail, whose muscles receive innervation from motor neurons outside the ventral cord, did occur, but were uncorrelated with each other. This was not due to a complete interruption of communication between the head and the tail, because a light touch of the head caused the tail to curve immediately. This is the expected reaction of the tail to a touch of the head during the initiation of a backward movement [36]. An explanation for the uncorrelated motion of head and tail may be found in the fact that they are lacking the input from the stretch receptors located in the motor neurons of the midbody.

Although the results of our simulations and most experimental evidence are consistent with the proposed mechanism—global control of the interneurons transformed into a rather complicated spatiotemporal patterns of muscular activation by hard-wired stretch receptors—we would
like to call for caution. There are still some facts that do not naturally fit into this picture. We call attention merely to one of them, concerning the inhibitory neurons. To appreciate the problem, we recall that among the seven classes of somatic motor neurons, five are excitatory and two are inhibitory. As we have mentioned previously, all members of a given class of excitatory neurons are used either for forward or for backward movement. In contrast, the inhibitory neurons are used for forward and for backward locomotion. A plausible function for them is to delimit the excitation delivered by the excitatory neurons to the regions where the latter make neuromuscular junctions and thus to prevent the spread of the excitation across the whole syncytium of electrically coupled muscle cells. In our simulation, we have assumed that the inhibitory neurons have this function, and this is consistent with their morphology. In our model there is, however, no function for the observed oscillations of the intracellular potential of these cells [31].

Stretton and collaborators [24] suggested that these cells function in a circuit of coupled neuronal oscillators that is used for the propagation of muscular waves. Besides some theoretical arguments against this model [22], new experimental evidence does not support it. This evidence consists in laser ablation of all somatic muscle cells of the midbody of freshly hatched C. elegans, without interfering with the neurons. It was found (Russell, R. L.: Personal communication) that this operation not only makes the midbody flaccid (due to the absence of muscle cells), but it also has the effect that the movements of the head are no longer correlated with the movements of the tail, as in the previously described case when the neurons of the midbody had been destroyed. If muscular waves were propagated by the activity of coupled neural oscillators in the midbody, one would expect that ablation of muscle cells would not interfere with the communication between head and tail and that therefore a controlled phase relationship between head and tail movements would be conserved.

Although these results seem to support the stretch receptor hypothesis, the presence and function of stretch receptors does not explain the observed oscillations in the inhibitory motor neurons. This may indicate the occurrence of some phenomenon that we do not yet understand.

Despite the generally excellent correspondence of known features of homologous neuron classes between C. elegans and A. lumbricoides the latter has functionally and anatomically identified synapses from the inhibitory motor neurons to the excitatory motor neuron, which the former apparently does not have, although this difference may be due to the meth-
ods used to define a synapse in C. elegans [24]. Other reasons for caution are the observations concerning the pharynx [17], which show that non-neural parts of the nematode body can assume functions that are usually ascribed to the nervous system. On the other hand, the tentative conclusion of Avery and Horvitz [17] that most of the C. elegans nervous system is dispensable in hermaphrodites under laboratory conditions is certainly not true for the control of locomotion, the system we model in our work. This is shown by the laser ablation experiments, which make it clear that the somatic motor neurons as well as the ventral cord interneurons are essential for the generation of the muscular waves [36].

Now that we have been given the complete wiring diagram of an entire animal, what else could a modeler ask for? It should have become clear from the preceding that there are still many things that are not known. For instance, the only information about synapses available in the present wiring diagram is their existence and whether a given synapse is chemical or electrical. Nothing is known with certainty about the strength of the synaptic connection, or whether it is excitatory or inhibitory, let alone the time course of its activation, the ions involved, etc. In a sense, the wiring diagram of the C. elegans nervous system is like the wiring diagram of an electronic device in which the numerical values of the parameters of all components are unknown. In order to make progress with the modeling, we used indirect methods and made simple assumptions to determine these parameters, all of which have to be known for a detailed simulation. As an example for an indirect method, we would like to cite our hypothesis that γ-aminobutyric acid neurons are inhibitory and cholinergic neurons are excitatory. Since the same relationships have been found for the homologous classes in A. lumbricoides, we are quite confident about this hypothesis, but, of course, direct verification is needed. Another example is the hypothesis of the presence of stretch receptors for which evidence so far is indirect. A direct electrophysiologic measurement of the intracellular voltage as a function of elongation of the neuron would be needed to test this assumption. As an example for making the simplest assumption, we have postulated that all synapses have the same strength (or, for electrical synapses, the same conductance per membrane area). This is probably an oversimplification and recent experimental evidence [9] showing the capability of C. elegans of behavioral learning indicates a certain synaptic plasticity in its nervous system and, therefore, differential change of its synapses. Nevertheless, in the absence of any data, the assumption of identical synapses seems to be a reasonable one.
Let us now discuss some open problems that can and should be attacked, either with the presently available data or after more data have become available through future experiments. The detailed simulation has been limited so far to the neural circuitry which is responsible for forward locomotion. This circuitry should be gradually extended to comprise larger parts of the C. elegans nervous system. It is a long way until we can hope to make detailed realistic models of the most complicated parts of the nervous system, including the nerve ring, but it does not seem to be a goal that is impossible to attain.

It is of interest to compare the approach described in these comments with other studies of behavioral simulation. We have tried to constrain the simulation as much as possible by subjecting the simulated system to experimentally known restrictions. A very different approach was chosen by Braitenberg [37] who, in an elegant monograph, constructed a series of formal organisms without explicitly taking into account biologic constraints. He showed that surprisingly complex behavior results from a few, quite simple built-in rules. Obviously, Braitenberg was inspired by biologic systems, but his approach shows that some nontrivial behaviors are not restricted to biologic systems and may be easier to understand if seen in a more general framework. This work elucidated the fact that behavioral patterns that seem very complicated to analyze can be surprisingly simple to understand when they are, instead, synthesized ("law of uphill analysis vs. downhill invention").

We would like to mention two other studies that are situated between Braitenberg's and our work.

One is the work on Periplaneta computatrix, which is a computer model of the cockroach P. americana [38]. The model P. computatrix is an artificial insect that exists as a computer program. It wanders, follows edges of objects, and feeds when its nutritional energy content is low. It finds simulated food by being attracted to it through a sensory mechanism that detects the direction from which simulated odor signals come.

By a judicious choice of small simulated groups of neurons but without taking explicitly into account the details of the circuitry of the real cockroach, all these tasks can be accomplished, and the simulated insect behaves in many respects as a real insect does. The artificial insect seems to learn tasks just as advanced robots do, and interesting insights into adaptive behavior may be gained.

The other study is the work of S. R. Lockery et al. This is the only other simulation of a part of the nervous system of C. elegans of which we are
aware [39]. The authors of this study take an intermediate approach by constructing a network that is constrained to some extent by the known anatomy of *C. elegans*, but in which the neural geometry is neglected and in which the synaptic strengths are determined by an optimization algorithm. The model successfully reproduces some aspects of the chemotactical behavior of the nematodes.

We believe that all mentioned approaches will be useful for enhancing our understanding of the problem of intelligent or at least survivalist behavior.

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References


Aims and Scope

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