A Worm's-Eye View of the Brain

BY RICHARD RUSSELL

Our world is full of complicated machines (computers, for example) composed of very large numbers of fairly simple elements. The nature of these elements to some extent determines how these machines work, but more important is the way in which these elements are connected together. For instance, the same electronic components can be used to make a phonograph amplifier, a television set, or a Moog synthesizer; the difference lies in the way their components are wired together.

The same general principle, of course, applies to our own nervous systems. Our brains are made up of a very large number of nerve cells (probably about ten billion), but the number of cell types is apparently fairly small. What enables different parts of our brains to receive visual stimuli, to integrate these into a picture of the viewed object, and to react to that object with fear or pleasure or arousal is the way in which these cells are connected together.

But what are the principles by which these all-important cellular connections are established, and how do they actually work? The answers to these questions are still largely unknown, primarily because of the extreme complexity of the vertebrate brain. Although considerable progress can be made by asking general questions about the functions of different parts of the brain (see, for example, the articles by James Olds and Roger Sperry on pages 12 and 29), we are still a very long way indeed from a "circuit diagram" of the vertebrate brain.

There is a quite reasonable expectation, however, that some of the principles used in mammalian brain circuit design may also be used in the design of much simpler nervous systems, for which there *are* reasonable prospects of obtaining a circuit diagram. Several laboratories, ours included, are now studying such simple systems in the hope of revealing some of these principles.

Our organism of choice, the small soil nematode (roundworm) *Caenorhabditis elegans*, was first recognized as virtuous for this purpose by Sydney Brenner of the Medical Research Council Laboratory of Molecular Biology in Cambridge, England. Like other more familiar nematodes (for example, the classical invertebrate-zoology-course nematode, *Ascaris lumbricoides*, or the trichinosis agent, *Trichinella spiralis*), *C. elegans* has a simple nervous system, with fewer than 300 nerve cells. Unlike these other nematodes it is extremely small—only about one millimeter long—and chooses to live in the soil rather than as a parasite.

Despite the simplicity of its nervous system, *C. elegans* shows a variety of behaviors; it moves with a sinusoidal gliding movement in which its 92 longitudinal muscle cells are exquisitely coordinated; it occasionally reverses, moving backward with equal coordination; it feeds on surrounding bacteria through the pumping action of a muscular pharynx; and when its gut becomes overly full, it defecates by using special muscle cells in its tail. It is attracted to some chemicals, repelled by others, and responds also to mechanical and thermal stimuli. Other behaviors, including a specialized mating behavior, a "social" clumping, and an acrobatic act of balancing on the tail, are exhibited in certain developmental stages or under certain environmental conditions.

One of the interesting general questions which arises in considering *C. elegans* is how such a variety of behaviors can be executed by such a simple nervous system. In order to determine how the component nerve cells are "wired up" to perform these behaviors, we have begun with an anatomical reconstruction in which the small size of *C. elegans* has been a distinct advantage.

Our goal in this reconstruction has been to obtain a complete anatomical analysis of the nervous system including the shapes, branchings, and positions of all the nerve cells, together with their all-important interconnections. We must go about this by using the electron microscope for the simple reason that the interconnections, or synapses, are in general so small that they can only be resolved in adequate detail by the electron microscope. Fortunately *C. elegans* is so small that its whole nervous system can fit in one field of view in this instrument, thereby greatly simplifying the task. Since the electron microscope also requires very thin specimens for its electron beam to penetrate, we must cut our animals up into many thin sections, examine each, and then use all to reconstruct a three-dimensional representation of the nervous system.

Much of our initial effort has gone toward establishing techniques for handling the large number of sections which must be cut and examined for this job. One of these techniques, for instance, is a movie-making process

devised by Randle Ware, a postdoctoral fellow. In this process, thin sections are cut serially (from a fixed and embedded animal), photographed in the electron microscope, and then rephotographed in alignment so that each becomes one frame of a movie film. When projected, this film gives the impression of an "optical trip" through the nervous system, and we use it as a convenient way of determining the detailed shapes of individual nerve cells.

Another technique, just being set up now, uses this sort of movie in connection with a computer graphics display terminal to map the positions of synaptic connections between nerve cells. Individual frames of the movie are projected onto the oscilloscope screen, and a computer-controlled light spot is used to mark and record the positions of synaptic connections. These positions can be easily retrieved from the computer memory and very efficiently used to determine the patterns of interconnection among nerve cells. For this phase of our work we are collaborating with Drs. Gilbert McCann and Ken-Ichi Naka of Caltech's information sciences group.

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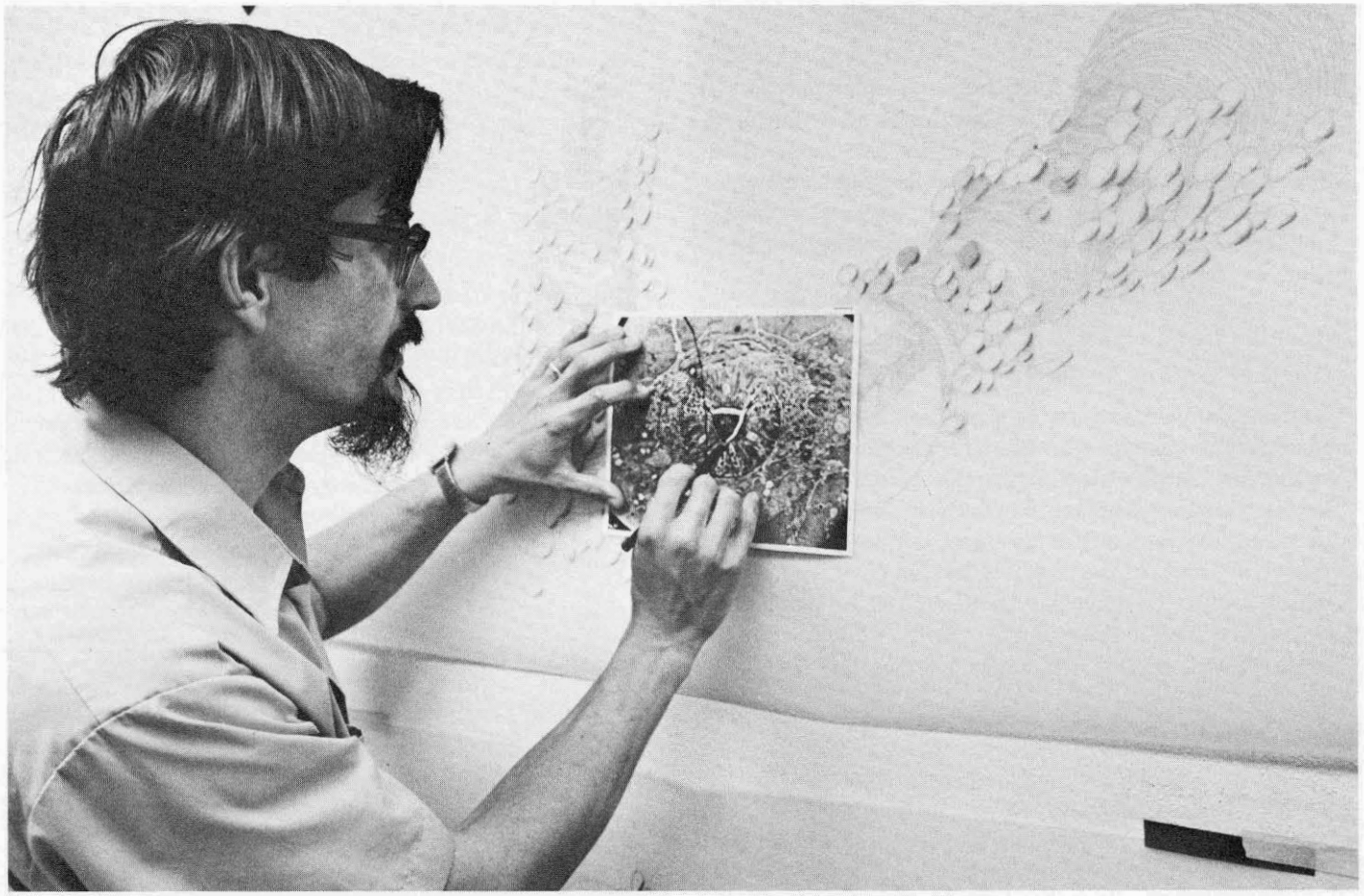
With these techniques in hand or in progress, we have begun our anatomical analysis by concentrating on one



Richard Russell, assistant professor of biology, outlines nerve pathways on a photograph of a cross-section of the small soil nematode. By repeating this process on sections cut serially along the length of the worm, he can build up a three-dimensional "circuit-diagram" of its nervous system. The diagram on the wall, a work in progress, shows the position of nerve cell bodies in the head of the worm.

part of the nervous system, the sensory part. This is located near the front of the animal, in a relatively confined space, and has proven well suited to anatomical analysis. We have determined the shapes of all of the 68 cells in this part of the nervous system, with particular emphasis on the shapes of the sensory endings by which these cells detect chemical signals, touch, and temperature. Homologies with the structures of sensory endings of known function in other animals have been detected and used to predict the functions of some of the sensory cells. Other cells, to judge by their lack of synaptic contacts, seem to play the role of accessory, non-nervous cells, despite their intimate structural involvement in the sensory endings. A remarkable degree of symmetry has been found, and also a high degree of structural reproducibility from animal to animal, testifying to the tight controls that must be exerted over the formation of this simple system in development.

Recently we have begun to analyze the synaptic contacts of the sensory nerve cells, and already two surprises have confronted us. First, some of the sensory nerve cells make direct synaptic contact with muscle cells which move the animal's head, and we are particularly intrigued to know what role such a simple reflex arc can play in governing behavior. Second, even the central nerve ring, the integrative structure which contains many sensory cell synapses, shows a marked degree of symmetry which will greatly facilitate its eventual structural analysis.

At this point in our structural analysis, some potentially generalizable principles are already emerging; first, in many respects the system appears to be constructed out of simpler cell assemblies, repeated several times and arranged symmetrically in the animal. This principle is very reminiscent of the highly reiterated structures of more complicated nervous systems such as the visual system of dipteran flies like *Drosophila*, or the cerebellum of vertebrates. Second, the degree to which nervous structure can be reproduced from animal to animal is really quite impressive; not only are nerve-cell bodies almost always in exactly the same positions in different animals, but also the various axons which comprise a nerve bundle are usually in quite characteristic positions within the bundle. Third, it appears that quite simple connections like sensorimotor reflex arcs can be included within much more complicated overall structures.

Our experience so far suggests that as our anatomical analysis continues, more and more principles of this sort will continue to emerge, particularly when we analyze synaptic connections and begin to reveal the structures of some nerve-cell circuits. Initially, of course, the circuits we reveal will simply be anatomically feasible ones, without any evidence that they are actually used in controlling behavior. However, a number of techniques can be used to test the functions of these circuits. For instance, we should be able to eliminate single cells or groups of cells by laser microbeams and thereby determine their functional roles. And there are indications that some neural transmitter compounds may be used by a very small number of nerve cells, whose functions could be selectively eliminated by drugs which block the action of these transmitters.

Perhaps the most comprehensive method, however, involves the use of mutations. Because it is a self-fertilizing hermaphrodite with a very short generation time, *C. elegans* is very well suited for the isolation of genetic mutants. We have already isolated over 300 independent mutants with behavioral alterations, including some which specifically affect the sensory nervous system. Because some of these mutants produce specific and reproducible changes in the structure of the nervous system, they are very useful for testing ideas on how the system functions; if an idea incorrectly predicts the behavioral consequences of a mutational change in nervous structure, then the idea must be scrapped or modified. (Seymour Benzer's article on page 6 discusses other uses of mutations affecting nervous function.)

Once the true functioning circuits of the *C. elegans* nervous system have been revealed by these techniques, the principles of circuit design used in this system should become clear, and it should then be possible to determine whether, as we strongly suspect, these same principles will apply in the construction of more complicated nervous systems like our own. □