Action yields reaction; the humble crayfish provides an elegant model for studying the integration of all the available, varied forms of sensory information so as to fit each reaction to each action.

ACTIVITIES OF A NERVOUS SYSTEM

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The *functioning of the nervous system of the* freshwater crayfish has been the primary object of investigations by the neurophysiology group at Caltech since 1934. The more we learn about different nervous systems, the more it appears that they all use the same kinds of tricks to deal with specific problems. But why do we concentrate on an animal that is so far off the main line of human evolution? The main reason, of course, is the limited number of nervous elements in the crayfish, which, though not as smart as many other animals, still shows a considerable variety of actions. Our experience has proved that it is possible to show in a number of cases that one certain nervous element, which can be readily identified in each specimen, is always involved in a given activity.

Our work started with studies of the motor axons innervating the muscles, and we found each muscle to be controlled by a very small and constant number of nerve fibers. Some muscles receive only a single motor fiber, most others two, and the legs a maximum of four. Not only are these numbers constant but each fiber also performs a special task that results in a specific type of contraction. We now know that these differences can depend on many factors—the differences in effect of the two motor fibers on a single muscle fiber being only one. But our original view, that each particular muscle acquires properties which make it especially adapted for its particular task, remains unchallenged. In many cases the additional presence of one or two peripheral inhibitory fibers—which can suppress to a greater or lesser extent the contractions initiated by the motor fibers—provides another way to vary the contraction according to circumstances.

When these observations were made, it was widely accepted that—as in vertebrate striated muscle fibers—any one muscle fiber was innervated at a single locus, the endplate, by a motor fiber branch. In the crustacean muscle, instead, each fiber receives many endings (multiterminal innervation), usually from more than one axon (polyneuronal innervation). Now it is known that these also occur in vertebrate smooth muscles, like those in the walls of the stomach, intestines, and blood vessels.

There are, of course, far fewer motor elements in the crayfish than in a frog or man, and this is part of the reason why the crayfish and all its relatives can manage with a greatly reduced number of neural elements in the central nervous system. It is estimated that the crayfish has several hundred thousand neurons, while man has about ten billion.

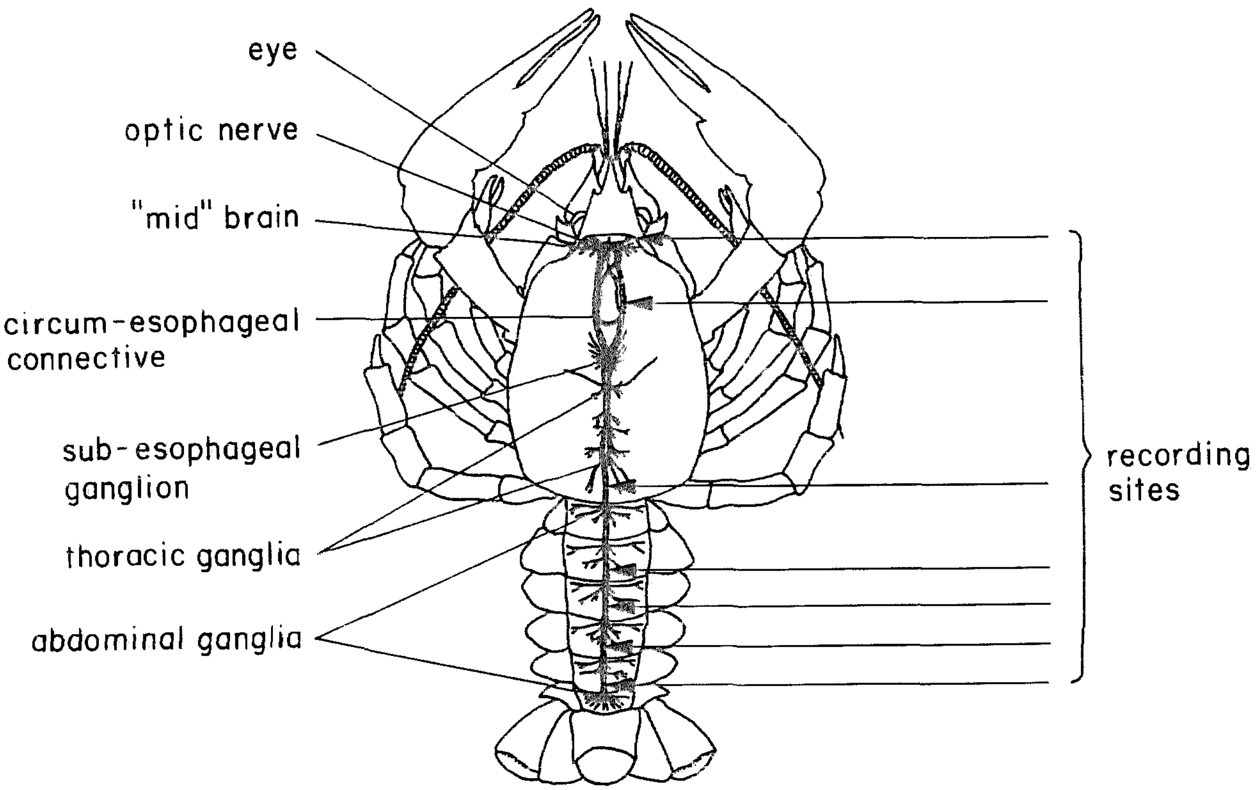
On the sensory side the crayfish is better provided than on the motor side. For instance, many hairs on the exoskeleton are innervated by one or two nerve fibers, whereas the compound eyes and the equilibrium organs (statocysts) respond with thousands of elements. In certain cases a great economy is evident. The function of the abdominal stretch receptors is especially impressive in this respect. There are only four receptors in each segment. The four are organized in two symmetrical pairs that, between them, signal the position and the speed of flexion of the abdominal segment behind the one they occupy. The ones that indicate position, the slow stretch receptors, have proved to be very useful for the study of many problems relating to the genesis of nerve impulses by mechanoreceptors. Because of the very small amount of tissue involved, these receptors are also favorable for a study of drug actions. In addition, they are among the few known sense cells that are innervated by an inhibitory fiber from the central nervous system that can "set" their sensitivity.

The axons of these sensory cells illustrate another aspect of the structure of the central nervous system. Upon entering it, the axons divide into two branches, one going forward to the brain and the other backward to the last ganglion. They thereby distribute their information throughout the whole system. Other sensory fibers, though more restricted, also have branches to neighboring ganglia. These extensions of the primary sensory fibers provide for integration of sensory events from more than one segment, which is of importance for the ultimate reaction to stimuli.

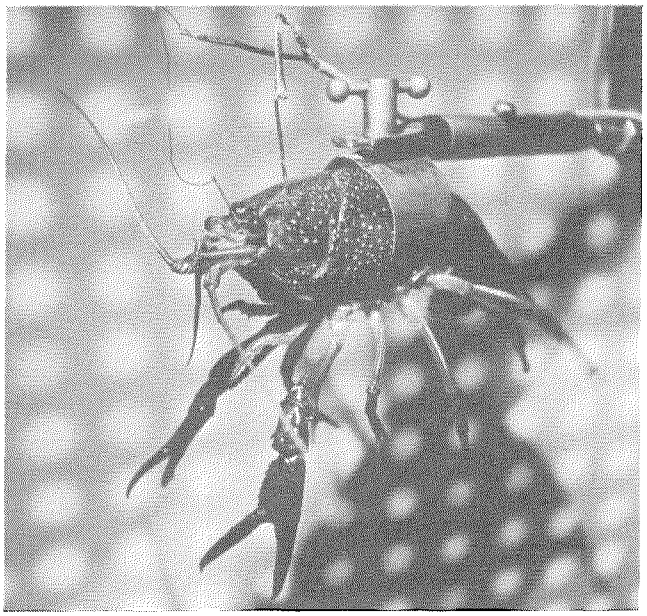
The integration of sensory events can be studied by recording the impulses caused by sensory stimuli in single interneurons—those elements of the nervous system that transform incoming impulses from sensory fibers to inputs for other interneurons or, more directly, for output fibers such as the muscle motor fibers.

The crayfish has proven to be particularly suitable for such studies. Its central nervous system (below) is much less condensed than in most other relatives, such as crabs, in which all thoracic and abdominal ganglia are fused. There are a number of levels in the crayfish where interneurons can be prepared for study. After removal of the sheath, the connectives can be split into small bundles containing one or a few reactive fibers. An interneuron will react, in contrast to most sensory fibers, to a relatively large body area—as, for example, to the touch of all hairs on one-half of a dorsal abdominal segment. Other interneurons have even larger fields, often consisting of equivalent areas on many neighboring body segments. For instance, one reacts to the hairs on the three peripheral parts of all five thoracic legs. This principle culminates in the sensory field size of an interneuron which reacts to touching the animal anywhere. As a consequence, stimulation of any one spot on the animal's surface will activate as many as 20 interneurons. The same information is thus channeled in many parallel pathways.

A plausible view of how this comes about would be that those interneurons with relatively small sensory fields provide the input for those with large fields—but this is generally not the case. Instead, the large field fibers receive input in many ganglia. This is shown by the fact that impulses in these fibers,



The central nervous system of the crayfish is especially suited for study because it is much less condensed than most of its relatives. The arrows indicate those levels of the system at which impulses have been recorded from interneurons.



Electrodes are implanted in the eye of the crayfish to record nerve impulses resulting from various stimuli.

passing a given location, travel forward when the back is stimulated and backward if the stimulated area is in front of the recording site.

Interneurons with other tasks are also present. There are, for instance, a number of interneurons that appear to have no sensory input, but that discharge continually by themselves before, as well as after, sensory isolation. Their functional significance is, as yet, problematic. "Activity" fibers discharge vigorously only when the animal struggles. "Command" fibers, when stimulated, cause coordinated body movements.

Our present investigations are mainly concerned with the optical apparatus. To obtain single-unit responses from the nerve tract between the brain and the four ganglia in the eyestalk, we push an insulated needle into the optic nerve (above). Its fine, bare tip will pick up impulse discharges of a single unit. A rather unexpected finding is that, in all species used, there are more fibers in the optic nerve which signal events from the brain to the ganglia than the other way around. Even when the very numerous primary sensory fibers, running in a tight bundle and activated by hair touch anywhere on the head region, are discounted, the statement still holds. This indicates strongly that the optic ganglia are the main location for the integration of visual and other inputs. The results of our research confirm this. First, there is no evidence for any fibers, primary or interneuronal, which have small visual fields. Though such negative evidence can never be considered completely conclusive, it is very likely that, during the many experiments that have been performed, such fibers would have been observed had they existed, since they obviously must be numerous. Second, all fibers reacting to visual input are also influenced by inputs coming from the brain, but in various ways.

One class of interneurons, consisting of 14 members, signals the light intensity falling on the eye. Each member responds to increases in light intensity over a very specific retinal area and is inhibited by illumination of all other areas. The fibers are all, therefore, influenced by any small area of the eye. However, since their excitatory fields overlap, light in any given spot will increase the discharges in several and decrease them in all others. This is another example of the principle of parallel computation.

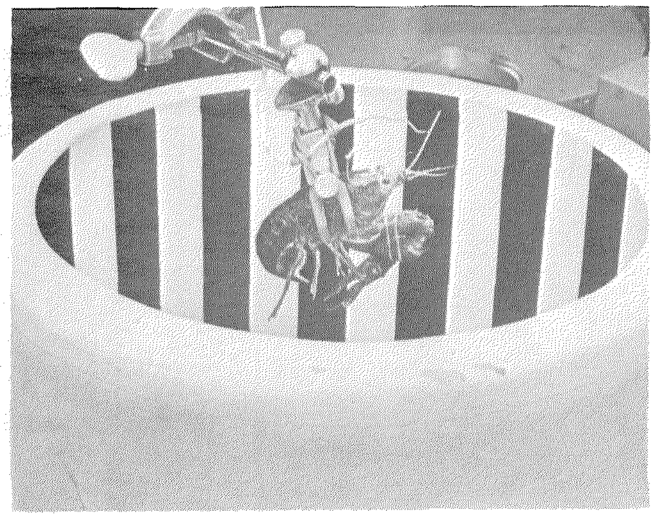
The responses of all these "sustaining" fibers are influenced by the level of the "excited state." Thus the same light exposure causes fewer impulses when the animal is quiet than when it moves its appendages. But when an excited state is present in total darkness, it does not by itself cause these fibers to fire impulses.

One is tempted to describe this process as being comparable to "paying more attention" to the visual stimulus. But this need not mean that the ultimate reaction to the stimulus is greater, for it may be, instead, a compensation for a lowered reactivity in the input-output chain (itself caused by the excited state) and thus a homeostatic mechanism.

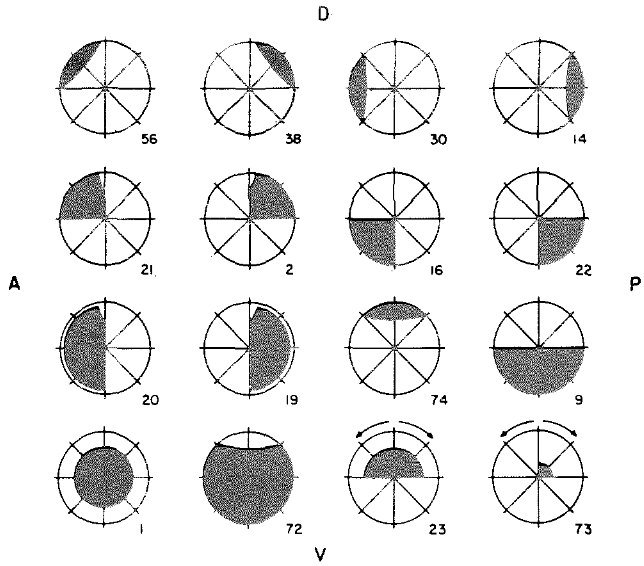
The excited state has other effects. This is illustrated by the increase in impulses in the motor fibers to some of the muscles which move the outer eyecup. These muscles provide for the adjustment of the eye's position to changes in visual and gravitational conditions. Another muscle, otherwise quite similar but responsible for the eye withdrawal reflex (comparable to our blink reflex), is in no way influenced. The six muscles whose motor fibers do show the excited state are often called the eye retractors, since, when all are active, they retract the eyecup, bringing it closer to the body. This is exactly what happens when an excited state develops, causing the often considerable increase in the firing frequency of all their motor fibers. But in addition each muscle serves for much more subtle adjustments. These are being studied in detail since they present us with an excellent subsystem for studying input-output relationships.

The six muscles are arranged in three pairs, each pair involved in a specific task, in a push-pull relationship. The first pair moves the eyecup forward and backward. The two sets of motor fibers show this antagonistic arrangement. When there is an increase in the frequency of impulses in the one, inhibition occurs in the other. The specific input for these fibers is wholly visual and derives from both eyes. Identical reactions are obtained both when the animal is rotated in a striped drum and when the stripes are rotated around the animal (below). Rotating the animal in the dark or in a totally white surround does not cause changes, proving that the equilibrium organs, or statocysts, have no influence on this pair of muscles. In order to affect the discharge rate of a given fiber in the same way, stripes must pass over one eye from front to back and over the other from back to front. There is, therefore, no change when the animal walks in a straight line, but only when he turns.

The second set of motor fibers regulates the position of the eye in the vertical plane. Here the effect of body rotation in darkness is at least as strong as under general illumination. The eye, which is turned downward by rotation of the animal, moves upward, and the impulses in the motor fiber to the responsible muscle increase in frequency until the eye looks straight down. There are two other ways by which a similar but smaller increase occurs on visual stimuli—turning stripes over the eye surface with a vertical, down-to-up component, or illuminating the other eye. When any of these factors is reversed, the same fiber is inhibited and its antagonist activated.



Studies of the eye muscle motor fibers are made with this experimental setup that allows rotation of either the animal or the striped drum in which it is suspended.



The excitatory visual fields of the 14 sustaining fibers in the crayfish eye are indicated by black areas. The fields of the two space-constant sustaining fibers, 0 23 and 0 73, are shown (lower right) for the animal's normal position.

The third set of motor fibers innervates muscles for eye rotations. These are influenced by way of the statocysts when the animal is turned head over heels, again in an antagonistic relationship. They also show the two types of visual input. Rotation of stripes produces an effect when the stripes are at right angles to the longitudinal axis of the body and are thus seen especially by the eye rims. Illumination causes an effect in only two areas of either eye, namely the front rim and back rim—the areas covered by the sustaining fibers 0 14 and 0 30 (above). Here the same areas of the two eyes work synergistically and against those on the opposite side. (For stripes turning in intermediate directions between the three described, all three systems are activated, and the resulting eye position is that place determined by the relative strength of contraction in all six muscles.)

The fact that it is possible to drive the eye muscles by moving stripes shows that there must be a way by which the crayfish can note them. Though this might be done by way of the sustaining fibers, the fact that the reactions are so independent of the intensity of illumination and the amount of contrast (as well as several other considerations) shows that such is not the case. We have found, in all species investigated, a class of interneurons which reacts specifically to moving objects, and these fibers share, with similar fibers of the frog and rabbit, insensitivity to level of illumination and to amount of contrast. In the crayfish the most commonly observed movement fibers have been called "jittery," since they respond maximally to small dark objects moving in an irregular manner over their sensory fields. A large dark object such as a black cardboard is responded to only when its border enters the field of vision, unless the movement is stopped and then renewed.

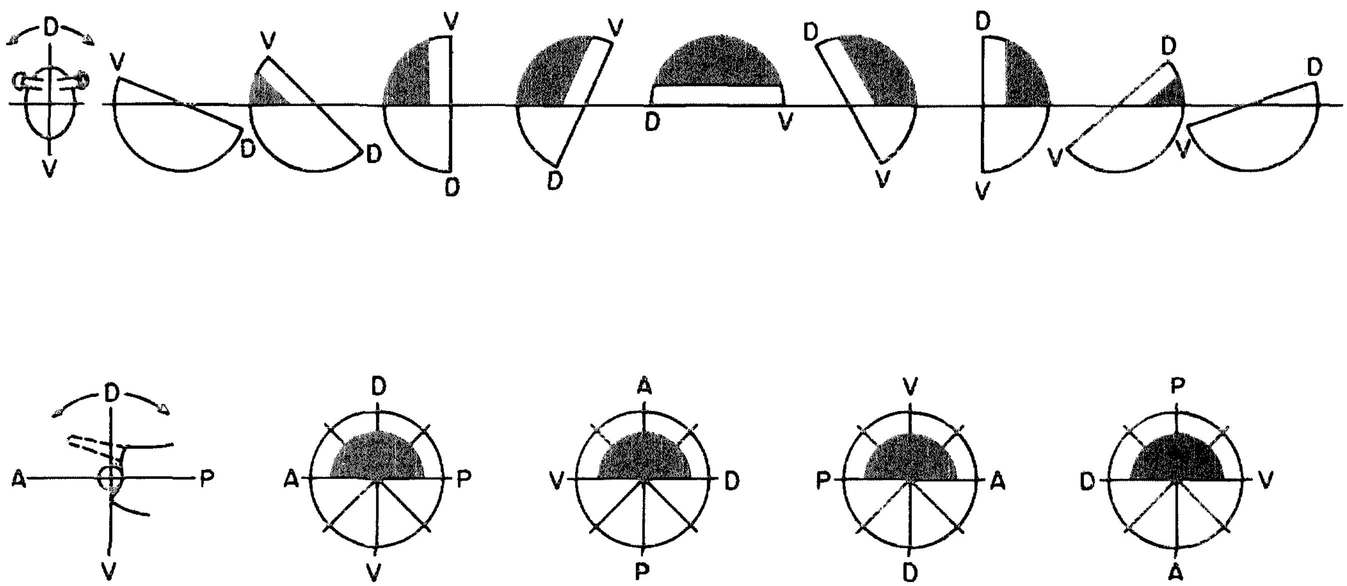
The movement fibers have fields similar to those of the sustaining fibers. This is especially apparent in the rock lobster, which has two additional movement fiber types. The 23 fields of the 23 members of the sustaining class of fibers are all represented by the 23 members of the jittery movement class, whereas for the other two classes respectively, 21 and 14 members with corresponding fields have been found. Therefore, visual events in one area can be signalled by four fiber classes. It is only in crabs that a type of movement fiber has been regularly obtained which reacts with a continuous discharge to a moving striped pattern. All other movement fibers have a more or less pronounced "habituation"—that is, sooner or later, they will stop reacting to a repeatedly presented stimulus.

In certain output channels the reaction to a few known interneurons can be inferred. In the case of the influence of illumination of the front and back eye rim areas, the participation of the four interneurons giving the sustaining discharges is likely. But since these same areas are also read by dimming fibers (a type that gives the inverse response from sustaining fibers), either might be the sole or the second source.

To decide about these matters, it would be necessary to exclude one or the other fiber type from reacting, which is technically impossible. It is therefore of interest that, on the basis of the known properties of still another class of optic interneurons, the space-constant fibers, such a relationship can be inferred with much greater confidence.

In the crayfish and rock lobster there are some fibers in each of the main fiber classes which are not considered real members of those classes because they have an additional feature. The visual field of these neurons is dependent upon the activity of the statocyst fibers. In the crayfish a space-constant sustaining fiber is present which, in the normal body and eye position, has a visual field that is limited to the inner upper half of the retina. The receptive field changes both its size and location, however, when the eye looks in a different vertical direction. The location of the field turns in a direction opposite to that of the animal when he is rotated head over heels (below).

When the animal is rotated around its long axis, however, it is the field size that changes from half-moon shape to full, quarter, and new moon, depending on how much of the circle of ommatidia is above



The visual field (shown in black) of a space-constant sustaining fiber changes with rotation of the crayfish along its longitudinal axis (top of diagram) and along its transverse axis (bottom).

and below the true horizon. In most other instances of space constancy, the maximum field, rather than being restricted to the middle part of the eye, covers nearly the total surface.

This relationship exists for two movement fibers found in the crayfish and for all four types of space-constant fibers found in the rock lobster. This means that the lobster's sustaining fiber, unlike that of the crayfish, does not become completely blind when the eye is turned to the ground. This is because the outer ommatidia in the eye rim are still about 5°-10° above the horizon.

In the crayfish the two space-constant movement fibers are both large and rather easily found. One has all the properties of a jittery movement fiber, whereas the other reacts strongly but with considerable habituation to quickly approaching objects. When a crayfish is highly responsive to optical input, the type of stimulus that triggers the jittery movement fibers also causes the so-called defense reflex. This reflex, which can be obtained by stimulation of a single interneuron in the commissure, consists of a raising of both claws and of the anterior part of the body, and is an expression of "aggressiveness." This fiber is a member of the group of command interneurons mentioned previously. When the animal is turned in space, this reflex is obtained only by stimulating the part of the eye within the receptive field of the space-constant jittery movement fiber. Therefore the latter is undoubtedly the most important, if not the exclusive, visual input channel for the reflex. Similarly, it appears that the fast space-constant movement fiber is the main trigger for activating the flight reflex, by which the animal "escapes" by swimming backward. This reflex is mediated by the two medial giant fibers, which originate in the brain. So long as the animal is in its normal position, the approach must be in the dorsal eye part. But when the crayfish sleeps (lying on one side), the reflex is also elicited by an object quickly approaching the lower eye half, which will now trigger the space-constant fiber.

Some years ago the prediction was made that if the functioning of any central nervous system is ever to be understood, that of the crayfish might well be the first. At present, though this goal has certainly not been reached, there is reason to remain optimistic. It may be significant that a viewpoint developed at Caltech over the years, as a result of our experimental findings, is closely akin to that of Konrad Lorenz's ideas about the relationships between different behavioral acts.

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Briefly, our viewpoint is that the central nervous system has a very democratic type of organization, in which at many places "yes" and "no" as well as "perhaps" votes are continuously cast. The results of these polls are expressed as an activation of certain command fibers, which will determine the actual performance. But in order that a coordinated act will follow, it is necessary that inhibition suppress all those effects which are incompatible. This can result from the additional activation of inhibitory central fibers which are known to be present. Peripheral mechanisms are also able to negate or change these central commands, up to the very last voting booths, by way of neuromuscular inhibitory nerve fibers. The one command which appears to be the least changeable at lower levels is the one for the flight reflex, which from a functional point of view is understandable. Associated with this supremacy is a "high" threshold, such that the occurrence of this command needs many more "yes" votes, and these in a tight temporal cluster, than do the more modifiable ones.

For the future it will be important to gather more and more information about the relationships between the different known subsystems and to complete as much as possible the survey of reactive neuron types. We can now implant electrodes which can record signals from a single known unit for days on end. This technique may materially contribute to our understanding of what is going on. So far it appears that for short-term experimentation the results obtained with these free-moving animals check well with those obtained previously. However, not only will we find out what changes in responsiveness take place, but it will also be easier to assign possible functional relationships between the activity of a given interneuron and the behavior of the animal. We have thus collected over the years a considerable number of the bits and pieces, which makes it possible in some instances to see how they fit together. But whether continuation along these lines will eventually solve all of the problems of how this central nervous system computes its many output reactions is unpredictable. □