Analysis of Restricted Neural Networks

Connections between identified nerve cells are studied anatomically and physiologically in invertebrates.

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In an article that appeared in *Science* in 1959 (*1*), T. H. Bullock described the events of the preceding decade in neurophysiology as a "quiet revolution." His account emphasized the contributions that came from the application of microelectrode techniques to peripheral and central neurons and summarized the kinds of activity found in excitable cells. To judge from the paucity of additions in the intervening years, the quiet revolution may now be about over. Further skirmishes will certainly modify what we have learned, and important activity will continue in the area of membrane mechanisms. But the student of nervous integration can list with some assurance of completeness the major ways in which nerve cells communicate with one another.

More and more, therefore, neurobiologists have begun to inquire about the arrangement and the origin of neural connections. Such connections are difficult to study in complex brains, because astronomical numbers of cells are involved in each class of operation. The assignment of function in such systems is essentially statistical: one can attribute a role to neurons of a given *type*, but not to individual cells.

Neuronal connections may, however, be precisely specified in a number of invertebrate preparations. In contrast to the brains of vertebrates, these smaller central nervous systems are composed of specific neurons that may be distinguished from all others on the basis of position, size, and connections. Such "identified cells" can be studied repeatedly, in one preparation after another, once their anatomical and physiological constancy has been established.

These cells are large, and therefore make appropriate targets for microelectrode penetration and for new anatomical methods that are selective for particular neurons. They also offer an opportunity to use biochemical techniques in parallel with electrophysiological ones, and therefore provide promising material for studies on such important topics as plasticity and learning. In this article our emphasis is on the use of such systems for interpreting the connections that underlie simple behavioral acts.

Organization of Invertebrate Ganglia

Most invertebrate central nervous systems consist of a number of cellular aggregates (ganglia) joined together by bundles of parallel neuron processes (connectives). In contrast with analogous areas of vertebrate central nervous systems, the ganglia consist of a rind of cell bodies (somata) surrounding a central core of intertwined processes, the neuropile (Fig. 1A). The somata are usually monopolar, and receive no synaptic connections. Instead, junctions are between branches within the neuropile, which form a dense feltwork of axon profiles in electron micrographs (Fig. 1B). Though some central nervous structures in arthropods have regularly stratified neuropile, that of most ganglia has a disorderly appearance (*2*).

Since the somata are distant from sites of input and from outgoing axons, they are often relatively uninvolved in the processes of excitation and transmission, though they may passively mirror distant events. Blockage of the electrical responses by hyperpolarization in such somata may leave the rest of the cell still capable of normal excitation; in some experiments, cell bodies have even been removed without physiological deficit. In lateral giant fibers of crayfish, which have been reconstructed anatomically and are well understood physiologically, the potential seen in the soma when an axonal impulse is set off is less than 5 millivolts. The soma and a substantial length of its neurite do not participate in electrical activity. Experiments in which a current-passing microelectrode is used to depolarize the soma and a second one is used to record the potential changes indicate that the soma membrane cannot even be excited electrically (*3*).

Zones of synaptic impingement and of impulse initiation are thus spread along cell processes within neuropile, instead of being arranged in some orderly relation to the soma, as they are in vertebrates. The more distal portions of the branches appear to be the areas in which presynaptic endings are especially dense. Impulses are initiated in the more proximal branches or along the main axon, by a combination of synaptic depolarization and all-or-none impulse activity on the part of certain branches (*4*). Many crayfish interneurons have branches in several ganglia, and such cells conduct impulses in both directions (*5*). Multiple sites of impulse initiation can exist within a single ganglion, and the interactions between propagated events are complex. The essentially linear array of synapses along the set of processes, and the potentiality for unitary action on the part of major branches, means that these cells are more regionally subdivided than those in the best-studied vertebrate systems (*6*).

These findings have been made by penetrating the neuron processes in neuropile with fine microelectrodes. The difficulty with this procedure is that the position of the microelectrode tip is not known, so that one can identify cells only on the basis of physiological criteria. It would be preferable to work with the large, readily identified somata on the surface of the ganglia, but many of these are, like the lateral giant soma, relatively isolated from electrical activity. Some large motor neuron somata are more involved in the excitation process: im-

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pulses occurring in branches and in the main axon invade the somata, though they are incidental to the process of reflex transmission. Subthreshold potentials correlating with synaptic activation and with the active response of distant parts of the cell may also be recorded there (*7*).

In the neurons of molluscan ganglia, the somata—though they are devoid of synapses—are usually invaded by impulses. Moreover, the view they permit of synaptic events is much better than that provided by arthropod somata. Synapses are located nearby, along the main axon or on short branches. Single impulses in presynaptic neurons often produce large synaptic potentials, and this helps in the analysis of neuronal connections. The large size of the cell bodies and their variegated pigmentation have also helped to make gastropod mollusks, in particular the sea hare *Aplysia,* favored material for work with restricted networks (*8*).

A disadvantage of these molluscan preparations for such work is that their peripheral motor systems are poorly understood. Many of the cells that have been identified by recording in ganglia send processes out through connectives, but it is not known whether they are motor neurons. Even axons that can

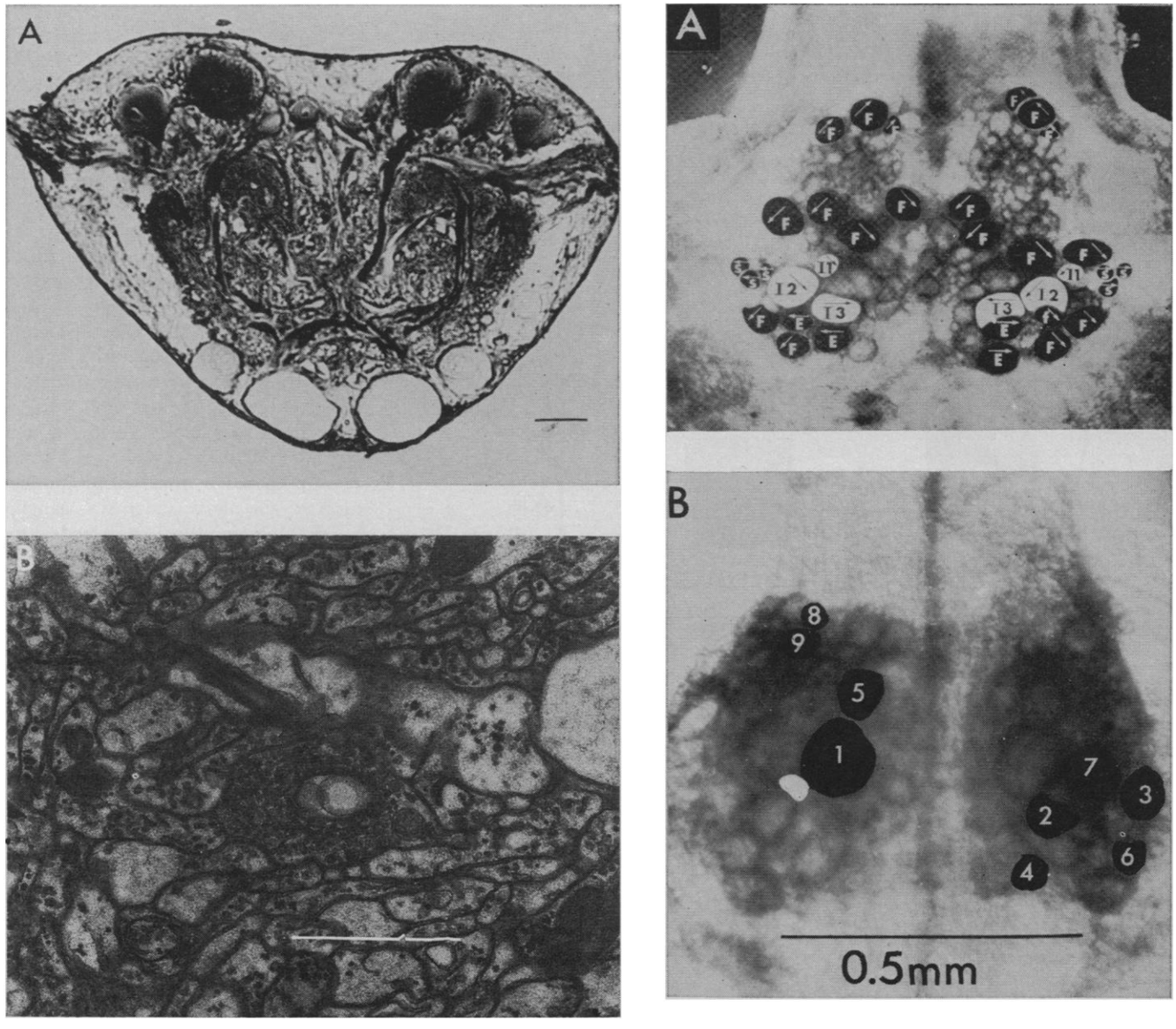

Fig. 1 (left). (A) Cross section through the third abdominal ganglion of the crayfish, ventral side uppermost (10-micron section, fixed in alcoholic Bouin's solution and stained with reduced silver; calibration, 100 microns). The somata of several large motor neurons are visible on the ventral surface, and profiles of the lateral and medial giant fibers are visible on the dorsal surface; between them is the neuropile, with several tracts of fibers crossing through it. (B) Electron micrograph of a region of neuropile in the third abdominal ganglion. (Fixed in glutaraldehyde and embedded in Epon; calibration, 1 micron.) Fig. 2 (right). Maps of the third abdominal ganglion in (A) lobster and (B) crayfish. In A (from *11*), the somata of fast flexor motor neurons are indicated (*F*); cell I_2 is the peripheral inhibitor to the fast flexors. In B, only cells supplying this group of muscles have been mapped for comparison. The cell corresponding to I_2 is indicated in white; the numbers 1 to 9 designate identified somata of the fast flexor motor neurons that exit by way of the third roots. The somata on the left cross; somata 8 and 9 supply the next anterior segment, and correspond to two of the three anterior cells in A. Soma 1 is the motor giant neuron; it and its neighbor, 5, correspond to the similarly placed but relatively smaller pair in A. The ipsilateral group is in the same general area as in A, but the distribution and size relations are quite different.

be traced to the periphery may intercept a peripheral plexus of nerves rather than innervate the muscles directly. For this reason motor neurons have not yet been identified in *Aplysia*, though connections between presumed interneurons have been worked out thoroughly.

The motor systems of arthropods, on the other hand, consist of large muscle fibers with motor nerve endings distributed densely along them. The innervation of whole muscles is sparse: most receive only two or three neurons, and many are innervated by a single axon. Thus the motor output to behavioral units can be analyzed in terms of identified cells, either by recording directly from motor nerves or by implanting electrodes in the muscles of intact, freely moving animals.

Neurogeography

In several ganglia it has been possible to examine the relationship between the position of identified somata and the connections they make. This procedure was first undertaken in the visceral ganglion of *Aplysia*, where some 30 cells and clusters have been identified and connections between many of them have been defined (*9*). The connections made by specific cells, as well as their size and position, appear to be quite constant in different individuals. Kandel and his associates have also been able to demonstrate regional differentiation with respect to important membrane properties. In *Aplysia*, certain interneurons have a double action: they depolarize and excite some postsynaptic neurons, while hyperpolarizing and inhibiting others. That the two effects are caused directly by branches of the same cell can be shown by the fixed time relationships between simultaneously recorded events in the postsynaptic cells, and by the fact that the responses can be mediated by the same transmitter substance (*10*). Cells of the left rostral quarter ganglion tend to receive inhibitory connections, and cells of the right caudal quarter ganglion tend to receive excitatory connections, from the same group of double-action interneurons (*9*). From this result it is clear that either receptor sites or membrane responses are regionally segregated.

Similar maps have been prepared for neuron somata in arthropod ganglia. The methods used differ from the primarily electrical ones used in *Aplysia*, since the cell bodies are in more distant contact with active areas in the neuropile, and are also smaller and less readily distinguished visually. Takeda and Kennedy (*7*) first identified motor neuron somata in crustacean ganglia by penetrating the cells with a microelectrode, stimulating them, and correlating efferent root impulses with the soma potentials. Otsuka, Kravitz, and Potter (*11*) prepared a much more complete map of the third abdominal ganglion of the lobster; they combined direct and indirect stimulation techniques in intact preparations with chemical analysis of the identified somata, taking advantage of the fact that excitatory and inhibitory efferent neurons produce different transmitters (Fig. 2A). Cells that proved to be inhibitory in function contained much higher amounts of γ-aminobutyric acid (the putative inhibitory transmitter) than motor neurons did. Interestingly, the inhibitory cells for three functionally unrelated muscles were found to be clustered together; this suggests that these cells might have a common developmental lineage and hence a related biochemical competence. One of us (A.S.) has recently constructed a similar map for the homologous abdominal ganglion in the crayfish (Figs. 2B and 3). Though crayfish and lobsters are in different families, they display clear homologies at the level of single identified cells: both types of giant fibers and a number of the identified motor neurons clearly correspond in the two species. The ganglionic maps, however, are not similar. The somata of the major fast abdominal flexor mo-

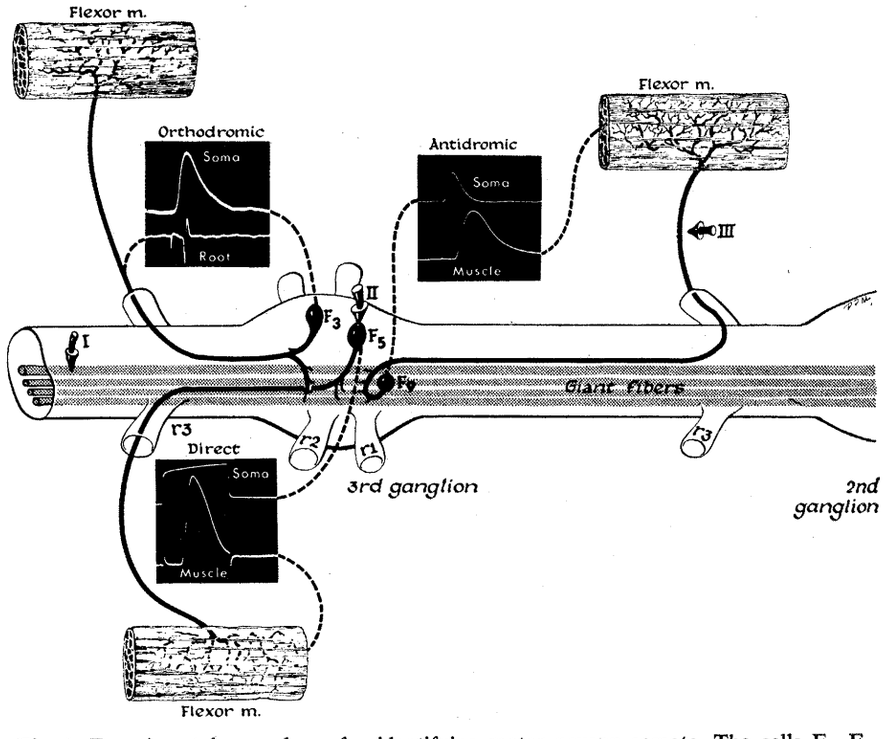

Fig. 3. Experimental procedures for identifying motor neuron somata. The cells F_3, F_5, and F_9 are shown in approximately their correct relationship. Symbols *I*, *II*, and *III* indicate sites of stimulation. Single isolated giant fibers (*I*) were sources of orthodromic excitation. Intracellular microelectrodes in motor neuron somata (*II*) were used to pass depolarizing currents for direct excitation, and suction electrodes were used on the appropriate roots (*III*) to stimulate motor neurons antidromically. The heavy dashed lines indicate the recording sites. Suction electrodes were used to record *en passant* from the roots, and intracellular microelectrodes were used to record soma potentials and postjunctional activity in the muscle fibers. Records derived by different procedures are given for the three cells. That for F_3 shows a soma potential and an efferent spike in the ipsilateral third root, resulting from a single stimulus to the giant fiber (*I*). The record for F_5 shows (upper trace) a direct depolarizing pulse delivered to the soma and recorded with a second microelectrode and (lower trace) the resulting junctional potential in the appropriate muscle, recorded simultaneously. The record for F_9 shows simultaneous responses in the muscle and soma resulting from electrical stimulation of the next anterior contralateral third root (*III*).

tor neurons occupy quite different positions in the two animals, and the size order of identified somata is drastically altered.

Maps of the positions of somata have also been made for the cockroach and for an annelid, the leech. For the cockroach, Cohen (*12*) has used an ingenious, entirely anatomical technique. When a motor axon is severed, its soma develops a perinuclear ring that can be detected by stains selective for ribonucleoprotein. This feature may be used to identify motor neuron somata, by establishing that a particular cell sends its axon out in a given peripheral nerve. The positions of the identified somata are at least as constant as those of neurons in crustacean ganglia. The leech ganglion presents the special advantage of having large sensory cells with central somata. The distribution of their peripheral processes has been determined in physiological experiments by Baylor and Nicholls (*13*), and they are of enormous potential value in experiments on the development of central connections.

In summary, the soma maps that have been constructed so far suggest that very orderly developmental processes distribute the ultimate products of the ganglion cell lineage. Soma position is relatively constant in a given species, and in bilaterally symmetrical ganglia the correspondence in position of identified "partner" neurons is quite precise. On the other hand, while relative axon size and connection pattern appear fairly stable phylogenetically, the position and size relationships of somata are not. We cannot, at this point, do more than guess what factors govern "neighbor relations" in the soma maps. Clusters based upon membrane properties and upon biochemical competence have been identified, and these undoubtedly relate to some shared aspect of the developmental history of the cluster—whether lineage or environment we cannot be sure. Whatever the rules that govern the form of soma array, they do *not* appear to be rules of connection. Neighbors may be tonic and phasic, with entirely separate inputs; they may serve reciprocally acting muscles, or unrelated ones. There is, in fact, no need to assume that the position of a soma has anything to do with the geography of its processes. Since the cell bodies are usually remote from the events of conduction and transmission, their position places few constraints upon the connections made by their outgrowths. All that can be said of somata, really, is that at one time they produced the functionally important processes to which they remain attached and for which presumably they provide metabolic support. The outcome—that the resulting structure appears to lack order—does not suggest that the connections between processes are unspecific.

Neuron Structure and Cell Constancy

Since the position of a cell body does not specify its connections, maps defining soma arrangement are obviously of limited usefulness. One needs maps of entire neurons, but they must be *specific* neurons, and the classical anatomical techniques lack the required selectivity. Some beginnings have been made with the conventional methods of silver staining and serial reconstruction. By following processes from previously mapped somata, connections between specific cells have been identified in crayfish (*7*) and in *Aplysia* (*14*), and a comparison of the structure of major branches in a few identified crayfish cells gave preliminary indications that their anatomical arrangement was constant (*15*).

A technique recently developed by Stretton and Kravitz (*16*) makes it feasible for the first time to map the processes of a chosen cell. The dye procion yellow can be injected iontophoretically into somata; it diffuses reasonably rapidly, stays within the cell into which it is injected, and survives fixation. It displays a readily detected fluorescence, and is yellow enough to be visible within the cell under ordinary illumination during injection. The original developers of this technique analyzed the morphology of some identified cells in the second abdominal ganglion of the lobster by plotting the position of fluorescent axon profiles in serial sections. Their reconstructions of a particular cell in different preparations supported the idea that a given unit has a precisely determined layout of major branches.

In our own experiments we have microinjected cells by way of their isolated axons. After allowing an appropriate time for diffusion, which may take several days, we fix the ganglion, clear it, and examine the whole mount in the fluorescence microscope (Fig. 4). Axonally injected cells are identified by matching filled somata with the previously constructed map. This procedure gives an excellent general picture, particularly if photographs are taken from more than one angle. The ganglion can then be embedded, serial-sectioned, and reconstructed by transposing the fluorescent profiles from each section onto a sheet of transparent plastic. If the sheets are stacked up and separated according to scale, a skilled artist can translate the resulting model into an accurate three-dimensional drawing.

With this method we have analyzed the morphology (i) of medial and lateral giant fibers in the third abdominal ganglion of crayfish, (ii) of a smaller identified interneuron in the sixth ganglion, and (iii) of all ten identified flexor efferent neurons. Our results fully support those of Stretton and Kravitz concerning the constancy of branches, and suggest several additional conclusions. First, the extent of electrical involvement of a soma varies with the length and thickness of the process connecting it to the rest of the cell. So far the somata of interneurons have turned out to be separated from axon and major branches by a long, thin process. Such an arrangement severely attenuates passively propagated axonal potentials viewed in the soma. Motor neuron somata, on the other hand, are often joined by relatively thicker and shorter processes to the rest of the cell; such somata may be invaded by impulses, and when they are adequately depolarized by means of an intracellular electrode, an impulse is propagated out along the axon. There are also differences in the ability of soma and neurite membrane in different cells to produce active electrical responses. Second, the results suggest that the complex systems of branches put out by a neuron in a given ganglion may be for the purpose of receiving input rather than of distributing output. The median giant fibers pass through the abdominal ganglia without sending off any branches whatever; yet we have shown by physiological experiments that these fibers activate most of the ten efferent neurons supplying the fast flexor muscles in each half-segment (*7*). Unless these junctions are all electrotonic, which seems unlikely in view of the absence of large areas of contact, chemical transmitter must be liberated from the apparently undifferentiated axis cylinder rather

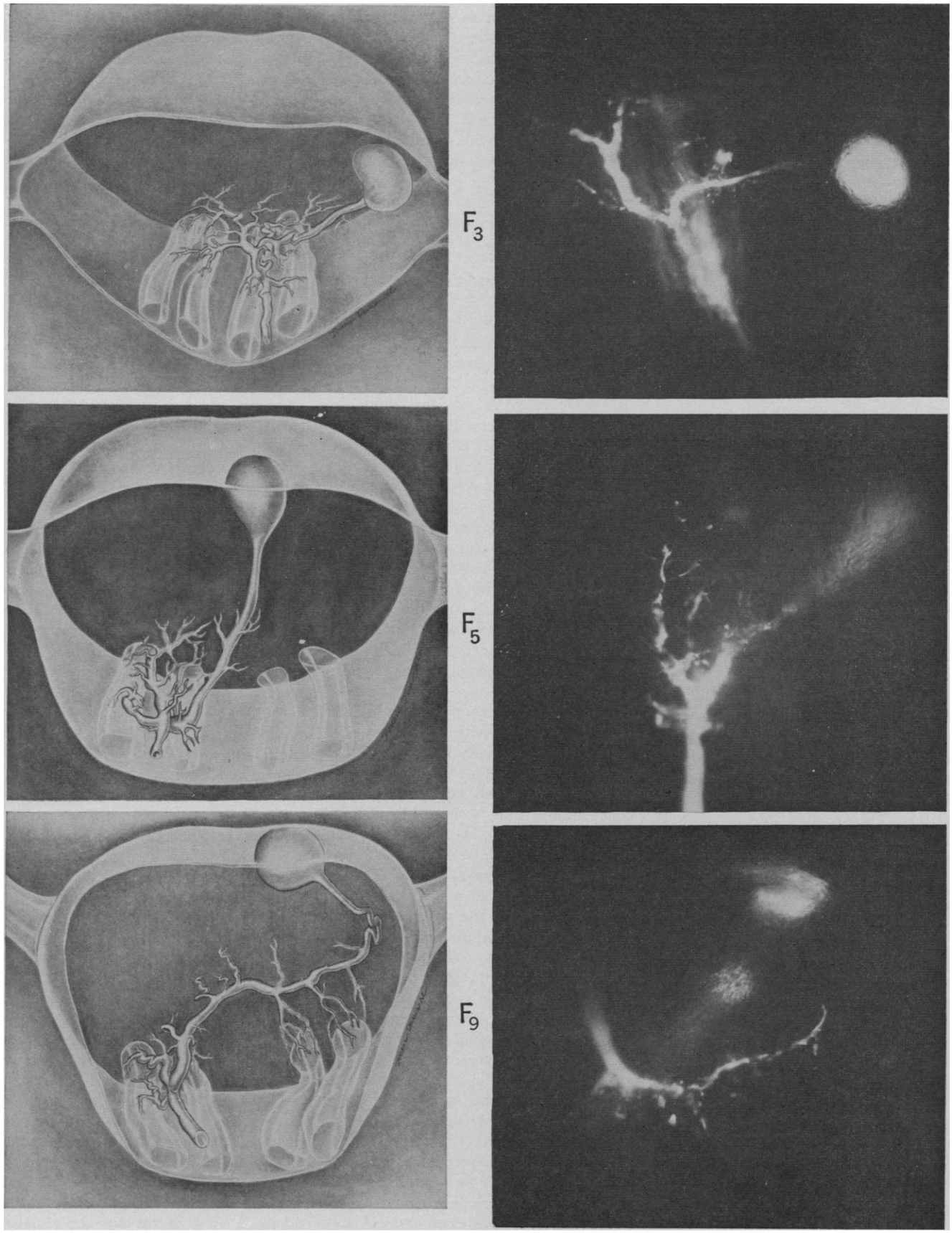

Fig. 4. Morphology of flexor motor neurons F_3, F_5, and F_9 determined by the dye-injection method. (At right) Photographs of a whole mount as viewed in the fluorescence microscope; (at left) reconstruction of the cell, made from serial sections of the same preparation (for a description of the method, see text). The orientation of the photographs and of the reconstruction is similar except in the case of F_9; for that cell, the photograph is from the dorsal surface.

than from conventional branching terminals.

Some somata are on the same side as their axonal processes, whereas the axons of others cross the midline and exit on the opposite side. Crossing is sometimes associated with physiological interaction at the point of decussation; this is true in the case of three inhibitory cells in crayfish and lobster abdominal ganglia (*11*, *17*), and in the case of the lateral giant cells (*3*). Some motor neurons and interneurons, on the other hand, cross without demonstrable interaction.

Analysis of Specific Connections

Both the lateral and medial giant fibers are presynaptic inputs to the motor neurons that innervate fast abdominal flexor muscles in the crayfish; this system is the basis for the familiar quick backward swimming used for escape. By injecting dye into the motor axons in the third root, we have been able to map the processes of all fast flexor motor neurons. The somata of these cells had already been located by intracellular stimulation and recording, as described above. The method is illustrated in Fig. 3, in which three different cells are shown. Cells F_3 and F_5 exit caudally; the latter crosses and the former does not. Cell F_9 exits rostrally, by way of the contralateral third root. The position of each axon was demonstrated by intracellular stimulation and recording from the appropriate root or muscle, or both, and by stimulation of the axon in the root while recording from the soma and the appropriate muscle simultaneously.

Each of these cells can be discharged by electrically stimulating single giant fibers that have been isolated in a distant segment (*7*, *18*). The detailed morphology of F_3, F_5, and F_9, reconstructions of which are shown in Fig. 4, suggests that they should respond differently to the two kinds of giant fibers. The reconstructions (Fig. 4, left) show that each motor neuron sends major branches to individual giant fibers, consistent with their importance as inputs. Neurons F_3 and F_9 connect with all four giant fibers, while F_5 clearly sends branches only to the homolateral lateral and medial giant fibers. If the dye fills all branches and if the only connections between the presynaptic axons and the motor neuron are direct, several physiological predictions can be made. The lateral giant fibers are cross-connected in each ganglion, so that stimulating one of them will always fire both; all three motor neurons should therefore respond to stimulation of either of the lateral giant fibers. The median giant fibers, however, have only a labile anterior cross-connection, which was eliminated in these experiments. Neurons F_3 and F_9 should therefore be activated by either medial giant fiber, whereas F_5 should be driven only by the homolateral one.

Figure 5 shows that these predictions are borne out. Simultaneous recordings from homologous somata on the two sides of the ganglion (Fig. 5, left) revealed that the F_3 and F_9 cells on both sides responded to one impulse in a single median giant; but when recordings were made in the same way from the F_5 cells, only the homolateral soma responded. Both the directness of the anatomical connections and the short latency of the soma responses suggest that the connections are monosynaptic. The lack of a response in F_5 (and in another cell that makes visible contacts only with the homolateral giant fibers) indicates that direct contact of motor neuron branches with the giant fiber is a requirement for physiological interaction. We are thus in a position to analyze synaptic contacts defined by both physiological and anatomical means.

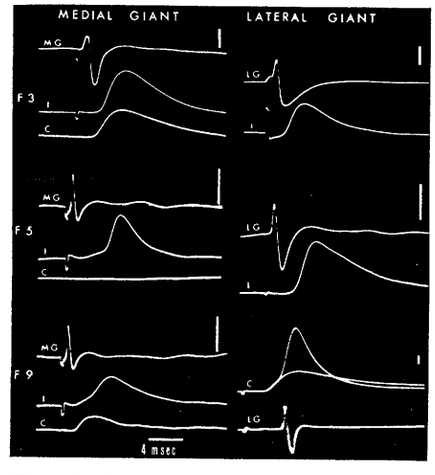

Fig. 5. Responses of identified motor neurons to giant-fiber stimulation. In each record one trace (*MG* or *LG*) is from an electrode monitoring the response of the giant fiber to the stimulus, and the other (or others) is from microelectrodes located in the ipsilateral (*I*) or contralateral (*C*) somata of (top) F_3, (middle) F_5, and (bottom) F_9. Since both lateral giant fibers are cross-connected and fire together, only the response from one cell is illustrated. In the traces at bottom right the lateral giant was stimulated repetitively at a rate of 30 stimulations per second; the superimposed sweeps illustrate the division of the soma response into two components (see *7*). In the record at lower left the response of the ipsilateral F_9 contains both components, while that of the contralateral cell contains only one. Neuron F_5 does not respond at all to the contralateral median giant, as would be predicted from the reconstruction in Fig. 4. Vertical calibration lines indicate 5 millivolts.

The dye-injection techniques obviously raise a number of hopes for the future, since they enable one to combine anatomical and physiological approaches to identified cells. For example, one should be able to find a dye that combines the property of electron density with the properties of mobility and retention, thus producing a selective stain for the electron microscopy of identified cells. The dye-injection methods can also be made more useful in light microscopy through combination with other methods. The Nauta stains for degenerating fibers have recently been applied successfully to arthropod material (*19*). It should be possible to map the distribution of degenerating terminals upon a neuron marked by dye injection in order to find out whether sensory fibers from different peripheral sources end in different regions, and to examine the physiological significance of any such localization. Finally, it should be possible to make *paired* injections of synaptically related cells, using two dyes with distinguishable fluorescence.

Physiological Evidence for Fixity of Connections

The classical anatomical methods show that the parts of a given cell occupy a given space, but they cannot describe the connections that cell makes. Wiersma and his collaborators (*20*) first showed that physiologically determined connections can be used to make unique identifications within a sizable population of neurons. By dissecting fine filaments from the central connectives in the abdomen, he made single-fiber preparations, located the unit in space, and determined its connections with incoming sensory fibers by mapping the areas on the body surface which could excite it. Wiersma identified over 100 units, each of which consistently combined a particular location,

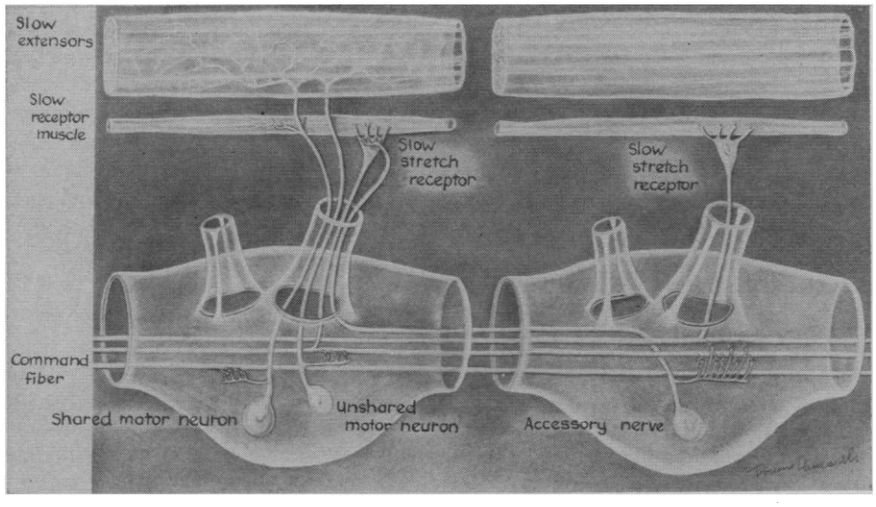

Fig. 6. Diagrammatic representation of connections among identified cells that mediate postural extension reflexes in the crayfish abdomen. The command fiber shown activates the shared motor neuron only; the stretch receptor activates the accessory nerve in its own segment of entry and in adjacent segments; the accessory nerve inhibits the stretch receptor cell peripherally (see text).

a size as approximated by the amplitude of its spike, and a set of connections as determined by the map of its receptive field. Each interneuron combined input in a unique way. One, for example, might respond to stimulation of a group of hairs on the dorsal surface of abdominal segments 4, 5, and 6; a second, to the equivalent areas on all abdominal segments; a third, to hairs on the entire ipsilateral half of the body surface; and so forth. A given sensory area was multiply represented, but each time in a unique combination with others.

At almost the same time, Arvanitaki and Tauc were finding that specific cells in *Aplysia* were constant in position and input (*8*). The number of identified cells for *Aplysia* has expanded rapidly, and connections between units can be identified by correlating impulses in one with synaptic events in a number of others. Since the cells are all large enough to be impaled with microelectrodes under visual control, several units can be examined for effects while one is stimulated. Such procedures have revealed a striking ubiquity of connections, enabling Kandel and his colleagues (*21*) to work out networks involving sizable numbers of interconnected cells.

"Command" Neurons

Just as the input connections of a nerve cell can be used to specify its uniqueness, so can its output. Some neurons in invertebrates produce widespread motor effects in the animal when stimulated. For example, single impulses in the giant fibers of crayfish activate numbers of motor neurons in each segment; a single neuron produces the entire defensive reflex (*22*); and still others trigger cyclical beating of the abdominal appendages (*23*). In the nudibranch mollusk *Tritonia*, identified cells release complex turning or swimming movements when stimulated (*24*). Such responses may result from single impulses; more commonly, a repetitive discharge is required to produce the output. Not only do interneurons control special motor actions of this kind; they appear to be basic elements in the central nervous control of all movement. In experiments on command interneurons in crayfish in our laboratory, it has been established (i) that continued activity in one cell alone is sufficient to evoke such behavior as abdominal flexion or extension, or appendage movement, the rate of movement being a function of discharge frequency; (ii) that the motor output is reciprocal—that is, while motor neurons innervating the moving muscle are active there is simultaneous inhibition of the antagonist; and (iii) that each command interneuron produces a unique spatial array of motor output, so that various geometrically different movements may be encoded by "labeled lines" (*25*).

The outputs of single command interneurons can be very complex. For example, a single cell located near the ventrolateral margin of the abdominal connectives in crayfish produces a complex, rhythmic series of movements in the appendages of the tail when the cell is stimulated at constant frequency. The behavior involves several groups of muscles: by simultaneous recording from the motor nerves that innervate these muscles, Larimer and Kennedy (*26*) have shown that the output is highly stereotyped and cyclical, with dozens of motor neurons each discharging in a specified phase of the cycle. Such rigidly patterned "motor scores" have been described for other behavior in arthropods, notably by Wilson (*27*) in the flight system of the locust. In the crayfish behavior described above the motor score is released by a single cell, with a fixed position; the output has nearly the same form in animal after animal, and it is unchanged even after total deafferentation of the preparation. As with other motor scores, the output derives its form from central connections and does not depend upon peripheral feedback.

Although few of the prominent interneurons that respond readily to sensory stimulation have any motor effects, some cells may be found that produce output and also are excited by stimulation of the animal. The motor influences produced by these interneurons resemble those produced by the interneurons' own effective input, suggesting that they are intermediate elements in the transmission of intersegmental reflexes. Comparisons of the distribution of output and input in interneurons that occupy different segments show that there is a tendency toward congruence: that is, if a cell has its strongest input in a given segment it is likely to have its most powerful output there as well (*28*). Cases have been found, however, in which neurons activated in only one ganglion by sensory stimuli have a more broadly distributed motor influence. Conversely, cells occasionally receive input in several segments but produce output in only one.

Analysis of Specific Networks

An ultimate objective of the work with restricted networks is to describe behavioral acts in terms that account for each participating unit. Among the examples that could be cited to illustrate the level of current progress are the following networks, for which such descriptions have been achieved: (i)

interconnected sets of about a dozen identified neurons in *Aplysia* (*21*); (ii) a gastropod mollusk eye that contains only five cells, each connected to the four others by way of inhibitory synapses (*29*); (iii) the four central giant fibers in crayfish and the ten phasic flexor efferents with which they synapse in each abdominal segment. Unfortunately, none of these networks has both an identified behavioral output and a known input.

A network which has both, though it presents other difficulties, is that controlling postural extensor muscles in the crayfish abdomen. These muscles are controlled by five motor neurons, and are equipped with a stretch receptor (*30*) that signals flexion of the segment. The stretch receptor neuron is associated with a muscle strand that receives efferent innervation from some of the motor neurons supplying the working extensors. When the segment is flexed, the receptor cell is excited, and connections between it and one of the motor neurons produces a reflex extension that resists the imposed change (*31*). The same afferent discharge produces an intersegmental inhibitory reflex, activating special efferent inhibitory neurons that innervate the stretch receptor and serve to reduce its firing rate (*32*).

In work by Fields (*33*) and by Fields, Evoy, and Kennedy (*34*), the cells involved in these actions have been identified. These, with their connections and their relationship to some controlling elements, are shown diagrammatically in Fig. 6. The importance of the specific connections is as follows. First, the stretch receptor afferent connects *only* to a specific extensor motor neuron, which is an especially effective one: it innervates a large percentage of the muscle fibers and produces unusually large junctional potentials. It *never* innervates the receptor muscle. The result is a strong reflex action which avoids positive feedback due to receptor-cell reexcitation. Second, the inhibitory reflex spreads strongly to more anterior segments. This distribution results from the facts (i) that the connections made by the stretch receptor afferents are strongest in the segment of entry, and (ii) that the efferent inhibitory neuron, the accessory nerve, exits from the ganglion anterior to that in which its soma and branches occur. The polarity of spread will produce a series of dependent changes in the conformation of anterior segments when any one segment is flexed. Third, the connections made by command interneurons select between those extensor motor neurons that innervate the working muscles alone and those that innervate the stretch receptor muscle as well. Commands of the former type do not affect the postural servomechanism. Commands of the latter type, however, produce simultaneous shortening of the receptor muscle. If the segment is shortening against a load, the contraction in the receptor muscle will lead that in the working muscles, and the receptor cell will discharge; if the segment is not loaded, the muscles will shorten together and little tension will be produced in the receptor muscle. Central excitation of these particular motor neurons thus can produce "load-compensated" movements, in which incremental motor outflow proportional to the load is generated by the reflex arrangement between the stretch receptor and the unshared motor neuron (*35*). These conclusions are all relevant to the behavioral output, and all of them depend upon knowing the connections between *specific* cells, rather than between classes of cells.

Limitations

Unfortunately, none of the animals that have been most successfully used in the work on small systems of identified neurons is favorable material for genetic study, and none has a developmental pattern that appears promising for experimental intervention. Invertebrates with large neurons often have inaccessible early developmental stages, and most of them are difficult to rear in the laboratory. While large size is an asset to the neurophysiologist, it usually is positively correlated with generation time, which provides additional obstacles for genetic analysis. Eventually, it will probably be necessary to correlate neurophysiological and developmental or genetic information from different organisms, by using homology; it is unlikely that we shall be relieved of this necessity by the fortuitous appearance of a neurobiologist's *Drosophila*.

Even if we were, we would have to deal with a different question: Are findings from small networks really applicable to more complex systems? It is frequently argued that connections between the much larger numbers of cells in vertebrate brains are made in an essentially probabilistic way. A corollary of this view is that there are no unique cells, and that identical functions may be performed by more than one set of elements.

One of the arguments advanced to support this view is that mammals sometimes appear to suffer the loss of substantial parts of their brains without noticeable behavioral deficit. However, monkeys or people that have been subjected to section of the corpus callosum appear visually "normal" until they are tested in an apparatus which allows them to perform independent visual-motor tasks with the two halves of their brains (*36*). With less drastic surgical procedures, exposure of the loss might require very subtle tests indeed. Even if a part of the brain could be removed without measurable effect, this would not demonstrate an original equivalence of function: parts of biological systems often exhibit regulative behavior—that is, they respond to imposed change with activities that are not a part of their repertoire in the intact mechanism.

Detailed studies of regions of the mammalian nervous system, furthermore, are revealing increasing differences among the members of neuronal populations. Such improvements in our knowledge may ultimately reveal sets of individually unique cells with determined connections, such as one finds in simpler systems. Since we cannot distinguish formally between the alleged randomness of the connections and our own ignorance about them, the issue will have to await further experimental insights.

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Atoms for Peace Awards

Six scientists are honored for their contributions in development of peaceful uses for the atom.

Robert E. Marshak

The award ceremony this year assumes a very special character. The Trustees had originally intended to take this occasion to present the last Atoms for Peace Awards to both the elder statesman of the peaceful atom, President Eisenhower, and to a relatively younger group of scientists and engineers whose recent achievements illustrate in striking and contrasting ways the peaceful uses of atomic energy. While President Eisenhower is unfortunately no longer with us, Dr. Killian's eloquent words confirm the validity of our original conception, namely to honor a group of scientists and engineers whose contributions will deeply influence the directions of research and practice for years to come in the field of atomic energy.

The selection of the six recipients of the last Atoms for Peace Award was not a simple task, and, when I complete my summary of individual contributions, I believe that it will become quite clear why the Trustees take so much satisfaction in the choices that they made. First, consider that the power and the terror and the beneficence of atomic energy all flow from the tremendous strength of the so-called nuclear force. A knowledge of the properties of the nuclear force is therefore basic to an understanding of the fission of heavy nuclei by neutrons and the fusion of light nuclei with each other.

Two Awards in Nuclear Structure Theory

It was in connection with an attempt to explain certain properties of heavy nuclei that Professors Aage Bohr and Ben Mottelson developed their famous unified collective nuclear model. The unified collective model of the nucleus reconciles in a beautiful way the shell model, developd in 1949 by Maria Mayer and J. H. D. Jensen, and the liquid drop model, developed as early as 1936 by Niels Bohr. The idea of Aage Bohr and Ben Mottelson was to retain the essential features of the shell model for the protons and neutrons inside the nucleus but to argue that, because of the collective action of the nucleons, the surface of the nucleus behaves like that of a liquid drop. By recognizing the interplay of independent particle and collective modes of motion, Bohr and Mottelson were able to explain a wide range of nuclear phenomena, such as the intrinsic deformation of heavy nuclei and the enhancement of quadrupole transitions in such nuclei. The Bohr-Mottelson theory also explains subtleties in the fission process and in the theory of superheavy nuclei. Apart from its own intrinsic importance, the work of Aage Bohr and Ben Mottelson inaugurated a new era in nuclear structure theory which has had, and will continue to have, far-reaching consequences for our basic understanding of the processes involved in the controlled release of fission energy.

Professor Aage N. Bohr was born in Copenhagen, Denmark, in 1922 and holds a Ph.D. from the University of Copenhagen. After World War II, he spent several years at the Institute for Advanced Study in Princeton and at Columbia University before returning to the Institute for Theoretical Physics of which his father, Niels Bohr, was director. In 1963, Aage Bohr succeeded his father as director of this institute,

The author, Distinguished University Professor of Physics at the University of Rochester, is a trustee for the Atoms for Peace Awards. This article is the text of an address presented on 14 May 1969 at the Atoms for Peace Award ceremony in Washington, D.C.