hepatitis virus appears to be based on a single experiment in which the original investigator questioned the validity of the results (*7*). The properties of the agent which we have studied in previous experiments are that it is at least partially heat labile (*2, 3*), ether sensitive [according to data from Dr. Melnick's group (*3*)], and that it bands on density gradient ultracentrifugation in cesium chloride at a density of 1.210 ± 0.05 (*8*).

Failure of pooled human gamma globulin and serum from convalescent marmosets (*2*) to protect marmosets against experimental hepatitis has been cited as the second reason why the marmoset disease could not have been produced by human hepatitis virus (*3*). The experimental design used in our studies involves parenteral transmission, and detection of disease not by clinical but by laboratory criteria. In an analogous human situation, namely post-transfusion hepatitis and detection of both icteric and anicteric cases by serial serum enzyme follow-up, there is little evidence, if any, for protection by pooled gamma globulin. Previously infected marmosets are, however, almost solidly resistant to reinfection (*2, 3*). Susceptibility rates of marmosets in given experiments are high (*1*), and if this disease were due to a virus indigenous to marmosets one would expect more resistance to infection in randomly selected animals caught in the wild.

The third objection is based on the development of apparent hepatitis in control animals. This has not been observed in our laboratory [and at least one other (*9*)], in either inoculated or uninoculated control animals, nor have we seen the development of hepatitis following the repeated hepatic trauma of needle biopsy. The transitory elevations of serum enzyme in one of our inoculated controls, with a concurrent liver biopsy *not* showing hepatitis, stresses the importance of correlated biochemical and morphological observations in these studies.

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3 June 1969

Excitatory and Inhibitory Motoneurons in the Central Nervous System of the Leech

Abstract. *The locomotion and reflex responses of the leech are brought about by muscles that are arranged in a regular, simple pattern in the body wall and that flatten, shorten, lengthen, or bend the animal. In the segmental ganglia, it is possible to recognize by morphological and physiological criteria the individual motoneurons that cause contractions and relaxations of these muscles.*

Invertebrate preparations have been used extensively for the study of problems that at present seem too complex for analysis in the vertebrate brain [see, for example (*1, 2*)]. The medicinal leech offers particular advantages for an analysis of the integrative mechanisms that underlie purposeful movements. These accrue principally from the rigorously segmented form of the animal's body and nervous system. Each of the segments over most of the length of the worm is equipped with a similar set of muscles and a ganglion containing only about 350 nerve cells. In a relatively small population of neurons such as this, one can hope to recognize individual nerve cells, determine their function, and eventually, perhaps, establish the way in which sensory cells, interneurons, and motor cells are connected to produce coordinated movements of the animal. One might, for example, learn what cells are involved when the animal shortens in response to a noxious stimulus applied to the skin, what cells enable him to swim rhythmically, and whether inhibition of muscles occurs at the periphery.

It is already known that 14 sensory cells in each ganglion provide the animal with information about cutaneous mechanical stimuli. Their receptive fields have been mapped and their synaptic connections with each other in the central nervous system (CNS) have been traced (*2*). In this report it is shown that individual motor cells can also be identified. Each motor cell, like a sensory neuron, occupies a characteristic position in the ganglion and possesses a specific set of properties by which it can be recognized in animal

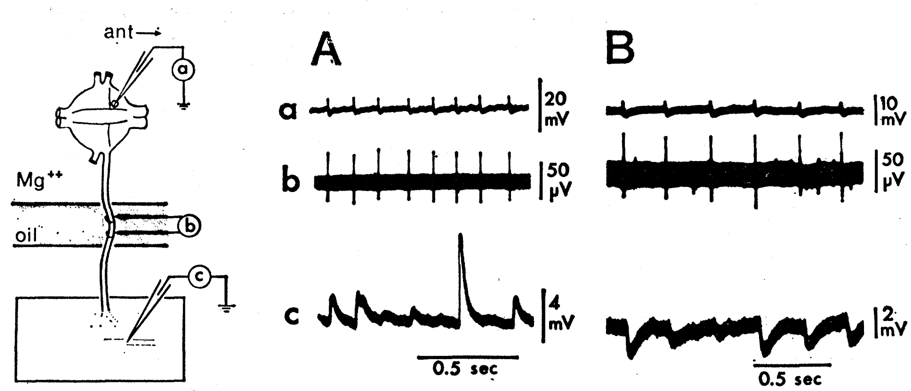

Fig. 1. Evidence that the axon of a motor cell body leaves the ganglion through a root and innervates muscle fibers. (A) Excitatory cell; (B) inhibitory cell. At left is a diagram of the experimental arrangement. The lines drawn within the ganglia in this diagram and in Fig. 2 represent the "packet" margins (see *2*) which serve as convenient landmarks for locating cells. The motor neuron is stimulated by passing a depolarizing current through the recording electrode (*a*). Each impulse in the motor cell's axon is monitored in the root (*b*). At the neuromuscular junction, each action potential in the motoneuron sets up a junction potential in the muscle fiber (*c*); *ant*, anterior.

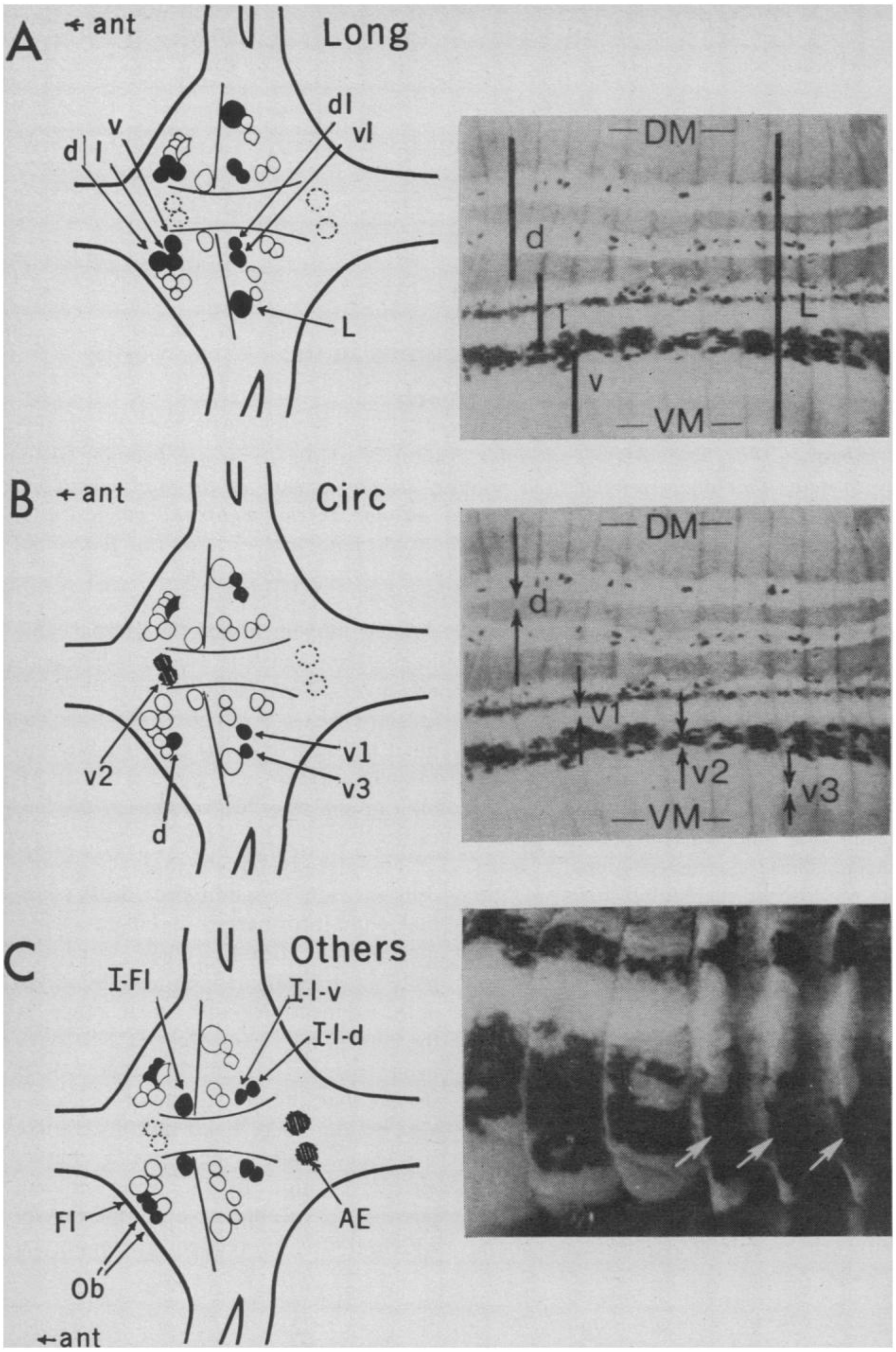

Fig. 2. Positions of motoneurons within the segmental ganglion and their fields of innervation at the periphery. (Left) In each of the three diagrams of the ganglion (dorsal aspect) the whole population of identified motoneurons has been drawn in outline, and individual cells have been inked in as follows. The cells that are outlined with dotted lines lie on the ventral side of the ganglion. (A) Cells innervating longitudinal (*Long*) muscle; *ant*, anterior. Each cell innervates fibers in a particular region of the body wall and is labeled accordingly; *d*, dorsal; *l*, lateral; *v*, ventral; *dl*, dorsolateral; and *vl*, ventrolateral. The cell labeled *L* supplies all of the longitudinal fibers on one side of the body. (B) Cells innervating circular (*Circ*) muscle (labeling as above); *v1*, *v2*, and *v3* refer to three cells with ventral territories. (C) Cells innervating oblique (*Ob*), flattener (*Fl*), and annulus erector (*AE*) musculature; also inhibitory cells causing relaxation of longitudinal muscle (*I-l-v*, *I-l-d*), and of flattener muscle fibers (*I-Fl*). (Right) (A) The approximate position in the circumferential direction of territories of motoneurons innervating longitudinal muscle. Territory width is indicated by a black stripe drawn on the skin of the body wall which has been cut to one side of the dorsal (*DM*) and ventral (*VM*) midlines and laid out flat (see text). (B) Position of territories of motoneurons innervating circular muscle. (C) Photograph of six annuli on the skin taken while the annulus erector motoneuron was being stimulated. The motoneuron caused the three annuli on the right (arrows) to be erected into ridges.

after animal. Together the 34 motor cells so far identified in each ganglion can account for the innervation of all the muscle groups in the body wall.

In preliminary experiments it was suspected that a neuron had a motor function if stimulation of its cell body caused contractions or relaxations in a group of muscle fibers. In itself, however, this is not enough to determine the function of the cell since activity in sensory cells can also cause contractions through reflex mechanisms. It was, therefore, essential to establish whether the cell being investigated was directly connected to the active muscle fibers or whether other neurons were interposed. A typical experiment is shown in Fig. 1A. The arrangement of the electrodes and the preparation are shown diagrammatically at the left of the figure. An excitatory motoneuron was impaled by a microelectrode attached to a probe that enabled current to be passed into the cell while simultaneously recording from it. To exclude the possibility of synaptic interactions in the CNS, the ganglion (but not the periphery) was bathed in 20 m*M* Mg^{++}, which blocks chemical synapses in the leech as in other preparations (*3*). The cell was stimulated by a steady depolarizing current; each action potential recorded in the cell body (trace a) was followed by an impulse in the axon of the cell (that emerges through the contralateral root) (trace b), and by an excitatory junction potential in the innervated muscle fiber (trace c). Many motor cells acted in this way, giving rise to contractions, but others, such as that shown in Fig. 1B, gave rise to inhibitory junction potentials and a visible relaxation of the muscle. It can be concluded that these cells send their axons out of the ganglion to innervate muscle fibers either directly or through an additional peripheral synapse that follows in a one-to-one manner.

Other experiments were consistent with these results; for example, stimulation of the appropriate root set up an antidromic action potential that could be recorded in the cell body. A number of the motor cells were injected through the microelectrode with a fluorescent dye, Procion Yellow M4RS (*4*), which outlined the cell processes. Such injections directly confirmed that the axon of the cell emerged through the root and make it very unlikely that the stimulated cell was driving a second cell through a synapse, either chemical or electrical, in the ganglion. Some of the motor cells supplied subcutaneous muscle fibers that were not accessible for penetration with microelectrodes. Since junction potentials could not be recorded from these fibers, contractions were observed under the dissecting microscope while the cell bodies were being stimulated in 20 m*M* Mg^{++} and recordings were being made from the roots.

The muscles that the identified motor cells supply are arranged simply in three layers in the body wall: (i) immediately under the skin a layer of circular muscles runs circumferentially; (ii) deep to this lie two layers of obliquely oriented fibers, spiraling around the animal in opposite directions; and (iii) deeper still lies a thick layer of powerful longitudinal muscle fibers. In addition to these sheets that make up the body wall, there is a group of subcutaneous fibers that raise the skin into ridges (see arrows in Fig. 2C, right) and another group of fibers which traverse the coelom from dorsum to ventrum and flatten the animal. Leech muscle fibers are similar to those of other annelids (*5*) and of crustacea in that they are innervated diffusely rather than at one discrete end-plate region. Thus, the fibers develop tension in a graded manner, depending on the level of depolarization achieved by excitatory junction potentials; muscle action potentials are observed on occasion, but they are not necessary for the development of tension.

All of the excitatory and inhibitory motoneurons that have been identified are shown in diagrams of the ganglion on the left of Fig. 2. Each motoneuron innervates a field confined to one of the muscle layers and always located in the same circumscribed position in the body wall. In Fig. 2 on the right, the positions of the fields of innervation of some of these cells are shown in relation to skin markings. One half of the body wall has been stretched out flat and photographed from the skin side; the long axis of the animal runs horizontally, as do the pigment bands, and the annuli can be seen running vertically (five annuli comprise one segment).

Each of the cells labeled with small letters in Fig. 2A, left, supplies longitudinal fibers running in a different part of the circumference of the segment. In Fig. 2A, right, black lines indicate the circumferential extent of three of these territories. The area that contracts is slightly longer than one segment, but the length of the individual muscle fibers is not yet known. Presumably these cells are involved in bending the animal up, down, or to one side.

In contrast, the large motor cell labeled *L* in Fig. 2A innervates the whole extent of the sheet of longitudinal muscle in the segment (indicated by the black line extending from the dorsal to the ventral midline in Fig. 2A, right). The excitatory junction potentials produced by cell *L* are large and give rise to a powerful shortening of the segment. In addition, this cell is connected to its homolog on the other side of the ganglion by an electrical synapse so that the impulses occur in both cells with a high degree of synchrony. One can speculate that this pair of *L* cells in every segment is used when the whole animal rapidly shortens, for example in response to a noxious stimulus applied to the skin. Since cell *L* and a cell supplying a restricted area of longitudinal muscle can both cause junction potentials in the same fiber, longitudinal fibers can receive innervation from at least two different motoneurons.

In Fig. 2B, left, are shown the cells which innervate circular muscle fibers; on the right, arrows point to the center of the territories of these cells. (The circumferential extent of these territories, as judged by watching contractions through the microscope, is about the length of the arrows; the longitudinal extent is about nine annuli.) The remaining motor cells, seen at left in Fig. 2C, innervate the oblique, flattener, and annulus erector muscles; inhibitory cells supplying the longitudinal and flattener musculature are also shown. The cell labeled *AE* causes the skin to be raised into ridges along the annuli as in Fig. 2C, right. The photograph shows one edge of a territory of an *AE* cell; the three annuli on the right (arrows) are within the territory and have erected in response to stimulation of the *AE* cell body.

The 28 excitatory cells described above appear to constitute a major fraction of the excitatory motoneurons in the ganglion. Together they supply all of the muscles, and an extensive search of the ganglion so far has failed to reveal additional cells that directly initiate muscular contraction when stimulated in the presence of Mg^{++}. At this stage one can infer which individual cells are active when, for example, the animal turns in a certain direction while lengthening and flattening his body. The role of the six inhibitory cells in the control of the muscular movements is not clear, but they may facilitate the elongation of the slowly relaxing longitudinal muscles.

Experiments can now be made to trace the synaptic connections between the sensory and motor cells within a ganglion and between adjacent ganglia. In this way one might begin to analyze the pathways by which the leech performs its limited repertoire of movements.

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21 May 1969

Visual Motion Perception: Experimental Modification

Abstract. *If a human observer fixates a moving spiral pattern for 15 minutes, a negative aftereffect of motion is perceived when he inspects a stationary spiral 20 hours later. The illusory motion is seen only when the stationary test stimulus falls upon the portion of the retina which had been stimulated by real motion. Thus previous stimulation can cause a relatively long-term modification of vision.*

In the classic aftereffect of visual motion the observer views a repetitively moving pattern for a short time, the motion of the pattern is then stopped, and an illusion of the pattern moving in the opposite direction is experienced (*1*). Under the usual conditions the aftereffect dissipates within a few seconds, and perhaps for this reason the effect was considered by 19th-century physiologists as a "motion afterimage,"