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Specific Modalities and Receptive Fields of Sensory Neurons in CNS of the Leech

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THE CENTRAL NERVOUS SYSTEM of the leech consists of a chain of discrete segmental ganglia; at either end of the animal several of these are fused together to form the larger head and tail ganglia. The segmental ganglia are strikingly similar to one another (9, 14), a reflection of the simplicity of the animal's body. Thus, apart from the specialized mouth and tail suckers, most of the worm is made up of almost identical segments (3, 16, 21). The leech therefore offers a number of advantages for the study of problems that at present seem too complex for analysis in higher animals. For example, it appears that connections within ganglia and between ganglia are made with great precision at the level of specific neurons; the neurons are relatively few in number and many are large enough to be recognized by several criteria. Thus the general problem of neuronal specificity can be studied experimentally.

In this paper, the first of two, we have made use of the repeating segmental pattern of the nervous system to investigate the sensory system and its organization and also questions regarding the specificity of neurons for certain sensory modalities. As a beginning we have attempted to answer the following questions: *1*) Do neurons that perform a particular function have the same shape, position, electrical properties, and synaptic connections in each ganglion? This is what one might expect from studies made on other invertebrate ganglia (18, 27, 33). *2*) What are the synaptic connections that underly reflex coordination between adjacent ganglia? Are they stereotyped? *3*) How constant is the field of innervation of one ganglion? Is part of each segment doubly innervated or are there sharp boundaries and no overlap between adjacent body segments (24, 28)?

It will be shown here that the pattern of cutaneous innervation is remarkably constant from segment to segment and from animal to animal. Each ganglion contains several sensory cells which can be readily identified and which are driven by different adequate stimuli applied to the skin. The receptive fields of these cells are laid out in relation to natural skin markings. Preliminary accounts of some of these observations have been published elsewhere (5, 6).

METHODS

Preparation and electrical recordings

Medicinal leeches (*Hirudo medicinalis*), which ranged in length from 5 to 10 cm when fully extended, were used. Before experiments the animals were kept at 4 C in distilled water containing about 1–10% leech Ringer solution (see below). Earlier papers have described the appearance of leech ganglia, their dissection from the animal, and the techniques used for impaling single neurons (15, 20, 26). Microelectrodes were pulled on an Industrial Science Associates micropipette puller and were filled with 3 M KCl by boiling under reduced pressure. When synaptic potentials were to be observed, the KCl was replaced by 3 M K-acetate to prevent the reversal of inhibitory postsynaptic potentials (IPSPs) that resulted from diffusion of Cl^- into the cell (unpublished observations). The microelectrodes, whose resistances ranged from 30 to 70 megohms, were connected to probes arranged in modified bridge circuits which enabled currents to be injected into cells while recordings were made of their activity (12). The time constant of the recording circuit was 80 μsec with a 50-megohm electrode. Electrical recordings of activity in nerve trunks were made with a suction electrode or with a pair of platinum wires in mineral oil.

A fuller account of the morphology of the ganglia and the methods of identifying various cells will be given below (see RESULTS). In many experi-

ments the function of a cell and its field of innervation were determined. The preparation in these cases consisted of a number of body-wall segments, connected to their ganglia by nerve roots on one side. A diagram of the dissection is shown in Fig. 1 and a photograph of the preparation in Fig. 10. The viscera were removed, and the skin and underlying muscle could then be pinned out in a chamber with a glass bottom. The ganglia were held about 1 mm away from the body wall; this made it possible to view cells in the ganglia with dark-field illumination and to record from the roots with external electrodes. The body wall and ganglia were bathed in fluid of the following ionic composition, which approximates that of the animal's own blood: (mM) NaCl, 115; KCl, 4; $CaCl_2$, 1.8; tris maleate neutralized with NaOH, 10; glucose, 10. In solutions containing high Mg^{++} (used to block synaptic transmission), NaCl was replaced by an osmotically equivalent amount of $MgSO_4$. When necessary, the composition of the bathing fluid flowing into the chamber could be changed by turning a tap while recording intracellularly. Preparations survived for 12 hr or more in Ringer fluid at room temperature (19 C). Leeches can survive for many days in Ringer fluid with no sign of change in the properties of the cutaneous afferents in the skin.

Mechanical stimuli were applied to the skin by a stylus mounted on a Leitz micromanipulator. For reproducible, light touch to activate T cells (see below) the stylus was moved by a piezoelectric element (32) driven by a stimulator. The tip of the stylus used varied with the type of experiment. For precise localization of touch receptors a broken glass microelectrode with a tip of about 20 μ was used; for stronger stimuli the tip was larger, up to to 200 μ.

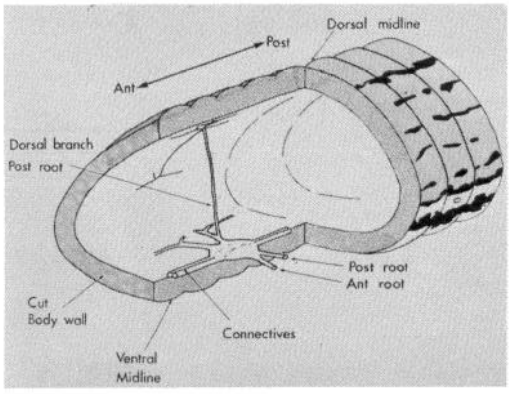

FIG. 1. Diagram of part of a leech to show relation of ganglion and nerve roots to the body wall. The preparation, when dissected, consists of skin and underlying muscle pinned out flat with the ganglion held away as shown in Fig. 10.

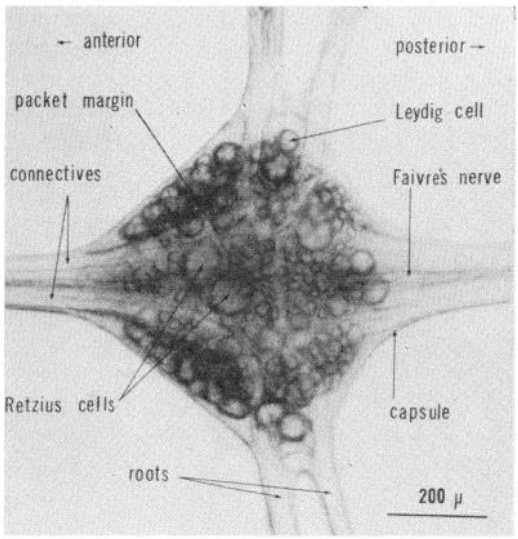

FIG. 2. Photomicrograph of a segmental ganglion seen from its ventral aspect with transmitted light. The paired connectives and Faivre's nerve run to adjacent ganglia, the roots to the body wall. Individual cells can be clearly seen; some have been labeled here and others in Fig. 3. The packet margins are defined by the boundaries of individual glial cells (see text and ref. 20).

Histology

Permanent whole mount preparations of methylene blue-stained ganglia were made, using a modification of the method described by Addison (1). Several milliliters of 0.2% methylene blue in water were injected into the gut of a leech and the animal was left overnight. The ventral nerve cord was then exposed and kept moist under a thin film of Ringer solution, allowing the blue color to "develop." Suitably stained ganglia were removed and placed in ice-cold saturated ammonium picrate for 1 hr. They were transferred without rinsing into ice-cold 8% ammonium molybdate and left for 1 hr. They were then washed for 1 hr in running tap water, dehydrated rapidly in ice-cold 90% and absolute ethanol, and placed in xylene to clear. Finally they were mounted under cover slips in mounting medium contained in a shallow chamber, the sides of which were formed from broken cover slips. Omission of the ammonium picrate caused the cell bodies to lose their stain but left certain fibers stained heavily.

RESULTS

Description of CNS of the leech

Figure 2 shows the ventral aspect of a typical ganglion, from the middle part of the animal. The principal features that one can identify through the dissecting microscope

are: *1*) The connectives, consisting of two bundles of axons that run to adjacent ganglia. Each connective contains several thousand unmyelinated axons less than 4 μ in diameter. A third more slender bundle, known as Faivre's nerve, lies between the connectives; it is seen more easily at higher magnification from the dorsal aspect. *2*) An anterior and a posterior root on each side. The axons in the roots, again unmyelinated, supply the body wall and underlying viscera. The 5th and 6th ganglia from the head send an extra nerve to the genitalia. *3*) A connective tissue capsule, containing scattered smooth muscle fibers. It surrounds the whole CNS and is covered by a layer of endothelial cells (not visible at this magnification). In situ, the CNS lies within a blood sinus; all exchange between blood and neurons or glial cells occurs by diffusion across the capsule since there are no capillaries within the CNS. *4*) The neuronal perikarya. It has been estimated that there are 350 nerve cells/ganglion (20) but the exact number is not known. There are more cells in several ganglia at each end of the nerve cord. The cells are segregated into 6 discrete groups or "packets," each of which is associated with 1 large glial cell. The margins of the packets provide convenient landmarks that help in the recognition of individual neurons. The positions of cells are relatively constant, so that with experience one can identify by inspection about 30 cells on the ventral aspect of a ganglion. Among these are the two giant Retzius cells (see Figs. 2 and 3; see also refs. 11 and 15) and the cells labeled T, P, and N in Fig. 3. The neurons are unipolar and the synaptic connections are made within a central neuropil which lies dorsally and cannot be seen in Fig. 2. There are no synaptic endings on the perikarya. *5*) Glial cells; a single glial cell occupies the space between the neuronal perikarya within a packet. Two other glial cells are associated with the neuropil and one with each connective. The spaces between neuronal and glial membranes are about 150 A wide and serve as channels for the movement of ions and small molecules (26).

Identification of individual neurons

The size, shape, and position of a nerve cell provide valuable clues and, in certain cases, unmistakable evidence for its identification. For example, the 2 giant Retzius cells can be recognized immediately in every ganglion. Other criteria for recognition are provided by the electrical properties of the cells. Thus, the 14 cells labeled T, P, and N, which are the subject of this paper, all have highly distinctive action potentials of at least 60 mv that overshoot by more than 15 mv (Fig. 3). This clearly sets them apart from the other neurons in the ganglion, for, with the exception of the Retzius cells and the paired Leydig cells near the roots, no other neurons have been found to give overshooting action potentials. Instead, the action potentials of most cells are small (5–30 mv), presumably because the cell bodies are not actively invaded by impulses. The Retzius cells and the Leydig cells cannot be confused with the T, P, or N cells because their action potentials are smaller (ca. 50 mv), longer lasting, and often show a second component which is transmitted electrotonically from activity in the contralateral neuron of the pair.

The cells with large overshooting action potentials can be further subdivided into groups T, P, and N as shown in Fig. 3 (the significance of this nomenclature will become apparent later). The cells of a group lie close to one another, have similar sizes and shapes, and exhibit identical action potentials. Thus the three T cells are smaller in diameter than the N or the P cells; they have action potentials up to 70 mv in amplitude and about 2 msec in duration; they tend to fire in bursts (see upper trace T, Fig. 3) and can discharge at 200/sec during a maintained depolarization. The two neurons labeled P are larger than both T and N cells and show still larger action potentials with a 4 msec duration (middle trace P, Fig. 3). They are silent unless stimulated. The cells labeled N have action potentials up to 100 mv in amplitude and 4 msec in duration; these impulses have even larger undershoots. The N cells tend to fire spontaneously at low frequency immediately after penetration, eventually becoming silent unless stimulated.

The action potentials of T, P, and N cells all show positive afterpotentials (i.e., hyperpolarizations) that summate with repetitive firing. The summation of positive afterpotentials may lead to hyperpolarizations of over 20 mv. During such a hyperpolarization the undershoot of the action potential becomes smaller and is followed by a slow depolariza-

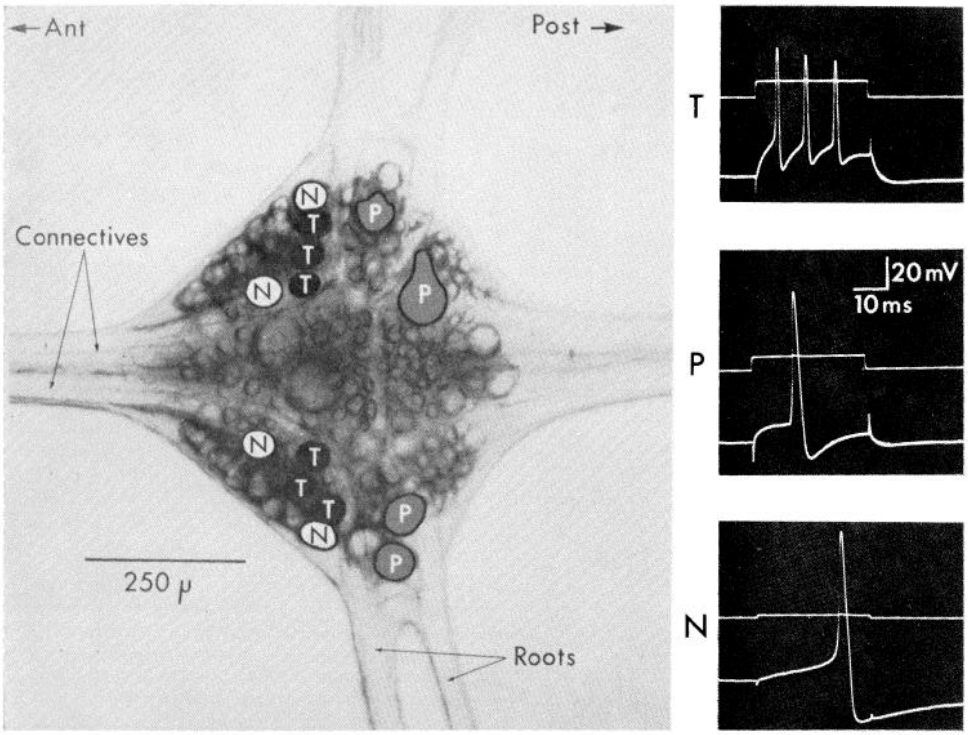

FIG. 3. Action potentials of identified cells. Photograph on the left is the same as that seen in Fig. 2 but with T, P, and N cells labeled. On the right are intracellular recordings of action potentials elicited by passing depolarizing current through the microelectrode. The T cells fire repeatedly during a maintained depolarization; the N cells fire spontaneously and have a large undershoot (see text). Current injected is monitored on the upper trace and the calibration is 5×10^{-9} amp.

tion (see, e.g., Fig. 7 below). The significance of the "afterpotential," its effects on IPSPs and impulse propagation, and its underlying ionic mechanism will be discussed in a later paper (unpublished observations).

Identification of cells stained with methylene blue

The cells classified as T, P, or N according to the criteria described above can also be recognized in ganglia stained with methylene blue. Preparations of this sort allow one to see the branches of a cell and follow their course by focusing up and down through the thickness of the ganglion.

Retzius (29) made extensive studies of the morphology of neurons in methylene blue-stained ganglia of the medicinal leech and related species. There are numerous other histological studies (e.g., Apáthy (4), Sánchez (31), Havet (17)) but his seem the most systematic and detailed. Retzius' descriptions of incoming sensory fiber arborizations, as well as nerve cells and their branching patterns, apply with remarkable fidelity to preparations made in this study. For example, in Retzius' drawing (Fig. 4*A*) the fiber labeled pf'a enters the ganglion through the anterior root, branches, and loops across the midline of the ganglion to form terminals in the neuropil contralateral to the site of entry. Another fiber labeled pf^2 enters the posterior portion of the ipsilateral neuropil. Fibers are seen which clearly correspond to these in Fig. 4*B*, a photomicrograph of one of our preparations. Except at the level of the finest terminals there is a clear correspondence between the two pictures. A peculiarity of the methylene blue technique is that the stain may be highly selective. In some preparations many cells are stained (Fig. 4*C*); in others only the two giant Retzius cells become blue.

Occasionally the dye appears selectively in three cells in the anterolateral packets of each side. That these cells are the same as the T cells and those labeled nz^5 by Retzius can be deduced from the good correspondence be-

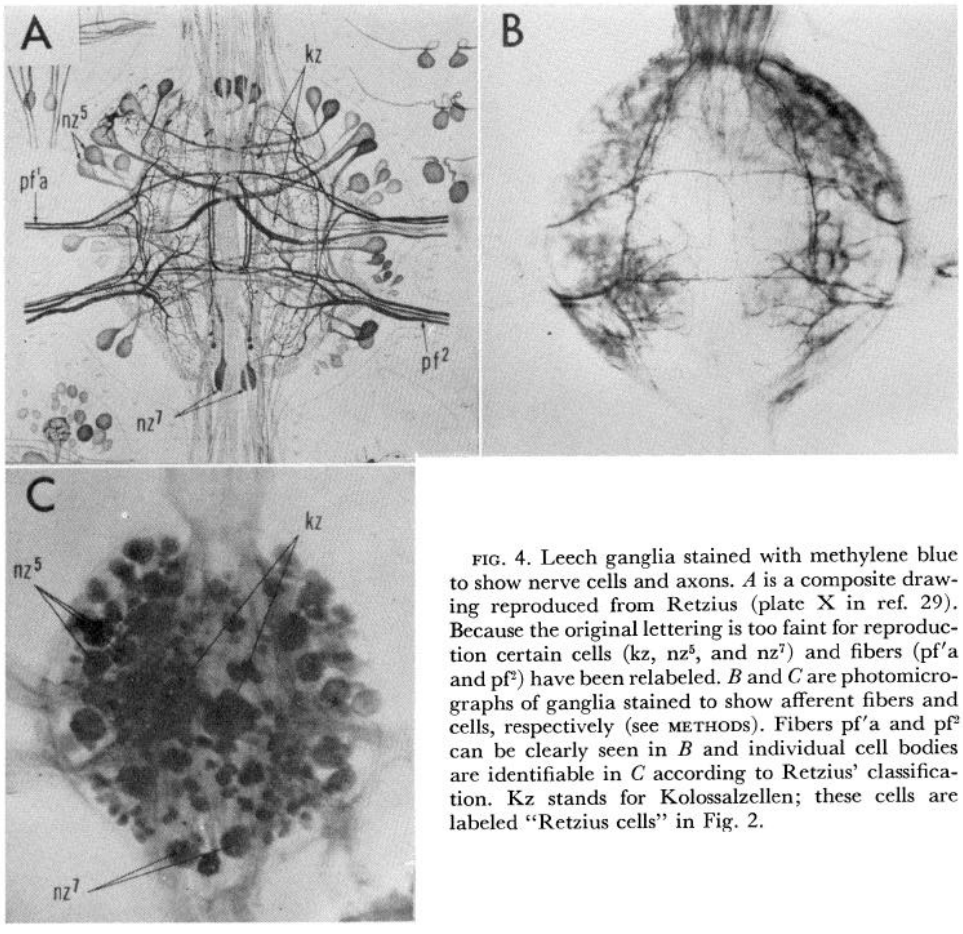

FIG. 4. Leech ganglia stained with methylene blue to show nerve cells and axons. *A* is a composite drawing reproduced from Retzius (plate X in ref. 29). Because the original lettering is too faint for reproduction certain cells (kz, nz^5, and nz^7) and fibers ($pf'a$ and pf^2) have been relabeled. *B* and *C* are photomicrographs of ganglia stained to show afferent fibers and cells, respectively (see METHODS). Fibers $pf'a$ and pf^2 can be clearly seen in *B* and individual cell bodies are identifiable in *C* according to Retzius' classification. Kz stands for Kolossalzellen; these cells are labeled "Retzius cells" in Fig. 2.

tween their shapes, sizes, and positions, as seen in Figs. 3, 4, and 5. The positive identification as T cells has been made by recording intracellularly from stained preparations, when the characteristic action potentials are observed. The correspondence with Retzius' nz^5 is also confirmed by the courses taken by their axons; unlike most other cells in the ganglion, two of the nz^5 cells send a process down both the anterior and posterior ipsilateral roots; the third cell traverses only the posterior root. In four preparations we have been able to follow axons of the three T cells and have observed the same pattern of branching as shown in Fig. 5, *right*. A minor difference between our results and those of Retzius is that in our experiments it is usually the most lateral T cell rather than the middle one that sends its process down only the posterior root. The P and N cells cannot be positively identified in Retzius' pictures, but they can be stained with methylene blue. The lateral cell of each pair sends a process to both the anterior and the posterior roots. Numerous preparations have been compared to Retzius' drawing, and the correspondence has always been close for a variety of other cells as well.

We have concluded that Retzius' drawings apply to our animals and can be used to predict connections, which can then be confirmed by mapping electrically. It will be shown later that the branching patterns of T, P, and N axons traced by this technique are in agreement with the histological findings (see Fig. 14, below).

Responses of identified neurons to cutaneous stimuli

The histological observations in the preceding section show that cells labeled T, P, and N send one or more processes down the ipsilateral roots to the periphery. Accordingly they could be either sensory or motor in function. It will

be shown here that the cells can all be driven by appropriate mechanical stimuli applied to the skin; they do not innervate muscles or glands and therefore are not motoneurons. Although when stimulated the T, P, and N cells cause muscular contractions, the movements are brought about reflexly; the responses have a long and variable latency and disappear when synaptic connections within the ganglion are blocked by high concentrations of Mg^{++} (see below).

Figure 6 illustrates the responses of each type of cell to various forms of cutaneous stimuli. The neurons labeled T fire in response to light touch of the skin surface. Brief indentations of 30 μ or less or eddies in the solution bathing the skin cause the neurons to respond. The sensory discharge to a step indentation is rapidly adapting and usually ceases within a fraction of a second. As might be expected, a tactile stimulus moving in the receptive field gives rise to a maintained discharge, whose frequency can be graded by varying the rate at which the stimulus moves over the skin or by varying the rate at which the skin is indented at a point. There was no evidence of differential sensitivity to the direction of a moving stimulus.

The cells labeled P respond only to more marked deformation of the skin. Their discharge is slowly adapting and often lasts 10–20 sec or more during maintained pressure. Again the frequency can be graded with the extent of the indentation. The mechanical threshold has not been precisely determined but it is clear that light touch is ineffective, whereas 7 g applied through a blunt stylus 200 μ in diameter is quite adequate to produce a high-frequency discharge. Compressing an annulus (see Fig. 10) with blunt forceps is also effective.

The N cells require still stronger mechanical stimuli. About 21 g applied through 200-μ-diameter stylus is more than sufficient to cause a maintained discharge, but again the threshold has not been determined precisely. The stimulus which gives the highest frequency and best maintained discharge of N cells is a radical deformation produced by pinching the skin with forceps, cutting it, or poking a pin through it. The N cells, like the P cells, are slowly adapting and often continue to fire after the stimulus has been removed.

The stimuli applied to the skin were inevitably cruder and less accurate for P and N

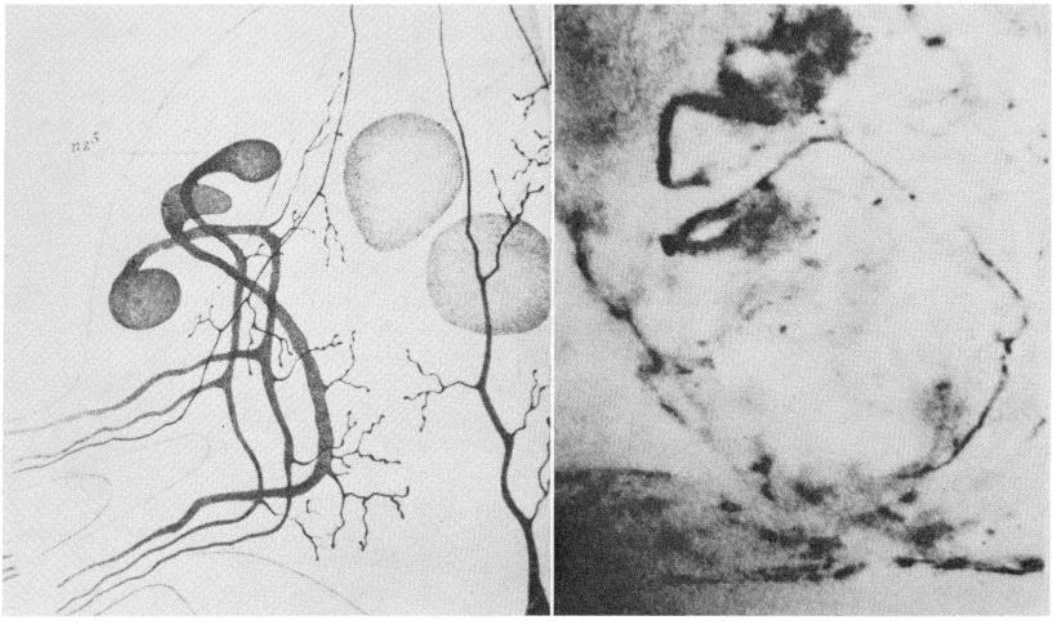

FIG. 5. Cells and axons stained with methylene blue. Retzius' picture on the left (reproduced from Fig. 2, plate 8 in ref. 29) shows the three nz^5 cells sending axons down the ipsilateral roots. The middle cell has a single axon in only the posterior root. This cell and its axon coursing to the posterior root are seen in the photomicrograph shown on the right. Because the preparation is a whole mount the focus appears blurred, and the other two nz^5 cells and their axons cannot be traced.

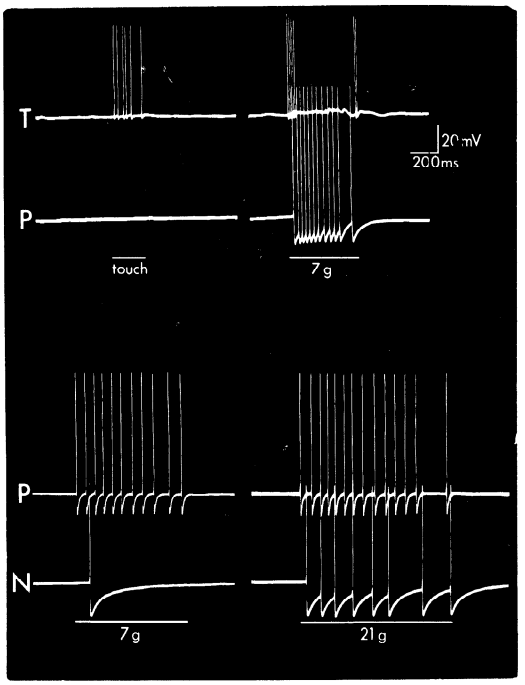

FIG. 6. Intracellular recordings from T, P, and N cells to illustrate their responses to cutaneous mechanical stimuli (see Fig. 10 for photograph of the preparation). *Upper traces*: simultaneous recordings from T and P cells during natural stimulation. On the left, touching the skin lightly (<0.5 g) caused the T cell to fire, whereas the P cell remained silent. On the right, the skin was indented by a 200-μ-diameter stylus attached to a 7-g spring; this stimulus caused a maintained discharge of the P cell and rapidly adapting discharges of the T cell at application and release of the stimulus. *Lower traces*: simultaneous records from P and N cells. On the left, the 7-g stimulus fired only the P cell (the single N cell action potential occurred "spontaneously" as part of a regular rhythm of firing). On the right, a 21-g stimulus applied in the same way fired both cells. Note that P and N cells adapt slowly, whereas the T cell adapts rapidly.

cells than for T cells. Nevertheless the differences in the threshold stimuli for these cells are consistent from one preparation to the next and can be readily detected. Thus P and N cells never respond like a T cell to a light touch, and the N cells always require a stronger stimulus than the P cells. Several other forms of stimuli have been tried and found not to fire the three groups of cells; these include *a*) changing the osmotic pressure of the fluid in contact with the skin surface from the tonicity of distilled water to that of a saturated sucrose solution, *b*) changing the pH of the fluid from 2–12, *c*) varying the skin temperature from 2 to 40 C, *d*) stretching and kneading the body-wall musculature, and *e*) changing the intensity of the light incident on the skin through a range which causes behavioral responses and a sensory discharge in the connectives leaving the ganglion. The adequate mechanical stimulus must be delivered to the skin, not to the underlying muscle and connective tissue. Thus, if the muscle and connective tissue under the skin are dissected out, preserving some of the cutaneous innervation intact, the cells still respond to mechanical stimulation of the skin. Removal of the skin from the body wall, however, abolishes all sensory responses.

All the T, P, and N cells could be driven by electrical stimuli applied to the appropriate region of the skin through the tip of a suction electrode. Usually pulses of 5 msec or less were adequate with the tip negative (approx. 5 v); electrical stimuli were more precise than mechanical for delineating fields of innervation for P and N cells. It seemed likely that terminals rather than main branches of the axon were activated by this procedure because of the congruence of fields mapped independently by the two forms of stimuli (see below Fig. 13 and ref. 25). If the main branches of the axons had been activated by the electrical stimuli, responses should have been obtained from outside the field, along the path of the nerves leading to it. No such responses could be obtained with stimuli twice as strong as those effective inside the field.

Evidence that identified cells are sensory

It is clear at this point that the three groups of neurons can be driven by specific forms of natural, cutaneous stimulation. Thus each group, defined by morphological and electrical criteria, has a specific adequate stimulus. T, P, and N, of course, refer to touch, pressure, and noxious mechanical stimuli, respectively. One must, however, establish whether these are true sensory cells, i.e., the cell bodies of axons that conduct impulses into the ganglion from the skin. Or are they driven synaptically in the neuropil by sensory neurons that lie in the periphery? To distinguish between these alternatives simultaneous recordings have been made of the incoming action potentials in the nerve roots and the impulses in the cell bodies. Figure 7 illustrates an experiment of this type for a touch cell. Light touching of the skin (lower, left) gave

rise to a series of unitary potentials in the root, each followed at short latency by an action potential in the cell body. This one-to-one correspondence is maintained at frequencies of up to 200/sec for touch cells. When the cell was stimulated directly through the microelectrode (upper, right) the same unitary potential appeared in the root but its polarity was reversed because propagation was occurring in the opposite direction. The identity of this axon as that of the touch cell has been confirmed by a "collision" experiment using precisely timed stimuli delivered by the piezoelectric element (Fig. 8). Here the orthodromic action potential set up by touch failed either to propagate or to be set up during the refractory period of an antidromic action potential produced by direct stimulation of the cell. Finally, the presence of branched axons in two of the three touch cells (see Fig. 5)

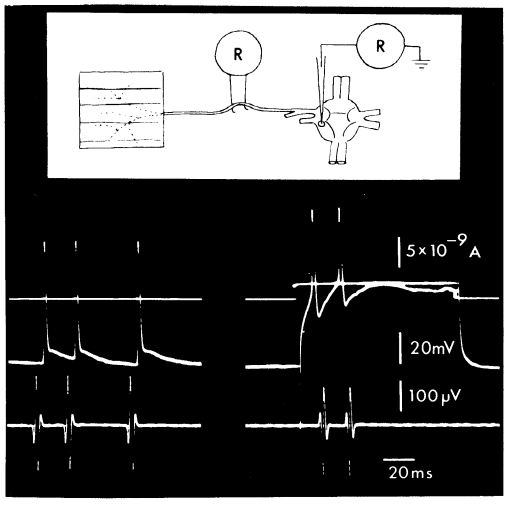

FIG. 7. Simultaneous recordings from touch cells and axons. The drawing above shows arrangement of external electrodes on the root, between the ganglion and the skin (left). In the recordings (below) the lower trace is from the root, the upper trace is from a touch cell. When natural stimuli were applied by stroking the skin gently with a stylus (left-hand series of records), an impulse in the root preceded each action potential in the cell. Note the absence of other action potentials in the root. When the cell was stimulated directly by injecting current through the microelectrode (right-hand series of records), the unitary potential that followed in the root was reversed in polarity. In the left-hand records the "undershoot" that usually follows the intracellularly recorded action potential has been reduced in size and is followed by a slow depolarization. This occurs during the hyperpolarization that follows previous repeated firing (see text).

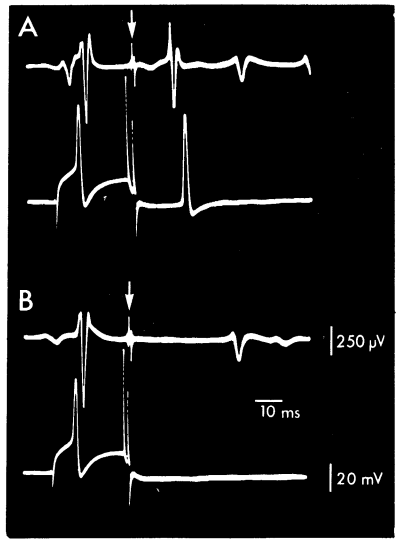

FIG. 8. Occlusion of antidromic and orthodromic impulses in the axon of a touch cell. Preparation was similar to that in Fig. 7. For precise timing and reproducibility the mechanical stimuli were delivered by a stylus attached to a piezoelectric element. External recording from the intact root was monopolar. *A*: simultaneous recordings from the root (above) and a touch cell (below). Direct stimulation of the cell with a depolarizing current pulse produced an action potential, followed by an action potential in the root. Just after the direct stimulus, the skin was touched, using the piezoelectric element (note artifact, marked by arrow); the touch set up a unitary potential in the root, followed by an impulse in the cell. *B*: same as *A*, but with interval between stimuli critically shortened. Impulses no longer appeared in response to touch, because of refractoriness; action potentials either collided in the root or failed to be set up. The result shows that the root axon which responded to touch is a process of the touch cell. Small action potentials are presumably occurring in efferent fibers.

suggests that impulses entering orthodromically through one root might emerge antidromically by an axon reflex through the other. That this is the case is shown in Fig. 9. When, however, the cell was hyperpolarized by passing current through the microelectrode, the impulse became blocked and failed to invade the other root. These are the results that one would expect if the axon of the touch cell were giving rise to the unitary potential recorded in the root. We do not yet know whether the hyperpolarization that follows repetitive firing (see above) can also block impulse transmission through the "axon reflex" pathway.

Similar results have been obtained with P and N cells. Unitary action potentials in the roots always preceded action potentials in the

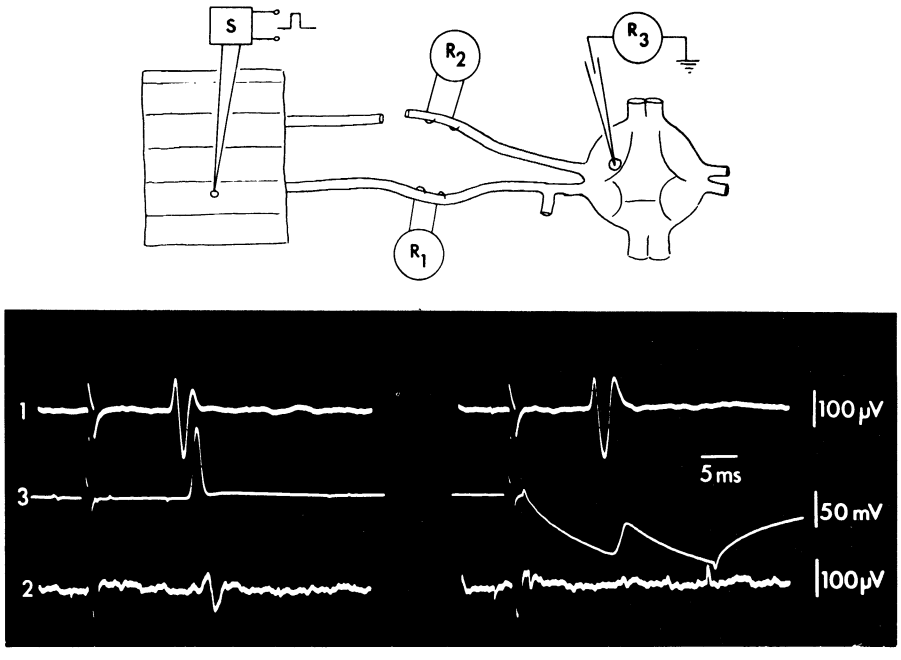

FIG. 9. "Axon reflex" in the branches of a touch cell. Diagram shows the preparation, the external and intracellular electrodes, and the piezoelectric element used to touch the skin. On left, a brief touch (note artifact) caused an action potential in the posterior root (record 1), followed by action potentials in the cell body (record 3), and anterior root (record 2). When the cell body was hyperpolarized by current injected through the microelectrode (record 3, right), the impulse failed to invade the soma actively, producing instead a local potential of longer duration. Coincident with this block, the unitary potential failed to appear in the anterior root (lower trace, 2). Note that once again a single unitary potential appears in the root in response to touch, as if only one fiber were activated by touching this area of skin. Experiment was made in 15 mM Mg^{++}, which had been applied to the whole preparation 1.5 hr earlier.

cells during cutaneous stimulation; the same unitary potentials became inverted and followed impulses initiated directly in the cell body. The fibers carrying information about pressure or noxious stimuli gave rise to smaller action potentials in the roots than those of the touch cells, presumably because they were of smaller diameter.

While these results show that the axons of the identified neurons conduct centripetally as a result of natural stimulation applied to the ipsilateral skin, they do not enable one to decide whether the cells are first- or second order sensory neurons. Thus there might be peripheral mechanoreceptor neurons whose processes form synaptic connections with the terminals of T, P, or N cells within the skin. To test for this possibility preparations have been bathed in Ringer fluid containing 20 mM of Mg^{++} for periods of up to 12 hr. This procedure blocks chemical synapses within the ganglion. The neuromuscular junctions are also blocked; stimulation of an entire root or a motoneuron no longer causes a contraction of the musculature of the body wall (A. E. Stuart, unpublished). Under these conditions the cells and their axons continue to fire in response to the adequate mechanical stimuli with no detectable change in threshold or maximum rate of firing. For example, the touch cells can continue to follow brief 30-μ indentations of the skin delivered at 200/sec. Three possibilities remain: *1*) there might be no peripheral synapse; *2*) Mg^{++} might not reach the synapse; and *3*) the synapse might be electrical, in which case it would not necessarily be affected by Mg^{++}. From the physiological evidence one can, however, conclude that the cells are sensory in function, even if they should turn out to be second-order neurons.

Receptive fields

Each of the cells has a particular region of the skin from which it can be driven—its re-

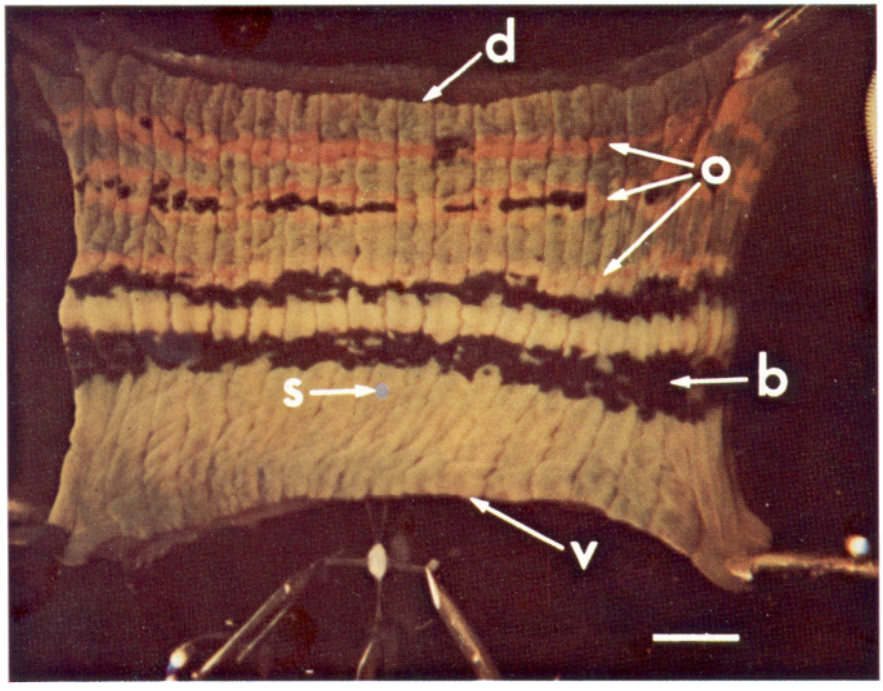

FIG. 10. Part of the body wall of the leech pinned out with a segmental ganglion attached by its roots (D, dorsal midline; v, ventral midline). Dorsal skin is dark and is marked by three longitudinal orange stripes (○). Laterally there is a black stripe (b). Annular margins run circumferentially. Central annulus is marked by a sensilla (s-retouched), in the ventral part of the skin near the ganglion. Scale, 2 mm.

ceptive field. The fields lie in a relatively constant relation to certain natural skin markings. Since the markings provide a convenient two-dimensional coordinate system on the skin surface they will be described first.

The skin of a typical segment is marked by a series of five rings, or annuli, extending around the circumference of the body (Fig. 10). Each ring has a raised center portion flanked on both sides by grooves. The ganglion is situated at the central annulus of a segment, which is marked by a series of sensillae (small spots of unknown function), one of which is prominent on each side of the ventral midline. Hence, position in the longitudinal dimension of the body wall can be defined in terms of annuli. Position in the circumference of a given annulus can be related to the longitudinally oriented pigment bands in the skin. A black stripe runs through the light-green ventrolateral skin, while three orange stripes traverse the darker dorsal skin.

The receptive fields of the three touch cells in a typical preparation are shown in Fig. 11. A single cell innervates a territory that is roughly oval in shape and consists of a part of the central annulus (vertical arrows) and three or sometimes four annuli on each side. The field of one cell therefore overlaps those of its homologues in adjacent ganglia to a variable extent. However, we have invariably found that the central annulus is innervated by only one ganglion; in general this is also true of the annulus on either side of it, but, as shown in Fig. 11, these may receive some innervation from adjacent ganglia. The three touch cells divide the skin surface into roughly equal parts around the circumference of half the body wall. The most lateral touch cell in the ganglion innervates dorsal skin, the middle cell innervates ventral skin, and the medial cell innervates lateral skin. The boundary between ventral and lateral skin fields is consistently found in the region of the lateral black stripe, while that between lateral and dorsal cells usually occurs midway between the two lateral orange stripes.

Within a receptive field there are hundreds of sensitive spots from which a touch cell can be driven by a minimal skin deflection, i.e., they have a low threshold for deformation. These spots are surrounded by regions that drive the cell only when stimulated more strongly. When using threshold deflections of

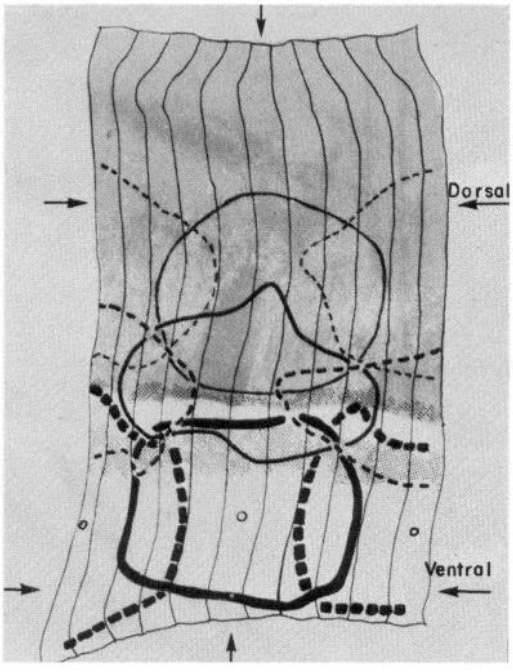

FIG. 11. Receptive fields of touch cells drawn on a tracing of the skin. Horizontal arrows indicate the dorsal and ventral midlines of the skin; vertical arrows mark the central annulus, in which the sensilla appears as a small circle. Boundaries of the receptive fields of the three touch cells are shown as solid lines of different thickness. Interrupted lines show the fields of cells in adjacent ganglia. Fields were mapped successively for the nine cells using light touch and marking the positions from which responses could be obtained on an enlarged photograph of the skin. Cells could be driven most effectively by touching the center of their fields; fewer action potentials were produced by stimulation at the edges of the fields (see Figs. 12 and 15). An annulus' width is approximately 1 mm.

a 20-μ stylus, movements of 50 μ in any direction from a spot may cause responses to disappear. The sensitivity of individual spots is similar throughout the field but the density varies with position. The distribution of the receptors within the field of a lateral touch cell is shown in Fig. 12. Individual spots were not counted; instead an estimate was made from the number of action potentials produced by traversing the skin with a stylus held in a manipulator. The stylus was moved over each annulus within the field at several different positions, with traverses in both directions (posterior to anterior and anterior to posterior). The rate of the traverse was gauged subjectively. The results were reproducible from trial to trial irrespective of direction. For example, stroking the central part of the central annulus repeatedly gave 10 impulses (SE of mean, ± 1 impulse, 6 trials); after 1 hr a similar stimulus applied at the same position once again gave rise to 10 impulses (SE of mean ± 2 impulses, 6 trials). Each point in Fig. 12 is the mean number of action potentials obtained in at least 6 traverses. The results indicate that the receptors are most abundant in the central part of the field; the density decreases gradually toward the periphery along both the longitudinal and the dorsoventral axes. Accordingly, regions of overlap receive scant innervation from each of 2 cells. We do not yet know whether the total innervation of a region of overlap is equal to or different than that of a region innervated by a single cell.

The P and N cells also have clearly circumscribed fields that are oval in shape and more or less symmetrical about the central annulus. One side of a segment of the body wall is supplied by only two cells for a given modality; the field of each cell covers roughly half the total area. Thus an individual P or N cell innervates a larger area than a T cell. The lateral N and P cells in the ganglion are driven from the ventral field, whereas the medial N and P cells are driven from the dorsal field. The extent of overlap of adjacent receptive fields is similar to that described for T cells. For example, a field usually extends longitudinally over 7 annuli but may include 8 or occasionally 9 annuli (never 10). Figure 13 illustrates a typical example of the dorsoventral overlap of the receptive fields of 2 P cells.

Fields were also determined by applying electrical stimuli to the skin through a suction electrode, as described previously. Such stimuli were more convenient than mechanical stimuli (pinching or squeezing the skin) for mapping the fields of the two pairs of neurons driven by pressure and noxious stimulation. The excellent correspondence between fields determined by mechanical and electrical stimuli (Fig. 13) suggests that sensory endings are activated in both cases. The boundaries were as sharp with the electrical as with the mechanical stimuli. Increasing the current by a factor of 2 or 3 produced no increase in the size of the field (25).

For each modality the dorsal field is innervated by the cell which sends its axon

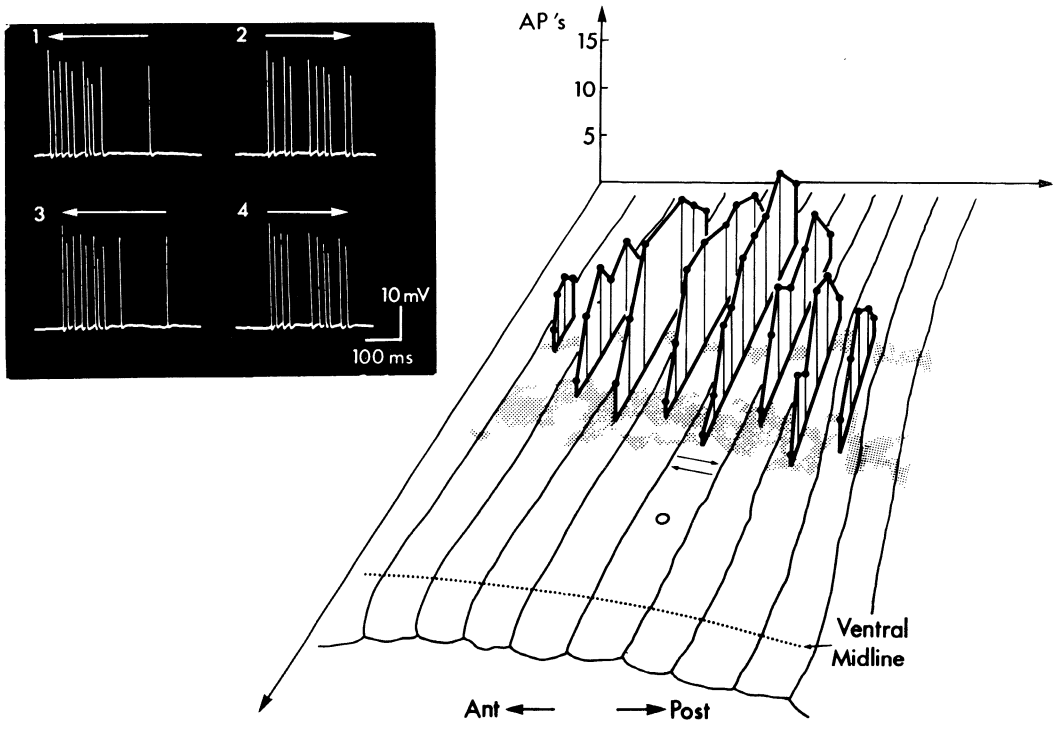

FIG. 12. Density of receptors within the receptive field of a lateral touch cell. Intracellular recordings were made (*left*) while the skin of an annulus was stroked lightly from left to right (2 and 4) and from right to left (1 and 3). Each response consisted of 10 action potentials occurring at similar intervals for one direction of stimulation. Results are plotted on the right as the mean number of action potentials evoked by stroking for each position on the skin. The number can be read off on the scale. Edges of the field give rise to fewer action potentials and presumably contain fewer endings that drive the cell, since the sensitivity of an individual spot is about the same throughout the field. An annulus' width is approximately 1 mm.

down only the posterior root. The axons of these cells leave the posterior root through a large dorsally directed trunk which winds around the gut before entering the deep surface of the body wall. The cells with the ventral and lateral fields however (two touch cells, one P, and one N cell) send processes out of both the anterior and posterior roots. These relations are shown diagrammatically in Fig. 14.

The following experiments were made to determine the contribution of the fibers in each root to the fields of the cells. First the field was mapped, next the anterior or the posterior root was cut, and finally the field was remapped. With the anterior root cut, the central annulus and the annuli anterior to it were completely denervated. Ten such experiments were made in which records were taken from the ventral and lateral touch cells. In every experiment it was found that the axons running through the posterior root innervated only annuli posterior to the central annulus. The fields supplied by axons in the anterior root were delineated after cutting the posterior root (11 experiments). In general, these fields stop at the same boundary and do not include annuli posterior to the central annulus. However, this boundary is not so constant, and the anterior axon of the lateral touch cell usually innervates small areas of one or two posterior annuli (9 of 11 experiments). Rarely (3 of 11 experiments) the anterior axon of the ventral touch cell also spreads posteriorly over one annulus. These results show that individual branches of the same cell respect one another's territory with minimal overlap. In contrast to the situation at boundaries between the fields of adjacent cells, the density of receptors does not decrease at the central edge of the field of one branch of a cell, where it abuts on the territory of another branch of the same cell. Figure 15 shows the large number of sensitive spots

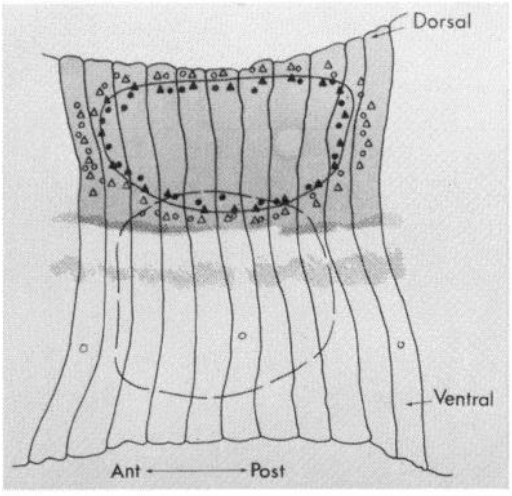

FIG. 13. Receptive fields of cells driven by pressure. Dorsal and ventral midlines are indicated by arrows and the central annulus by a sensilla. The dorsal field was defined by mechanical (●) and electrical stimuli (▲) applied to the skin. Failures to evoke an action potential are indicated by open symbols. The ventral field was mapped only by electrical stimuli. An annulus' width is approximately 1 mm.

present at the most anterior annulus supplied by the posterior branch of the ventral touch cell. It is clear that while this annulus is at the edge of the territory supplied by the posterior branch of the axon, it has far more endings in it (from the axon in question) than the other two annuli forming the posterior part of the receptive field.

There is a similar subdivision in the fields of the cells that innervate dorsal skin (laterally situated touch and pressure cells and the medial cell driven by noxious stimuli). They each send a single axon dorsally through the nerve trunk that runs around the gut (see Fig. 14). The trunk breaks up into branches as shown in Fig. 16. The posterior annuli are innervated by posterior branches, whereas the central annulus and those annuli anterior to it are innervated exclusively by the anterior branches (1, 2, 3 in Fig. 16). The finer details of the organization of the receptive fields will be described in a later paper (unpublished observations).

DISCUSSION

Functional organization of ganglion

The results show that the position, branching pattern, and function of each of a group of 14 sensory neurons in a ganglion are highly constant. Indeed any property which might influence the function of a cell as a neuron appears to be fixed. Thus each cell of a group has a characteristic set of functional properties that include its size, position in the ganglion, intracellular action potential configuration, adequate stimulus, branching pattern, and receptive field. In a later paper it will be shown that the synaptic interconnections of these cells are also highly specific and invariant (unpublished observations). One might predict, though there is not yet evidence, that the group of cells of a given sensory modality should all liberate the same transmitter substance and have identical receptors to transmitters. The cells are assigned very efficiently to accomplishing tasks, for there is no duplication of cells and relatively little overlap in the receptive fields (see below).

The specificity with which the attributes of a cell are determined is probably unique to the nervous system. In other organs, such as the liver or skin, many neighboring cells have similar or identical structures and functions. In the nervous system, however, adjacent cells that appear superficially quite similar may perform very different functions. There is no reason to think that the nervous system of the leech is unusual in the degree of its cellular specificity. Studies on other invertebrates (see 18, 27, 29, 33) and vertebrates (13, 30) in which individual cells can be identified reveal similar invariance and specificity.

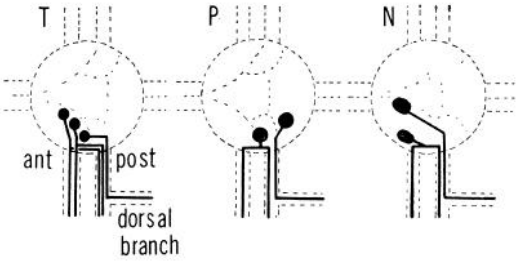

FIG. 14. Schematic diagram of the positions of T, P, and N cells and the course of their axons down the roots. For each type, one cell sends an axon solely down the dorsal nerve branch which arises from the posterior root (see Fig. 1); its ramifications are shown in Fig. 16. Pathways were determined from methylene blue-stained preparations, by recording electrically (as shown in Figs. 7, 8, and 9) and by observing the effects of cutting various roots and branches (e.g., Figs. 15 and 16).

Identified neurons as sensory elements

It is clear that the neurons described are sensory in function. They send processes into the ipsilateral roots and these axons conduct into the ganglion impulses which arise in response to natural stimuli. There are many well-known examples of invertebrate sensory neurons whose cell bodies are situated outside the CNS. However, the T, P, and N cells do not conform to the widespread notion that in the invertebrates the cell bodies of all sensory neurons are located in the periphery (see 7). Further, Alexandrowicz and Whitear (2) had proposed on anatomical grounds that certain sensory cell bodies are present within the CNS of crustacea, and there seems to be no compelling evidence against the idea that similar neurons occur in other invertebrate ganglia. It follows then, that in an invertebrate ganglion a cell body that sends a process into a peripheral nerve is not necessarily a motoneuron.

It is possible that the neurons described are not first-order sensory cells but instead are driven by primary transducing elements across a peripheral synapse not blocked by Mg^{++}. Since chemical synapses within the leech ganglion (unpublished observations), at neuromuscular junctions (22) and at numerous other sites, are known to be blocked by this ion (19), such a synapse would have to be either electrical in nature or inaccessible to Mg^{++}. Only morphological evidence can decide this question definitely. Anatomical observation should also reveal the structure of the sensory endings in the skin and perhaps show whether the differences in modality and adaptation have a structural basis.

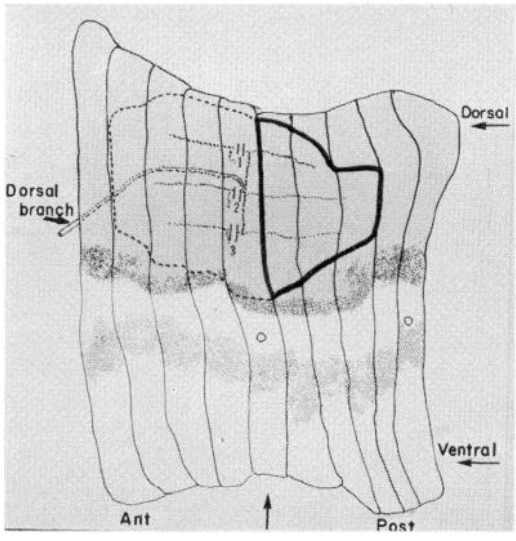

FIG. 16. Subdivision of the receptive field of a touch cell innervating dorsal skin. Horizontal arrows mark the dorsal and ventral midlines and the vertical arrows the central annulus. The dorsal branch of the posterior root (see Figs. 1 and 14) and its ramifications are shown under the skin. After cutting the anterior branches 1, 2, and 3, the field became reduced to the area indicated by the heavy line, stopping abruptly at the edge of the central annulus. This suggests that different factors regulate the distribution of sensory endings within the central region of a field and its periphery, where overlap occurs with the fields of other neurons.

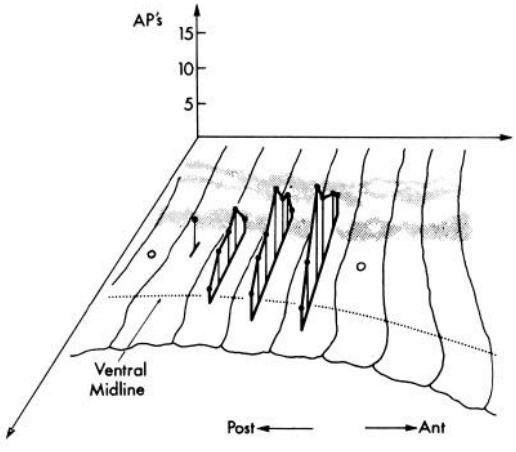

FIG. 15. Density of receptors within the posterior half of the receptive field of a ventral touch cell, determined with natural stimuli and plotted as in Fig. 12. The anterior root was cut before mapping the field. Note the boundary at the central annulus, marked by a sensilla, and the large number of action potentials generated from this region, in contrast to the numbers at the edge of the field. An annulus' width is approximately 1 mm.

The question arises whether these 14 neurons convey all the information about touch, pressure, and noxious mechanical stimuli to the segmental ganglia. Numerous fibers which presumably have cell bodies located in the periphery also provide input to the ganglion (see 29, Fig. 4). However, the modalities that these fibers subserve are not known; they might be connected to peripheral receptors activated by a variety of stimuli. Leeches have been observed to respond to changes in temperature and illumination and are strongly attracted by certain odors, including human sweat (21). On the other hand, fibers with peripheral cell bodies might also convey additional information about mechanical defor-

mation of the skin. If so, the axons must be small and their conduction velocity slow, since records taken from the whole intact roots reveal afferent impulses in only the T, P, and N fibers. Figures 7 and 8 show that any other action potentials, if present, are submerged in the noise. It can be concluded that T, P, and N neurons constitute the only rapidly conducting cutaneous mechanoreceptor input. The situation is in certain respects similar to that found in the sensory input coming from mammalian skin. Here, too, large fibers are driven by touch, pressure, and noxious stimuli; the receptors for the different modalities have different adaptation characteristics (8). In the mammal, however, we know that smaller unmyelinated fibers also conduct impulses in response to mechanical stimuli (10); at present one cannot, for the reasons given above, state whether this is the case in the leech.

Various naturally occurring stimuli might activate the different types of sensory cells. The touch cells are so sensitive that they are caused to signal by movement of water over the skin. They might play a part in the reflex regulation of swimming movements. The pressure cells would probably fire if the leech were to drag its body over a rough surface or through some narrow opening. The N cells would only be activated by far more drastic stimuli such as a bite or a crush or perhaps the suction applied by another leech. Each of the three types of stimuli gives rise to quite different reflex effects in the animal, and it will be of interest to see which motoneurons are activated reflexly by the different stimuli (see below).

Organization of receptive fields

The most striking feature of the pattern of cutaneous innervation is orderliness. Each cell innervates a clearly defined territory on the skin. This area overlaps longitudinally and dorsoventrally with the fields of cells in neighboring ganglia and in the same ganglion. The degree of overlap is variable but only within precise limits. Thus in more than 30 experiments we have not seen a central annulus to be innervated by more than 1 ganglion. In fact, the net overlap is considerably smaller than it appears from Figs. 11 and 13 because the density of innervation falls off steeply toward the edge of a field. This decrease in density of innervation suggests that the presence of innervation may in some way prevent additional nerve fibers from invading a territory and forming additional endings in it. The idea is reinforced by the finding that the outlines of the field seen in Figs. 11, 12, and 14 are oval. These observations seem analogous to those made on skeletal muscles, where motor nerve axons cannot form end plates on a muscle fiber that is already innervated, although they can do so on a denervated fiber (23).

There is a marked contrast between the type of boundary found between adjacent fields and that between subdivisions of the same field. Thus the posterior branch of a ventral or lateral touch neuron never invades the central annulus; here the innervation stops abruptly at the annular margin instead of decreasing gradually, as it does at the periphery of the field. The same has been found to be true for individual branches of the cells that innervate dorsal skin. A region with a high density of receptors (e.g., in the middle of the field) can be innervated by two different branches of the same neuron with little or no overlap. It will be shown in a later paper that the individual fields are subdivided in a highly orderly and constant manner and resemble a quilt in their organization (unpublished observations). We have at present little idea of the specific mechanisms that regulate the spatial extent of an axon in the skin. It would appear as though a fiber might "repel" other branches more strongly if they arise from the same cell than if they come from a homologue, and not at all if they come from a cell with a different modality. On the other hand, differentiations of the skin itself or the underlying tissues might play a role in specifying innervation. Whatever the mechanisms, it seems clear that they give rise to patterns of innervation correlated with the presence of pigmentation and other skin markings (such as the sensillae present in the central annulus). It should be possible to distinguish between alternative hypotheses by studying reinnervation of skin after operations. Such experiments are in progress.

The arrangement of receptive fields might allow the animal to recognize the position and direction of movement of cutaneous mechanical stimuli. We do not know how the CNS analyzes the incoming information, or the degree to which position can be discriminated

between adjacent fields or even within parts of the same field. Are different reflex effects produced by stimuli in overlap and nonoverlap regions? It would be of interest to determine the pattern of projection of the sensory neurons on each other and on higher order cells within the CNS. With the limited number of cells present it should be possible to explain aspects of the behavior of a leech in terms of the function of its sensory neurons, interneurons, and motoneurons.

SUMMARY

The cell bodies of 14 neurons that are sensory in function have been identified in the segmental ganglia of medicinal leeches. They can be distinguished from other neurons by their sizes, shapes, and positions. In addition they have distinctive electrical properties and action potentials which provide a basis for further classifying them in 3 groups.

Each of the three groups of sensory cells responds to a different mechanical stimulus applied to the skin of the ipsilateral body wall. On each side of a ganglion three cells respond to a light touch, two to maintained pressure, and two to more severe noxious stimuli such as pinching or squeezing. The time course of adaptation is characteristic for each type of cell.

The axons run to the periphery by way of ipsilateral nerve roots and conduct afferent impulses. Several lines of evidence suggest that they are either first-order sensory neurons or second-order neurons driven in a one-to-one manner by a peripheral synapse.

These 14 cells constitute the sole rapidly conducting elements for conveying information about touch, pressure, and noxious mechanical stimuli to the CNS.

The receptive fields of the 14 sensory cells are discrete and constant in size, shape, and position. Their boundaries are related to natural skin markings, so that one can, by simple inspection, locate the field of a particular neuron. The fields are overlapped at their margins by the fields of homologous cells in neighboring ganglia and in the same ganglion. Within the areas that are doubly innervated, the density of sensory endings contributed by each cell is decreased, as if some form of competition between cells were occurring.

The receptive fields are further subdivided into discrete areas innervated by the different branches of a single cell. These boundaries, unlike those between adjacent fields of different cells, are abrupt and show less overlap. Factors that might regulate the spread of sensory endings in the skin are discussed.

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REFERENCES

1. ADDISON, W. H. Neurological technique. In: *McClung's Handbook of Microscopical Technique*, edited by Ruth McClung Jones. New York: Hafner, 1950, p. 346–388.
2. ALEXANDROWICZ, J. S. AND WHITEAR, M. Receptor elements in the coxal region of decapoda crustacea. *J. Marine Biol. Assoc. U.K.* 36: 603–628 1957.
3. ALLEN, B. M. The topography of organs in typical segments of *Hirudo*. *Biol. Bull.* 3: 161–164, 1902.
4. APÁTHY, S. Das leitende Element des Nervensystems und sein topographischen Beziehungen zu den Zellen. *Mitt. Zool. Statist. Neapol.* 12: 495–748, 1897.
5. BAYLOR, D. A. AND NICHOLLS, J. G. Receptive fields, synaptic connections and regeneration patterns of sensory neurons in the C.N.S. of the leech. In: *Physiological and Biochemical Aspects* of *Neural Integration*, edited by F. D. Carlson. New York: Prentice-Hall, 1968, p. 3–16.
6. BAYLOR, D. A., NICHOLLS, J. G., AND STUART, A. E. Synaptic connexions of paired homologous neurons in the C.N.S. of the leech. *J. Physiol., London* 189: 10–11P, 1967.
7. BULLOCK, T. H. AND HORRIDGE, G. A. *Structure and Function in the Nervous Systems of Invertebrates*. San Francisco, Calif.: W. H. Freeman, 1965.
8. BURGESS, P. R. AND PERL, E. R. Myelinated afferent fibres responding specifically to noxious stimulation of the skin. *J. Physiol., London* 190: 541–562, 1967.

9. COGGESHALL, R. E. AND FAWCETT, D. W. The fine structure of the central nervous system of the leech, *Hirudo medicinalis*. *J. Neurophysiol.* 27: 229–289, 1964.
10. DOUGLAS, W. W. AND RITCHIE, J. M. Mammalian nonmyelinated nerve fibers. *Physiol. Rev.* 42: 297–334, 1962.
11. ECKERT, R. Electrical interaction of paired ganglion cells in the leech. *J. Gen. Physiol.* 46: 573–587, 1963.
12. FEIN, H. Passing current through recording glass micropipette electrodes. *IEEE Trans. Biomed. Engr.* 13: 211–212, 1966.
13. FURSHPAN, E. J. AND FURUKAWA, T. Intracellular and extracellular responses of the several regions of the Mauthner cell of goldfish. *J. Neurophysiol.* 25: 732–771, 1962.
14. GRAY, E. G. AND GUILLERY, R. W. An electron microscopical study of the ventral nerve cord of the leech. *Z. Zellforsch.* 60: 826–849, 1963.
15. HAGIWARA, S. AND MORITA, H. Electrotonic transmission between two nerve cells in the leech ganglion. *J. Neurophysiol.* 25: 721–731, 1962.
16. HARANT, H. AND GRASSÉ, P. P. Classe des Annélides achètes ou hirudinées ou sangsues. In: *Traité de Zoologie*, edited by P. P. Grassé. Paris: Masson, 1959, vol. 5, p. 471 593.
17. HAVET, J. Structure du système nerveux des annélides (Nephelis, Clepine, Hirudo, Lumbriculus, Lumbricus). *Cellule* 17: 63–136, 1900.
18. KANDEL, E. R., FRAZIER, W. T., AND COGGESHALL, R. E. Opposite synaptic actions mediated by different branches of an identifiable interneuron in *Aplysia*. *Science* 155: 346–349, 1967.
19. KATZ, B. AND MILEDI, R. A study of spontaneous miniature potentials in spinal motoneurones. *J. Physiol., London* 168: 389–422, 1963.
20. KUFFLER, S. W. AND POTTER, D. D. Glia in the leech central nervous system: physiological properties and neuron-glia relationship. *J. Neurophysiol.* 27: 290–320, 1964.
21. MANN, K. H. *Leeches (Hirudinea)*. New York: Pergamon, 1961.
22. MARTIN, A. R. Quantal nature of synaptic transmission. *Physiol. Rev.* 46: 51–66, 1965.
23. MILEDI, R. Formation of extra nerve-muscle junctions in innervated muscle. *Nature* 199: 1191–1192, 1963.
24. MILL, P. J. AND KNAPP, M. F. Efferent sensory impulses and the innervation of tactile receptors in *Allolobophora longa* ude and *Lumbricus terrestris linn. Comp. Biochem. Physiol.* 23: 263–276, 1967.
25. MOUNTCASTLE, V. B., DAVIES, P. W., AND BERMAN, A. L. Response properties of neurons of cat's somatic sensory cortex to peripheral stimuli. *J. Neurophysiol.* 20: 405–434, 1957.
26. NICHOLLS, J. G. AND KUFFLER, S. W. Extracellular space as a pathway for exchange between blood and neurons in the central nervous system of the leech: ionic composition of glial cells and neurons. *J. Neurophysiol.* 27: 645–671, 1964.
27. OTSUKA, M., KRAVITZ, E. A., AND POTTER, D. D. Physiological and chemical architecture of a lobster ganglion with particular reference to gamma-aminobutyrate and glutamate. *J. Neurophysiol.* 30: 725–752, 1967.
28. PROSSER, C. L. Impulses in the segmental nerves of the earthworm. *J. Exptl. Biol.* 12: 95–104, 1935.
29. RETZIUS, G. *Biologische Untersuchungen, Neue Folge II*. Stockholm: Sampson and Wallin, 1891.
30. ROVAINEN, C. M. Physiological and anatomical studies on large neurons of central nervous system of the sea lamprey (*Petromyzon marinus*). I. Müller and Mauthner cells. *J. Neurophysiol.* 30: 1000–1023, 1967.
31. SÁNCHEZ, D. El sistema nervioso de los herudíneos. *Trab. Lab. Invest. Biol. Univ. Madr.* 10: 1–143, 1912.
32. SATO, M. Response of Pacinian corpuscles to sinusoidal vibration. *J. Physiol., London* 159: 391–409, 1961.
33. TAUC, L. Transmission in invertebrate and vertebrate ganglia. *Physiol. Rev.* 47: 521–593, 1967.