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PHYSIOLOGICAL PROPERTIES AND RECEPTIVE FIELDS OF MECHANOSENSORY NEURONES IN THE HEAD GANGLION OF THE LEECH: COMPARISON WITH HOMOLOGOUS CELLS IN SEGMENTAL GANGLIA

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SUMMARY

A study of the head ganglion of the leech was made to compare the properties of specific sensory cells in this ganglion with those of homologous neurones in the segmental ganglia.

1. In the head ganglion, cells were identified that had electrical properties, sensory modalities and adaptation properties similar to those of touch (T), pressure (P) and nociceptive (N) cells in the segmental ganglia. The cell bodies of these neurones were situated in characteristic positions that could be correlated with those in the segmental ganglia. Several lines of evidence suggested that they were primary sensory neurones. Fewer T, P and N neurones were identified in the head ganglion than would be expected from its six constituent segmental ganglia.

2. The receptive fields of identified T, P and N cells were situated on the external surface of the head and the interior of the mouth with considerable overlap. They were generally smaller in size than those situated on the main part of the body. The receptive fields were also displaced anteriorly so that some of them were situated in segments anterior to those of the innervating cells.

3. The morphology of the sensory cells in the head ganglion was studied by intracellular injection of horseradish peroxidase. The general branching characteristics of the cells and the structural appearance of their processes resembled those of homologous cells in the segmental ganglia. However, the routes taken to the periphery by some of the cells were not constant from head ganglion to head ganglion. This variability was confirmed by electrophysiological evidence, and differed from the constancy seen in segmental sensory cells.

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4. The results indicate that sensory cells in the head ganglion resemble homologous cells in the segmental ganglia with respect to their organization and physiological properties.

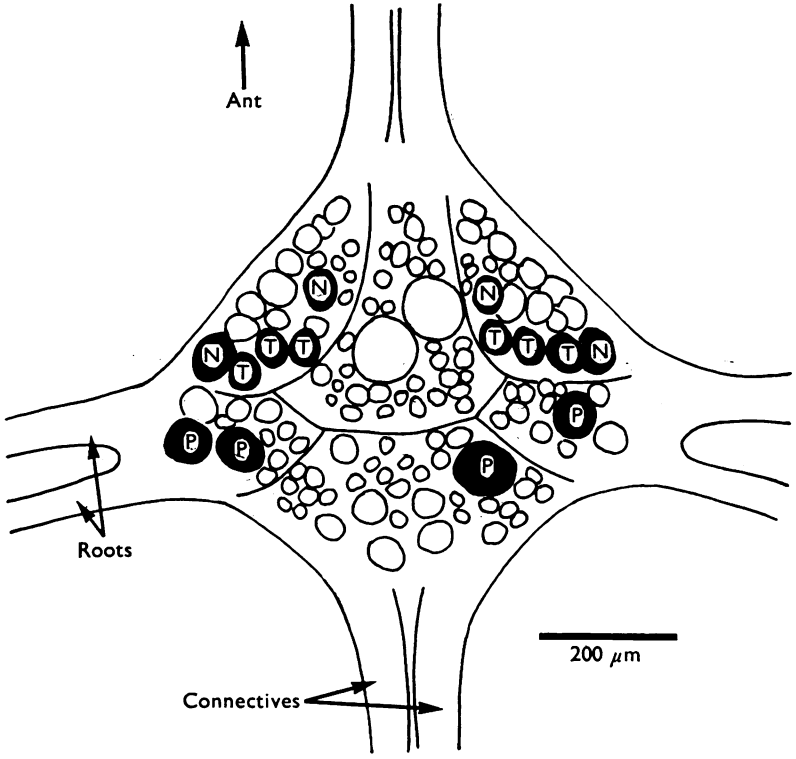
INTRODUCTION

The central nervous system of the medicinal leech, *Hirudo medicinalis*, consists of a chain of twenty-one segmental ganglia along the length of the animal and the larger head and tail ganglia at the two ends. Each segmental ganglion is bilaterally symmetrical and contains only about 350 neurones, which can be identified under the microscope and penetrated with micro-electrodes. The simple and stereotyped organization of the segmental ganglia has made them a favourable preparation for examining the basic properties of nerve cells and their integrative activities (see Nicholls & Van Essen, 1974). Individual sensory and motor neurones have been identified in these ganglia, and based on the properties of synaptic connexions between these cells it is possible to explain the neural mechanisms underlying simple reflexes such as shortening in response to cutaneous stimulation (Nicholls & Purves, 1970, 1972; Muller & Nicholls, 1974), or more complex behaviour such as swimming (Kristan, Stent & Ort, 1974*a*, *b*; Ort, Stent & Kristan, 1974).

Despite early anatomical studies (Retzius, 1891; Sánchez, 1909, 1912), the organization of the head ganglion remains largely unknown, other than that it is composed of six fused segmental ganglia (Mann, 1961). Since the head of the leech has structural specializations such as eyes, jaws and a mouth with sucker, it is of interest to determine whether the head ganglion is still organized in the same manner as the segmental ganglia. One question would be whether this ganglion contains cells with the same properties and functions as those in the segmental ganglia. In terms of development this raised the more general question of whether homologous neurones exhibit stereotyped characteristics regardless of their environment in the developing nervous system. Some evidence from electrophysiological (Kuffler & Potter, 1964; Wilson & Lent, 1973; Kleinhaus & Prichard, 1974) and histochemical studies (Marsden & Kerkut, 1969; Rude, 1969) have suggested that this might indeed be the case. Moreover, although each segmental ganglion has some degree of autonomous control over its own segment, it receives inputs from the head ganglion through the interconnecting connectives. Thus, in order to understand fully the neural basis of behaviour in the animal it will be necessary to establish the functional relationship between the segmental ganglia and the head ganglion.

An attempt has been made to identify cells in the head ganglion which are homologous to the touch, pressure and nociceptive cutaneous sensory neurones in the segmental ganglia (Nicholls & Baylor, 1968). These sensory

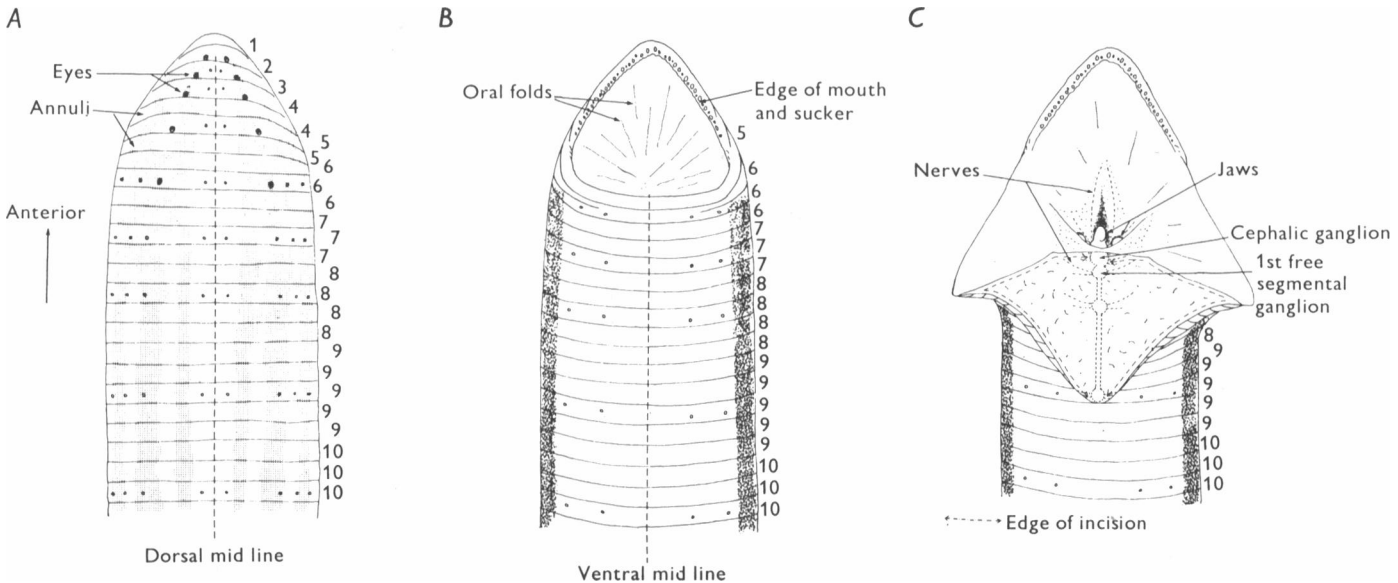
neurones have been selected for study because they are already well characterized in the segmental ganglia. For example, they have constant locations (Text-fig. 1), characteristic electrical properties and circumscribed receptive fields (Nicholls & Baylor, 1968; Baylor & Nicholls, 1969*a*, *b*; Yau, 1976). Their synaptic connexions with each other and with identified motor neurones have also been studied (Baylor & Nicholls, 1969*c*; Nicholls & Purves, 1970, 1972; Muller & Nicholls, 1974). It will be shown that such cells can indeed be identified in the head ganglion, and their receptive fields and morphology will be described.



Text-fig. 1. Drawing of a leech segmental ganglion seen from its ventral side. Typical positions of touch (T), pressure (P) and nociceptive (N) mechanosensory cells are shown. The large neurones in the centre of the ganglion are the Retzius cells. Not all cell bodies situated on the ventral side of the ganglion are drawn.

METHODS

The general experimental arrangement and recording techniques have already been described elsewhere (Nicholls & Baylor, 1968). The head ganglion was dissected out of the animal by opening both the ventral and dorsal body walls of the head;



Text-fig. 2. Segmentation and topography of the leech head. *A*, dorsal view. The number on the right side of each annulus indicates the segment to which it belongs. Note the progressive loss of annuli from segment 8 to segment 1. In segment 9 and posteriorly there are five annuli per segment. *B*, ventral view. The mouth and its internal folds are shown. The oblique orientation of the mouth obliterates the ventral aspect of segments 1 through 4. *C*, ventral view. The ventral body wall is opened to display the surface features of the mouth in more detail. The ventral nerve cord is also exposed to show the head ganglion and the segmental ganglia.

care was taken to avoid damaging the circumoesophageal ring of the ganglion. The ganglion was pinned to a shallow bath coated with Sylgard resin and viewed under darkfield illumination. Intracellular recordings were made with 4 M-K acetate electrodes of resistance 50–120 MΩ. Nerve roots were recorded from or stimulated extracellularly with either suction electrodes or paired platinum hooks in mineral oil.

In experiments to determine the function and the field of innervation of a cell, the head ganglion and the first few unfused segmental ganglia were dissected out together with the head region of the animal. Text-fig. 2 shows the segmentation on the head, the folds at the mouth and the relation of the head ganglion to other body structures. To stimulate the endings of a touch cell, a fine glass stylus (tip diameter about 50 μm) was moved either piezoelectrically or manually on the skin. The receptive fields of pressure cells were mapped using either mechanical stimulation or focal electrical stimulation applied to the skin through a blunt glass micropipette (tip diameter approximately 50 μm) filled with leech Ringer solution. Earlier work (Nicholls & Baylor, 1968) has shown that the receptive fields mapped independently by mechanical and electrical stimulation coincide. The receptive fields of nociceptive cells were mapped by squeezing the skin with fine forceps.

Almost all experiments were conducted in normal leech Ringer fluid, containing (mM): NaCl, 115; KCl, 4; $CaCl_2$, 1·8; Tris maleate (buffered to pH 7·4 with NaOH), 10; glucose, 9. For experiments conducted in Ringer containing Mg^{2+}, $MgCl_2$ was substituted for an equivalent concentration of NaCl.

Cell morphology was studied by injecting horseradish peroxidase (HRP) intracellularly. The electrodes for injection contained 1–2 % HRP (Sigma type VI) in 0·2 M-KCl solution. The injection method and histological procedures were the same as those described by Muller & McMahan (1976), except for the following modifications. (i) Before penetrations into cells the HRP-filled electrodes were bevelled against 0·3 μm corunda paper (Thomas) mounted on a rotating disk. Bevelled electrodes had low resistance (50–100 MΩ) and tips both large enough (approximately 1 μm) for pressure injection and sufficiently sharp for stable intracellular recording. (ii) Ganglia containing injected cells were usually left at 4° C in normal Ringer for about 12 hr before fixing. For studying projections of long and slender cell processes, especially those of pressure and nociceptive cells, the ganglia were left in 50–75 % hypotonic Ringer for the same period of time.

General description of head ganglion

Pl. 1 shows the ventral aspect of the head ganglion. It can be distinguished into two parts: the suboesophageal ganglion and the supra-oesophageal ganglion. The suboesophageal ganglion is formed by the fusion of ganglia in segments 3, 4, 5 and 6 (referred to as subganglia 3, 4, 5 and 6). Each subganglion still retains certain characteristics of an unfused segmental ganglion, such as the arrangement of cell packets and the possession of a pair of ventro-centrally located giant neurones, the Retzius cells (Kuffler & Potter, 1964; Wilson & Lent, 1973; Kleinhaus & Prichard, 1974). The supra-oesophageal ganglion, on the other hand, has a quite different appearance, although it is formed by the fusion of ganglia in segments 1 and 2. The circumoesophageal connectives consist of bundles of axons that run between the suboesophageal and the supra-oesophageal ganglia. There are seven nerve roots on each side of the head ganglion. Three of these roots are ventral and they all emerge from the suboesophageal ganglion; the other four roots are dorsal, of which two are from the suboesophageal ganglion, one from the supra-oesophageal ganglion and the remaining one from the circumoesophageal connective. Since an unfused segmental ganglion gives off two roots on either side, the fact that the head ganglion gives off only seven roots on each side although it is composed of six segmental ganglia suggests that

root fusion has also occurred during development. This is supported by the evidence that one or more of the roots have more than one rootlet (e.g. root VI in Text-fig. 3).

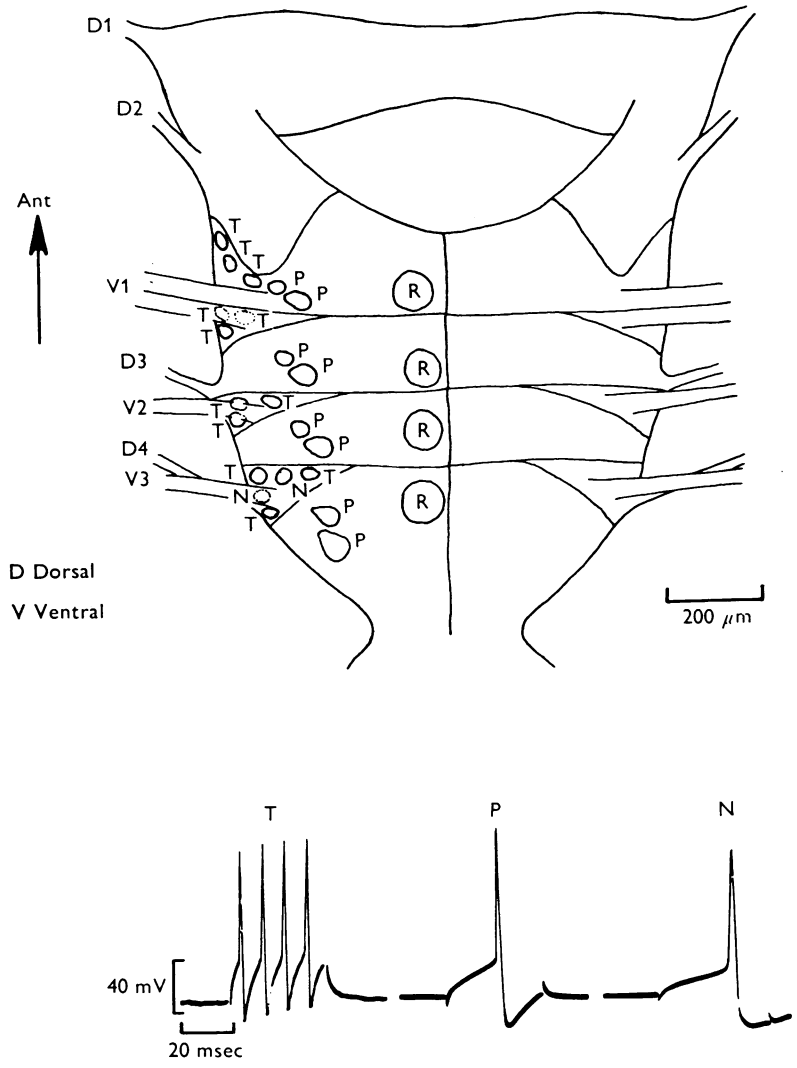
The head ganglion is almost twice as thick as the segmental ganglia. This is in part due to a comparatively larger neuropile as observed in cross-sections of the ganglion. The cell packets, however, still have a single layer of cells as in the segmental ganglia (Coggeshall & Fawcett, 1964). Although cell counts have not been made, visual examination of the ganglion has indicated that each subganglion may have about the same number of cells as a typical segmental ganglion. Individual cells, however, are smaller in size than those in the segmental ganglia.

RESULTS

Identification of mechanosensory neurones

By intracellular recording it was possible to identify those cells in the head ganglion which had electrical properties corresponding to those of segmental touch (T), pressure (P) and nociceptive (N) cells (Nicholls & Baylor, 1968). In Text-fig. 3, the identified cells have been labelled T, P or N based on this criterion. The T cells gave action potentials up to 80 mV in amplitude and about 2 msec in duration; they tended to fire in bursts and could discharge at up to 200/sec during a maintained depolarization. At rest, their membrane potentials were frequently interrupted by inhibitory synaptic potentials. The P cells gave larger (as much as 100 mV) and longer lasting (about 4 msec) action potentials than the T cells. They were silent unless stimulated, and showed delayed rectification to subthreshold depolarization. The action potentials of N cells were also about 4 msec in duration and they usually had larger undershoots than both T and P action potentials. They tended to fire spontaneously at low frequencies immediately after penetration, eventually becoming silent unless stimulated. The overshooting action potentials of these cells distinguished them from most other neurones in the head ganglion, which gave action potentials not exceeding 20 mV, probably owing to failure of the impulses to invade the cell body.

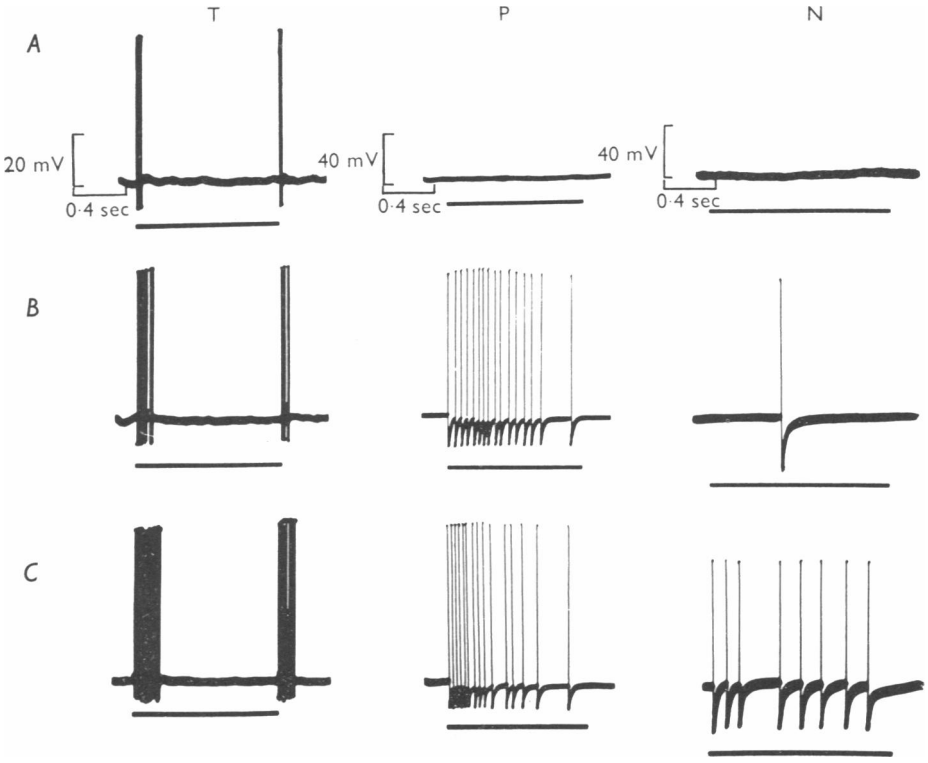
These T, P and N cells in the head ganglion corresponded in position to those of the segmental ganglia: the T and N cells were situated in the anterolateral packets and the P cells in the posterolateral packets of each subganglion. Three T and two P cells were identified on either side of each subganglion of the suboesophageal ganglion, agreeing with their numbers in the segmental ganglia. On the other hand, although two N cells were identified on each side of subganglion 6, none could be recognized in subganglia 3, 4 and 5. No cells with T, P and N electrical properties could be found in the supraoesophageal ganglion. All the neurones that were recorded from in this ganglion gave action potentials not exceeding 10 mV. Whether homologues of T, P and N cells exist in the supra-oesophageal ganglion remains to be answered.



Text-fig. 3. Top: diagram of the head ganglion to show the locations of identified T, P and N cells. The same cells are present on the contralateral side of the ganglion. The cells labelled 'R' are Retzius cells. Bottom: intracellular recordings of action potentials elicited in the T, P and N cells by passing depolarizing current through the micro-electrode. They were identical in configuration to those recorded from segmental T, P and N cells (see Nicholls & Baylor, 1968).

Evidence that the identified cells are sensory neurones

The T, P and N cells in the suboesophageal ganglion could all be driven by specific cutaneous mechanical stimuli which corresponded to those required for segmental touch, pressure and nociceptive cells (Text-fig. 4).



Text-fig. 4. Intracellular recordings from cephalic T, P and N cells to illustrate their responses to cutaneous mechanical stimuli. The horizontal bar underneath each record indicates the time during which the stimulus was applied. *A*, light touch on the skin with a stylus caused T cells to discharge, but not P and N cells. The T cells were rapidly adapting and discharged briefly at the cessation of the stimulus. *B*, pressing on the skin with a stylus elicited responses from both T and P cells, but not N cells. The P cells were slowly adapting and fired as long as the stimulus lasted. *C*, squeezing the skin with forceps caused discharges from all the cells. The N cells were also slowly adapting and sometimes continued to fire after the stimulus was removed.

A T neurone fired in response to light touch on the skin and showed rapid adaptation; at the release of the indentation there was usually another brief discharge. A maintained discharge could be achieved by repetitive indentation of the skin at a point or by a stimulus moving over its recep-

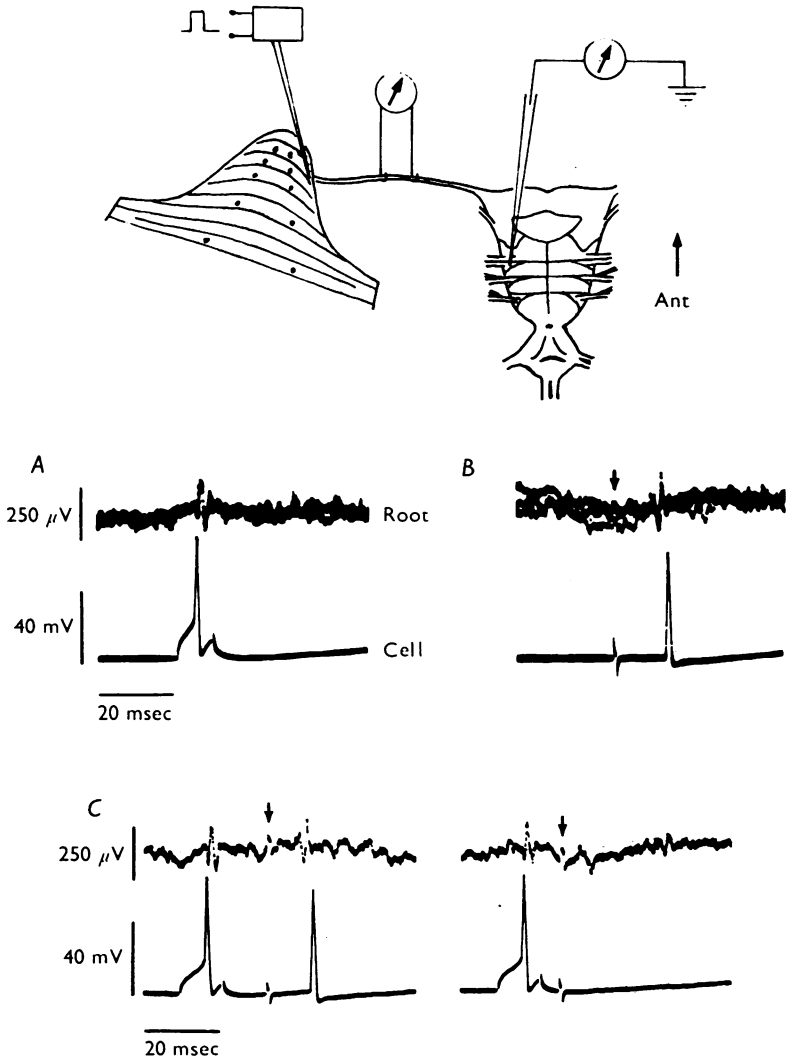
tive field. The P cells did not respond to light touch but only to more marked deformation of the skin, such as pressing on it. They did not discharge at frequencies as high as those of T cells, but their response adapted slowly and they often fired for many seconds to maintained pressure. The N cells required still stronger mechanical stimuli to fire. A good stimulus was to pinch the skin with forceps. Like the P cells, they adapted slowly and often continued to fire after removal of the stimulus. The stimuli applied to the skin in these experiments were not sufficiently refined to determine the absolute thresholds of the T, P and N cells, but they were comparable to those of segmental touch, pressure and nociceptive cells.

Other forms of stimuli have also been tested, but they failed to elicit responses from the cells. These included: (i) changing the tonicity of fluid in contact with the skin from that of distilled water to that of a saturated sugar solution, (ii) changing the pH of the fluid from 4 to 10, (iii) varying the skin temperature from 4 to 40° C, (iv) shining light on the skin, and (v) adding blood serum to the fluid in contact with the mouth.

The cells' responses to mechanical stimulation were not abolished by the presence of up to 20 mM-Mg^{2+} in the perfusing Ringer fluid. Since Mg^{2+} is known to block chemical synapses in the leech central nervous system and neuromuscular junctions (Nicholls & Purves, 1970; Stuart, 1970), this suggested that there were unlikely to be central or peripheral chemical synapses mediating the responses, unless they were inaccessible to Mg^{2+}. Moreover, simultaneous recordings from a nerve root and the cell body indicated that when a cell was stimulated directly through the intracellular electrode, an action potential could always be recorded from the root at a short and constant latency (Text-fig. 5*A*). Focal stimulation on the skin gave rise to an action potential of the same configuration but opposite polarity in the root which was followed at the same latency by an action potential in the cell body (Text-fig. 5*B*). It was also possible to stimulate the cell at both the soma and the periphery so that the outgoing and incoming action potentials annihilated each other by collision (Text-fig. 5*C*). These cells thus appeared to be primary sensory cells, conveying information about touch, pressure and noxious stimuli to the central nervous system. They will be referred to as cephalic touch, pressure and nociceptive cells.

Receptive fields

The receptive fields of the cephalic mechanosensory cells were situated on the ipsilateral side of the head. They differed from segmental receptive fields in ways which reflected certain structural characteristics of the head. One of these is a progressive change in segment size. In a typical body segment there are five annuli, with each segmental ganglion situated just

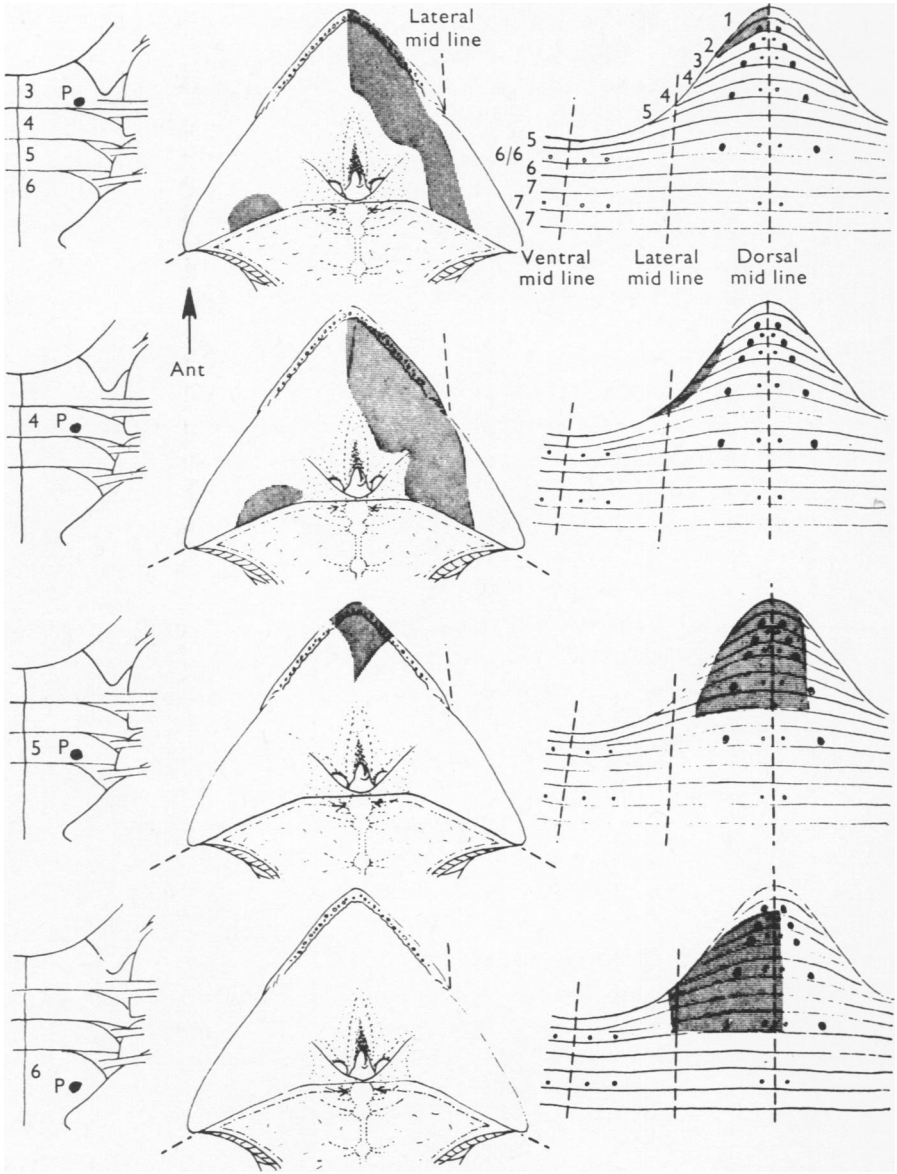


Text-fig. 5. Simultaneous recordings from a cephalic T cell (intracellular) and a nerve root (extracellular). The experiment indicated that the cell sent a process through the root to innervate the skin. *A*, an action potential elicited in the cell body was followed at short delay by an impulse in the root. *B*, touching the skin (marked by arrow) generated an impulse in the root and also an action potential in the cell body. *C*, the outgoing and incoming impulses annihilated each other when initiated within a critical time interval, suggesting they propagated in the same fibre. The results shown in *A*, *B* and *C* were unchanged in the presence of external Mg^{2+} (up to 20 mM). Experiments on cephalic P and N cells gave similar results.

underneath the ventral surface of the central annulus. Toward the head region the segments gradually lose annuli (Hanke, 1948; Mann, 1952). This is illustrated in Text-fig. 2. In segment 1, there is only one annulus; segment 2, one annulus; segment 3, one annulus; segment 4, two annuli; and so on. From segment 9 on, each segment has five annuli. Notwithstanding this change, the size of each annulus stays more or less constant. Another characteristic in the head is that the oblique orientation of the mouth and sucker obliterates the ventral aspect of segments 1 through 4 (Text-fig. 2*B*). There are various foldings inside the mouth, but any division of this region into individual segments is not obvious (Text-fig. 2*C*).

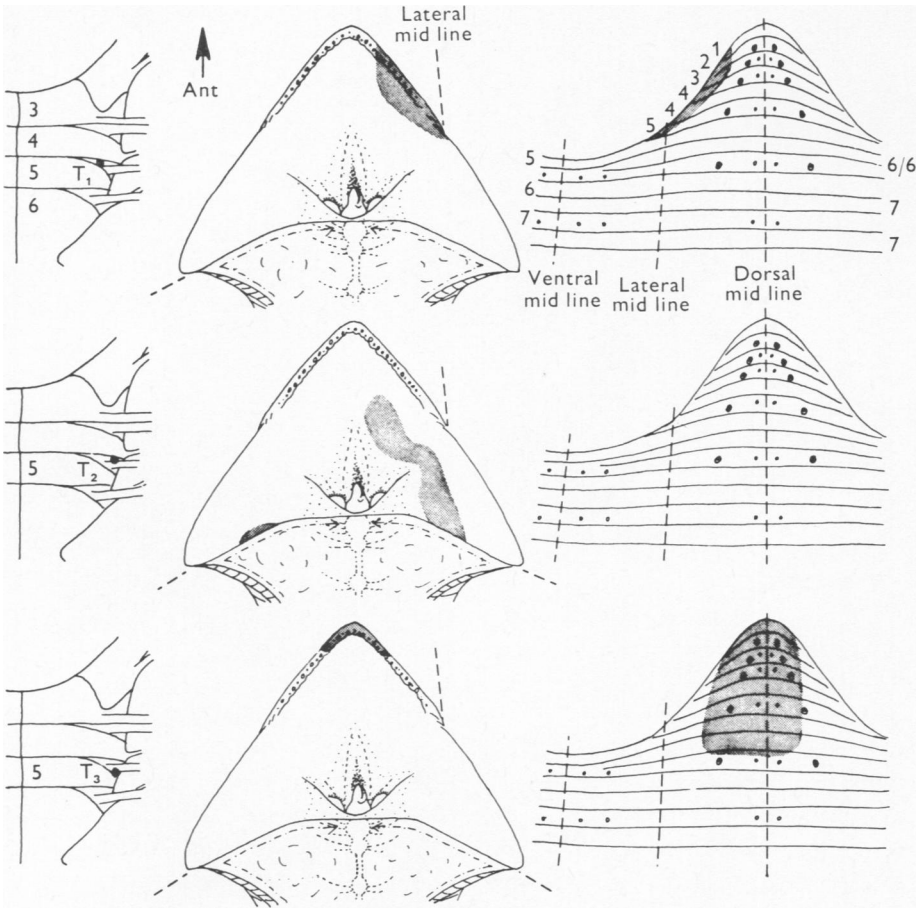
Text-fig. 6 shows the receptive fields of four cephalic pressure cells which were homologous to the medial pressure cell in a segmental ganglion (cf. Text-fig. 1). These receptive fields covered not only the external body surface but also the interior of the mouth. With respect to the pressure modality the dorsal skin of the head was innervated almost exclusively by the medial pressure cells, as is true in a typical segment further down the body (Nicholls & Baylor, 1968). The dorsal receptive fields still resembled their segmental counterparts in being quite uniformly shaped and with their anterior and posterior boundaries roughly following annular margins (Yau, 1976). In contrast, the receptive fields inside the mouth had distorted boundaries, which might in part be explained by the complicated topography of the mouth interior. Whether a receptive field was on the external body surface or in the mouth, it was always a continuous area rather than being made up of disconnected patches. It was also usual for a receptive field on the dorsal body surface to extend into the mouth. This might be expected since the mouth interior develops as an invagination of the ectodermal body wall (Mann, 1961).

Unlike the situation in the main part of the body (Yau, 1976), the receptive fields on the head were often not centred on the segments where the cell bodies were located but were displaced anteriorly. For example, the receptive field of the medial pressure cell in subganglion 6 extended from segment 6 to the anterior margin of segment 3, and that of the medial pressure cell in subganglion 3 hardly covered its own segment at all (Text-fig. 6). The situation was actually more complex because the receptive fields extended into the mouth, where there were no segmental demarcation lines to indicate the locations of the receptive fields. It did appear, however, that the anterior displacement of the receptive fields on the dorsal skin had caused the receptive fields of the first two or three segments to shift deep into the mouth. Another characteristic was that although the more anterior segments in the head rapidly decreased in size, the absolute sizes of the receptive fields did not decrease proportionally. This resulted



Text-fig. 6. Receptive fields of the medial pressure cell in subganglia 3, 4, 5 and 6. The receptive field of a cell was not centred on the segment to which the cell belonged, but was displaced anteriorly. Although the segments gradually decreased in size in the anterior direction, the fields did not decrease proportionally. Note the extensive overlaps between adjacent receptive fields.

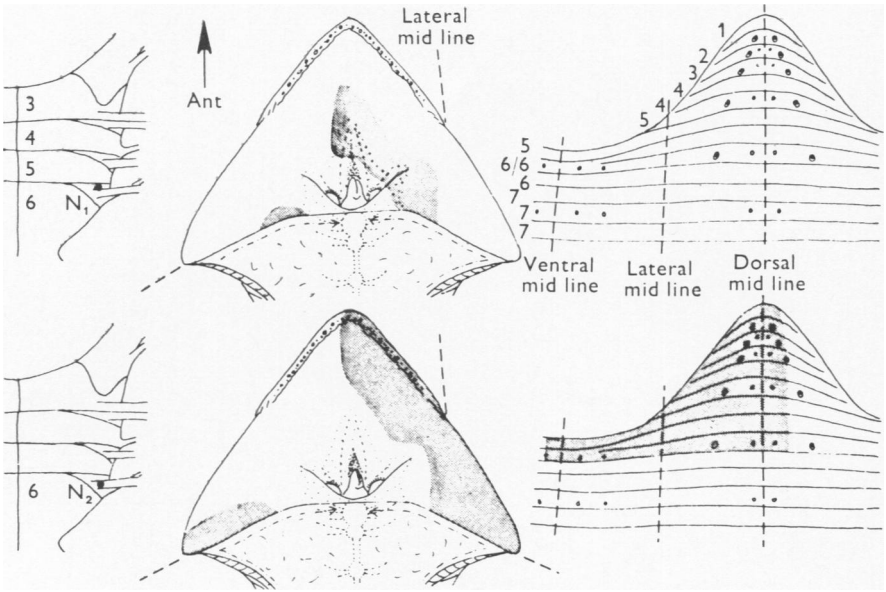
in considerable overlap, and even complete superposition, of receptive fields of sensory cells in adjacent subganglia because some receptive fields spanned as many as four segments. In comparison, the segmental receptive fields span only two to three segments, and their overlaps are generally less extensive (Yau, 1976).



Text-fig. 7. Receptive fields of the three touch cells in subganglion 5. They showed the same characteristics as the receptive fields of pressure cells but were smaller in size.

The receptive fields of cephalic touch cells had very similar characteristics (Text-fig. 7). These were, however, smaller in size than those of pressure cells, probably because there were more touch cells than pressure cells to divide up the territory. The receptive fields of nociceptive cells were different (Text-fig. 8). One of the two identified nociceptive cells

innervated the entire head region both on the dorsal skin and in the mouth; the other innervated a rather restricted region close to the oesophagus. The fields of these two nociceptive cells were thus sufficient to cover the whole head.

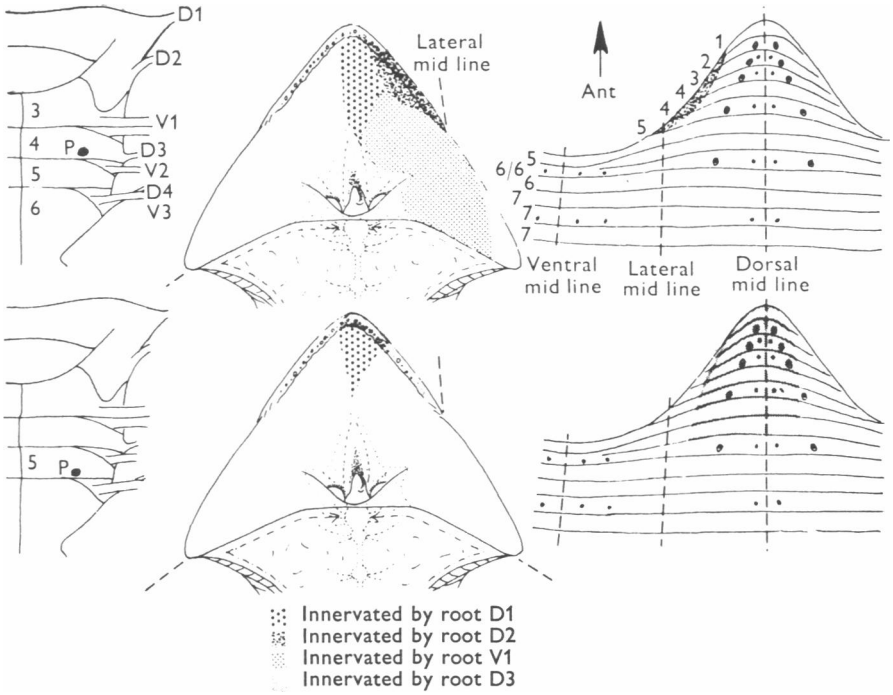


Text-fig. 8. Receptive fields of the two cephalic nociceptive cells. Note the extensiveness of the receptive field of N_2. The fields of N_1 and N_2 together covered the entire head region.

As in other body segments (Nicholls & Baylor, 1968), sensory endings were not uniformly distributed over a receptive field. This was most apparent for the touch receptive fields, on which there were distinct sensitive spots surrounded by areas of higher threshold. Each of these spots probably represented a sensory ending. The relative distribution of sensitive spots was estimated by moving a stylus uniformly over the receptive field and noting changes in firing frequency of the cell. Such experiments indicated that the most densely innervated areas were situated at the tip of the head and the edge of the mouth, in accord with the expectation that these areas are particularly important in stimuli detection.

The receptive field of a cell could be divided into subfields each of which being innervated by a different nerve root. This was done by first mapping the receptive field and then noting changes in its boundaries as the field was reduced by cutting one nerve root after another. Text-fig. 9 depicts the subfields of two cephalic pressure cells. Like those of segmental sen-

sory cells (Nicholls & Baylor, 1968; Yau, 1976), the subfields of a cephalic sensory cell had little overlap with each other. For a given sensory cell, one or more subfields were usually larger than the others. Correlations with morphology (next section) indicated that the large subfields of a cell



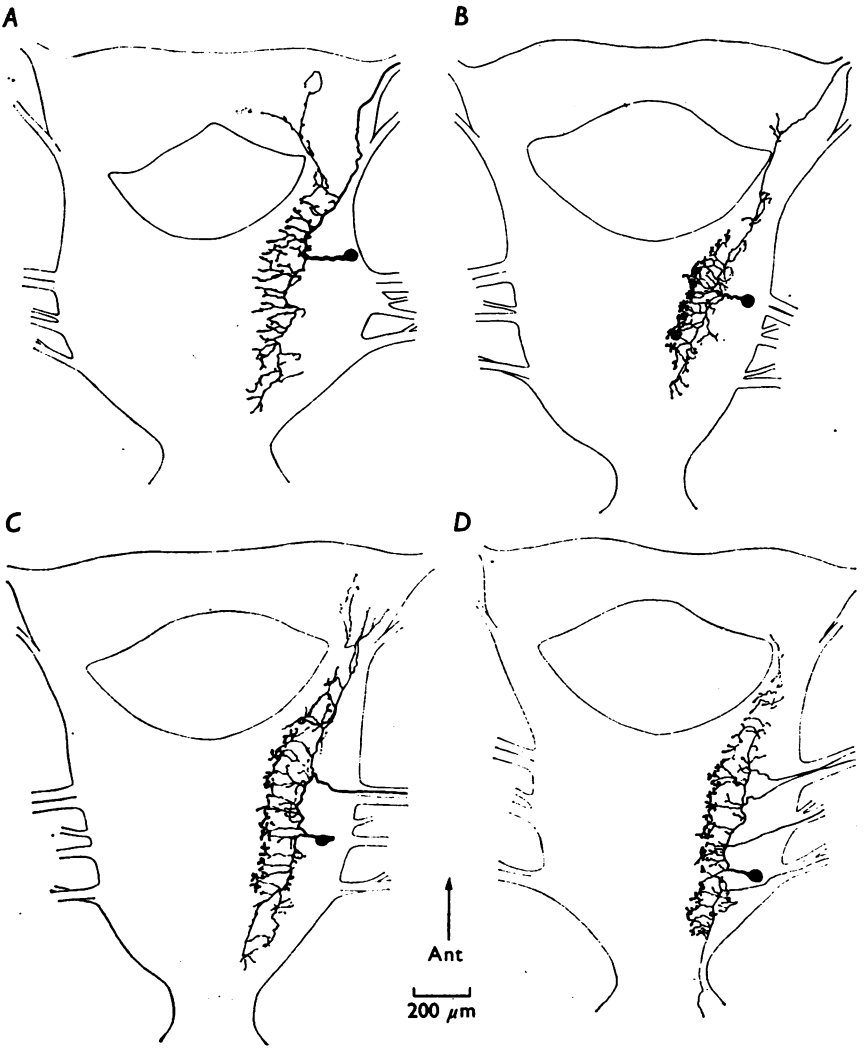
Text-fig. 9. Subfields of the medial pressure cell in subganglia 4 and 5. They were mapped by noting changes in size of the receptive fields after severing the nerve roots one after another. Note the difference in size between the subfields of a cell. There was negligible overlap between adjacent subfields.

were innervated by its large root branches, and the small subfields innervated by its small root branches. Although the location and the size of a cell's receptive field were largely constant from animal to animal, one or more of the small subfields were sometimes absent. This was associated with some inconstancy in the branching of the sensory cells to be described below.

Morphology of cephalic sensory cells

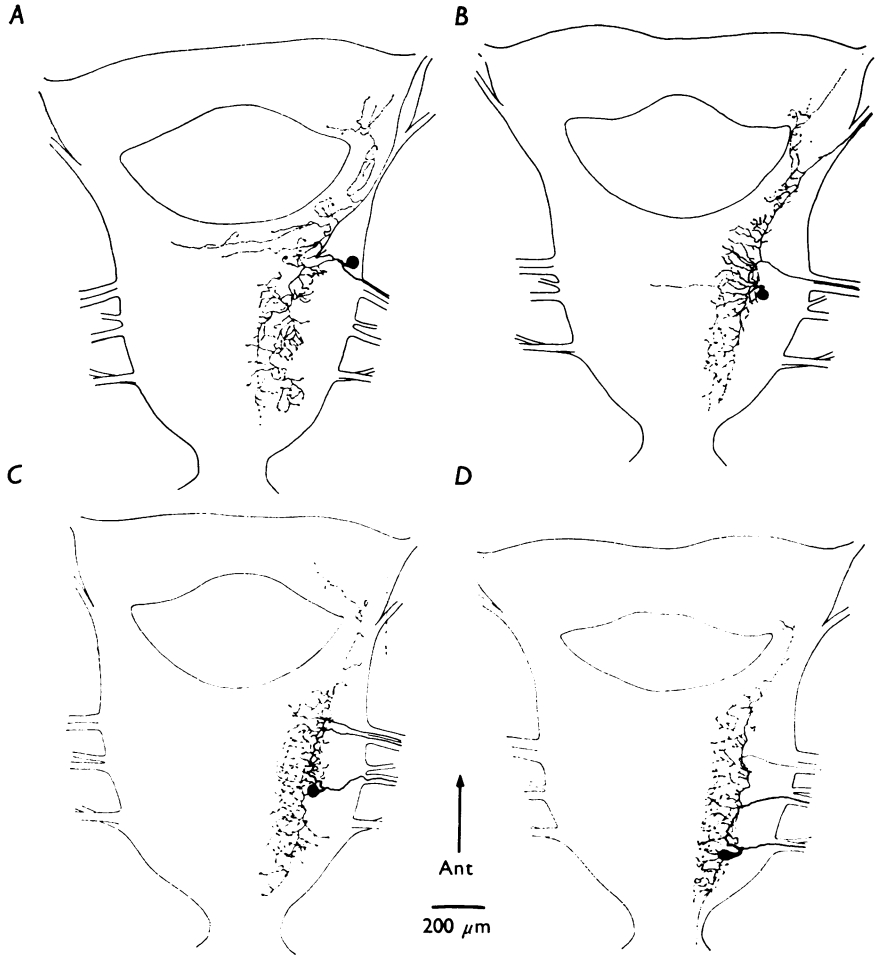
Pl. 2 shows a touch cell injected with horseradish peroxidase, and Text-fig. 10 shows camera lucida drawings of four injected touch cells in subganglia 3, 4, 5 and 6 respectively. As in the segmental ganglia (Nicholls & Purves, 1970; Muller & McMahan, 1976; Yau, 1976) the stem

process of a cell entered the neuropile and soon branched into numerous processes; eventually one or more branches left the ganglion through the nerve roots. Regardless of the subganglion in which a touch cell was located, its meshwork of arborizations extended throughout the ipsilateral neuropile of the suboesophageal ganglion. One or more branches from a cell usually entered the ipsilateral circumoesophageal connective and gave



Text-fig. 10. *A*, *B*, *C* and *D*, camera lucida drawings of touch cells in subganglia 3, 4, 5 and 6 which were injected with horseradish peroxidase. Each cell branched throughout the suboesophageal ganglion regardless of its location.

off a few processes in the supra-oesophageal ganglion. The extent of ramification in this ganglion was, however, relatively scant. In subganglion 6, and sometimes subganglion 5 as well, the cells usually sent a branch which

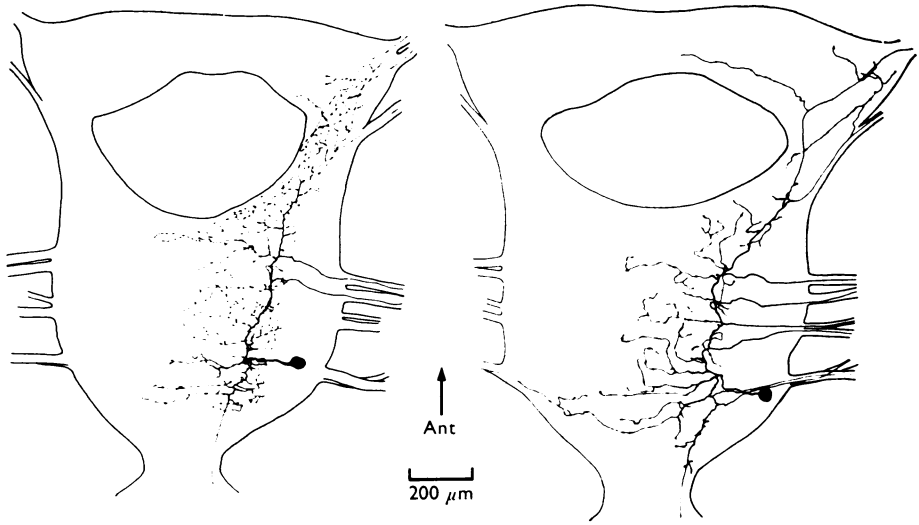


Text-fig. 11. *A*, *B*, *C* and *D*, camera lucida drawings of the lateral pressure cell in subganglia 3, 4, 5 and 6 which were injected with horseradish peroxidase. Their extents of arborization were similar to those of touch cells, but with processes finer and more profuse.

entered the posterior connective and arborized in the first and second free segmental ganglia (segmental ganglia 7 and 8). Sometimes branches went to the periphery through the roots of these ganglia as well. The general branching of pressure and nociceptive cells was similar to that of touch cells, except one or more of their processes usually crossed the mid line of

the head ganglion and branched in the contralateral neuropile (Text-figs. 11, 12). There was, however, no evidence of any branches leaving the ganglion through the contralateral nerve roots.

Except for their more extensive arborizations, the cephalic touch, pressure and nociceptive cells resembled the segmental cells in the morphology of their processes (Muller & McMahan, 1976; Miyazaki & Nicholls, 1976; Yau, 1976). For instance, the touch cells had short but robust processes that were studded with prominent 'bouton-like' structures. There



Text-fig. 12. Camera lucida drawings of the cephalic nociceptive cells injected with horseradish peroxidase. Their processes were more slender and longer than those of touch and pressure cells.

is evidence from electon microscopy (Muller & McMahan, 1976) that these structures correspond to synaptic terminals. The pressure cells had thinner processes and less prominent 'bouton-like' structures. The nociceptive cells had the thinnest processes, but these were very long and covered more territory in the neuropile than those of touch and pressure cells.

The dispositions of the cells' root branches revealed in injected cells and verified by electrophysiological recording experiments are summarized in Table 1. In this table, the roots are labelled according to Text-fig. 3. One characteristic common to all cells was that they sent branches to either the roots associated with their own subganglion or those more anterior, but rarely to the roots posterior to their location. This was in accord with the finding that the receptive fields of these cells were displaced anteriorly. The two nociceptive cells were unique in that although both of them were situated in subganglion 6, the medial one sent branches

to the anterior three roots and the lateral one to all seven roots. These branching patterns were also reflected by the locations and the sizes of their receptive fields.

TABLE 1. Distribution of branches of cephalic mechanosensory cells in the nerve roots of the head ganglion. Results from intracellular horseradish peroxidase injection

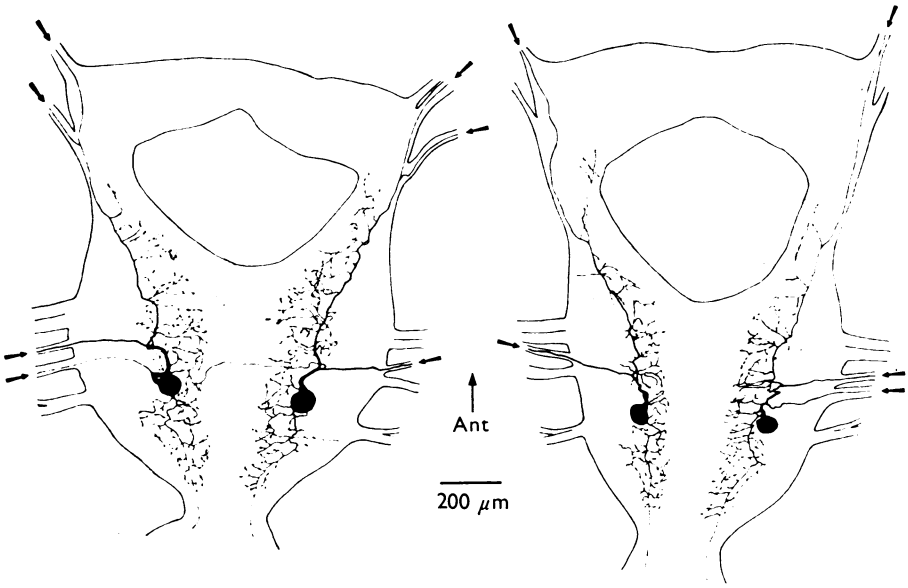
Cell/Root*	D1	D2	V1	D3	V2	D4	V3
Subganglion 3							
Lateral P (6)	5	5	4	—	—	—	—
Medial P (11)	11	8	10	—	—	—	—
T_1 (3)	3	—	—	—	—	—	—
T_2 (3)	3	—	—	—	—	—	—
T_3 (3)	3	—	—	—	—	—	—
Subganglion 4							
Lateral P (8)	4	8	8	—	—	—	—
Medial P (23)	21	22	22	—	—	—	—
T_1 (1)	—	1	—	—	—	—	—
T_2 (3)	3	2	—	—	—	—	—
T_3 (2)	2	2	—	—	1	—	1
Subganglion 5							
Lateral P (12)	2	5	12	1	3	—	—
Medial P (22)	20	16	—	22	3	—	—
T_1 (3)	—	—	3	—	—	—	—
T_2 (6)	1	1	5	—	1	—	1
T_3 (6)	—	—	6	—	—	—	—
Subganglion 6							
Lateral P (12)	—	—	12	—	12	—	12
Medial P (27)	—	—	—	27	—	25	2
T_1 (12)	—	—	11	—	11	—	8
T_2 (10)	—	—	8	—	10	—	5
T_3 (7)	—	—	—	7	—	7	—
Lateral N (12)	10	8	12	9	11	11	12
Medial N (3)	3	3	3	—	—	—	—

For each category of cells, the number of injected cells studied is given in brackets, and individual numbers in the body of the Table indicate how many of these cells sent a process or processes through a given root. T_1, medial position; T_2, anterolateral position; T_3, posterolateral position. These were, however, only average positions.

* Root designations according to Text-fig. 3.

The general morphology of a given sensory cell was similar from animal to animal, but the disposition of its processes, in particular the root branches, did not stay constant in different animals (Table 1). For example, although the medial pressure cell in subganglion 5 invariably sent a

branch to root D3, its other branches could leave the head ganglion by way of roots D1, D2, V2 or a combination. Examination of its morphology showed that the branch which consistently entered root D3 also had the largest diameter (Text-fig. 13). Such relationship between variability and branch size, that the large branches of a cell almost always went to



Text-fig. 13. Camera lucida drawings of peroxidase-injected medial pressure cells on contralateral sides of subganglion 5 in two head ganglia. The arrows indicate the roots which contained processes from these cells. This Figure shows that a cell did not consistently send branches into certain roots. See Table 1 for summary.

certain roots but the small branches could choose among others or be absent altogether, was exhibited by other cells. This variability has not been observed in segmental touch, pressure and nociceptive cells (Nicholls & Baylor, 1968; Yau, 1976). From Table 1 it is obvious that the variability was not random over all the roots for a given cell, and in some cells the branching was quite constant. Correlations between branch size and subfield size also suggested that large subfields were innervated by the large and usually constant root branches and small subfields by the small and more variable root branches. This might explain why the receptive field of a cell stayed roughly invariant in size and location despite the variability in branching.

DISCUSSION

Sensory organization of the head ganglion

The results show that the head ganglion shares similar characteristics in its organization with the segmental ganglia. It possesses a mechanosensory system which consists of cells with the same electrical properties, locations and modalities as the touch, pressure and nociceptive cutaneous sensory cells in the segmental ganglia. All of these cells have been identified in the suboesophageal ganglion, which is formed by the fusion of four subganglia each of which still resembles a distorted segmental ganglion in appearance. In each subganglion three touch and two pressure cells are present on either side, in agreement with their number in a segmental ganglion. On the other hand, homologues of the two segmental nociceptive cells could only be identified in the most posterior subganglion. Since the receptive fields of these two cells already encompass the entire head region, however, there might in fact not be additional nociceptive cells in the head ganglion, implying that there is less emphasis on fine localization of noxious stimuli than for other modalities. It has still to be answered whether synaptic connexions similar to those already characterized in the segmental ganglia (Baylor & Nicholls, 1969*c*) exist among these mechanosensory cells in the head ganglion. Preliminary experiments have shown that, as in segmental ganglia (Baylor & Nicholls, 1969*c*), there is electrical coupling between touch cells in the same subganglion and in adjacent subganglia (K. W. Yau, unpublished).

The supraoesophageal ganglion remains an unknown. It bears no resemblance to the segmental ganglia in appearance, and no homologues of segmental mechanosensory cells could be identified in the present study. Histochemical studies (Marsden & Kerkut, 1969; Rude, 1969) have indicated that this ganglion might be developmentally set apart from the rest of the central nervous system because there is no correspondence between its monoamine-containing cells and those in the other ganglia. Other studies by Hagadorn, Bern & Nishioka (1963) and Hagadorn (1966*a*, *b*) have suggested that the supra-oesophageal ganglion has significant neurosecretory activity. These workers identified a neurohaemal organ, which is a storage-release site for neurosecretory substances, on the posterolateral aspect of this ganglion. It would be of interest to follow the development of the ganglion and discover the fate of the would-be mechanosensory cells.

Receptive fields and morphology of cephalic sensory cells

Like their segmental homologues, the touch, pressure and nociceptive sensory neurones in the head ganglion have circumscribed receptive fields

on the ipsilateral side of the animal's head. Apart from their more complicated geometry in the mouth, these receptive fields have an orderly arrangement that is constant from animal to animal. In association with reduced segment sizes in the head, these receptive fields are smaller than those which cover the main part of the body. However, the overlaps between adjacent receptive fields are extensive, as they are elsewhere (Yau, 1976). Certain regions on the head are innervated by as many as four sensory cells of the same modality. This multiplicity of innervation is caused partly by the fact that although the segments decrease sharply in size toward the head extremity the corresponding receptive fields shrink less rapidly, resulting in their cover of more segments. Another cause is that towards the head region the positions of the receptive fields are shifted more and more anteriorly relative to the segments which contain the innervating cells, resulting in a concentration of innervation on the head by many sensory cells. This might be important functionally because the animal's behaviour suggests that the head is its main stimulus detector. The finding that the receptive fields cover the mouth suggests that the mechanosensory cells in the head ganglion probably play a significant role in locomotion and feeding. Both of these activities are initiated by probing around of the protruded mouth which is followed by sucking on the chosen substrate surface.

The morphology of the touch, pressure and nociceptive cells in the head ganglion is comparable to that of segmental cells with respect to both the way they branch and the structural appearance of their processes (Muller & McMahan, 1976; Miyazaki & Nicholls, 1976; Yau, 1976). The finding that some of the cells send branches to the periphery through the roots associated with more anterior subganglia rather than their own subganglion suggests that differentiation of the head ganglion into individual segments and subganglia may not be as well defined as its components suggest. This is further supported by the variability in branching of many of the cells, which occurs not only from animal to animal but also on contralateral sides of the same head ganglion. In other words, it makes no difference whether a cell sends a process to the periphery through one nerve root or another, as long as the process gets to the right locality in the periphery.

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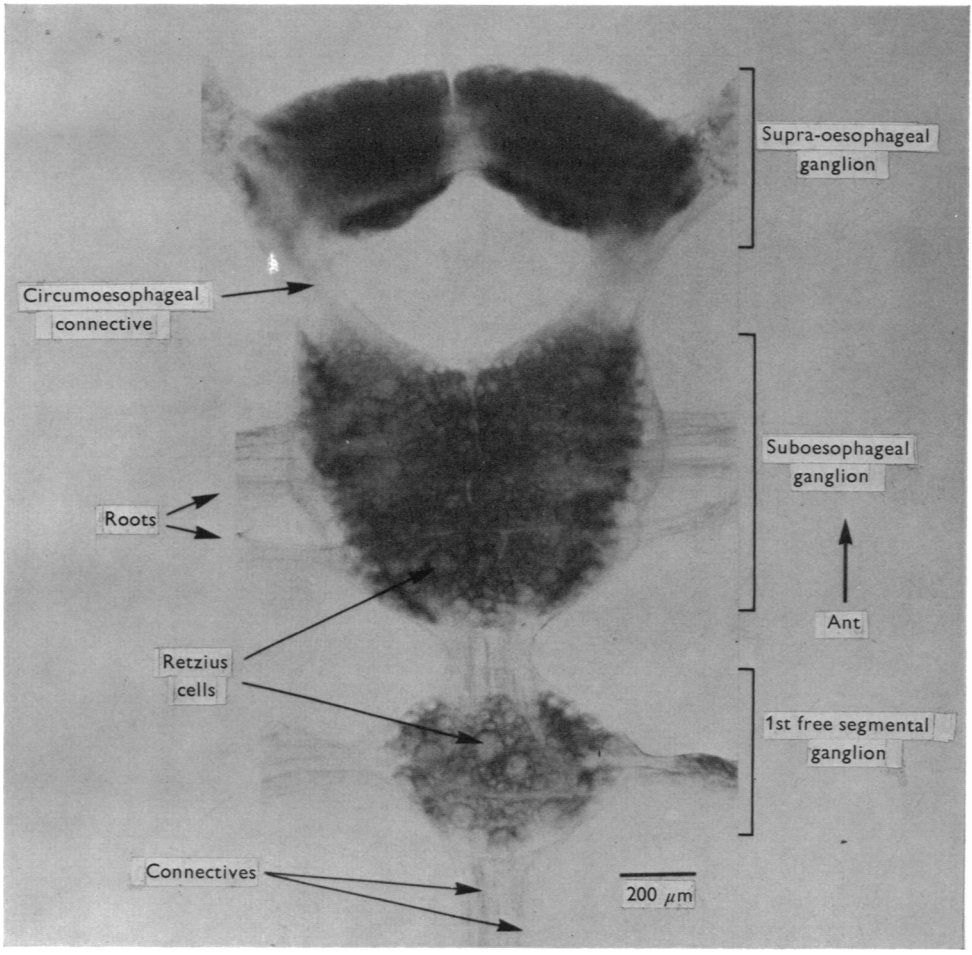
EXPLANATION OF PLATES

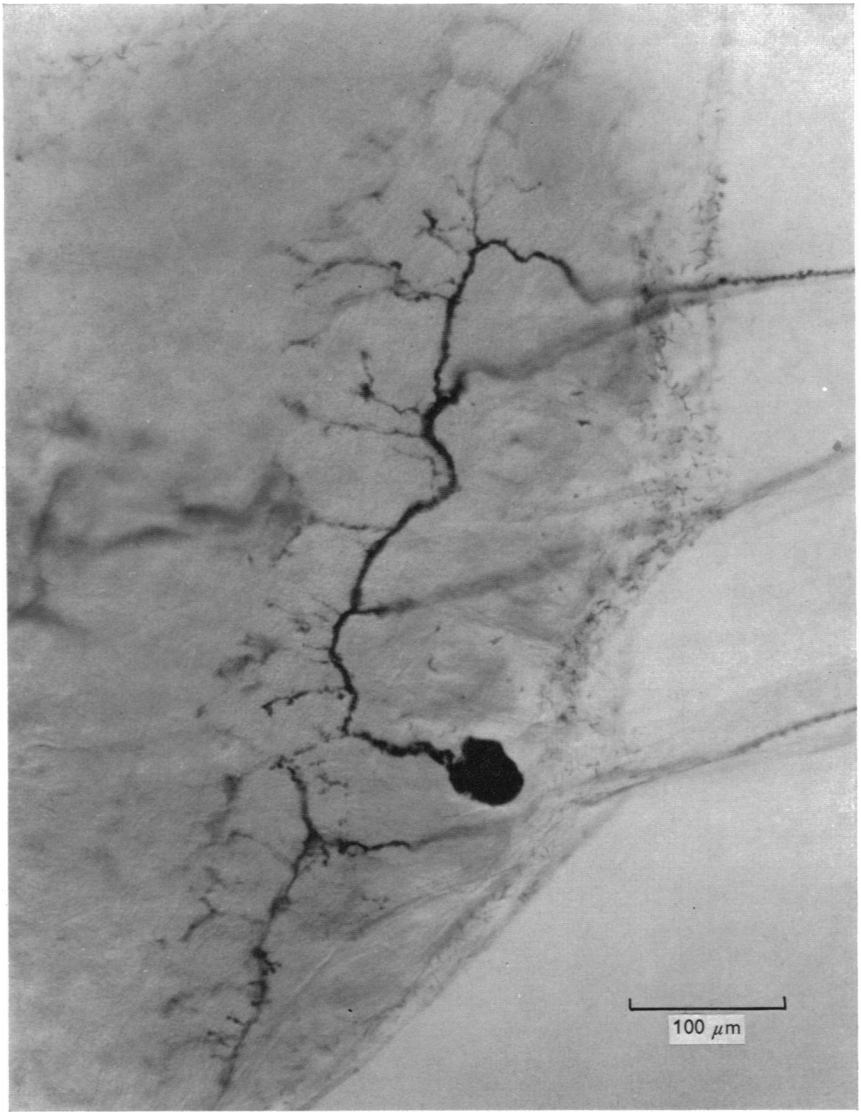
PLATE 1

Photomicrograph of the ventral aspect of the head ganglion and the first free segmental ganglion. The boundaries between the four constituent segmental ganglia (subganglia) of the suboesophageal ganglion are vaguely visible. The four pairs of Retzius cells in these subganglia can be seen situated close to the mid line, as in the segmental ganglia. The supra-oesophageal ganglion, however, bears no similarity to the segmental ganglia in appearance.

PLATE 2

Photomicrograph of a touch cell in subganglion 6 which was injected with horseradish peroxidase. The preparation was a whole mount, so not all parts of the cell were in focus. Four branches left the ganglion through three roots.


Supra-oesophageal ganglion
Circumoesophageal connective
Suboesophageal ganglion
Roots
Ant
Retzius cells
1st free segmental ganglion
Connectives
200 μm


100 μm