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Distribution and Morphology of Nociceptive Cells in the CNS of Three Species of Leeches

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ABSTRACT

The present study describes the segmental variation in the distribution and morphology of nociceptive neurons (N cells) in the central nervous system of the leech.

N cells of midbody ganglia can be segregated into lateral and medial types. We show that monoclonal antibodies specific for N cells can distinguish between the two populations. The monoclonal antibodies were used to map the complete distribution of the cells along the nervous cord. There are two pairs of medial and lateral nociceptive neurons in the midbody ganglia, one pair of the medial type in the sex ganglia (5 and 6), and a pair of the lateral type in ganglia 20 and 21. The caudal brain is without nociceptive neurons. This distribution was confirmed by electrophysiological means.

The morphology of N cells in different parts of the nervous system was investigated by intracellular horseradish peroxidase (HRP) injections. In the terminal segmental ganglia the N cells showed extensive arborizations in the head and tail brains and, contrary to N cells in the midbody ganglia, their arborizations spanned more than three segments. N cells are absent in the tail brain, but the N cells of ganglia 20 and 21 were shown to innervate the entire caudal region. The basic morphology of all N-cell homologues was found to be very similar for three leech species.

In the sex ganglia the pair of N-cell homologues were examined in *Haemopis, Hirudo,* and *Macrobdella.* The results showed a progressive modification in the three species of the cell's morphology, peripheral projections, and physiological responses, possibly correlated with the evolution and complexity of the sexual organs.

HRP injections and monoclonal antibody staining revealed that a common feature of N-cell homologues is the presence of processes that tightly surround the cell soma of other cells. This suggests that N cells may have other functional properties in addition to being primary sensory neurons.

Key words: segmental variation, homologous neurons, mapping, monoclonal antibodies, horseradish peroxidase

The leech is a segmented animal with a nervous system which is embryonically derived from a fixed number of 33 ganglionic precursors. The most anterior ganglion in the adult nervous system is the supraoesophageal, which has a different embryological origin from the rest of the CNS (Weisblat et al., '80). The remaining 32 ganglia are serially homologous. The first four ganglia of these are fused to form the head brain, and the last seven caudal ganglia are fused to form the tail brain. In between these terminal structures are 21 unfused segmental ganglia which are linked by connectives. The connectives are composed of two large lateral nerve bundles and a smaller medial bundle, Faivre's nerve.

Within each ganglion a number of neurons with constant morphological and physiological properties can be identified on the basis of size and position. The identifiable neu-

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rons include the mechanosensory cells T, P, and N (Nicholls and Baylor, '68) and various moto- and interneurons such as the L cell (Stuart, '70), the AE cell (Stuart, '70), the S cell (Frank et al., '75), and the Leydig cell (Keyser et al., '82). However, within the homologous structures of the nerve cord, there is considerable variation: (1) The number of neurons in the segmental ganglia varies (Macagno, '80). For example, ganglion 6 contains almost twice as many neurons as a standard ganglion, whereas ganglion 21 contains only about one-third the number. (2) Certain cells associated with a particular function are found only in a restricted number of ganglia (Stent and Kristan, '81). (3) Some identified cells have been found to have segmental differences in their morphology and physiology (Shafer and Calabrese, '81; Beleslin, '77; Weeks, '82). It is of considerable interest to know how this variation in distribution and physiological parameters of identified cells correlates with the anatomy and behavior of the animal.

For mapping the distribution of the neurons we applied immunohistochemical techniques using monoclonal antibodies (Kohler and Milstein, '75). Recently, monoclonal antibodies have been shown to be able to recognize antigens restricted to specific subsets of neurons both in invertebrates (Zipser and McKay, '81) and vertebrates (Sternberger et al., '82; McKay and Hockfield, '82). This allows complete maps of antigenically related cell types to be generated using the antibody as a molecular marker (Zipser, '82). In this paper we report the staining pattern of three antibodies which specifically recognize a particular cell type in the leech, the primary sensory neurons for noxious stimuli, the N cells. Each standard segmental ganglion has four N cells which can be identified by their characteristic physiology and morphology (Nicholls and Baylor, '68; Muller and McMahan, '76). However, the four cells are not identical and can be divided into a medial (Nm) and a lateral (Nl) type. The two populations differ from one another in their receptive fields (Blackshaw et al., '82), their extrasynaptic receptors (Sargent et al., '77), and their synaptic output (Blackshaw et al., '82; Kleinhaus and Johansen, unpublished observations). This study extends these observations by showing that the two types of N cells carry antigenically distinct molecules which can be recognized by monoclonal antibodies. The distribution of the two populations along the nervous cord is mapped, and the morphology and physiology of N cells in the specialized ganglia is described.

MATERIALS AND METHODS

Leeches of the three species *Haemopis marmorata*, *Hirudo medicinalis*, and *Macrobdella decora* were obtained from commercial suppliers and kept in spring water at 6°C.

Monoclonal antibodies

The monoclonal antibodies Lan 3-2 and Lan 4-2 were generated by B. Zipser and R.D.G.M. and have previously been described (Zipser and McKay, '81; McKay et al., '83). By immunizing mice with homogenized adult nerve cords from *Hirudo* following the procedure of Zipser and McKay ('81) 3G8 was raised. The hybridoma line has been recloned three times in soft agar and shown to be a monoclonal IgG by an Ouchterlony double-diffusion assay.

For immunocytochemical staining whole nerve cords of leeches were dissected in leech Ringer (Nicholls and Baylor, '68), pinned out in sylgard dishes, and fixed in 4% paraformaldehyde, 0.05 M phosphate buffer, pH 7.4, for 30 minutes. The connective capsules on the ganglia were broken with fine forceps, and the ganglia were permeabilized for better antibody penetration by xylene extraction. The nerve cords were incubated overnight directly in hybridoma supernatant containing 2% Triton X-100. After washing in phosphate buffer, nerve cords were incubated for 2 hours in a HRP-conjugated second antibody (goat antimouse; Cappel 1:30) before reaction with 3,3'-diaminobenzidine (0.03%) and H_2O_2 (0.01%). The reaction was allowed to proceed until an appropriate staining density was obtained. The preparations were dehydrated in alcohol, cleared in xylene, and embedded as whole mounts in Depex (BDH Chemicals Ltd.)

Electrophysiology

The intracellular recording techniques have been described elsewhere (Nicholls and Baylor, '68). Electrodes filled with 4 M potassium acetate having resistances between 20 and 50 MΩ were used. Ganglia were dissected in leech Ringer (Nicholls and Baylor, '68) and pinned out in sylgard-coated dishes. The cells were identified by their size, position, and electrical parameters as described by Nicholls and Baylor ('68) and Keyser and Lent ('77).

The mapping of receptive fields was performed by making a dorsal incision through the body wall and pinning out skin preparations containing several segments with all connectives and peripheral roots intact. Over each ganglion in the ventral midline, a small piece of skin was removed to allow for intracellular recording from the mechanosensory cells. Their fields were either probed by pinching the skin with forceps or by applying electrical stimuli of about 10 V, 1-msec duration, through a suction electrode. To diminish local reflexes and eliminate chemical synaptic interactions, the Ringer in these experiments was modified to contain 20 mM $MgCl_2$.

HRP injections

HRP injections were performed as described by Muller and McMahan ('76). The electrodes were filled with 3% HRP (Sigma type VI), 0.2 M KCl, and 0.2% fast green and bevelled to have resistances of 50–60 MΩ. Following injection, the ganglia were incubated in culture media (L15 with 5% fetal calf serum) for either 3 hours when only the intraganglionic branching pattern was examined, or for 12–24 hours when intersegmental and peripheral projections were investigated. The preparations were fixed with 1% glutaraldehyde, 1% paraformaldehyde in Ringer for one-half hour, rinsed in 8% sucrose, and reacted with 0.5% benzidine dihydrochloride in the presence of 0.01% H_2O_2 until sufficient staining intensity was obtained. The reaction was stopped with a brief rinse in 8% sucrose, and the ganglia were incubated in 15% sodium nitroferricyanide for 4 minutes. The resulting preparations were dehydrated in absolute alcohol, cleared in xylene, and embedded as whole mounts in Depex.

RESULTS

Immunohistochemical mapping of nociceptive neurons

Complete maps of neurons stained with three different monoclonal antibodies were made by incubating the entire leech nervous cord in the antibody. Figure 1A shows the staining pattern of the antibodies on standard segmental

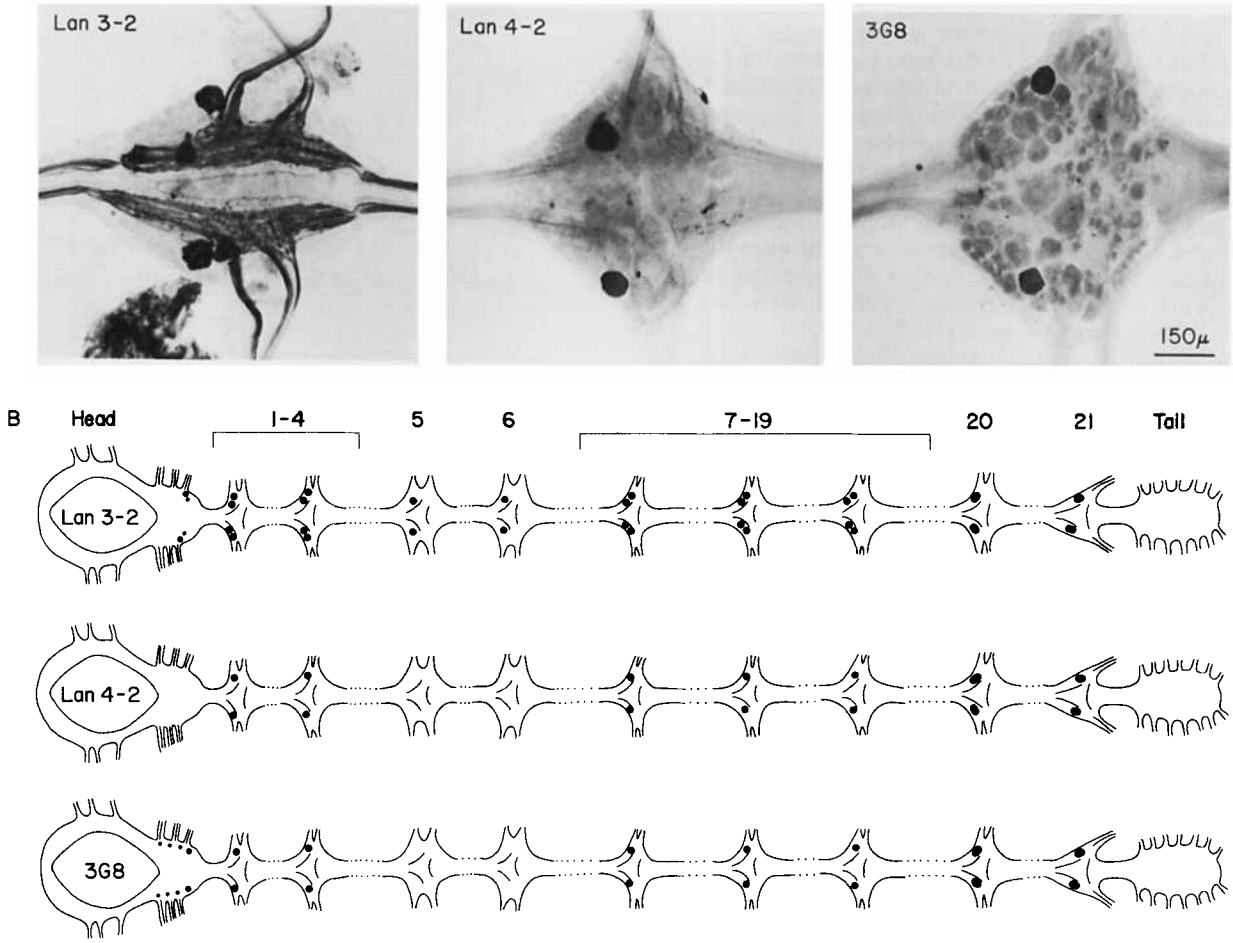


Fig. 1. The staining pattern of three monoclonal antibodies which specifically recognize nociceptive cells. A. Photographs of midbody ganglia stained with the monoclonal antibodies. Lan 3–2 stains all four nociceptive cells in *Haemopis.* Lan 4–2 stains only the lateral N cells in *Haemopis;* 3G8 stains the two lateral N cells in *Hirudo.* B. Diagram of the complete distribution of the cells stained by Lan 3–2, Lan 4–2, and 3G8. The staining pattern of ganglia 1–4 and 7–19 are identical, and therefore only representative ganglia are shown.

ganglia. In all three cases the identity of the neurons recognized by the antibody was confirmed by a double-labeling technique (Zipser and McKay, '81; Zipser, '82).

Lan 3-2. This antibody binds to all four nociceptive neurons in *Haemopis* (Fig. 1). Recently, a map of this antibody was presented by Zipser ('82). We confirm these results except that in our hands we have obtained specific staining of a pair of neurons in ganglia 5 and 6 in more than 75% of our preparations. In this study we present additional evidence that these cells are N-cell homologues with respect to their morphology and shape of action potentials. Therefore, we have incorporated these cells into the map.

In addition to binding to the cell soma of the nociceptive neurons, Lan 3–2 binds to fascicles of axons (Fig. 1A) which occupy stereotyped positions in the connective (Hockfield and McKay, '83). In the related species *Hirudo* and *Helobdella* the antibody crossreacts only with axon fascicles; no cell bodies are stained (McKay et al., '83). Lan 3–2 has been shown to bind to surface glycoproteins with molecular weights between 90,000 and 130,000 daltons (McKay et al., '83).

Lan 4-2. This antibody stains only the lateral pair of N cells in the standard midbody ganglion of *Haemopis* (Fig. 1). Contrary to Lan 3–2 no cells are stained in the head ganglion or in ganglia 5 and 6. Lan 4–2 stains fascicles in a fashion similar to Lan 3–2 (Hockfield and McKay, '83).

3G8. This antibody was raised against *Hirudo.* It shows a staining pattern of the lateral N cell similar to that of Lan 4–2 (Fig. 1). However, it stains two cells in each subganglion of the head brain, and it binds only to cell bodies. The 3G8 does not crossreact with *Haemopis.*

Comparison of the three maps reveals that only Lan 3–2 stains a pair of cells in ganglion 5 and 6, whereas all three antibodies bind to the single pair of N cells in ganglia 20 and 21. This suggests that the N cells found in the sex ganglia are of the medial type, whereas the N cells in

ganglia 20 and 21 are of the lateral type. This hypothesis has been further strengthened by experiments with *Macrobdella* (Johansen et al., '84) where the medial and lateral N cells can be shown to have different responses to the application of the local anesthetic procaine. The response of the N cells in ganglia 5 and 6 and 20 and 21 followed the predictions made from the antibody staining pattern.

In all three cases the caudal brain was devoid of any antibody binding to cell bodies. It seems unlikely that this should be due to poor penetration or slight alterations of the antigens for all three antibodies. Furthermore, no cells with N-like electrical properties could be identified in a search with electrophysiological techniques, although T and P cells could be found. Therefore, these observations suggest that the caudal brain is without nociceptive neurons.

Yau ('76) identified four N cells in the head brain which were all found in subganglion 4. This agrees well with the binding pattern of Lan 3–2. However, Lan 4–2 has not been seen to stain cells in the head, whereas 3G8 binds to two neurons in each subganglion. So in contrast to the simple correlation of antibody staining and the distribution of N cells in the midbody ganglia, the fused head ganglion exhibits a more complex pattern of staining. This discrepancy between the antibody staining patterns can have various reasons, the most likely of which is an actual difference in the distribution of the antigenic determinant among the cells. Additional electrophysiological studies are required to further characterize these neurons. In the rest of this paper we confined our studies to N cells in the segmental ganglia.

Morphology and physiology of nociceptive neurons

Midbody ganglia. Each midbody ganglia has two lateral and two medial N cells. Figure 2A,B shows the morphology of these cells in *Haemopis.* No major difference in morphology between the lateral and medial N cell could be detected. As described for *Macrobdella* (Johansen et al., '84), both N cells send two branches out in each ipsilateral connective. Occasionally processes are seen entering the contralateral connective and Faivre's nerve (Fig. 2B). Otherwise the branching pattern is very similar to the original description of N-cell morphology in *Hirudo* by Muller and McMahan ('76). The electrophysiological properties of N cells from the standard midbody ganglia for the three species *Haemopis, Hirudo,* and *Macrobdella* were found to be indistinguishable by Keyser and Lent ('77).

Ganglia 1 and 2. Yau ('76) has described the branching pattern of the cephalic N cells. Contrary to the other sensory modalities (touch and pressure) which have the normal number of sensory cells in each subganglion, N cells were found only in subganglion 4, which was suggested as indicating a lesser emphasis on detection of noxious stimuli in the head region. We have found by long-term incubation of HRP-injected neurons that the head region is extensively innervated also by the N cells located in ganglia 1 and 2 (Fig. 3A,B). The process from the N cells in ganglion 1 leave the head ganglion through all the peripheral nerves including those originating from the supraoesophageal ganglion (Fig. 3A). Some branches extend into the supraoesophageal ganglion. Figure 3C,D shows the arborizations of a medial N cell in the head ganglion. The processes cross the midline and branch in the contralateral neuropil. The arborizations extend out in the contralateral roots and can be followed past the first branching point.

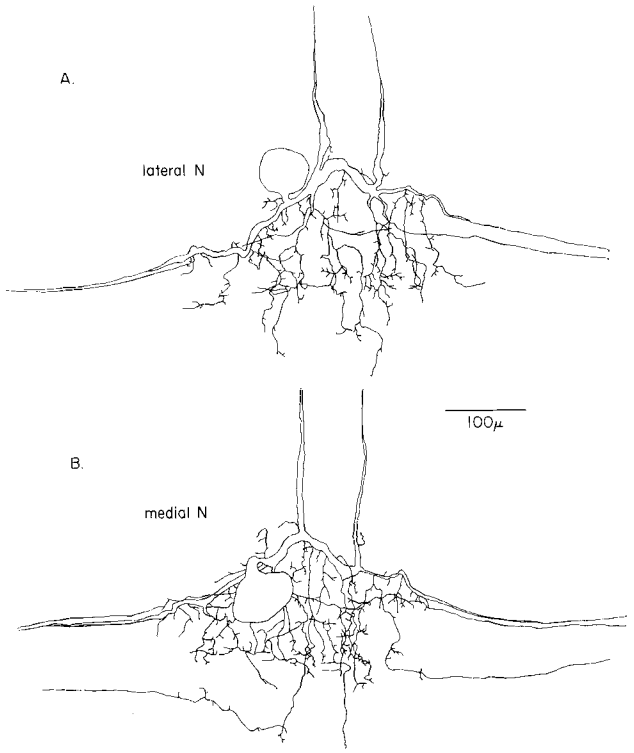


Fig. 2. Camera lucida drawings of HRP-injected midbody nociceptive neurons in *Haemopis.* A. Lateral N cell. B. Medial N Cell.

The processes from the N cells in ganglion 2 extend through ganglion 1 into the head where they have peripheral projections in all the head nerves (Fig. 3B). The N cells in both ganglia 1 and 2 have projections through both ipsilateral nerve roots of the neighboring ganglion (Fig. 3A,B) and branches in the neuropil.

By recording from N cells in ganglion 1 with the peripheral roots of all segmental ganglia cut, it was confirmed by stimulating the skin of the head that the projections of the cells through the brain nerves were associated with a functional receptive field for noxious stimuli in the head region.

These results show that the head region is not innervated solely by cephalic neurons, but is innervated by projections of N cells located in the proximal segmental ganglia as well. The cells have arborizations far more extensive than the mechanosensory cells located in standard midbody ganglia, which typically span only three segments (Blackshaw, '81).

Ganglia 5 and 6. Ganglia 5 and 6 innervate the sexual organs (Zipser, '79). This is correlated with changes in the number of cells present in the ganglia (Macagno, '80) and in the branching patterns of their peripheral nerves. The morphology and innervation of the genitalia vary considerably between species, as is illustrated in Figure 4B–D for the three species *Hirudo, Macrobdella,* and *Haemopis.* The preparations were made in the winter during the animals' nonreproductive state. The extent and complexity of the sexual organs clearly differ. *Hirudo* and *Haemopis* have two distinct sex nerves arising from the anterior roots of

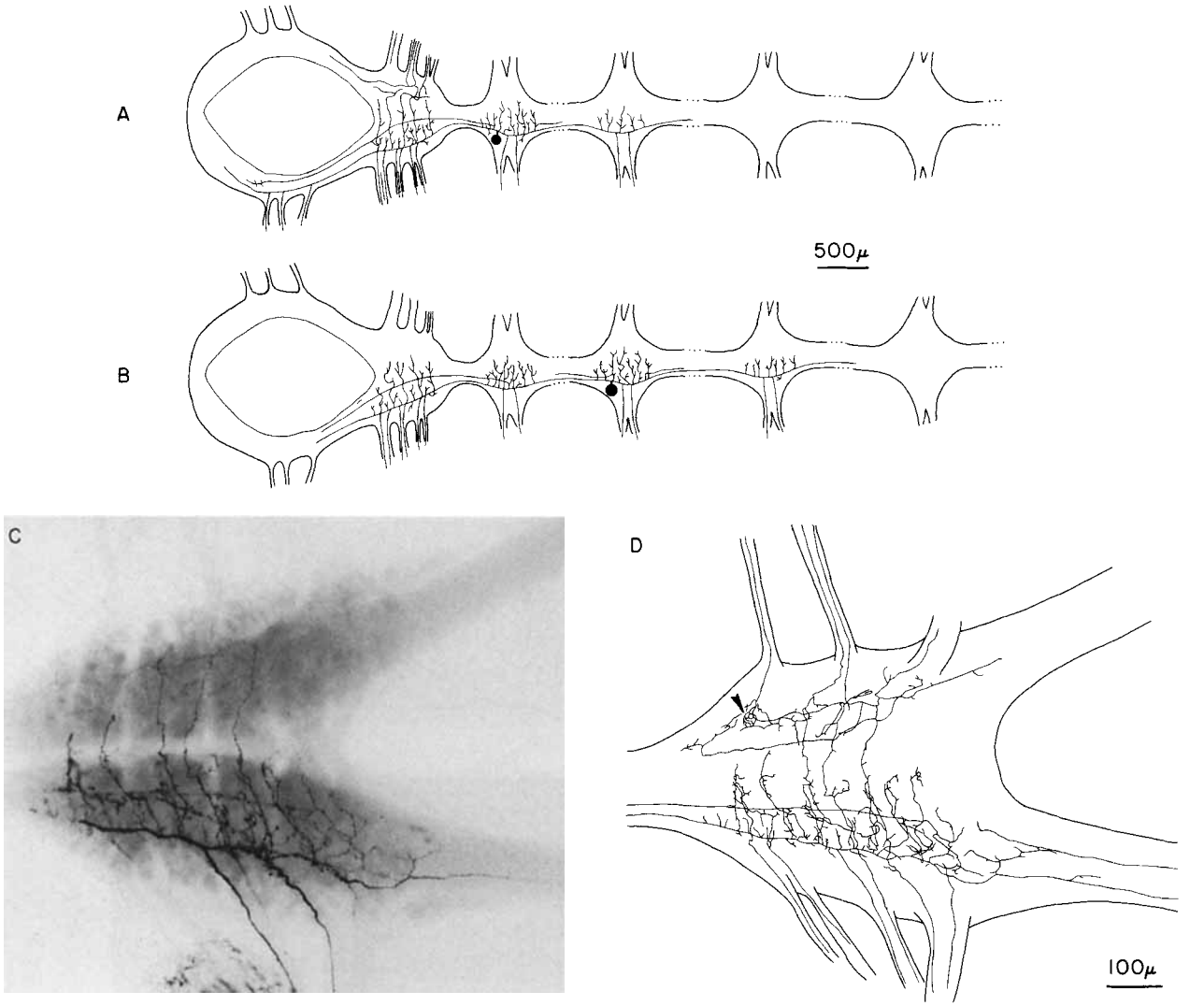


Fig. 3. A. Diagram of the typical branching pattern of N cells situated in ganglion 1. B. Diagram of the arborizations of N cells located in ganglion 2. C. Photograph of the arborizations in the head brain of an HRP-injected medial N cell located in ganglion 1. D. Camera lucida drawing of C. Arrowhead indicates processes which tightly surround the soma of an unidentified cell. C and D are of the same magnification.

both ganglia 5 and 6, which innervate the male genitalia (Fig. 4A,B,D). The posterior root of ganglion 5 has an additional nerve running to the penis sheath. The female genitalia are innervated from ganglion 6 by a branch of the posterior root. *Macrobdella* lacks any distinctive sex nerves and the sexual organs are innervated by thin and inconspicuous branches of the roots.

The mapping with monoclonal antibodies suggested the presence of only one pair of N homologous neurons (Nsex) in ganglia 5 and 6. This was confirmed by an electrophysiological search in all three species in which each were found to have only one pair of neurons with the action potential characteristics typical of midbody ganglia nociceptive neurons (Fig. 4A). However, properties of the neurons for the three species varied from one another in several aspects.

In *Macrobdella* the peripheral neurites branch out through both roots and all their collaterals (Fig. 4A). Johansen et al. ('84) have shown that the cells' morphology is indistinguishable from midbody N cells and that the cells respond to noxious stimuli applied to both the skin and the gut wall.

In *Hirudo* there are projections only in the sex nerves and the anterior roots' primary branch (Fig. 4A). Sometimes a very limited receptive field can be obtained on the ventral skin, but this is not a consistent finding. However, the skin of the two segments containing the sex ganglia are not without noxious sensory innervation. The N cells of the adjacent ganglia 4 and 7 have expanded secondary receptive fields which cover the skin areas of segments 5 and 6, respectively (data not shown). The morphology of the cell (Fig. 5C) is indistinguishable from that of other N cells in *Hirudo*.

In *Haemopis* the cell has processes only in the sex nerves (Fig. 4A). Figure 6A shows the projections of a Nsex cell in the sex nerves of ganglion 5 to the penis sheath, and Figure

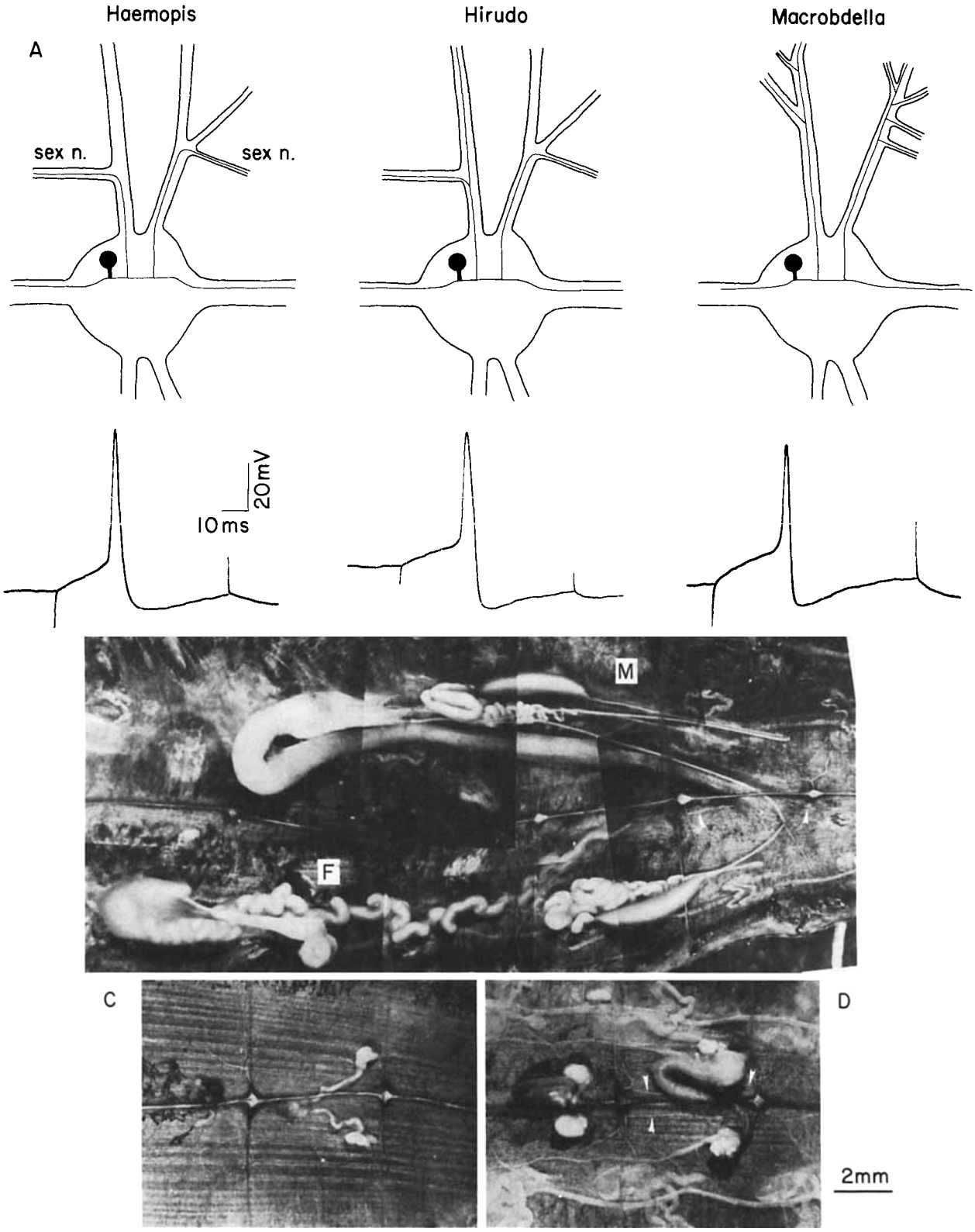
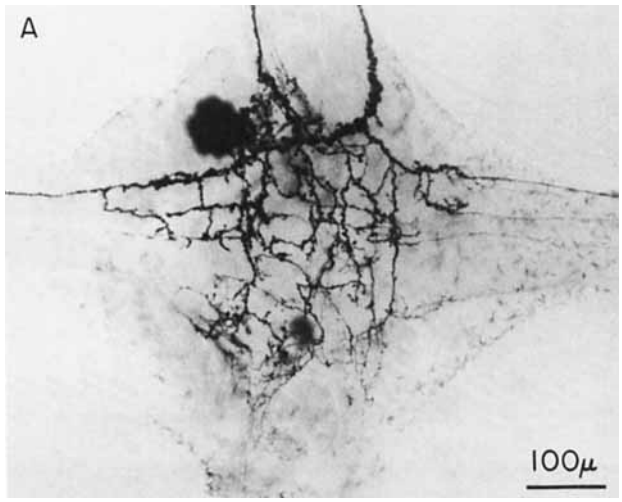


Fig. 4. A. Diagram of the peripheral projections of the N cells located in the sex ganglia (5 and 6) of *Haemopis, Hirudo,* and *Macrobdella.* Recordings of the action potentials from Nsex in the three species are shown underneath. B. Photograph of the sexual organs of *Haemopis.* The male genitalia (M) consists of a muscular penis surrounded by a penis sheath. The penis is connected to two sperm vescicles by a pair of ejaculatory ducts. The male gonopore is located between ganglia 5 and 6. The female genitalia (F) consists of a vagina, whose gonopore is situated between ganglia 6 and 7. C. The sexual organs of *Macrobdella.* Note the absence of distinctive sex nerves. D. The sexual organs of *Hirudo.* Arrowheads indicate the sex nerves originating from ganglia 5 and 6 in *Haemopis and Hirudo.*



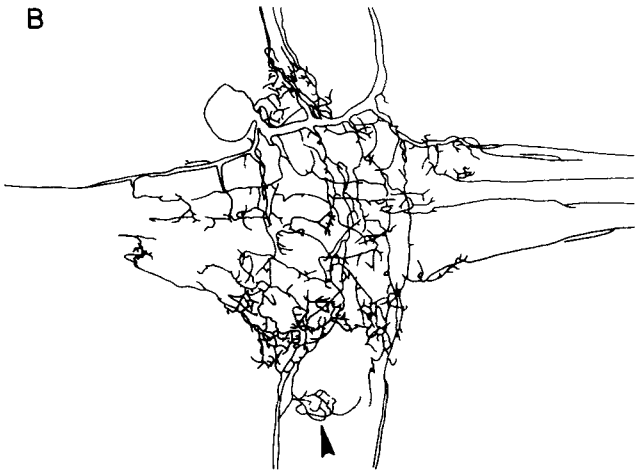
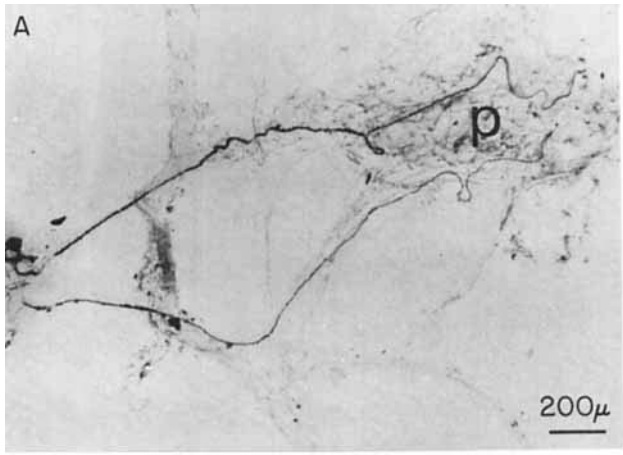


Fig. 5. Morphology of N-cell homologues in ganglia 5 and 6. A. Photograph of an HRP-injected N cell in ganglion 6 of *Haemopis*. B. Camera lucida drawing of A. Arrowhead indicates processes which tightly surround the soma of an unidentified cell. C. Camera lucida drawing of an HRP-injected N cell from ganglion 5 in *Hirudo*.



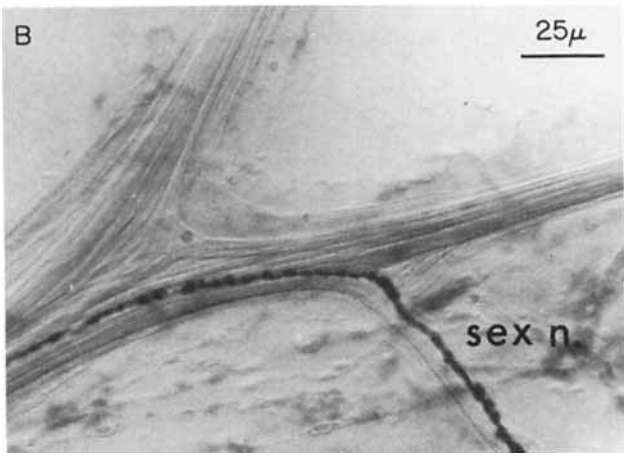


Fig. 6. A. The peripheral projections of an N-cell homologue in ganglion 5 of *Haemopis*. The axons project only in the sex nerves and run to the penis sheath (p), where they branch extensively. B. Photograph taken with Nomarski optics of the posterior root of ganglion 6 in *Haemopis*. The sex nerve originates close to the branch point of the root, where it divides into the anterior and posterior branch. The axon from an HRP-injected N-cell can clearly be seen to project through the sex nerve only.

6B shows the projections from the cell entering the sex nerve where it branches off from the posterior root. No other neurites entering the other branches can be seen. It has not been possible to obtain a response in the cell from either noxious stimuli to the male genitalia or the skin. The morphology of the cell compares well to the general N-cell pattern (Fig. 5A,B) except that elaborate arborizations in the contralateral neuropil occur and that several branches are seen to usually enter the lateral connectives and Faivre's nerve. Processes leaving the contralateral roots are often observed.

These results suggest that the described pair of cells found in the sex ganglia of the three species are true N-cell homologues: (1) The shape of their action potentials is very similar to those of the midbody N cells; (2) the general morphology of the cell compares to the usual N-cell pattern; and (3) in *Macrobdella* and *Hirudo* (but not in *Haemopis*) responses to noxious stimuli to the skin have been observed.

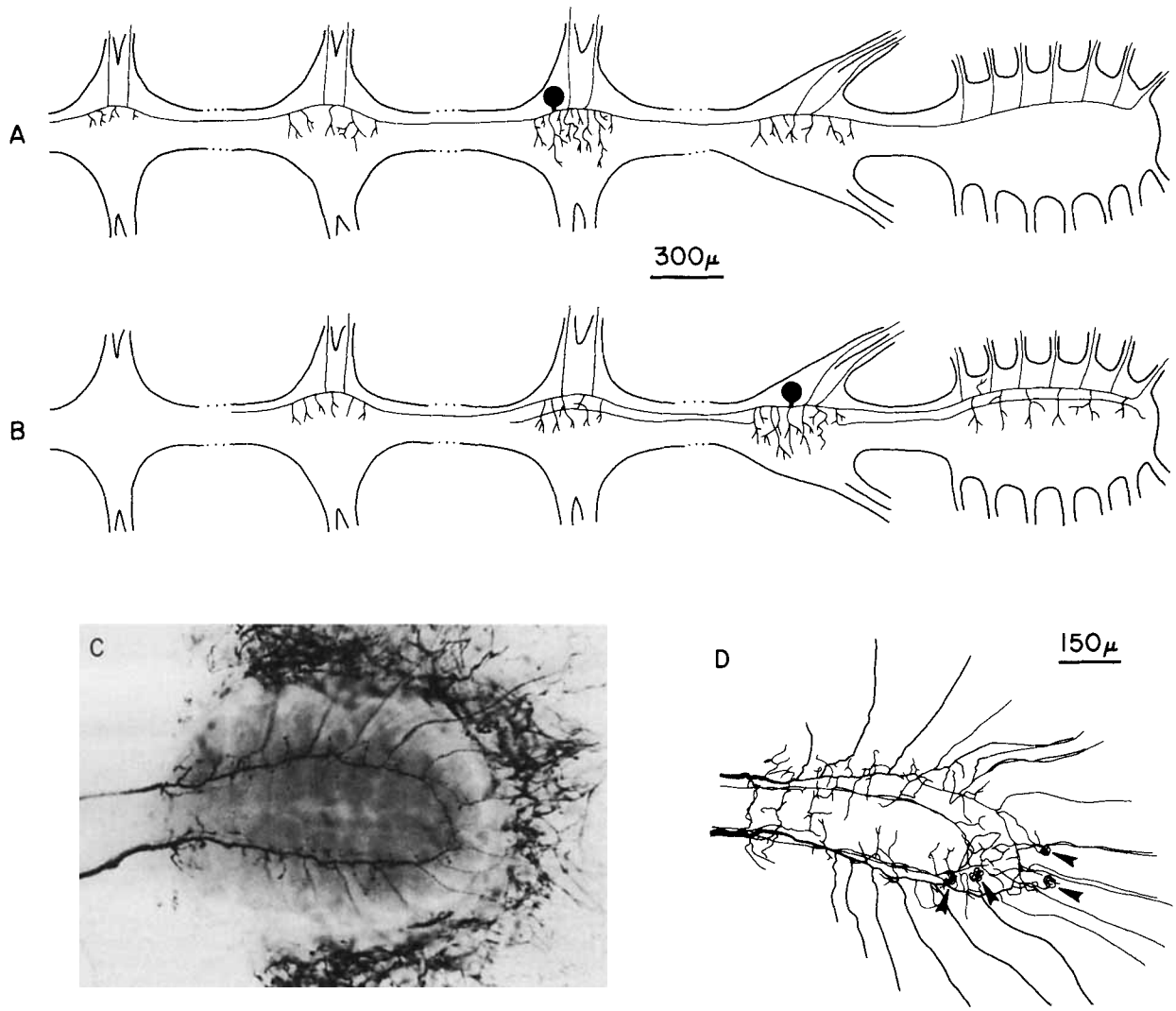


Fig. 7. A. Diagram of the typical branching pattern of N cells situated in ganglion 20. B. Diagram of the arborizations of N cells located in ganglion 21. C. Photograph of the arborizations in the tail ganglion of two HRP-injected N cells in ganglion 21. D. Camera lucida drawing of C. Arrowheads indicate processes which surround the soma of several unidentified cells. C and D are of the same magnification.

Ganglia 20 and 21. The maps of cell bodies binding Lan 3–2, Lan 4–2, and 3G8 previously described suggested that the caudal brain was devoid of nociceptive neurons and that only one pair of nociceptive neurons was present in each of ganglia 20 and 21. This was confirmed by electrophysiological recording from cells in the two ganglia. It seemed unlikely that the caudal part and the posterior sucker of the animal should be without capability to detect noxious stimuli, so we investigated the possible innervation of these areas by the N cells in ganglia 20 and 21. HRP injection of the neurons showed that both N cells (Fig. 7A,B) send processes into the caudal brain and that they have branches in all seven tail nerves. Figure 7C,D shows the detailed arborizations in the tail ganglion of the two N cells from ganglion 21 in *Hirudo.* In addition to the main projection, a finer secondary process running in another plane of the ganglion is usually found. The secondary arborizations are not as extensive as those seen in the head brain for the N cells of ganglia 1 and 2. The proximal processes span at least two more ganglia, and neurites leave each of these ganglia's ipsilateral roots (Fig. 7A,B).

To confirm the innervation of the posterior sucker of processes from N cells in ganglia 20 and 21, we mapped their receptive fields. The field indicated in Figure 8 is the skin area where stimulation evoked invading action potentials in the cell body. Attenuated potentials, probably due to conduction block, could sometimes be obtained by stimulation proximal to the depicted fields. The number of annuli are reduced in the last segments of the animal. The posterior sucker can be demonstrated to be innervated through processes in the connective between ganglion 21 and the tail brain by cutting the peripheral roots of ganglion 20 and 21 (Fig. 8). In this case responses from the posterior sucker only are obtainable. A sharp border between fields on opposite sides of the ventral midline of the posterior sucker was found.

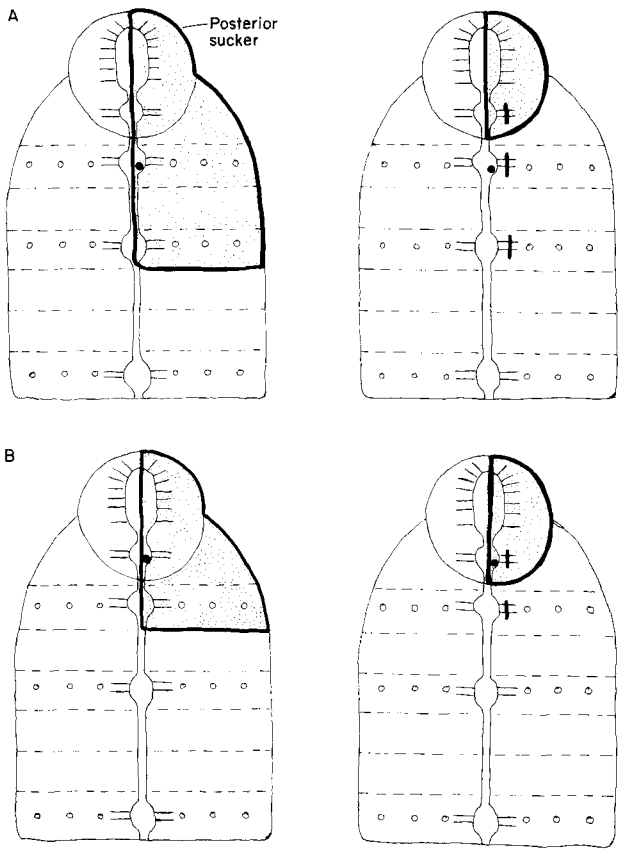


Fig. 8. The receptive fields of N cells in ganglia 20 and 21. A. Left: The field of N(20) with all peripheral roots intact. Right: The field obtained after the peripheral roots have been cut. B. Left and right: The receptive field of N(21) with peripheral roots intact and cut, respectively.

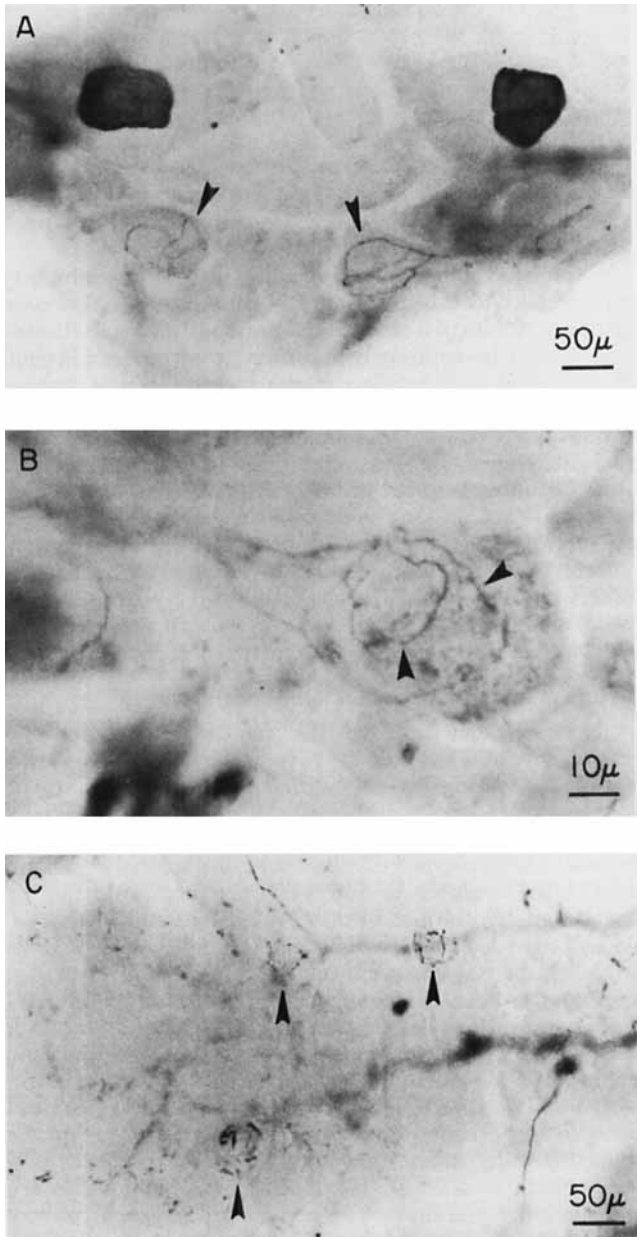


Fig. 9. A. Preparation stained with Lan 4–2 in *Haemopis*. Arrowheads indicate stained processes which surround the soma of two medial P cells. B. Ganglion stained with Lan 3–2 in which antibody-positive processes (arrowheads) are closely associated with the soma and axon hillock of a P cell. *Haemopis*. C. Photograph of a tail brain in which the somata of three cells are surrounded by processes originating from two HRP-injected N cells in ganglion 21. *Haemopis*. Arrowheads indicate stained processes.

Wraparounds. Figure 9A,B shows neurites stained with Lan 4–2 and Lan 3–2, respectively, which tightly surround the soma and axon hillock of P cells (identifiable by their size and position). This wraparound is not restricted to P cells. What presumably is N and Leydig cells have also been observed to be associated with the wraparound. That this phenomenon is due to processes originating from the N cells can be inferred from the HRP injection studies. As is indicated by the arrows in Figures 3D, 5B, and 7D, these wraparounds are consistently seen on cells in the neighboring ganglia of filled N cells. We have observed wraparounds in the same ganglion as the filled cell only in the case of Nsex, where the wraparounds occur on the contralateral side (Fig. 5B). Figure 9C shows three unidentified cells in the caudal ganglia surrounded by processes from the two N cells whose somata are located in ganglion 21. The phenomenon has previously been reported for midbody N cells by Muller et al. ('78). Our results suggest that this is a characteristic feature shared by all the homologous N cells along the nervous cord.

DISCUSSION

The nervous system of the leech is segmental and derived from embryonically homologous ganglia. However, the adult nervous cord is segregated into several distinct regions. We have investigated the segmental variation in distribution and morphology of a particular cell type, the nociceptive neurons. We used specific monoclonal antibodies against these cells to map their distribution. The results showed that the full set of four nociceptive neurons were found in ganglia 1–4 and 7–19. Only one pair was found in ganglia 5 and 6 and 20 and 21, whereas no nociceptive neurons seemed to be present in the caudal ganglion. This pattern was confirmed by electrophysiological means. In

addition, all the mapped cells were identified as N-cell homologues by their morphology and physiological properties. The staining pattern obtained suggested that the two N cells in the sex ganglia were of the medial type, whereas the pair present in ganglia 20 and 21 were of the lateral type. The distinction between lateral and medial N cells along the cord has been confirmed by pharmacological means (Johansen et al., '84). Monoclonal antibodies could in this way distinguish between two molecularly heterogeneous types of cells within a single sensory modality.

In the head ganglion the results show some ambiguity with respect to the number of N cells present. However, mapping results of a monoclonal antibody for a specific cell type should be cautiously interpreted without additional physiological confirmation of the identity of the stained cells, because the antigenic determinant recognized by the antibody may be very small and shared by several different molecules expressed by unrelated cells. On the other hand, lack of staining does not necessarily mean that a particular cell type is not present. As demonstrated by the results of the present study and by Sargent et al. ('77), there can be heterogeneity on the molecular level between neurons of an otherwise seemingly homogeneous cell type. In this case the agreement of the results of Lan 3–2 with those of Yau ('76) suggest that there are only two pairs of N cells present in the head ganglion.

The branching pattern of the N cells varies considerably along the nervous cord. Especially in the anterior and posterior segmental ganglia the N cells show extensive arborizations, spanning several segments and innervating the entire head and tail region. Wallace ('80) has reported similar results for AE and T cells, and presented evidence that neurons in embryonic leeches arborize in more ganglia than in the adult leech. The N-cell homologues in the sex ganglia and in the head brain (Yau, '76) also show variations of the basic pattern of N-cell morphology.

Intersegmental variation in the properties of identified neurons has been demonstrated for several other invertebrate preparations (Mittenthal and Wine, '78; Ghysen, '78; Bate et al., '81). Developmental studies suggest that the variation is dependent on the neuron's segmental determination and position (Ghysen et al., '83; Bate et al., '81) and that the adult axonal branching pattern of neurons can be shaped both by differential outgrowth and by loss of processes (Bate et al., '81). The mechanisms behind this are still virtually unknown. The application of specific monoclonal antibodies as markers in early embryonic development when electrophysiological recording is not feasible may provide means of elucidating these questions.

The sex ganglia are specialized segmental ganglia comprised of twice as many neurons as the standard midbody ganglia. This is probably due to the presence of neurons specifically associated with the function and innervation of the sexual organs. For example, four such neurons controlling penile eversion have been described by Zipser ('79). The sexual organs and their associated ganglia show species differences in gross morphology and number of neurons. In this study we report on species differences in the properties of identified homologous neurons. The N-cell homologues of ganglia 5 and 6 show a progressive degree of modification in the following order of the species: *Macrobdella, Hirudo, Haemopis.* However, in all three species the electric properties and major morphological features of the cells are indistinguishable from midbody nociceptive neurons, thus justifying the classification of the cells as N-cell homologues. The data obtained from the three species with regard to their peripheral branching patterns and physiological responses indicate that the N-cell homologues in ganglia 5 and 6 have undergone a progressive specialization and that the trend may be correlated with the degree of evolution and complexity of the reproductive system.

The N cells have been shown to be primary sensory neurons for nociceptive stimuli by a variety of tests (Nicholls and Baylor, '68). This study shows that a common feature of N-cell homologues is to have processes that tightly surround the cell soma of other cells. In addition to the cells already mentioned in the results section, the lateral N cell, but not the medial, has coiled terminals on the peripherally located Hoover cells (Blackshaw et al., '82). The functional importance of these processes is unknown. In the leech as in other invertebrates no functional synapses have been found on the cell soma (Coggeshall and Fawcett, '64; Gerschenfeld, '73). The cells that receive surrounding processes from the N cells all have electrically excitable somata. It is possible that the N cells in some way modulate the excitability of these neurons, perhaps through neurosecretion.

The possibility exists that N cells have other functions in addition to the sensory and that the two kinds of N cells may be functionally different. In the phylogenetically more primitive glossophiinid leech *Haementeria* the segmental ganglia have a smaller number of cells (Macagno, '80) and only one pair of N cells is present in each ganglion (Kramer and Goldman, '81). It is therefore feasible that a pair of N cells with additional specialized properties has evolved from the original single pair of N cells.

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