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A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF THE SACRAL PARASYMPATHETIC NUCLEUS AFTER LABELLING PRIMARY AFFERENT AND EFFERENT ELEMENTS WITH HORSERADISH PEROXIDASE

The Ohio State University PH.D. 1984

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A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF THE SACRAL PARASYMPATHETIC NUCLEUS AFTER LABELLING PRIMARY AFFERENT AND EFFERENT ELEMENTS WITH HRP

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

GARY M. MAWE, B.S.

* * * * *

The Ohio State University

1984

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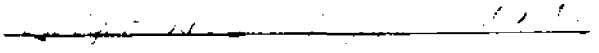

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Department of Anatomy

For Dad and Mom

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INTRODUCTION

Recent electrophysiological and anatomical studies have contributed a great deal to our understanding of the sacral autonomic reflex pathways, especially with regard to innervation of the distal colon and urinary bladder (for a review see deGroat, et al., 1981). Electrophysiological experiments have determined that neural pathways mediating micturition and defecation are distinct. Defecation involves a spinal reflex arc that includes unmyelinated (c-fiber) axons in its afferent and efferent limbs (deGroat and Krier, 1978). Following transection of the spinal cord at low thoracic levels, this response recovers very rapidly from an initial period of spinal shock. After recovery, the electrophysiological characteristics of the defecation reflex are unchanged, indicating that it is normally mediated at the spinal level.

Micturition is mediated by spinal and spino-bulbo-spinal reflex pathways utilizing lightly myelinated and unmyelinated afferent axons and lightly myelinated efferent axons (Barrington, 1931; Kuru, 1965; deGroat, 1975). Electrical stimulation of bladder afferent fibers elicits a biphasic response in vesical postganglionic parasympathetic

axons (Milne and deGroat, 1979; deGroat, et al., 1979). The early response is initiated by small myelinated (A-delta fiber) afferent axons, and has an average latency of 100 msec. The late response, which has a smaller amplitude and is undetected in some animals, has an average latency of 190 msec and is elicited by unmyelinated afferent fibers (C-fibers). Transection of the spinal cord at low thoracic levels eliminates both responses. However, approximately one week after the transection, the long latency C-fiber response recovers (Milne and deGroat, 1979; deGroat, et al., 1979). It is interesting to note that the recovery period of this reflex corresponds to the onset of automatic micturition, characteristic of spinal animals and humans. These data, along with other observations, indicate that the short latency response involves a spino-bulbo-spinal pathway, and that the long latency response occurs at the spinal level.

Retrograde transport of horseradish peroxidase (HRP) has been used to study the morphology of the sacral parasympathetic nucleus (SPN) in the cat as well as several other species (cat: Nadelhaft et al., 1980; deGroat et al., 1978; Sato et al., 1978; Yamamoto et al., 1978; rat: Hancock and Peveto, 1979; Nadelhaft and Booth, 1982; dog: Petras and Cummings, 1978; and monkey: Nadelhaft et al., 1983). The SPN of the cat is comprised of two separate cell groups in the intermediolateral gray of sacral spinal

segments one, two, and three, which are arranged in the form of an inverted "L" (Nadelhaft et al., 1980). A dorsal band (DB) of cells oriented mediolaterally in lamina V innervates the colon. The bladder is innervated by a lateral band (LB) of cells arranged dorsoventrally in lateral lamina VII.

Studies using transganglionic transport of HRP, following application of the enzyme to the pelvic nerve, have shown that dorsal root afferents from pelvic viscera project to the region of the SPN (Morgan et al., 1981; Nadelhaft et al., 1983). Most of these projections are collaterals from afferent axons in Lissauer's Tract which pass medially or laterally in lamina I along the edge of the dorsal horn. Axons passing medially, described by Morgan et al. (1981) as the medial collateral pathway (MCP), arborize in lamina X and medial lamina V, while those passing laterally, the lateral collateral pathway (LCP), arborize in lateral laminae V and VII. These axons are located in close proximity to dendrites of SPN preganglionic neurons. However, due to the long central delays (45-70 msec) described for both the colon and bladder reflexes (deGroat and Krier, 1976; Milne and deGroat, 1979), it has been suggested that only polysynaptic rather than monosynaptic connections exist in cats with an intact neuraxis (Morgan et al., 1981; deGroat et al., 1981).

In the present study, HRP was applied to the cut ends of dorsal and or ventral sacral roots. This technique provides more detailed visualization of the morphology of afferent and efferent neural elements. Application of HRP to the central ends of cut dorsal rootlets fills axons of all calibers and allows for observation of the distribution of primary afferent axons and their swellings at the light microscopic (LM) and electron microscopic (EM) levels (Beattie et al., 1978; Light and Perl, 1977, 1979; Proshansky and Egger, 1977). HRP applied to cut ventral rootlets diffusely fills motoneuron and preganglionic neuronal somata and dendrites resulting in a Golgi-like labelling of these neurons (Light and Metz, 1977; Liuzzi, et al., 1983).

The improved resolution provided by these techniques has allowed an approach to the following questions: 1) What is the laminar and geometric extent of preganglionic neuron dendrites in the cat sacral spinal cord?; 2) Do neurons in the SPN receive direct input from primary afferent fibers?; 3) What are the cytological features of primary afferent synaptic terminals exist in the SPN?; 4) Do primary afferent axons terminate directly on preganglionic neurons?; and 5) Do ventral root afferent fibers terminate on neurons in the SPN? Preliminary accounts of these experiments have been reported (Mawe et al., 1983, 1984 a, b).

METHODS

Thirty eight adult mongrel cats were used in this project; most were females ranging between 5 and 7 pounds. Animals were anesthetized initially with suritol, intubated and respirated artificially. Supplemental intravenous injections of pentobarbital (55 mg/Kg) were administered as needed to maintain a deep level of anesthesia. Core temperature and end tidal carbon dioxide levels were monitored throughout the procedure. The sacral spinal cord was exposed laterally by removal of the 5th and 6th lumbar laminae, transverse processes and pedicles. The lateral approach was employed rather than a standard laminectomy because it provides direct access to ventral as well as dorsal roots and allows for precise matching of dorsal and ventral rootlets for labelling. Animals were paralyzed with Pavulon (0.1 cc/4.5 Kg) during manipulation of the roots.

Surgical manipulation of dorsal roots only was performed in 13 cases, ventral roots only in 10 cases, and both dorsal and ventral roots in 15 cases. Rootlets were gently dissected from the surface of the spinal cord and cut 2-3 mm from their entrance or exit. After gentle washing of the cut ends with distilled water, chips of dry,

concentrated HRP were applied to the rootlets. While keeping the field as dry as possible, HRP was reapplied as necessary for 30 minutes to one hour. At this point, excess HRP was flushed from the sites of application, the cord was covered with gel-foam, the wound was closed in layers, and deep anesthesia was maintained for an additional 10-20 hours. The cats were sacrificed by intracardiac perfusion with 1L of saline containing an initial 30 mg bolus of xylocaine followed by 2L of an aldehyde fixative prepared in Sorenson's phosphate buffer (1% glutaraldehyde, 3% paraformaldehyde; pH 7.3; 1410-1430 mOsm). The tissue was then removed and placed in fresh fixative for 3-6 hours followed by overnight immersion in a 30% sucrose solution in phosphate buffer.

Sections were cut transversly or parasagittally (2 cases) at 60 um on a Vibratome (Oxford) and placed in trays with nylon screen bottoms. The sections were then rinsed once in phosphate buffer, blotted on paper towels, and placed in 5% cobalt choride for intensification of the reaction product (Adams, 1977). Following blotting and four rinses in phosphate buffer, the sections were incubated at 37 degrees centegrade in 0.05% diaminobenzidine (DAB) for 20 minutes. Four ml of 0.06% H202/100ml of DAB solution were then added for 5-15 minutes and the tissue was gently agitated on a rotator. Sections were intermittently examined at low power to monitor the magnitude of the

reaction product formation. When labelled structures such as axons and dendrites could be clearly defined, the tissue was rinsed three times in phosphate buffer to discontinue the reaction and minimize reaction product formation in structures containing endogenous peroxidase and in blood vessels.

Alternate sections were mounted on chrome-alum coated glass slides and counterstained for Nissl substance. Remaining sections were placed in 1% osmium tetroxide for 1 hour, stained en block with uranyl acetate, dehydrated in graded alcohols, and flat embedded in Maraglas resin between two sheets of Aclar (Allied Chemical Company) (Mawe et al., 1983a). These sections were examined light microscopically and were then trimmed and glued to blank Beem capsules for sectioning on a Reichert OMU-2 ultramicrotome. One micron thick sections were taken and stained with toluidine blue. Ultrathin sections were picked up on Formvar coated slotted grids, stained with uranyl acetate and lead citrate, and were examined with a Phillips 301 electron microscope.

DATA ANALYSIS:

Sections counterstained with cresyl-violet as well as sections embedded in Maraglas were examined at the LM level on a Leitz Orthoplan microscope with bright field and differential interference contrast optics. Selected

sections were photographed. Camera lucida drawings at several magnifications were made of labelled PGNs and primary afferent fibers within the SPN.

In order to characterize the distribution of preganglionic neurons within the SPN, maps of the locations of all labelled S2 PGNs were made from three cases with an analog X-Y plotter (Houston Instruments, 2000 recorder) interfaced to a Leitz Orthoplan microscope. These maps were plotted on camara lucida drawings of every third section. Counts of labelled preganglionic neurons were also made during this process. In order to avoid the possibility of counting the same cell in two adjacent sections, corrections were made using the formula $N=N'(t/t+a)$, in which N is the corrected number of cells, N′ is the number of cells counted, "t" is the section thickness, and "a" is the average cell diameter (see Abercrombie, 1946; Nadelhaft et al., 1980). Measurements of average cell diameter (minor axis) were made on camara lucida drawings prepared using a 63X oil objective.

Prior to electron microscopic analysis, the vibratome sections were carefully documented at the LM level. Camera lucida drawings of the sections were done to locate labelled neurons and axons, fiber bundles and blood vessels. The sections were also photographed with black and white, and color slide film. The area of interest was then carefully trimmed from the section under a dissection

microscope and glued to a blank Beem capsule for ultramicrotomy. Drawings made from one micron sections were overlayed upon drawings of the 60 um vibratome sections for definite identification of labelled neurons. The 1 um drawings were then used as maps to locate labelled profiles during electron microscopic examination of subsequent ultrathin sections. Ultrastructural sampling was limited to the SPN by constant reference to these drawings. Myelinated axons passing ventrally from the dorsal columns into the intermediomedial gray region served as a medial boundary for sampling in the DB region. (Figures 36-39 illustrate a labelled PGN which was photographed in a 60 um section, a one micron section, and with the electron microscope.)

The initial classification of presynaptic profiles was based on the shapes of synaptic vesicles. These were divided into three categories: round (or spherical), pleomorphic, and flattened. Criteria for the categorization of vesicle containing terminals were based on those formulated by Palay and Chan-Palay (1974). Presynaptic profiles classified as round vesicle terminals contained almost exclusively spherically shaped vesicles. Pleomorphic vesicle terminals contained both spherical and more or less flattened vesicles in different proportions. Terminals of the flattened vesicle category contained almost exclusively flattened vesicles. On occassion, terminals with uniformly oval vesicles were observed. Since the longitudinal

orientation of vesicles and mitochondria in these termials were the same, the shape of vesicles was attributed to stretching of the formvar substrate. Such terminals were classified as spherical. Terminals were further classified on the basis of the presence and number of dense core vesicles (DCVs).

Postsynaptic elements were classified as somatic, dendritic or axonic. Sizes of dendritic profiles were determined by measuring cross sectional diameter in electron micrographs. Characterization of postsynaptic elements containing vesicles was the same as described for presynaptic elements. Pre- and postsynaptic densities were not used as a criterion for terminal classification because reaction product in labelled profiles often precluded accurate assessment of thickness of these features.

RESULTS

LIGHT MICROSCOPIC OBSERVATIONS

Light microscopic results similar to and predictive of some of those described here have been reported previously (e.g. Nadelhaft, et al., 1980; Morgan, et al., 1981). However, technical differences exist between this study and previous reports. In the present study, HRP was applied to spinal roots rather than more peripherally to the pelvic nerve. This procedure, while sacrificing the specificity of identifying peripheral sources of efferent and afferent elements, provides more complete labelling and thus better resolution of structural detail. Also, in this study light microscopic results were used as a basis for electron microscopic examination of the same tissue. In light of possible differences in the results provided by these techniques, a relatively detailed description of light microscopic observations will be presented.

After application of HRP to ventral roots S1, 2, and 3, motoneurons and preganglionic neurons (PGNs) are labelled (Figs. 1 and 2). Sacral PGNs are located laterally in the intermediate gray region and base of the dorsal horn, and have their major dendritic spread in the

transverse plane. Neurons of the dorsal band (DB) typically are mediolaterally oriented (fig. 3, arrows), and PGNs of the lateral band (LB) typically are oriented dorsoventrally (fig. 2 and fig. 4, arrows). In this tissue, the reaction product in the cells differed in appearance: many PGNs and almost all motoneurons had dark diffuse reaction product (fig. 5, cell 1); some PGNs in the DB and most PGNs in the LB region had light diffuse label with granules (fig. 5, cell 2); and some cells in the LB and most in the DB contained only granular label (figure 5, cell 3). In neurons with granular label, dendrites could not be traced very far from their somata. However, dendrites of diffusely labelled neurons could often be traced hundreds of micrometers from their cell bodies (figs. 9-13).

Number and distribution of PGNs

Analysis of PGN distribution and numbers was limited to the S2 spinal segment which previously has been shown to contain the majority of cat sacral PGNs (Nadelhaft et al., 1980). A case in which all sections were prepared for light microscopy was used for cell counts and distribution analysis. In this case, HRP was applied to all S2 ventral rootlets as well as the ventral rootlets of caudal S1 and rostral S3. The number of labelled cells in S2 was 913. This figure was obtained after using the correction factor described in the methods section. Labelled cells in the

dorsal and lateral bands have slightly different diameters: DB cells have a mean minor axis of 13.3 um with an S.D. of 2.7 um (n=50); the mean minor axis for the LB cells was 17.2 um with an S.D. of 4.6 um (n=50).

The transverse and longitudinal arrangements of labelled S2 PGNs within the SPN are illustrated in figures 6 and 7. Figure 6 is a composite drawing of a series of cross-sections organized from caudal to rostral, with PGNs from three 60 um sections indicated on each cord section. In general, the distribution of PGNs is consistent throughout the S2 segment with the LB forming the back and the DB forming the base of an inverted "L". In some sections the separation between the LB and DB is fairly distinct (e.g. fig. 6, section F). Cells of the LB are primarily located in the lateral aspect of Rexed's (1954) lamina VII, although some cells were also found in laminae V as well as in the lateral funiculus (fig. 6, sections C and F). Neurons of the DB are situated mainly in lamina V. However, labelled DB cells were often found in dorsal lamina VII and laterally in lamina I. The extent of lamina VI is minimal at this level of the spinal cord (figure 8; Rexed, 1954).

The rostro-caudal distribution of labelled PGNs in S2 is illustrated in figure 7. Although fewer cells were labelled caudally, there was a relatively even distribution of neurons without obvious periodicity.

Morphology of PGNs.

The shape of PGNs in the sacral spinal cord varies with their location within the SPN. Three general groups of PGNs can be described: 1) neurons of the DB located laterally, in or adjacent to lamina I (cell A, fig. 8); 2) neurons in the DB located ventral and medial to those in group 1 (cell B, fig. 8); and 3) neurons of the lateral band (cell C, fig. 8). Neurons of the first group were almost always observed to be fusiform and bipolar, with one dendrite extending dorsolaterally, parallel to the lateral edge of the dorsal horn in lamina I. The other primary dendrite passed ventromedially across the base of the dorsal horn into laminae V and VII. Dendrites of these neurons demonstrated very little branching. Examples of this type of neuron are illustrated in figure 4 (open block arrow), figure 9 (cell 1), and figure 31.

Most neurons in the DB were classified in the second group. Neurons in this category are mediolaterally oriented, are fusiform or oval in shape, and may be bipolar or multipolar (figs. 9, cell 2; fig. 10). Dendrites which passed medially usually took a horizontal course and often extended contralaterally into laminae V and VII (figs. 9 and 10). Some medially extending dendrites projected dorsally into lamina IV. Laterally extending dendrites of

these DB neurons usually take a dorsolateral course along the lateral edge of the dorsal horn (figs. 9, cell 2; fig. 10), although some follow fascicles of dendrites into the lateral funiculus.

The third group, constituting the neurons of the LB (fig. 2;c in fig. 8; figs. 11, 12, and 13), is the most heteromorphous group of the three. Neurons in the LB are oriented dorsomedially, parallel to the lateral edge of the intermediate gray matter. Cell bodies of these neurons are fusiform, oval, or triangular (fig. 2, open block arrow) in shape, and although some exibit bipolar dendritic patterns (fig. 12), most are multipolar (figs. 11 and 13). Dendrites of LB neurons which extend dorsally from the cell body either course along the gray-white border into lamina I (fig. 11, open block arrow) or dorsomedially into the dorsal band region (fig. 13, block arrow). In some cells, a single dorsally extending dendrite bifurcates into both regions (fig. 12, open block arrow and block arrow). Dendritic branches of LB neurons which extend laterally into the lateral funiculus either pass horizontally into the lateral funiculus (fig. 13) or dorsolaterally into the dorsolateral funiculus. A bundle of such dendrites is indicated by the block arrow in figure 2. Dendrites which course ventrally from LB neurons can be traced along the lateral edge of the intermediate gray matter and ventral horn (figs. 11 and 13).

Axons of neurons in all three groups emerge from the soma or a proximal dendrite and pass ventrally along the lateral edge of the ventral horn (figs. 9-12, A) to enter the ventral root with fascicles of motoneuron axons. Labelled axons were examined for the presence of collateral branches which re-enter the SPN, but none were observed. Other fine axon-like processes were seen entering the lateral funiculus after emerging from PGN primary dendrites and axons.

Afferent distribution in the SPN

Following application of HRP to the dorsal roots of the three sacral segments, primary afferent fibers of all calibers were labelled. In the 13 cases in which the enzyme was applied only to the dorsal rootlets, no labelled neurons were seen. The LCP and MCP described by Morgan et al, (1981), were easily identified along the lateral and medial edges of the dorsal horn (fig. 16). The LCP, which is the most prominent contingent of afferent fibers at this level of the spinal cord, arises from the Tract of Lissauer. It is a fascicle of axons, 30 to 70 um wide in transverse section, which passes ventrally along the lateral border of the dorsal horn in lamina I. The density of the LCP varies when observed in transverse sections (compare fig. 15 with figs. 17 and 18). One source of such variability is a periodic fasciculation of axons as they

pass ventrally through lamina I (figs. 22 and 23, open block arrows). The periodicity, which is most easily observed in sagittal sections becomes more obvious as the fibers approach the SPN (figs. 23, 24, and 26). Occasionally fascicles of LCP axons leave the main bundle and course ventrally in the lateral funiculus, often following cords of neuropil in the reticulated region of the dorsal horn to reenter the intermediate gray matter (fig. 14, arrow). The LCP most commonly expands into a broad terminal field in lateral lamina V (figs.14 and 18). Some of the axons continue ventrally into the LB region (fig 14, arrowheads) where they extend as far as the intermediolateral nucleus described by Rexed (fig. 8, IML). In some sections, a fascicle of axons could be followed medially across the base of the dorsal horn projecting into the dorsal gray commissure (fig 15, arrowheads). In fortuitous sections some of these axons could be traced to a terminal field in lateral lamina III and IV of the contralateral spinal gray (fig. 20). Other axons passing medially in dorsal lamina VII veer dorsomedially to terminate in medial lamina V (figs 16 and 17). As the LCP enters the dorsal aspect of the SPN, fibers spray into the neuropil (figs. 14 and 18) and exhibit frequent varicosities (fig. 18; fig. 19, arrowheads). Such varicosities probably represent boutons en passant (e.g. Beattie et al., 1978).

Ventral root afferents.

Following application of HRP to the ventral root alone, small fascicles of fine fibers were observed entering the SPN from the ventral aspect (fig. 35) and became varicose (fig. 27) These labelled fibers, which are qualitatively similar to dorsal root afferent axons, are thought to be ventral root afferent axons. They were seen continuing into lamina I along the lateral and dorsolateral edges of the dorsal horn (figs. 28 and 29). Labelled fibers were frequently encountered in Lissauer's tract as well (Beattie et al., 1984).

Afferent-PGN interactions.

In cases in which injury filling of both dorsal and ventral roots was performed, swellings on labelled axons of the LCP were frequently observed in close apposition to the somata and dendrites of labelled PGNs. Spindle-shaped PGNs in the DB situated in lamina I and dorsolateral lamina V (e.g. fig. 8, cell A) were seen embedded in fascicles of labelled LCP axons (figs. 26, 30 and 31). This group of PGNs was the most heavily contacted by dorsal root afferents. However, varicosities on afferent axons were also observed in apposition to labelled preganglionic neurons located more ventrally in the DB and the dorsal aspect of the LB (figs. 32 and 33).

Following injury filling of the ventral root alone, varicose axons also appeared to be in close apposition to labelled PGNs (figs. 34 and 35). These appositions of putative ventral root afferent fibers were most often observed in the lateral band region.

ELECTRON MICROSCOPIC OBSERVATIONS

Sections embedded in plastic which had well labelled afferent fibers, PGNs, or both, were chosen for electron microscopic examination so that direct correlations could be made between the LM and EM observations. An example of a labelled cell that was observed in a 60 um thick plastic section is shown in figure 36. It was identified in subsequent 1 um thick (fig. 37) and ultrathin (figs. 38 and 39) sections. In toluidine blue stained 1 um sections, diffusely labelled profiles appear light brown, whereas granular reaction product in labelled cells appears as dark brown grains within the cytoplasm. At the EM level, diffusely labelled profiles are characterized by electron dense reaction product which was typically aggregated on membrane surfaces (e.g. D in fig. 43) and, less frequently, dispersed in the cytoplasm (e.g. fig. 63) (also see Beattie et al., 1978). Diffuse plus granular label was frequently encountered. In these cells. In addition to that just described, reaction product was also observed in lysosomal elements, vesiculotubular elements, and occasionally within

Golgi cisternae (figs. 38 and 39). Reaction product in membrane bound inclusions was the only type of label observed ultrastructurally in cells exhibiting only granular label.

Neuropil of the SPN

When examined in 1 um and ultrathin sections, the SPN is characterized by small neurons, large numbers of unmyelinated axons and relatively few myelinated axons (fig. 40). Nuclei of SPN neurons are typically oval with aggregated chromatin dispersed throughout and often heaviest along the nuclear membrane (figs. 38 and 65). Indentations of the nuclear membrane were frequently observed. In the somata, parallel cisterns of rough endoplasmic reticulum and polysomes exist singly or in clusters were a ubiquitous feature (e.g. fig. 38). In ultrathin sections, the Golgi apparatus was observed in several locations in the cytoplasm. Synaptic terminals, which were sometimes observed in clusters, contacted the somata of neurons in the SPN (figs. 41 and 66). Most of the somatic surface, however, was covered by glia (figs. 38 and 41). Occasionally, dendrites and axons were apposed to the cell surface (fig. 41).

Dendritic profiles in the SPN range from approximately 0.1 um to 6.5 um in diameter, as determined from measurements of dendritic profiles (n=259 from 3 cases)

with synaptic contacts taken from electron micrographs of the SPN. However, very few dendrites in the 3.0 um to 6.5 um diameter range were observed. The predominance of small dendrites is reflected in the large percentage of terminals on dendrites measuring less than 3.0 um in diameter (figs. 48, 49, 50, and 69). Dendrites and somata occasionally contain spherical and dense core vesicles (fig. 64, open arrows).

In transverse sections, large fascicles of unmyelinated axons were seen entering the SPN from the dorsolateral aspect (Fig. 40, LCP) and were frequently observed to be labelled in dorsal root cases (Mawe et al., 1984). These axons often contain rows of clear spherical and dense core vesicles. Small myelinated axons are evenly dispersed throughout the SPN and often contain reaction product following application of HRP to the dorsal or ventral roots. Some bundles of unmyelinated axons pass rostrocaudally in the SPN. Large myelinated fibers were usually found in bundles oriented rostrocaudally near the lateral border of the gray matter (figs. 2 and 40, asterisks). These bundles of axons frequently separate cords of neuropil containing PGNs and their dendrites from the main portion of the nucleus. Other large myelinated axons were seen in small mediolaterally oriented fascicles (fig. 40, block arrows) passing from the lateral funiculus through the SPN into the deep dorsal horn and intermediate

gray.

Axon terminals were seen throughout the SPN in contact with somata, dendrites, spines, and other axon terminals. Presynaptic terminals in this nucleus typically contained round or pleomorphic vesicles and very often contained DCVs as well. Very few terminals with flattened vesicles were seen.

Labelled PGNs

Preganglionic neurons of the second sacral segment, which were labelled by application of HRP to ventral rootlets, were examined at the EM level. The ultrastructure of such neurons in the SPN is similar to that described for all neurons in this region. Axosomatic synapses on PGNs occurred alone or in directly apposed groups of two or more terminals (fig. 41). These terminals contained round or pleomorphic vesicles, and often DCVs.

Although dendrites of PGNs ranged from <0.5 um to 4.5 um in diameter as measured on electron micrographs, the majority of them measured between 0.5 um and 2.0 um. Occasionally, labelled profiles which appeared to be dendrites exhibit swellings containing large numbers of spherical vesicles (fig. 42, block arrow).

Terminals on labelled dendrites (n=193 from 3 cases) contained round (79.7%; figs. 42, and 43) or pleomorphic vesicles (20.3%; figs. 44, 45, and 46). Those containing

round vesicles often contained DCVs as well (66%; fig. 43) and were occasionally found in association with postsynaptic dense bodies (fig. 43, arrowheads). Terminals with pleomorphic vesicles often contained DCVs as well (50%; fig. 44, term. 1, and figs. 45 and 46). No postsynaptic dense bodies were seen in association with these terminals. The presence and number of DCVs in terminals synapsing on PGNs is shown graphically in figure 47 (hatched bars).

The postsynaptic distribution of terminals on PGNs was also examined quantitatively (figs. 48 and 49). The most frequent sites of terminal-PGN interactions were on somata and on dendrites ranging from 0.5um to 1.5 um in diameter. This finding was not surprising since most labelled dendrites were in this size range. An analysis of the postsynaptic distribution of round versus pleomorphic vesicle terminals on labelled PGNs revealed that round vesicle terminals were most often found on the somata and more distal dendrites (fig. 49, open bars). Terminals with pleomorphic vesicles had a similar predilection for somatic contact sites, but were more frequently encountered on more proximal dendrites (fig. 49, hatched bars).

Labelled afferents

Labelled S2 dorsal root afferent terminals were

examined at the EM level following application of HRP to the dorsal or dorsal and ventral roots. Primary afferent terminals (n=241 from 3 cases) preferentially distribute to small diameter dendritic elements in the neuropil, with 81% of the population terminating on profiles less than 1.0 um in diameter (fig. 50; figs. 51-56). Few labelled terminals were encountered on somata (fig. 50; figs. 64 and 65). All dorsal root afferent terminals that were examined contained clear spherical vesicles and 66% of these contained DCVs as well (fig. 47, open bars; fig. 54).

Occasionally, profiles which were postsynaptic to labelled afferent terminals contained round (n=5; fig. 59) or pleomorphic (n=5; figs. 57, 58, and 60) synaptic vesicles. These postsynaptic profiles may be axon terminals, or type 2 dendrites (Gobel, 1976), or both. One or more DCVs were present in three of the five postsynaptic profiles with round vesicles and four of the five with pleomorphic vesicles. Small clusters of vesicles were sometimes observed in the postsynaptic profile (figs. 57 and 58), but that was not always the case (figure 59). In two of the ten examples, synaptic vesicles were clustered adjacent to the postsynaptic membrane near the region of the synaptic complex suggesting the possibility of reciprocal synapses (figs. 58 and 59, arrows).

Labelled terminals were also observed in the SPN following injury filling of the ventral roots alone with

HRP (figs. 61, 62, and 63). These terminals (n=18 from 1 case), although present in much smaller numbers, are qualitatively similar to axon terminals of dorsal root origin. Twenty eight percent of the terminals contained only clear spherical vesicles while 78% also contained DCV's (figs. 61, 62, and 63). Terminals on dendrites less than 0.5 um in diameter accounted for 44.5% of the population; another 44.5% of the terminals synapsed on dendrites with diameters ranging from 0.5-1.5 um.

Afferent-PGN interactions

Labelled afferent terminals were observed synapsing on labelled PGN's after application of HRP to both the dorsal and ventral roots (figs. 64-68). In corroboration with the LM results, these interactions were most often seen in the dorsolateral aspect of the SPN. Of 23 dorsal root afferent terminals apposed to PGN's, 30% were located on the somata (fig. 69; figs. 64 and 65). It is interesting to note that six of seven dorsal root afferent terminals that were observed synapsing on somata in the SPN contacted PGNs. Labelled terminals contacting labelled dendrites less than 1 um in diameter constituted 43% of the population (figs. 66; and 69, hatched bars). The identity of some postsynaptic profiles could not be definitely determined, e.g. the serial sections shown in figures 67 and 68. Fifty six percent of the dorsal root afferent terminals

contacting PGNs contained DCV's (fig. 70, hatched bars).

Labelled terminals were also seen adjacent to labelled neurons after application of HRP to the ventral rootlets alone (figs. 71 and 72). Terminals of this type (n=7) contacted dendrites less than 1 um in 57% of the cases (fig. 69, open bars) and contained clear spherical vesicles with 85% of the population also containing DCVs (fig. 70, open bars). While in some cases a synaptic complex was not apparent (fig. 71), in other instances a complex was clearly seen (fig. 72).

DISCUSSION

The reaction product

When HRP is applied to a cut axon, passive diffusion occurs within the axoplasm for a limited distance (approximately 10 mm; Light and Perl, 1979a) and can appear as light or dark diffuse reaction product at the light microscopic (LM) level depending on the density of reaction product present. The axon can also package horseradish peroxidase (HRP) and transport it for long distances back to the cell body and into the dendrites with the resultant reaction product appearing granular at the LM level and as dense membrane bound inclusions at the electron microscopic (EM) level (Lavail and Lavail, 1974; Broadwell et al., 1980; Tsukita and Ishikawa, 1980; Mawe et al., 1983).

In the current experiment, three types of retrograde label in preganglionic neurons (PGNs) were observed after application of HRP to ventral rootlets. First, granular label was most often seen in dorsal band (DB) neurons and in some of the lateral band (LB) neurons. Second, in some DB and a large proportion of LB neurons, light diffuse plus granular label was found. Third, dark diffuse label was observed in some LB neurons and nearly always in ventral

horn motoneurons. When dark diffuse neurons were examined ultrastructurally, they were found to contain membrane bound reaction product as well, which was masked at the LM level by the high density of diffuse reaction product. Similar categories (Keefer, 1978) and distributions (Light and Metz, 1979) have previously been described for retrogradely labelled neurons in the ventral horn.

Such qualitative differences in the reaction product may be caused by several variables. One potential variable is distance of the somata from the HRP application site. Ventral horn motoneurons, which are closest to the ventral rootlets, label very densely; neurons of the DB, which are furthest away from the site of application, exhibit mainly granular and light diffuse plus granular label; and PGNs of the LB, which are located at an intermediate distance exhibit all three types of label. Axon diameter may also influence the labelling pattern. Motoneurons have the largest axons and are the most darkly labelled, whereas, the DB neurons have the smallest axons (deGroat et al., 1982) and are the most lightly labelled. Lateral band neurons have axons diameters which fall into an intermediate range and they demonstrate all three types of reaction product. Axon diameters might influence the amount of HRP entering the cell with larger axons simply providing larger channels for diffusion of HRP from the application site. Alternatively, larger axons may take longer to seal

their membrane defect than small axons, and thereby provide longer exposure times of the axoplasm to the high concentration of extracellular HRP at the application site. However, all three forms of labelled PGNs are sometimes situated in close proximity to one another within the SPN (eg. fig. 5). Such variation could be due to different concentrations of HRP in the microenvironments of individual axons, or possibly the angle at which individual axons are cut. For example a 45 degree cut would result in a larger membrane defect than a perpendicular cut. Also, axons of closely adjacent PGNs may have different axonal sizes or may exit the spinal cord via different rootlets resulting in different distances between HRP application and the somata.

Primary afferent fibers entering the spinal cord via the dorsal and ventral roots were labelled in the present study by anterograde diffusion of HRP. Afferent axons of all diameters were visualized at the LM level as densely labelled fibers with en passant and terminal varicosities. At the EM level, labelled afferents and their synaptic terminals are characterized by electron dense reaction product which is observed in the cytoplasm and aggregates on the surfaces of membranes.

Preganglionic neurons

In the present study, injury filling of axons with HRP proved to be a very effective means of labelling sacral PGNs. Spatial distribution, cell counts, and orientations of PGNs reported here are consistent with results provided by application of HRP to the pelvic nerve in the cat (e.g. Nadelhaft et al., 1980). In addition, a more detailed resolution of PGN dendritic arborizations was made possible by the current technique even in the presence of overlapping labelled primary afferent fibers. Also, injury filling of PGNs with HRP yields a reaction product that is easily visualized at the EM level, and thus has allowed for the first time an ultrastructural description of synaptic profiles terminating on identified sacral PGNs.

In the cat, PGNs in the SPN are situated in the form of an inverted "L" in the intermediolateral gray region of the cat sacral spinal cord. Lateral band neurons form the back and dorsal band neurons form the base of the "L". This pattern was also described in peripheral nerve studies (Yamamoto et al., 1977; Morgan et al., 1979; deGroat et al., 1979; Nadelhaft et al., 1980). In this species, viscerotopic organization of sacral PGNs has been described anatomically (Nadelhaft et al., 1980) and physiologically (deGroat et al., 1982). Neurons innervating the large intestine are located in the DB region, while PGNs innervating the urinary bladder are located in the LB. Such

viscerotopic division of the SPN was not seen in the rat (Hancock and Peveto, 1979; Nadelhaft and Booth, 1982) or in the monkey (Nadelhaft et al., 1983).

In the current experiment, the number and distribution of PGNs paralleled very closely the pattern of PGN labelling following peripheral nerve applications of HRP. The number of labelled PGNs counted in S2 from a representative case in this study is well within the range of S2 PGN counts reported by Nadelhaft et al. (1980) following application of HRP to the pelvic nerve. Also consistent with previous reports were the orientations of neurons identified as PGNs in the present study with the major extent of the dendritic tree being in the transverse plane, and the major axis of DB neurons being mediolateral and that of LB neurons being dorsolateral. No periodicity of labelled neurons was seen in the rostrocaudal plane in this study, nor was it reported after applying HRP to the pelvic nerve in the cat (Nadelhaft et al., 1980). However, Brown and Nolan (1979) described clusters of neurons within the cat SPN in pyridine silver stained material. In light of the present results and the results of retrograde transport studies it is doubtful that these neurons were PGNs.

The dendritic arborizations of neurons in the DB were observed to extend medially into the contralateral commisural gray and laterally and dorsally in lamina I,

almost to the apex of the dorsal horn. Dendrites of neurons in the LB extended laterally into the lateral funiculus, dorsally along the lateral edge of the dorsal horn, dorsomedially into lamina V and ventrally along the lateral aspect of the ventral horn. Similar patterns of dendritic arborization were described in the cat after labelling the pelvic nerve (Nadelhaft et al., 1980). However, when PGNs are labelled by peripheral nerve application of HRP, dendrites are granularly labelled and very difficult to distinguish from overlapping transganglionically labelled primary afferent fibers. With the present technique, diffusely labelled dendrites can be followed for long distances even when they are embedded in labelled fascicles of afferent fibers.

Brown and Nolan (1979) presented an ultrastructural description of the types of synaptic terminals located within the SPN. These investigators analyzed data for synapses on dendrites of all sizes, and somata ranging from 20-30 um in diameter. As reported in the present study, Nolan and Brown indicated that dendrites in the SPN measured less than 6 um in diameter and most were less than 2.0 um. They described terminals containing clear spherical vesicles, clear vesicles plus DCVs, and flattened vesicles. These investigators classified terminals as containing granular vesicles only if they had at least three DCVs. In the current experiment the same categories of terminals

were observed with the exception that the example given by Brown and Nolan of a flattened vesicle terminal (Brown and Nolan, 1979, fig. 11) corresponds to terminals classified as pleomorphic in the present study. Because PGNs could be identified specifically at the EM level in the current study, the variety and distribution of terminals presynaptic to PGNs was examined. In the current experiment, of the terminals on the somata and dendrites of sacral PGNs, 79.7% contained round vesicles, 23.3% contained pleomorphic vesicles; 59.6% of these contained at least one DCV as well. In order to compare the random population of terminals reported by Nolan and Brown to the population of terminals presynaptic to PGNs reported here, the current data were recalculated. Results from the present study indicate that 59.2% of the observed terminals synapsing on PGNs contained only spherical vesicles, 27.7% contained at least three DCVs, and 13.6% contained pleomorphic vesicles. Corresponding figures for Nolan and Brown's data are 65.5% spherical, 26.25% granular, and 5.7% flattened vesicle terminals. This comparison indicates that the population of terminals synapsing on PGNs is similar to the population of terminals synapsing within the SPN on unidentified profiles. Data from the present experiment indicate that terminals containing pleomorphic vesicles tend to terminate on more proximal dendrites than terminals containing spherical vesicles.This tendency was also

evident in the data reported by Nolan and Brown. As was also observed by Nolan and Brown, postsynaptic dense bodies were a frequently encountered feature in the SPN. In the present study, postsynaptic dense bodies were also observed in labelled PGNs.

Possible sources of terminals synapsing on PGNs include descending projections from the brain stem, propriospinal neurons, and local interneurons. Dense projections to the SPN from the dorsolateral pons have been demonstrated in several species (cat: Holstege and Kuypers, 1982; rat: Loewy et al., 1979; opposum: Martin et al., 1979; and monkey: Westlund and Coulter, 1980). This region of the brainstem contains noradrenergic neurons (Dahlstrom and Fuxe, 1964; Jones and Moore, 1974; Poitras and Parent, 1978). Westlund et. al. (1982), have presented strong evidence suggesting that the noradrenergic terminals in the SPN originate in the dorsolateral pons. Another source of descending input to the SPN is the raphe nucleus and surrounding medial reticular formation of the medulla oblongata (Holstege and Kuypers, 1982; Martin et al., 1982a, 1982b). Serotonergic terminals, which have been demonstrated in the SPN (Dahlstrom and Fuxe, 1965) probably arise from this source. Descending monoamine projections may account for a proportion of the DCV containing terminals encountered in the current study (Hokfelt, 1967, Bak, et al., 1969).

Interneurons, which provide an important link in the polysynaptic pathways that mediate micturition and defecation (deGroat et al., 1979, 1981; Milne et al., 1978), and propriospinal neurons are also probable sources of unlabelled terminals synapsing on PGNs. Coordination of sacral PGN firing with somatic input to striated muscles and sympathetic input to the urinary bladder and large intestine are essential for efficient voiding of waste material. Some of these interactions occur at the spinal level (Thor et al., 1983).

Primary afferent fibers provide an additional source of direct synaptic input to sacral PGNs (Mawe et al., 1984). Terminals of labelled dorsal and ventral root afferent axons were observed synapsing on labelled PGNs in the present study. These interactions will be discussed in greater detail below.

Dorsal root afferents.

In the present study, the dorsal root afferent distribution to the SPN was described. Briefly, most labelled fibers entering the SPN pass in the LCP and display several trajectory patterns within the SPN. The majority of fibers end in the dorsolateral portion of the nucleus. Others extend from this region medially across the base of the dorsal horn, some continuing beyond the midline to terminate in lateral lamina IV of the contralateral

dorsal horn. A small contingent of fibers pass from the LCP ventrally into the region occupied by LB neurons. Using transganglionic transport of HRP from the pelvic nerve, Morgan et al. (1981) in cat, and Nadelhaft et al. (1983) in monkey, have demonstrated that a large proportion of axons in the LCP are of visceral afferent origin. In cat (Morgan et al., 1978; Ueyama et al., 1984) and in monkey (Ropollo et al., 1982), axons labelled transganglionically from the pudendal nerve were also found in the LCP. Light and Perl (1979b) injected single axons which were activated by stimulation of cutaneous high threshold mechanoreceptors in the tail of a cat. Some of these axons, which may travel to the spinal cord via the pudendal nerve, course in the LCP. Recent immunocytochemical studies have revealed that LCP axons are heavily labelled with antibodies to vasoactive intestinal polypeptide (Basbaum and Glazer, 1983; deGroat et al., 1983), substance P, and cholecystokinin (deGroat et al., 1983). All of these peptides are known to be present in dorsal root ganglion neurons. Immunoreactive axons were observed to arborize heavily in the dorsolateral aspect of the nucleus with some extending through the DB region and a very few in the LB area. This pattern as comparable to the dorsal root afferent distribution described above.

The periodicity observed in HRP labelled LCP axons was also observed in axons labelled with antibodies to substance P and cholecystokinin (Lowe et al., 1981; deGroat et al.,

1983).

Under oil immersion, individual labelled axons within the SPN were seen as thin black fibers with varicosities throughout their course. Several investigators have suggested that varicosities such as these represent sites of synaptic contact (Proshansky and Egger, 1977; Brown et al., 1977; Ishizuka et al., 1979; Light and Perl, 1977, 1979). Ultrastructural studies of primary afferent fibers diffusely filled with HRP support this suggestion (Beattie et al., 1978, 1979; Liuzzi et al., 1983; Mawe et al., 1984). Ultrastructural examination of these fibers in the current experiment indicates that labelled primary afferent terminals within the SPN contain clear spherical vesicles and often DCVs as well. This is consistent with the results of Nolan and Brown (1981) who used anterograde degeneration to identify primary afferent terminals. In the present study, dorsal root terminals with and without DCVs were seen on somata, but most contacts were axodendritic with the majority terminating on dendritic profiles less than 0.5 um in diameter. Nolan and Brown (1981) observed only terminals containing DCVs synapsing on somata within the SPN, and both types synapsing on dendrites. The discrepancy between the results of the present study and those of Nolan and Brown may be due to sampling differences or could be attributable to differences in the techniques employed. In addition to the greater difficulty of locating and

identifying degenerating versus HRP labelled terminals, the time-course of degeneration varies with fiber diameter (Sprague and Ha, 1964; LaMotte, 1977). Nolan and Brown consistenty used a five day survival time and therefore may have overlooked terminal degeneration which was not optimally visible five days after axotomy. Injury filling of axons with HRP on the other hand, labels the full fiber-diameter spectrum of afferent fibers and their terminals within a survival time of 10-20 hours (Beattie, et al., 1978, 1979).

Although some of the labelled dorsal root afferents examined in this study terminated on somata and dendrites of labelled PGNs, most contacted unlabelled, unidentified profiles. It is likely that many of these afferents are terminating on interneurons associated with micturition and defecation, as well as neurons which relay information rostrally to other levels of the neuraxis. The dorsolateral aspect of the SPN, which receives the most pronounced primary afferent input, contains populations of interneurons that can be activated or inhibited by vesicle and intestinal sensory fibers (Milne et al., 1978; deGroat et al., 1981). Many of these neurons recieve input from mechanoreceptive and nociceptive afferents in the pudendal nerve as well.

Evidence for the presence of rostrally projecting neurons in lateral lamina V which receive direct afferent

input also exists. Carstens and Trevino (1978) showed that there are neurons in lamina V of the cat spinal cord which project directly to the thalamus. Physiological data indicate that in monkey there are spinothalamic neurons located in the dorsolateral SPN which respond to both visceral and cutaneous afferent stimuli (Milne et al., 1981). Kuru and Takase (1947) noted that in humans, cells in this region underwent chromatolytic changes following anterolateral tract cordotomies which disrupted bladder sensations. Application of HRP to cut axons in the dorsolateral funiculus between the T13 and L2 segments of the cat spinal cord results in bilateral retrograde labelling of neurons in the dorsolateral SPN (deGroat et al., 1981). Although the destination of these rostrally projecting axons is unknown, their cells of origin are well situated to receive direct afferent input and therefore may account for some of the unlabelled profiles which are postsynaptic to labelled primary afferent terminals.

In the current study, some labelled dorsal root afferent terminals were presynaptic to profiles which contain round or pleomorphic synaptic vesicles and sometimes DCVs. Nolan and Brown (1981) reported that a small number of afferent axoaxonic synapses exist in the SPN. It is difficult to determine whether the terminals observed in the present study represent axoaxonic contacts, or interactions between primary afferent fibers and vesicle

containing dendrites (type 2 dendrites, Gobel, 1976), or some of each. Gobel (1976) illustrated similar contacts in the substantia gelatinosa of the cat spinal trigeminal nucleus which were identified as type 2 dendrites. In the present study, postsynaptic profiles containing vesicles were rarely observed to be postsynaptic to other profiles. Possible reciprocal synapses with the primary afferent terminals exist in the SPN as were reported by Gobel (1976) in the substantia gelatinosa. Functional implications of these connections are unclear.

Ventral root afferents

Several lines of evidence suggest that ventral root afferent axons were labelled in the course of the present study. When HRP was applied to the ventral roots alone, thin labelled fibers, often in fascicles, were frequently observed at the LM level coursing along the lateral edge of the ventral horn and arborizing in the SPN; some continue dorsally along the lateral edge of the dorsal horn and arborize in lamina I or enter the tract of Lissauer. Within the SPN, such fibers displayed varicosities which were sometimes seen in close apposition to somata and dendrites of labelled PGNs, especially those located in the lateral band region. Upon ultrastructural examination of tissue in which the ventral roots were labelled with HRP, reaction product was visible in terminals that contained clear

spherical vesicles and clear spherical vesicles plus DCVs. These terminals were observed to synaptically interact with labelled PGNs as well as unlabelled profiles in the neuropil. Labelled terminals with similar ultrastructural features were also noted in lamina I along the lateral edge and at the apex of the dorsal horn; labelled unmyelinated and lightly myelinated axons were also seen passing through lamina I and coursing rostrocaudally in Lissauer's Tract (Beattie et al., 1984).

Possible origins for labelled fibers and terminals within the SPN include recurrent collaterals, presynaptic dendrites, and ventral root afferents. Physiological evidence strongly suggests that recurrent collaterals of PGN axons exist and that these collaterals exert an inhibitory influence on PGNs via interneurons (deGroat and Ryall, 1968; deGroat, 1976). Since many of the contacts observed in the present study were on identified PGNs, it is unlikely that these terminials are from the population of collaterals postulated from physiological results (deGroat and Ryall, 1968; deGroat, 1976). Although those terminals observed in the SPN not in direct contact with the PGNs represent a plausible source of recurrent inhibition, careful LM examination of labelled PGN axons in the present study revealed very few possible examples of labelled recurrent collaterals. The collaterals that were observed passed laterally into the lateral funiculus.

Labelled fibers with varicosities could not be traced back to labelled neurons either, but in some cases were traced to parent axons that pass along the lateral edge of the ventral horn, a course which ventral root afferents might be expected to take. This suggests that the collaterals described in physiological experiments were not labelled in the current study.

It should be noted that recurrent collaterals of motoneurons were observed in the ventral horn. Although motoneuron collaterals did not project in the direction of the SPN, fine dendritic extensions from motoneurons in the region of Onuf's nucleus were traced into the SPN. Swellings on these fine dendrites were occasionally observed in close apposition to labelled PGNs (unpublished observations). It is doubtful that this source of input to the SPN accounts for the terminals in question because the morphological characteristics of elements traced back to motoneuron dendrites are different. The labelled fibers take a more tortuous course and are fasciculated in the SPN and superficial dorsal horn. In addition, they do not exhibit the ultrastructural characteristics previously described for presynaptic dendrites (e.g. Rall et al., 1966; Ralston, 1968; Lund, 1969; Gobel, 1976).

In recent years, accumulating evidence has indicated the presence of sensory fibers entering the spinal cord by way of the ventral roots. Approximately 30% of the axons in

lumbosacral ventral roots are unmyelinated (Coggeshall et al., 1974; Applebaum et al., 1976), and of these half are thought to be PGN axons and the other half are considered ventral root afferents (Applebaum et al., 1976). Physiological evidence indicates that ventral root afferent fibers include unmyelinated and lightly myelinated axons and have both somatic and visceral receptive fields (Clifton et al., 1975; Coggeshall and Ito, 1977). Two thirds of the ventral root afferents in S3 and Cx1 ventral roots have visceral receptive fields in the pelvis and most of the remaining ventral root afferent fibers were activated by cutaneous nociceptive stimuli (Clifton et al., 1976). Approximately seventy percent of the L7 and S1 ventral root afferents in the cat have somatic receptive fields, most of which are nociceptive. The remaining 30% of the L7 and S1 ventral root afferents fibers have receptive fields in pelvic viscera including the bladder and colon (Coggeshall and Ito, 1977).

It is reasonable to speculate that ventral root afferent fibers conveying cutaneous nociceptive and visceral afferent information may terminate in regions of the spinal cord which receive similar input from the dorsal roots. In the lumbosacral spinal cord such input from dorsal roots has been shown to distribute primarily in the superficial dorsal horn and SPN (Light and Perl, 1979; Morgan et al., 1981; Nadelhaft et al., 1983). Degeneration

studies of ventral rhizotomized animals have indicated that ventral root afferents terminate in the dorsal horn (Dimsdale and Kemp, 1966; Mikeladze, 1966) and in the intermediolateral gray (Mikeladze, 1966). In a more recent study, Light and Metz (1978), using injury-filling of ventral roots, illustrated labelled fibers passing dorsally from the ventral root exit region to arborize within the dorsal horn.

Terminals and fibers which were labelled in the present study following application of HRP to the ventral root alone correlate well with previously reported physiological and morphological data regarding ventral root afferents. As mentioned previously, the labelled fibers are of small caliber and distribute within the SPN and laminae I and II. At the EM level, labelled unmyelinated and lightly myelinated axons were seen in these same regions as well as the tract of Lissauer which has been shown to contain nociceptive and visceral afferent fibers which enter the spinal cord via the dorsal roots (Light and Perl, 1979b; Morgan et al., 1981). Labelled terminals, which are morphologically indistinguishable from dorsal root afferent terminals, were observed contacting unlabelled and labelled neuronal profiles within the SPN and unlabelled profiles in lamina I. It is concluded that labelled terminals observed in the SPN and lamina I are of ventral root origin.

Direct afferent-efferent interactions

Monosynaptic connections between primary afferent fibers and sacral PGNs have been postulated by several investigators (deGroat et al., 1981; Morgan et al., 1981; Nolan and Brown, 1981; Nadelhaft et al., 1983). Following application of HRP to the pelvic nerve, a pronounced overlap between afferent and efferent elements has been demonstrated at the LM level in the cat (Morgan et al., 1981) and monkey (Nadelhaft et al., 1983). Unfortunately, this technique does not provide the resolution necessary to examine interactions between single afferent fibers and PGNs at the LM level, and ultrastructral identification of afferent terminals labelled by transganglionic transport of HRP is dificult. Nolan and Brown (1981) used the technique of anterograde degeneration to describe primary afferent synaptic terminals within the SPN. However, conclusions regarding monosynaptic interactions could not be drawn because PGN profiles were indistinguishable from other neurons in the region.

Evidence from electrophysiological experiments performed on cats with intact spinal cords suggest that micturition and defecation reflex pathways are exclusively polysynaptic (deGroat et al., 1981). Stimulation of afferents from the bladder and colon results in long latency responses (bladder: 60-75 msec; colon: 45-60 msec) in postganglionic fibers on the surfaces of these organs.

However, in chronic spinal animals, short latency reflexes (7-25 msec) which may be monosynaptic are capable of initiating micturition (deGroat and Ryall, 1969; deGroat et al., 1981).

The technique employed in the present study has allowed a more discrete examination of the morphology of PGNs, primary afferents, and their interactions, than application of HRP to peripheral nerves. Also, the technique used here is well suited for sequential light and electron microscopic examination of labelled elements. At the LM level, varicosities on labelled primary afferent fibers were often seen in close apposition to somata and dendrites of labelled PGNs. Ultrastructural examination revealed labelled terminals synapsing on the somata and dendrites of labelled PGNs. As noted previously, similar interactions were observed between proposed ventral root afferent terminals and PGNs.

The monosynaptic connections described here provide a possible morphological substrate for the short latency response recorded in chronic spinal cats. DeGroat and his colligues (1981; 1983) have suggested that the appearance of the short latency response observed in chronic spinal cats may be due to anatomical reorganization of dorsal root afferent systems as a consequence of removal of the suprasegmental input to the SPN. In light of the current observations of a monosynaptic input to the PGNs in normal

animals, perhaps unmasking of an already existant connection might be a more parsimonious explanation for the physiological results. Dorsal root sprouting may also contribute to the full development of such physiological alterations (Thor, et al., 1982; Murray and Goldberger, 1974).

FIGURE LEGENDS

FIGURES 1-4

Figures 1-4 illustrate neurons which were labelled by application of HRP to sacral ventral roots. Figure 1 is a low power micrograph which demonstrates the locations of motoneurons and PGNs within the gray matter of an S2 section. Bar=500 um. Figure 2 is a higher power micrograph of lateral band PGNs in the region indicated in figure 1. The solid block arrow is pointing to a group of labelled PGN dendrites passing dorsalaterally into the white matter. A triangularly shaped PGN is indicated by the open block arrow. The asterisk indicates a bundle of myelinated axons. Bar=50 um. Labelled dorsal band neurons are shown in figure 3 (arrows). Bar=100 um. Labelled lateral band neurons are indicated by the arrows in figure 4. The open block arrow in figure 4 is pointing to a dorsolaterally situated dorsal band cell.

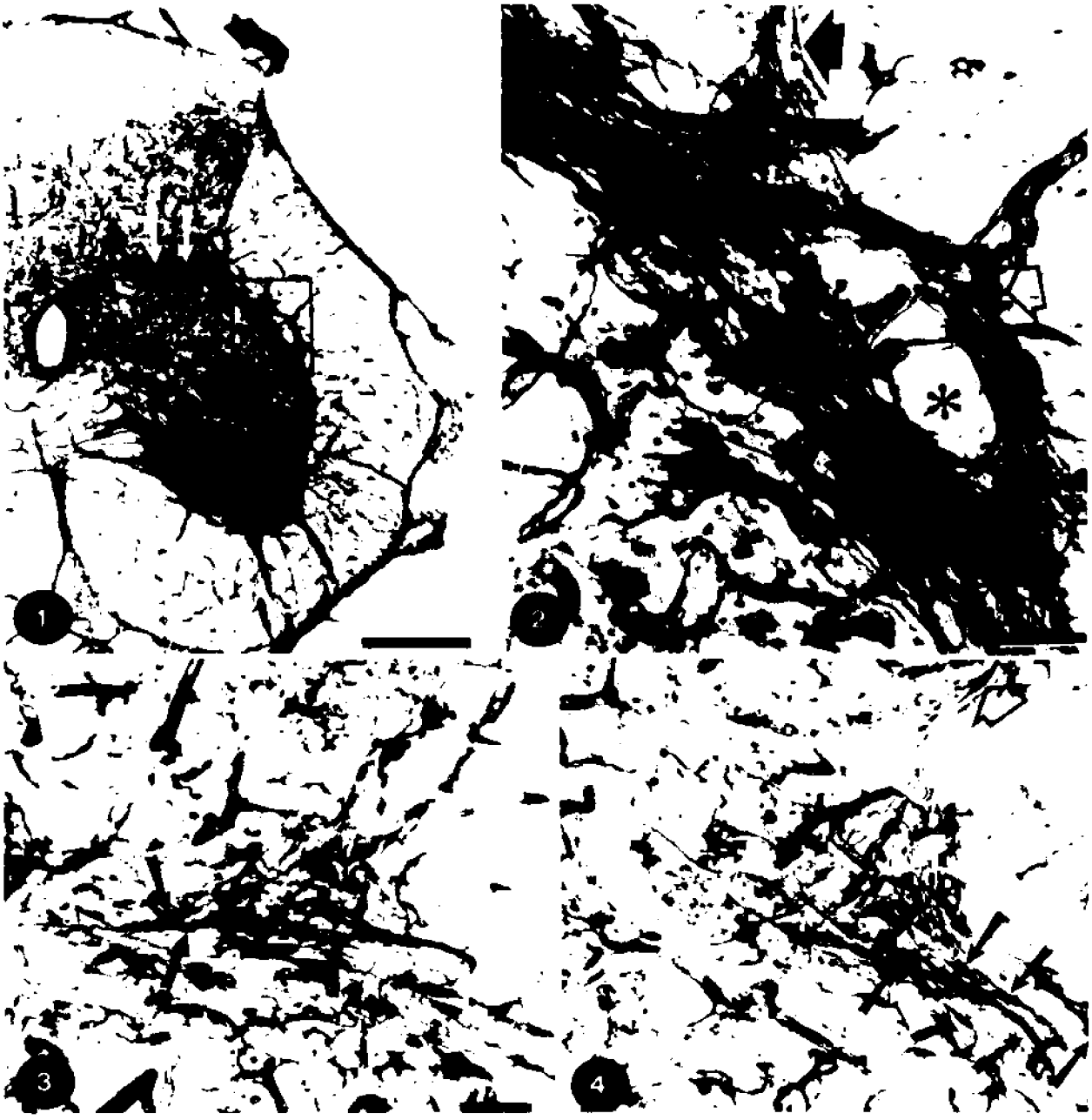

1
2
3
4

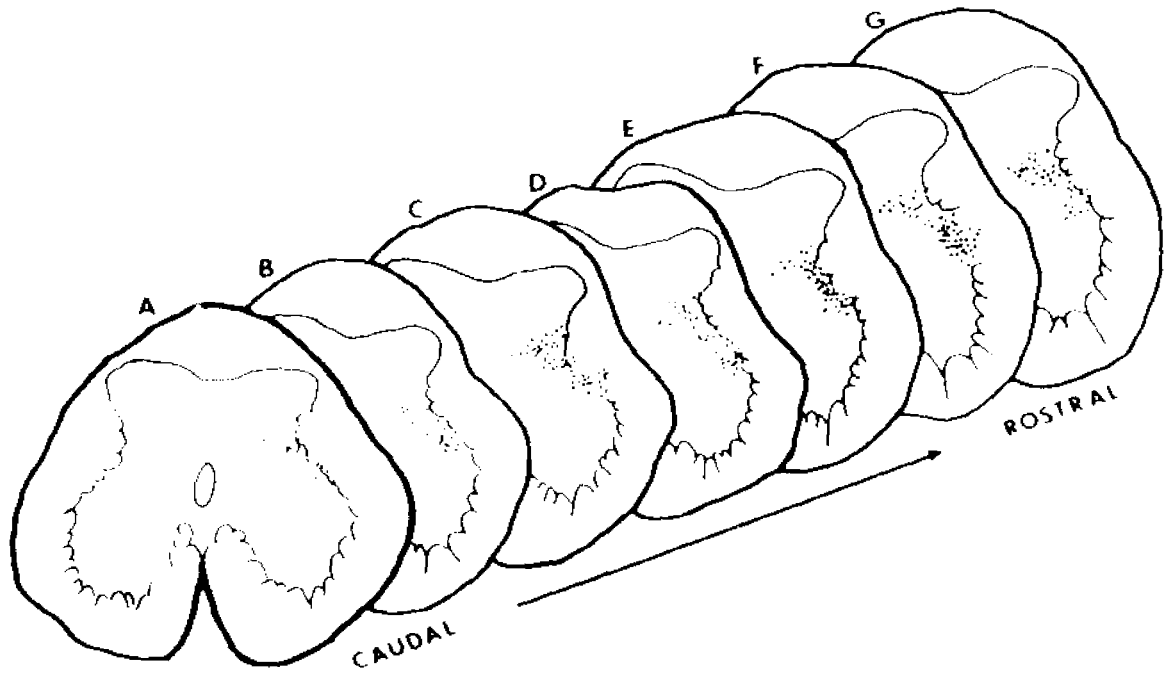
FIGURE 5

The three types of neuronal reaction product are shown in figure 5. These include a dark diffusely labelled neuron (cell 1), a diffuse plus granularly labelled neuron (cell 2), and a granularly labelled neuron (cell 3). A labelled blood vessel (BV) is also indicated. Bar=20 um.

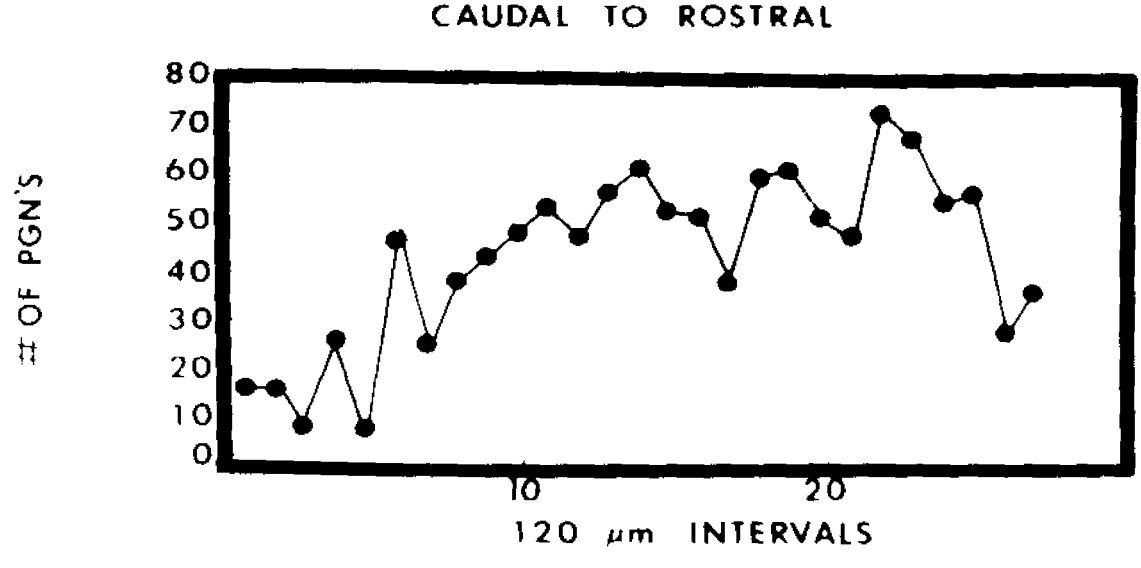

1
2
BV
5

FIGURES 6 and 7

Figure 6 illustrates the rostrocaudal distribution of labelled cells in S2 after HRP application to the ventral rootlets. Figure 7 is a plot of labelled cell counts from the same case.


A
B
C
D
E
F
G
CAUDAL
ROSTRAL

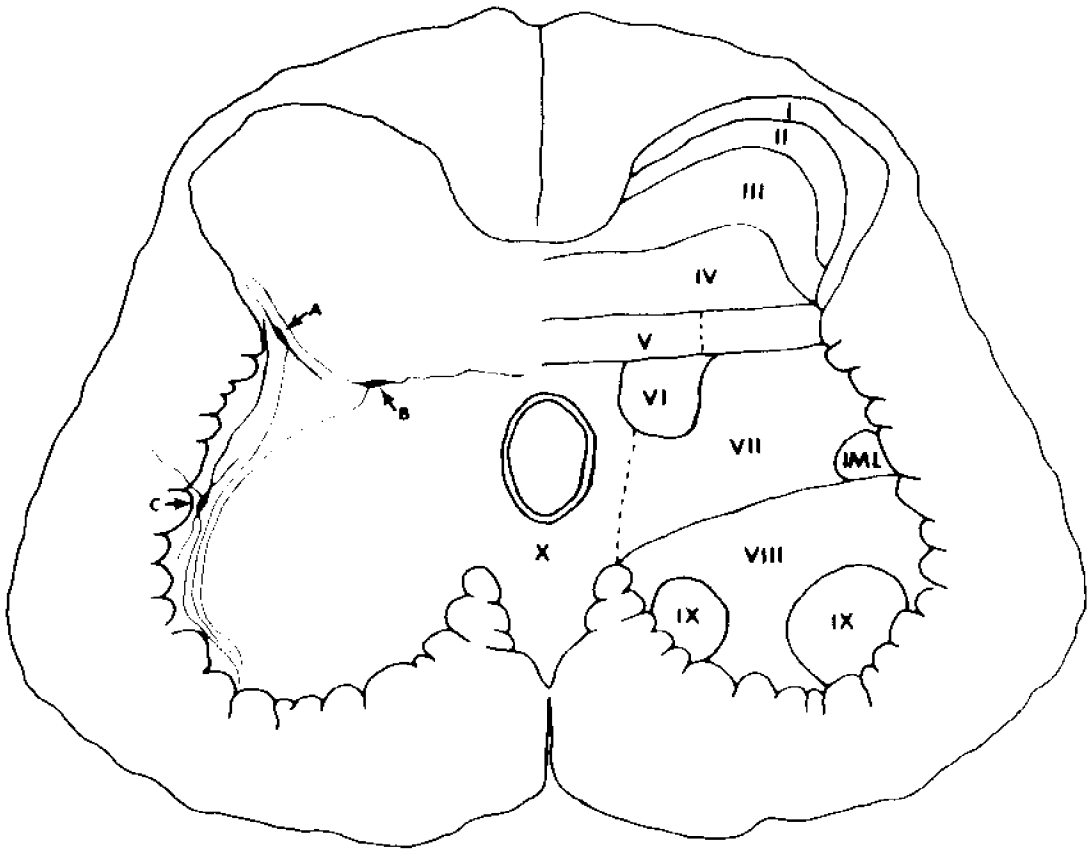
6


CAUDAL TO ROSTRAL
OF PGN'S
80
70
60
50
40
30
20
10
0
10
20
120 μm INTERVALS

7

FIGURE 8

The arrangement of Rexed's laminae at spinal level S2 is illustrated on the right. The three types of PGNs (A, B, and C) are shown on the left.

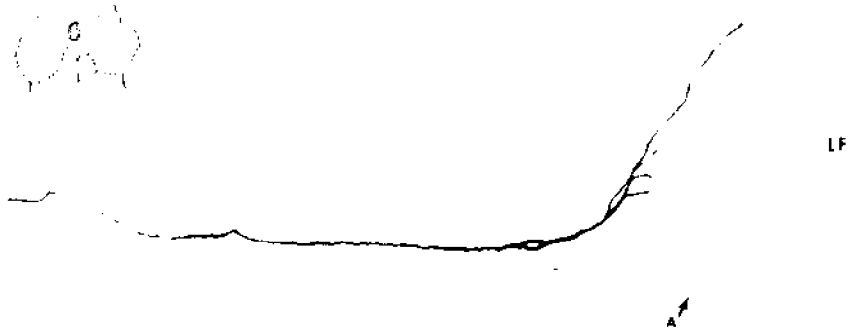


8

FIGURES 9 and 10

Figures 9 and 10 illustrate representative dosal band PGNs. (A, axons; cc, central canal; LF, lateral funiculus).

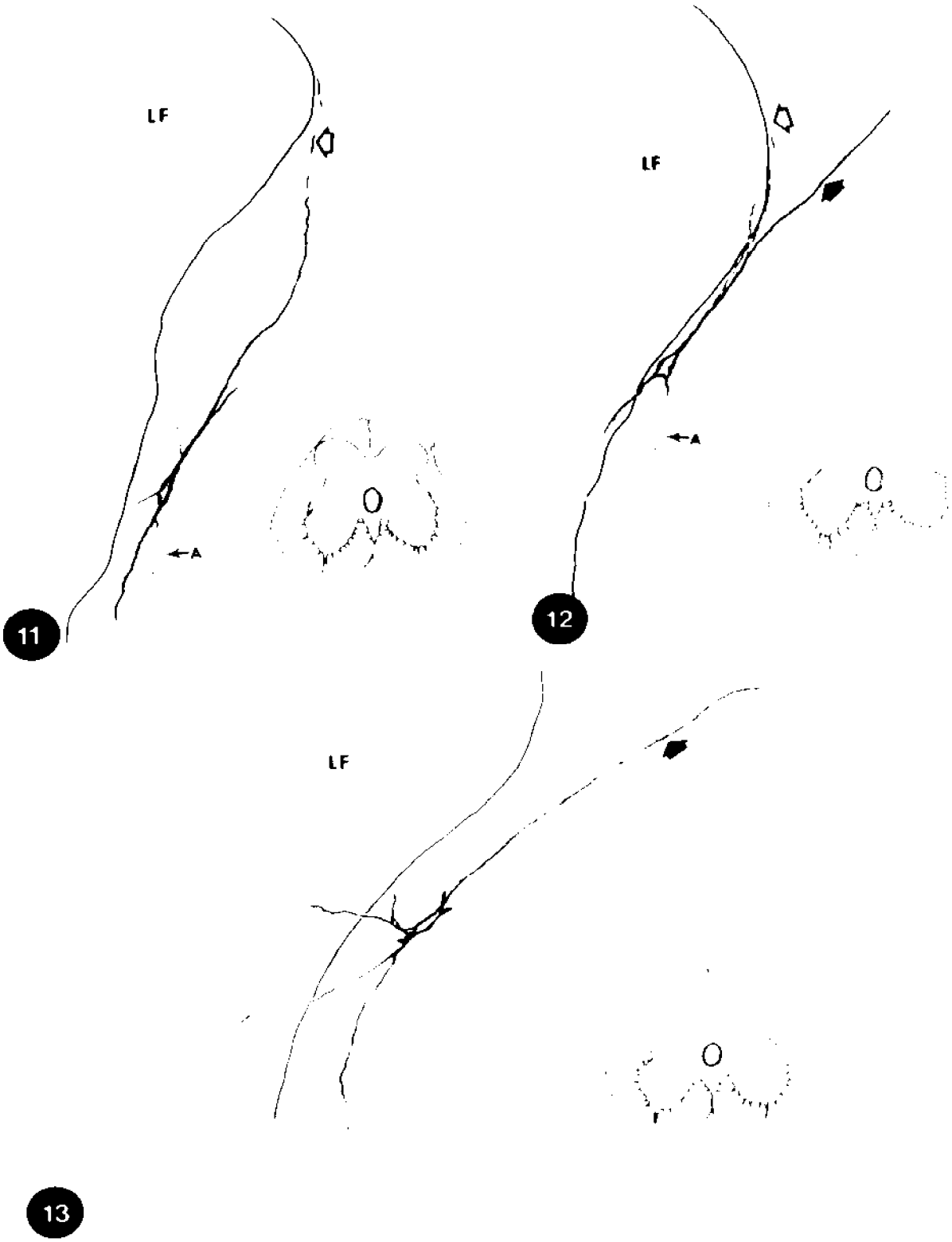
9



10

FIGURES 11-13

Figures 11, 12, and 13 illustrate lateral band PGNs. (A, axons; LF, lateral funiculus).


LF
A
11
LF
A
12
LF
13

FIGURES 14-19

Figures 14-19 show the LCP primary afferent distribution within the SPN. In figure 14, LCP axons pass ventrally along the lateral border of the dorsal horn and in a fascicle through the white matter (arrow) to enter the dorsolateral aspect of the SPN where most of the axons terminate. Labelled afferent fibers continue into the LB region (arrowheads). Bar=100 um. Figure 15 illustrates LCP axons continuing medially from the dorsolateral SPN to the midline of the spinal cord (arrowheads). Bar=100 um. Afferent fibers which pass medially and dorsally are seen in figure 16 (bar=200 um.) and at higher power in figure 17 (bar=50um.). Figures 18 (bar=50 um.) and 19 (bar=10 um.) illustrate the appearance of individual labelled afferent fibers. Arrowheads in figure 19 indicate varicosities on labelled axons. (cc, central canal)

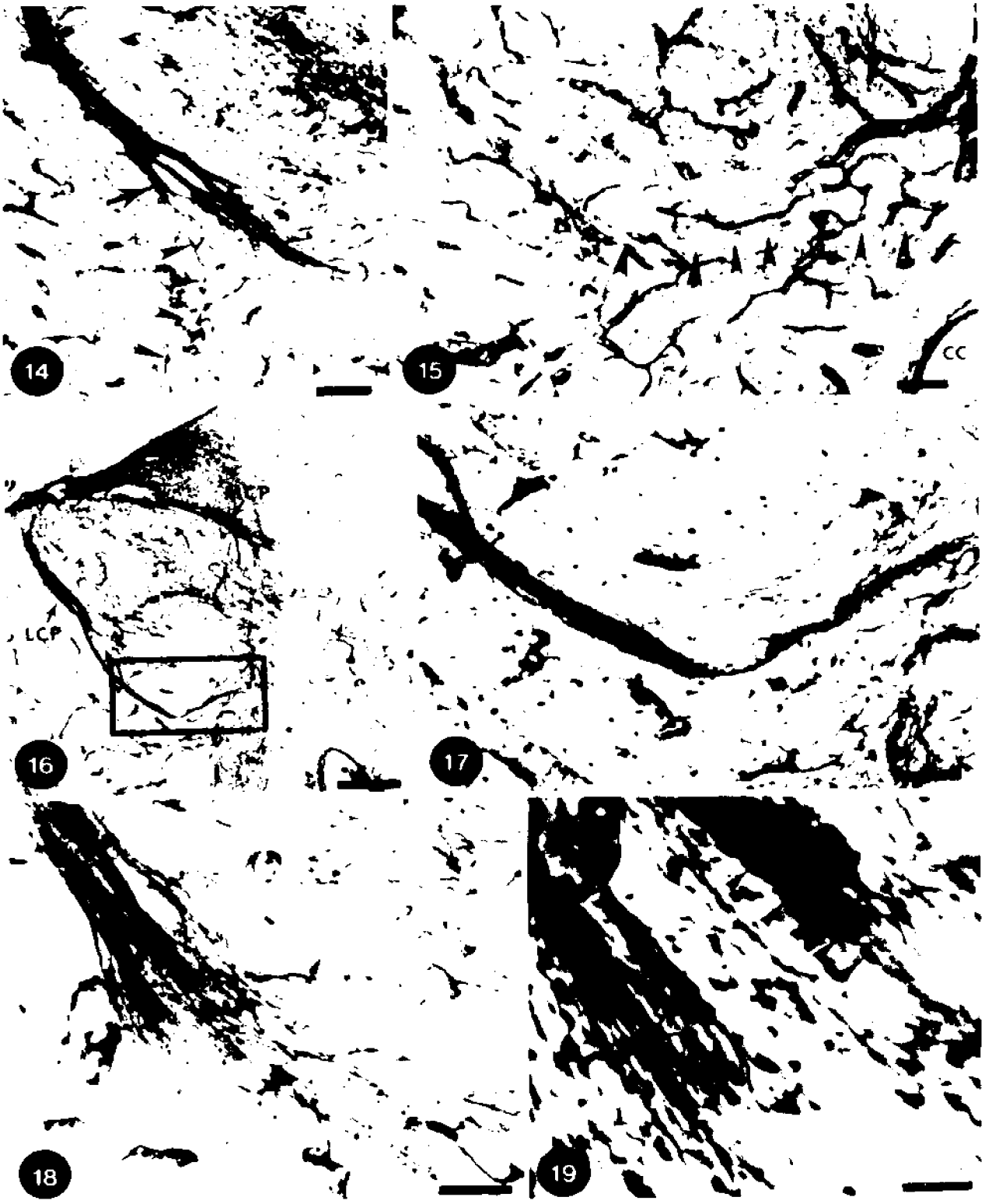

14
15
CC
LCP
16
17
18
19

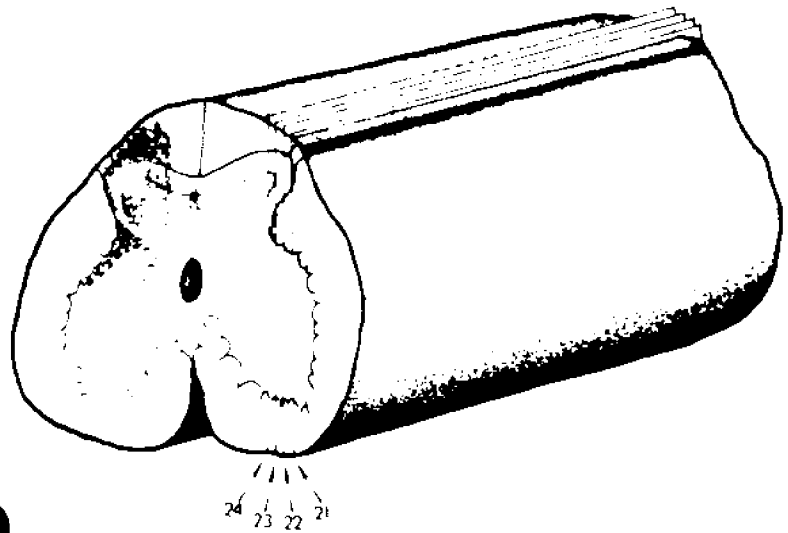
FIGURE 20

Figure 20 is photomicrograph of primary afferent axons which were labelled by application of HRP to the contralateral dorsal rootlets. The block arrow indicates an area of arborization of these axons which corresponds to the region indicated by the block arrow in the inset. Bar=100 um.

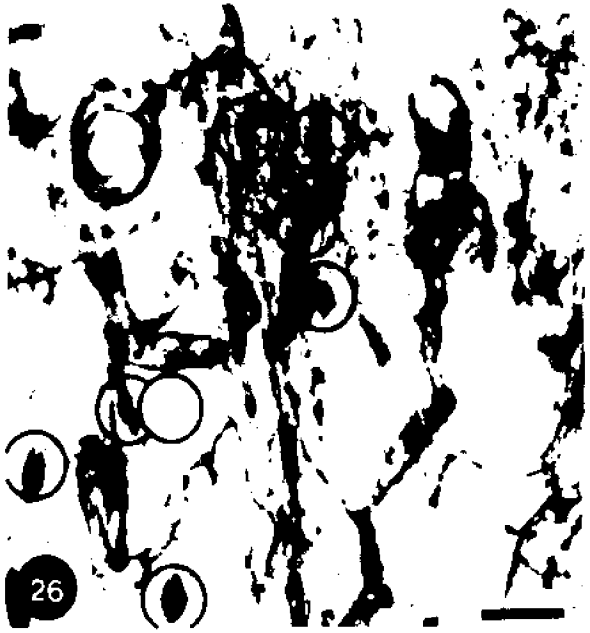

20

FIGURES 21-26

Figures 21-24 are photomicrographs of parasaggital sections at S2 following application of HRP to dorsal and ventral roots. Open block arrows indicate LCP axons which fasciculate as they pass ventrally towards the SPN. PGNs are circled. Bar=200 um. Figure 25 shows the planes of section represented in figures 21-24. Figure 26 is a higher power photomicrograph of the area indicated in figure 24. Note the overlap between a fascicle of afferent fibers and labelled PGNs (circles). Bar=50 um.



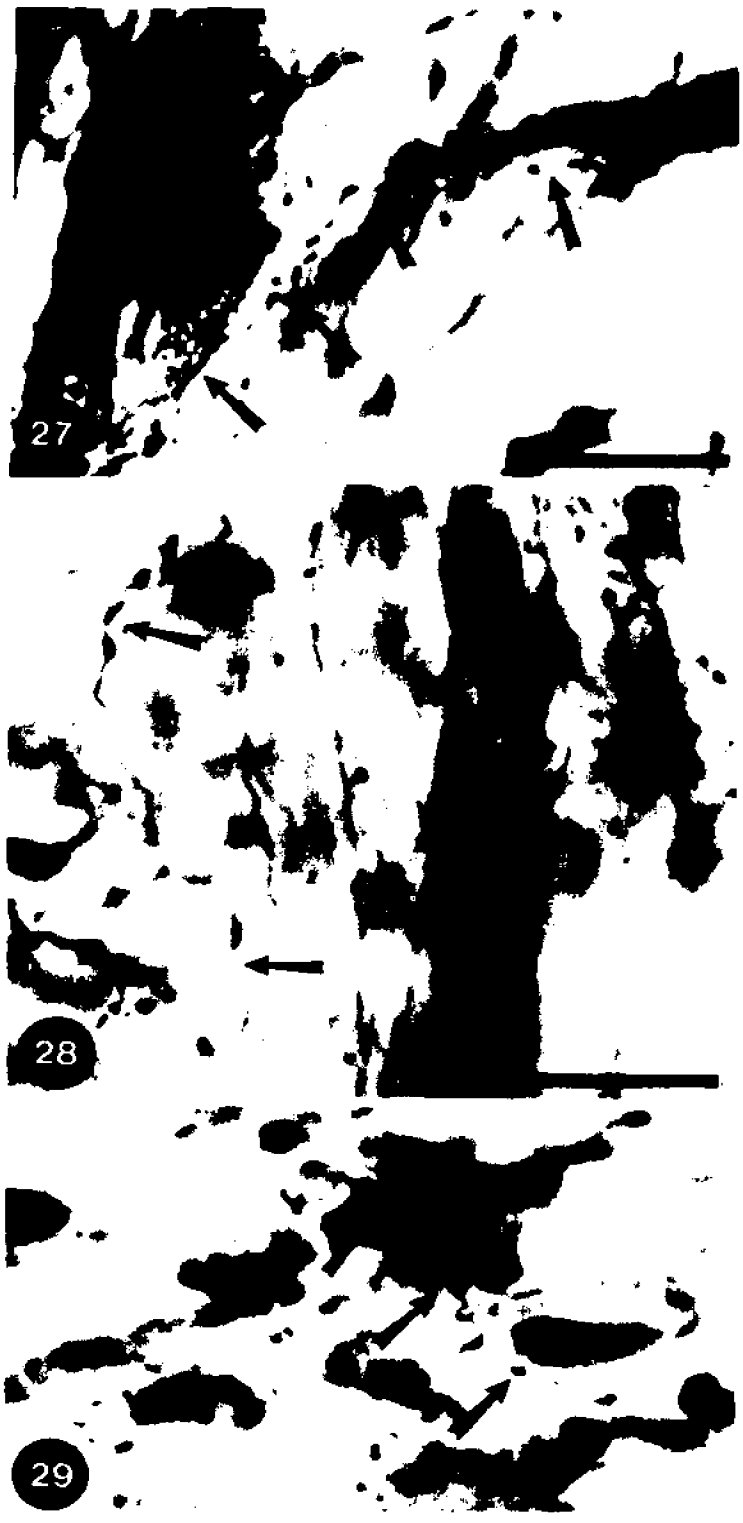
25



26

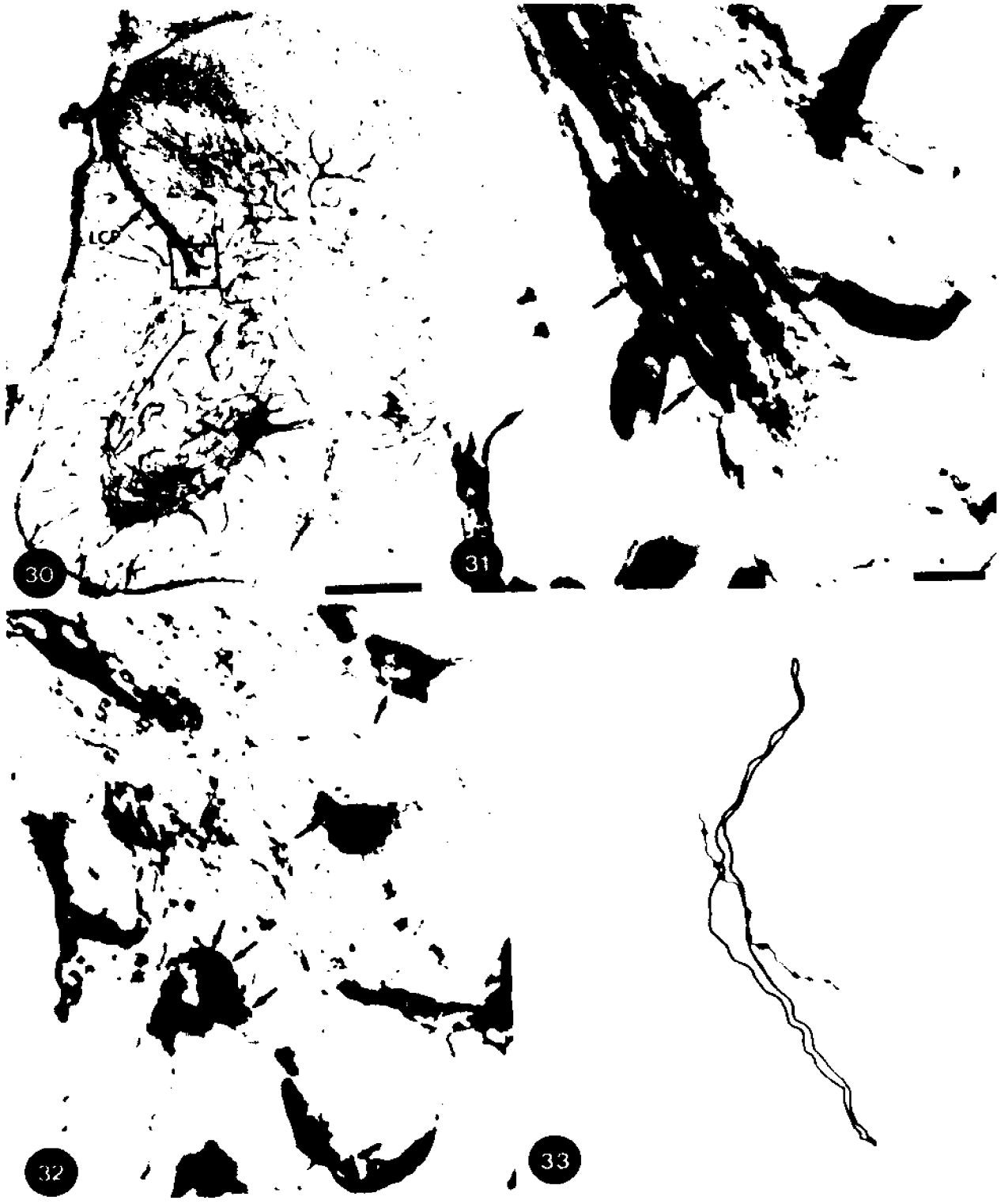
FIGURES 27-29

Thin fibers with varicosities, which were labelled after application of HRP to the ventral roots alone, are indicated by arrows in figures 27-29. Figure 27 is a photomicrograph of the LB region of the SPN. Figure 28 shows fibers in lamina I along the lateral edge of the dorsal horn, and figure 29 is from lamina I adjacent to the Tract of Lissauer. Bars=20 um.


27
28
29

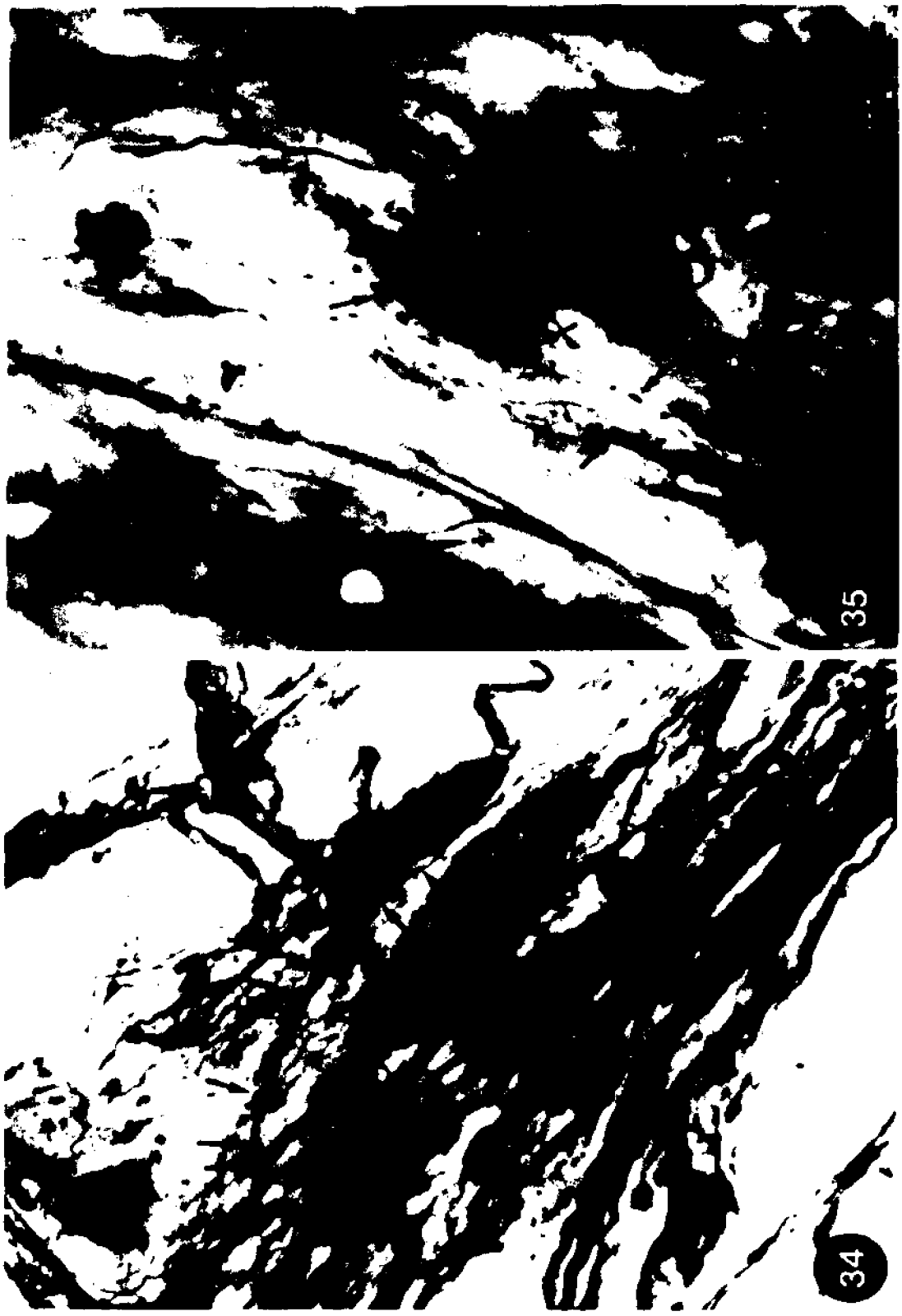
FIGURES 30-33

Figures 30-33 illustrate interactions between primary afferent fibers and sacral PGNs. Figure 30 is a low power photomicrograph illustrating a large fascicle of SPN axons entering the SPN. Bar= 500 um. Close appositions between these afferent fibers and PGNs (arrows) are seen in figure 31, which is a higher power micrograph of the area indicated in figure 30. Bar=20 um. Arrows in figure 32 indicate swellings on primary afferent axons which are apposed to PGNs. Figure 33, which is a camera lucida drawing of the neuron indicated by a block arrow in figure 32, illustrates a labelled fiber as it passes along the soma of a PGN.


30
31
32
33

FIGURES 34 and 35

Figures 34 and 35 illustrate fibers with varicosities (arrows), which were labelled after application of HRP to the ventral roots alone, in close apposition to labelled PGNs. Bars=20 um.


35
34

FIGURES 36-39

Figures 36-39 illustrate a single neuron which was observed and documented at the LM level, then sectioned and observed electron microscopically. Figure 36 shows this dark, diffusely labelled neuron as seen in a 60 um thick, plastic embedded section. The block arrow serves as a reference point on the neuron for figures 37 and 38. Small diameter afferent fibers intersect with a diffusely labelled dendrite (arrow). Another dendrite of the neuron is indicated by the small arrows. Bar=50 um. Figure 37 is a 1 um thick section through the neuron. It appeared light brown with labelled granules. The difference between an unlabelled dendrite (curved arrow) and the labelled dendrite (arrow) is clearly shown. A region of numerous labelled granules is indicated by the arrowhead. Bar=50 um. Figures 38 and 39 are electron micrographs through soma and dendrite of this same neuron. Reaction product is seen in numerous membrane bound inclusions. The open block arrow in figure 38 is in the nucleus of the glial cell indicated by the open block arrow in figure 37. Bar=1.0 um.

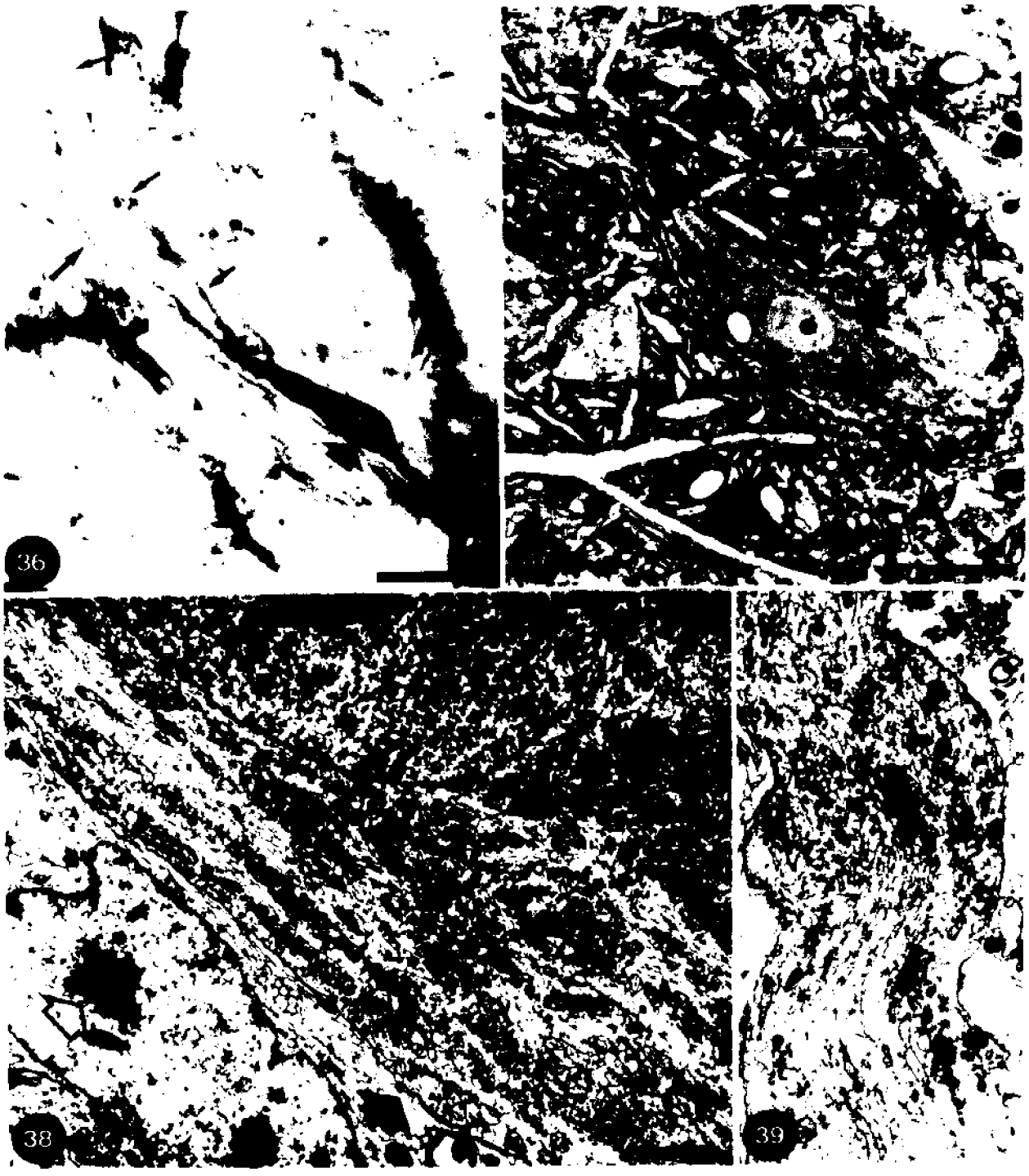

36
38
39

FIGURE 40

Figure 40 is a photomicrograph of a 1 um thick section including the lateral funiculus (LF) and a portion of the SPN. The LCP, which includes large numbers of unmyelinated axons is indicated. Arrows indicate the locations of labelled PGNs. Bar=100 um.

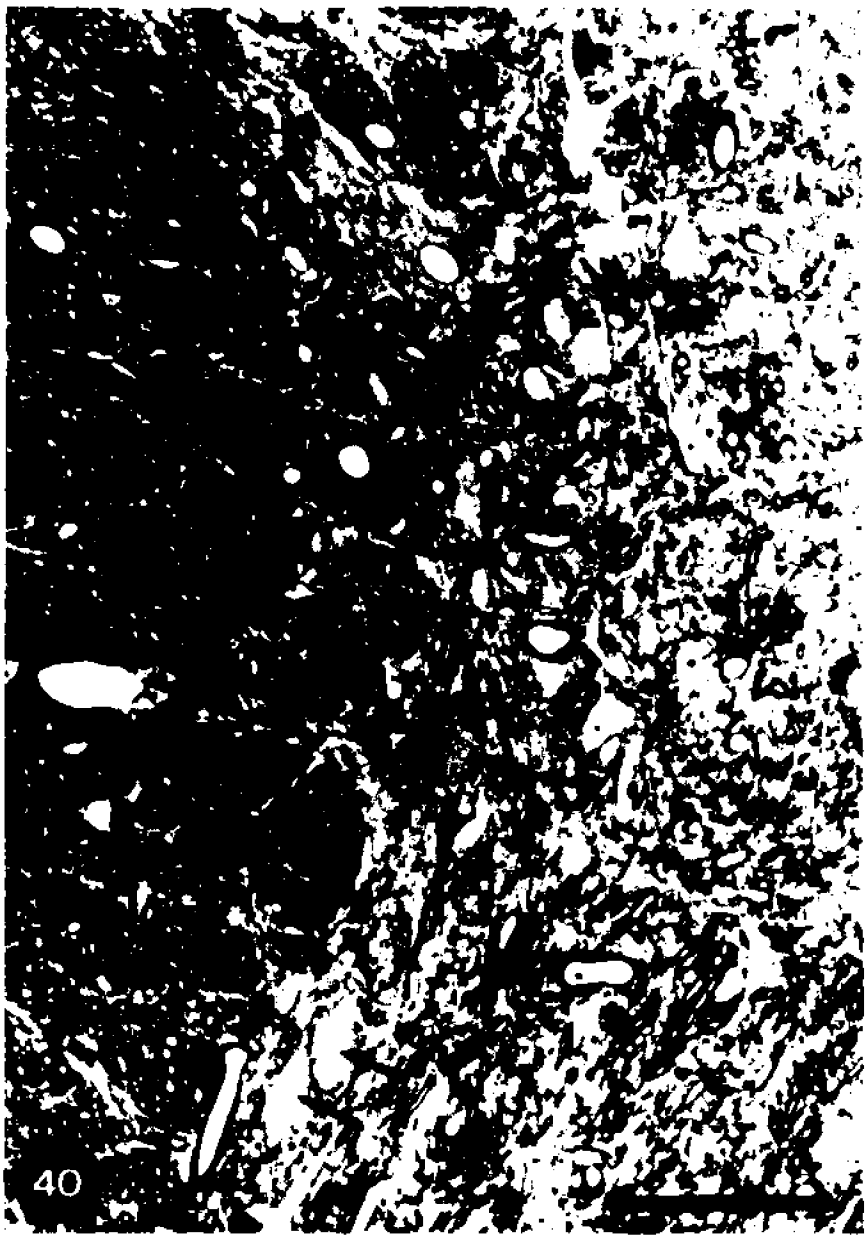
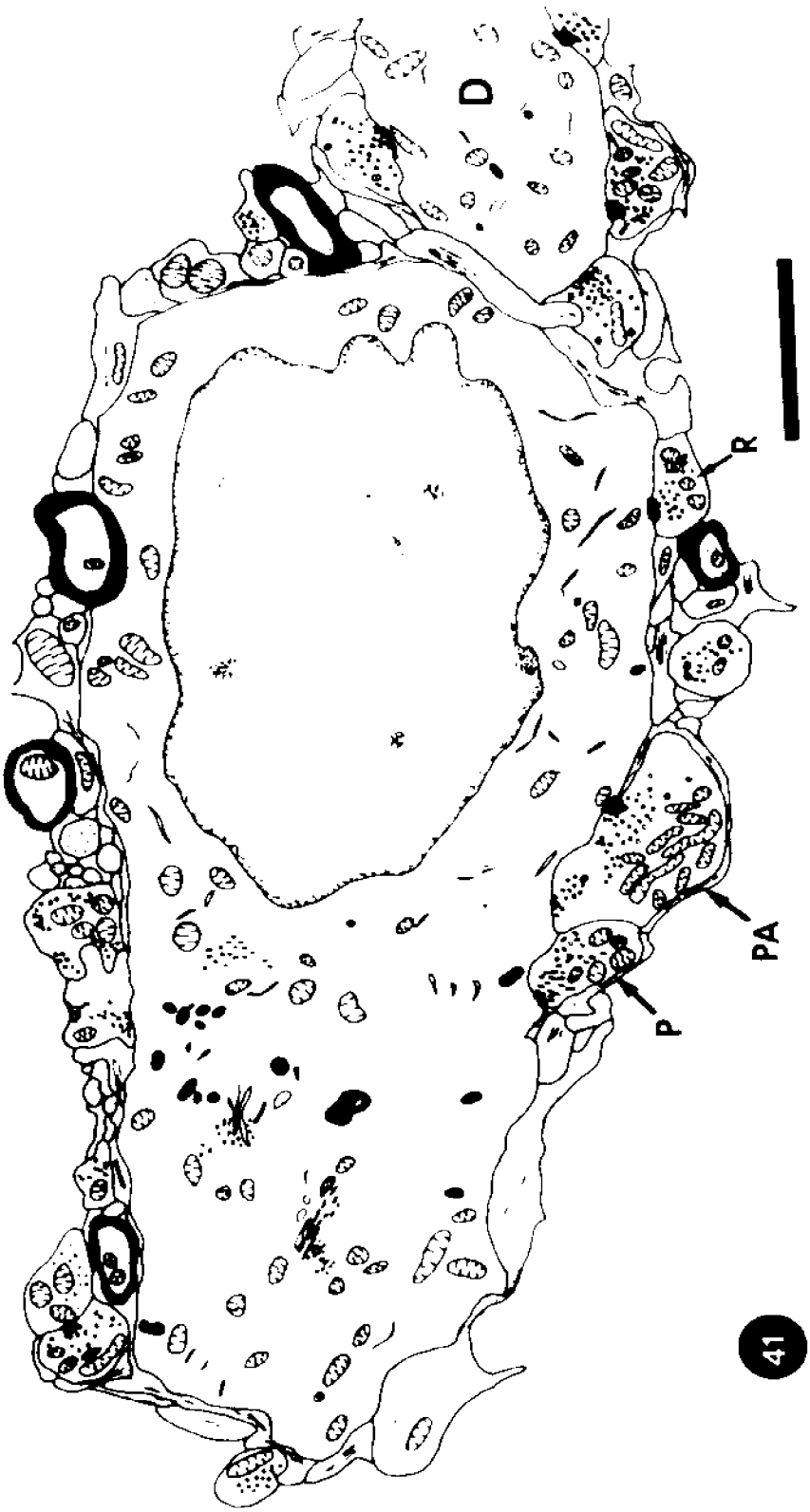

40

FIGURE 41

A drawing of a labelled PGN reconstructed from electron micrographs is seen figure 41. Terminals synapsing on the soma include a pleomorphic vesicle containing terminal (P), a terminal containing round vesicles (R), and a primary afferent terminal (PA) containing round vesicles and DCV's. Also directly apposed to this neuron are glial fibers, myelinated axons, dendrites and terminals not synapsing on the neuron in this section. A dendrite of this neuron (D), separated in this section by a glial process, can also be seen. Bar=2 um.



41

FIGURES 42-46

Figures 42-46 illustrate unlabelled terminals synapsing (double arrows) on dendrites (D) of labelled PGNs. Terminals in figures 42 and 43 contain spherical vesicles; terminals in figures 44-46 contain pleomorphic vesicles. Terminals in figure 42, #2 in figure 43, #1 in figure 44, and figures 43 and 44 contain DCV's as well. The open block arrow in figure 42 indicates a labelled myelinated axon, the closed block arrow indicates a labelled vesicle containing profile which may be a dendrite. Arrowheads in figure 43 indicate postsynaptic dense bodies. Bar=0.5 um.

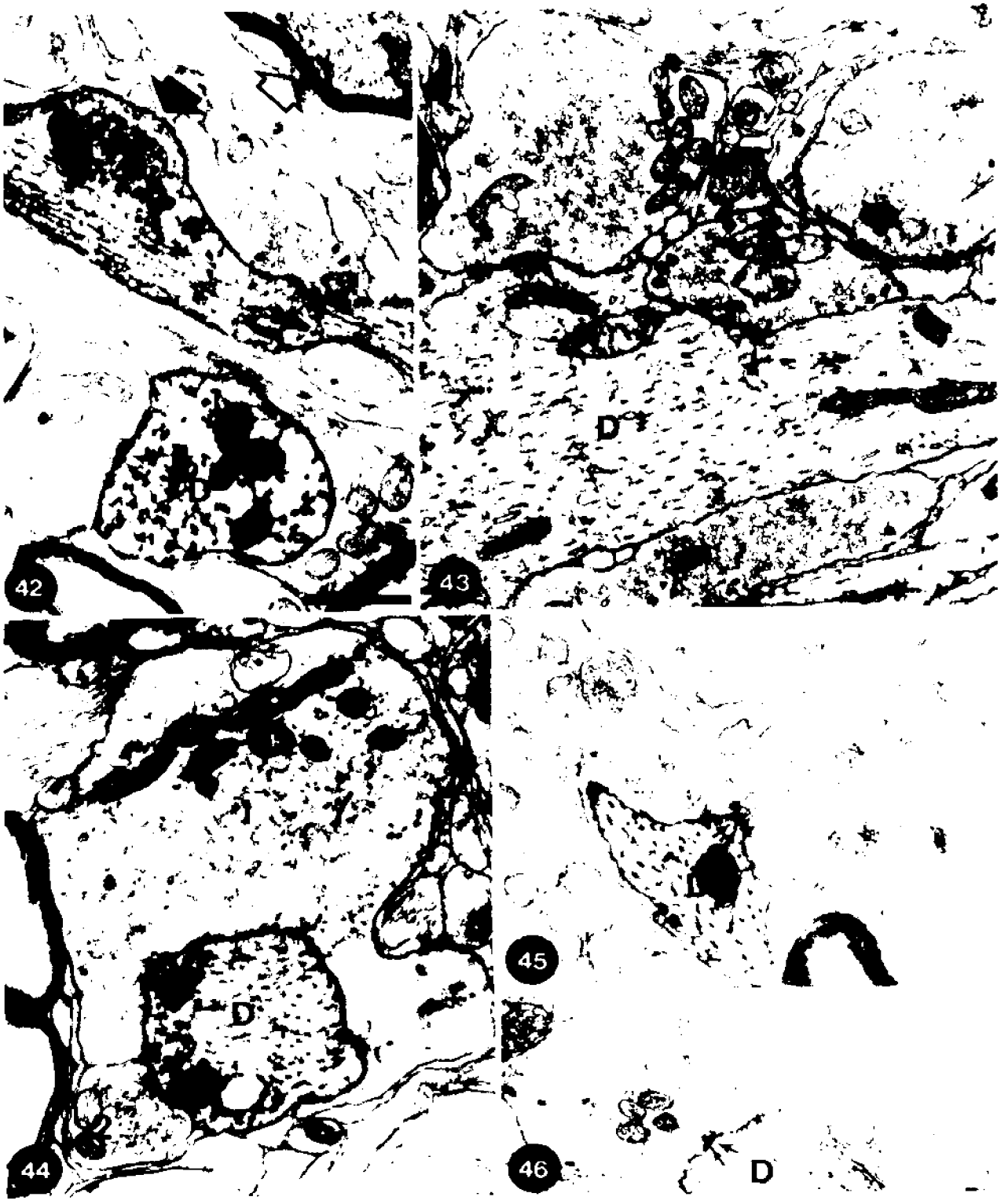

42
43
D
44
D
45
46
D

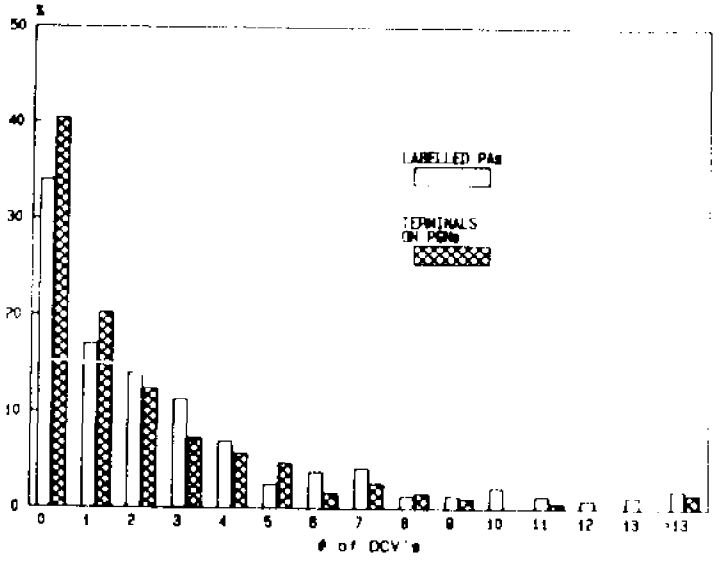
FIGURE 47

Figure 47 is a bar graph indicating proportions of terminals containing different numbers of DCVs. Labelled primary afferent terminals (n=241) are represented by open bars. Terminals on labelled PGNs (n=193) are represented by hatched bars.

FIGURE 48

The postsynaptic distribution of terminals synapsing on labelled PGNs is demonstrated by the bar graph in figure 48.

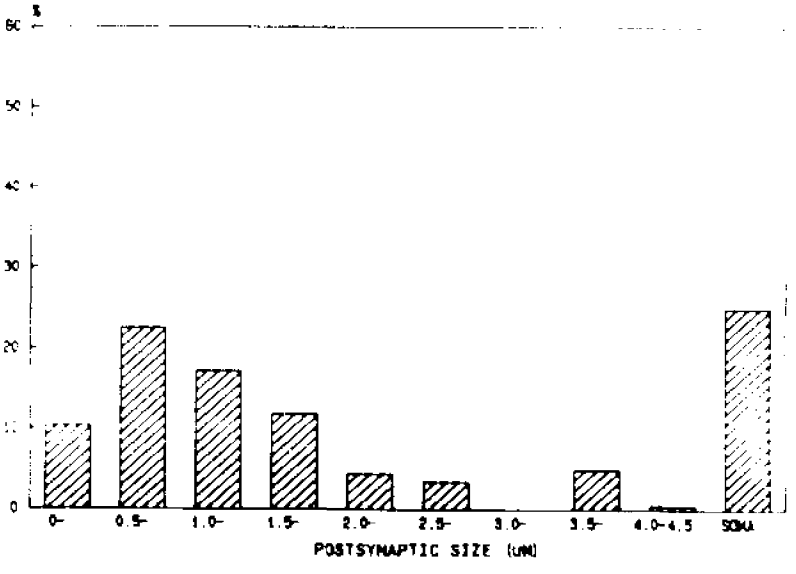
DCV's/TERMINAL



47

POSTSYNAPTIC DISTRIBUTION

terminals on labelled PGNs



48

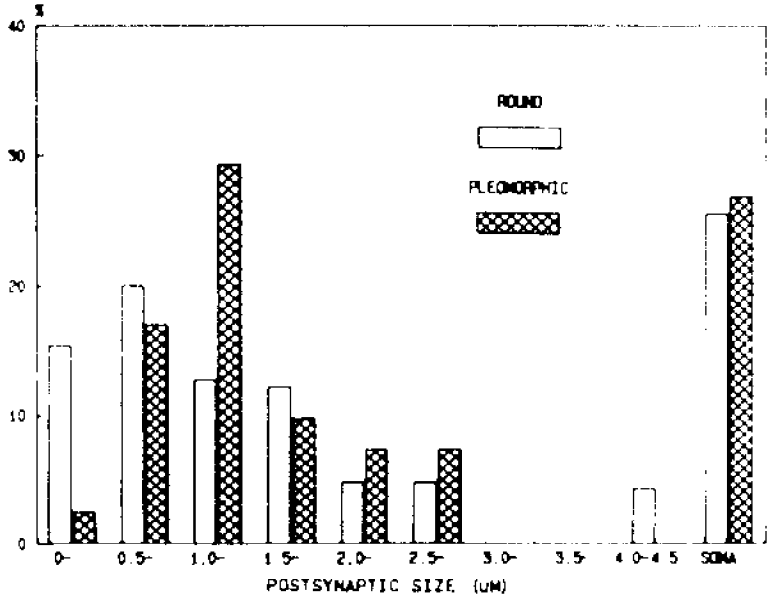
FIGURE 49

A comparison of the postsynaptic distribution of round (n=154) versus pleomorphic (n=39) vesicle containing terminals is illustrated in figure 49.

FIGURE 50

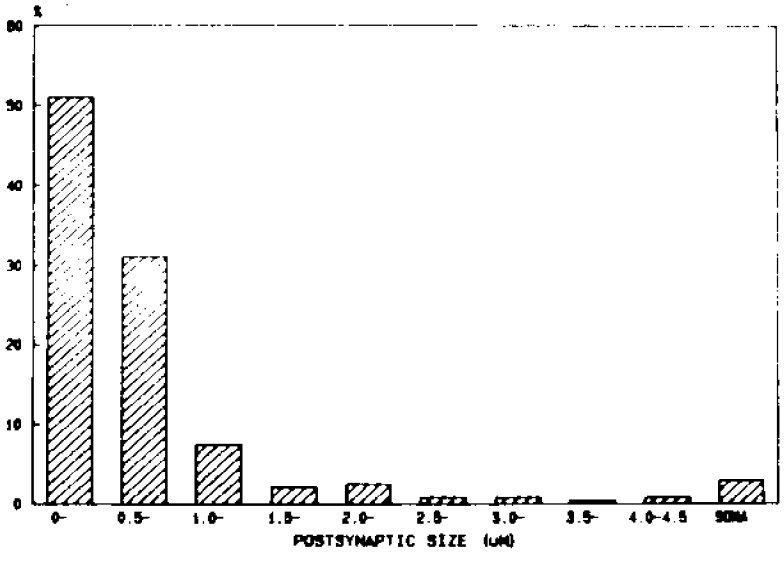
The postsynaptic distribution of labelled primary afferent terminals (n=241) is illustrated in figure 50.

POSTSYNAPTIC DISTRIBUTION
round VS pleomorphic



49

POSTSYNAPTIC DISTRIBUTION
labelled PA terminals



50

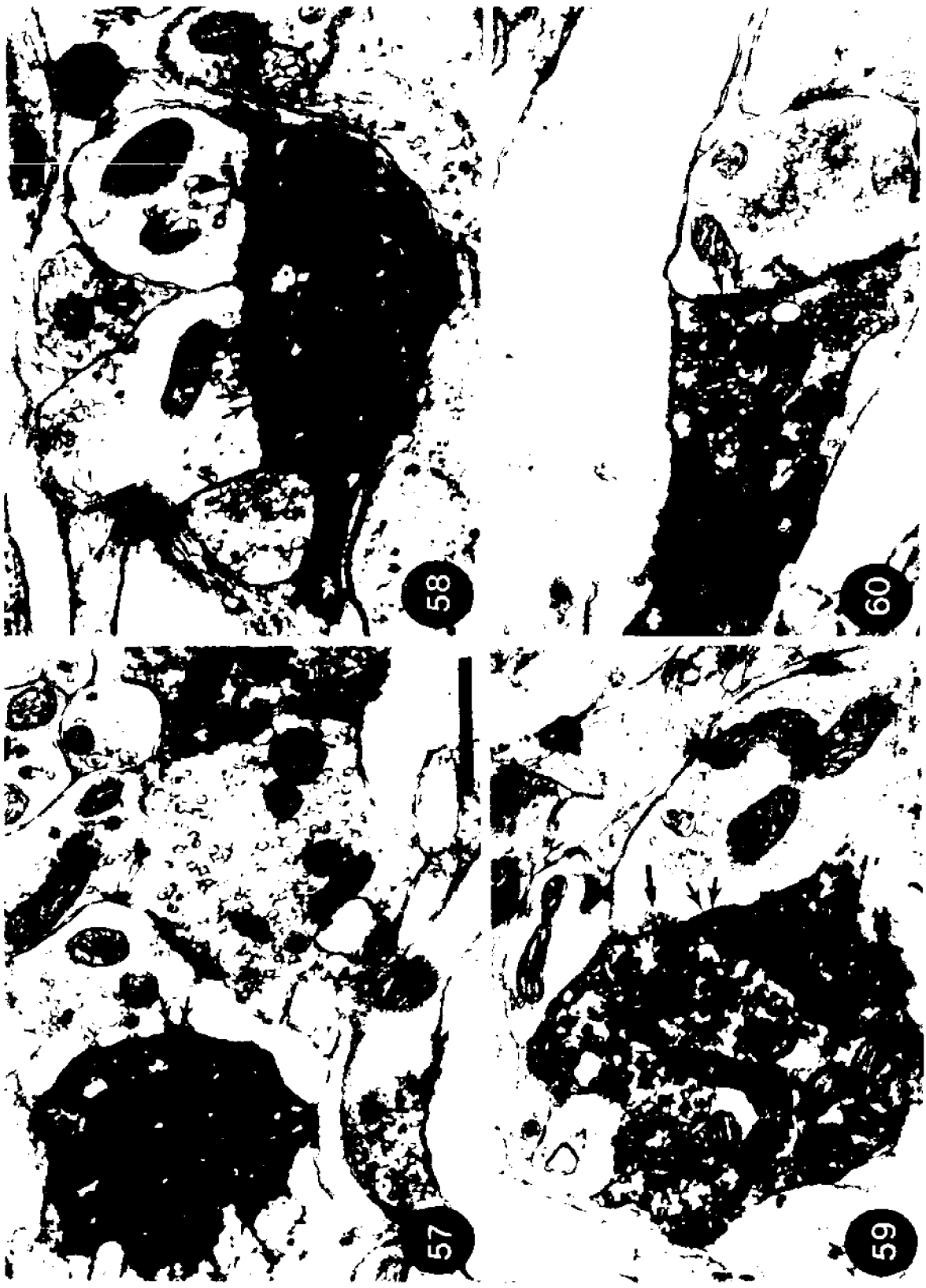
FIGURES 51-55

Figures 51-55 illustrate labelled dorsal root afferent terminals synapsing (double arrows) on unlabelled dendritic profiles. Such terminals contain round vesicles or round vesicles plus DCV's (fig. 54). The terminal in figure 51 is synapsing on the shaft and spine (arrow) of an unlabelled dendrite. A postsynaptic dense body is indicated by the arrowhead in figure 52. Bar=0.5 um.


51
52
53
54
55
56

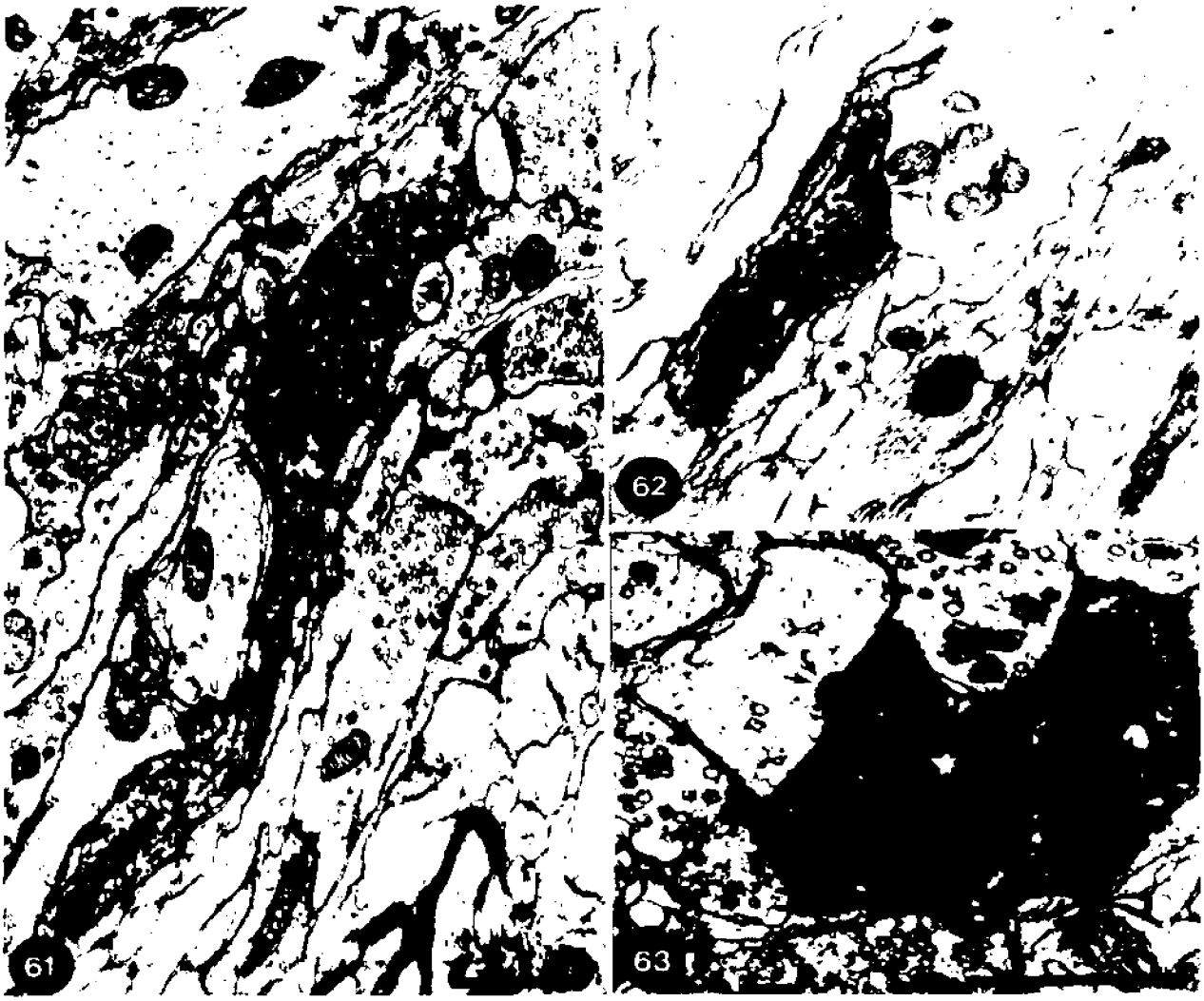
FIGURES 57-60

Labelled terminals synapsing (double arrows) on unlabelled profiles containing vesicles are illustrated in figures 57-60. The postsynaptic profiles in figures 57, 58, and 60 contain pleomorphic vesicles; the postsynaptic profile in figure 59 contain round vesicles. Single arrows in figures 58 and 59 indicate regions of possible reciprocal synapses. Bar=0.5 um.


58
60
57
59

FIGURES 61-63

Terminals which were labelled following application of HRP to the ventral root alone are seen synapsing (double arrows) on unlabelled dendritic profiles in the SPN in figures 61-63. Bars=0.5 um.


61
62
63

FIGURES 64-68

Synaptic interactions (double arrows) between labelled dorsal root afferent terminals and labelled PGNs are shown in figures 64-68. Figures 64 and 65 illustrate axosomatic contacts; reaction product containing cisternae and lysosomes are indicated by arrows. The open block arrows in figure 64 indicate DCVs in the soma of the PGN. Figure 66 shows a labelled afferent teminal synapsing with a labelled PGN dendrite. Serial sections of a labelled terminal contacting a labelled profile which may be an unmyelinated axon are shown in figures 67 and 68. The density and widened cleft indicated by double arrows in figure 67 correspond to the region containing a cluster of synaptic vesicles in figure 68 (double arrows). The open block arrows in figures 67 and 68 indicate the same dendrite in each section. Bars=0.5 um.

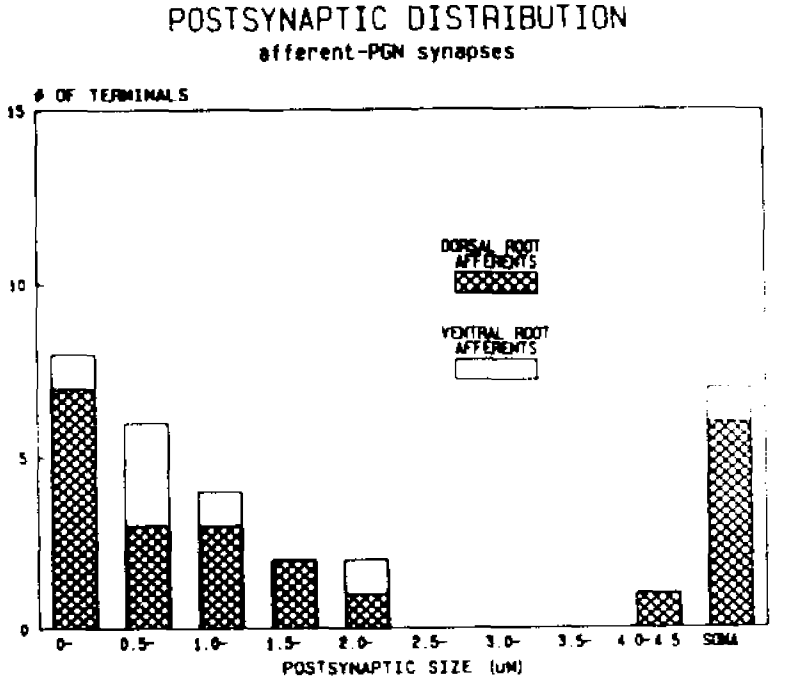

64
65
66
67
68

FIGURE 69

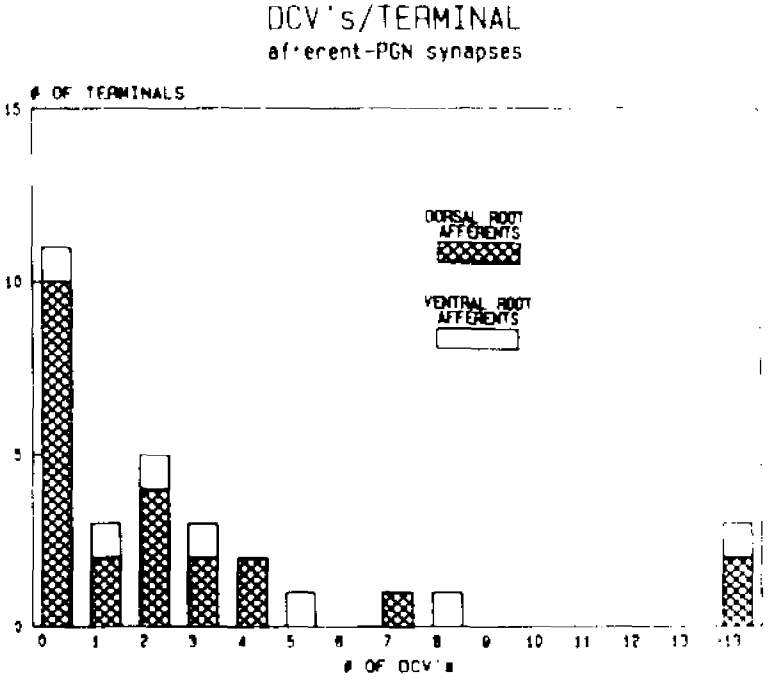
The postsynaptic distribution of labelled dorsal (hatched bars) and ventral (open bars) root afferent terminals contacting PGNs is illustrated in figure 69.

FIGURE 70

Figure 70 illustrates the number of afferent terminals synapsing on PGNs with different numbers of DCVs. (dorsal root afferents, hatched bars; ventral root afferents, open bars).


POSTSYNAPTIC DISTRIBUTION
afferent-PGN synapses
OF TERMINALS
15
10
5
0
DORSAL ROOT AFFERENTS
VENTRAL ROOT AFFERENTS
0-
0.5-
1.0-
1.5-
2.0-
2.5-
3.0-
3.5-
4.0-4.5
SOMA
POSTSYNAPTIC SIZE (uM)

69


DCV's/TERMINAL
afferent-PGN synapses
OF TERMINALS
15
10
5
0
DORSAL ROOT AFFERENTS
VENTRAL ROOT AFFERENTS
0
1
2
3
4
5
6
7
8
9
10
11
12
13
>13
OF DCV's

70

FIGURES 71 and 72

Figures 71 and 72 illustrate terminals labelled by ventral root application of HRP which are directly apposed to (figure 71), or synapsing (figure 72, double arrows) on PGN dendrites. The open block arrow in figure 71 indicates a labelled unmyelinated axon. Reaction product containing in inclusions are indicated by arrows in figure 71. Bar=0.5 um.


72
71

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