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Peptide- and Non-Peptide-Containing Unmyelinated Primary Afferents: The Parallel Processing of Nociceptive Information

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Peptide- and non-peptide-containing unmyelinated primary afferents: the parallel processing of nociceptive information

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[Plates 1–3]

Primary afferent C fibres can be subdivided into a number of subgroups on the basis of cytochemistry or receptor binding characteristics. Numerous peptides have been localized to dorsal root ganglia, yet these appear to be only found in approximately 50% of small perikarya. A large proportion of the remaining small cells do not contain peptides but are identifiable in rodents by their content of a fluoride resistant acid phosphatase. Attempts have been made to correlate particular biochemical types with particular receptive field profiles, with rather modest success. As an alternative we suggest, principally from an analysis of skin afferents, that peptide- and non-peptide-containing afferents are two distinct C fibre pathways innervating similar peripheral structures and conveying similar information, but to different areas within the dorsal horn. Morphological evidence also suggests that these two subsystems form either glomerular or simple synaptic arrangements in the dorsal horn. The significance of parallel pathways for the processing of nociceptive information is briefly discussed.

1. Introduction

It has been widely assumed that the enormous amount of biochemical differentiation seen among primary afferent C fibres correlates with the different receptive field profiles reported for these sensory neurons. A recent re-examination of this question, however, revealed considerable numerical difficulties with such a simple physiological–cytochemical correlation (Lynn & Hunt 1984). We suggest here that most of the available data can be fitted into an alternative framework; that peptide- and non-peptide-containing pathways represent parallel afferent systems carrying similar information to different areas of the superficial dorsal horn. This is illustrated diagramatically in figure 1.

2. Two modes of termination for C fibres within the dorsal horn

Approximately 70% of all primary afferent fibres are unmyelinated C fibres (Nagy *et al.* 1983). A proportion of these and certain small diameter myelinated A δ fibres are thought to carry the information related to nociception (Cervero & Iggo 1980). Autoradiographic tracing experiments with tritiated proline injected into the dorsal root ganglion revealed that in the rat the majority of primary afferent termination is within the first two layers of the dorsal horn (Nagy & Hunt 1983), that is the marginal layer (layer I) and the substantia gelatinosa (layer II) which is further sub-divided into inner (II_i) and outer (II_o) sub-layers. Closer examination reveals that the majority of termination is within the outer portion of layer I and the inner portion of layer II although some termination is found throughout the dorsal horn.

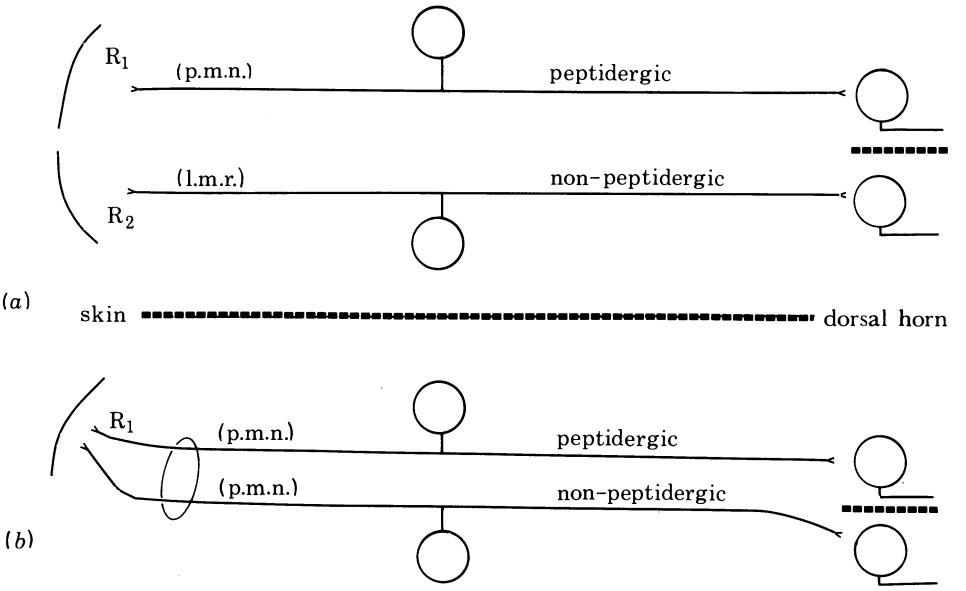

FIGURE 1. A summary diagram of the two hypothetical modes of primary afferent C fibre organization. Case (*a*) represents the first, i.e. peptide- and non-peptide-containing neurons have distinctive non-overlapping receptive field properties. Case (*b*) is the alternative hypothesis, that there are parallel peptide- and non-peptide-containing pathways carrying the same information to different areas of the dorsal horn. An example explained in the text is given in brackets. P.m.n.: polymodal nociceptive neuron; l.m.r.: low threshold mechanoreceptive neuron: both are found within the saphenous nerve innervating the skin.

The termination of C and A δ fibres within the superficial dorsal horn was also investigated following injection of the enzyme horseradish peroxidase into the dorsal root ganglion (Nagy & Hunt 1984). At the earliest survival times only C fibre terminations were labelled but in a Golgi-like fashion. Three tiers of C fibre termination were seen within layers I, II_o and II_i (figure 2). At longer survival times (seven days) C fibres arborizations were no longer visible and A δ terminal patterns were seen. These were localized to layers I, II_o and the border zone between layers II and III. Observations with the electron microscope in rat (Ribeiro-da-Silva & Coimbra 1982; Hunt & Nagy, unpublished observations), monkey (Ralston 1979) and cat (Snyder 1982) revealed that there were a number of distinct modes of synaptic termination within the superficial layers of the dorsal horn. The most conspicuous was a glomerular type of termination with a central primary afferent axon terminal surrounded by a number of small dendritic profiles. As this type is destroyed in rat by neonatal application of the drug, capsaicin (Ribeiro-da-Silva & Coimbra 1984) it is assumed to be derived from C fibres. A second type of ending which may form glomerular of scalloped endings is thought to be derived from A δ fibres and is found primarily within layers I and II_o and within the border zone between layers II and III, that is not within layer II_i (Réthelyi *et al.* 1982; Ribeiro-da-Silva & Coimbra 1984). A rather less well-defined group of primary afferent endings contains a variety of organelles including usually dense core vesicles but which do not form glomerular synaptic complexes. These are probably also C fibre terminals and are limited to layers I and II_o. In summary (figure 2) layer II_i receives only a C fibre input, axons terminating usually as the central elements in synaptic glomeruli while layers I and II_o have a mixed Aδ–C fibre input. This group of C fibres does not usually form glomeruli. Primary afferent termination is highly

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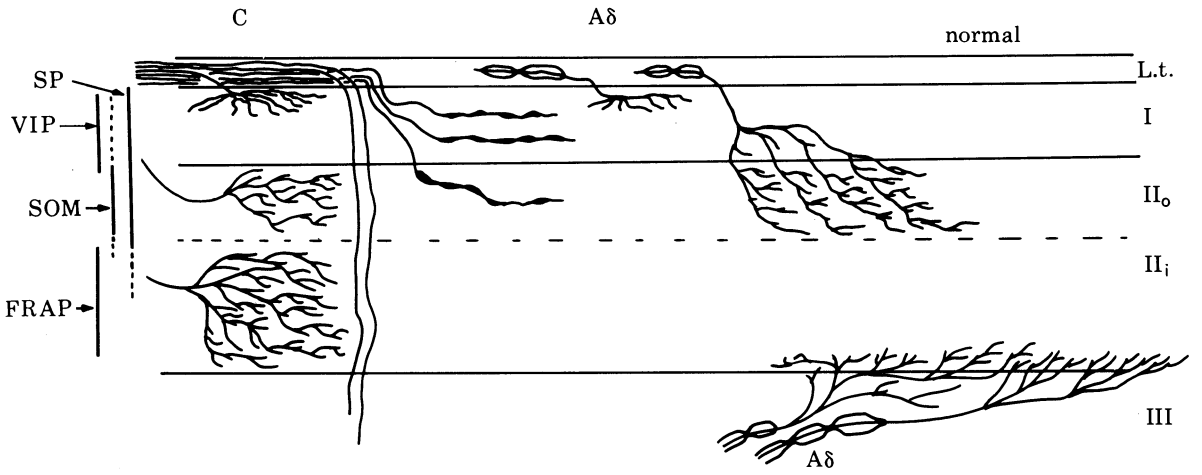

FIGURE 2. A diagrammatic representation of C and Aδ primary afferent arborizations within the superficial dorsal horn. These were reconstructed from a study that exploited the differential labelling patterns seen following injection of horseradish peroxidase into dorsal root ganglia (Nagy & Hunt 1983). The termination field of the various peptides and FRAP containing primary afferents within the dorsal horn is shown on the left. (From Lynn & Hunt (1984), with permission.) VIP: vasoactive intestinal polypeptide; SP: substance P; SOM: somatostatin; FRAP: fluoride-resistant acid phosphatase; LT: Lissauer's tract; I: marginal layer; II: substantia gelatinosa.

topographic within layer II but extends across several segments within layer I (Nagy & Hunt 1983). There is, therefore, evidence to suggest that there are two groups of C fibres terminating in distinct areas of the dorsal horn, and with different synaptic relationships.

3. THE ORGANIZATION OF THE DORSAL HORN

The internal structure of the dorsal horn is complex. The most accessible summary of the internal structure has been provided by Gobel (1979) following on from earlier Golgi studies (for review see Cervero & Iggo 1980). Spino-thalamic projection neurons are located in layers I and V. There are two principal types of interneuron within the substantia gelatinosa; the stalked cell and the islet cell. Stalked cells are thought to project into layer I, while islet cells have a limited axonal arborization and are thought to communicate primarily through the dendritic release of neurotransmitter. Both types of cell may be contacted by primary afferent fibres (Gobel *et al.* 1980).

A large number of peptides and other putative neurotransmitter substances have been localized in interneurons but not projection neurons of the dorsal horn. Of the principal interneuronal types, subgroups of stalked cells have been shown to contain enkephalin, glutamic acid decarboxylase (the synthetic enzyme for the neurotransmitter GABA), and possibly neuropeptide Y, while certain subgroups of islet cells may also contain enkephalin and neurotensin (Hunt *et al.* 1982; Bennett *et al.* 1982). It is also becoming clear from intracellular recording studies that morphological type does not predict the receptive field profile (Bennett *et al.* 1980). This may suggest that there are sub-populations of stalked and islet cells with distinct cytochemical and physiological profiles.

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4. Primary afferent neurons: histochemical distinctions

C fibres are thought to be derived from small diameter perikarya within the dorsal root ganglion (Lieberman 1976) and terminate in two distinct fashions within the dorsal horn as outlined above. These two groups of C fibres can also be distinguished on the basis of their peptide content. In rodents those not containing peptides for the most part contain a fluoride-resistant acid phosphatase (FRAP) of unknown function (Coimbra *et al.* 1974), and there appear to be roughly equal proportions of FRAP and peptide-containing small perikarya within the dorsal root ganglion. On cytochemical grounds the axons of these two groups of cells also terminate in distinct territories within the dorsal horn. FRAP positive termination within the lumbar and cervical enlargements is predominantly within deeper proportions of the substantia gelatinosa, layer II_i (Coimbra *et al.* 1974; Csillik & Knyihar 1978). Whereas peptide containing fibres, regardless of whether they contain somatostatin, substance P, cholecystokinin, calcitonin gene-related peptide or vasoactive intestinal polypeptide-like immunoreactivity, terminate within layers I and II_o of the dorsal horn (Gibson *et al.* 1981; Hunt *et al.* 1982; Rosenfeld *et al.* 1983). At the electronmicroscopic level most FRAP is restricted to the glomerular type of axonal endings (Coimbra *et al.* 1974), while peptide containing terminals seem to be of the dense core vesicle containing variety and are rarely found in glomeruli (Barber *et al.* 1979). There is some evidence to suggest that substance P containing primary afferents may directly contact spino-thalamic neurons in layer I (Priestley & Cuello 1983), whereas this route is not open to the deeper lying glomerular type of non-peptidergic, FRAP containing primary afferent. From the distinct termination pattern of peptidergic and non-peptidergic primary afferents within the dorsal horn it was predicted that FRAP and peptides could not co-exist within the same dorsal root ganglion cell. This was in fact shown to be the case (Nagy & Hunt 1981) although a very small amount of co-existence cannot be ruled out. In a study demonstrating this lack of co-existence we counted twice as many FRAP neurons as substance P positive neurons although this was without colchicine treatment to build up peptide levels (Nagy & Hunt 1981).

We have also examined the question of whether these two systems, of peptide- and non-peptide-containing neurons are exclusively unmyelinated fibre pathways. Briefly, rat sciatic nerve was ligated and 18 hours allowed for the build-up of peptide levels proximal to the ligation. The accumulation of peptide within fibres was then examined with the electron microscope. Exactly the same procedure was repeated for FRAP containing C fibres. In both cases we were unable to find evidence for either substance P or FRAP containing myelinated fibres. Peptide and FRAP were found exclusively in small groups of unmyelinated fibres present within larger bundles of unmyelinated axons (figure 3, plate 1) (Rossi & Hunt, unpublished observations).

5. Peripheral targets of unmyelinated sensory fibres

The hypothesis outlined in the introduction suggests that peripheral target areas are innervated by both peptide and non-peptide containing primary afferents. To test this directly we examined peripheral tissues for either their content of substance P containing fibres (or other peptide containing fibres) and for the possible dual innervation by FRAP containing sensory neurons. Substance P and other peptides were examined on thick sections of fixed target tissues,

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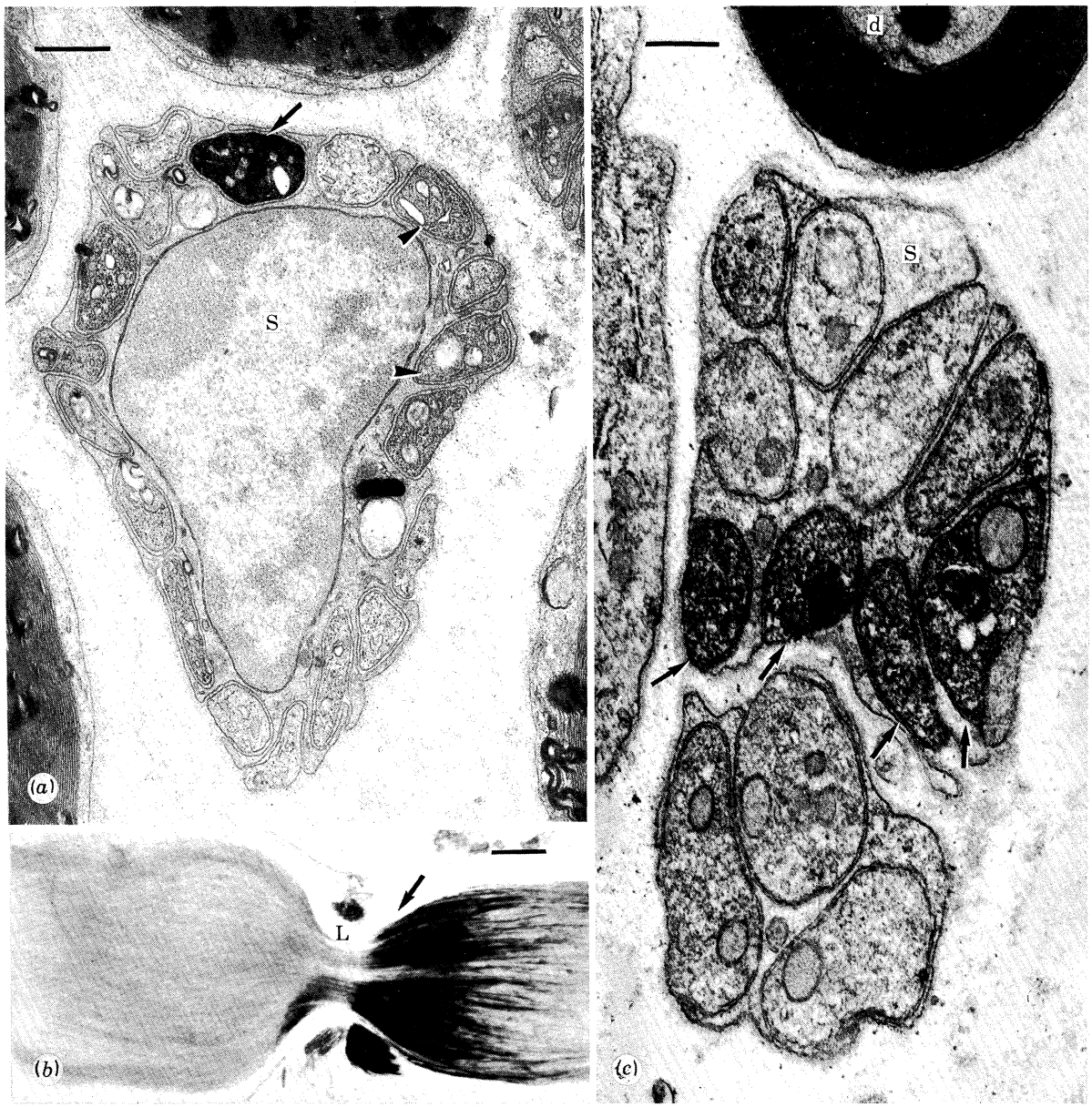

FIGURE 3. (*a*, *c*) Electron-microscopic observations made on the sciatic nerve following ligation as in (*b*) and subsequent immunohistochemical staining for substance P (*c*) or histochemical staining for acid phosphatase (*a*). Acid phosphatase accumulates proximal to the ligation shown in (*b*). At the electron-microscopic level (*a*) only unmyelinated fibres (arrow) contain acid phosphatase along with a number of less heavily stained unmyelinated profiles (arrowheads). Similar nerves stained for substance P (*c*) were also found to contain stained unmyelinated profiles but no myelinated fibres were labelled. A small number, but never all, of the fibres in a bundle related to one Schwann cell were stained with either marker. (From Rossi & Hunt, unpublished observations.) Scale bar: (*a*), 0.45 μm; (*b*), 200 μm; (*c*), 0.30 μm.

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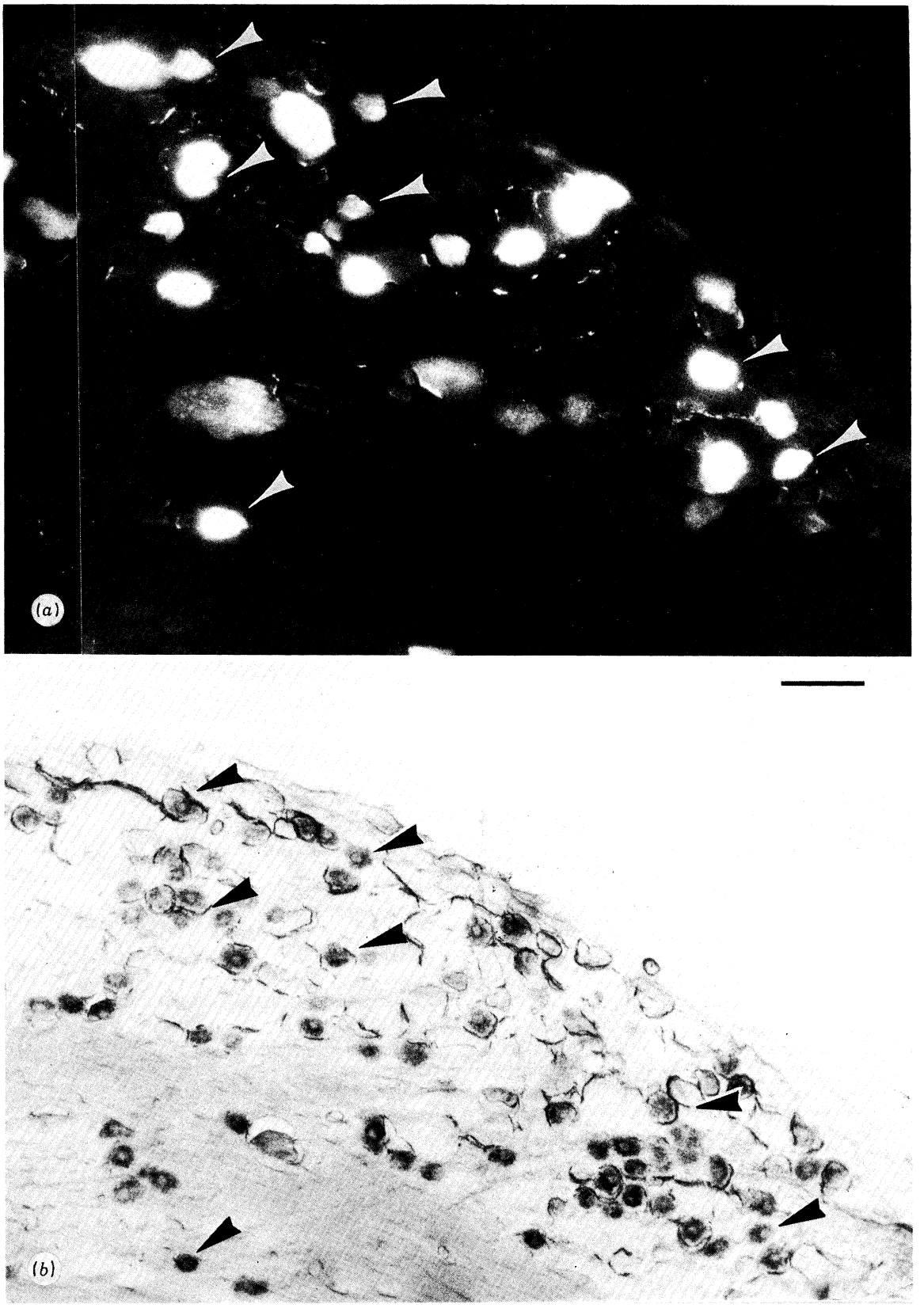

FIGURE 4. (*a*) The retrograde labelling of dorsal root ganglion (L5) cells following injection of True Blue into the lateral hairy skin of the root. (*b*) The same section subsequently stained for FRAP. A number of double labelled neurons are indicated by arrowheads. Scale bar = 70 μm. (From Rossi and Hunt, unpublished observations.)

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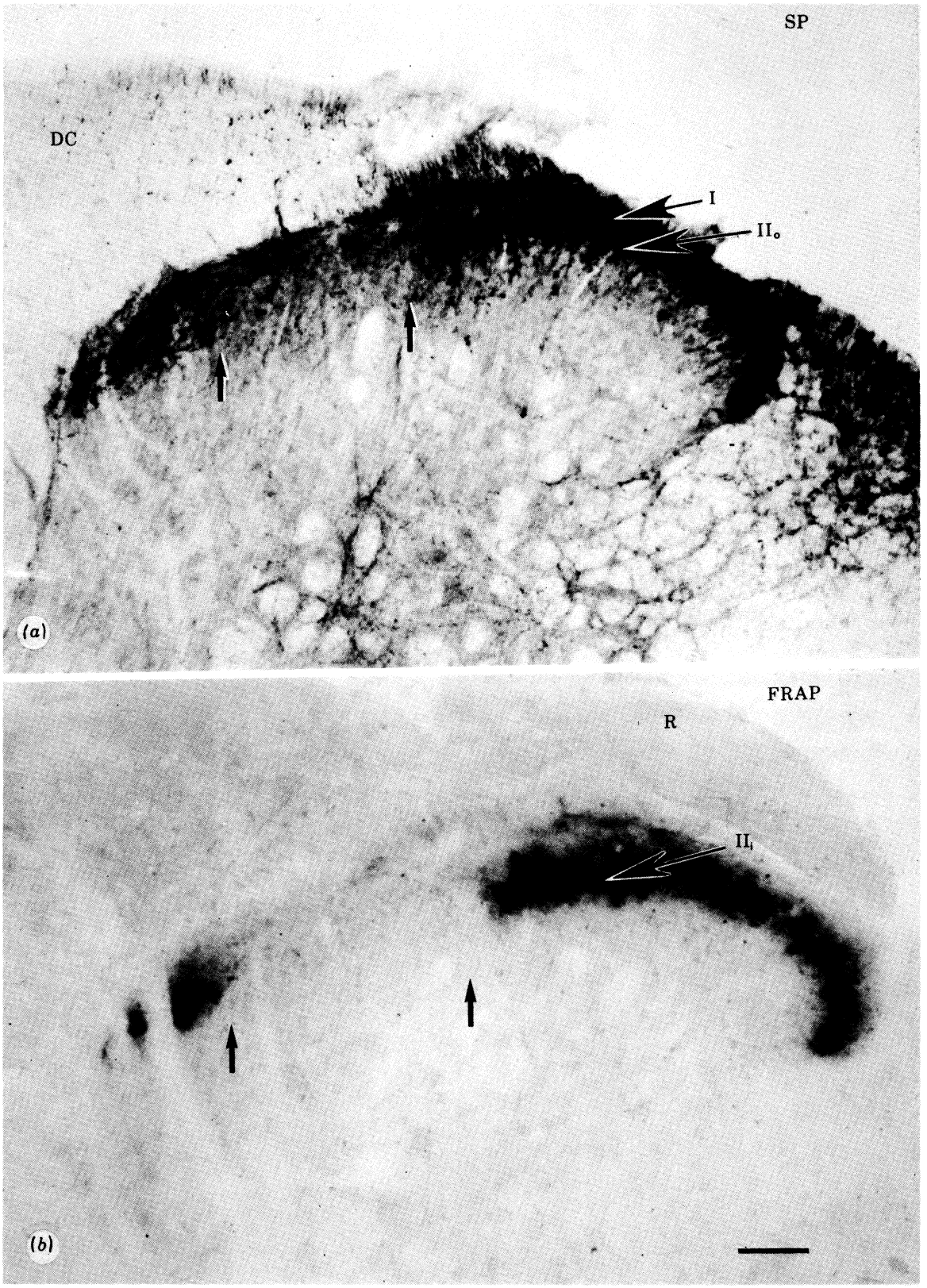

FIGURE 5. The dorsal horn stained for substance P (*a*) or FRAP (*b*) 14 days after section of the saphenous nerve at the thigh level. Drop-out of substance P or FRAP is indicated in the area between the arrows. R: dorsal root; DC: dorsal columns. Scale bar: 120 μm. (From Rossi and Hunt, unpublished observations.)

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such as muscle, skin or joints, using immunohistochemical techniques. FRAP is not visible at the single fibre level, however, and the problem must be approached indirectly. This involved injecting the dye True Blue (1%) into target issues and allowing time for the retrograde movement of the dye within primary sensory neurons. After a sufficient survival time ganglia were removed and examined for fluorescent labelling with True Blue and subsequently processed for acid phosphatase histochemistry to assess the degree of double labelling of sensory neurons. Finally we made use of the observation that damage to the peripheral process of a dorsal root ganglion cell results in changes in the central process of the same cell within the dorsal horn (Csillik & Knyihar, 1978; Barbut *et al.* 1981). This is seen as changes in FRAP and peptide levels within layers I and II of the spinal cord.

Immunohistochemical analysis of peripheral tissue revealed that substance P was found primarily within the dermis and to a lesser extent the epidermis and around blood vessels throughout the limb (Cuello *et al.* 1978). There was no indication of any substantial innervation of muscle. This analysis was confirmed by double labelling studies with the use of True Blue. Injection of True Blue into muscles labelled neurons in the dorsal root ganglia that did not contain substance P or to any great extent, FRAP. However, injections of True Blue into hairy skin of the foot resulted in extensive labelling of small perikarya within the appropriate dorsal root ganglion (figure 4, plate 2). Approximately 50% of the small cells labelled contained FRAP. Finally section of the saphenous nerve, a purely cutaneous nerve, results in loss of both substance P and FRAP from the dorsal horn. The loss of substance P and FRAP was topographically similar (figure 5, plate 3) (Rossi & Hunt, unpublished data).

Taken together with the observation that 80% of saphenous C fibres supplying hairy skin are polymodal nociceptors (Lynn & Carpenter 1981; Fleischer *et al.* 1983) then either there has been considerable bias in the cytochemical or physiological sampling or both FRAP and substance P containing sensory neurons, which innervate skin, have similar receptive field properties.

6. Parallel processing and analgesia

If there were two C fibre pathways by which information related to nociception can enter the dorsal horn then analgesia would result most profoundly from interference with both of these pathways and their postsynaptic targets. One of the most potent methods of obtaining analgesia is by the intrathecal perfusion of opiates over the spinal cord (Yaksh & Rudy 1976). Opiate receptors that are thought to mediate this response are found by autoradiographic methods throughout layers I and II of the dorsal horn but without any noticeable sub-layering (Young & Kuhar 1979; Ninkovic *et al.* 1981, 1982; Hunt *et al.* 1982). Some of these receptors are thought to be on primary afferents and indeed opiate receptors have been found over small perikarya within dorsal root ganglia (Ninkovic *et al.* 1982). It was also subsequently shown in the monkey that some of these opiate-receptor-positive small perikarya also contained substance P and equally importantly that equal numbers of neurons not containing substance P also possess opiate receptors (Hunt & Ninkovic 1983). Loss of opiate receptor following dorsal root section has been detected throughout layers I and II and is not restricted to superficial areas of peptide containing primary afferent termination or to the deeper areas of non-peptidergic termination (Ninkovic *et al.* 1981, 1982). This would suggest that both peptide- and non-peptide-containing primary afferent neurons possess opiate receptors and therefore may both be involved in the mediation of analgesia by exogenously applied opiates.

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7. Conclusions

It is suggested that there are two parallel C fibre primary afferent pathways carrying similar sensory information into different areas of the dorsal horn. The evidence supporting this hypothesis is largely derived from observations on the innervation of the skin but may be equally applicable to other peripheral target tissues such as muscle, joints and viscera but about which we have at present rather limited cytochemical information. Sensory pathways from the skin have been identified by virtue initially of their peptide content. Peptide-containing fibres terminate superficially within dorsal horn layers I and II_o, are in a position to directly contact spinothalamic neurons and rarely form synaptic glomeruli. The pattern of distribution is similar to that of small diameter myelinated (Aδ) fibres, which are in many cases the axons of high threshold mechanoreceptive sensory neurons (Réthelyi *et al.* 1982). Non-peptide-containing C fibres, including those containing FRAP, terminate deep within the substantia gelatinosa of the cervical and lumbar enlargements, are not in a position to directly influence the majority of spinal projection neurons, and terminate in a characteristic glomerular type of synaptic arrangement. Certain neurons within each population may possess opiate receptors and it is proposed that opiate induced analgesia results in part from a pharmacological action on both C fibre subsystems. This suggests that the assessment of the analgesic potency of antagonists to peptides such as substance P will have to take into account the existence of alternative nociceptive C fibre pathways.

The role played by the peptide- or non-peptide-containing afferents in sensory mechanisms at the level of the spinal cord is unknown. The release of peptides from primary afferent terminals within the dorsal horn may result in the generation of the slow postsynaptic potentials that have frequently been described (Iversen 1984). Peptides could be released in concert with other more 'classical' neurotransmitter substances but would remain a unique feature of this pathway. Neurotransmitter candidates for the non-peptide-containing C-fibre pathways have included ATP and glutamate but the evidence is inconclusive (see Jessell, this volume).

The interpretation suggested here may also have consequences for our understanding of events that occur peripherally. If axon terminals containing substance P and FRAP occur in close proximity within the skin and around blood vessels they may interact synergistically in the mediation of vasodilatation or extravasation (Lembeck & Gamse 1982). Similarly the central events and pathology that may underlie chronic pain states will have to take into account the different contribution made by the peptide- and non-peptide-containing C fibre sensory pathways in nociception.

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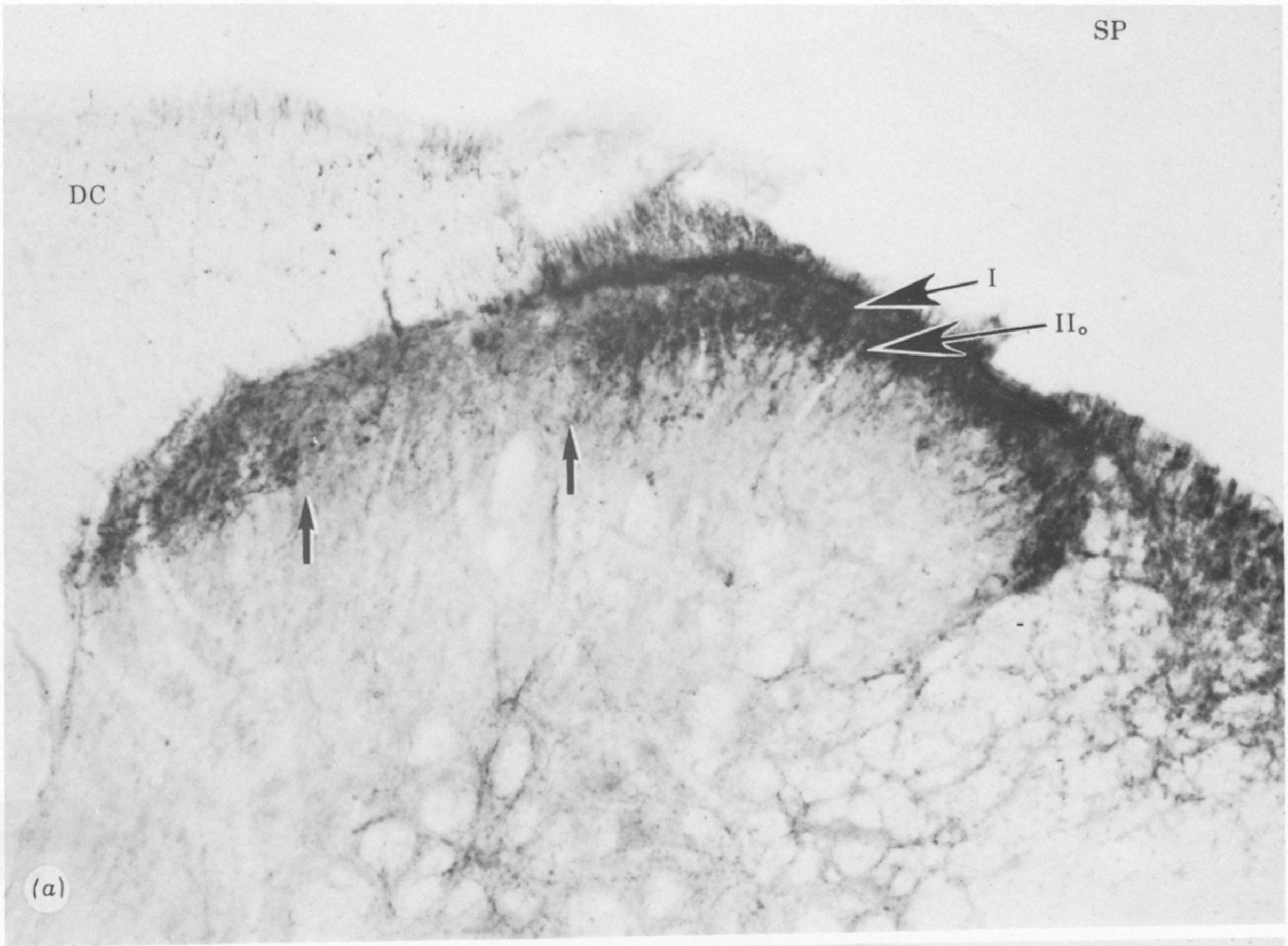

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