THE MORPHOLOGY, SOMATOTOPY AND PLASTICITY OF HINDLIMB LOW THRESHOLD CUTANEOUS PRIMARY AFFERENTS IN THE DORSAL HORN OF THE RAT LUMBAR SPINAL CORD.

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ABBREVIATIONS.

HRP	horseradish peroxidase
HFA	hair follicle afferent
RA	rapidly adapting afferent
SAI	slowly adapting type-I afferent
SAII	slowly adapting type-II afferent
SCT	spinocervical tract cell
PSDC	postsynaptic dorsal column
DRG	dorsal root ganglion
RF	receptive field
SG	substantia gelatinosa
AChE	acetylcholinesterase
FRAP	fluoride resistant acid phosphatase
TMP	thiamine monophosphatase
SP	substance P
SOM	somatostatin
CCK	cholecystokinin
VIP	vasoactive intestinal polypeptide
CGRP	calcitonin gene related peptide
DYN	dynorphin
ENK	enkephalin
SK	substance K
AVP	argenine vasopressin
GAL	galanin
BOM	bombesin
OXY	oxytocin
Mab	monoclonal antibody
Sa	saphenous
Su	sural
LS	lateral sural
PC	posterior cutaneous
Ti	tibial
STT	spinothalamic tract
P/PND	postnatal day
E	embryonic day

ABSTRACT.

The morphology of the collateral arborizations of hair follicle, (HFA's, n=38), rapidly adapting, (RA, n=14) and slowly adapting type-I, (SAI, n=6) afferents innervating hindlimb skin were studied by intraaxonal injection of horseradish peroxidase (HRP) in adult rats. Each physiological class of afferent possessed complex, simple and blind-ending collaterals based on numbers of boutons and terminal branch pattern. Each afferent had a distinct morphology, laminar location and dimensions depending on their peripheral receptive field (RF) location.

The location of the central terminal field of each afferent was reconstructed and somatotopic maps constructed for each afferent type. Overlap of central terminal fields was extensive between afferents within the same cutaneous nerve but it was restricted to blind, and on some occasions simple, collaterals between afferents from different cutaneous nerves. The spatial organization of the central terminals of cutaneous primary afferents formed a coarse somatotopic map of overlapping terminals whereby a region of dorsal horn had a maximal, but not exclusive, input from a particular skin area. This data was used to test the morphological and somatotopic consequences of neonatal deafferentation.

Neonatal administration of capsaicin on the day of birth resulted in dorsally directed sprouting of HFA's, but not RA afferents, into lamina II without altering the gross morphology or somatotopic organization of primary afferent collaterals.

Neonatal peripheral nerve section also resulted in sprouting, more complex than that following capsaicin treatment. Intact afferents adjacent to a denervated region sprouted from their normal terminal areas into denervated regions. When a particular functional class of afferent sprouted into an area containing terminals from another afferent class, the morphology of the sprouted terminal was appropriate to the new target area, rather

than to its own functional class. This indicates that the central rather than the peripheral target determines the terminal growth pattern.

In conclusion, the pattern and morphology of low threshold primary afferents is not fixed but can be altered by peripheral manipulations at birth.

CHAPTER 1 : GENERAL INTRODUCTION.

The pathways and patterns of terminations of primary afferent fibres in the central nervous system (CNS) are of great importance in the understanding of somatosensory transmission and pain. The central conduction of sensory information from the periphery has at least two different functions: to specify the characteristics of the stimulus in terms of sensory modality and to inform about stimulus position. The frequency of firing and activity of the primary sensory neurons provide detailed information about the stimulus (Burgess and Perl, 1973) and the pattern of firing of individual primary afferents will also depend on the physiological or pathophysiological state of the tissue (Sessle, 1987; Devor, 1988). In the dorsal horn of the spinal cord the dorsoventral position of the primary afferent terminals excited by a stimulus provides some information about the sensory modality of the stimulus, while the rostrocaudal and mediolateral positions of the afferent terminals provides information about the stimulus location on the body surface.

The exact site and distribution of afferent terminals determines the nature of the information received by different groups of second order neurons. In the CNS, cells in the vicinity of the primary afferent terminals have the opportunity for monosynaptic input, while distant cells may receive the information via distal dendrites or interneurons and so the timing of their responses will be different to that of nearby neurons. Physiological studies have indicated that afferent fibres of different sensory modalities are connected to separate groups of second order neurons. For example, hair follicle afferents (HFA's) make synaptic contacts with spinocervical tract cells (SCT's, Brown and Noble,1982; Brown et al., 1987a; Maxwell and Rethelyi, 1987) while slowly adapting type-I (SAI) afferents do not excite SCT cells but make monosynaptic contacts with other lamina IV

cells (Brown et al., 1973; Tapper et al., 1973; Tapper and Wiesenfeld, 1980, 1981). Cells in lamina II apparently receive time-locked monosynaptic input from A-delta and C fibres (Christensen and Perl, 1970; Kumazawa and Perl, 1976; Fitzgerald and Wall, 1980). Golgi and HRP studies have shown that cells in lamina IV also receive, presumably A-delta and C, monosynaptic primary afferent input but via their distal dendrites in lamina II (Light and Kavookjian, 1988; Todd, 1989). The distribution of primary afferents also determines the somatotopic organization of the second order neurons, whereby each point of the periphery (peripheral receptive field, RF) is represented at a particular site along the mediolateral and rostrocaudal extent of the dorsal horn (Wall 1960; Bryan et al., 1973; Brown and Fuchs, 1975; Koerber, 1980; Cervero and Tattersall, 1984; Light and Durkovic, 1984; Ritz et al., 1985; Swett and Woolf, 1985; Sorkin et al., 1986; Wilson et al., 1986; Woolf and Fitzgerald 1986; Chandler et al., 1988; Wilson and Snow 1988c; Pubols et al., 1989). In this way RF's of anatomically adjacent neurons from a continuous, but distorted map of the skin surface. This map is represented at each level of the neuraxis (Kandel and Schwartz, 1985); for example at the level of the dorsal column nuclei (Maslany et al., 1988a,b), cuneate nucleus (Crockett et al., 1988) and somatosensory cortex (Krubitzer and Kaas 1988; Snow et al., 1988).

1.1 METHODS OF INVESTIGATION OF PRIMARY AFFERENT TERMINALS.

Anatomical, physiological and neurochemical methods are available to trace the central course of primary afferent terminals and over the past 15 years a wealth of information has been provided about the termination sites in the CNS.

1.2 ANATOMICAL METHODS.

Golgi studies by Cajal (1909) of primary afferent terminals provided the basis of our knowledge today. More recent Golgi studies (Scheibel and Scheibel 1968; Hamano et al., 1978; Beal, 1979; Beal and Bicknell, 1981; Rethelyi, 1981; Cruz et al., 1987; Beal et al., 1988) have provided further details of cutaneous afferent morphology.

Degeneration techniques after cutting dorsal roots have been used to trace the pathways, location and the ultrastructure of terminal boutons (Lamotte, 1977; Ritz et al., 1985; Kapadia and Lamotte, 1987; Lamotte and Kapadia, 1987; Pubols and Bowen, 1988; Pubols and Foglesong, 1988). Cutting peripheral nerves, which results in the transganglionic degeneration of central terminals, has also been used to trace the central connections of particular nerves (Bondok and Sansone, 1984; Culberson and Brown, 1984; Arvidsson and Ygge, 1986; Giesler et al., 1988; Ygge, 1989).

Tritiated amino acids injected into the dorsal root ganglia (DRG) are transported to the central terminals and the site and distribution of these terminals can be seen with autoradiographic techniques (Lamotte, 1977; Ralston and Ralston, 1979, 1982; Rethelyi et al., 1979; Snyder, 1982).

The enzyme horseradish peroxidase (HRP) has proved to be a very powerful tool in elucidating the central connections of primary afferents. It can be crushed into the dorsal roots or peripheral nerves and it is taken up and transported by the damaged axons to its central terminals where its presence can be determined histologically and observed by light and electron microscopy (Proshansky and Egger, 1977; Light and Perl, 1979a; Gobel and Falls, 1979; Gobel et al., 1981; Cervero and Connell, 1984a,b; Coimbra et al., 1986; Imamura et al., 1986; Beattie et al., 1987; Baron et al., 1988; Casale et al., 1988; Liu and Hu, 1988; Pfaller and

Arvidsson, 1988; Bolton et al., 1989 a,b,c; Neuhuber and Zenker 1989).

HRP labelling has demonstrated the central terminal distribution of muscle nerves (Mysicka and Zenker, 1981; Ammann et al., 1983; Craig and Mense, 1983; Abrahams et al., 1984; Bakker et al., 1984; Nyberg and Blomqvist, 1984; Abrahams and Swett, 1986; McKenna and Nadlehaft, 1986; Mense and Prabhakar, 1986; Nishimori et al., 1986; Capra, 1987; Molander and Grant, 1987; Craig et al., 1988; Rivero-Melian and Grant, 1988; Shigenaga et al., 1988; Mense and Craig, 1988; Arvidsson and Rappana, 1989; Neuhuber and Zenker, 1989); visceral afferents (De Groat et al., 1978; Morgan et al., 1981, 1988; Neuhuber, 1982; Nadlehaft and Booth, 1984; Kuo and De Groat, 1985; Janig and McLaughlin, 1986;Kneupfer et al., 1988) and cutaneous nerves (Koerber and Brown, 1980, 1982; Fitzgerald and Swett, 1983; Smith, 1983; Ygge and Grant, 1983; Abrahams et al., 1984; Shigenaga et al., 1984; Kauz and Rethelyi, 1985; Molander and Grant, 1985, 1986; Nyberg and Blomqvist, 1985; Swett and Woolf, 1985; Abrahams and Swett, 1986; Woolf and Fitzgerald, 1986; Fitzgerald, 1987; Florence et al., 1988, 1989; Maslany et al., 1988a,b; Nyberg, 1988; Rice et al., 1988; Swett et al., 1988; Bolton et al., 1989 a,b,c; Rasmusson, 1988, 1989; Ygge, 1989; Brown et al., 1989; Culberson and Brushart, 1989).

Undamaged nerve terminals take up HRP from the surrounding tissues and this is improved if the HRP is conjugated to wheat germ agglutinin (WGA-HRP) which is actively transported to the central terminals (Carson and Mesulam, 1982; Molander and Grant, 1985, 1987). Other lectins can be used and these are taken up and transported at different rates (Robertson and Arvidsson, 1985; Robertson and Grant, 1985; Rivero-Melian and Grant, 1988). However, one of the most important applications of HRP to afferent morphology has been the technique of intracellular injection into single physiologically identified axons (Brown et al., 1977). This will be discussed in detail later (see section 1.6).

1.3 HISTOCHEMICAL METHODS.

Histochemical methods using the chemistry of primary sensory neurons have been used to map their central terminals. The enzyme fluoride resistant acid phosphatase (FRAP) has been shown to be present in the small B-type neurons of rodents and is transported to their central terminals where it disappears following dorsal root and peripheral nerve section (Coimbra et al., 1984; Kniyhar et al., 1986) and this property has been used to map the distribution of the terminals of different peripheral nerves (Devor and Clayman, 1980). More recently, thiamine monophosphatase (TMPase), a form of FRAP, has been used to produce more precise results (Kniyhar et al., 1986; Bezzagh et al., 1986). Carbonic anhydrase is restricted to the large A-type DRG neurons (Sommer et al., 1985) while other enzymes such as adenosine deaminase and glutaminase are also restricted to small type B neurons (Cangro et al., 1985; Nagy and Daddona, 1985) and these may be used in the future to examine the central terminations.

Peptides such as substance P (SP), somatostatin, (SOM) cholecystokinin (CCK), calcitonin gene related peptide (CGRP), vasoactive intestinal polypeptide (VIP), substance K (SK), dynorphin (Dyn), enkephalin (ENK), oxytocin (OXY), argenine vasopressin (AVP), galanin (GAL), and bombesin (BOM) are all found in primary afferents and colocalise in varying combinations with each other (Dalsgaard et al., 1982; Price, 1985; Jessell and Dodd, 1986). Their distribution is observed by immunocytohistochemical methods (Hokfelt et al., 1975) and can be used to map terminal fields since peripheral nerve section (Barbut et al., 1981) and capsaicin treatment (Jansco et al., 1981) deplete the central terminals of peptides. Although it has proved difficult to associate particular peptides with particular categories of small afferents (Leah et al., 1985a) because upto 4 different

peptides can exist in a sensory neuron (Leah et al., 1985b; Plenderleith et al., 1986; Garry et al., 1988) it has been shown that the chemical expression of primary afferents is characteristic of the peripheral target they innervate (O' Brien et al., 1989).

Other studies using cytoplasmic and cell surface markers show that different populations of DRG neurons express different carbohydrate structures on their surface. Molecular markers that correlate with the large light and small dark cell populations have been identified (Dodd and Jessell, 1985). Monoclonal antibodies (Mab) directed against the 200Kd neurofilament protein selectively label large light cells while the filamentous antigen Mab/C11 is restricted to small and intermediate DRG neurons and exhibits little or no overlap with neurons that express Mab (Dodd and Jessell, 1986). Monoclonal antibodies raised against lactoseries carbohydrates selectively label small and intermediate neurons with neurons restricted to the superficial dorsal horn (laminae I-II) while Mab's directed against globose carbohydrates identify neurons with terminals in laminae I and III-IV, suggesting globose series antigens are restricted to high threshold mechanoreceptors or low threshold cutaneous receptors (Dodd and Jessell, 1985). RT 97 is another monoclonal antibody against neurofilament protein and exclusively labels large A type DRG neurons (Lawson et al., 1984; O' Brien et al., 1989) while the antibody 2C5 labels a subset of the small DRG cells (Lawson et al., 1985). As yet these antibodies have not been used to study primary afferent central terminals.

1.4 ELECTROPHYSIOLOGY.

Electrophysiological methods have also been used to map the central terminals. Stimulating the primary afferent terminals in the CNS and recording extracellularly from single afferents in the dorsal roots, DRG, or peripheral nerve and mapping the sites of lowest

threshold for antidromic stimulation identifies the presumed sites of terminals (McMahon and Wall, 1985). Primary afferent terminals are difficult to record from in the CNS, although it can be done with spike triggered averaging (Meyers and Snow, 1986). Technically, it is easier to record the responses from second order cells to afferent input. For example, if a cell is shown by electrophysiological techniques to receive monosynaptic connections, it is likely that the afferent terminates in the region of the cell body or proximal dendrites (Brown et al., 1987a). Another method used to map afferent terminals is to use negative (N) waves. The area of peak negativity in the spinal cord corresponds to the area where most interneurons are activated by the volley. The current flows into sinks produced by the somata and dendrites by excitatory post-synaptic potentials (EPSP's) and action potentials and these areas of maximum negativity are presumed to be areas of densest terminations from the incoming afferent fibres (Beall et al., 1977; Schouenberg, 1984).

1.5 SUMMARY OF AFFERENT TERMINATION SITES FROM THESE METHODS.

The results of these various anatomical and histochemical methods have shown a correlation between the termination position in the dorsal horn and the functional type of primary afferent fibre such that C-fibres are known to terminate within laminae I and II, with the very smallest diameter fibres (unmyelinated) terminating in lamina II and slightly larger unmyelinated fibres and some small myelinated fibres terminating in lamina IIi. A-delta fibres innervating high threshold mechanoreceptors are found to terminate in laminae I and V while low threshold A-delta afferents and the myelinated A-beta low threshold cutaneous mechanoreceptors are known to terminate ventral to lamina II in laminae III-VI in cats, rats, monkeys and racoons. (Schiebel and Schiebel,

1968; Lamotte, 1977; Proshansky and Egger, 1977; Hamano et al., 1978; Gobel and Falls 1979, 1981; Light and Perl, 1979a; Rethelyi et al., 1979; Ralston and Ralston, 1979; Mense et al., 1981; Dodd and Jessel, 1986; Kniyhar et al., 1986; Cruz et al., 1987).

1.6 INTRACELLULAR STAINING OF PRIMARY AFFERENTS WITH HRP.

The enzyme HRP has been used in neurobiology as a marker for the location of the cells of origin of distant axon terminals because it was taken up by the terminals and retrogradely transported to the cell bodies (see section 1.2). Thus, this enzyme seemed a good candidate for an intracellular marker since its presence could be detected by a histochemical reaction. In May 1975, the first successful results of the intracellular injection of HRP into spinal cord neurons were recovered by Alan Brown and his colleagues. The results showed densely stained somata, dendrites, axons and collaterals which were easily observed under the light microscope and fine collateral branches that could be traced to what appeared to be synaptic boutons. This was indeed a revolutionary new technique which combined the power of the morphological detail seen in Golgi studies with the capacity to fully explore the physiological properties (modality, RF properties) with the microelectrode before filling with HRP by passing positive current through the microelectrode. The HRP was then transported to the central terminals and then the anatomical distribution of a physiologically characterized afferent could be determined. This technique has been used to identify muscle afferents (Brown and Fyffe, 1978, 1979; Brown, 1981; Mense et al., 1981; Hongo et al., 1987; Kiersted and Rose, 1988; Hoheisel et al., 1989) cutaneous afferents in the brainstem (Hayashi, 1982, 1985a,b; Kalia and Richter, 1985a,b, 1988a,b; Fyffe et al., 1986; Chiaia et al., 1987; Crockett et al., 1988; Jacquin et al., 1984, 1986a,b, 1988; Maslany et al., 1988a,b; Renehan et al.,

1988a,b), cutaneous low and high threshold afferents in the spinal cord (Brown et al., 1977, 1978, 1980c, 1981, 1987a; Brown, 1981; Egger et al., 1981; Brown and Noble, 1982; Light and Perl, 1979b; Maxwell et al., 1982, 1984, 1985; Semba et al., 1983, 1984, 1985; Bannatyne et al., 1984; Meyers and Snow, 1984; Ralston et al., 1984; Sakada et al., 1985; Snow and Meyers, 1985; Fitzgerald and Woolf, 1987; Maxwell and Noble, 1987; Woolf, 1987; Brown et al., 1988; Culberson et al., 1988; Sonty et al., 1988; Wilson and Snow, 1988b; Koerber et al., 1989; Shortland et al., 1989a,b) Intracellular filling of physiologically characterized DRG's (Hoheisel and Mense, 1986; Sugiura et al., 1986,1988) and dorsal horn neurons (Snow et al., 1976; Light et al., 1979c; Brown et al., 1980a,b; Bennett et al., 1980; Fyffe, 1981; Maxwell et al., 1983, 1984b; Woolf and Fitzgerald, 1983; Ritz and Grenspan, 1985; Egger et al., 1986; Woolf and King, 1987; Light and Kavookjian, 1988) has also been demonstrated.

The combination of electrophysiological identification and anatomical staining has proved to be one of the most important techniques recently developed. It has provided detailed information of structure-function relationships and of the organization of afferents in the CNS. The pioneering work of Brown and his colleagues, R. Fyffe, R. Noble, P.K. Rose and P.J. Snow, has provided important information about the organization of afferents in the spinal cord. These results showed that each physiologically defined type of cutaneous afferent unit has a distinctive spatial distribution and morphology of terminal arborizations in the dorsoventral plane. In the cat and rat, HFA's distribute terminal boutons to laminae IIi-IV (Brown et al., 1977; Meyers and Snow, 1984; Woolf, 1987; Shortland et al., 1989a), RA's distribute boutons to laminae IIi-V (Brown et al., 1980c; Woolf, 1987; Shortland et al., 1989b) while Pacinian capuscles (PC's) distribute boutons to laminae III-VI (Brown et al., 1980c) and Slowly adapting type-I (SAI's) terminals are found within laminae III-VI (Brown et al., 1978; Woolf, 1987). High threshold

A-delta mechanoreceptors distribute their terminal boutons to laminae I and V (Light and Perl, 1979b; Hoheisel et al., 1989) while low threshold A-beta cutaneous mechanoreceptors terminate in lamina III (Light and Perl, 1979b); cutaneous C-fibres are located in laminae I-II (Sugiura et al., 1986).

The principle aim of this thesis has been to take advantage of this intracellular staining method to investigate, firstly, the normal morphology and somatotopic organization of the central terminals of individual physiologically characterized cutaneous low threshold mechanoreceptors innervating the rat hindlimb and secondly to investigate the plasticity of these termination patterns following neonatal manipulations of the peripheral nervous system.

The results from normal rats supplement information from earlier physiological and anatomical studies in the rat (Molander et al., 1985; 1986; Swett and Woolf, 1985; Woolf and Fitzgerald, 1986) and allow comparisons with other similar studies in the cat (Brown et al., 1977, 1978, 1980c, Light and Perl, 1979a,b; Koerber and Brown, 1980, 1982; Light and Durkovic, 1984, Meyers and Snow, 1984, Snow and Meyers, 1985; Snow and Wilson, 1985, 1988; Brown et al., 1988 Culberson et al., 1988). They were also used as control material for the analysis of morphological changes in mechanoreceptor afferent terminals following two kinds of neonatal deafferentation: (1) systemic administration of capsaicin at birth and (2) neonatal nerve section and ligation on the day of birth. Intraaxonal injection of HRP into single identified cutaneous afferents in these animals allowed investigation of the detailed morphological changes in central terminals that are produced by such neonatal interventions and demonstrate the ability of intact primary afferents to change their normal termination pattern in the face of altered input.

CHAPTER 2: MORPHOLOGY AND SOMATOTOPY OF LOW THRESHOLD CUTANEOUS PRIMARY AFFERENTS.

2.1 INTRODUCTION.

The intraaxonal HRP staining technique has shown that the collateral axons of coarse and fine primary afferents terminate in different regions of the dorsal horn of the spinal cord such that coarse (A-beta) and some fine (A-delta) myelinated fibres from cutaneous receptors arborize mainly in laminae III-V (Light and Perl, 1979b; Brown, 1981; Woolf, 1987) while other fine myelinated (A-delta) fibres from mechanical nociceptors terminate in laminae I and V (Light and Perl, 1979b) and C-fibres terminate predominantly in laminae I and II (Sugiura et al., 1986).

Intraaxonal staining of primary afferents has not only confirmed that there is a specificity in the termination sites of different classes of afferent fibres but has also shown that collaterals axons of different classes exhibit some degree of specificity in their branching patterns (Brown, 1981; Ralston et al., 1984; Maxwell and Rethelyi, 1987). Hair follicle afferents exhibited the "flame shaped arbors" originally described by Scheibel and Scheibel (1968) which formed an uninterrupted sheet running, in cat, rat and monkey, rostrocaudally in the dorsal horn (Brown et al., 1977; Light and Perl, 1979b; Woolf, 1987; Shortland et al., 1989a) while rapidly adapting afferents showed a different morphology, having collateral arbors restricted to the medial 1/4 of the dorsal horn, running rostrocaudally in the dorsal horn but as an interrupted sheet of terminals (Brown et al., 1980c; Woolf, 1987). Slowly adapting type-I afferents (SAI's) have a C or L-shaped curving collateral branch which ramified to produce the terminal arbor and the rostrocaudal distribution of collateral arborizations was also different: adjacent arbors rarely overlapped (Brown et al., 1978; Woolf, 1987). Different species of animal have shown morphological similarities

between similar functional classes of cutaneous afferent; for example HFA's are similar in cat, rat and monkey (Ralston et al., 1984; Woolf, 1987) while morphological differences exist for other functional classes such as the cat and rat SAI afferents (Woolf, 1987).

The spatial arrangement of the central terminals of cutaneous low threshold primary afferent neurons in the dorsal horn of the spinal cord is such that a continuous map of the skin surface is formed in the CNS. Such a somatotopic organization of the central terminals of cutaneous afferents within the dorsal horn has been demonstrated by bulk labelling of thoracic (Ygge and Grant, 1983), forelimb (Nyberg and Blomquist, 1985, Florence et al., 1988, 1989; Pfaller and Arvidsson, 1988; Brown et al., 1989; Culberson and Brushart, 1989; Ygge, 1989) and hindlimb afferents (Koerber and Brown, 1980, 1982; Molander and Grant, 1985, 1986; Swett and Woolf, 1985; Woolf and Fitzgerald, 1986; Lamotte et al., 1989) and muscle afferents (Molander and Grant, 1987; Rivero-Melian and Grant, 1988) by using the transganglionic transport of HRP and its conjugates (see section 1.2). Such peripheral nerve and skin labelling experiments have shown that afferents that innervate contiguous skin areas have terminals that occupy contiguous regions of spinal cord in the horizontal plane and that each cutaneous nerve has its own terminal area within the dorsal horn with little (Molander and Grant, 1985; Lamotte et al., 1989) or no (Swett and Woolf, 1985) overlap between adjacent nerve territories. This somatotopic organization is also seen in the brainstem nuclei (Maslany et al., 1988a,b; Nyberg, 1988; Rice et al., 1988; Rasmusson, 1989). The data from these studies suggests that the site of the peripheral RF of an afferent is encoded by its mediolateral and rostrocaudal position within the dorsal horn.

Taken together with the results from intraaxonal studies it can be shown that there are two superimposed patterns in the dorsal horn, one related to somatotopy in the mediolateral plane and one related to receptor type in

the dorsoventral plane.

Until now, study of somatotopy has been restricted to bulk labelling of populations of afferents, in contrast to detailed morphological studies on single afferents. However, in order to study how individual primary afferents are packed together within the dorsal horn it is necessary to look at somatotopy using the technique of injecting HRP into single physiologically characterized afferents. Within an individual nerve teritory there are many hundreds of afferents, which must be packed in such a way that central overlap is inevitable. The nature of this overlap will be a critical factor in the receptive field organization of second order cells in the dorsal horn. Therefore, the aim of the first part of this thesis has been to compare the position of the peripheral RF of a group of cutaneous afferents with the morphology and position of their central terminals arborizations in the dorsal horn. To do this, single low threshold cutaneous afferents (HFA's, RA's, SAI's) innervating different areas of the rat hindlimb have been studied.

2.2 METHODS FOR THE INTRAAXONAL INJECTION OF HRP INTO LOW THRESHOLD CUTANEOUS AFFERENTS.

2.2 (i) ELECTROPHYSIOLOGY.

The experiments were performed on 75 Sprague Dawley rats (180-450g). Under urethane anaesthesia (1.5g/kg), the trachea and one carotid artery were cannulated. The rats were then transferred to a frame and stabilized by means of ear and hip bars. They were then decerebrated by aspiration of all the cranial contents rostral to the midbrain via two holes made in the parietal bone. The cavity was filled with loosely packed cotton-wool and then the animals were paralyzed with gallamine (0.5ml of 40mg/ml Flaxedil, May and Baker) and artificially ventilated. The heart rate, expired pCO_2 and rectal temperature were monitored throughout the experiment and

kept within the normal physiological range. The heart rate signal was monitored by pin electrodes in the forepaws connected to an E.C.G. amplifier displayed on an oscilloscope and counted on a rate meter. The heart rate was kept between 350-550 beats per minute and if necessary maintained with saline administration in 0.5 ml doses. The rectal temperature was monitored by a negative feedback circuit operating a heating element positioned 15 cm away from the animal and kept within the range 34-37^{0}C. The end expiratory pCO_2 was sampled from the tracheal cannula and fed to a Beckman Medical gas analyzer LB2. The end expiratory pCO_2 was adjusted by altering the rate of ventilation and the tidal volume and maintained between 3% and 5%.

The lumbar enlargement was exposed by a dorsal laminectomy removing vertebrae T13-L3 and the vertebral column was stabilized using two spinal clamps applied to the T12 and L4 vertebrae and hip clamps. The tail was stretched out and held taut and a plaster cast was applied around the hips and hip bars further stabilizing the vertebral canal and spinal cord during the application of cutaneous stimuli to the hindlimb. The dura and pia mater were removed and a curved metal saddle was inserted under the spinal cord to support it and aid intracellular recording (Woolf and King, 1987). The exposed spinal cord was covered with agar (3%) and a small hole made in the agar which was filled with warm paraffin oil.

Recordings were made medial to the dorsal root entry zone and lateral to the central vein in the lumbar enlargement (segments L3-L5) using thin walled (1.2 mm diameter) glass electrodes filled with 5-10% HRP (Sigma type VI or Boeringer HRP) in a Tris/KCl buffer (pH 7.7, impedances 20-80 Mohms). Searches were made for low threshold cutaneous afferents with receptive fields (RF's) on the hindlimb as the microelectrode was tracked through the spinal cord in 4um steps (using a Burleigh Inchworm microdrive system). The hindlimb was brushed with fine camel hair brushes and the extracellular responses

recorded on an oscilloscope. As the microelectrode approached an afferent axon the extracellular spikes became larger and the microdrive stepper was then advanced forward in 2um and a capacitance "buzzer" used to intracellularly penetrate the afferent axon. This was visualized on the oscilloscope by a drop in the membrane potential of 20-40 mV, and intracellular spikes of 40-60 mV with an after hyperpolarization present on the action potential. When an afferent was successfully impaled the position of the RF was assigned to a particular nerve territory (as defined from the peripheral nerve map produced by Swett and Woolf, 1985) and the RF properties of the afferent was carefully mapped with blunt probes and fine camel hair brushes. HRP was then injected into the afferent by passing positive current, 2-10nA, 150 ms depolarizing pulses every 200 ms for 2-10 minutes. A bridge balance facility on the DC amplifier allowed the potential recorded by the electrode to be checked during the passage of HRP. A rapid return of the DC voltage to the baseline indicated that the electrode had fallen out of the afferent axon in which case the HRP injection was terminated immediately to prevent HRP leakage into other axons. Initially only 1 afferent filling on each side of the cord was attempted per animal, but as a somatotopic pattern for the afferents emerged then more than one afferent filling, usually of different functional classes and distant receptive fields (eg. a HFA and a RA) was attempted on each side of the cord. In these cases, it was noted whether the second afferent impaled was recorded rostral or caudal to the first afferent. The conduction velocity of the afferent was measured by stimulating the centre of the receptive field (5mA, 500uS, 1Hz) with pin electrodes.

2.2 (ii) HISTOLOGY.

The animals were perfused with 500 mls of chilled saline followed by a 1000 ml mixture of 1.25%

glutaraldehyde, 1% paraformaldehyde in 0.1M phosphate buffer pH 7.4, a minimum of 2 hours following HRP iontophoresis into a characterized afferent. Lumbar segments L2-L6 were identified by tracing the sciatic nerve from the sciatic notch and identifying the L3, L4, L5 and L6, dorsal roots into which the sciatic nerve is distributed (depending on whether the plexus is prefixed or postfixed, Janig and McLachan, 1986) and then isolating each dorsal root and inserting insect pins between adjacent root entry zones; the lumbar spinal cord was then removed and a pin inserted in the ventral half of the spinal cord, running rostrocaudally through the right hand side of the spinal cord so that identification of the left and right sides of the cord was possible on free floating sections. The cord was stored overnight at 4^0C in 0.1M phoshphate buffer with 20% sucrose (pH7.4). Serial transverse sections (50 um) were cut at -16^0C on a cryostat and dry mounted onto gelatinized slides (1%) or cut on a freezing microtome and stained free floating before mounting onto gelatinized slides. The slides were incubated in a catechol-p-phenylenediamine mixture (Hanker et al., 1977) and then left to dry for two hours before being rapidly dehydrated through alcohols to prevent leakage of the reaction product. The sections were cleared in Histoclear (National Diagnostics) and mounted in DPX (BDH Chemicals Ltd). They were observed under the light microscope and camera lucida reconstructions of the afferent arborizations (X16 or X25 magnification lenses) were made from all the transverse sections on tracing paper. Adjacent sections of the terminal arbor were superimposed so that the complete rostrocaudal extent of an arbor could be visualized on a single tracing. Also the position of axon collaterals along the rostrocaudal extent was noted and measurements of the number and dimensions of collateral arborizations were made.

Under dark-field illumination, the ventral border of the substantia gelatinosa could be clearly identified. This boundary was marked on the tracings and afferent

terminals which extended beyond this boundary into the substantia gelatinosa could be clearly identified. In all cases, those terminals that were located in the substantia gelatinosa were located in the ventral part which shall be termed lamina IIi throughout the text. No allowance was made for shrinkage but a shrinkage factor of 12-16% can be expected (Lux et al., 1970).

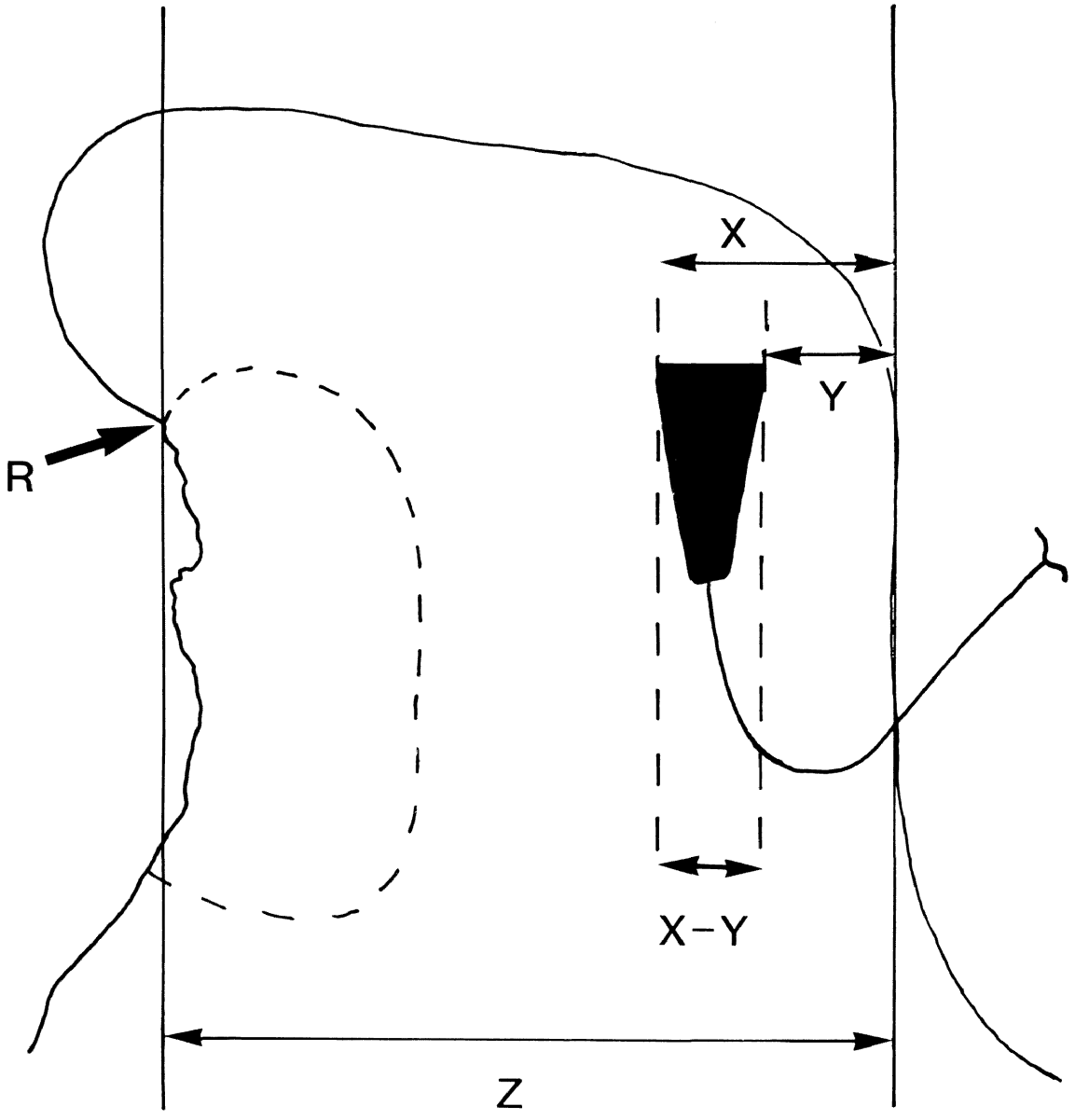
The dimensions of the arborizations were measured in the mediolateral direction as the distance from the most lateral to most medial bouton, in the dorsoventral direction as the distance from the most dorsal to most ventral bouton and in the rostrocaudal direction as the distance from the most rostral to most caudal bouton. For overlapping arbors the rostrocaudal dimensions was measured as the distance between adjacent collaterals. Volumes were calculated by multiplying the dimensions for each individual arbor.

The laminar boundaries used for describing terminal arbor positions are those according to Molander et al. 1984.

2.2 (iii) CONSTRUCTION OF PLAN VIEWS OF COLLATERAL ARBORIZATIONS AND SOMATOTOPIC MAPS.

In order to plot the relative positions of the central terminal arborizations recorded in different animals, and to take into account the inter animal variations in the sizes of the lumbar segments (Molander and Grant, 1985), the width of the dorsal horn was normalized using the medial border of the dorsal horn as an absolute marker for all measurements. The lateral border was defined for the purpose of these measurements as the border parallel to the medial border at the junction of the neck of the dorsal horn and the reticulated area (R fig. 1 legend for further details). The location of the collateral arbor could then be located at the same relative mediolateral position whatever the size of the cord.

FIGURE 1: The normalization of a terminal arbor within the dorsal horn. The two solid vertical lines represent the medial and lateral borders of the dorsal horn. The medial border is taken as the medial edge of the grey matter parallel to the dorsal column, while the lateral border is taken as the border parallel to the medial border at the point where the reticulated area joins the neck of the dorsal horn (R); the distance between the two borders being Zum. The distance X represents the distance from the medial border to ther most lateral bouton in the arbor; the distance Y represents the distance from the medial border to the most medial bouton, X-Y giving the mediolateral width of the arbor and (X-Y)/Z x 100% giving the mediolateral width in the dorsal horn.


X
Y
R
X−Y
Z

In order to reduce possible errors in the rostrocaudal direction due to variations in the size of individual lumbar segments (Swett & Woolf, 1985) the rostrocaudal position of an afferents central terminals was plotted with respect to the L4/L5 boundary mark. The lengths of the individual lumbar segments were measured in 35 animals and the mean values for the lengths of L3, L4 and L5 were 2200±20, 2550±86, 2875±90 um (x±SEM) respectively which showed there was only a small variation in the segment size across the population, justifying the above method.

2.3 RESULTS.

2.3.1 GENERAL PROPERTIES OF LOW THRESHOLD MECHANORECEPTORS AND THEIR CENTRAL TERMINALS.

From the seventy five animals a total of 173 intracellular penetrations of cutaneous primary afferents were attempted. Of these, 84 penetrations failed to result in labelling primary afferent collateral arborizations due to blockage of the electrode, insufficient time in the afferent axon or premature death of the animal, while 31 afferents were partially filled as judged by the extent and intensity of staining. In the remaining 58 penetrations, good intracellular staining with HRP was achieved resulting in an intensely stained stem axon and darkly stained collateral arborizations.

Of these 58 low threshold cutaneous afferents, thirty eight afferents responded with rapid adaption to movement of guard and tylotrich hairs on different parts of the hairy skin of the hindlimb and were classified as hair follicle afferents (HFA's). Six afferents were classified as type-1 slowly adapting mechanoreceptors (SAI's, two of which had receptive fields on the hairy skin and four on the glabrous skin). The remaining fourteen afferents were classified as rapidly adapting cutaneous mechanoreceptors (RA's) on the plantar surface of the hindpaw and toes

(six of which had RF's on the paw pads and eight on the toes). The RF's of all the primary afferents were clearly located within the peripheral nerve boundaries (except in one case) defined by Swett & Woolf (1985). All the afferents studied had estimated conduction velocities in the range 13-61 ms^{-1} ($x=31.08\pm1.15$; SEM) which was within the A-fibre range observed in the rat by Lynn and Carpenter (1982) but is likely to be an underestimate because of the method used to test them, since the conduction velocity changes along the course of the primary afferent fibre (Waddell et al., 1989).

The hair follicle afferents (HFA's) responded to movement of the guard and tylotrich hairs with a brief discharge of spikes, typically 3 or 4 spikes but sometimes more in sensitive units and the frequency of firing was higher when the hairy skin was stroked in the direction opposite to the lie of the hairs. All the hair units studied were rapidly adapting. There was no response to maintained displacement of the hairs.

Slowly adapting type-1 hairy afferents responded in a different way than did ordinary HFA's. When a maintained stimulus was applied to the hairs the response adapts quickly at first, but then more slowly. There was little sensitivity to skin stretch. Glabrous SAI afferents discharged in an irregular fashion to a maintained stimulus, similar to that of hairy SAI's. There was no spontaneous activity in these units.

There are two types of RA unit innervating the glabrous skin of the foot, RA (Krause corpuscles)and pacinian corpuscles which are differentiated on the basis of adaptiveness to a ramp stimulation, sensitivity to sinusoidal mechanical stimuli and conduction velocity. The RA units recorded in this study produced a high frequency discharge which adapted rapidly to indentation of the skin and in some units to light brush of the skin. There was no maintained firing response to maintained indentation of the skin. Some units were more sensitive to mechanical stimulation than others. The response

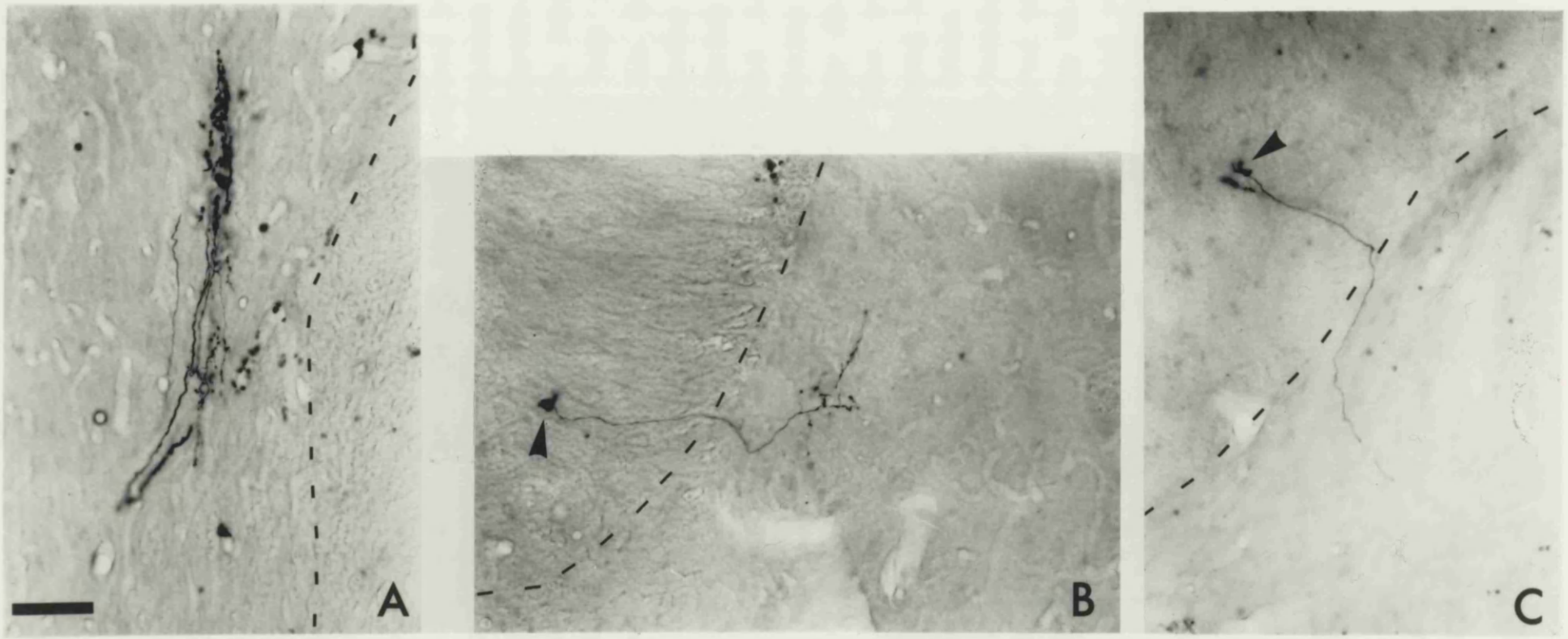
properties of all the afferents studied were similar to those previously described in the cat and rat (Brown and Iggo, 1967; Janig et al., 1968; Iggo and Ogawa, 1977; Lynn and Carpenter, 1982).

All the low threshold cutaneous mechanoreceptors studied entered the spinal cord via the L3, L4, L5 dorsal roots and then the axon bifurcated in the white matter into ascending and descending stem axon branches, the rostral branch running in the dorsal columns towards the dorsal column nuclei and the caudal branch travelling caudally, in the dorsal columns. On no occasion were non-bifurcating primary afferent fibres recovered, and this is in accordance with the findings of Rethelyi and Szentagothai (1973).

Afferents displayed three distinct types of collateral arborizations: complex, simple and blind-ending (Plate 1) as previously described by Woolf (1987) and Shortland et al., (1989a) in the rat and by Meyers and Snow (1984) in the cat. These were common to HFA's, RA's, and SAI afferents alike. Complex arborizations had extensive third or higher order dense networks of terminal axon branches and large numbers of en passant and terminal boutons (Plate 1A). These were located in the centre of an axon's rostrocaudal extent and overlap was common between adjacent terminal arborizations generating a narrow mediolaterally restricted sheet of terminal boutons within the dorsal horn for HFA's, but less so for SAI afferents and rarely in RA afferents. Simple collateral arborizations were located rostral and caudal to the complex terminal arborizations. They never overlapped with each other and had a reduced terminal branching pattern with few boutons (Plate 1B). The blind-ending axon collaterals had a severely reduced branching pattern and no boutons (Plate 1C) and were always located at the most rostral and caudal extremes of an axons rostrocaudal extent. Blind-ending collaterals did not always penetrate into the grey matter but sometimes terminated in the white matter. The numbers and types of

PLATE 1.

Photomicrographs of 50um thick transverse sections of lumbar spinal cord illustrating an example of a complex (A), a simple (B) and a blind-ending (C) collateral following intraaxonal injection of HRP into a physiologically characterized primary afferent. The dashed lines represent the outline of the dorsal horn and the arrows point to the stem axon in the dorsal column. Scale bar 100um.


A
B
C

these collaterals are shown for each class of afferent in Table 1. The distribution of these different arbors was independent of the injection site.

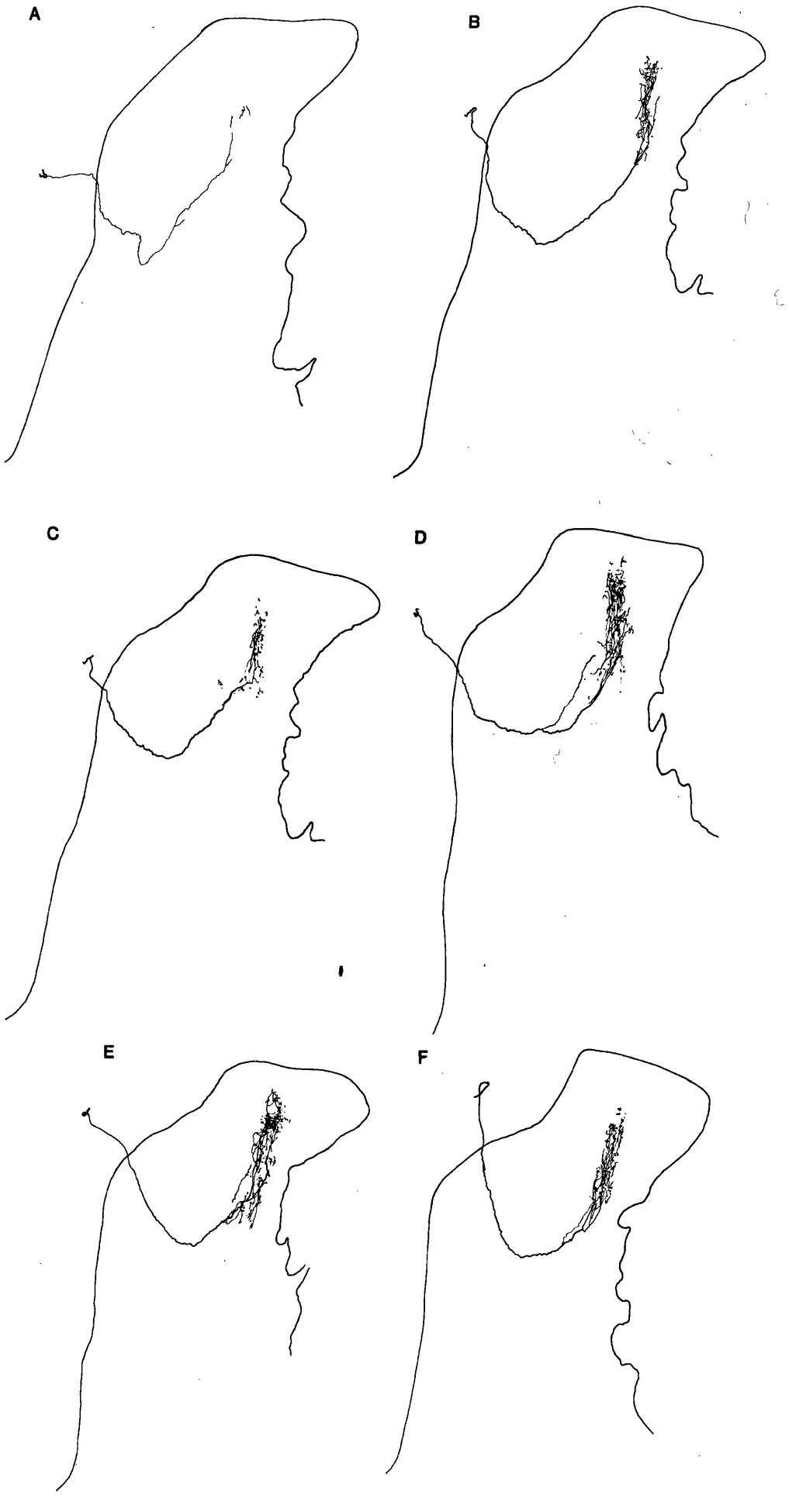
2.3.2 HAIR FOLLICLE AFFERENTS (HFA's).

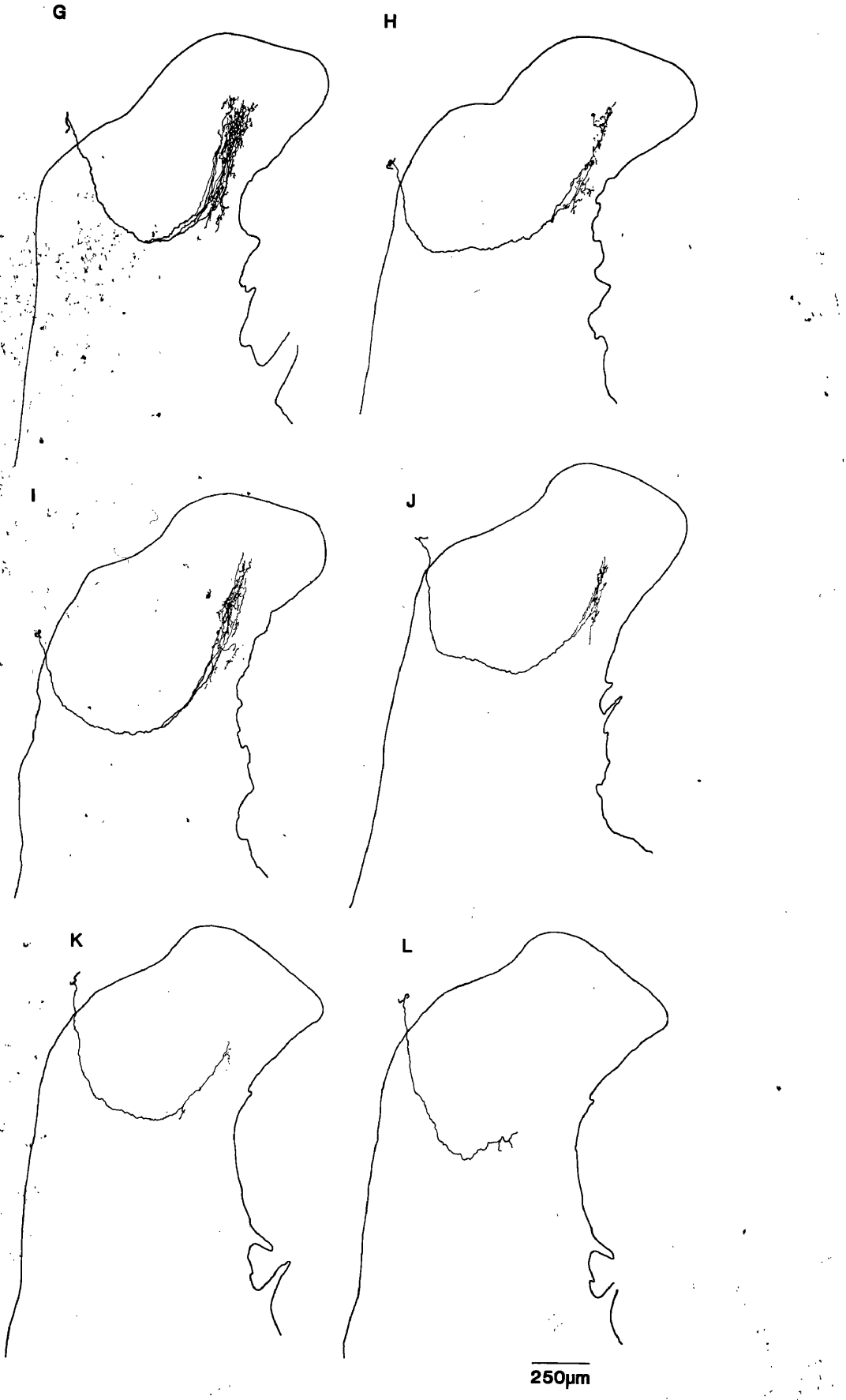
The central terminals of 38 well filled HFA's with receptive fields on different parts of the hairy skin of the hindlimb were recovered after histological processing. Fourteen of these had receptive fields on the lateral leg, seven on the medial leg, six on the dorsum of the foot and eleven on the toes. The size of the peripheral receptive fields of the different afferents was not uniform. Those on the dorsum of the foot and toes had receptive fields that were smaller than those located on the medial and lateral hindleg. All afferents innervating the foot and toes had small receptive fields (1-5 mm in diameter) responding to light brush of the small bristle (guard) hairs. Some afferents had receptive fields which crossed the glabrous-hairy border of the skin and in these afferents, light touch of the glabrous portion of the receptive field without any indication of hair deflection elicited a rapidly adapting response. The receptive fields of these afferents tended to be oblong with the long axis parallel to the hairy-glabrous skin border. Medial and lateral leg HFA receptive fields tended to be larger (3-15 mm in diameter) and light brush of the guard or tylotrich hairs produced a rapidly adapting discharge. However, in two afferents the receptive field was very small, responding to movement of a single tylotrich hair. The HFA's had an average conduction velocity of 30.03 ± 1.52 ms^{-1} (n=40, $\pm$SEM).

2.3.2 (i) MORPHOLOGY OF LATERAL LEG HAIR FOLLICLE AFFERENTS TERMINALS.

Figures 2 & 3 show the complete rostrocaudal extent of the collaterals innervating lateral leg HFA's with

FIGURE 2: Camera lucida reconstructions of twelve adjacent terminal arborizations from rostral (A) to caudal (L) of a lateral leg HFA whose receptive field was located on the lateral thigh.


A
B
C
D
E
F


G
H
I
J
K
L
250µm

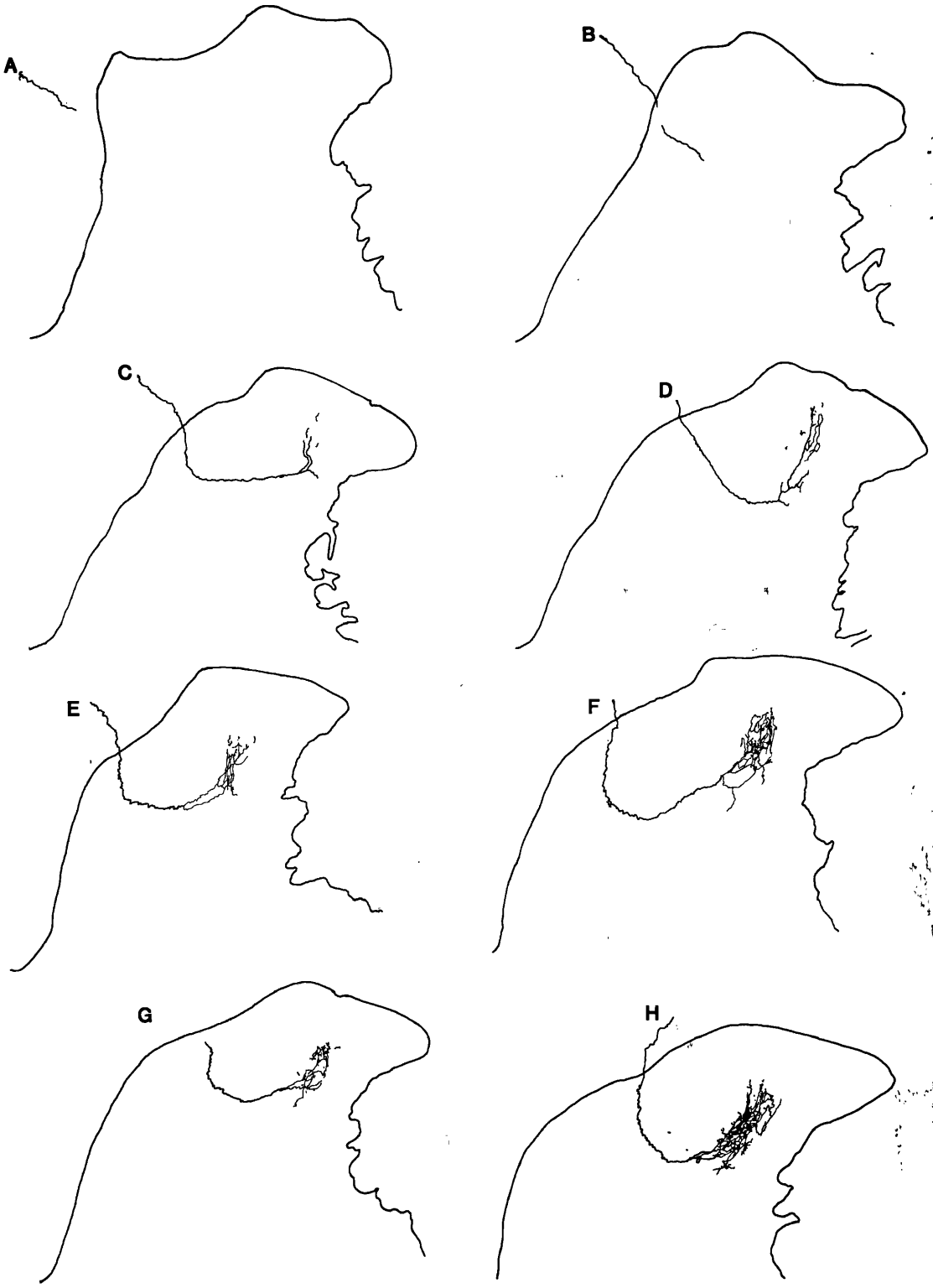

A
B
C
D
E
F
G
H

FIGURE 3: Camera lucida reconstructions of fourteen adjacent terminal arborizations from rostral (A) to caudal (N) of a lateral leg HFA whose receptive field was located on the lateral calf.

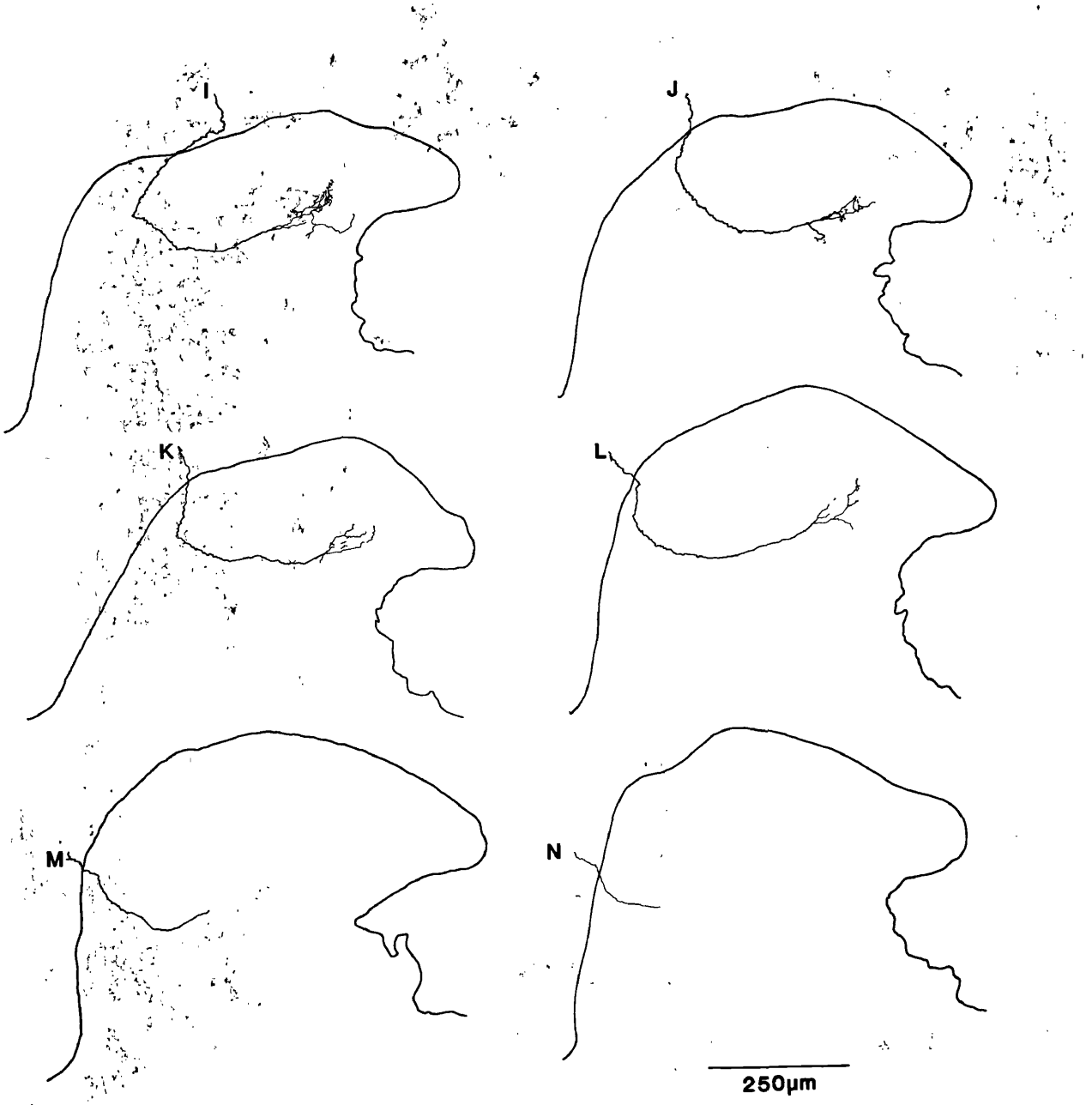
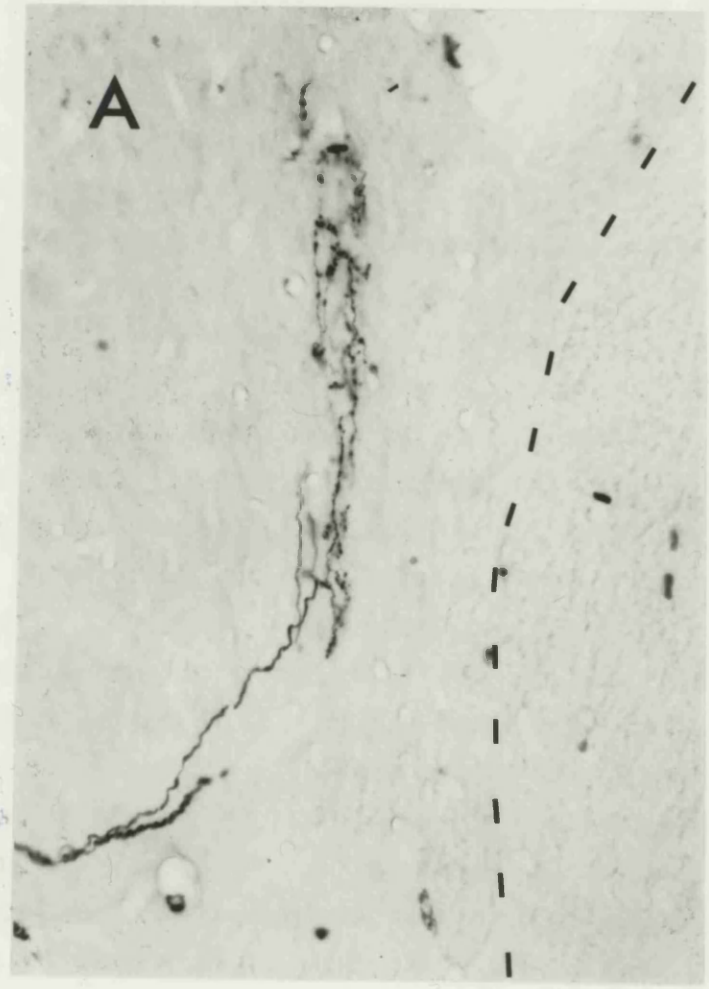
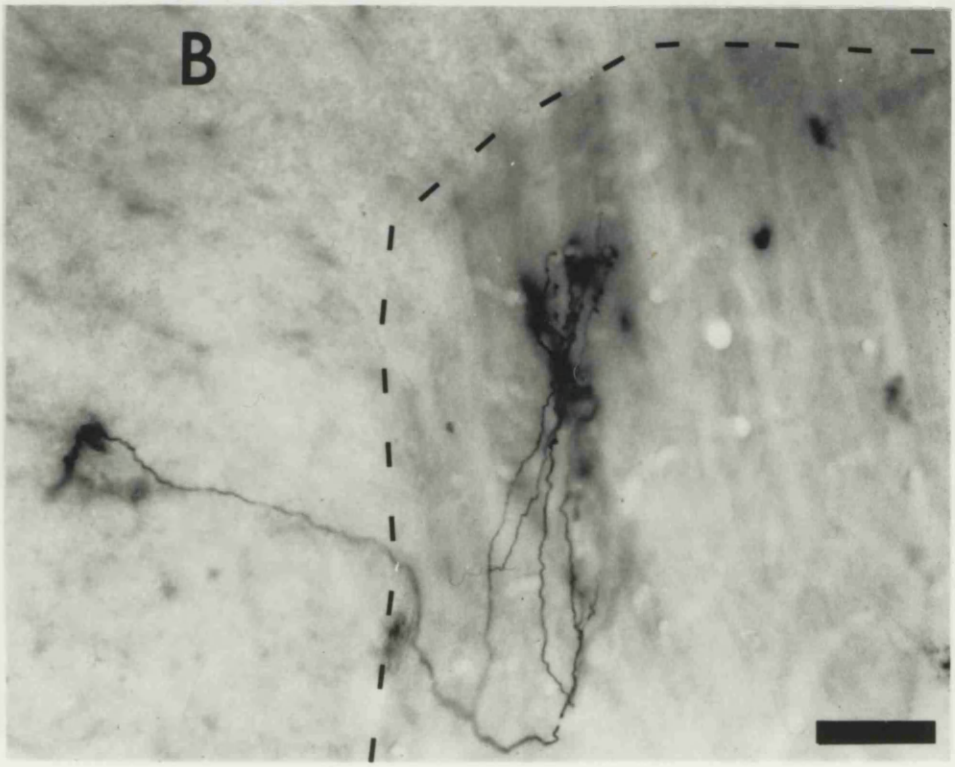


PLATE 2.

Photomicrographs of 50um thick transverse sections of lumbar spinal cord illustrating examples of HFA complex arbors. A is from a lateral leg HFA and B is from a medial leg HFA. Scale bar 100um.


A


B

peripheral RF's above (Fig. 2) and below the level of the knee (Fig. 3). The morphology of HFA arbors above and below the level of the knee were essentially identical. Lateral leg afferents were characterized by having a U-shaped curving collateral axon which ramified to produce the characteristic flame-shaped arbors (previously described in Golgi and HRP studies by Scheibel and Scheibel, 1968; Brown et al.,1977; Woolf, 1987) in the lateral quarter of the dorsal horn. Blind-ending collaterals were located rostral and caudal to the simple arbors which were, in turn, located rostral and caudal to the complex arborizations, examples of which are shown in Plates 1A, 2A, and Figs. 2B-G, 3F-H. The terminal arborizations had a distinctive and characteristic morphology with the terminal boutons of the complex arbors located within laminae IIi-IV (Figs. 2D,E) but the boutons of simple arbors never extended above lamina III. Often, the lateral leg complex arbors exhibited a small ventrolateral projection of terminal boutons (Fig 2.C-E, G-J, 3F-J) which terminated in the reticulated area of the dorsal horn. These were not seen in medial leg, dorsal foot or toe afferents.

One of the most distinctive features of HFA arborizations was the overlap between the terminal branches of adjacent collaterals. Overlap was only observed between adjacent complex arborizations (56/64) and did not occur between adjacent complex and simple arborizations. The overlap was most prominent in the middle of an axons' rostrocaudal extent and commonly produced an uninterrupted terminal sheet running in the rostrocaudal direction in the dorsal horn (Figs. 15-19). Occasionally the sheet was interrupted for a short distance, producing more than one terminal sheet but all were exactly aligned in the same mediolateral position in the dorsal horn (Fig. 15). The rostral and caudal ends of the overlapping complex arbors displayed an abrupt tapering off in the number of boutons over the last 100-150um.

2.3.2 (ii) MORPHOLOGY OF MEDIAL LEG HAIR FOLLICLE AFFERENTS TERMINALS.

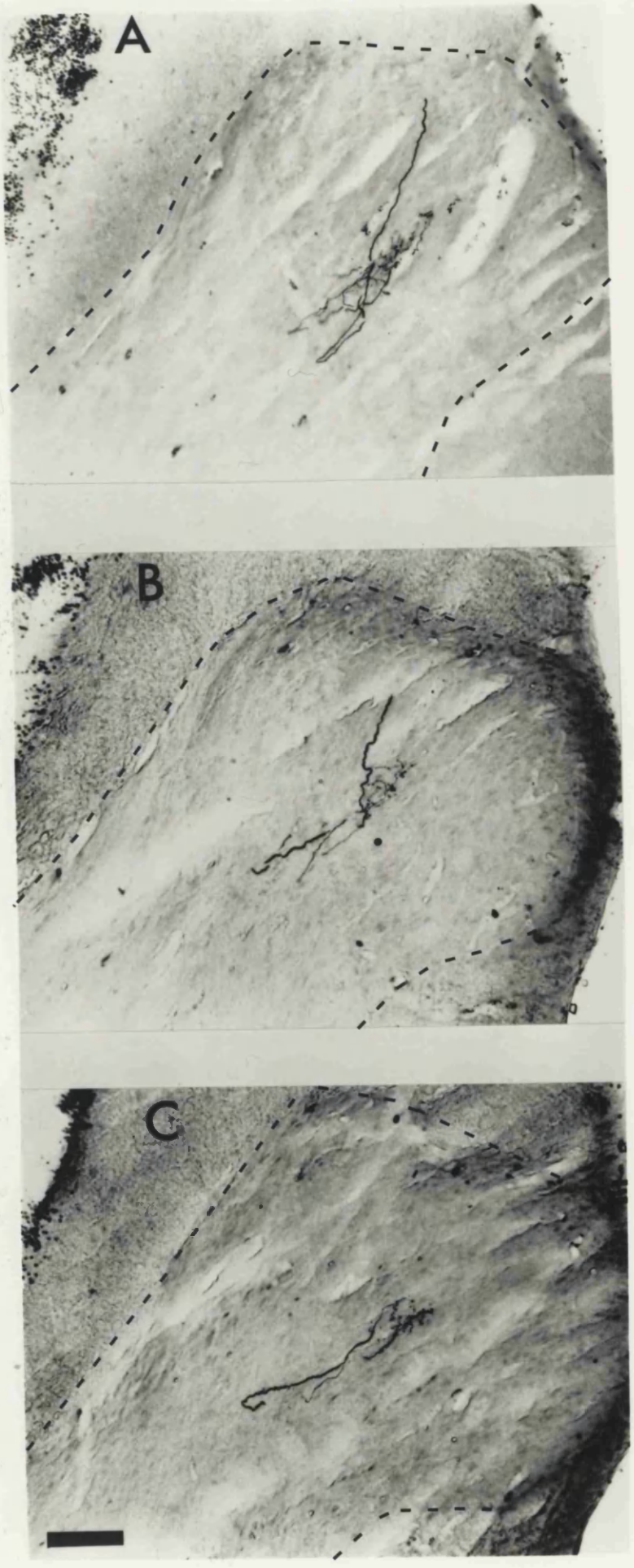
The morphology of the terminal arborization of a typical medial leg HFA is illustrated in figure 4 and plates 2B & 3. Like lateral leg HFA's, medial leg HFA's had a 'U-shaped' curving collateral which ramified to produce the classical flame-shaped arbors. The terminal boutons of the complex arbors (Fig. 4C-E, Plate 2B) were located within laminae IIi-IV but simple arbors extended only as far dorsally as lamina III. In some cases (Fig. 4 H-K) the more caudal simple collateral arborizations departed from the characteristic U-shape collateral, and instead dived downwards in the medial dorsal horn to arborize in deeper laminae. This occurred for 9/12 simple arborizations, the other 3/12 had a simple flame-shape as illustrated in fig. 4A,B. All the complex arborizations produced flame-shaped arbors which terminated in the medial quarter (Fig. 4) to middle of the dorsal horn depending on their rostrocaudal positioning within the lumbar enlargement (Fig. 20). As with the lateral leg HFA's 27/31 of the adjacent complex arborizations overlapped to produce sheets of terminals running rostrocaudally within the dorsal horn (as illustrated in the plan view of Figs. 15,16 & 20).

2.3.2 (iii) MORPHOLOGY OF DORSAL FOOT HAIR FOLLICLE AFFERENTS TERMINALS.

Figure 5 shows 6 complex arbors from dorsal foot HFA's and shows that the morphology was similar to the afferents previously described in that dorsal foot afferents exhibited the characteristic flame-shape arbors. Approximately 50% of the collaterals were of the complex type but only 10% of the arbors were of the simple type (Table 1) and 3/6 of the afferents had no simple arbors, only complex and blind-ending (perhaps reflecting inadequate dye filling). However, all the simple arbors

PLATE 3.

Photomicrographs of three consecutive 50um thick transverse sections of lumbar spinal cord from caudal (A) to rostral (C) of a saphenous HFA complex arbor. The dashed lines represent the outline of the dorsal horn. Scale bar 100um.


A
B
C

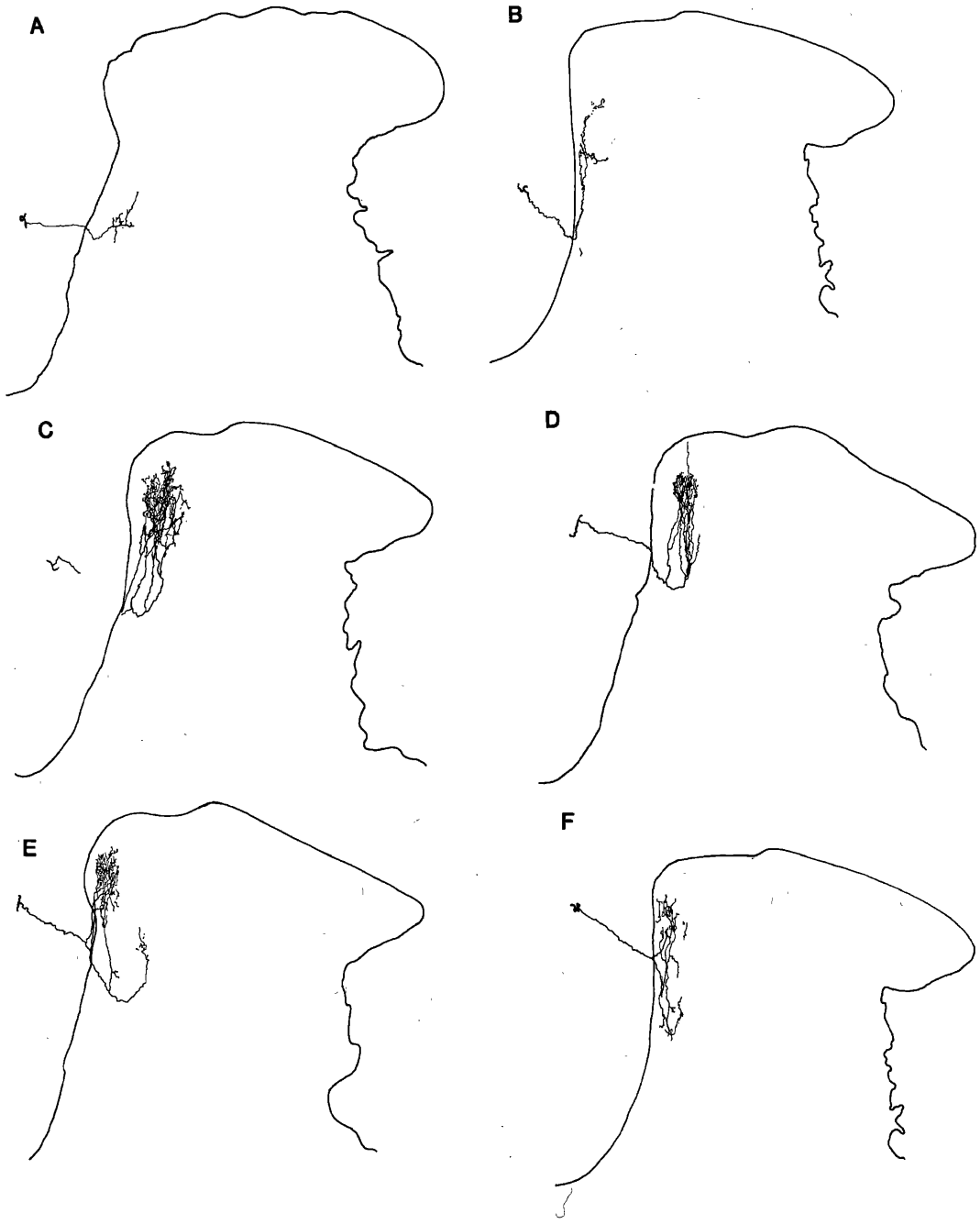

A
B
C
D
E
F

FIGURE 4: Camera lucida reconstructions of twelve adjacent terminal arborizations from rostral (A) to caudal (L) of a medial leg HFA whose receptive field was located on the calf.

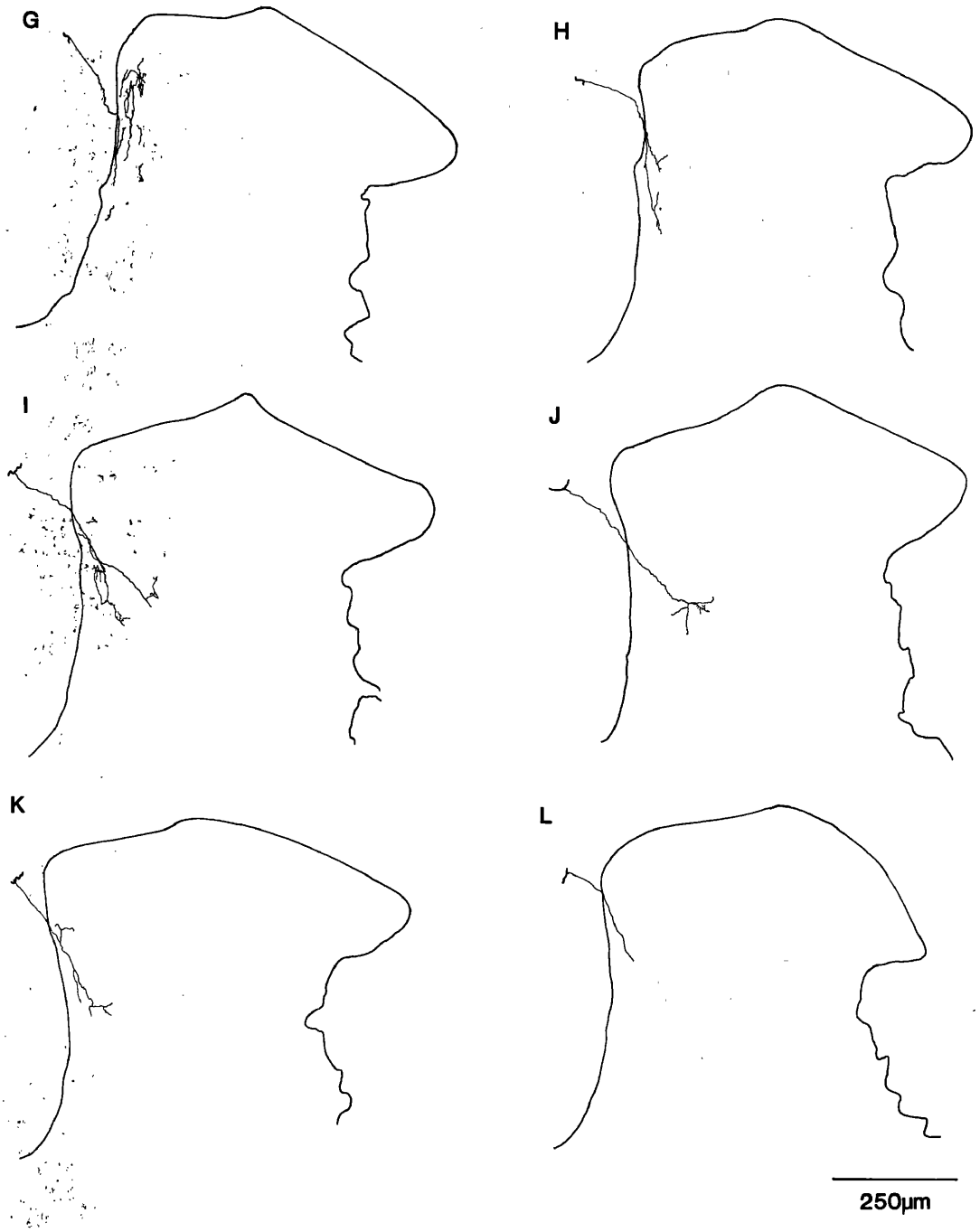
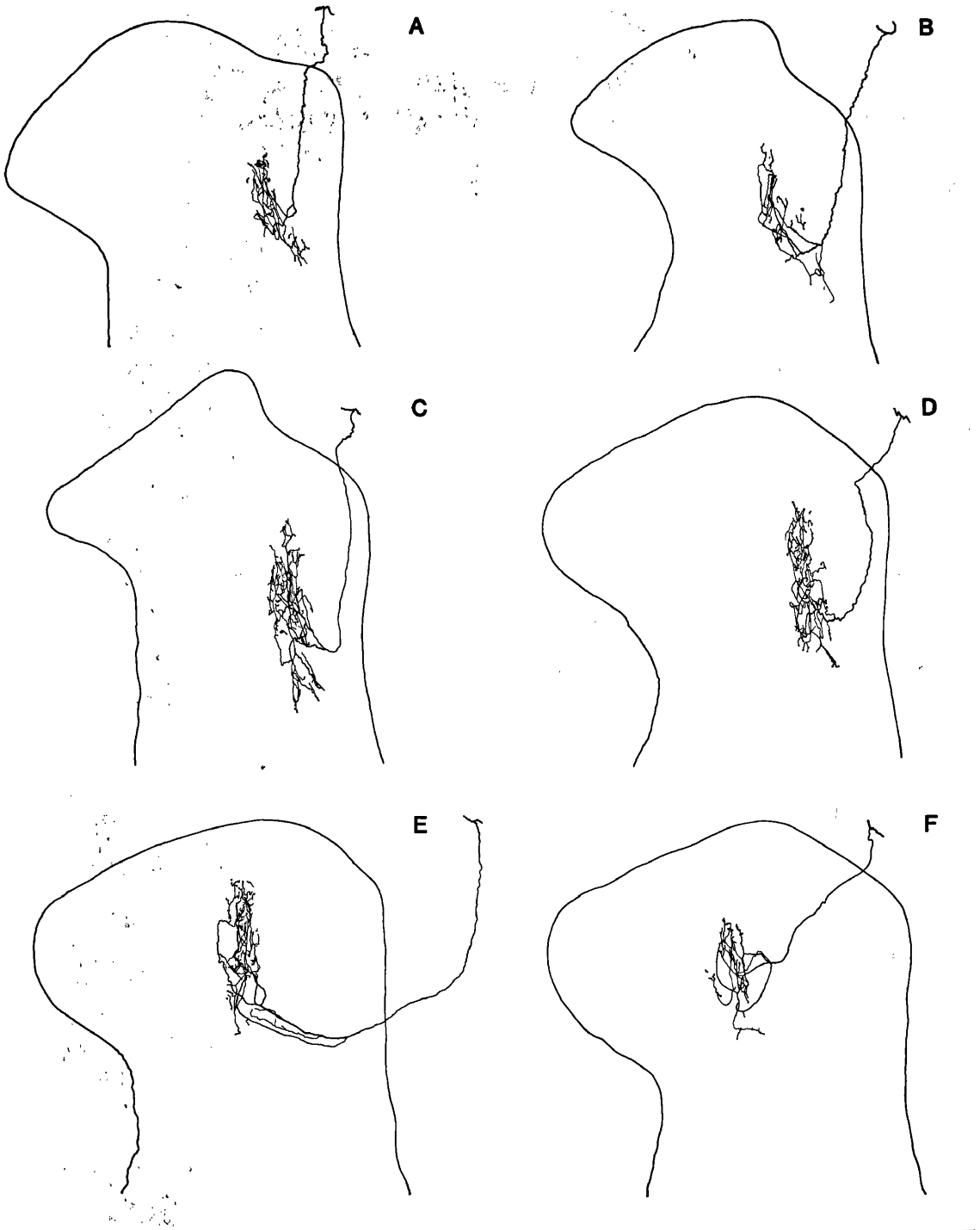


FIGURE 5: Camera lucida reconstructions of six complex terminal arborizations from HFAs innervating the dorsal foot. Scale bar 250um.



did show the flame-shape morphology. The terminals of the complex arbors were found in the middle of the dorsal horn and 17/20 complex arbors overlapped with adjacent arbors to produce rostrocaudally running sheets of terminals (see Figs. 16,21).

2.3.2 (iv) MORPHOLOGY OF TOE HAIR FOLLICLE AFFERENTS TERMINALS.

Toe HFA's tended to show considerable variation in the morphology of adjacent collateral arborizations in a single afferent. Six of the eleven toe afferents had peripheral receptive fields only on the hairy skin of the toes. Three of these afferents had collaterals which clearly departed from the flame-shaped arbors seen previously. Examples of these are shown in figs. 6 and 7. The rostral collaterals did appear to have the flame-shaped morphology (Figs. 6A,B; 7A-C) but their more caudal collaterals have a distorted shape (Figs. 6E-G; 7F-H). The mediolateral position of the terminals boutons, as for all the other HFA's described, remained constant throughout the rostrocaudal extent even though the form changed. The more caudal collaterals tended to show a slight ventral shift in terminal bouton position. Boutons were distributed from laminae IIi-IV, with the majority of boutons found in laminae IIi-III in the more rostral arbors and in ventral laminae III-IV in the caudal arbors. The other three toe HFA's with receptive fields only on the hairy skin had terminal arbors with the normal flame-shape (Figs. 8 and 9).

Five toe afferents had a receptive field that extended from the hairy skin onto the glabrous surface and they showed a unique feature common only to this type of afferent: terminal branches that ran ventromedially to arborize in laminae IV-V (Figs. 10 and 11). As the axon collateral descended through the medial dorsal horn, it bifurcated sending some branches ventromedially towards laminae IV-V, while the majority of terminal branches

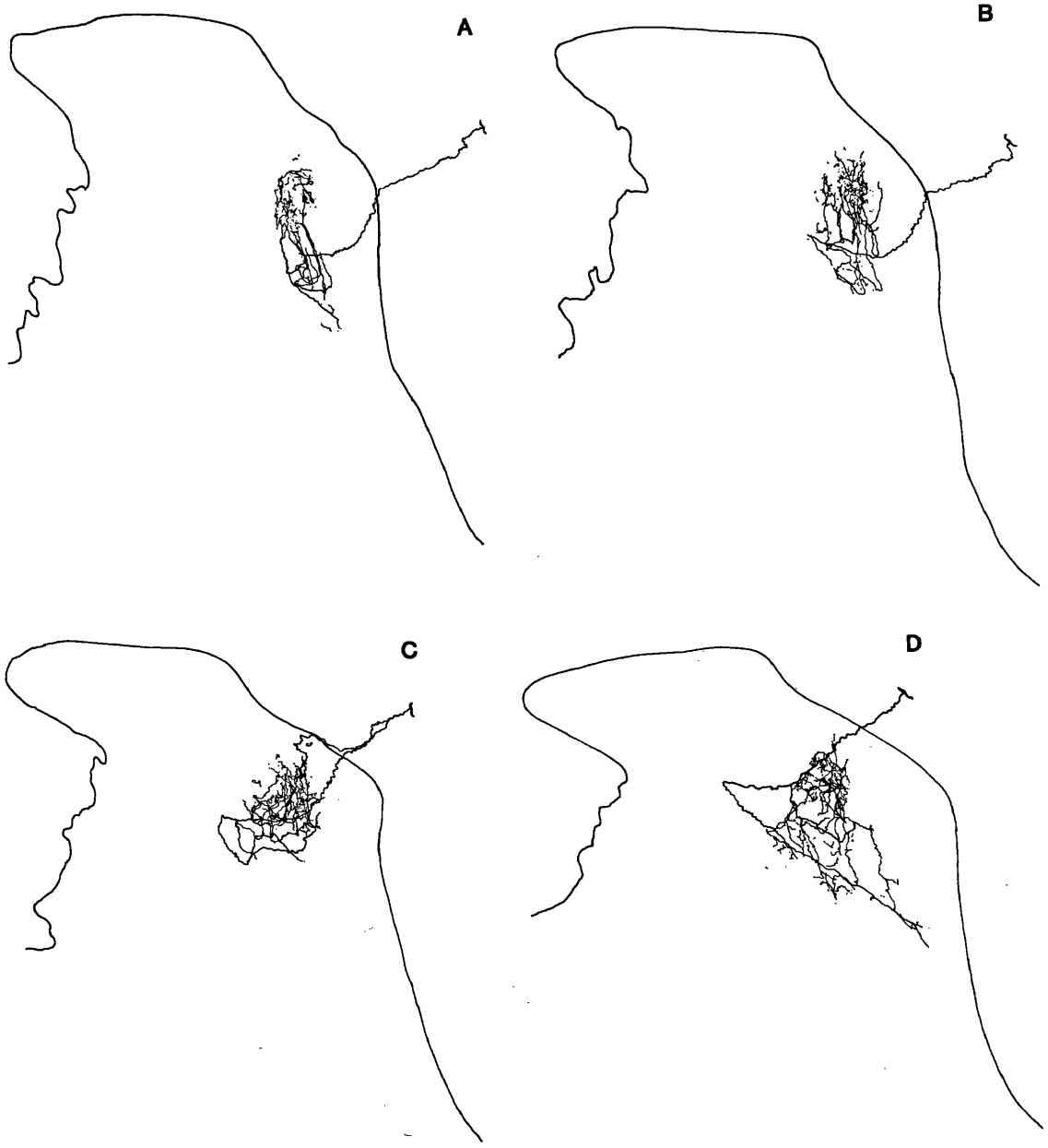

A
B
C
D

FIGURE 6: Camera lucida reconstructions of seven adjacent terminal arborizations from rostral (A) to caudal (G) of a toe afferent whose receptive field was located on the dorsomedial surface of toe 3 including the glabrous-hairy border.

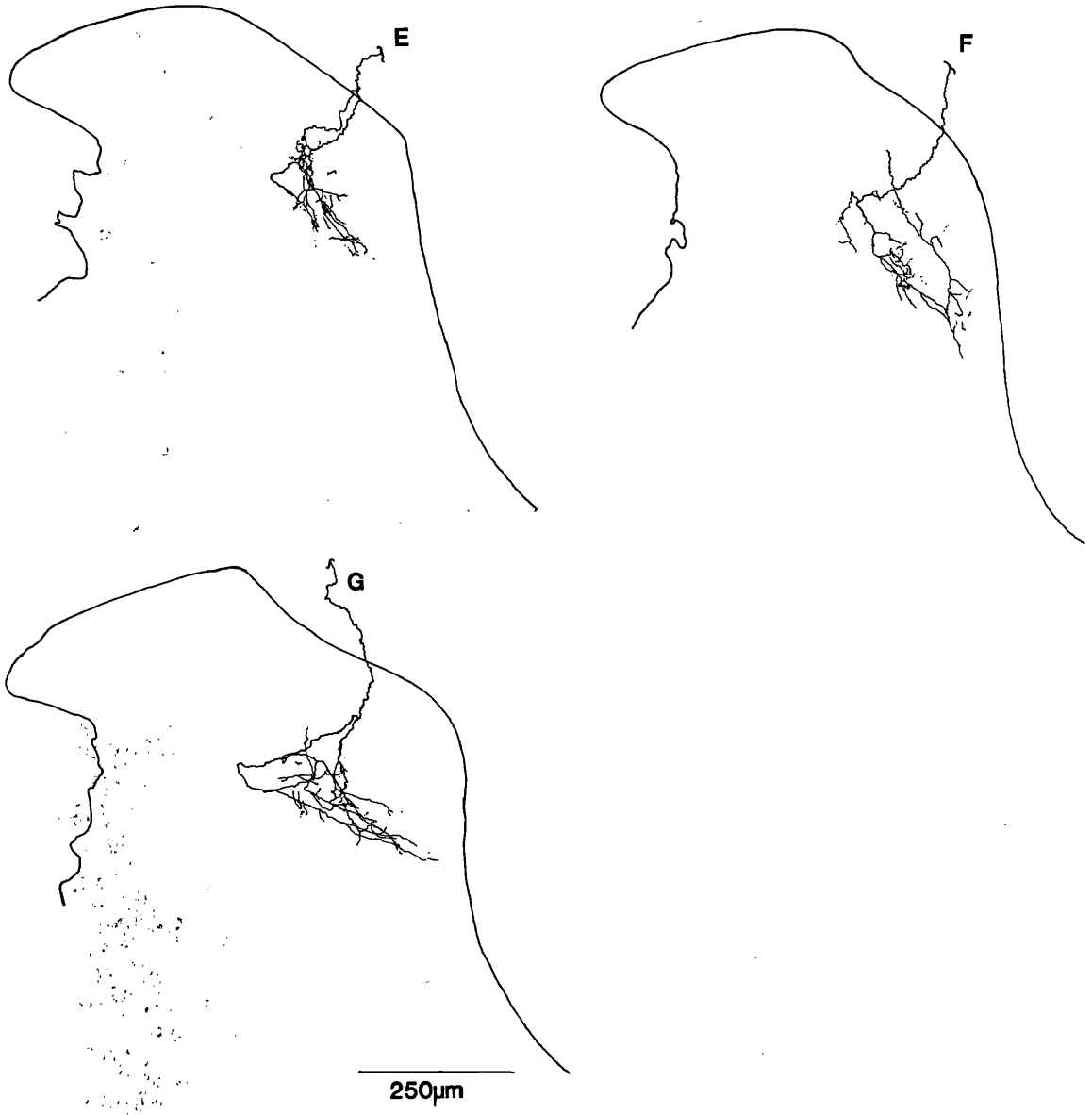
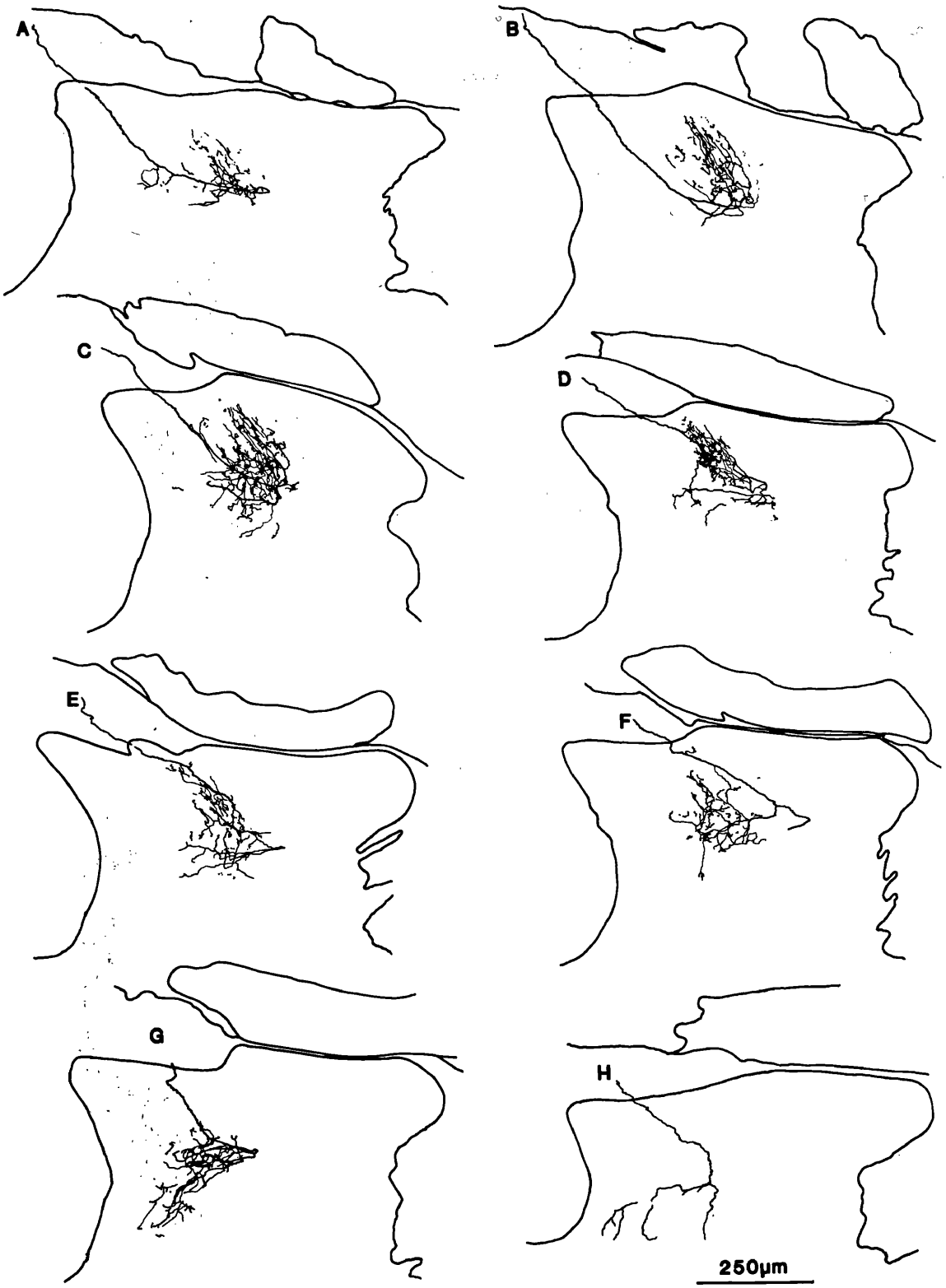


FIGURE 7: Camera lucida reconstructions of eight adjacent terminal arborizations from rostral (A) to caudal (H) of a toe HFA whose receptive field was located on the lateral surface of toe 5.



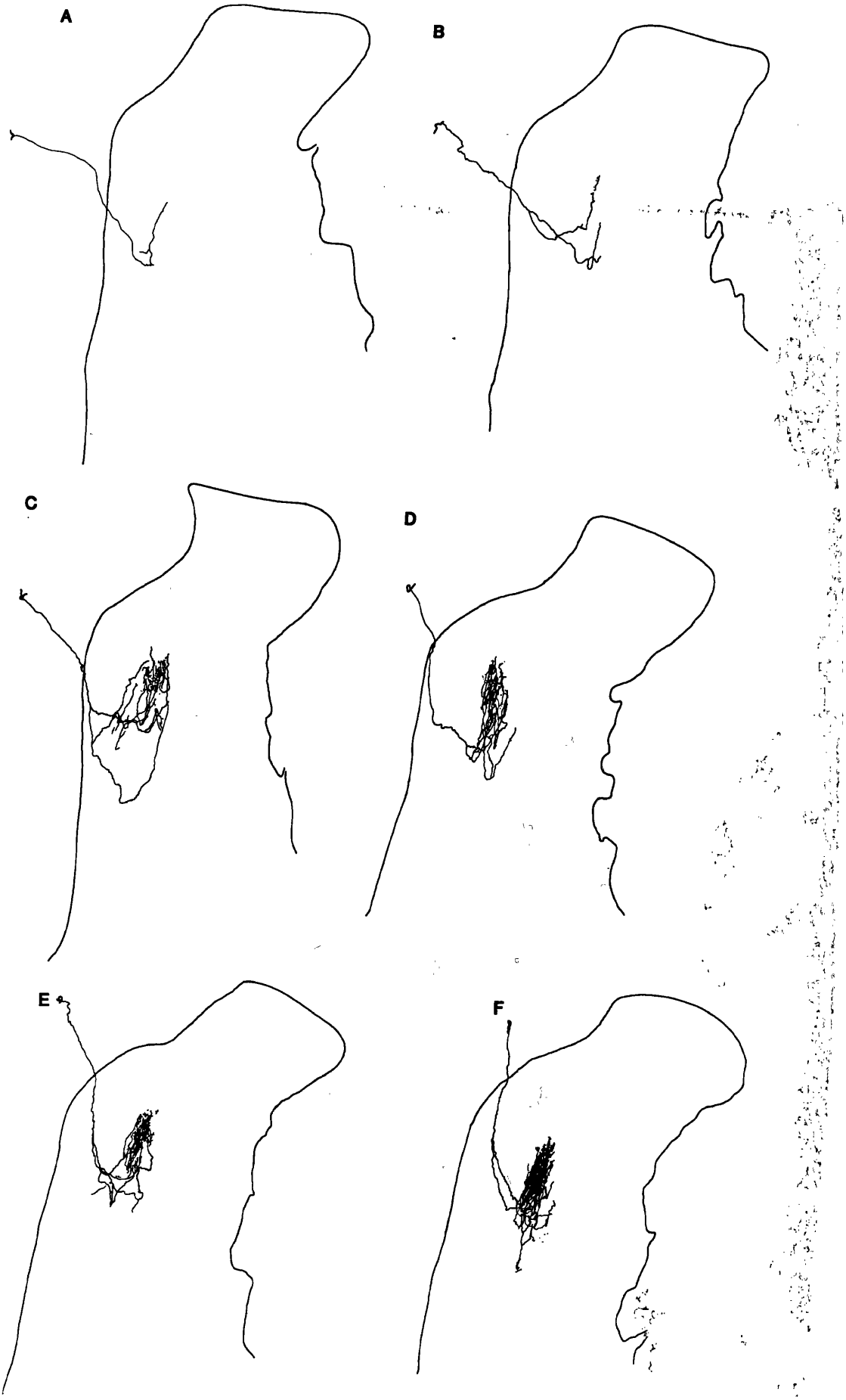

A
B
C
D
E
F

FIGURE 8: Camera lucida reconstructions of ten adjacent terminal arborizations from rostral (A) to caudal (J) of a HFA whose receptive field was adjacent to the dorsal toe nail of toe 2.

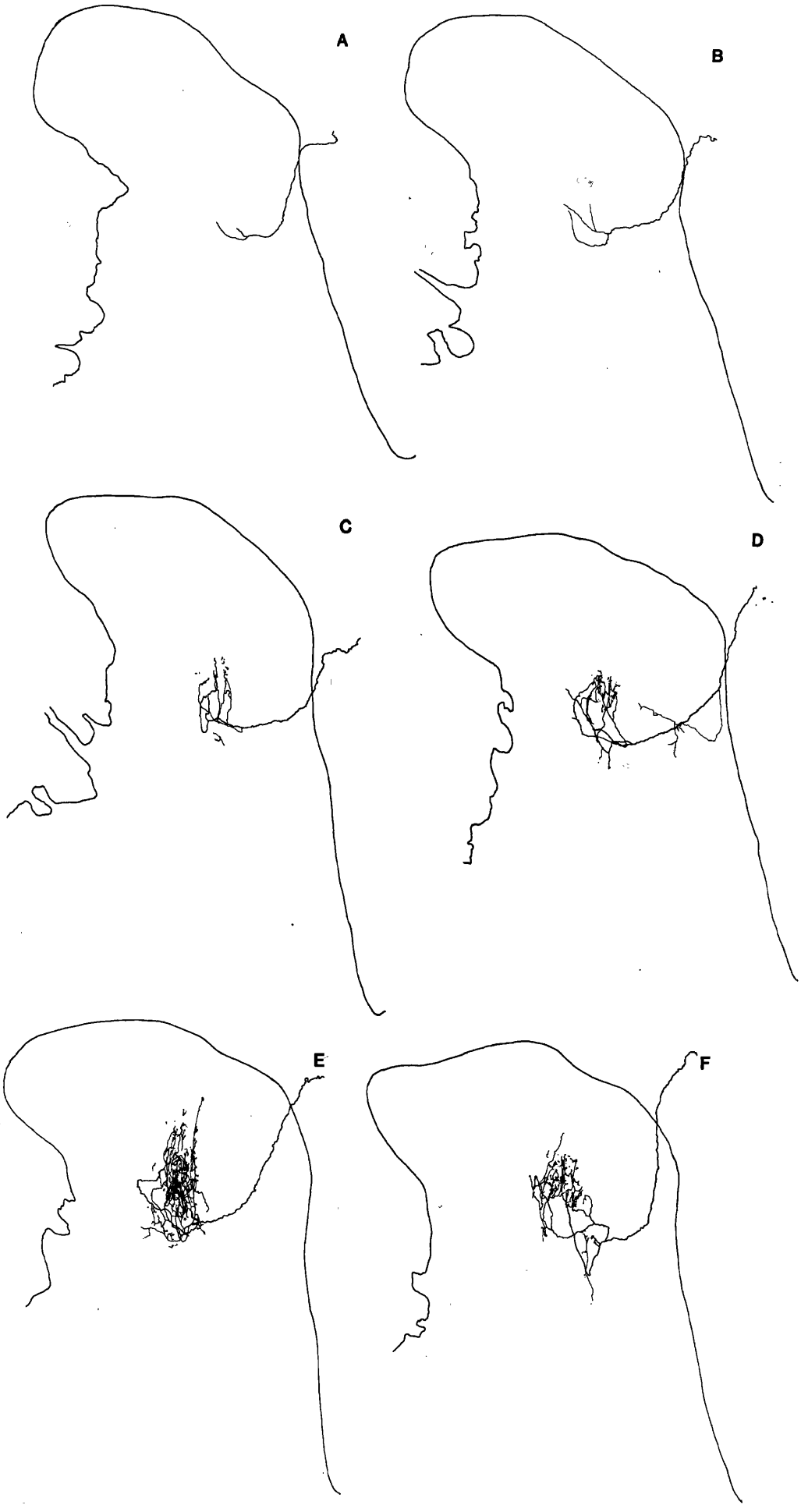
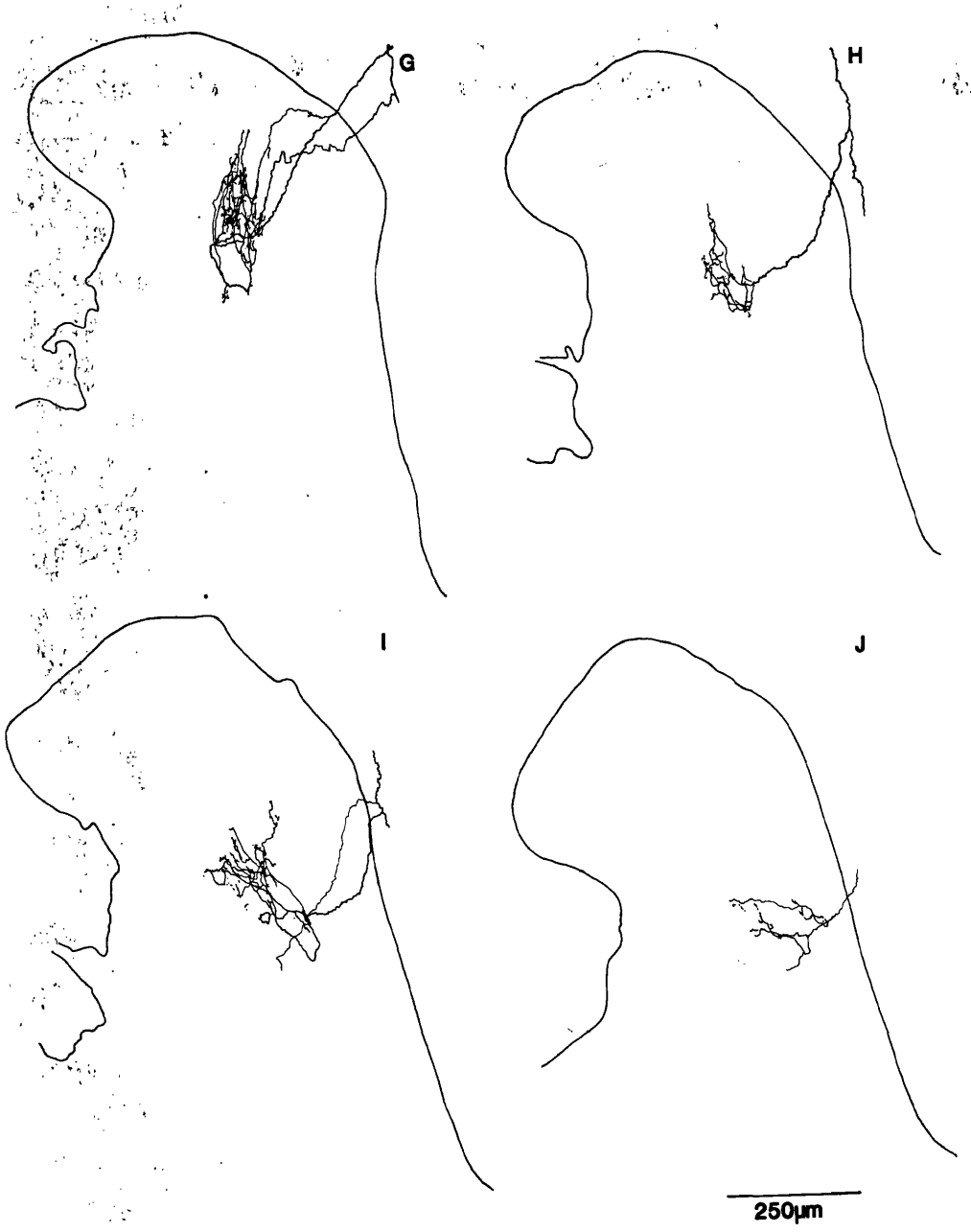

A
B
C
D
E
F

FIGURE 9: Camera lucida reconstructions of ten adjacent terminal arborizations from rostral (A) to caudal (J) of a toe HFA whose receptive field was located on the medial surface of toe 4.



terminated dorsolaterally in laminae IIi-III as flame-shaped arbors (Figs. 10 C-G, 11). The ventral terminal arbors were relatively simple in structure and had fewer boutons than did their dorsal counterparts. As with the hairy only toe afferents, adjacent complex collaterals of hairy-glabrous skin afferents had overlapping terminal arbors (17/23 glabrous-hairy and 28/32 hairy only complex arbors overlapped), but this was restricted to the dorsal terminal arbors; the ventromedial arbors never overlapped. However, like their dorsal arbors, the positioning of the ventromedial arbors was in strict mediolateral register. Figure 12 shows the rostrocaudal extent of an afferent innervating the medial glabrous-hairy border of the heel of the foot, with some collaterals showing these unique arborizations (Fig. 12E,F).

2.3.2 (v) COMPARISION OF THE ARRANGEMENT OF HAIR FOLLICLE COLLATERALS WITH DIFFERENT PERIPHERAL RECEPTIVE FIELDS.

Table 1 shows that there were essentially no differences in the organization of collateral axons with receptive fields on the lateral leg above or below the level of the knee, each having similar numbers of collaterals per afferent, longitudinal length of complex arbor in the cord and length from most rostral to caudal collateral. Medial leg afferents had slightly fewer collaterals per afferent but had similar numbers (and percentages) of complex, simple and blind-ending arborizations. The intercollateral spacing of collaterals was also slightly shorter compared to lateral leg afferents. The length of the terminal sheet was slightly shorter than that of lateral leg afferents, as was the distance from the most rostral to caudal collateral. Dorsal foot afferents had the lowest density of collaterals and shortest distance in the cord of terminal arbor sheet and distance from rostal to caudal collateral. Toe afferents had, on average, the highest numbers of collaterals per afferent but the lowest percentage of

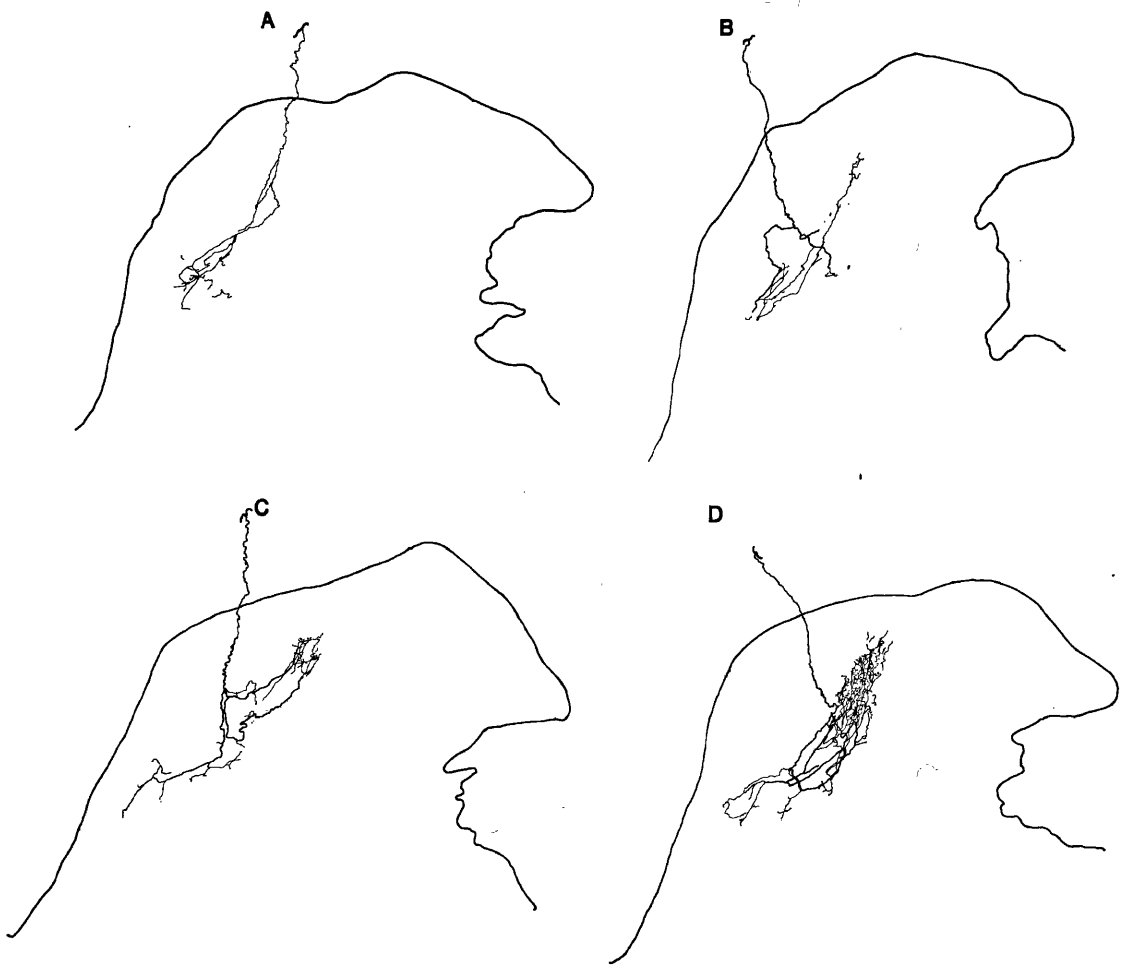

A
B
C
D

FIGURE 10: Camera lucida reconstructions of eight adjacent terminal arborizations from rostral (A) to caudal (H) of a toe afferent whose receptive field was located at the medial glabrous-hairy border of toe 4.

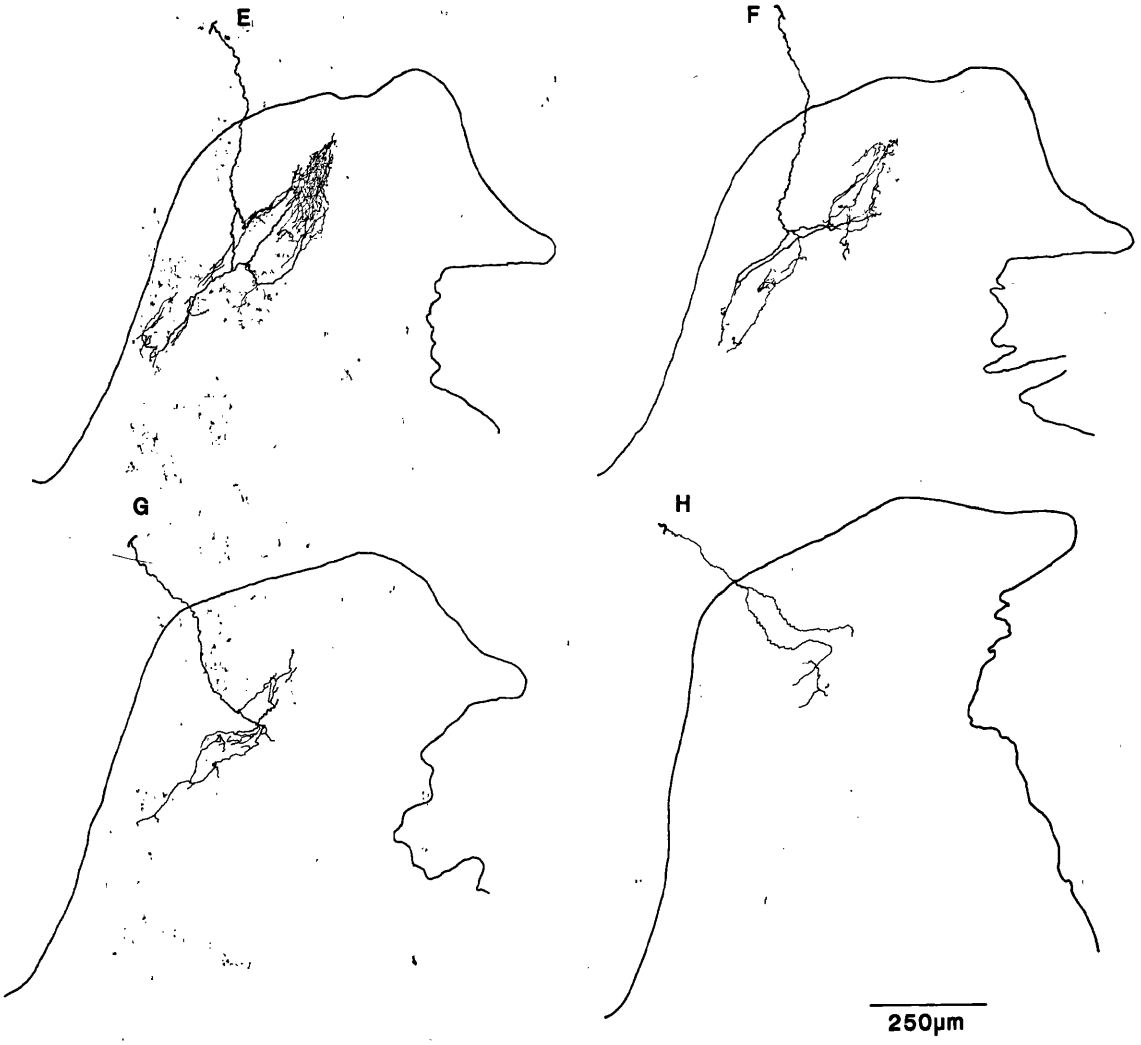
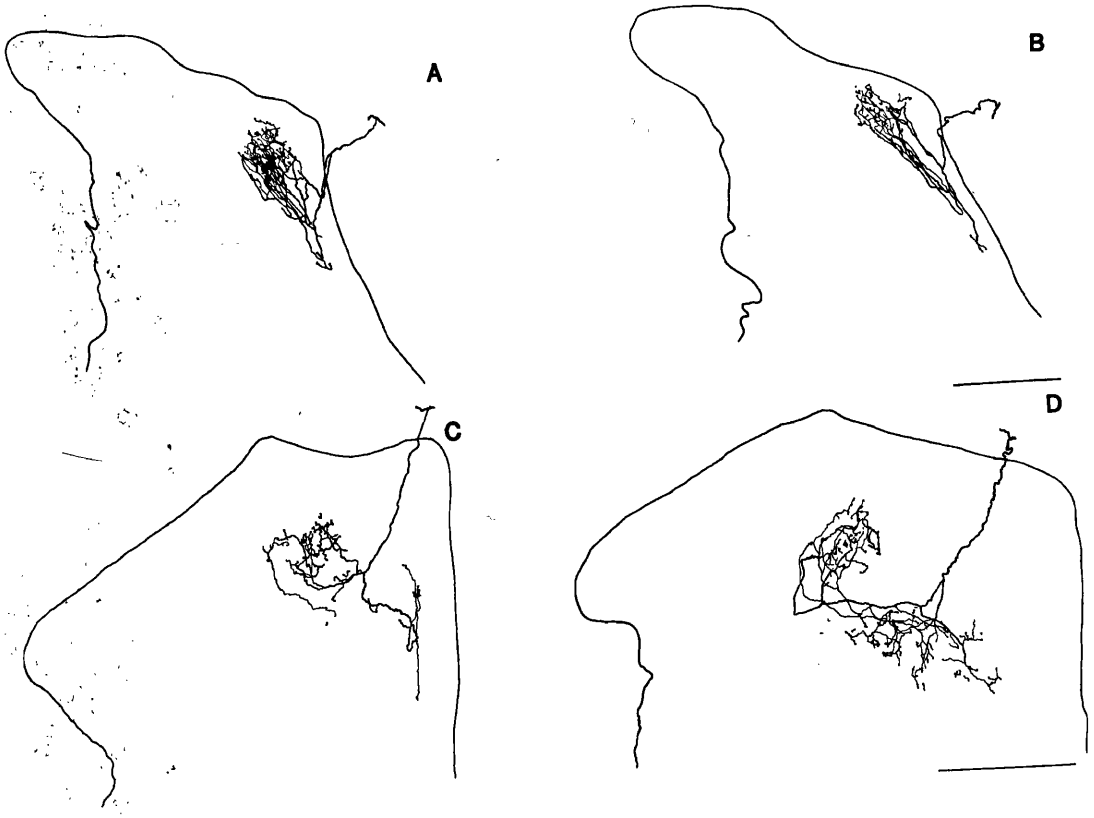


FIGURE 11: Camera lucida reconstructions of complex arborizations of afferents with receptive fields that cross the glabrous-hairy border. A & B are from an afferent whose receptive field was located on the medial side of toe 2; C & D are from an afferent whose receptive field was located on the lateral side of toe 5. Scale bars 250um.



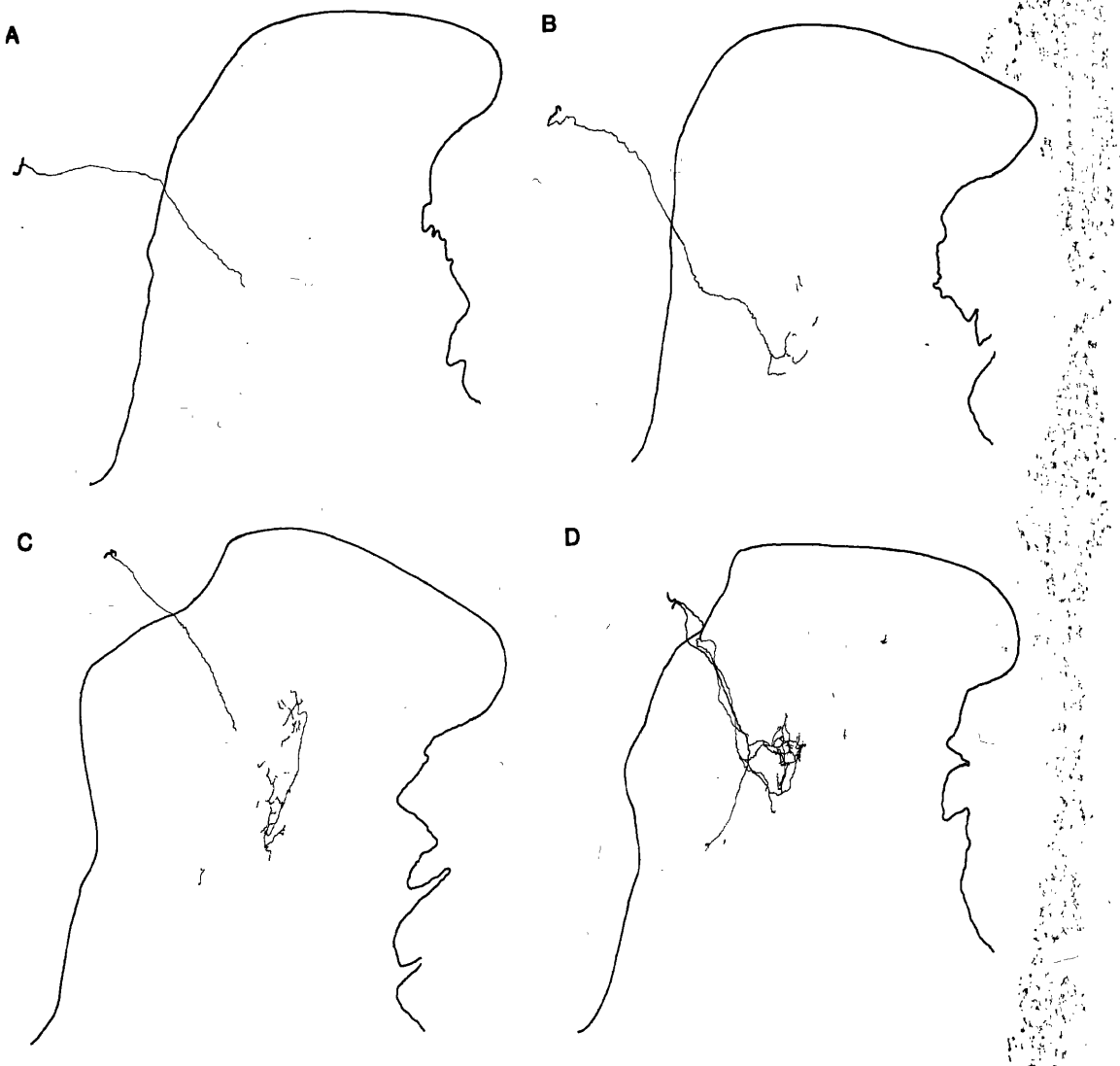

A
B
C
D

FIGURE 12: Camera lucida reconstructions of eight terminal arborizations from rostral (A) to caudal (H) of an afferent whose receptive field was located on the medial glabrous-hairy border of the foot. .

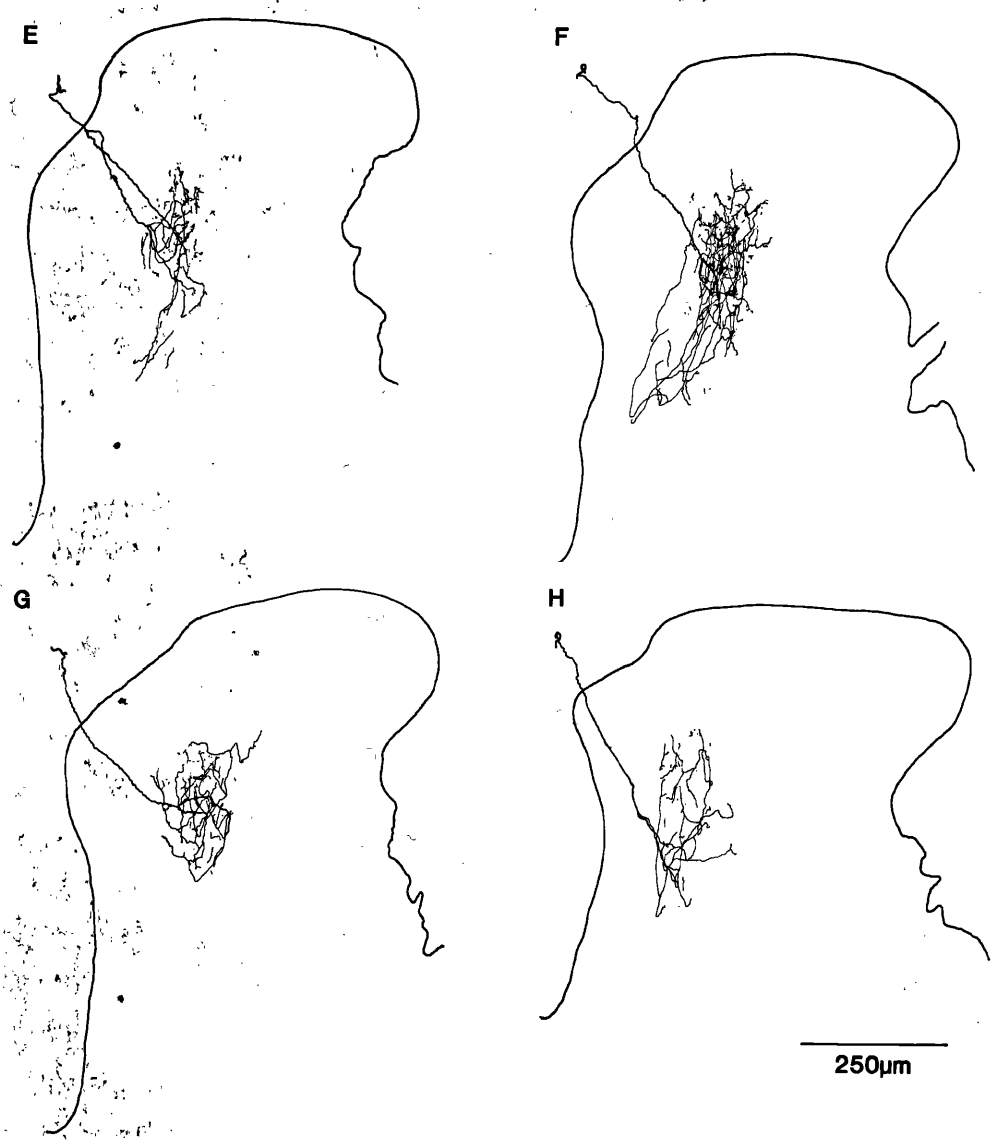


TABLE 1 Regional Difference IN HAIR FOLLICLE AFFERENT CENTRAL TERMINALS

	LATERAL LEG					
Afferent Type	Above Knee	Below Knee	Whole Leg	Medial Leg	Dorsal Foot	Toes
No. Afferents	9	5	14	7	6	11
No. collaterals/Afferent	9.33 ± 1.12	9.2 ± 1.48	9.29 ± 0.87	8.57 ± 1.39	6.83 ± 0.75	10.91 ± 0.76
No./afferent Complex	4.78 ± 0.97(51%)	4.6 ± 0.60 (50%)	4.51 ± 0.52 (49%)	4.43 ± 0.53 (52%)	3.33 ± 0.49(49%)	5.0 ± 0.67 (46%)
collaterals Simple	2.2 ± 0.38(17%)	2.0 ± 0.48 (22%)	1.79 ± 2.60 (19%)	1.71 ± 0.89 (20%)	0.67 ± 0.21(10%)	1.27 ± 0.30 (12%)
Blind	3.0 ± 0.80(32%)	2.6 ± 1.03 (28%)	2.86 ± 0.51 (32%)	2.43 ± 0.53 (28%)	2.83 ± 0.7 (41%)	4.64 ± 0.54 (42%)
Ending						
Length of afferent from most rostral to caudal collateral (mm)	3.08 ± 0.53	3.23 ± 0.47	3.04 ± 0.25	2.70 ± 0.30	2.29 ± 0.35	3.26 ± 0.29
Length of overlapping terminal arbor sheet (mm)	1.49 ± 0.19	1.46 ± 0.25	1.47 ± 0.14	1.29 ± 0.10	1.08 ± 0.17	1.34 ± 0.16
Intercollateral distance (μm)	3 51 ± 19	3 89 ± 33	3.70 ± 17	3.49 ± 33	3.69 ± 34	3.11 ± 17
Projection to lamina II:	24/42 (57%)	10/22(45%)	34/61(53%)	7/31 (23%)	3/41(7%)	12/55 (22%)
to IV/V	-	-	-	3/31 (10%)	-	25/55 (45%)

x+sem

complex arbors (46%) and highest percentage of blind-ending (42%) collaterals. A general pattern for the complex terminal arbor sheet emerged in that those overlapping sheets that lay in the lateral dorsal horn had the longest length followed by those in the middle of the dorsal horn (belonging to the toes) while those terminating in the medial dorsal horn had the shortest length (Table 1, fig. 15). This pattern also occurred for the intercollateral distances and for the length from most rostral to caudal collateral (lateral dorsal horn afferents were greater than middle dorsal horn afferents which were greater than medial dorsal horn afferents). It is interesting to note that the numbers (and percentages) of blind-ending collaterals was always greater than the numbers of simple arbors.

Table 2 presents data on the dimensions and volumes of the terminal arborizations from HFA's with different peripheral RF's. The most striking difference was the strict mediolateral compression of the terminal arbors of lateral leg afferents compared to other HFA's innervating other areas of the hindlimb. Lateral leg afferents which terminate in the lateral part of the dorsal horn had arborizations that were two thirds of the mediolateral width of the arborizations of medial leg, dorsal foot and toe afferents (Fig. 13) which terminate in the middle and medial portions of the dorsal horn. Significant differences were found for individual simple, complex and terminal arborizations when the mediolateral widths of lateral leg afferents and other HFA's from other hindlimb regions were compared ($P<0.05$ unpaired t-test) and significant differences in the rostrocaudal and dorsoventral dimensions of terminal arbors were also observed between lateral and medial leg HFA's. Lateral leg HFA's arbors also occupied the smallest volumes of cord and had significantly smaller volumes than those of toe arborizations. However, when the average dimensions/ afferent were statistically compared then significant differences ($P<0.05$) were found only in the mediolateral

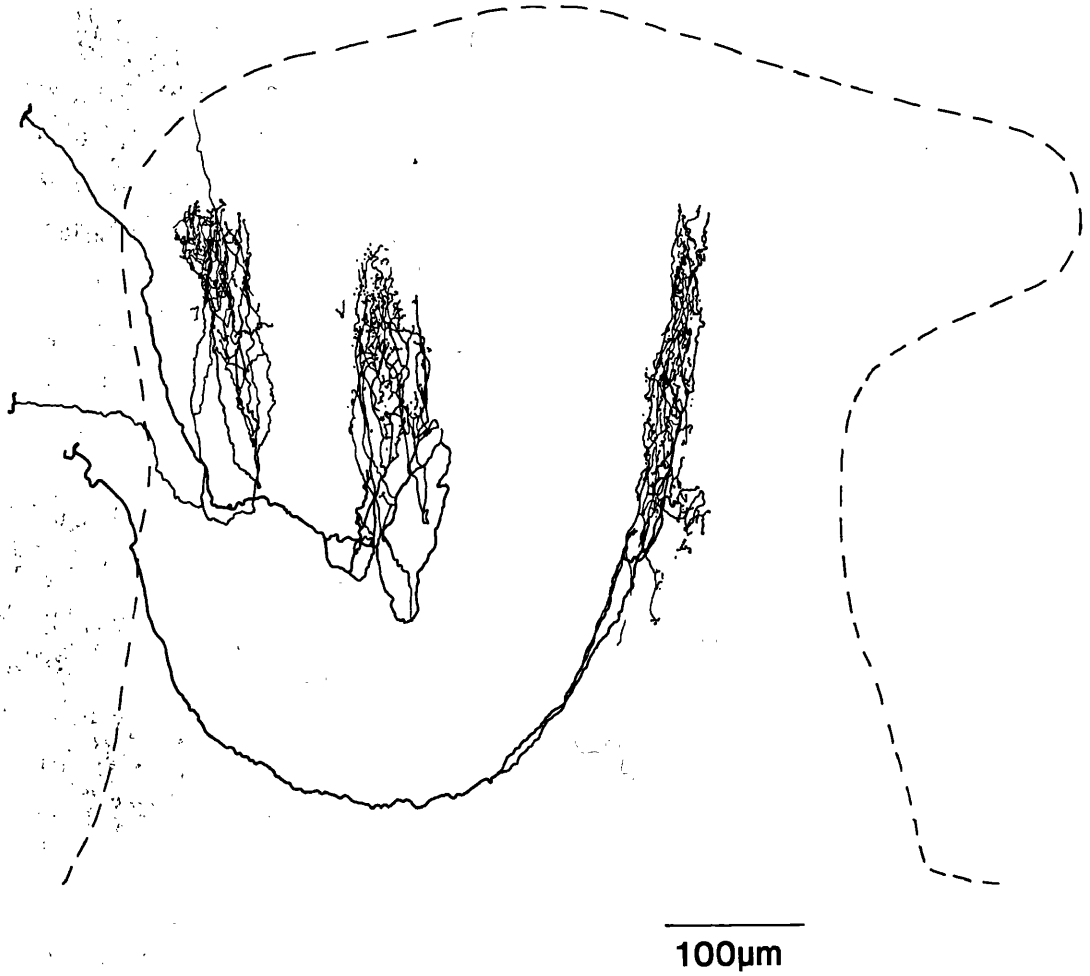
TABLE 2 DIMENSIONS OF TERMINAL ARBORIZATIONS OF HFA's INNERVATING DIFFERENT REGIONS OF THE RAT HINDLIMB.

	Lateral leg			Medial leg			Dorsal foot			Toes		
	C	S	C+S	C	S	C+S	C	S	C+S	C	S	C+S
N	64	21	85	30	12	42	20	4	24	55	14	69
ML (um)	53±2*	34±4*	48±2*	87±6*	56±6*	79±5*	79±6*	35±4	72±6*	97±8*	66±12*	90±7*
DV (um)	150±10*	75±8	131±8*	187±18*	105±16	165±14*	171±23	98±49	159±22	155±9	100±13	143±6
RC (um)	323±18	197±21*	290±16*	263±20	117±11*	222±18*	288±21	138±38	263±22	278±20	164±19	255±17
VOL ($x10^{-3}ul$)	3.0±0.6*	0.7±0.4*	2.4±0.5*	5.1±0.7*	0.8±0.5	3.9±0.6	4.4±1.0	0.7±0.2	3.8±0.8	5.0±2.2	1.8±1.3*	4.3±1.7*

x±SEM
*=sig. diff P<0.05 level
ML=mediolateral, DV=dorsoventral, RC=rostrocaudal,
VOL=volume, C=complex, S=simple, C+S=terminal.

Comparisons made were; C v C, S v S, C+S v C+S for lateral leg v medial leg, dorsal foot and toes and also for medial leg v dorsal foot, toes and dorsal foot v toes. * represents sig. diff. only for lateral leg v other regions.

FIGURE 13: High magnification reconstructions showing the typical morphology of complex arborizations in a medial leg, a dorsal toe and a lateral leg afferent. The dashed line represents the outline of the dorsal horn. The most medial arborization is from Fig. 4D and the most lateral arbor is from Fig. 2I. Note the difference in mediolateral width between the most lateral arbor and the other two arbors.



and dorsoventral directions for some afferents (Appendix II, Table 2A).

Another way of analyzing HFA's with different peripheral RF's was to compare the arrangement and dimensions of collaterals according to the nerve territory to which the peripheral RF belonged. The saphenous nerve territory innervates the anterior medial aspect of the leg becoming narrower over the medial malleolus and running down the medial side of the foot and innervating the hairy skin of toes 1,2. The superficial peroneal nerve (SP) innervates the dorsal surface of the foot, lateral toe 2, all of toes 3,4 while the sural nerve (S) innervates toe 5, the lateral side of the foot and the lateral lower leg (below the knee). Above the knee, the lateral side of the leg is innervated by the lateral sural nerve (LS). The posterior cutaneous nerve (PC) innervates the posterior aspect of the medial side of the leg while the tibial nerve (Ti) innervates the glabrous skin of the hindpaw and a small patch of hairy skin over the achilles tendon area (Fig. 14).

Table 3 shows the arrangement of collaterals for the different nerve territories. The LS had the highest total of collaterals per afferent, the SA, Su, SP had similar numbers and the PC the fewest. The LS also had the highest percentage of complex collaterals while the Su had the lowest although the range between the different nerves was quite narrow (43-54%). The percentage of simple arbors was lower (10-20%), with the Su having the most and the PC the least. Again, there were more blind-ending collaterals than simple collaterals with the LS having the fewest and the PC the most. When the distance from most rostral to caudal collateral was compared, the SA nerve territory (medial terminals in the dorsal horn) has the shortest distance and the LS (laterally placed terminals in the dorsal horn) the longest distance. This was reflected in part by the intercollateral distances and length of overlapping terminal sheet. Nerve territories with laterally placed central terminals in the dorsal horn

FIGURE 14: The relationship between the peripheral cutaneous receptive fields on the rat hindlimb innervated by the nerve branches as seen from the lateral (LAT), volar and medial (MED) aspects and their central terminal fields in the superficial dorsal horn as defined by bulk labelling of the peripheral nerves with WGA-HRP. Taken from Swett & Woolf (1985). The pattern is similar to that seen in the deeper laminae of the dorsal horn (Woolf and Fitzgerald, 1986). The peripheral cutaneous nerve territories were defined by recording action potentials from the distal cut ends of the nerves while probing the skin to low threshold stimuli. The figurines do not represent side on views but try to represent the 3-dimensional peripheral innervation on a 2-dimensional drawing. There was little variation of the peripheral nerve borders betwen animals and the borders were clearly distinguishable. SA= saphenous, LS= lateral sural, S= sural, SP= superficial peroneal, PC= posterior cutaneous, T= tibial nerves. Taken from Swett & Woolf, 1985.

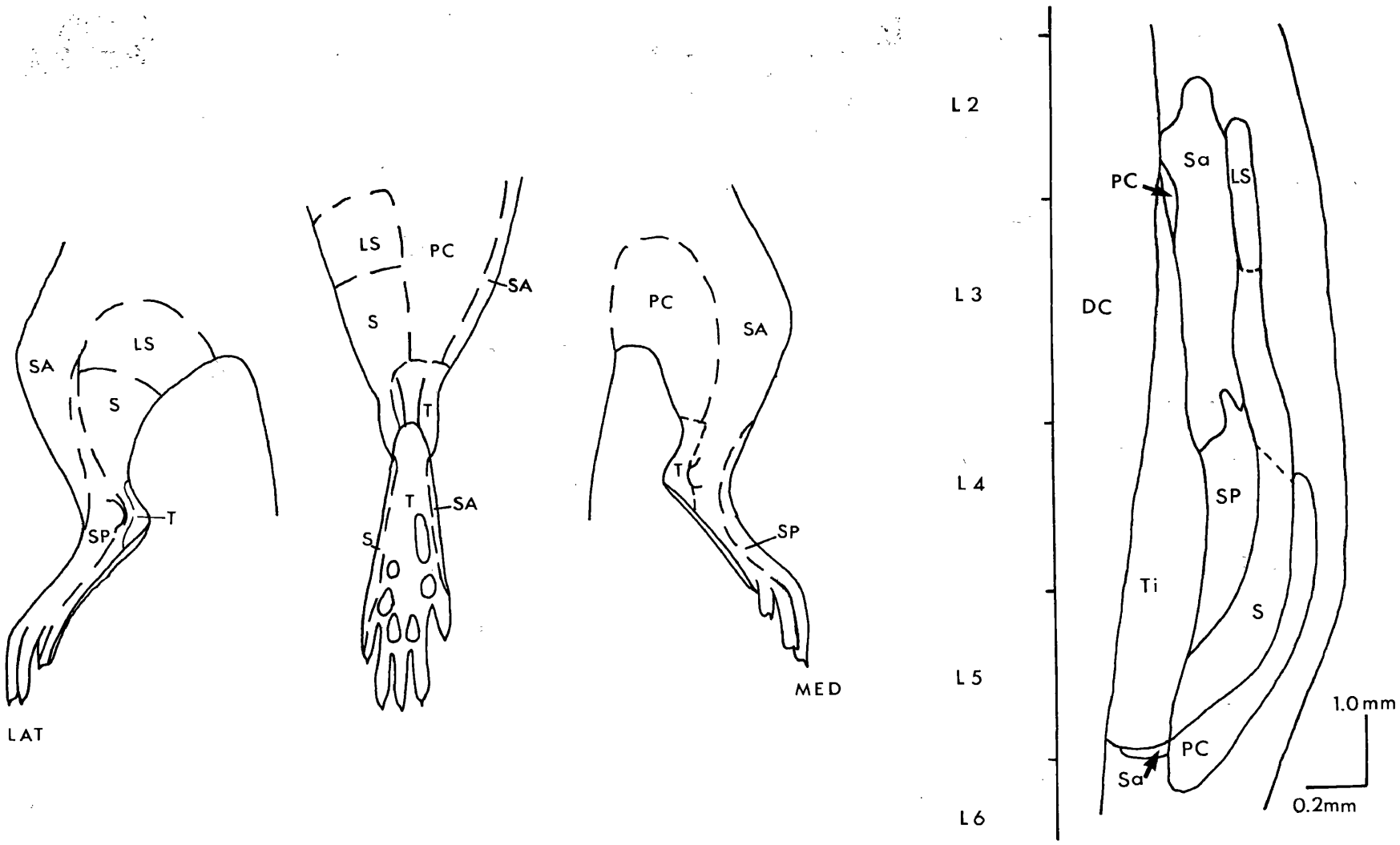

LS
PC
SA
S
T
SP
LAT
MED
L 2
L 3
L 4
L 5
L 6
DC
Sa
Ti
1.0mm
0.2mm

TABLE 3 ARRANGEMENT OF HFA COLLATERALS FROM DIFFERENT PERIPHERAL NERVE TERRITORIES

Nerve	SA	SP	SU	LS	PC
N	10	8	10	5	4
Total no collats.	86	74	97	54	30
No. collats /aff.					
total	8.6±1.0	9.25±1.25	9.70±0.97	10.80±1.74	7.50±0.87
complex	4.3±0.4	4.63±0.51	4.20±0.51	5.80±0.97	3.50±0.50
simple	1.7±0.6	0.88±0.35	1.50±0.40	2.20±0.38	0.75±0.48
blind-ending	2.6±0.4	3.88±0.81	3.90±0.67	2.80±0.80	3.25±1.10
length from most rostral to caudal collat. (mm)	2.76±0.27	3.11±0.44	3.09±0.29	3.35±0.59	2.74±0.47
intercollat. spacing (um)	342±26	343±25	345±21	334±25	382±30
projection to lamina IIi	7/43	4/41	12/42	17/40	5/17
length of o/l term. sheet (mm)	1.26±0.08	1.08±0.18	1.27±0.19	1.49±0.19	1.35±0.30

x±SEM

(Su, LS, PC) have a greater number of arbors extending into lamina IIi than medially placed central terminals (SA, SP).

Analysis of the pooled dimensions of complex, simple and terminal (complex and simple) arborizations from individual nerve territories (Table 4) showed that the SA, SP, Su nerve arborizations were all significantly wider than the LS arborizations and that the SA complex and terminal arbors were significantly wider than PC complex and terminal arborizations. SP complex arborizations were also significantly wider than PC complex arbors. SA complex arbors were significantly deeper than SP, & S terminal arbors. Rostrocaudally, PC complex and terminal arbors were significantly longer than those of the SA, LS, SP nerve arbors and also the LS simple arbors were significantly longer than those of SA simple arbors. All these dimensional differences were reflected in the volumes of dorsal horn occupied by the arborizations. SA complex and terminal arborizations had significantly larger volumes than the complex and terminal arborizations of SP and LS afferents. However, when the average dimensions /afferent for each nerve territory were compared then only the SA, LS and PC were significantly different ($P<0.05$) in the mediolateral direction, while the SA and SP afferents were significantly different in the dorsoventral direction (Appendix II, Table 4A). All significance tests were unpaired t-tests at $P<0.05$ level.

Tables 3 and 4 were similar to Tables 1 and 2 and showed that the mediolateral differences that existed for the central terminals of afferents with different peripheral RF's were also reflected by the nerve territories to which the peripheral RF belonged.

2.3.2 (vi) THE SOMATOTOPIC ORGANIZATION OF THE CENTRAL TERMINALS OF HAIR FOLLICLE AFFERENTS.

Figure 14 shows the central terminal distribution of the different peripheral nerves innervating the hindlimb

LE 4 DIMENSIONS OF TERMINAL ARBORIZATIONS OF HFA's FROM DIFFERENT
/E TERRITORIES.

/E:	SAPHENOUS		SUPERFICIAL PERONEAL			SURAL			LATERAL SURAL			POSTERIOR CUTANEOUS		
C	S	C+S	C	S	C+S	C	S	C+S	C	S	C+S	C	S	C+S
43	17	60	37	7	44	42	15	57	29	11	40	14	3	17
87±4*	55±5*	77±5*	75±4^	52±15^	71±5^	88±10˜	54±10˜	70±8˜	50±3˜*^	24±3˜*^	42±3˜*^	57±6*^	58±3	57±3*
200±2*	113±15	174±12*	124±10^	73±14*	115±9	146±10*	89±11	133±9*	169±21^	77±17	143±18	154±18	79±15	141±17
269±16^*	129±13	229±14^*	276±17^	129±21	251±16^	318±29	196±22	288±23	269±19#	195±35*	247±17#	350±40#^*	200±29	323±38#^*
5.2±0.7^*	0.9±0.2	4.1±0.6^*	2.9±0.4^	0.7±0.4	2.5±0.4	5.1±2.8	1.5±1.1	4.2±2.1	2.9±0.7*	0.4±0.3	2.2±0.5*	3.4±0.6	1.1±0.6	3.0±0.6

:M
ıediolateral, DV=dorsoventral,RC=rostrocaudal;
)lume (x10˜3µl) C=complex, S=simple C+S=terminal
˜,#= sig. diff. P<0.05 level

Comparisons made were: C v C, S v S, C+S v C+S, for each nerve territory. * = SA compared to all other nerves, ^ = SP versus all other nerves, ˜ = SU versus all other nerves, # = LS versus all other nerves.

of the rat as defined by bulk labelling with WGA-HRP while Fig. 15 shows the complete rostrocaudal extent of 8 intracellularly filled afferents with RF's as shown. When the spatial distribution of all the collaterals from an axon was analyzed, it was evident that the most rostral and caudal collaterals (which were simple or blind-ending) extended into areas of cord occupied by the complex terminal arbors of afferents with RF's in different nerve territories. Of the 131 blind-ending collaterals 56 (43%) showed overlap with other blind-ending, simple or complex arborizations and extended upto 2000um into adjacent nerve territories. Ten of the 54 (18%) simple arbors overlapped with other simple (2/10) or complex (8/10) arbors but they never projected more than 150um into the complex terminal area of afferents from another nerve territory. Only in 1 case (4 complex arbors) was overlap between complex arborizations of different nerve territories seen. This occurred for the dorsal toe 2 and medial glabrous-hairy toe 3 afferents. The toe 2 afferent has a RF which probably borders the boundary between the SA and SP nerve territories. Examples of this overlap are shown in Fig. 15 a,b. The 3 most caudal collaterals of the medial leg (SA) afferent extend into the Tibial (Ti) nerve territory (Fig. 15a) while the caudal collaterals of the upper lateral leg (LS) afferent were located in regions where the lower leg (S) afferent terminals were found and vice versa (Fig. 15b). Toe afferents also showed considerable overlap: the caudal collaterals from toe 3 extended into the complex terminal area of toe 5 (Fig. 15b) and those of toe 2 entered the toe 4 complex arbor territory (Fig. 15a) with a reciprocal distribution in the opposite direction.

When only complex collateral arborizations were analyzed then a somatotopic pattern with clearer boundaries emerged (Fig 16). A horizontal map of the dorsal horn through lamina III constructed from the afferents shown in Figs. 2-12 indicates this somatotopy. The complex arbors and 77% of the simple arbors with

FIGURE 15: Plan view through lamina III showing the entire rostrocaudal collateral distribution of 8 HFA's with RFs (indicated schematically by the illustrations which are viewed as either dorsal, ventral or side on images of the hindlimb in this and subsequent somatotopic map figures; these cartoon figurines should not be compared directly with Fig. 14) innervated by the saphenous (SA), sural (SU), lateral sural (LS) and superficial peroneal (SP) nerves. The shading represents complex and simple terminal arbors while the naked lines represent the blind ending collaterals. L2-L6 represents the lumbar segments; M, the medial grey border; L, the lateral grey border at the neck of te dorsal horn. The horizontal dashed lines represent the border boundaries between segments. The distance to the L5/6 and L3/4 borders from the L4/5 border is an average of this distancefrom allthe animals used to reduce interanimal variation in segment size. The afferent terminals have been plotted taking the L4/5 as an absolute boundary for their rostrocaudal extent. The mediolateral width of the arbors have been repesented as a percentage of the width of the dorsal horn as descibed in Fig. 1. Figs. a & b have been drawn for clarity but if superimposed overlap between different nerve territories can be seen, such that blind-ending collaterals from individual axons end in regionswhere complex terminal arborizations from other afferents occur.

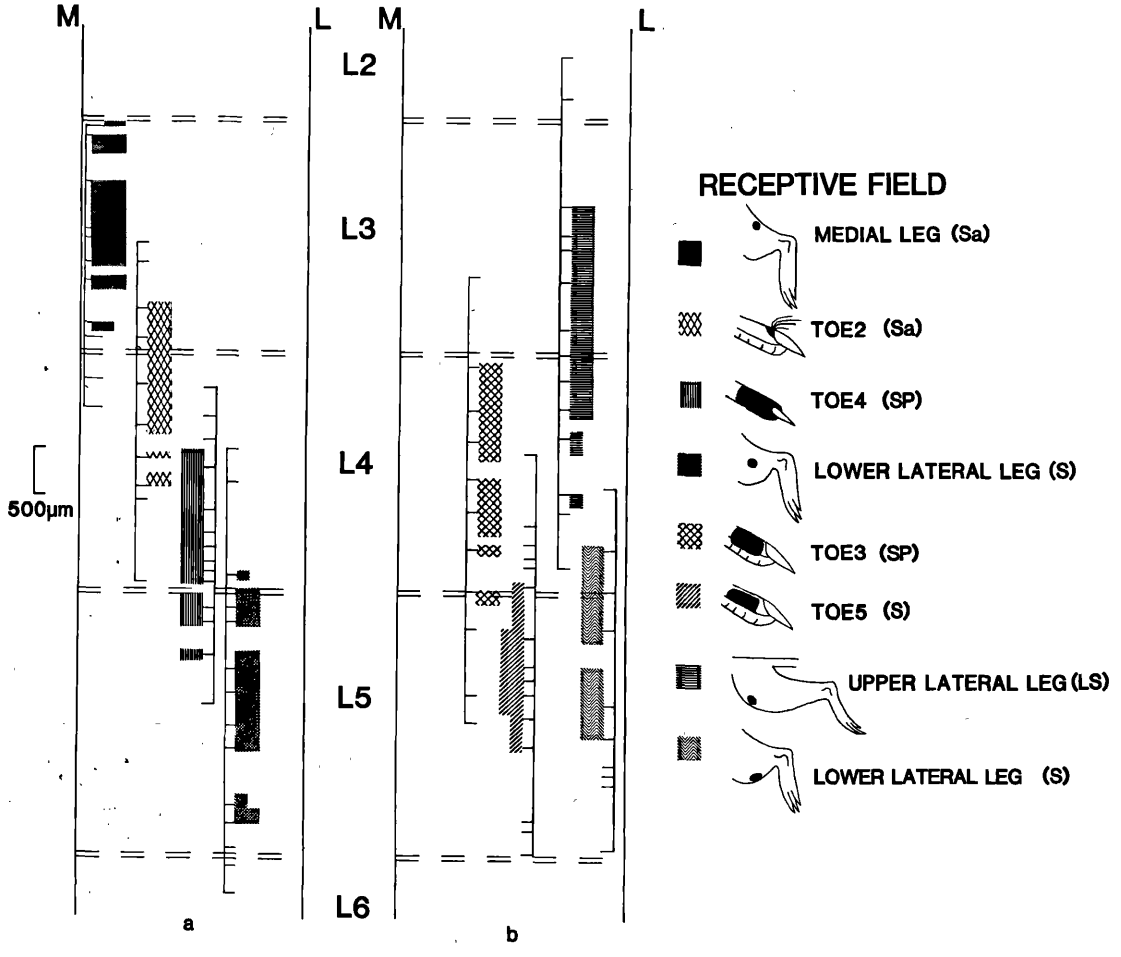
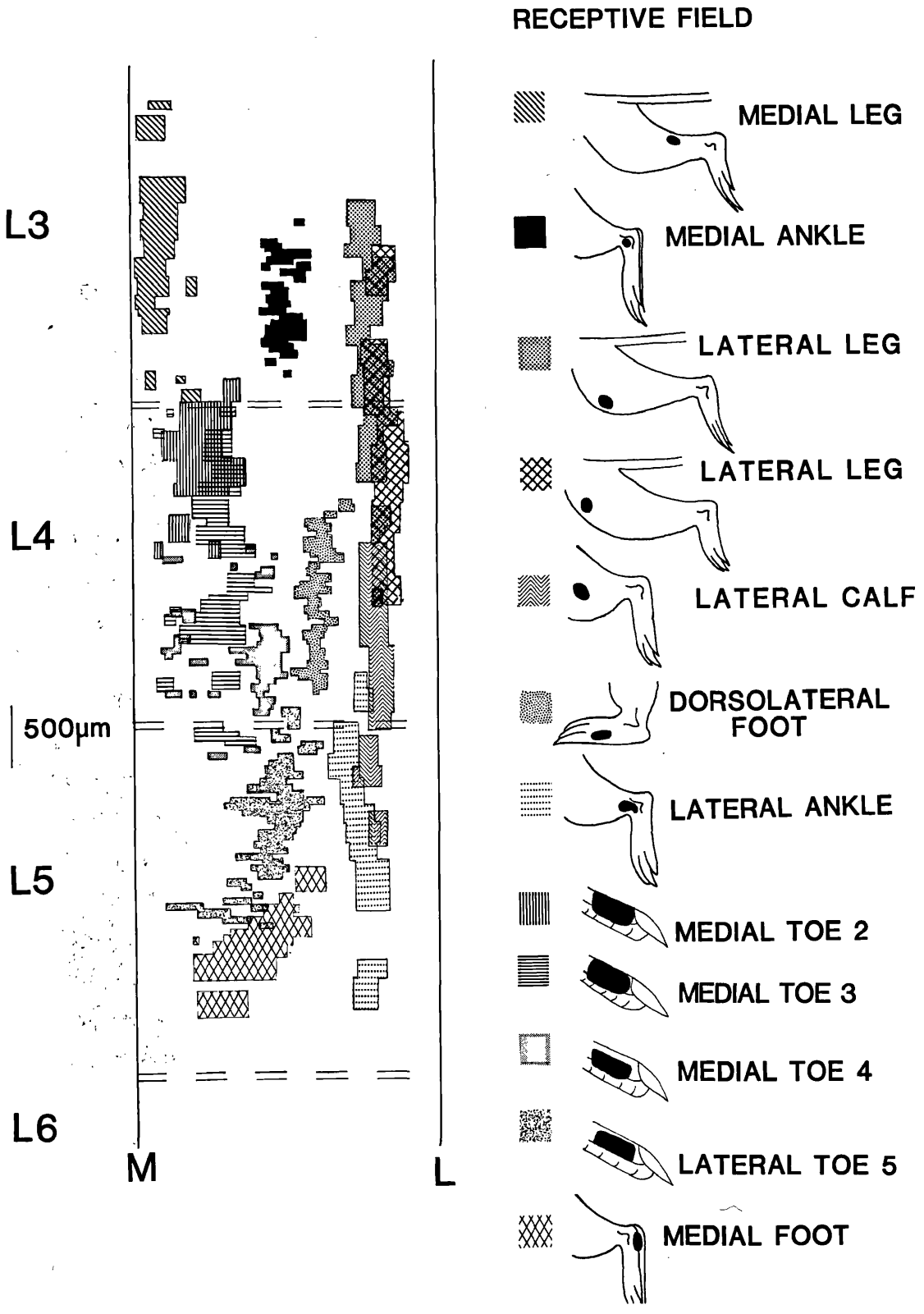

M
L
M
L
L2
L3
L4
L5
L6
500μm
a
b
RECEPTIVE FIELD
MEDIAL LEG (Sa)
TOE2 (Sa)
TOE4 (SP)
LOWER LATERAL LEG (S)
TOE3 (SP)
TOE5 (S)
UPPER LATERAL LEG (LS)
LOWER LATERAL LEG (S)

FIGURE 16: A plan view at the level of dorsal lamina III of the somatotopic arrangement of the central bouton containing arbors of HFA's with receptive fields on different parts of the hindlimb as shown. Abbreviations as shown in Fig. 15.



peripheral RF's in different nerve territories do not overlap; each was restricted to the central nerve territory of its own nerve (as defined by bulk transganglionic studies with HRP, Swett and Woolf, 1985; Woolf and Fitzgerald, 1986). For example, medial leg (SA) afferents were located medially from the lumbar L2/3 to the L3/4 border while the lateral leg (LS/S) afferents were represented in the lateral third of the dorsal horn from mid L3 to caudal L5 and dorsal foot afferents (SP) were located in L4 medial to the L4 lateral leg afferent and lateral to the L4 toe afferent terminals (Figs. 14,16).

Within an individual nerve territory there was, however, considerable overlap between the complex terminal arbors of afferents with non-overlapping peripheral RF's (Figs. 17-21). Afferents with non-adjacent RF's on the lateral leg above the knee (Fig. 17) have terminals located from mid-L3 to mid-L4 and a similar overlap was present for lower leg afferents (Fig. 18) except that the terminals were located from mid-L4 to caudal L5. The terminals from leg afferents innervated by the PC nerve also overlapped and were located throughout L5 (Fig. 19). Taking the lateral leg as a whole, there was a spatial somatotopic gradient of the terminals. As one proceeds caudally from L3 to L5, the RF's of afferents shift from lateral thigh to lateral calf to lateral ankle within the mediolaterally compressed overlapping terminal sheet, although within a given area such as the upper thigh (Fig. 17) the gradient was not continuous.

Figure 22 shows the central terminals of 7/11 afferents from different toes in the dorsal horn. Each toe has its own area of cord into which afferents from that toe terminate. Toes 2-5 were represented successively more caudally with toe 2 represented in caudal L3-rostral L4, toe 3 in mid L4, toe 4 in caudal L4-rostral L5 and toe 5 in mid-to-caudal L5 with little rostrocaudal overlap. There was a slight medial to lateral shift in the relative toe positions around the

FIGURE 17: A plan view showing the somatotopic organization of the terminal arborizations of HFA's with receptive fields on the lateral leg above the level of the knee. These afferents all lie within the lateral sural nerve territory. Abbreviations as in Fig. 15.

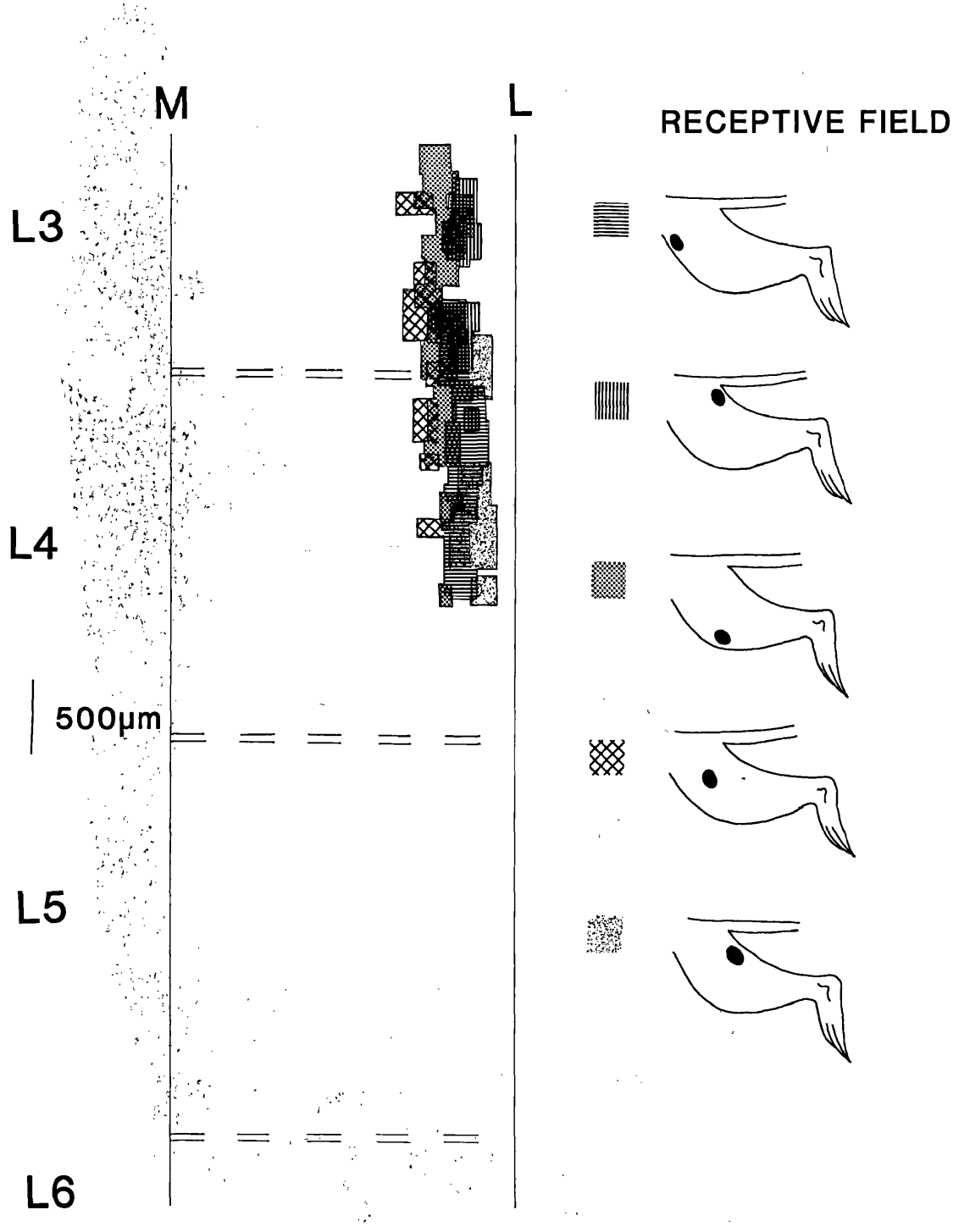


FIGURE 18: A plan view showing the somatotopic organization of the terminal arborizations of HFA's with receptive fields on the lateral leg below the level of the knee. These afferents are innervated by the sural nerve. Abbreviations as in Fig. 15.

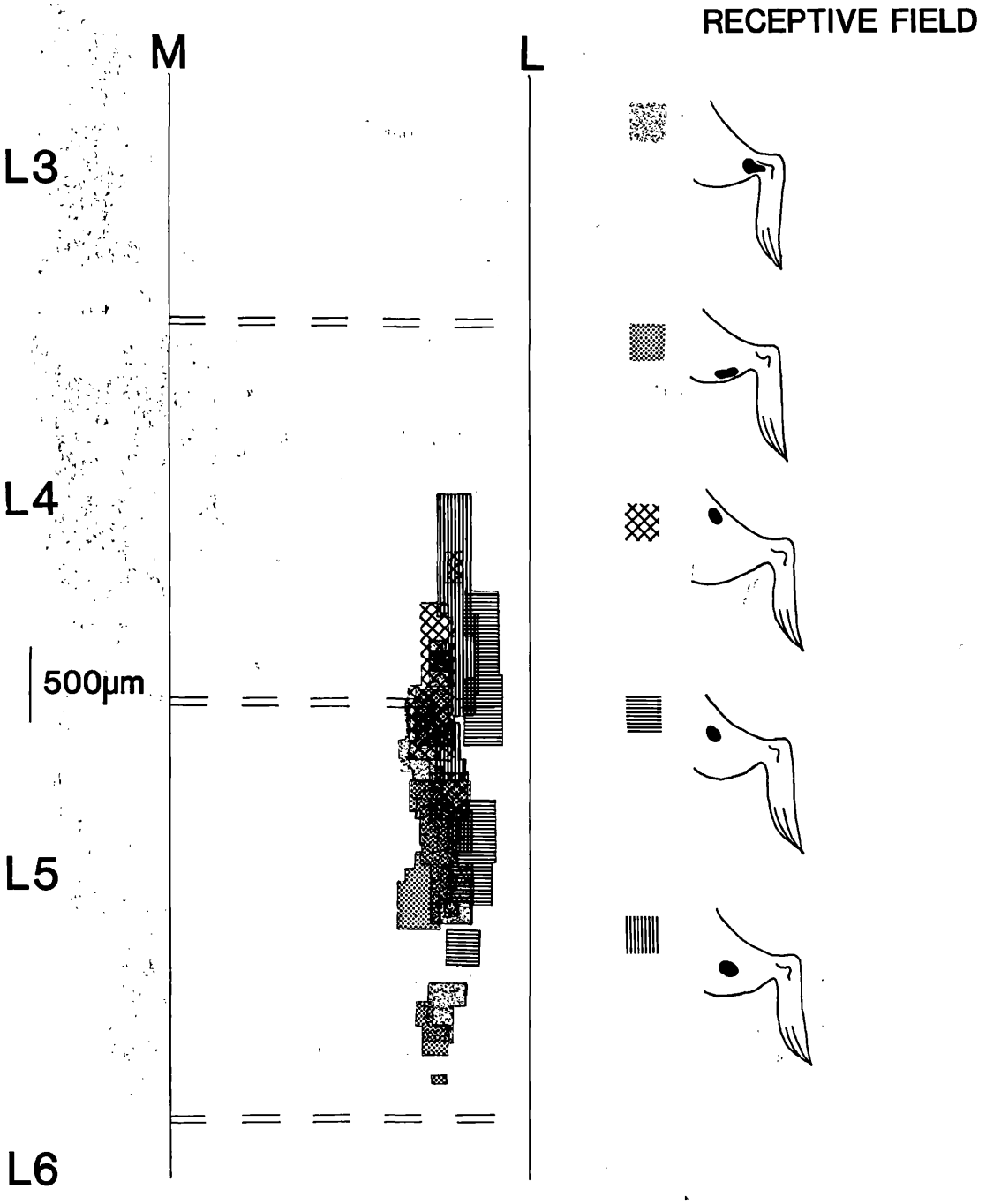


FIGURE 19: A plan view showing the somatotopic organization of the terminal arborizations of HFA's with receptive fields along the midline of the leg innervated by the posterior cutaneous nerve. Abbreviations as in Fig. 15.

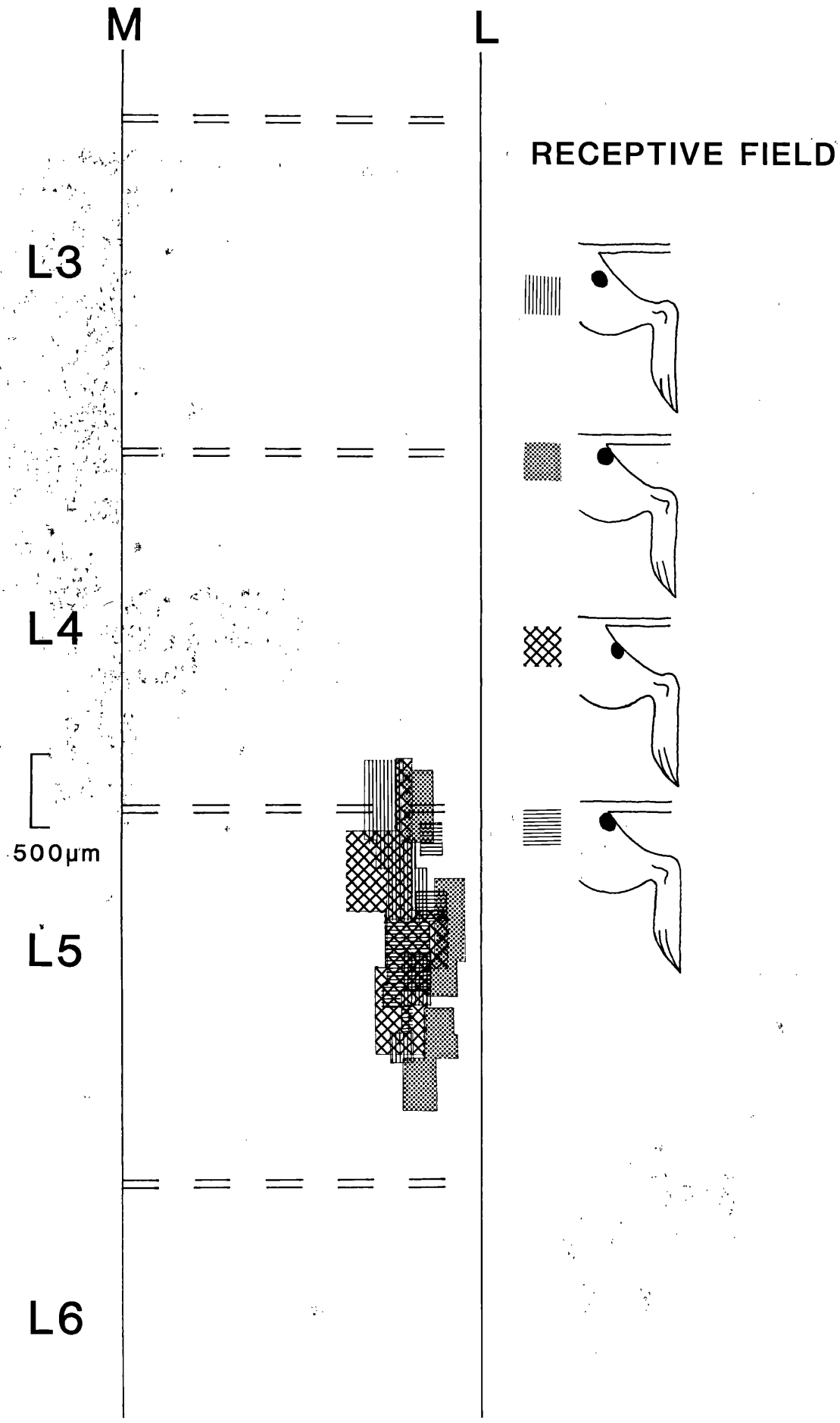

M
L
RECEPTIVE FIELD
L3
L4
500μm
L5
L6

FIGURE 20: A plan view showing the somatotopic organization of the terminal arborizations of HFA's with receptive fields as shown innervated by the saphenous nerve. All receptive fields are seen from the medial side. Abbreviations as in Fig. 15.

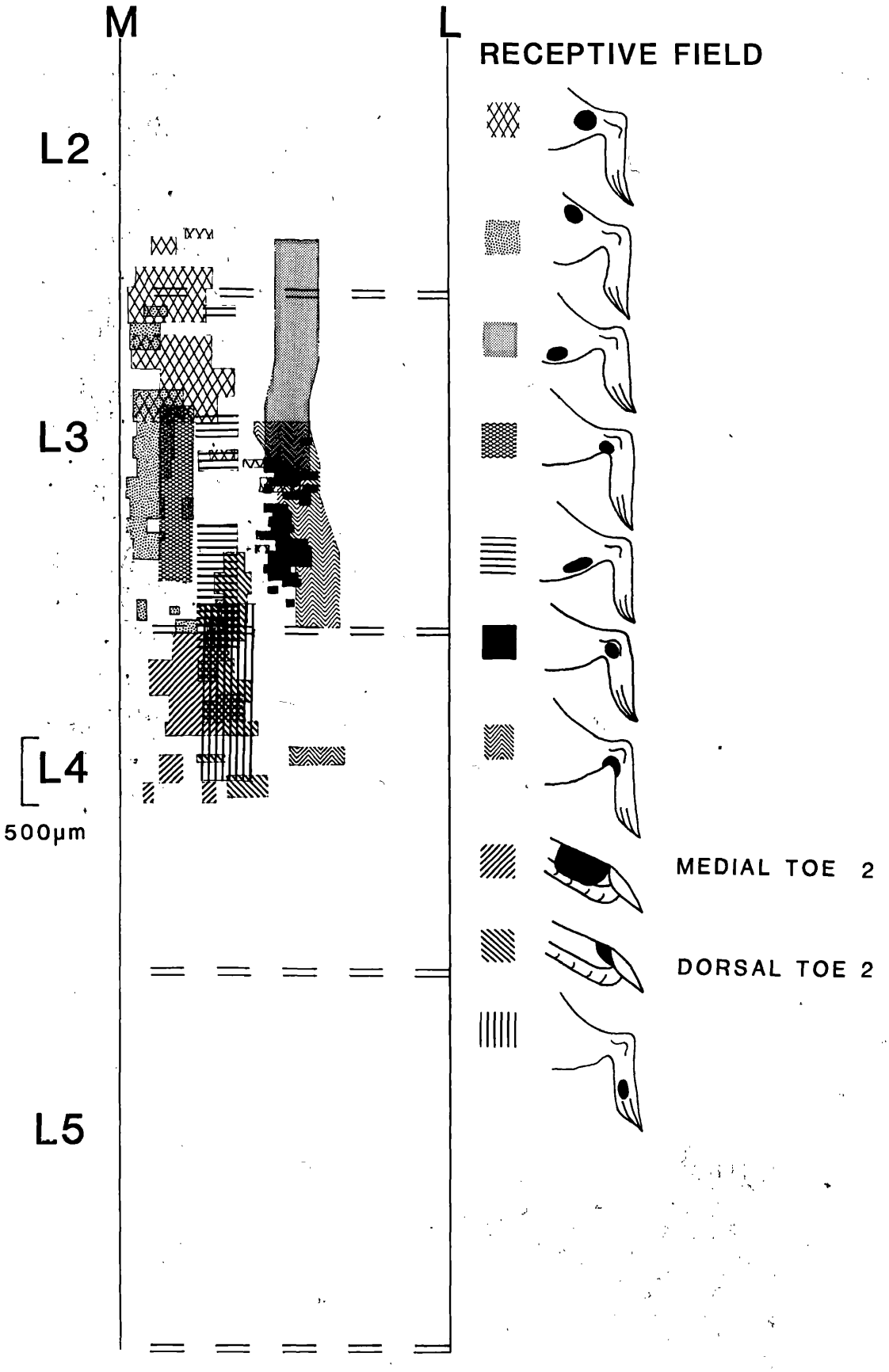
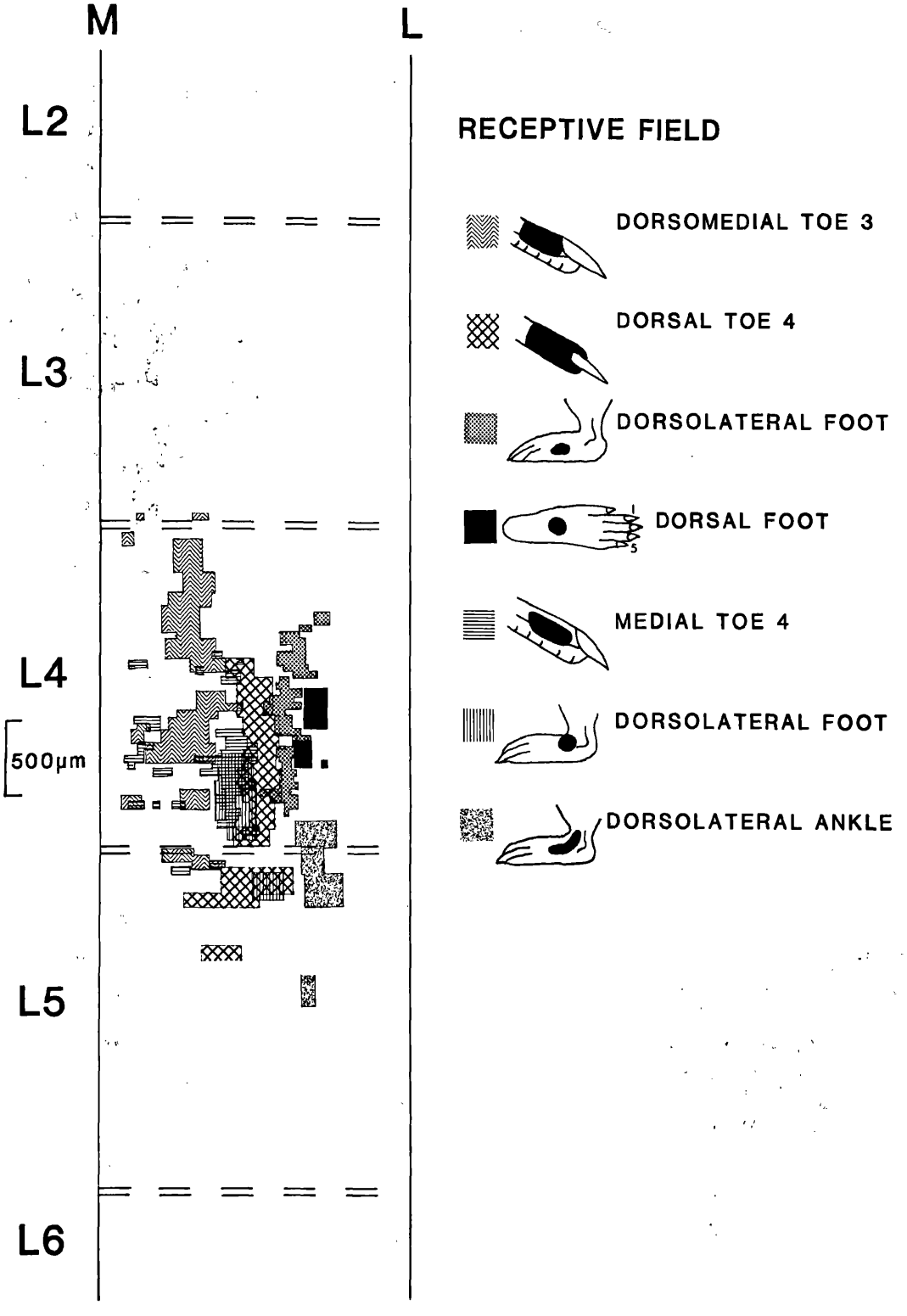

M
L
RECEPTIVE FIELD
L2
L3
L4
500µm
L5
MEDIAL TOE 2
DORSAL TOE 2

FIGURE 21: A plan view showing the somatotopic organization of the terminal arborizations of HFA's with receptive fields as shown innervated by the superficial peroneal nerve. Abbreviations as in Fig. 15.



L4/5 border and this occurred because the majority of the terminals of glabrous afferents are found in the medial half of the dorsal horn from L4 to L5 (Fig. 14), so that the position of the hairy toe afferents was lateral to the afferent terminals of glabrous skin afferents which predominate in this area of cord.

Within an individual toe territory it can be seen that afferents with slightly different RF's on the same toe have slightly different mediolateral positions at the same rostrocaudal level in the dorsal horn such that afferents with a RF having some glabrous input were located medial to those afferents which only had a hairy component to their RF (see toes 2,4 in Fig. 22, Fig. 23).

2.3.3 RAPIDLY ADAPTING AFFERENTS (RA's).

The stem axons, collateral branches and terminal arborizations of 14 rapidly adapting afferents were recovered after histological processing. Of these, 8 RA afferents had receptive fields on the toes, while the other 6 had peripheral RF's on the paw pads. The size and location of these receptive fields is shown in Fig. 24. On entering the spinal cord, all the afferents bifurcated into rostral and caudal stem axon branches with collaterals of varying complexity (simple, blind-ending and complex) being issued at varying distances along the rostrocaudal extent (Plates 4,5).

2.3.3 (i) MORPHOLOGY OF PAW PAD RA AFFERENTS.

Figure 25 shows the complete rostrocaudal extent of collaterals from a paw pad afferent. The simple collaterals (Fig. 25A-D, K-M) were located rostral and caudal to the complex terminal arborizations (Fig. 25E-J). Terminal branches and boutons were commonly issued from the main collateral branch and were usually located in deeper laminae than the complex arborizations although

FIGURE 22: A plan view of the somatotopic organization of the terminal arborizations of HFA's with receptive fields on the toes. Abbreviations as in Fig. 15.

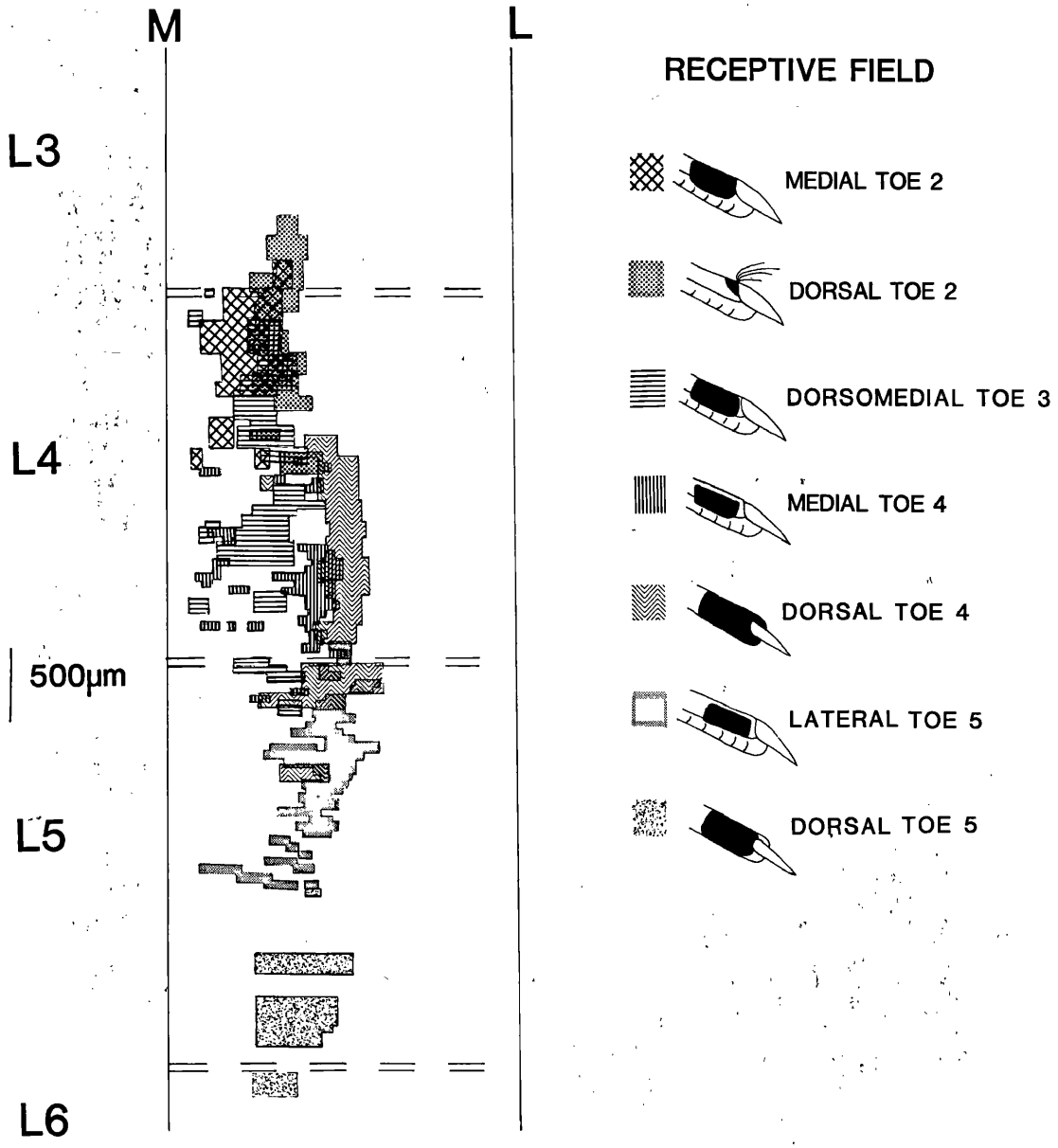


FIGURE 23: A plan view of the somatotopic organization of the terminal arborizations of HFA's with receptive fields on toe 5. LGH= lateral glabrous-hairy; LT= lateral toe; DT=dorsal toe. Abbreviations as in Fig. 15.

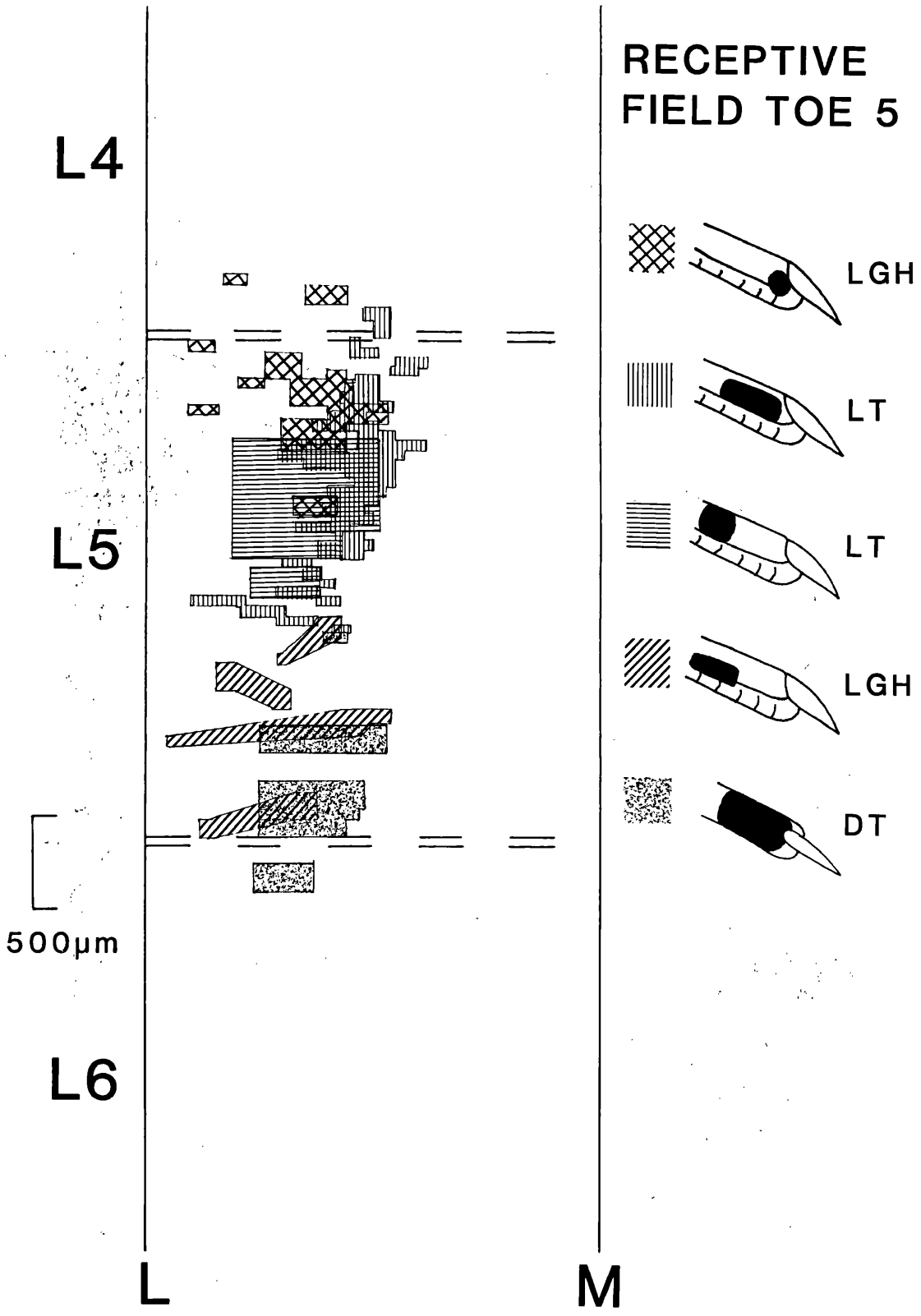


FIGURE 24: A plan view through lamina IV of the somatotopic arrangement of the central bouton containing arbors of rapidly adapting (RA) afferents with receptive fields (RF) on different parts of the glabrous surface of the hindpaw. Fig. a shows only toe RF's & Fig. b shows only paw pad RF's and have been drawn separately for clarity. Abbreviations as in Fig. 15.

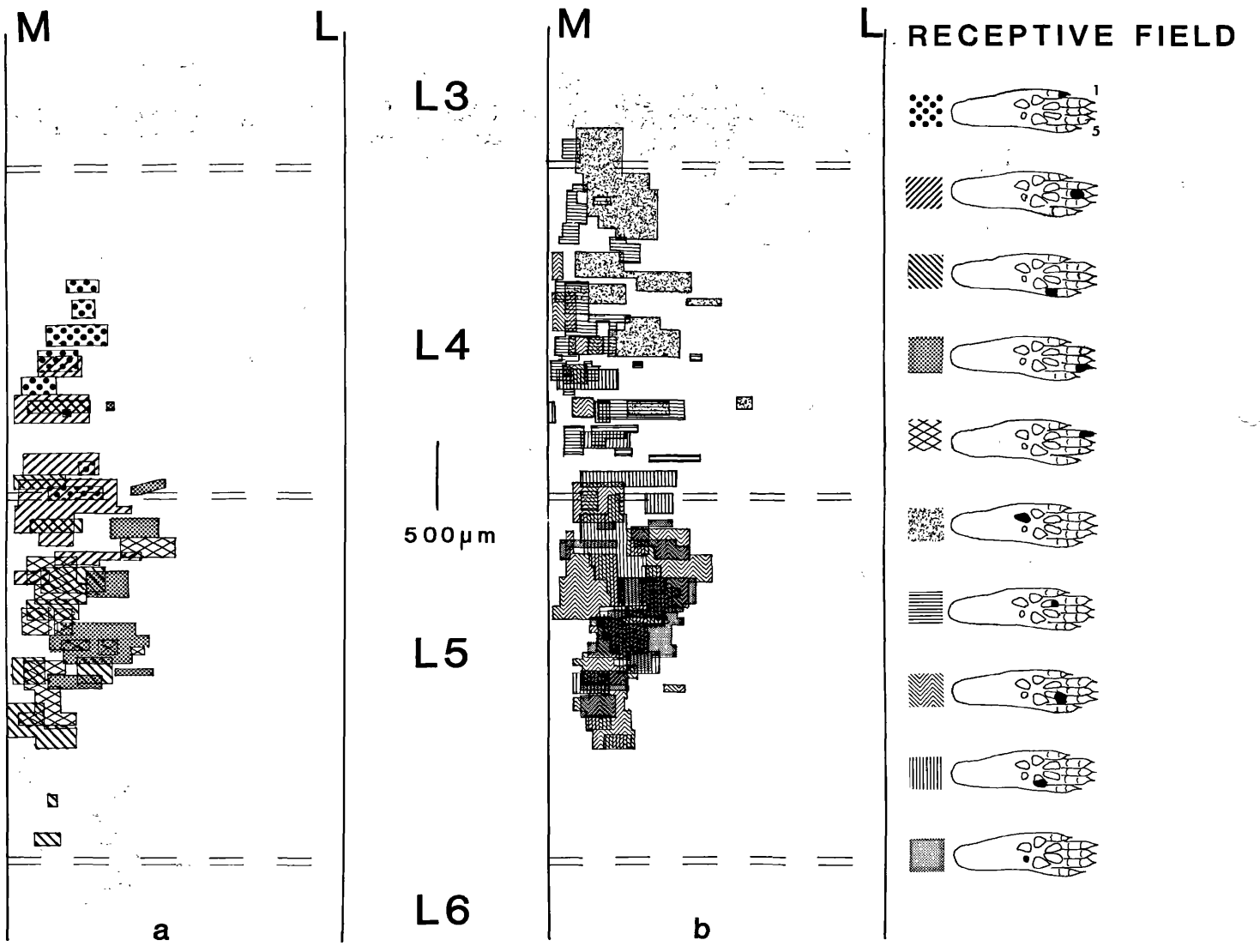
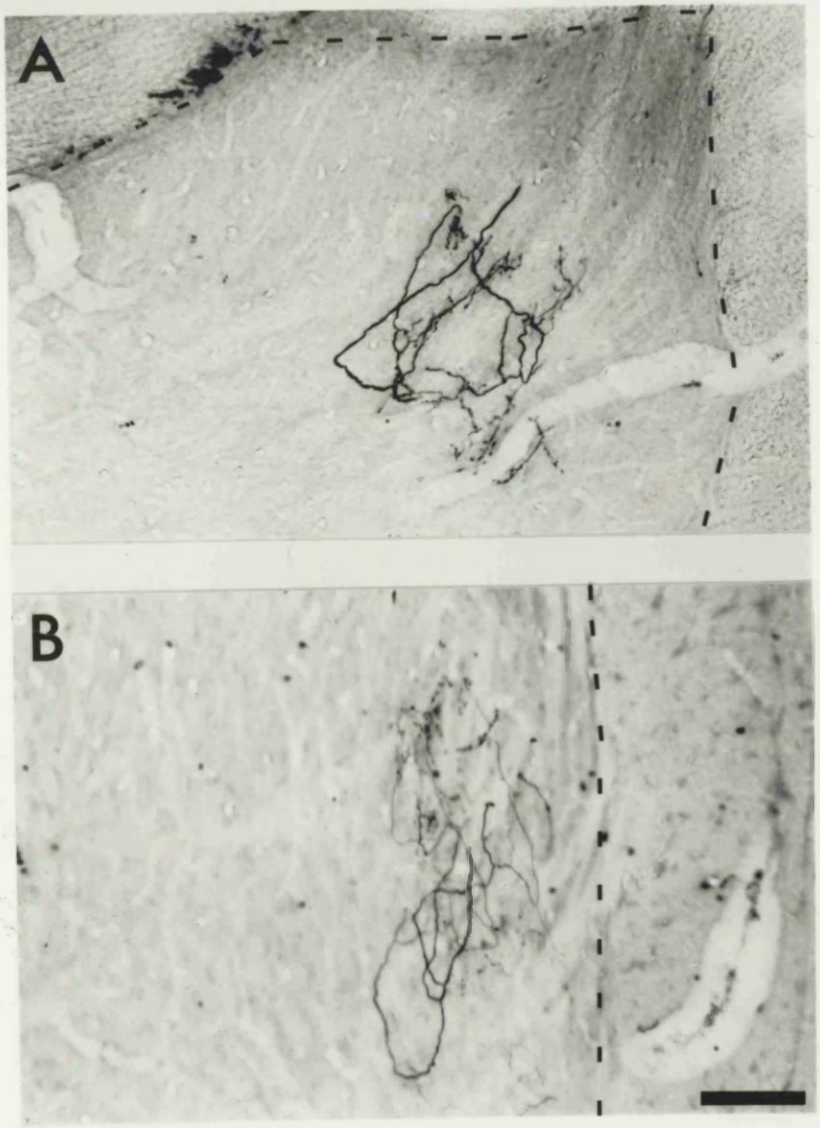

M
L
M
L
RECEPTIVE FIELD
L3
L4
500μm
L5
L6
1
5
a
b

PLATE 4.

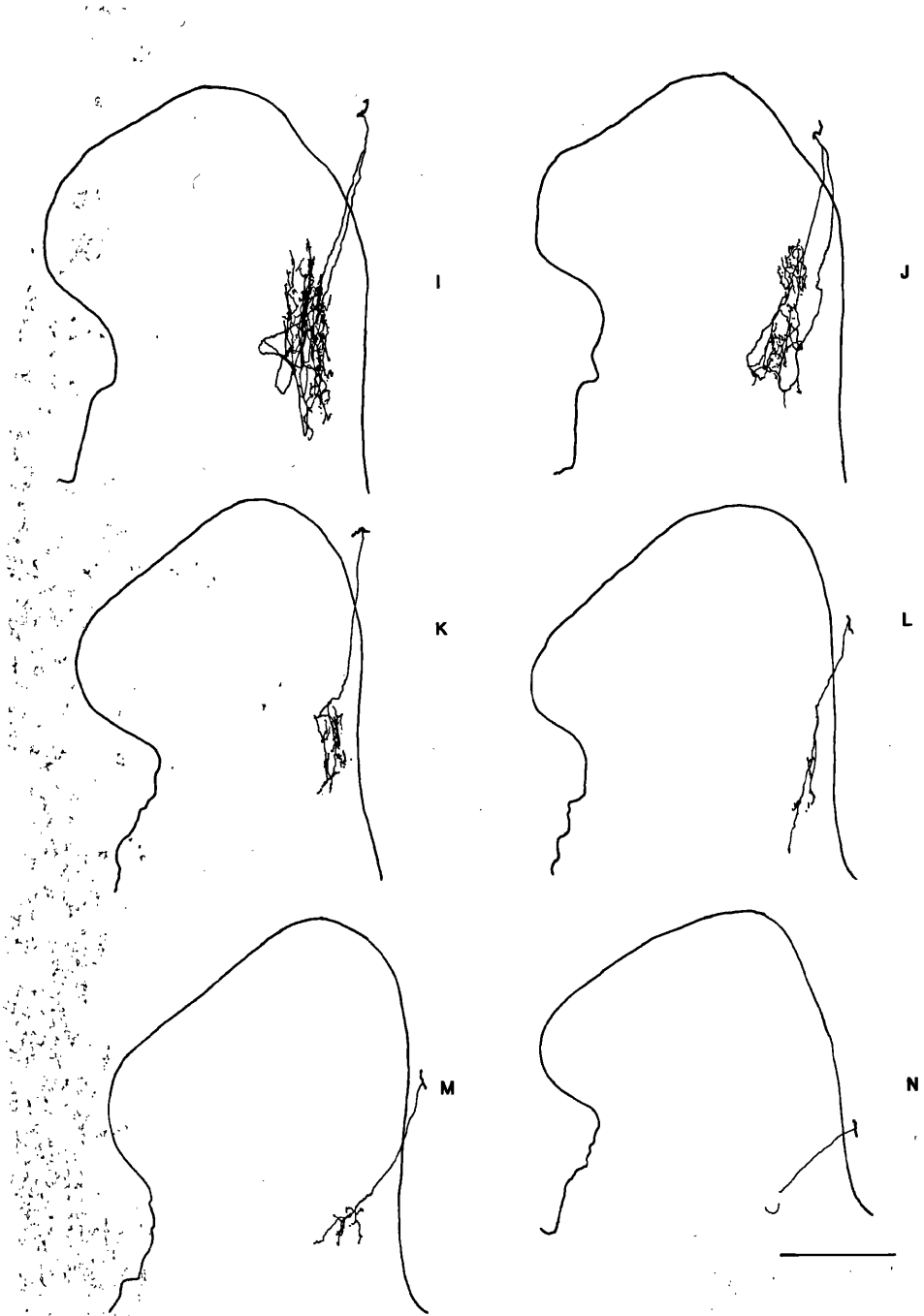
Photomicrographs of 50um thick transverse sections of lumbar spinal cord illustrating examples of RA afferent collaterals intraaxonally stained with HRP. A and B are of complex arbors from paw pad afferents; C is a complex arbor from a toe afferent. Dashed lines represent the outline of the dorsal horn. Scale bar 100um.


A
B


C


A
B
C
D
E
F
G
H

FIGURE 25: Camera lucida reconstructions of all the collateral arborizations from rostral (A) to caudal (N) of a RA afferent whose receptive field was located on the distal paw pad adjacent to toe 5. Scale bar 250um.



this was not always the case (see Fig. 33E,F). There was never any terminal overlap between adjacent simple arborizations. There were no consistent morphological features between simple arborizations within an individual paw pad afferent or between paw pad afferents (Fig.33E-H).

As with the simple arborizations, paw pad complex arborizations showed a marked variation in the morphology of individual terminal arborizations, (Plate 4A,B) although for some adjacent complex arborizations the morphology appeared quite consistent (Figs. 25F-I, 26C-E, 27C-D). In some cases the collateral axon branched dorsal to the terminal arborization (Figs. 25F, H-J, 26A,C, 27C) while in others the axon collateral looped ventral to the arbor and then ramified to produce the terminal boutons (Figs. 25E,G, 26E-F, 27D, 28A,B).

As for the terminals themselves, they were located at a more superficial laminar level compared to the simple arborizations. For most of the complex arborizations, terminals were distributed in laminae III-IV with an occasional terminal intrusion into ventral lamina II (Figs. 25F, 26C, 27C,D, 28B), with fewer boutons distributed deeper into lamina V (Figs. 25I, 26F). As well as this dorsoventral variation there was a marked mediolateral and rostrocaudal variation in the dimensions of individual arborizations (compare Figs. 26-28). Analysis of the dimensions of complex and simple arbors (Table 6) of paw pad afferents showed that the complex arbors were approximately three times as wide, twice as deep and one and a half times as long as the simple arbors and this was reflected in the volume of cord occupied by the arbors.

A new feature not previously described before for RA afferents was that for 4/6 afferents there was terminal overlap between adjacent complex collaterals. This may be why there was a consistent morphological shape between some adjacent complex arborizations (as shown in the plan view of Fig. 24b).

FIGURE 26: Camera lucida reconstructions of six adjacent complex arborizations from rostral (A) to caudal (F) of a RA afferent whose receptive field was on the surface of the paw pad adjacent to toe 2. Scale bar 250um.

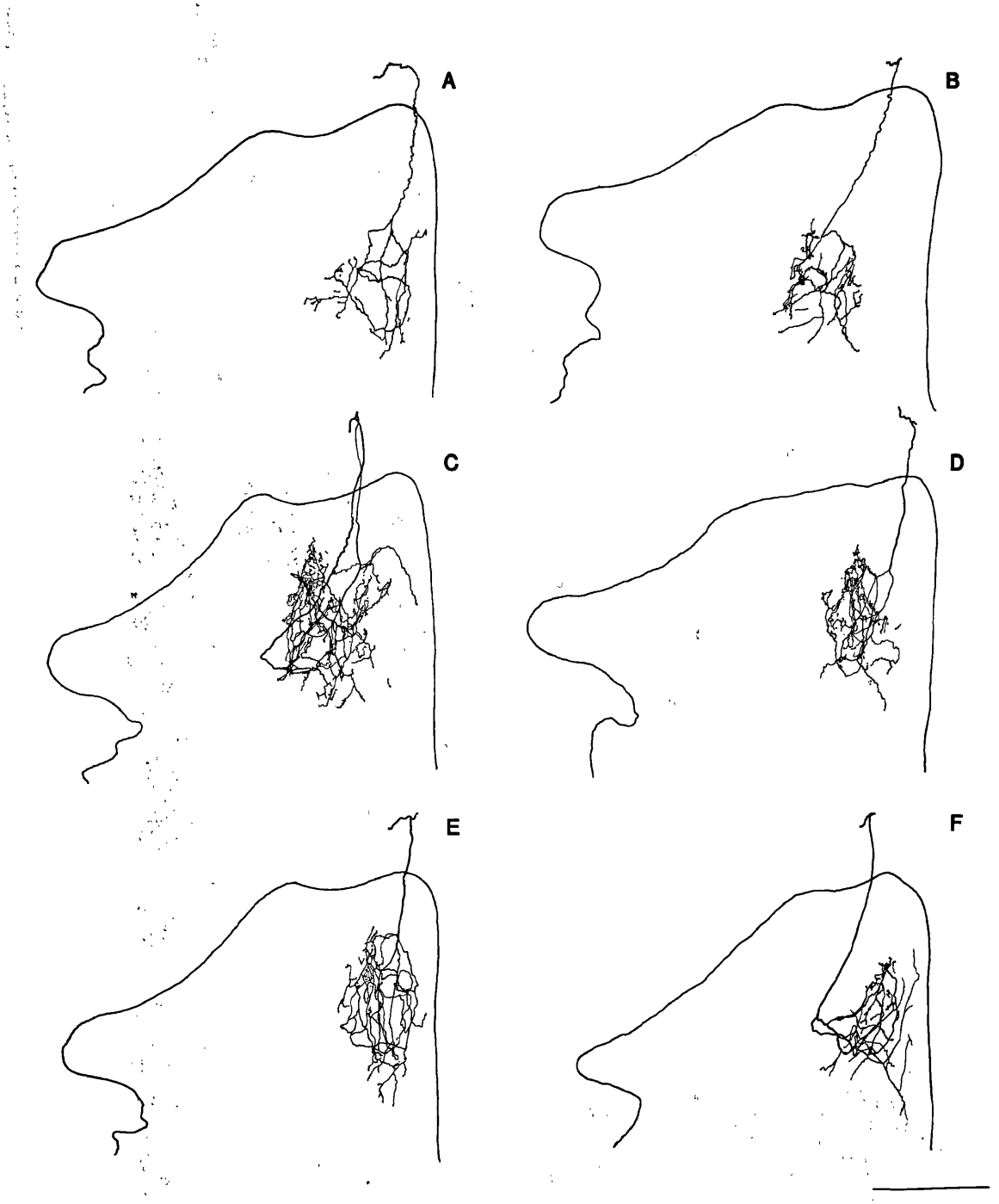


FIGURE 27: Camera lucida reconstructions of four adjacent complex arborizations from rostral (A) to caudal (D) of a RA afferent whose receptive field was located on the surface of the paw pad adjacent to toes 4 & 5. Scale bar 250um.

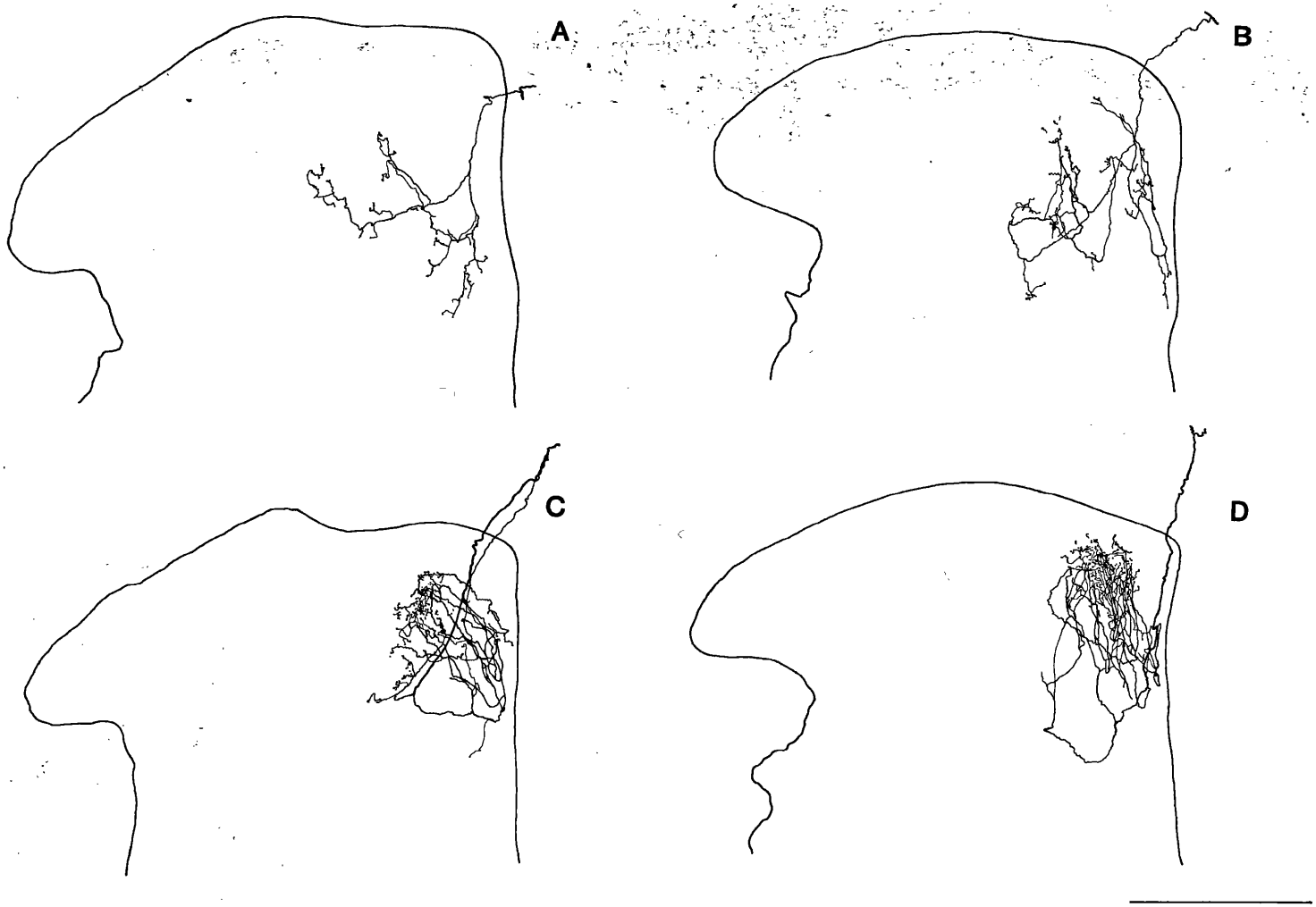
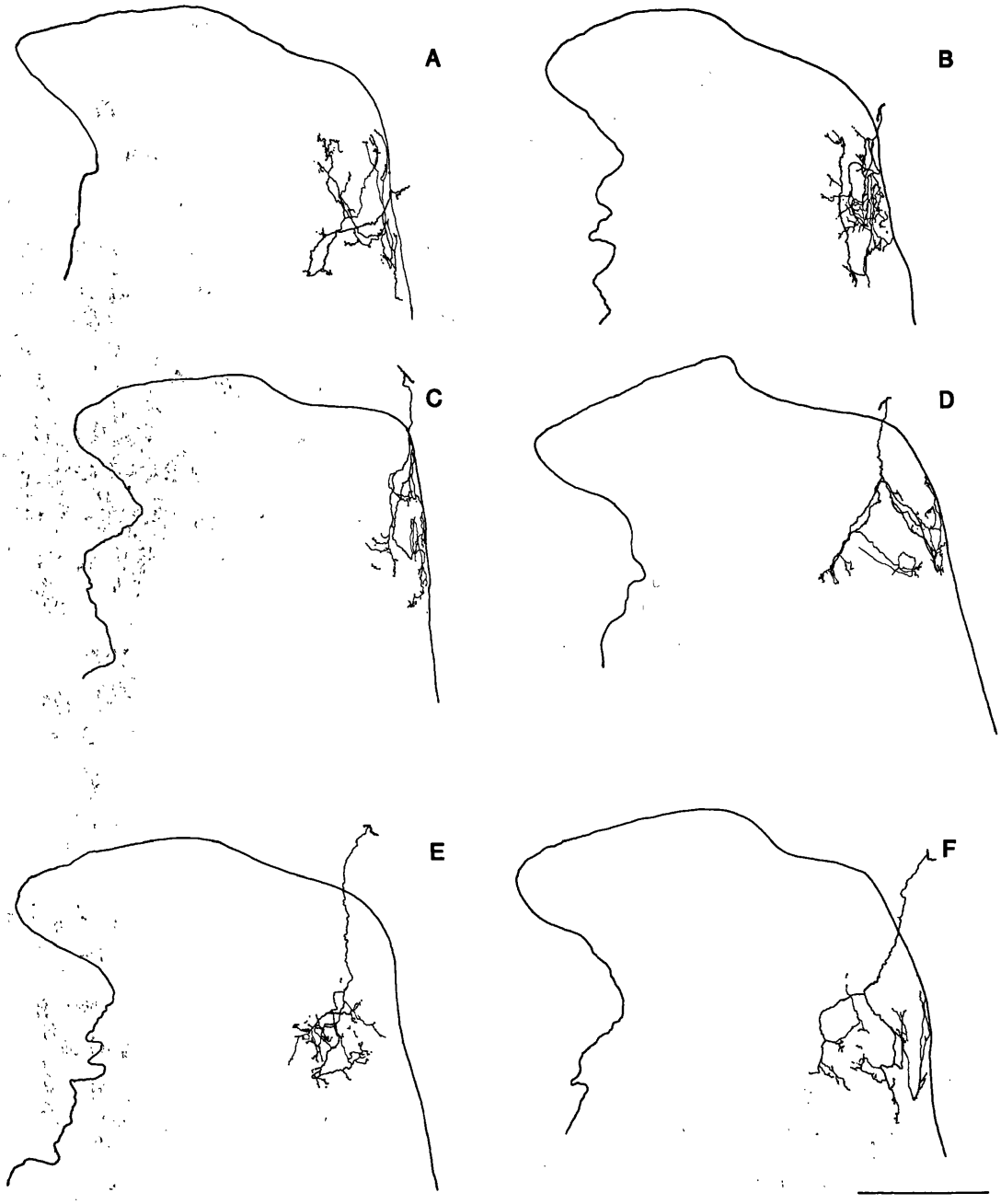

A
B
C
D

FIGURE 28: Camera lucida reconstructions of six adjacent complex arborizations from rostral (A) to caudal (F) of a RA afferent whose receptive field was located on the surface of the paw pad adjacent to toe 3. Scale bar 250um.



2.3.3 (ii) PAW PAD RA AFFERENT SOMATOTOPIC ORGANIZATION.

Figure 24b shows the plan views of the terminal arborizations of paw pad afferents with peripheral receptive fields as shown. It shows that there was considerable central overlap between the complex terminals of afferents with adjacent and non-adjacent peripheral RF's. Terminals were distributed from the medial border of the dorsal horn to the medial half of the dorsal horn throughout L4 and L5. The paw pad afferents showed some topographical organization with the proximal paw pad of toe 1 represented most rostally and (moving around to the foot) to the distal paw pad of toe 5 which was represented most caudally.

2.3.3 (iii) MORPHOLOGY OF TOE RA AFFERENT TERMINALS.

The complete rostrocaudal extent of the collaterals of a toe afferent is shown in Fig. 29. The blind-ending collaterals (Fig. 29A,B, I-K) were located at the rostral and caudal extremes of the axons rostrocaudal extent and the simple arbors (Fig. 29C,D, H) were located rostral and caudal to the complex arbors (Fig. 29E-G, Plates 4C,5). The simple arbors showed relatively little secondary or tertiary branching with the terminal branches and boutons being issued from the secondary collateral branches (Fig. 29C,D,H). Boutons were located quite deep in the dorsal horn in lamina IV mainly but some simple arbors had a few more dorsal branches in lamina III (Fig. 29D,H). Other examples of toe simple arbors are shown in Fig.33A-D.

Examples of the terminal arborizations of complex collaterals are shown in figs. 30-32 and Plate 4C. These show that there was considerably more variation in the morphology of individual terminal arborizations in and between adjacent complex arbors than was seen in paw pad complex arbors. In the majority of arbors, the collateral axon branched dorsal to the arborization (Figs. 29E, 30D-F, 31A,C,D, 32A,C-F). In others, the collateral branch

A
B
C
D
E
F

FIGURE 29: Camera lucida reconstructions of all the collateral arborizations from rostral (A) to caudal (K) of a RA afferent whose receptive field was located on the glabrous surface of the tip of toe 3. Scale bar 250um.

FIGURE 30: Camera lucida reconstructions of six complex arborizations from RA afferents whose receptive fields were on the glabrous surface of toe 5 (A&B), toe 2 (C&D) and toe 1 (E&F). Scale bar 250um.

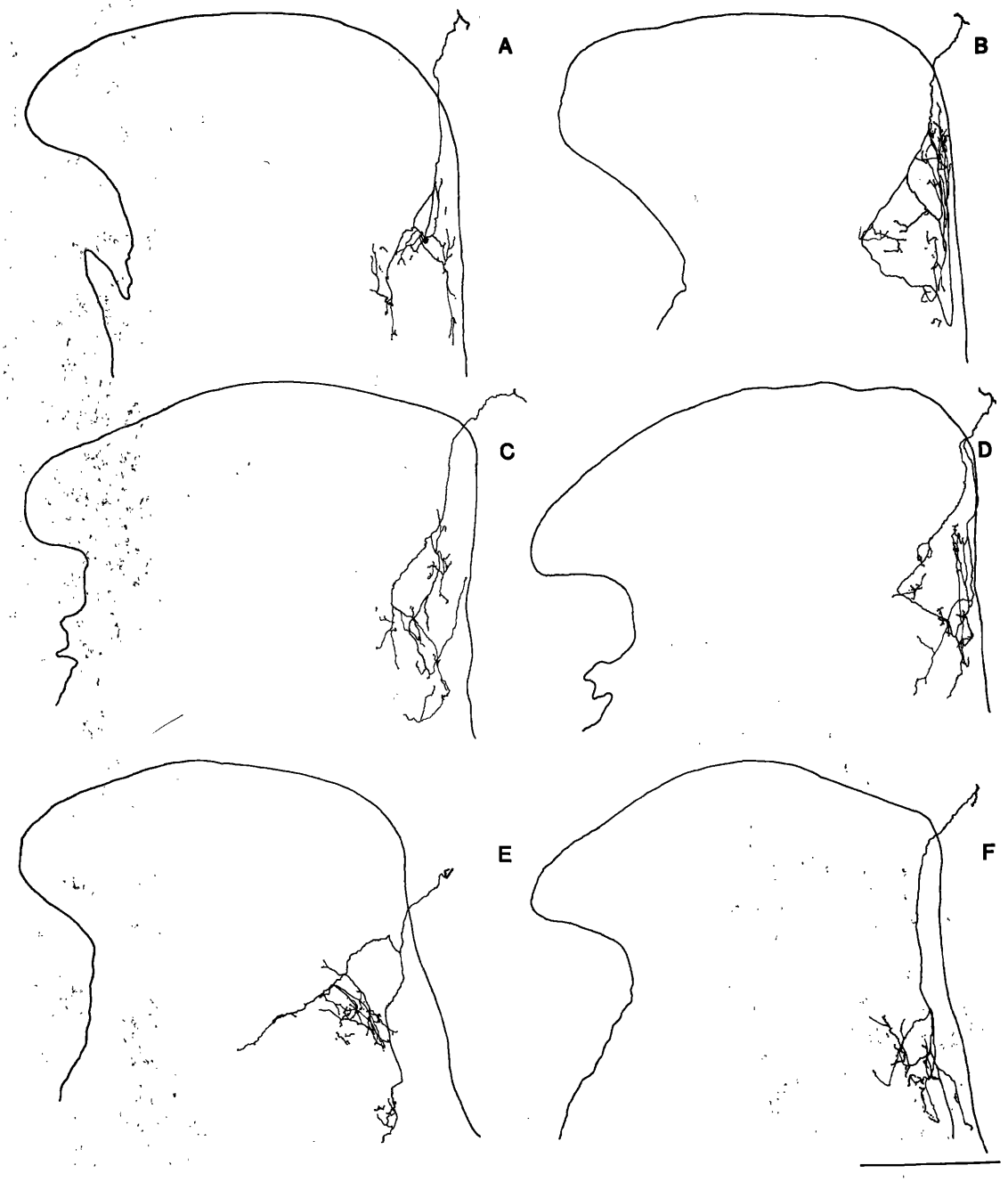


PLATE 5.

Photomicrographs of three consecutive 50um thick transverse sections of lumbar spinal cord from rostral (A) to caudal (C) of a toe RA complex collateral. The dashed lines represent the outline of the dorsal horn. The arrow points towards terminal boutons in lamina II_i. Scale bar 50um.

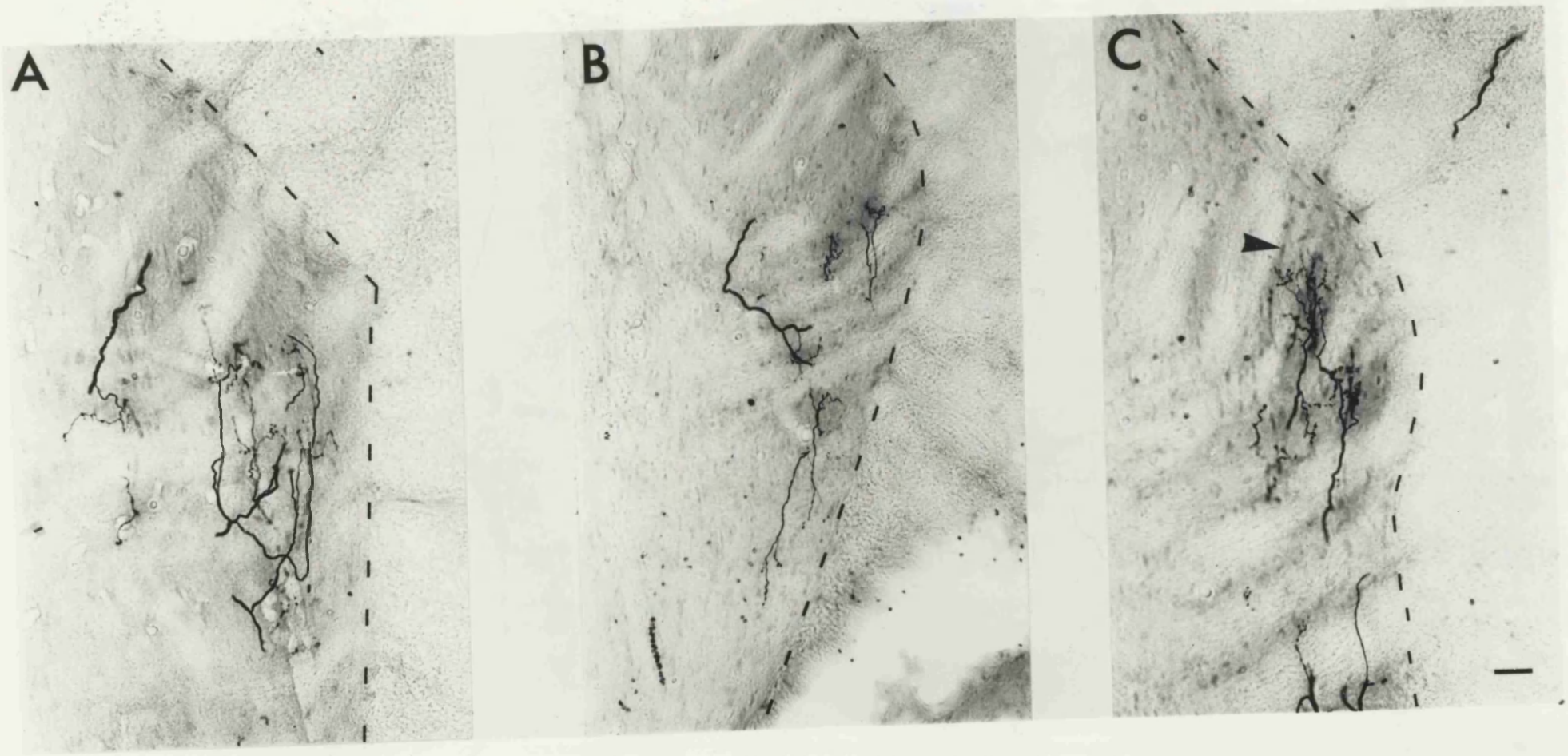

A
B
C

FIGURE 31: Camera lucida reconstructions of six adjacent complex arborizations from rostral (A) to caudal (F) of a RA afferent whose receptive field was located on the glabrous surface of toe 1. Scale bar 250um.

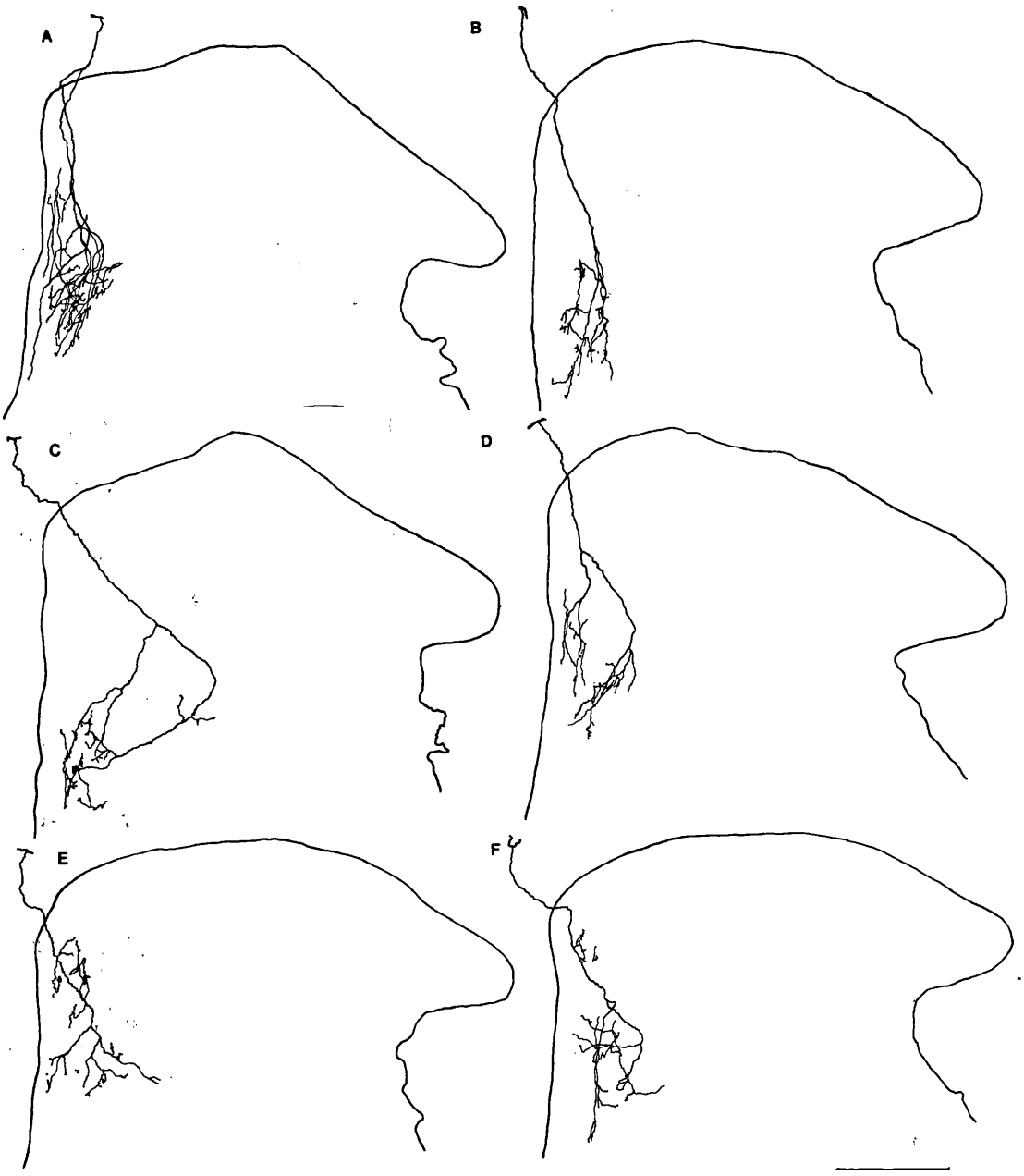


FIGURE 32: Camera lucida reconstructions of adjacent complex arborizations from rostral to caudal of RA afferents whose receptive field was located on toe 3 (A-D) and toe 4 (E-G). Scale bar 250um. Note the varied morphology of adjacent arborizations.

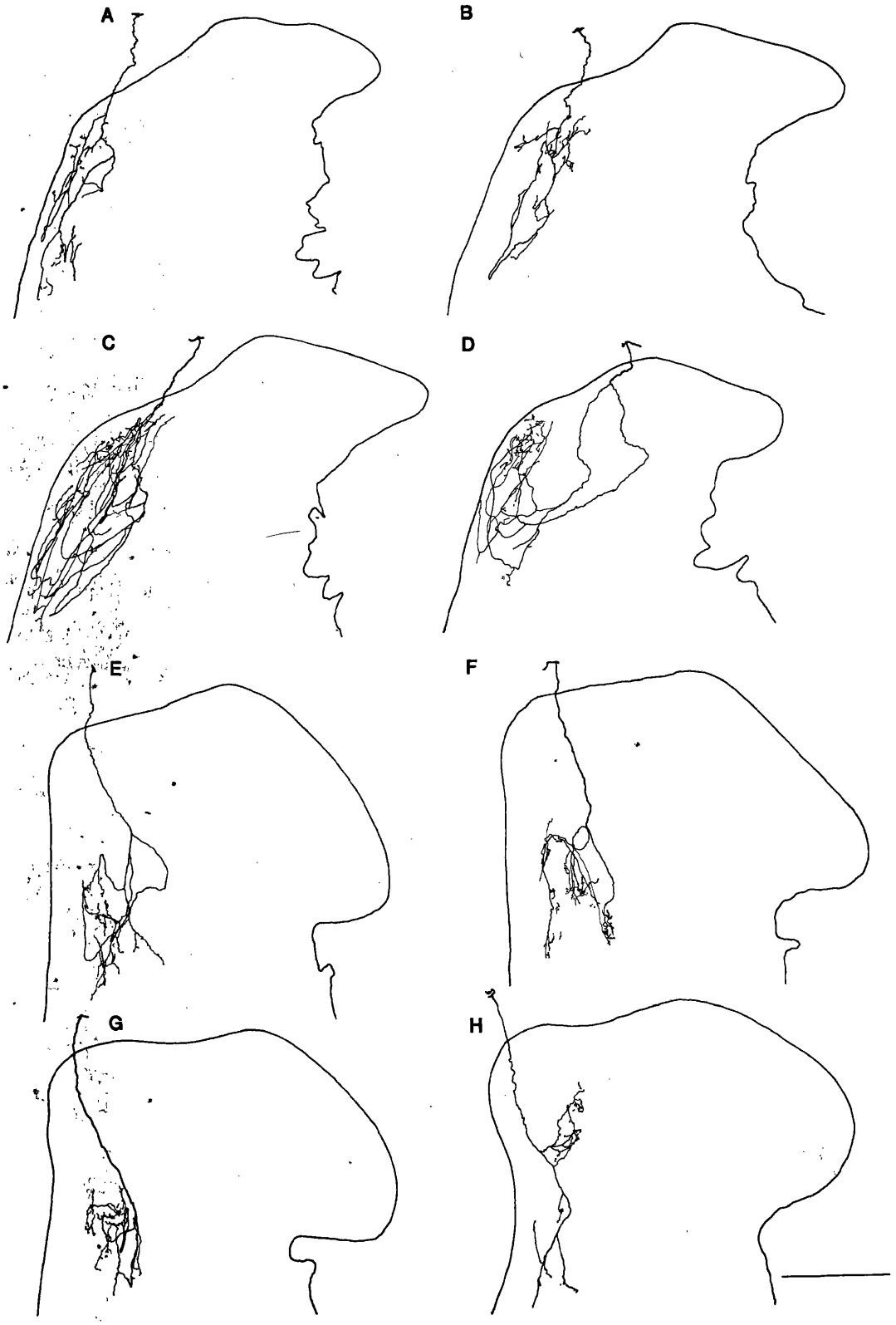
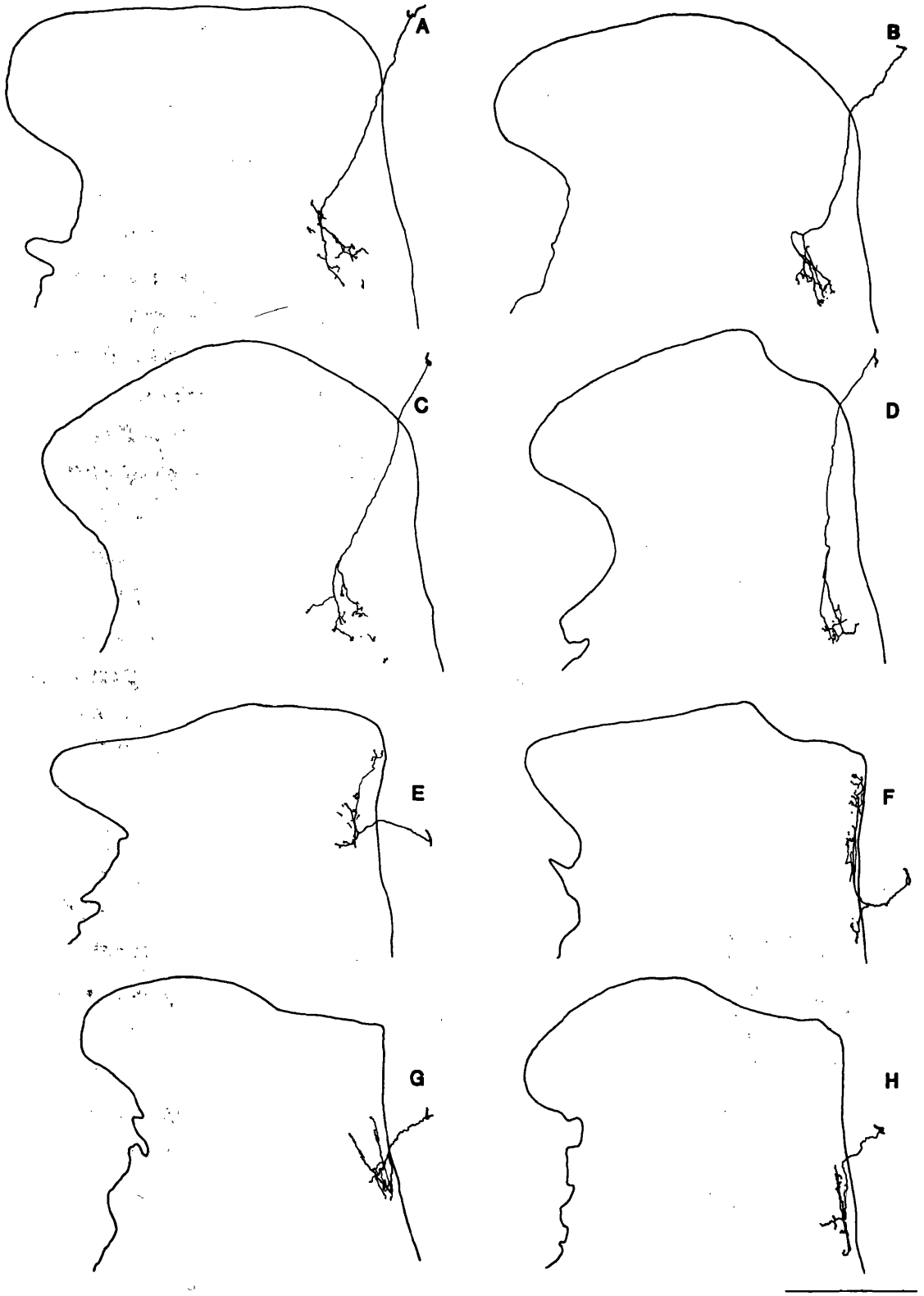

A
B
C
D
E
F
G
H

FIGURE 33: Examples of camera lucida reconstructions of simple arborizations from toe (A-D) and paw pad (E-H) RA afferents. Scale bar 250um.



curved round from the lateral side of the dorsal horn to produce an arborization close to the medial edge of the dorsal horn (Figs. 29G-H, 30B,D, 31C, 32D), while in a few the terminal branches were issued from the descending collateral branch (Figs. 30C,31E,F, 32H).

The dorsoventral distribution of the terminal arbors also differed from those of the paw pad afferents. The majority of the terminals were located deeper in the dorsal horn in, laminae IV-V, but some terminals were located in laminae III and occassionally in lamina IIi (Figs. 29F,G, 32D, Plate 5). The terminal arbors also had quite a restricted mediolateral width, the boutons being restricted to the medial 1/5-1/3 of the dorsal horn (Fig. 24a). Analysis of the dimensions of toe complex and simple arbors showed that there were differences between the two types: complex arbors being dimensionally larger overall but the differences were not as marked as in paw pad afferents and this was reflected by the volume of dorsal horn occupied by each (Table 6).

For 4/8 of the toe RA afferents there was some terminal overlap between adjacent complex arborizations but this was restricted to a maximum of 3 adjacent collaterals in one case and more commonly only involved 2 adjacent arbors. This may account for the more varied morphology of the terminal arborization of toe RA afferents.

2.3.3 (iv) TOE RA AFFERENT SOMATOTOPIC ORGANIZATION.

Figure 24a shows the plan views of the terminal arborization of toe afferents with peripheral RF's on the toes as shown. The terminals were distributed in the medial 1/3 of the dorsal horn from mid-L4 to caudal-L5 with the majority of afferent terminals present in the medial 1/4 of L5. Again, as with paw pads afferents, there was central overlap of complex terminals between afferents with adjacent and non-adjacent peripheral RF's. There did not, however, appear to be any topographic

organization of the central terminals of toe afferents so that there was no rostral to caudal progression in the spinal cord of toes 1-5 (as seen in HFA's).

2.3.3 (v) COMPARISONS BETWEEN PAW PAD AND TOE RA AFFERENT TERMINALS.

Comparison of the arrangement of collaterals of paw pad and toe RA afferents (Table 5) showed that paw pad RA's have more collaterals per afferent than toe afferents but that when the number of complex, simple and blind-ending collaterals were analyzed then the percentages were similar for the two groups, with toe afferents having a slightly higher percentage of complex and blind-ending collaterals than paw pad afferents. Paw pad afferents were on average 0.6 mm longer from most rostral to caudal collateral than toe afferents but had a slightly shorter intercollateral distance (perhaps not surprising when they have more collaterals per afferent than toe afferents).

Inspection of Figs. 25-32 gave the impression that the paw pad complex arborizations were larger than the toe complex arborizations and that paw pad simple arborizations appeared thinner than toe simple arborizations (Fig. 33). Analysis of the dimensions of the complex and simple arborizations (Table 6) revealed several interesting features. The mediolateral width of paw pad complex arbors were significantly wider than those of toe complex arbors and conversely toe simple arbors were wider than paw pad simple arbors ($P<0.05$ unpaired t-test) but that the overall width of terminal arborizations of paw pad and toe arbors was almost identical. The overall dorsoventral length of terminal arborizations of toe afferents was significantly longer than that of paw pads as was that for simple arbors, but not when only complex arbors were compared. Only the rostrocaudal length of paw pad complex arborizations were significantly longer than that of toe RA complex arborizations ($P<0.05$ unpaired t-test). The volume of

TABLE 5

ARRANGEMENT OF THE COLLATERAL BRANCHES OF RA AFFERENTS.

	PAW PADS	TOES
No. afferents	6	8
Total no. collaterals	78	85
No. collaterals/ afferent: total	13.0±1.53	10.6±0.49
complex	5.0±1.08	4.1±0.53
simple	4.3±1.08	2.9±0.74
blind-ending	3.7±0.80	3.4±0.53
intercollateral distance (um)	290±23	307±26
length from most rostral to caudal collateral (mm)	3.48±0.21	2.89±0.25
projection to II_i	8/30	5/35

x ±SEM

TABLE 6 DIMENSIONS OF THE TERMINAL ARBORIZATIONS OF RAPIDLY ADAPTING (RA) AFFERENTS.

	PAW PAD RA			TOE RA		
	C	S	C+S	C	S	C+S
N	30	26	56	35	23	58
ML (um)	127±11*	43±4*	88±8	99±6*	69±10*	87±6
DV (um)	210±13	100±8*	155±11*	259±14	157±24*	224±17*
RC (um)	260±15*	160±13	214±12	210±14*	189±15	202±11
VOL(ul) (X10^{-3})	7.3±0.2	0.7±0.5*	4.2±0.6	6.7±1.4	3.3±1.1*	5.4±1.0

X ± SEM
* = signif. diff. P<0.05 level
unpaired t-test.
ML=mediolateral, DV=dorsoventral, RC=rostrocaudal,
VOL=volume.
C=complex, S=simple, C+S=terminal

Comparisons made were: C v C, S v S, C+S v C+S,

cord occupied by complex paw pad arbors was slightly greater than that of toe complex arbors but the simple arbors were significantly smaller in volume compared to toe simple arborizations. However, analysis of the average dimensions /afferent (Appendix II, Table 6A) showed only significant differences ($P<0.05$) for the simple arbors of paw pad and toe RA afferents.

Superimposition of Figs. 24a and b clearly conveys the fact that afferents with adjacent and non-adjacent peripheral receptive fields have central terminals which overlap in the medial dorsal horn of L4-L5. If the paw pad and toe afferents are considered purely as a homogenous population of RA afferents, then there is no topographic organization of peripheral RF's of the different parts of the glabrous skin of the foot within the central terminal area of the tibial nerve in the dorsal horn.

2.3.4 SLOWLY ADAPTING TYPE 1 MECHANORECEPTORS.

After histological processing the central terminals of 6 SA type-1 mechanoreceptors, four innervating glabrous skin and two innervating hairy skin, were recovered (Plate 6). SAI afferents had a wide range in the laminar distribution of terminal boutons with boutons of hairy SAI arbors generally being located more superficially than those of glabrous SAI arbors. There was also a large variation in the mediolateral width of individual arbors within a single afferent. Like HFA's and RA's, SAI afferents exhibited complex, simple and blind-ending collaterals. Examples of the complete rostrocaudal extent of the collateral arborizations of a hairy SAI and a glabrous SAI afferent are shown in Figs. 34 and 35.

2.3.4 (i) MORPHOLOGY OF HAIRY SAI AFFERENT TERMINALS.

Hairy SAI arbors generally had a "flame-shaped"

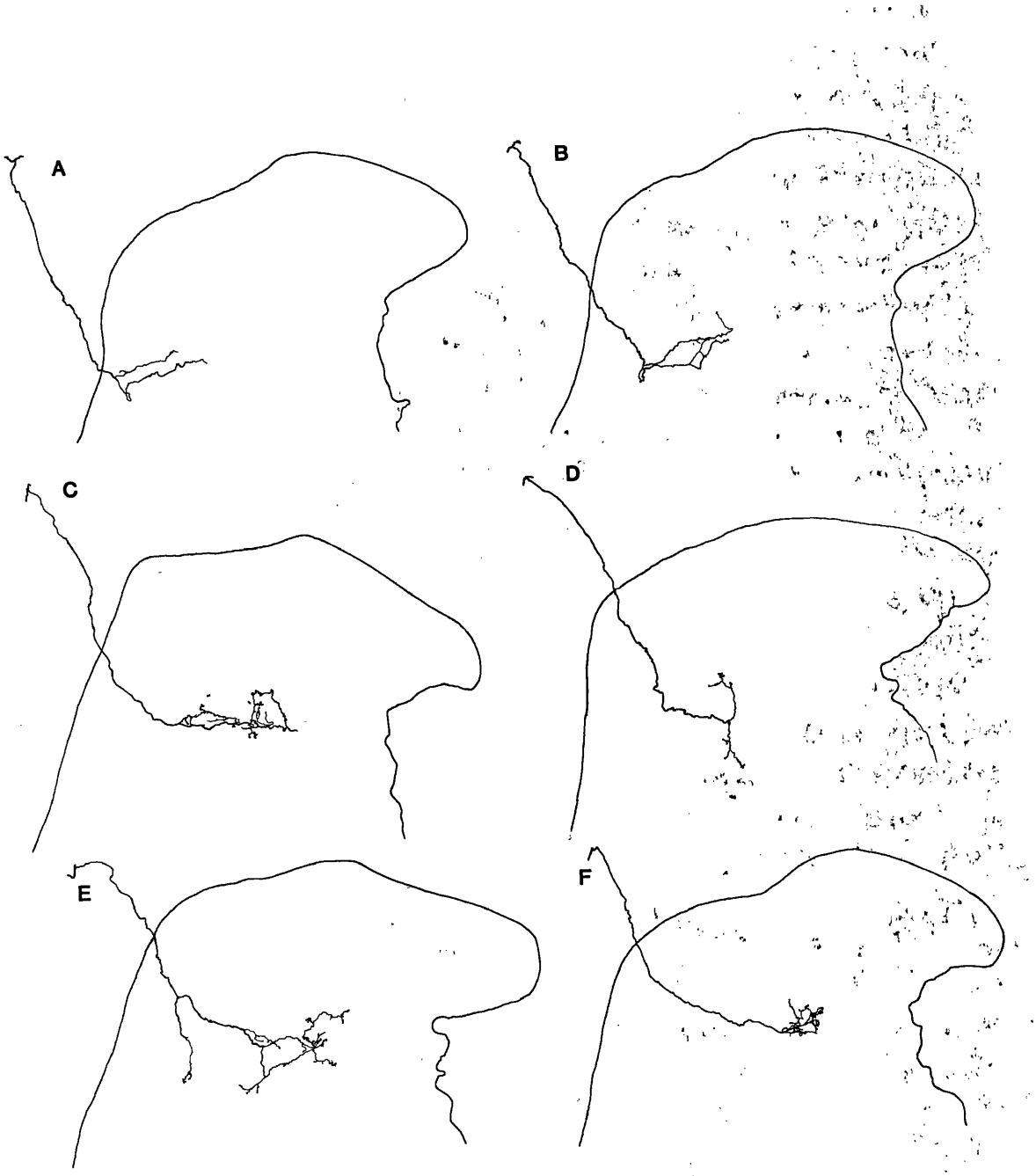

A
B
C
D
E
F

FIGURE 34: Camera lucida reconstructions of all the collateral arborizations from rostral (A) to caudal (L) of a slowly adapting type I (SA-I) afferent whose receptive field was located on the medial side of the shin. Scale bar 250um.

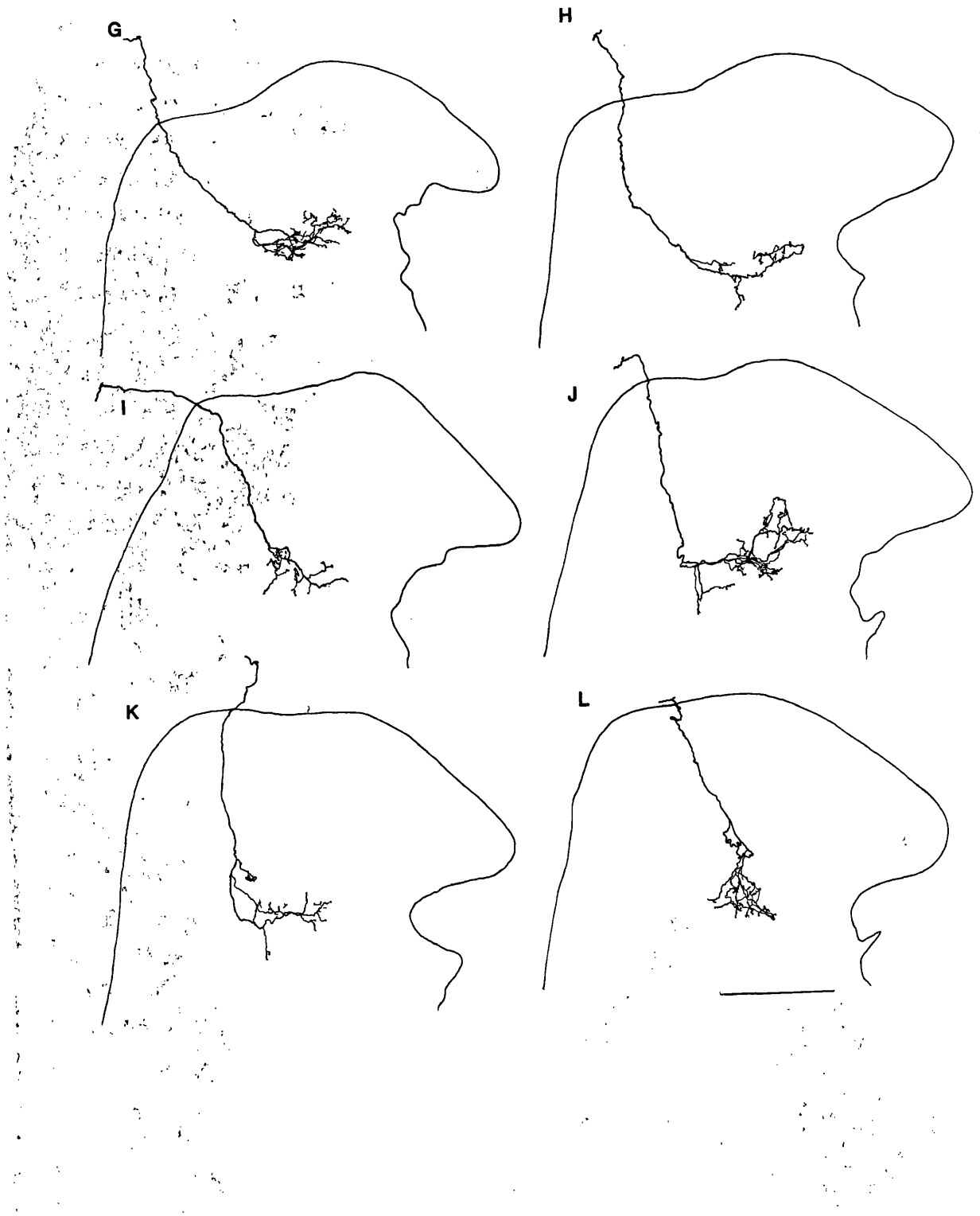


PLATE 6.

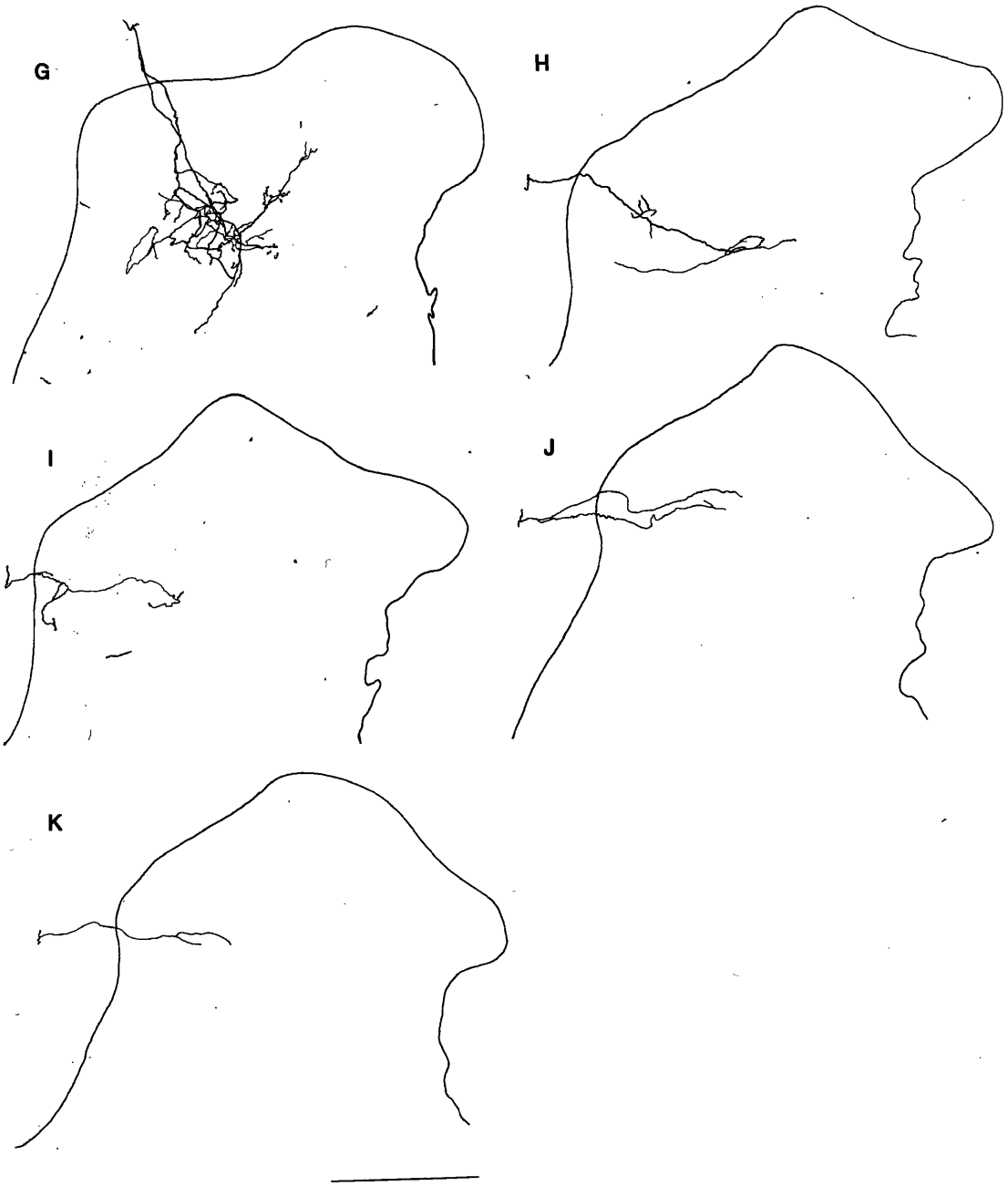
Photomicrographs of 50um thick transverse sections of lumbar spinal cord. A illustrates part of the complex collateral reconstructed in Fig. 34E; B shows 2 collaterals from different functional classes of primary afferents with different peripheral RF's. The left hand arrow points towards the stem axon of a SAI afferent whose terminal arbor is issued from the left hand collateral branch (and is reconstruced in Fig. 36C); the right hand arrow points towards the stem axon of a saphenous HFA whose terminal arbor appears above that of the SAI afferent in the grey matter. Note that this is an example of afferents with different RF's having overlapping central terminal fields. The dashed lines represent the outline of the dorsal horn. Scale bar 100um.

appearance although this shape was not always as apparent as in HFA's. In one afferent innervating the medial skin (Fig. 34), some of the terminal arborizations showed a loose flame-shape (Fig. 34C,G) while others showed a resemblance to the L-shaped collateral arborizations of SAI afferents seen in the cat (Fig. 34J-K). However, some arbors (Fig. 34D,E,H,I,K,L) clearly departed from this pattern, having ventrally directed terminal branches and boutons. There was no overlap between adjacent complex arbors in this afferent. The other hairy SAI afferent had a receptive field with physiological characteristics which showed a rapidly adapting as well as a slowly adapting component was involved in response to hair movement. Examples of 6 complex collateral arborizations from this afferent are shown in Fig. 36. The morphology of these arborizations were quite unlike those of the first hairy SAI afferent. There was no U or L-shaped curving collaterals as in the first SAI afferent, rather the collateral branch dived ventrally into the grey matter where upon it branched to arborize in laminae IIi-IV. The morphology of adjacent collateral arborizations did not show any consistent features in this afferent. However, there was overlap between some adjacent complex arbors in this afferent, (5/8) but they did not form the continuous overlapping rostrocaudal terminal sheet seen in HFA's.

2.3.4 (ii) MORPHOLOGY OF GLABROUS SAI AFFERENT TERMINALS.

The morphology of glabrous SAI arborizations was clearly different from the hairy SAI arbors (comparison of Figs. 34 and 35). The most striking observation was that the morphology of glabrous SAI afferent collaterals were much simpler in branching pattern and had fewer boutons in an arbor compared to collaterals of hairy SAI afferents. There were no consistent features between adjacent SAI-glabrous arborizations such as those seen in the hairy SAI's. The complex collateral arborizations of some glabrous SAI's had very wide mediolateral dimensions with

FIGURE 35: Camera lucida reconstructions of all the collateral arborizations from rostral (A) to caudal (K) of a SA-I afferent whose receptive field was located on the lateral surface of the paw pad adjacent to toe 3. Scale bar 250um.



boutons distributed to the more superficial laminae (III-IV, Fig. 35D-G), whilst complex arborizations from others had a more restricted mediolateral extension and terminated deeper in the dorsal horn (laminae IV-VI, Fig. 37), resembling RA arborizations in morphology rather than SAI arbors. Only in 1 case (Fig. 38 RF5) was any overlap between adjacent complex terminal fields seen in glabrous SAI afferents.

2.3.4 (iii) COMPARISON OF HAIRY AND GLABROUS SAI AFFERENT TERMINALS.

Inspection of Figs. 34-37 conveys the impression that glabrous SAI arbors were wider than hairy SAI arbors. Analysis of the pooled dimensions of the simple and complex arborizations revealed several interesting features (Table 8). Glabrous SAI complex arbors were indeed wider and deeper but not longer than their hairy counterparts, but this was not significantly different for any dimension measured ($P<0.05$ unpaired t-test). Glabrous SAI complex arbors occupied a greater volume of cord than hairy SAI arbors. When the simple arbors were compared, then the hairy SAI arbors were significantly wider but not longer or deeper than their glabrous counterparts ($P<0.05$ unpaired t-test). They were also significantly larger in the volume of cord occupied. This difference may be explained in the shape of the arborizations: Hairy SAI simple arbors have a flame-shaped appearance compared to the mediolaterally restricted "RA -type" appearance of the glabrous SAI simple arbors. When the dimensions of all the terminal arborizations (complex and simple) were compared, only the rostrocaudal lengths of SAI-hairy and glabrous arbors were significantly different ($P<0.05$ unpaired t-test). However, when the average dimensions /afferent were compared there were no significant differences ($P<0.05$) for any dimension measured (Appendix II, Table 8A).

The above observations may be correlated with the

FIGURE 36: Camera lucida reconstructions of six adjacent complex arborizations from rostral (A) to caudal (F) of a SA-I afferent whose receptive field was located on the dorsomedial surface of the foot. Scale bar 250um.

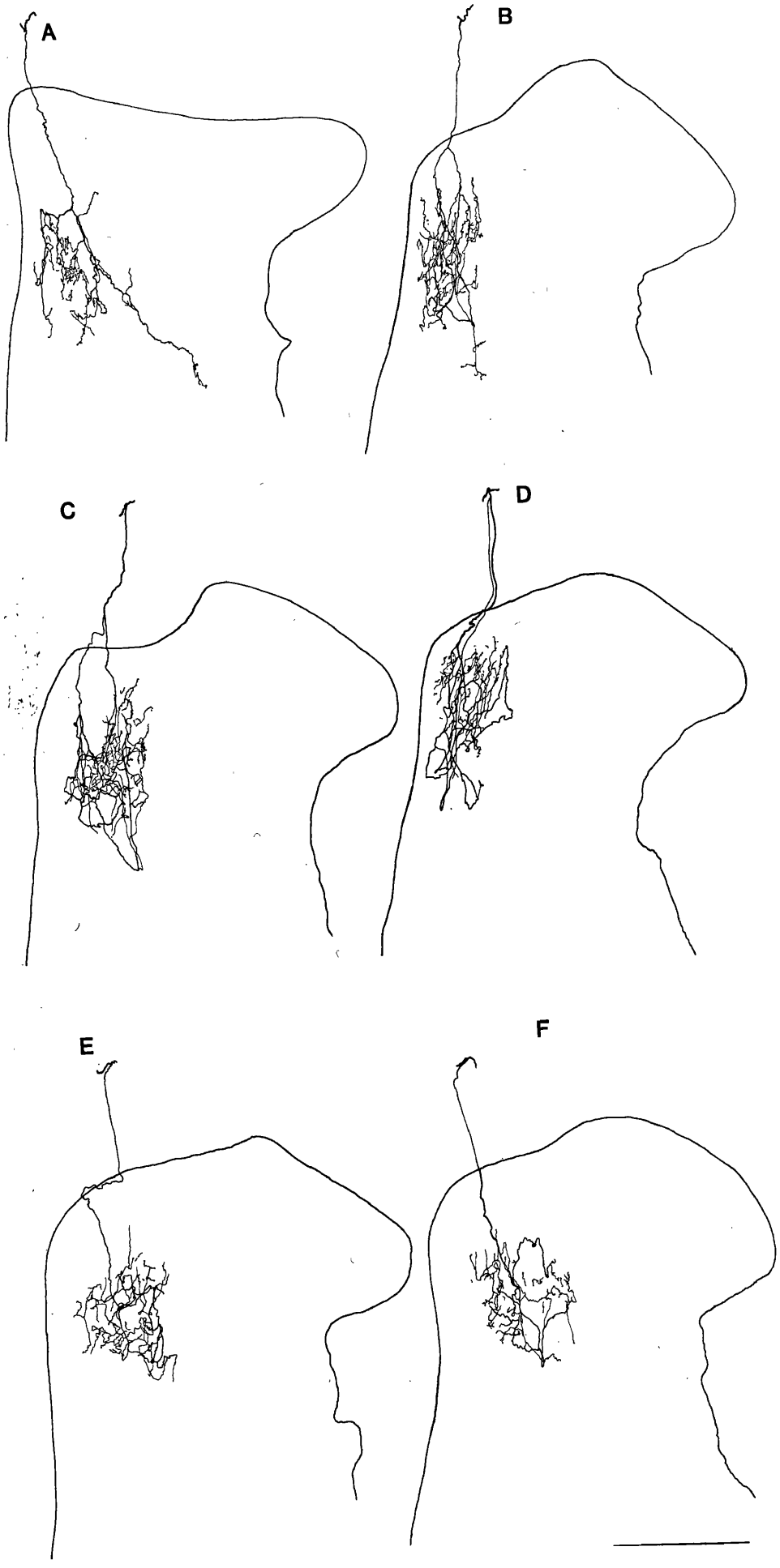

A
B
C
D
E
F

FIGURE 37: Camera lucida reconstructions of six adjacent complex arborizations from rostral (A) to caudal (F) of SA-I afferent whose receptive field was located on the lateral glabrous surface of the foot. Scale bar 250um.

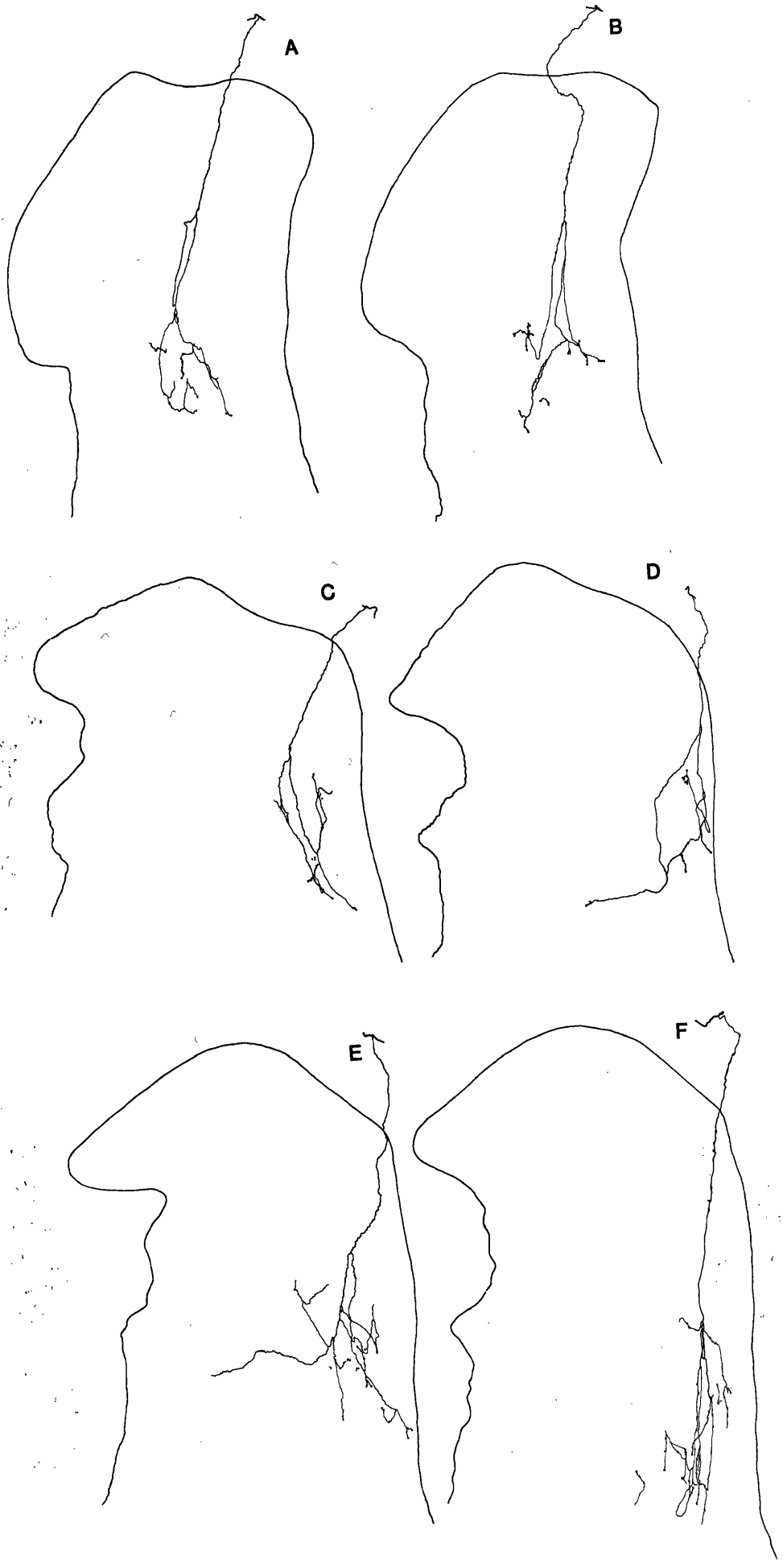

A
B
C
D
E
F

TABLE 7

ARRANGEMENT OF THE COLLATERAL BRANCHES OF SLOWLY ADAPTING MECHANORECEPTORS.

	HAIRY SAI	GLABROUS SAI
No. afferents	2	4
Total no. collaterals	25	37
No. collaterals/ afferent: total	12.50±0.50	9.25±1.71
complex	6.00±1.99 (48%)	3.25±0.95 (35%)
simple	5.00±1.99 (40%)	2.50±0.29 (27%)
blind-ending	1.50±0.50 (12%)	3.50±0.87 (38%)
intercollateral distance (um)	376±63	319±23
length from most rostral to caudal collateral (mm)	4.33±0.78	2.58±0.35
projection to II_i	2/12 (17%)	0
length of overlapping terminal sheet (um)	525±25 (n=2)	250 (n=1)

x ±SEM

finding that the intercollateral spacing of arborizations was greater for hairy SAI afferents compared to glabrous SAI afferents (Table 7) but is more likely to be due to the general observation made by Brown (1981) that afferents with arbors in the medial dorsal horn have collaterals which are more closely spaced than those afferents with terminal arborizations in the lateral dorsal horn.

Comparisons between hairy and glabrous SAI afferents in the arrangement of collaterals (Table 7) shows that hairy SAI afferents have a greater proportion of complex and simple terminal arbors and a smaller proportion of blind-ending collaterals compared to glabrous SAI afferents. However, the small population under analysis means that these particular results should be treated with caution.

2.3.4 (iv) SOMATOTOPIC ORGANIZATION OF SAI AFFERENT TERMINALS.

The somatotopic organization of the central terminals of the 6 SAI afferents in the dorsal horn is shown in Fig. 38. The afferents projected to the area of spinal cord expected from their receptive field position in a given peripheral nerve territory (Fig. 14). The medial dorsal foot afferent (Saphenous nerve innervated) is located rostrally in L2-L3, the medial shin (innervated also by the saphenous nerve) is represented in the middle to lateral third of the dorsal horn throughout L3 with some extension caudally into the adjacent superficial peroneal nerve territory. The afferents innervating the glabrous skin (tibial nerve) were all located caudal to the SAI-hairy afferents and were located in the medial half of the L4-L5. There was some central terminal overlap of afferents with non-adjacent peripheral RF's within a given nerve territory. A simple pattern for glabrous SAI afferents in the rostrocaudal direction emerged with the toes represented rostral to the paw pads which were

FIGURE 38: A plan view through lamina III of the somatotopic organization of the central bouton containing arbors of SA-I afferents with receptive fields as shown. Abbreviations as in Fig. 15.

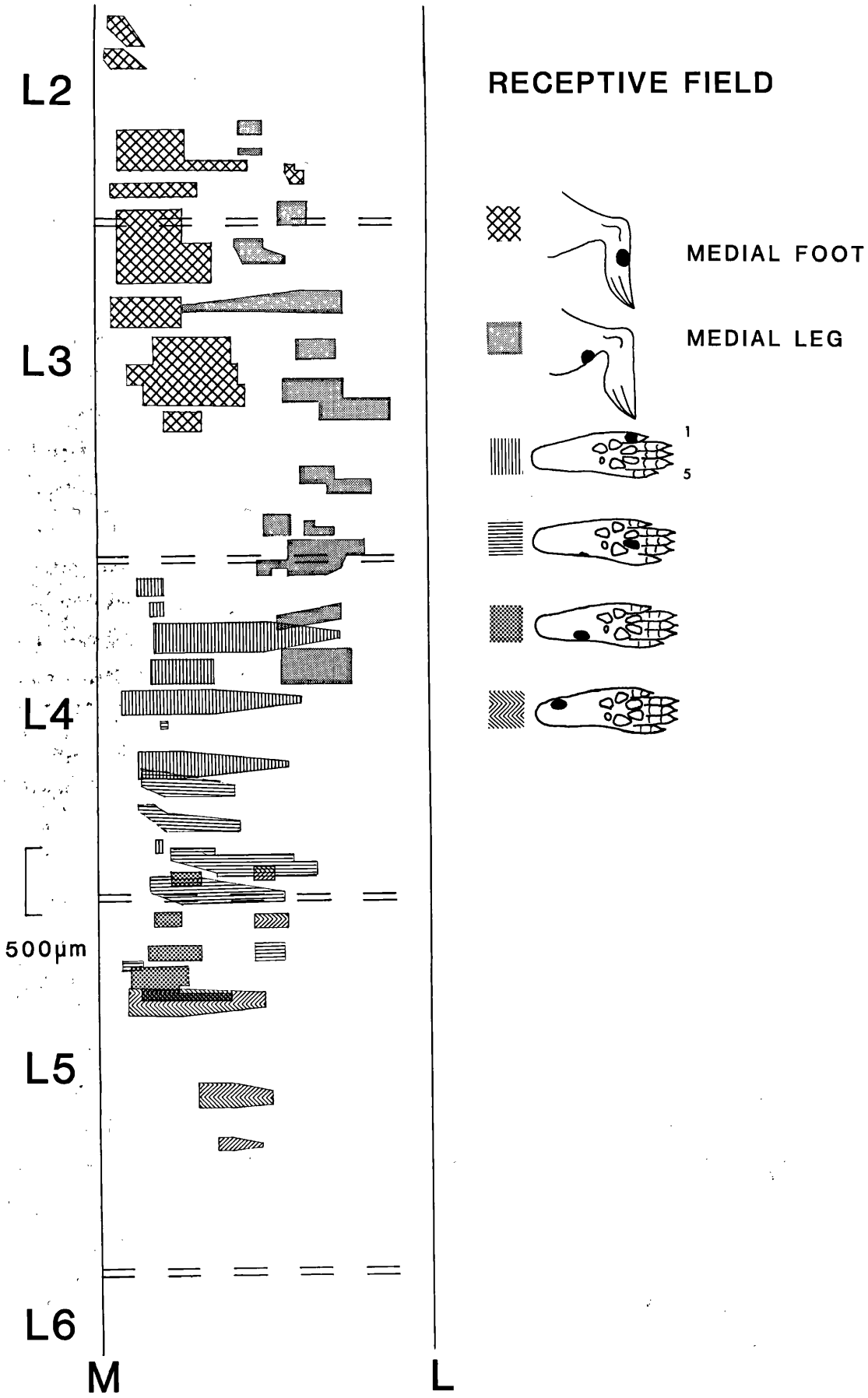

L2
L3
L4
L5
L6
500µm
M
L
RECEPTIVE FIELD
MEDIAL FOOT
MEDIAL LEG
1
5

TABLE 8 DIMENSIONS OF THE TERMINAL ARBORIZATIONS OF SLOWLY ADAPTING TYPE-I AFFERENTS.

	HAIRY SAI			GLABROUS SAI		
	C	S	C+S	C	S	C+S
N	12	10	22	13	10	23
ML (um)	156±16	112±28*	136±16	214±27	41±6*	139±24
DV (um)	204±27	129±15	170±18	270±13	84±18	189±28
RC (um)	254±27	220±24	239±12*	200±11	160±21	183±12*
VOL(ul) (X10^{-3})	8.9±2.5	3.4±0.5*	6.1±1.3	10.8±1.9	0.8±0.1*	6.5±1.5

X ± SEM
* = signif. diff. P<0.05 level
unpaired t-test.
ML=mediolateral, DV=dorsoventral, RC=rostrocaudal, VOL= volume.
C=complex, S=simple, C+S=terminal

rostral to the glabrous foot afferent which was, in turn, rostral to the glabrous heel afferent. However, this must be treated with some caution since there were only a small number of afferents represented.

2.3.5 COMPARISON OF LOW THRESHOLD HFA, RA AND SAI PRIMARY AFFERENTS.

In the rat, different functional classes of low threshold cutaneous mechanoreceptors have certain features in common and other features specific to their own class. Features in common included possession of complex, simple and blind-ending collateral arborizations (Table 9), although the percentages of the different types were different for each class of afferent. Also the terminals of all low threshold afferents were largely restricted to laminae III-IV and each class of afferent contained complex arbors with boutons in lamina IIi, although this was most common for HFA's but rarer in SAI's and RA arborizations.

Each type of afferent unit had its own characteristic axon collaterals, although the morphology of individual arborizations varied. Hair follicle afferents terminal arborizations had mainly "flame-shaped" arbors although toe HFA arbors departed from this shape somewhat (Figs. 7-12). The morphology of the individual arborizations of both RA and SAI afferents varied considerably amongst each other so that adjacent arbors had, in some cases, quite a different appearance. This occurred usually when the adjacent arbors were non-overlapping; adjacent overlapping RA and SAI arbors tended to have a similar morphology (Figs. 25-37).

The intercollateral spacing along a cutaneous axon depended more on the mediolateral position of its arbors in the dorsal horn than on the type of afferent unit. Hence, RA's had the shortest intercollateral distance in the dorsal horn while HFA's and SAI's had similar distances (Table 9) although the intercollateral distances

TABLE 9 THE ARRANGEMENT OF COLLATERALS OF LOW THRESHOLD PRIMARY AFFERENTS IN THE DORSAL HORN OF THE SPINAL CORD.

	HFA	RA	SAI
No. afferents	38	14	6
Total no. collaterals	351	163	62
No. collaterals/ afferent: total	9.24±0.51	11.64±0.78	10.33±0.88
complex	4.31±0.27 (47.3%)	4.57±0.64 (39.9%)	4.17±0.83 (40.3%)
simple	1.42±0.22 (15.4%)	3.50±0.64 (30.1%)	3.33±0.76 (32.3%)
blind-ending	3.48±0.30 (37.3%)	3.50±0.44 (30.0%)	2.83±0.70 (27.4%)
intercollateral distance (um)	343±11	296±15	343±24
length from most rostral to caudal collateral (mm) X±SEM	2.94±0.15	3.07±0.20	3.16±0.48

for medially placed HFA's were shorter than for laterally placed ones (Table 1). For SAI-glabrous afferents, the intercollateral distances were shorter than SAI-hairy afferent distances (Table 7).

As morphological criteria alone were sometimes insufficient to distinguish between different functional classes of afferent (for example a RA afferent and a SAI-glabrous afferent), analysis of the dimensions and arrangements of collaterals provided quantitative differences (Tables 9, 10). HFA's possessed the fewest collaterals per afferent, shortest length from rostral to caudal collateral, smallest mediolateral and dorsoventral lengths and smallest volume of cord occupied while RA's had the highest number of collaterals per afferent, shortest intercollateral spacing but were intermediate between HFA's and SAI's in length of afferent from rostral to caudal collateral, mediolateral dimensions and volume of cord occupied. SAI arbors had, on average, the largest mediolateral and dorsoventral dimensions and volume of cord occupied, longest distance from rostral to caudal collateral. Density of boutons and amount of overlap between adjacent arbors also provided differences between the HFA's RA's and SAI's: HFA's had the greatest density of boutons and showed the most overlap in the rostrocaudal direction between adjacent complex arbors, while SAI glabrous arbors had the least boutons. Also adjacent RA and SAI afferent collaterals overlapped rarely.

2.4 DISCUSSION.

2.4 (i) MORPHOLOGY OF DIFFERENT TYPES OF HAIR FOLLICLE AFFERENTS.

The morphology of individual HFA's innervating the hindlimb has been investigated in detail at the light microscopic level in the rat (Woolf, 1987), and cat (Brown et al., 1977; Brown, 1981) and monkey (Light and Perl,

TABLE 10 DIMENSIONS OF TERMINAL ARBORIZATIONS OF DIFFERENT TYPES OF PRIMARY AFFERENTS.

	HFA's			RA's			SAI's		
	C	S	C+S	C	S	C+S	C	S	C+S
N	116	54	220	65	49	114	25	20	45
ML (um)	76±3	45±9	69±3	108±6	55±7	84±5	186±22	77±18	138±20
DV (um)	158±7	86±14	143±13	245±13	127±16	181±13	238±30	107±17	180±22
RC (um)	294±11	155±18	263±9	232±11	174±14	207±8	229±19	190±23	210±12
VOL ($x10^{-3}$)	4.3±1.1	1.0±0.7	3.5±1.0	7.0±0.8	1.9±0.8	4.8±0.7	9.9±2.0	2.1±0.3	6.4±1.5

x±SEM

ML=mediolateral, DV=dorsoventral, RC=rostrocaudal,

VOL=volume, C=complex, S=simple, C+S=terminal.

1979b; Ralston et al., 1984) and at the E.M. level in the cat (Maxwell et al., 1982; Rethelyi et al., 1982; Ralston et al., 1984; Maxwell and Rethelyi, 1987). The results of this study confirmed previous observations that HFA's innervating the thigh and calf skin formed narrow sagittal sheets of flame-shaped arbors extending from laminae IIi-IV in the dorsal horn as originally described in Golgi studies by Scheibel and Scheibel (1968). Hair follicle afferents innervating the foot and toes have not been previously studied in the rat, although they have been intraaxonally stained in the cat (Meyers and Snow, 1984; Snow and Wilson 1988; Wilson and Snow, 1988b). They have broader terminal arbors with a more diffuse arrangement of terminal branches than other HFA's and toe afferents occasionally depart from the flame-shape whereas dorsal foot afferents do not. Hair follicle afferents which terminated in the medial and middle areas of the dorsal horn were significantly wider than those that terminate in the lateral part of the dorsal horn (Table 2, Fig. 13). Medial leg and dorsal foot arbors were about 50% wider while toe arbors were almost twice as wide as lateral leg terminal arbors and occupied up to 20% of the width of the dorsal horn. These wider medially located arbors have been seen in the cat (Brown et al., 1977; Brown et al.,1988) although the authors do not mention (or attach any significance to) the mediolateral differences.

There are several possible explanations for this. Firstly, there may be more room on the medial side of the dorsal horn for the afferents to terminate than laterally. Embryonically, the organization of the afferents can be interpreted as a reflection of the proximodistal gradient of the limb bud (Wall 1960). According to this theory, afferents on the proximal limb develop first and project laterally whereas those on the distal limb develop later and terminate medially (Benowitz et al., 1989). This may occur as the dorsal horn neuronal population develops from lateral to medial so that perhaps those afferents which first arrive are more tightly packed in the lateral dorsal

horn while the medial side develops later, possibly leaving more room for medially directed afferents.

Another possibility is that the medial side of the dorsal horn contains penetrating muscle (Brown and Fyffe, 1978, 1979; Hongo et al., 1987), joint (Craig et al., 1988) and skin afferents (Devor et al., 1986; Woolf, 1987) from the dorsal columns and as these pass through the medial dorsal horn, they physically push the arbor apart. A possible resulting effect of this could be that a sensory coding function for stimulus discrimination may be employed in the dorsal horn so that those areas of peripheral skin which need to be able to discriminate two closely opposed stimuli can do so within the dorsal horn by having a larger central terminal representation. The foot and toes, which have a small external area compared to the rest of the hindlimb, have a very large termination area, occupying the medial half of L4 and L5 in the dorsal horn, probably owing to the high afferent innervation density of the toes. A similar observation has been made in the cortex where specialised sensory areas such as the vibrissae barrel fields, forelimb, hindlimb have enlarged representations (Killackey et al., 1978; Sheperd, 1988).

Hair follicle afferents with a peripheral RF crossing the hairy-glabrous border have not been reported before in either cat or rat. However, the peripheral skin innervation of the cat paw is different from that of the rat. Glabrous skin is only present on the central paw pad and toe pads whereas the whole of the volar aspect of the rat paw is glabrous. Therefore, these glabrous-hairy afferents may represent a minor subpopulation of HFA's in the cat skin (if they are present at all). The split peripheral RF was also represented centrally as a split terminal field which crossed the boundary between the glabrous and hairy skin central representations. For the complex arbors of these glabrous-hairy afferents, this was seen as a dense broad terminal arbor which occupied up to 15% of the mediolateral extent of the dorsal horn, with the majority of boutons terminating in laminae IIi-III and

a smaller, simpler ventromedial terminal projection near the medial grey matter border in laminae IV-V. This ventromedial projection terminates in the area which, in L4/5 of the dorsal horn, receives input from glabrous cutaneous receptors (Woolf and Fitzgerald, 1986; Woolf, 1987; Shortland et al., 1989b).

All the afferents recovered in this study bifurcated into rostrally ascending and caudally descending stem axon branches from which collaterals were issued. This is in accordance with the findings of Rethelyi and Szentagothai (1973) and Hammano et al., (1978) but conflicts with those of Brown et al., (1977). Brown et al., (1977) reported that about 2/3 of the population of HFA's recovered in their study only had a rostrally ascending stem axon; Meyers and Snow (1984) found 37% (3/8) of their HFA's only projected rostrally. Both studies were performed in the cat. In the rat, Woolf (1987) found that 1/6 (17%) of the HFA's failed to bifurate. Non-bifurcating HFA's have not been reported in the monkey (Light and Perl, 1979b; Ralston et al., 1984). The presence of non-bifurcating HFA's may be a species specific property and is characteristic of cat HFA's but in the rat and monkey they are very rare. Electrophysiological experiments have shown that cutaneous afferents may project for long distances caudally in the spinal cord (Wall and Werman, 1976; Pfaller and Arvidsson, 1988; Wall and Shortland, unpublished observations) and this has also been confirmed by degeneration techniques (Imai and Kusama, 1969).

A characteristic feature of the majority of HFA collaterals was the flame-shaped appearance of the arbor in transverse sections (Brown et al., 1977; Woolf, 1987; Shortland et al., 1989a). Although the morphology is similar between different species, the dimensions and laminar projections do vary and this is not merely to do with the size of the animals. The mediolateral dimensions of rat HFA's were smaller than that of the cat (Woolf, 1987) and there were occassional terminal invasions of

lamina IIi by arborizations in the cat and monkey (Brown et al., 1977; Light and Perl, 1979b; Rethelyi et al., 1982; Ralston et al., 1984).

Thirty two percent of the complex arborizations in this study entered lamina IIi. This agrees with the developmental results of Beal et al., (1988) and with the intraaxonal studies of Woolf,(1987) and Shortland et al., (1989a) who found 44% and 36% respectively of complex arbors projected to lamina IIi. In addition, Shortland et al., (1989a) found that lateral leg HFA's had a higher incidence of invasion into lamina IIi compared with other peripheral skin regions. These arbors that enter lamina IIi were similar to the type-II HFA collaterals described developmentally by Beal et al., (1988) which are derived from G, T and D hair afferents. The morphology of these type-II HFA's (Beal et al., 1988) also closely matched the intracellular descriptions of D hair afferents described in the cat and monkey (Light and Perl, 1979b) which also terminated in lamina IIi, while the description of Beal et al's., (1988) type-1 HFA collaterals were similar to those of G and T hair afferents described by Brown (1981).

Complex arbors never entered laminae II_o or I (although I collateral out of 351 had a blind-ending terminal branch projecting to lamina I). Ths is an important point because it is at variance with the early results from the Golgi studies in neonatal dog and kitten by Ramon y Cajal (1909) and Scheibel and Scheibel (1968). The results from those neonatal Golgi studies disagree with the descriptions of HFA arborizations observed in the adult following iontophoretic or topical application of HRP. In the adult, HFA's demonstrated with HRP produce a single longitudinal column (lobule) and do not distribute terminals to adjacent lobules. Using the Golgi technique in newborn animals, Cajal (1909) and the Scheibel's (1968) showed HFA's which entered and arborized in more than one lobule and also penetrated throughout lamina II (Scheibel and Scheibel, 1968). Some were seen to extend

into the overlying white matter (Cajal, 1909), while in the adult HFA terminals were confined to lamina IIi. These discrepancies suggested that there may be a temporary expansion or overgrowth of HFA terminal fields in the immature animal which subsequently receded as the animal attains adulthood. Carefully timed Golgi studies of the development of HFA's in the rat (Beal et al., 1988) showed that HFA's grow into the dorsal horn in a ventrodorsal manner, often following the path of vasculature and reach their final termination site, lamina IIi or III, (depending on the type of HFA collateral) by postnatal day 5. In addition, there was no distribution of HFA's to more than one lobule. Beal et al., (1988) suggest that the discrepancies seen by Cajal (1909) and Scheibel and Scheibel (1968) may be species differences or the result of misinterpretations of "certain regularly occuring phenomenon" (Beal et al. 1988).

The terminals of HFA's were distributed in longitudinal sheets extending from laminae IIi-IV in the dorsal horn. The arrangement of these arbors can be closely correlated with the connectivity pattern of the fan shaped arbors of spinocervical tract neurons as described by Brown (1981) and Brown and Noble (1982). Electrophysiological experiments have also suggested that HFA's constitute the only monosynaptic input to these neurons (Brown, 1981; Brown et al., 1987a) while other electrophysiological experiments have demonstrated a polysynaptic input of Group III HFA's (A-delta) onto SCT cells (Brown et al., 1987b,c). HFA's have also been shown to terminate on the dentritic trees of postsynaptic dorsal column neurons which are also found in laminae III-V (Maxwell et al., 1985).

The results of the present study on the morphology of cutaneous HFA's in the lumbar spinal cord of the rat showed there were no contralateral projections to the dorsal horn. Evidence for cutaneous primary afferent fibres crossing to the contralateral dorsal horn has been shown by degeneration techniques (Culberson et al., 1979;

Ritz et al., 1985), Golgi impregnation (Scheibel and Scheibel, 1968; Hamano et al., 1978), anterograde bulk labelling (Rethelyi et al., 1979; Light and Perl, 1979a,b; Matushita and Tanami, 1983; Smith, 1983; Nunez et al., 1986), electrophysiological techniques (Smith, 1986) and by intracellular staining with HRP (Light and Perl, 1979b; Ritz et al., 1989). These have shown that while bilaterally projecting afferents are quite common at high cervical (C1-C4) and sacrocaudal (S3-Ca3) regions, they were rare in brachial and lumbrosacral regions. A feature of these afferents was that they often have a bilateral RF which crossed the dorsal or ventral midline of the body or tail (Culberson et al., 1979; Ritz et al., 1989).

The collateral spacing along an axon's rostrocaudal extent was found to be correlated to the mediolateral position in the dorsal horn and not with the afferent unit type. HFA fibres which terminated medially in the dorsal horn had collaterals more closely spaced than did those laterally, with HFA's terminating in the middle having an intermediate value (Tables 1 and 3). This was also seen between SAI-hairy and glabrous afferents (Table 7) and for RA afferents compared to HFA's and SAI's (Table 9). This feature has been seen also in the cat by Brown (1981). The reason for this has remained unclear but it may have something to do with RF transformation. If afferents give off approximately equal numbers of collaterals then those placed laterally should have a greater rostrocaudal domain than those placed medially. This is in fact the case for HFA's (Table 1). The length of the overlapping terminal sheet was shorter in the medial dorsal horn than in the lateral dorsal horn. The somatotopic maps generated from recording from DHN's have shown that in the lateral part of the dorsal horn a given location on the hindlimb (for example lateral thigh) was represented over a greater rostrocaudal distance than was the case for a single location (such as a toe) represented in the medial dorsal horn (Brown, 1981). The dentritic trees of DHN's also have their greatest dendritic spread in the rostrocaudal

direction and shortest ones in the medial dorsal horn (Brown et al., 1980b). This might imply that larger peripheral RF's have larger central terminal fields as suggested by Woolf (1987). However, the length of an overlapping terminal arbor sheet was the same for a RF encompassing a single tylotrich hair or a group of hairs (Shortland et al., 1989a). Perhaps these phenomena are due to differential growth patterns that causes individual arborizations to be wedged shaped, being longer laterally than medially (Brown, 1981).

The relative synaptic density of different collaterals at different rostrocaudal locations may contribute to the spatial focussing of representations of different skin areas in the dorsal horn so that each may have a zone of maximal input at the centre of its terminal zone (mediated by complex arbors) and less input at its periphery (Woolf, 1987). The degree of branching of collaterals at the rostral and caudal extremes and number of boutons were found to decrease in the present study, in line with previous observations in cat (Meyers and Snow, 1984) and rat (Woolf, 1987). It may be that the complex arbors represent the normal avenue of synaptic contact whilst simple and blind-ending arbors are the sites of somatotopially inappropriate (SIA) and ineffective synapses (Snow and Meyers, 1985). It has been shown however, that action potentials can invade some of these SIA projections (Meyers and Snow, 1986).

The possibility that the blind-ending collaterals may reflect inadequate dye filling cannot be excluded since they were located at the axon's rostral and caudal extremes, although these were independent of the injection site. Also, the stem axon could be followed beyond the most caudal or rostral blind collateral, making this unlikely. These blind-ending collaterals may represent the anatomical substrate for the long-ranging afferents originally described by Wall and Werman (1976) and were located in somatotopically inappropriate areas of spinal cord (Meyers et al., 1984). It is not known whether these

collaterals have the ability to excite dorsal horn neurons and it has been proposed that these synapses are normally held ineffective (Wall, 1977) but can be demonstrated by electrical stimulation of primary afferents (Mendell et al., 1978), or dorsal roots (Merrill and Wall, 1972). Recently, it has been shown that these collaterals may play a role in the collateral sprouting that occurs following neonatal peripheral nerve section in the rat (Fitzgerald and Woolf, 1987) and cat (Snow and Wilson, 1988; Wilson and Snow, 1988b) and could represent a potential site for similar sprouting to occur in the adult (Molander et al., 1988; Lamotte et al., 1989).

The origin of these blind-ending collaterals are unknown. One of the possible explanations of these collaterals is that they represent the remnants of connections that were effective during an earlier developmental stage but were then withdrawn later during the precise synapse formation between sets of neurons (Purves and Lichtman, 1985). However, bulk labelling of peripheral nerves in the neonate and adult in the dorsal horn have shown the projections to be remarkably similar (Fitzgerald and Swett, 1983; Swett and Woolf, 1985). This data has been taken to indicate that there was no exhuberant projections in afferent projections in the developing somatosensory system (Fitzgerald, 1985). However, there remains some serious concerns relating to the size of terminal projection zones of peripheral nerves from transganglionic labelling (see later). As yet no detailed studies into the origin of these blind-ending collaterals has been attempted and their origin remains to be determined.

2.4 (ii) MORPHOLOGY OF SLOWLY ADAPTING TYPE-I AFFERENT TERMINALS.

The morphology of individual SAI's innervating the hindlimb has been studied by light and electron microscopy in the cat (Brown et al., 1978; Egger et al., 1981; Semba

et al., 1983; Bannatyne et al., 1984; Ralston et al., 1984; Ritz et al., 1989), monkey (Ralston et al., 1984) and rat (Woolf, 1987). The results from these studies indicated that there were species differences in the morphology and laminar termination sites of SAI arborizations. In the cat, SAI's have a characteristic morphology which was consistent for the collaterals along an individual axon, being "C"-shaped for hairy SAI arbors and "C"- or "L"-shaped for glabrous SAI arbors (Brown et al., 1978). The morphology of monkey SAI afferents was similar to that of the cat (Ralston et al., 1984). However, this characteristic appearance was not present for rat SAI collaterals (Woolf, 1987). Woolf (1987) noted that there was a wide variation in the morphology of adjacent arborizations and that there were no characteristic features that enabled them to be morphologically distinguished from RA afferents.

The results of the present study confirmed this observation. Some hairy SAI afferents appeared to have an L-shaped collateral arbor while others did not (compare Figs. 34 and 36) and some glabrous SAI arbors closely resembled RA arbors (Fig. 37), while others did not (Fig. 35). However, there were differences in the morphology of SAI-hairy and glabrous arborizations that enabled them to be distinguished from each other. The most noticable of these was the density of boutons in an arbor: SAI-hairy arbors had a much higher density of boutons (and a more complex branching pattern) than did SAI-glabrous arbors (compare Figs. 36 and 37). This feature was also observed between cat SAI-hairy and glabrous afferents (Brown et al., 1978). The reverse case appeared to be seen for glabrous and hairy SAI arbors recovered by Woolf (1987) in the rat. In his Figs. 11 and 12, glabrous SAI arbors had more boutons than hairy SAI arbors. Woolf (1987) made no mention of differences between glabrous and hairy SAI arborizations. The majority of boutons in SAI-hairy arbors were "en passant" while SAI-glabrous boutons were "boutons terminaux" or boutons de passage, similar to the

arrangement in the cat (Brown et al., 1978).

Species differences were also seen in the dorsoventral positioning of terminal arborizations of SAI collaterals. In the monkey boutons were distributed to laminae III-IV (Ralston et al., 1984), laminae III-V in the cat (Brown et al., 1978; Egger et al., 1981; Ralston et al., 1984; Ritz et al., 1989) with the majority located in dorsal lamina IV and some projecting into laminae V-VI (Semba et al., 1983). In the rat, terminals were distributed mainly to laminae IV and V with occasional intrusions into laminae VI and III (Woolf, 1987). The results here showed that terminals from SAI collaterals were distributed over a much wider range, laminae IIi-VI but that this was somewhat dependant on the afferent type. SAI-hairy arbors tended to terminate in laminae IIi-IV, the majority of terminals being in laminae III-IV. This is also probably true in the cat. Brown et al., (1978) found that 9/13 SAI-hairy afferents terminated primarily in laminae III-IV while the remaining 4 glabrous SAI afferents arborized in laminae IV and dorsal V. Semba et al., (1983) recorded only from SAI-glabrous afferents and found boutons distributed from lamina III to lamina VI, with the heaviest concentration in lamina IV. They also found a decrease in size of the boutons from laminae IV-VI. The study of Woolf (1987) did not observe (or failed to mention) any dorsoventral difference in laminar arrangement between hairy and glabrous afferents probably due to the small sample size but inspection of his figs. 11 and 12 (Woolf, 1987) show that hairy arbor terminals appear more dorsally than glabrous terminals. In the present study the SAI-glabrous terminals were also located deeper in the dorsal horn, mainly laminae IV-V.

Differences in the terminal morphology and laminar arrangement of SAI-glabrous and hairy afferents will have functional implications in terms of the second order neurons that they innervate. Laminae III-VI contains cells of the SCT and postsynaptic dorsal column (PSDC) pathways as well as interneurons which may constitute part

of polysynaptic pathways such as the SCT system (Brown and Noble, 1982). Monosynaptic connections of SAI's to PSDC and unidentified dorsal horn neurons in laminae III-VI have been shown by anatomical (Maxwell et al., 1985) and electrophysiological (Brown et al., 1973; Tapper et al., 1973; Tapper and Wiesenfeld, 1980, 1981) while SCT cells receive no input from SAI afferents (Brown, 1981). Finally, SAI afferents from the plantar cushion of the cat are known to be involved in the plantar cushion reflex (Egger and Wall, 1971). At least two interneurons are thought to be involved in this reflex and DHN's in the medial dorsal horn of laminae III-IV and VI have been shown to respond to low threshold cutaneous stimulation of the plantar cushion, with those in the most medial portion of lamina IV of L7 being the likely first order DHN that receives SAI low threshold input (Egger et al., 1986).

There were other similarities and differences observed between cat and rat SAI afferents. As in the cat lumbrosacral cord, (Brown et al., 1978) the intercollateral spacing between adjacent arbors was shorter in rat SAI-glabrous afferents than for SAI-hairy afferents (Table 7) and occasionally adjacent arborizations overlapped. In cat sacrocaudal spinal cord, the intercollateral distance between adjacent SAI collaterals was half that seen in the cat lumbrosacral cord (Ritz et al., 1989), and approached the dimensions of SAI arbors seen in the rat lumbar cord (Table 9). This suggests that the DHN target cells are either more closely packed together or have shorter dendritic fields. Collateral overlap in the sacrocaudal cord was also observed (Ritz et al., 1989). In the present study, 6/45 (13%) of the complex arbors overlapped, the incidence being higher for hairy SAI afferents (20%) compared to SAI-glabrous afferents (3%). In the cat, not all SAI afferents bifurcated into rostral and caudal projecting stem axons (Brown et al., 1978) whereas in the rat all the SAI's bifurcated (Woolf, 1987; present study).

Rat SAI afferents had a higher number of collaterals

per afferent than did cat SAI's but the dimensions were larger in the cat (Brown et al., 1978). The dimensions and arrangement of collaterals of SAI afferents (Tables 7-9) were larger than those reported by Woolf (1987). These differences may be due to sample size (number as well as type of SAI afferent).

2.4 (iii) MORPHOLOGY OF RAPIDLY ADAPTING AFFERENT TERMINALS.

It has been shown that there are 2 kinds of RA mechanoreceptors innervated by myelinated fibres in the glabrous skin of the hindpaw in cats (Janig et al., 1968): pacinian corpuscles (PC) and rapidly adapting (RA, Krause corpuscles) afferents and these differ in their physiological response properties to mechanical stimuli (Iggo and Ogawa, 1977) and in the morphology and laminar position in the dorsal horn as revealed by light and electron microscopic studies (Brown et al., 1980c; Maxwell et al., 1984a; Ralston et al., 1984; Semba et al., 1984, 1985). Electron microscopy of the boutons of PC's and RA's in the cat have shown them to be similar in many respects, such as the presence of round clear vesicles, size and shape of boutons and synaptic associations with dendritic shafts or spines but that differences were observed in the number of contacts made and the degree of complexity of synaptic organization (Maxwell et al., 1984a; Semba et al., 1985).

Pacinian corpuscle collaterals distribute boutons to laminae III-VI, with a larger, dorsal projection to laminae III-IV than to laminae V-VI according to Brown et al., (1980c) while Maxwell et al., (1984a) and Ralston et al., (1984) reported a narrower dorsoventral range: laminae III-V. Semba et al., (1984) observed terminal arborizations in laminae IIi-VI distributed as two clusters, one in laminae III-IV and the other in lamina V. Rapidly adapting (Krause) afferents also have a narrower laminar distribution: mainly lamina III and dorsal lamina

IV (Brown et al., 1980c; Maxwell et al., 1984a). Semba et al., (1985) however, reported a greater laminar distribution, from lamina III to VI. Whereas these previous studies have been conducted in the cat, only one such study has been performed in the rat (Woolf, 1987). The results from this study found terminal distributed from laminae III-V, with the highest density in laminae IV-V. The results of the present study, confirmed those of Woolf (1987) but in addition provide evidence for terminal invasion into lamina IIi (Plate 5). Paw pad afferents mainly arborized in laminae III-IV while toe RA afferents arborized primarily in laminae IV-V.

In this study, both paw pad and toe RA's afferents were classified as rapidly adapting on the basis of adaptive response to light brush and indentation of the skin. Adaptiveness to ramp stimulation was not tested, so identification of RA afferents as PC's was not attempted. However, there were morphological differences between paw pad and toe RA afferents. Paw pad afferents arborized mainly in laminae III-IV with occasional terminal intrusions into lamina IIi and V. The paw pad simple arbors had a shorter dorsoventral length and appeared to be located at deeper laminar levels than complex arbors. For some paw pad afferents (2/6) there appeared to be a dorsoventral shift in the laminar terminal sites as the collaterals proceeded caudally while in others (4/6) there was no clear change of laminar terminal position. This dorsoventral shift as axon collateral boutons terminated more caudally has been seen in pacinian corpuscle afferents in the cat (Semba et al., 1984) and may reflect the change from complex to simple arbors.

The dimensions of paw pad arbors showed significant differences between complex and simple arbors compared to toe afferents, conveying the impression that they were larger than toe RA afferents, but in fact this was not so when all terminal arborizations (complex and simple), or when the average dimensions /afferent were considered (Table 6, Appendix II Table 6A). However, paw pad complex

arbors occupied upto 25% of the mediolateral width compared with about 15% for toe RA complex arbors. As in the cat, the dimensions of RA afferents were generally smaller in all directions (mediolateral, dorsoventral, rostrocaudal) compared to PC afferents (Brown et al., 1980c). The paw pad afferents all bifurcated into rostrocaudally directed stem axons which issued collaterals which overlapped for 20/30 (67%) of the complex arborizations. Overlapping arborizations in the cat have been observed for PC's by Brown et al., (1980c) but only for the arborizations which were located in laminae III-IV and not in laminae V-VI. However, Semba et al., (1984) have observed overlap between arbors in laminae III-IV, and V-VI but as two populations of terminals separated by a rostrocaudal gap of about 1.5mm.

In comparison to the paw pad afferents, the toe RA afferents recovered in this study had different morphological characteristics. The laminar termination site was mainly in laminae IV and V in keeping with the results of Woolf (1987). The invasion of lamina IIi by toe RA afferents has not been observed in previous studies in the cat or rat (Brown et al., 1980c; Maxwell et al., 1984a; Semba et al., 1985; Woolf, 1987), and although fewer toe RA arbors terminated in lamina IIi compared to paw pad afferents, there was little difference in the numbers between the two groups (Table 5). Previous studies by Brown et al., (1980c) concluded that RA afferents terminals were restricted to lamina III, resembling HFA afferents in the recurving nature of the collaterals and laminar site but differed from HFA's in that adjacent arbors were separated by a gap of 100-700um. They also differed in their mediolateral dimensions, being narrower than HFA's (Brown et al., 1980c). Aspects of this original description have been confirmed (Maxwell et al., 1984a) or argued against (Semba et al., 1985). Semba et al., (1985) found a more extensive dorsoventral terminal projection stretching from lamina III-VI with a diffuse dorsoventral distribution of boutons in the

rostrocaudal direction (ie. no dorsoventral-rostrocaudal shift) but a shorter extent of boutons in the rostrocaudal direction. Secondly, unlike Brown et al., (1980c), Semba et al., (1985) found that boutons were distributed almost continuously along the rostral to caudal axis with very few gaps so that adjacent collaterals overlapped. Semba et al., (1985) concluded that the differences seen were due to better filling of RA afferents and a larger sample size (7 versus 3 afferents of Brown et al., 1980c) reflecting a greater variation among functionally homogenous afferents. The results of Woolf (1987) in the rat concur with those of Semba et al., (1985) in laminar termination site (III-VI) but differ in that Woolf (1987) observed no overlap of adjacent collaterals in the rostrocaudal direction. In this respect the results are similar to those of Brown et al., (1980c) but the morphology of rat RA afferents was different, having no consistent features as seen by Brown et al., (1980c).

The results of the toe RA afferents recovered in this study show features similar to those seen by Semba et al., (1985) such as laminar positioning, and overlap between adjacent complex arborizations (12/35, 34%) and to those of Woolf (1987) in inter- and intra- arbor variation among RA afferents. Finally, unlike the results of Brown et al., (1980c) and Woolf (1987), all the toe RA afferents bifurcated into rostral and caudal branches.

The variability in the morphology of PC and RA arborizations and also in their laminar termination site will obviously have functional implications on the second order neurons that they innervate. In the cat, PC's distribute their information to two distinct laminar regions and there are differences in both terminal axon orientation and bouton density in these two regions which indicate that at least two different neuronal populations are receiving information. The boutons are ideally suited to interact with the dendrites of dorsal horn neurones that are found in laminae III-V (Schiebel and Schiebel, 1968; Proshansky and Egger, 1977). The target neurons for

RA afferents are also situated in laminae III-IV and may be similar to those of PC afferents, since it has been shown that both types of afferents make synapses with PSDC neurons (Maxwell et al., 1985), at least at the light microscopic level. No contacts have been observed with SCT cells for either RA or PC afferents (Brown, 1981; Maxwell et al., 1984b).

It is tentatively suggested that the paw pad RA afferents of the present study are similar to the PC's described in the cat (Brown et al., 1980c; Semba et al., 1984) while toe RA's are similar to Krause corpuscle afferents described by Brown et al., (1980c), Semba et al., (1985); and Woolf (1987) based on morphology and arbor dimensions. Pacinian corpuscles are known to exist in rat glabrous skin (Sanders and Zimmermann, 1986) although their RF's differ strikingly from those in the cat, monkey and man by being of small size with distinct RF borders. PC and RA receptors in rat produce a short latency discharge to skin indentation, but PC's elicit no further discharge with prolongation of the rise of indentation, characterizing them as very rapidly adapting acceleration detectors. However, the glabrous afferents recovered in this study were not tested for adaptiveness to ramp stimulation which unequivocally distinguishes between the two types. As paw pads and RA toe afferents share features such as overlap of collaterals and projections to lamina IIi, they may be subpopulations of a general RA afferent population. A greater sample of afferents, including glabrous afferents innervating the sole of the foot would be needed to verify this point and these afferents should be tested for PC properties.

2.4 (iv) COMPARISON WITH LOW THRESHOLD CUTANEOUS AFFERENTS IN THE BRAINSTEM.

In the spinal cord, the peripheral receptor appears to be an accurate predictor of the somatosensory primary afferent arbor shape in the dorsal horn (Light and Perl,

1979b; Brown, 1981; Ralston et al., 1984; Semba et al., 1985; Woolf, 1987; present study) while in the brainstem, structure-function relationships for trigeminal primary afferents (Hayashi, 1982, 1985a,b; Jacquin et al., 1984, 1986a,b, 1988; Chiaia et al., 1987), visceral afferents (Kalia and Richter, 1985a,b, 1988a,b), cutaneous and proprioreceptive afferents in the cuneate nucleus (Fyffe et al., 1986; Crockett et al., 1988) are less certain.

The results of these studies, while at odds with each other (Hayashi, 1985a,b versus Jacquin et al., 1986a,b), clearly demonstrated that non-vibrissae related nociceptive afferents were morphologically distinct from low threshold cutaneous afferents. In the trigeminal subnucleus interpolaris (SpVi) physiologically different classes of vibrissae afferents were morphologically indistinguishable (Hayashi, 1985a,b; Jacquin et al., 1984, 1986a, 1988) while morphological differences existed for different afferent classes in the medullary (SpVc) and cervical dorsal horn (Hayashi, 1985a; Jacquin et al., 1986b, 1988; Chiaia et al., 1987).

The morphology of HFA's and SAI's in the cuneate nucleus (Fyffe et al., 1986) and of visceral rapidly and slowly adapting afferents in nucleus tractus solitarius (nTs, Kalia and Richter, 1985a,b, 1988a,b) were also morphologically distinct from those types in the spinal cord, although certain similarities in collateral arrangement and bouton type existed for spinal cord somatic afferents and brainstem afferents (Kalia and Richter, 1985b, 1988b).

The results from brainstem afferent studies indicates that the peripheral receptor association does not always predict distinctions in central morphology as it does in the spinal cord. The most likely explanation for this is a qualitative difference in the fundamental principles of organization (Jacquin et al., 1986b) imposing topographical constraints which may override the qualitative morphological distinctions that exist for functionally defined spinal and non-vibrissae trigeminal

primary afferents. The evidence suggests that the structure of the central target may influence the pattern of termination. Nociceptive fibres bear no resemblence to hairy skin afferents in the medullary dorsal horn, whereas in SpVi they all look very similar (Jacquin et al., 1988). The former is a laminated structure whereas the latter is not. If this is a determining factor, then arbors in the other trigeminal subnuclei, oralis and principalis, should be morphologically similar. This has been shown in the rat (Hayashi, 1985a).

2.4 (v) SOMATOTOPIC ORGANIZATION.

The somatotopic organization of primary afferents has been investigated using bulk labelling of cutaneous nerves with HRP (Koerber and Brown, 1980, 1982; Smith, 1983; Ygge and Grant, 1983; Nyberg and Blomqvist, 1985; Swett and Woolf, 1985; Molander and Grant, 1986; Woolf and Fitzgerald, 1986; Nyberg, 1988; Rice et al., 1988; Brown et al., 1989; Culberson et al., 1989; Rasmusson, 1989; Ygge, 1989), intradermal injection of HRP (Molander and Grant, 1985; Kauze and Rethelyi, 1985; Florence et al., 1988, 1989; Maslany et al., 1988a,b), HRP injection into DRG's (Pfaller and Arvidsson, 1988), degeneration (Culberson and Brown, 1984) and electrophysiological (Heaney et al., 1984; Meyers and Snow, 1984; Meyers et al., 1984) techniques. Intraaxonal injection of HRP into single afferents provides an alternative and more detailed method of studying the somatotopic organization of a group of cutaneous afferents. Preliminary results have been documented for the cat (Brown et al., 1988; Sonty et al., 1988; Ritz et al., 1989) while a more detailed analysis has been carried out in the rat (Shortland et al., 1989a).

An unresolved question arising from bulk labelling studies is whether adjacent peripheral nerve territories have terminal fields which were partially- or non-overlapping in the dorsal horn. In the dorsal horn of

the spinal cord, minimal overlap has been observed between the central terminals of primary afferents by some (Koerber and Brown, 1980, 1982; Molander and Grant, 1985, 1986; Nyberg and Blomqvist, 1985; Florence et al., 1988, 1989; Pfaller and Arvidsson, 1988; Rivero-Melian and Grant, 1988; Brown et al., 1989; Lamotte et al., 1989) but not others (Ygge and Grant, 1983; Swett and Woolf, 1985; Woolf and Fitzgerald, 1986). However, when single afferents are labelled as in this study, upto 43% of the blind-ending (non-bouton containing) and 23% of the simple (low density bouton containing) collaterals of afferents of a particular nerve territory overlapped with the complex (high density bouton containing) arbors of afferents from a different nerve territory. The discrepancy arising from this result with that of bulk labelling methods is possibly due to transganglionically transported HRP accummulating in boutons and not in blind-ending collaterals. Hindlimb central terminal maps constructed from bulk labelling experiments in the rat (Swett and Woolf, 1985; Molander and Grant, 1986) would then largely be maps of the location of complex and simple arbors rather than of the full extent of all the collaterals of an axon. Swett and Woolf (1985) studying the innervation territories of primary afferents with WGA-HRP found that each labelled nerve in the dorsal horn occupied its own area with no overlap between adjacent territories and that this was reflected in the periphery where electrophysiological mapping of peripheral RF's of the nerves showed that the boundaries for most were sharply delineated (except for the one between the peroneal and tibial nerve). On the other hand, Molander and Grant (1986) who performed the same experiment of bulk labelling hindlimb nerves with HRP found considerable overlap between adjacent nerves in the dorsal horn, although the basic somatotopic map was similar to that of Swett and Woolf (1985). Possible reasons for the observed differences included diffusion of HRP into other regions other than those intended for uptake; differing

interpretations of laminar borders of the labelling (which were sometimes diffuse or were artefacts resulting from labelling fibres of passage), or from transneuronal labelling (Molander and Grant 1985, 1986). However, the results of the studies by both groups could be correct, the differences being due to the technical limitations of the methods used. A potential source of error which could have led to nerve territory overlap in the present study relates to the designation of a particular RF as lying within a given nerve territory since the borders of the nerve territories were not marked on the hindlimb or identified for every animal. The peripheral nerve boundaries used here were those defined by Swett and Woolf (1985) and these cutaneous borders have been shown to be consistent for a large number of animals examined using electrophysiological techniques. Furthermore, these borders have also been defined repeatedly in this laboratory using antidromic stimulation to produce neurogenic extravasation of peripheral nerves (Woolf and Fitzgerald, personal communication) and by others (Wiesenfeld-Hallin, 1988) and in these cases the peripheral territories were also found to be consistent between animals. As the majority of RF's recorded here clearly lay within a single nerve territory, not close to the border of adjacent nerve territories we can be confident that any error of assignations introduced into this study was low. Here it was shown that there was minimal terminal overlap between adjacent terminal arborizations from different nerve territories and that where this did occur it involved simple and blind-ending collaterals. Complex arborizations from one nerve territory were not seen to overlap with the complex arborizations from another nerve territory (except for one case and here the peripheral RF bordered two different nerve territories). It is to be expected that if a peripheral RF of an afferent is located at, or crosses the border of two different peripheral nerve territories then the central terminals of the afferent will be located at,

or across, the borders of the two nerve territories in the dorsal horn. This is seen in the case of the glabrous-hairy HFA's whose central terminals are located in both the glabrous and hairy areas of the spinal cord (across the border between the tibial and the saphenous, superficial peroneal or sural nerves).

More recently, it has been demonstrated that, at least for C-fibres, there are overlapping peripheral skin territories between adjacent pairs of nerves (Wiesenfeld-Hallin, 1988). This was visualized by plasma extravasation of Evans blue dye to antridromic C-fibre stimulation of hindlimb nerves. Overlap was greatest on the toes and dorsum of the foot and minimal on the volar aspect. This method, however, gives indistinct nerve borders due to the patchiness of leakage of the dye to the skin surface so the amount of overlap is open to question, but some corresponding overlap in the projection of rat hindlimb skin to the substantia gelatinosa has been observed (Molander and Grant, 1985).

Within a given nerve territory the situation was very different from that between nerve territories. Cutaneous afferents with non-adjacent RF's had central terminal fields that overlapped to a considerable extent in the rostrocaudal direction in the dorsal horn. The synaptic density of the terminal arbors of different collaterals may nevertheless contribute to a representation of different skin areas in the dorsal horn. Each afferent's collaterals, although overlapping with other axons terminals, had a zone of maximal bouton density which fell off relatively gradually as one moved along the longitudinal axis but very rapidly in the transverse axis. These zones of focussed synaptic input differed slightly for different afferents according to the location of their RF's. For example, the lateral leg HFA's showed a spatial gradient of their complex arbors, which lay in a narrow sheet in the same mediolateral plane from mid L3 to caudal L5, because as one moved from rostral to caudal there were terminals of afferents from the thigh, calf, and ankle

with different but overlapping longitudinally distributed zones of maximal input. A similar pattern was present for the paw pad RA afferents; as one proceeded from rostral L4 to caudal L5 the RF changed from the proximal paw pad of toe 1 to proximal paw pad toe 5.

Intradermal injection of HRP into fore- and hindlimb digits (Molander and Grant, 1985; Maslany et al., 1988b; Florence et al., 1988, 1989) or bulk labelling of digital nerves with HRP (Nyberg and Blomqvist, 1985; Culberson et al., 1989) has shown that each digit has its own discrete area of cord, the digits 1-5 being represented successively caudally with little overlap between adjacent digits. There was also a mediolateral separation in the projections of the dorsal and plantar surface of a particular digit (Molander and Grant, 1985; Nyberg and Blomqvist, 1985) with the dorsal surface being represented lateral to the plantar surface at the same rostrocaudal level in the cord. The present study using intraaxonal injection of HRP into characterized afferents has confirmed the rostrocaudal sequence of toes 2-5 in the dorsal horn for HFA's previously described by Molander and Grant (1985) with little rostrocaudal overlap and that afferents with a glabrous input to their RF were located medially to afferents with a purely hairy RF at the same rostrocaudal level. However, the observation that the glabrous skin of digits 2-5 are also represented successively caudally in medial dorsal horn (Nyberg and Blomqvist, 1985) has not been observed here, at least not for RA toe afferents. A possible explanation for this discrepancy lies in the fact that the nerves innervating the dorsal surface of the digits are supplied from three different nerves, the saphenous, peroneal, sural nerves, which have been shown to terminate in different areas of the cord (Swett and Woolf, 1985; Molander and Grant, 1986) and may therefore not be expected to overlap significantly. However, the digital plantar nerves of toes 1-5 are all branches of the tibial nerve, and as shown there was little or no detailed somatotopy within a

nerve territory such that adjacent and non adjacent RF's all overlapped.

Finally, let us considers the somatotopy of two different functional classes, RA's innervating the glabrous skin (Fig. 24) and HFA's innervating the toes and medial leg (Figs. 20,22). Superimposition of Figs. 20,22,24 conveys the impression that there was some mediolateral overlap between different functional classes. However, the differing laminar termination sites, laminae IV and V for RA afferents compared to laminae IIi and III mainly for HFA's (Woolf 1987 and present study) may give a false impression. However, mediolateral and dorsoventral overlap between RA and HFA afferents has been observed (personal observation) thus smearing the somatotopic organization and implies that convergence of functionally distinct afferents with seperate RF's onto the same postsynaptic neuron may occur. A smearing of the somatotopic pattern in the dorsoventral direction has been observed by bulk labelling methods (Woolf and Fitzgerald, 1986).

The central terminal overlap maybe related to the pattern of peripheral innervation. Hair follicles are innervated by more than one afferent, vellus hairs having 1-5 afferents per follicle, guard hairs 3-15, tylotrichs more than 20 afferents per follicle (Millard and Woolf, 1988). A single guard HFA will innervate a number of follicles over a widespread area (Lynn and Carpenter, 1982) and that area will be, in turn, innervated by a large number of different afferents, producing a mosaic of overlapping RF's. It was not surprising to find, therefore, that the central terminals of afferents with different RF's overlapped to a considerable degree. The relatively large size of the central arbors of individual afferents compared to the area of the dorsal horn devoted to the terminals of individual nerves which consists of many afferents ensures that overlap must occur to accommodate all the terminals. Despite this, there was an approximate 1:1 relationship between the amount of skin

surface innervated by a given peripheral nerve and the amount of surface area of superficial dorsal horn required to process this afferent input for hairy skin, while the glabrous skin afferents in the tibial nerve occupy proportionately more than twice the surface area of the dorsal horn than a comparable area of hairy skin, (Swett and Woolf, 1985) although this may be related to innervation density (Lynn and Carpenter, 1982).

However, at the single afferent level the central terminal area was not related to the peripheral RF area, so that the area occupied by the central terminals of, for example, a single tylotrich hair was similar to that from a group of hairs. Because the greatest somatotopic gradient is in the mediolateral plane it is not surprising that overlap is least in this axis. By inspection, the width of individual primary afferents ranges from 10-30% of the dorsal horn width, so that only a maximum of 10 non-overlapping afferents could fit side to side across the dorsal horn in the rat. In the cat this figure is less, only 5 or 6 non-overlapping afferents (Sonty et al., 1988). Because the absolute size of the rostrocaudal axis is so much greater than the mediolateral and because the somatotopic gradient in this axis is so much less steep, it is to be expected that the packing arrangement of central terminals is one of overlap in this plane.

The developmental arrangement of the packing of the central terminals raises intriguing questions. How does an afferent know where to terminate? What guides it to its correct location? The exact answers to these questions are, at the moment, unknown. What is known for cutaneous primary afferents is that from the outset they know where they are going to terminate within the dorsal horn. Primary afferents grow into the spinal cord in a developmentally timed sequence, (Smith, 1983; Fitzgerald, 1987) and terminate in the appropriately correct area of dorsal horn (Fitzgerald and Swett, 1983). Clearly, a sophisticated signalling system is required to form such a precise system. Perhaps special labels are involved such

as specific cell surface antigens (Dodd and Jessell, 1985) or trophic attractants are involved in afferent target recognition.

In the case of HFA's, evidence is now beginning to emerge about their development (Beal et al., 1988). HFA's enter the spinal cord on E19 via a characteristic U-shaped pathway and extends dorsally into the head of the dorsal horn. The head is divided into 8-12 compartments or lobules, each lobule containing only a single flame shaped arbor (Beal et al., 1988). These have been clearly observed in Golgi studies (Cajal, 1909; Scheibel and Scheibel, 1968). The flame-shaped arbors often follow the spinal cord vascular pattern which separates adjacent lobules and then during the early postnatal period form definitive arbors in laminae IIi-IV which reach their adult dorsal limit by Postnatal (P) day 5. There is no overlap between adjacent lobuli (Beal et al., 1988) as was once thought (Cajal, 1909) since the vasculature may separate them. This may be why there is such little overlap in the mediolateral plane for primary afferents from different nerve territories. The mediolateral extent of a nerve territory may be a certain number of lobuli wide and as adjacent lobuli do not overlap so neither do the nerve territories.

In the horizontal plane (parallel to the laminae borders) HFA lobules are rectangular in shape and by P5 they already overlap each other in the longitudinal direction (Beal et al., 1988). However, considerable growth of the spinal cord in the rostrocaudal direction has yet to take place, so that the arbors will continue to grow in length throughout the elongation period (upto about P30) of the spinal cord. For the arbors, this must be due to addition of axonal membrane throughout the arbor length rather than growth at the rostral and caudal ends since the arborization field is essentially established by P5 (Fitzgerald and Swett, 1983). It is not surprising to see therefore, considerable overlap in the rostrocaudal direction within a nerve territory since afferents

located in the same lobule mediolaterally can only grow rostrocaudally. The rostrocaudal "cut-off" point between adjacent nerve territories is somewhat obscure. Assuming that, like the adult, a developing HFA contains simple, blind-ending and complex arbors then minimal overlap would be expected to be present between the caudal collaterals of one nerve territory and the rostral collaterals of another. This may be so since exuberant projections have not been observed in the spinal cord (Fitzgerald, 1987). Nevertheless, the precision of this system can be easily disrupted during neonatal life by peripheral nervous system injury (see chapters 3 & 4).

2.5 SUMMARY.

The morphology of cutaneous low threshold primary afferents may be related to their peripheral innervation. For HFA's, flank afferents were different from toe afferents and these were different from glabrous-hairy afferents; paw pad RA afferents were different from toe RA afferents while SAI-hairy afferents were different from SAI-glabrous afferents. All low threshold cutaneous afferents terminated in laminae III-V but RA and SAI afferents also projected to lamina IIi. Centrally, afferents showed overlap between different nerve territories when one considered all the collaterals along a particular axon, but when only complex arbors were considered, there was no central overlap. However, within a nerve's central field, there was overlap between complex arbors of afferents with non-adjacent RF's resulting in a blurred representation of the periphery in the dorsal horn. The normal avenue of stimulus identification, however, may be through the synaptic boutons of the complex arbors which did nevertheless form a somatotopically organized zones of maximal input.

The spatial distribution of the central terminals of afferents is by itself insufficiently precise to provide accurate information about stimulus location but provides

a geometric framework for the generation of cutaneous RF's. Spatial analysis by the CNS must depend on the focussing of input in many afferents together with the consequent postsynaptic excitatory and inhibitory interactions on dorsal horn neurons.

CHAPTER 3: PLASTICITY IN SOMATOSENSORY SYSTEMS.

(A) CAPSAICIN TREATMENT.

3.1 GENERAL INTRODUCTION.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), the pungent ingredient of hot peppers, exerts its neurotoxic effects predominantly on unmyelinated (C) sensory fibres. Capsaicin has two sites of action on primary afferent neurons: the axon and the nerve terminals (Szolcsanyi, 1982), distinguishable by their time course (Lembeck and Donnerer, 1981). Its mode of action also depends on the age of the animal (neonate versus adult), method of administration (systemic, close arterial, intraperitoneal, intradermal, intrathecal, topical nerve or skin application) dose, and duration of the experiment (chronic versus acute), (Fitzgerald, 1983; Russel and Burcheil, 1984; Buck and Burks, 1986; Gamse et al., 1986; McMahon and Fitzgerald, 1986; Such and Jansco, 1986).

Capsaicin has the ability to both stimulate and cause degeneration of unmyelinated primary afferents fibres. In vitro preparations recording from dorsal root ganglion neurons (Marsh et al., 1987; Alreja et al., 1988; McLean and Barron, 1988) and from nociceptors (Dray et al., 1989), in vivo pharmacological preparations (Aimone and Yaksh, 1988; Sawynok et al., 1988), electron microscopy of C-fibres (Jansco, 1978; Jansco et al., 1977; Nagy et al., 1980) and of cultured DRG's (Hiura and Sakamoto, 1987a,b), intra- and extracellular recordings from nociceptor fibres (Wall and Fitzgerald, 1981; Fitzgerald and Woolf, 1982; Petsche et al., 1983; Lynn, 1984; Simone et al., 1989), and radioimmunoassays for nociceptive transmitters (Gamse et al., 1980, 1982; Nagy et al., 1980; Jansco et al., 1981; Papka et al., 1981; Wall and Fitzgerald, 1981; Schultzberg et al., 1982; Inomata and Nasu, 1984; Solodkin and Ruda, 1988) have all shown that capsaicin has profound effects in both adults and neonates.

3.1 (i) EFFECTS IN THE ADULT.

The effects of capsaicin on sensory neurons are clearly more profound in neonates than in adults. When adults are treated systemically with capsaicin there is a selective degeneration of a subpopulation of primary sensory neurons (Jansco et al., 1985; Ritter and Dinh, 1988) and a depletion of substance P (SP), cholecystokinin (CCK), vasoactive intestinal polypeptide (VIP), somatostatin (SOM) and fluoride resistant acid phosphotase (FRAP) from the dorsal horn (Jansco and Kniyhar, 1975; Gamse et al., 1981). There is also insensitivity to chemical pain but no change in heat pain thresholds (Fitzgerald, 1983), although close-by arterial injection of capsaicin excites mechanoheat sensitive nociceptors (Szolcsanyli et al., 1988) and intradermal injection causes sensitization of dorsal horn neurones to low threshold stimuli (Simone et al., 1989). Local application of capsaicin to a single nerve results also in the depletion of SP, CCK, SOM, CGRP, FRAP (Wall, 1985) probably as a result of inhibition of axoplasmic transport (Gamse et al., 1982; Wall, 1985). Topical application of capsaicin to the nerve results in substantial increases in noxious heat thresholds (Gamse et al., 1982) while reducing the number of noxious heat responsive dorsal horn neurons (Fitzgerald, 1982; McMahon and Fitzgerald, 1986) without affecting responses to mechanical noxious responses (Fitzgerald and Woolf, 1982). Nerve treatment also results in removal of afferent inhibition (Fitzgerald, 1982) although dorsal root potentials and primary afferent depolarization are both unaffected by nerve application whereas they are absent following neonatal capsaicin treatment (Wall, 1982, 1985; Wall et al., 1982b). In adults, the depletion of peptides and associated sensory deficits are sometimes reversible (Wall, 1985; Buck and Burks, 1986; Solodkin and Ruda, 1988). This is not the case in neonates where the

effects are lifelong.

3.1 (ii) EFFECTS IN THE NEONATE.

When administered neonatally, during the first few days of life, capsaicin can destroy 41-95% of sensory C-fibres at doses of 50mg/kg (Jansco et al., 1977; Scadding, 1980; Nagy et al., 1981, 1983; Lynn, 1984; Arvidsson and Ygge, 1986; Hiura and Sakamoto, 1987b; Lynn et al., 1987; Hiura and Ishizuka, 1989) while repeated neonatal doses (Hiura and Sakamota., 1987b) or higher doses (Lawson and Nickels, 1980; Lawson, 1981; Nagy et al., 1983; Nagy and Hunt., 1983) can destroy 6-34% of thinly myelinated A-delta fibres. However, even at maximal doses there appears to be approximately 5% of C-fibres which are resistant to capsaicin.

The neurotoxic action of capsaicin is possibly due to an excessive calcuim ion entry due to changes in the membrane permeability leading to neuronal death (Marsh et al., 1987; Wood et al., 1988). Morphometric analysis has shown that 28-78% of the small B-type DRG cells and 14-52% of larger sized (500-1950um^2) A-type neurons are destroyed while intrinsic neurons remain unaffected by neonatally administered capsaicin (Lawson and Nickels, 1980; Lawson, 1981; Nagy and Hunt, 1983; Nagy and Van der Kooy, 1983; Otten et al., 1983; McDougal et al., 1985; Arvidsson and Ygge, 1986; Hiura and Sakamoto, 1987b). The loss of some of the larger sized neuron indicates that some unmyelinated fibres originate from large sized neurons as identified by Hoheisel and Mense (1986). This loss of neurons results in a depletion of SP, SOM, FRAP, thiamine monophosphatase (TMP), VIP, CGRP, CCK from the DRG, peripheral nerve and central terminals in the spinal cord (Nagy et al., 1980, 1981; Jansco et al., 1981; Papka et al., 1981; Fitzgerald, 1983; Inomata and Nasu, 1984; McDougal et al., 1985; Skofitsch and Jacobowitz, 1985; Solodkin and Ruda, 1988; South et al., 1988; Hammond and Ruda, 1989). Some recovery of peptides has been observed

in the dorsal horn some months afer treatment (Solodkin and Ruda, 1988; Hammond and Ruda, 1989) presumably from the remaining unaffected afferents or intrinisic spinal cord cells. These changes leave the animals with a life long insensitivity to noxious chemical stimuli (Jansco et al., 1977; Fitzgerald, 1983; Nagy and Van der Kooy, 1983) and reduced sensitivity to noxious heat (Hunt, 1983; Nagy and Van der Kooy, 1983; Doucette et al., 1987) while leaving responses to noxious mechanical stimuli unchanged (Doucette et al., 1987).

In the dorsal horn, neonatal capsaicin-induced destruction of C primary sensory neurons, naturally leads to the destruction of C-fibre terminals from the substantia gelatinosa (Jansco et al., 1977; Ribero da Silva and Coimbra, 1984; Ribero da Silva et al., 1986). There are also postsynaptic changes such as a loss in number of spinothalamic tract (STT) neurons from lamina I (Saporta, 1986; Saporta and Jacobson, 1988) and changes in the dorsal horn physiology such as reduced primary afferent depolarization, increased receptive field size and disorganization of the somatotopic map of dorsal horn neurons (Wall et al., 1982a,b; Cervero et al., 1984).
Some of these behavioural and physiological effects may be due to a reorganization of connections or sprouting of surviving afferents in response to partial deafferentation. Several studies using bulk labelling and staining techniques have demonstrated an apparent reorganization of the remaining primary afferent terminals in the dorsal horn following neonatal treatment (Nagy and Hunt, 1983; Rethelyi et al., 1986; Beal and Knight, 1987). HRP application to cut dorsal roots (Rethelyi et al., 1986) or to DRG's (Nagy and Hunt, 1983) and Golgi impregnation techniques (Beal and Knight, 1987) have shown a dorsal extension of the intact A-fibre terminals into the deafferented substantia gelatinosa (SG). However, these studies tell us very little about the pattern of this new terminal growth.

The aim of this present study was to examine the

extent of sprouting of A-fibres afferents into the SG following neonatal C-fibre destruction at the single fibre level using intracellular labelling of individual physiologically characterized primary afferents with HRP.

3.2 METHODS.

3.2 (i) ADMINISTRATION OF CAPSAICIN.

Under ether anaesthesia, rat pups were injected subcutaneously with 0.1ml, 50mg/kg capsaicin (in 10% alcohol, 10% Tween 80, 80% saline) on the day of birth (postnatal day, PND, 0). They were then returned to the litter where they grew up and were weaned uneventfully until they reached adulthood.

3.2 (ii) ELECTROPHYSIOLOGICAL RECORDINGS.

On reaching adulthood, the rats were anaesthetized with urethane (1.5g/kg) and prepared for intracellular recording using the method described in detail in section 2.2 (i).

3.2 (iii) RECONSTRUCTION PROCEEDURES.

Camera lucida reconstructions and plan views showing the full rostrocaudal, mediolateral and dorsoventral area of afferent terminal arborizations were made. From these reconstructions, the centre point of the overlapping sheet of terminals of HFA's was determined and bouton counts were made from an area 100um either side of this point extending 200um ventrally from the top of the dorsal horn grey matter in a line parallel with the axis of orientation of the arborization in both capsaicin and control HFA's.

3.2 (iv) SHRINKAGE OF THE DORSAL HORN.

The shrinkage of the superficial dorsal horn (SDH) was studied using either acetylcholinesterase (AChE) or Cresyl fast violet according to the methods described in Paxinos and Watson (1982). Briefly, four control and four capsaicin rats were perfused with 1.25% glutraldehyde 1% parafamaldehyde in phosphate buffer (pH7.4) (section 2.2 ii) and the appropriate lumbar segments were identified, pinned, removed and cut at 80um for Cresyl fast violet and 50um for AChE staining. Sections were examined by light microscopy and measurements made (X135) of the area between the dorsal border of the dorsal horn and either the border between the outer and inner zones of lamina II, which was defined as the transition from the intensely stained, closely packed cells of IIo to the less compact inner zone (IIi) in Nissl material (Molander et al., 1984) or the ventral border of the densely stained AChE band. The measured area extended from a point 95um lateral from the medial border of the dorsal horn to a point 190um lateral to this point.

To check the effectiveness of capsaicin administration, at the end of the experiment animals were given 1ml of Evans blue via the carotid cannula and mustard oil applied to the hindpaw and the presence or absence of neurogenic extravasation observed. The resulting dye extravasation was scored over the following ten minutes on a scale of 0-10 where 0 was a failure of appearance of dye in the skin and 10 was a complete uniform colouration of the treated area. All untreated animals scored 10 using this method. Treated animals commonly scored between 2-6, indicating a substantial loss of C-fibres.

3.3 RESULTS.

3.3.1 CYTOLOGICAL ANALYSIS.

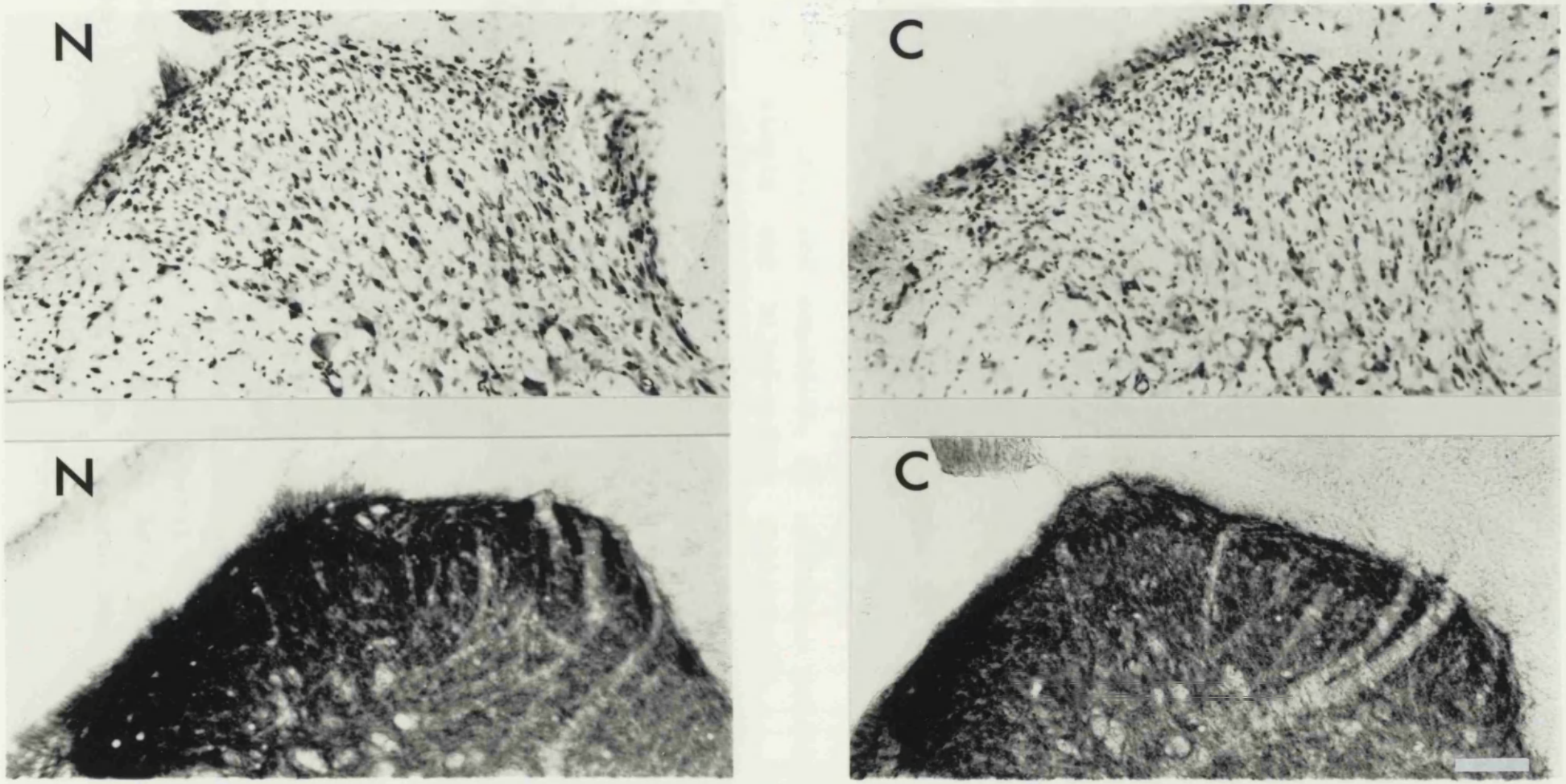
AChE staining was restricted to a dense band in laminae I-II of the dorsal horn and the motor neuron pools of the ventral horn in both control and capsaicin animals (Plate 7). To compare the sizes of these bands between the two groups, sample areas were measured (see methods) and compared. AChE labelled areas of spinal cord from sections pooled from 2 capsaicin animals (n=121 sections in total) were a mean of 25% and 27% smaller than in corresponding smaller than in corresponding spinal segments of 2 normal animals (n=118 sections in total). In the Nissl stained material (Plate 7), analysis of lamina II areas (see methods) of 68 sections from 2 capsaicin treated and 36 sections from 2 normal rats revealed mean area decreases of 8% and 23% in the capsaicin treated animals.

3.3.2 INTRACELLULAR ANALYSIS.

The central terminals of 11 HFA's and 3 RA's intracellularly injected with HRP in capsaicin-treated rats were recovered in the lumbar spinal cord after histological processing. Of the 11 HFA's, six had peripheral RF's on the leg and five on the hairy skin of the foot and toes. Of the RA's, two had RF's on the toes and one on the paw pad of the foot. The morphology of these capsaicin afferents were compared to intraaxonally stained control HFA's and RA's described in section 2.3.2, 2.3.3. As in control rats, the size of the capsaicin HFA's peripheral RF was not uniform, those on the proximal hindlimb being larger than those on the distal limb. However, there was no consistent difference in the RF size from comparable hindlimb areas in control and capsaicin rats both for HFA's and RA's. The receptive field properties of capsaicin afferents were indistinguishable

PLATE 7.

Photomicrographs of 50um thick Acetylcholinesterase stained (bottom panel) and 80um thick cresyl fast violet stained (top panel) transverse sections of lumbar spinal cord in control (N) and neonatally capsaicin-treated (C) rats. Scale bar 100um.


N
C
N
C

from those of control afferents and the conduction velocities of control and capsaicin treated afferents were also similar at 28.55±1.48 and 28.74±0.91 ms^{-1} (±SEM) respectively.

Both the control and capsaicin primary afferent axons bifurcated on entering the spinal cord into rostral and caudal projecting stem axons, with collateral arborization being given off at varying distances along the rostrocaudal extent. Overlapping terminal arbors were found in the middle of the HFA collaterals' rostrocaudal domain forming narrow saggital sheets of terminals (while this was rarely the case for control and capsaicin RA's). Arbors situated further rostrally or caudally along an axon's rostrocaudal extent (simple arbors) never overlapped in capsaicin and control HFA's and RA's.

3.3.2 (i) COMPARISON OF CAPSAICIN-TREATED AND CONTROL RAPIDLY ADAPTING AFFERENTS.

Both control and capsaicin RA afferents exhibited complex, simple and blind-ending collaterals. The morphology of the complex (Fig. 39) and simple terminal arborization of the capsaicin RA's was not obviously different from the control RA arborizations (comparison with Figs. 25-32). Terminals were distributed to the medial quarter of the grey matter (as in control RA's) in laminae III-IV with more boutons present in the deeper laminae rather than in the more superficial laminae. Only in 1 case (Fig. 39C) was a blind-ending terminal branch seen entering laminae IIo.

Analysis of the arrangement of collateral branches of capsaicin and control RA's (Table 11) showed that control RA's have slightly more collaterals per afferent than capsaicin RA's but that when the different types of collaterals were compared, the percentage of each class were similar as was the intercollateral distance between adjacent arbors. However, control RA's had a longer length of afferent stained (as measured from the most

FIGURE 39: Camera lucida reconstructions of eight complex arborizations of rapidly adapting (RA) afferents in rats that had been treated at birth with neonatal capsaicin. A-D are from a toe RA afferent while E-H are from a paw pad RA afferent. Scale bar 250um.

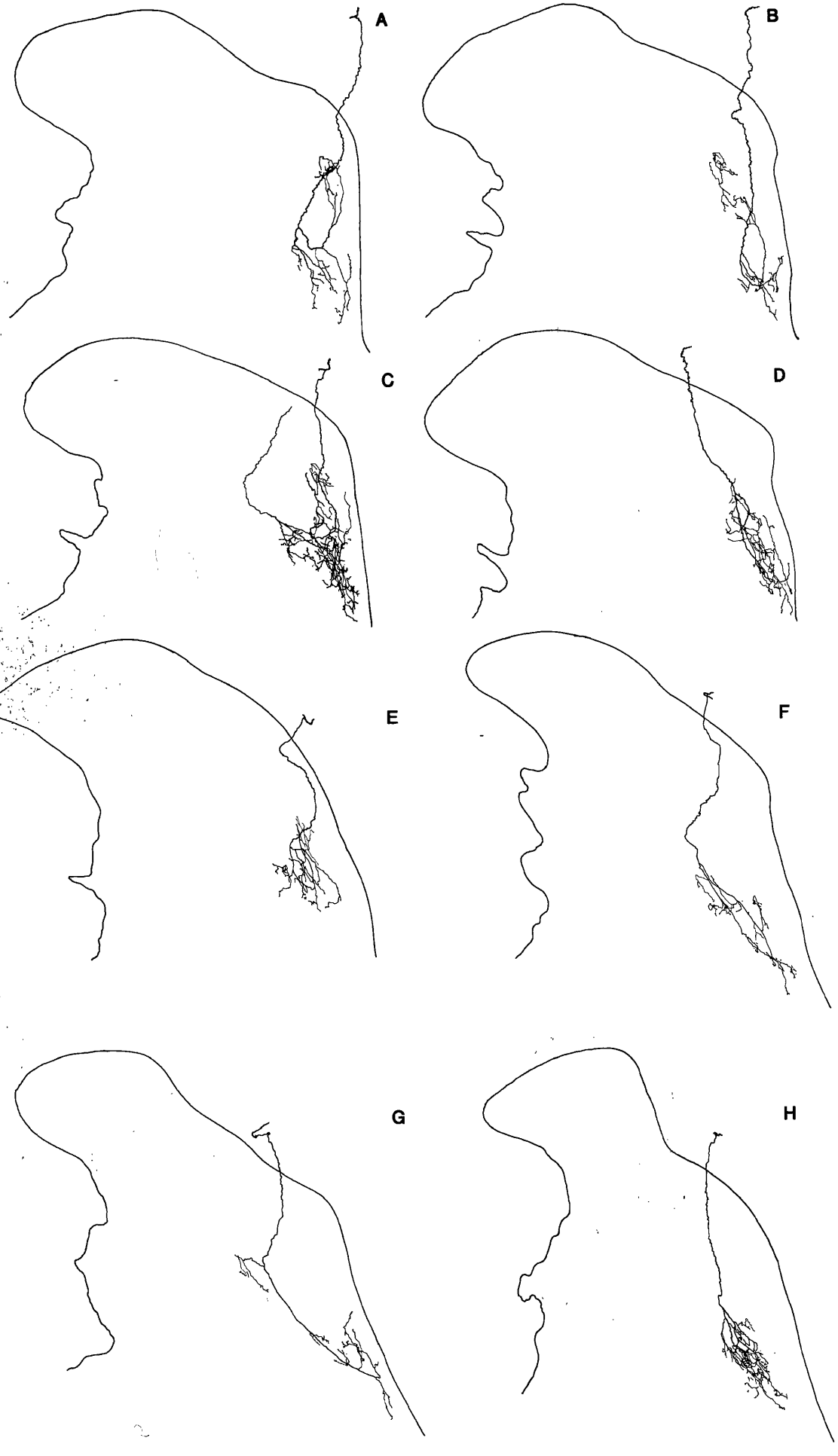

A
B
C
D
E
F
G
H

Table 11 The arrangement of collaterals of primary afferents in control and neonatally capsaicin treated rats.

	CAPSAICIN TREATED		CONTROL	
Type of Afferent	HFA	RA	HFA	RA
number of afferents	11	3	38	14
total number of collaterals	100	29	351	163
average no. collaterals/ afferent				
total	9.09±1.17	9.67±1.86	9.24±0.51	11.46±0.74
complex	4.64±0.75 (51%)	4.33±0.33 (44%)	4.37±0.27 (47%)	4.57±0.64 (40%)
simple	1.45±0.31 (15%)	2.67±1.20 (28%)	1.42±0.22 (15%)	3.5 ±0.64 (30%)
blind-ending	3.00±0.80 (34%)	2.67±0.88 (28%)	3.48±0.30 (38%)	350±0.44 (30%)
intercollateral distance (um)	340±29	290±21	343±11	296±15
length from most rostral to caudal collateral (mm)	2.77±0.34	2.52±0.54	2.94±0.15	3.07±0.2
length of overlapping terminal sheet (mm)	1.09±0.13	1.15±0.25	1.38±0.09	0.4±0.15

X±SEM

rostral to caudal collateral) than capsaicin RA's although there were many fewer capsaicin RA's to compare to the control RA's.

Analysis of the pooled dimensions of capsaicin and control RA's (Table 12) showed that the dimensions of the arborizations (complex and simple) were similar in the dorsoventral and mediolateral planes but significantly different ($P<0.05$ unpaired t-test) in the rostrocaudal direction when individual arbors were compared. When the average rostrocaudal dimensions /afferent was compared (Appendix II) then these values were not significantly different ($P<0.05$). However the volumes occupied by the arborizations were not significantly different ($P<0.05$). The difference in the rostrocaudal direction may be due to the fact that adjacent capsaicin RA collaterals overlapped more than control RA's but it was surprising considering that control RA's had a greater distance from most rostral to caudal collateral. However this result should be treated with caution when dealing with such a small population of capsaicin RA afferents.

The lack of change in morphology of capsaicin RA's and similarity of dimensions and arrangement of collaterals (Tables 11, 12) to control RA afferents suggests that neonatal capsaicin treatment has no effect on this group of primary afferents.

3.3.2 (ii) COMPARISON OF CAPSAICIN-TREATED AND CONTROL HAIR FOLLICLE AFFERENTS.

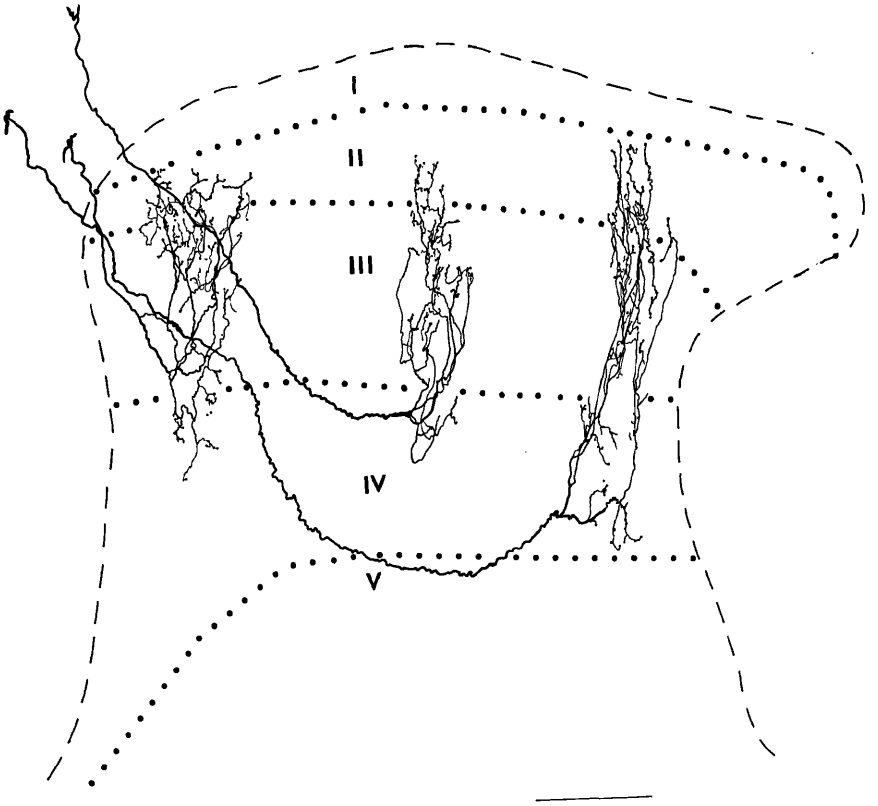
From camera lucida reconstructions (Fig. 40) it could be seen that neonatal capsaicin did not alter the characteristic morphology of HFA collateral arbors. Capsaicin afferents which had peripheral RF's on the leg and dorsum of the foot showed the typical "flame-shaped" arbors while hairy toe afferents exhibited a more variable morphology of collateral arborization, a pattern seen in the control toe afferents described in section 2.3.2 (iv).

Capsaicin HFA's displayed the same three types of

TABLE 12 DIMENSIONS OF THE TERMINAL ARBORIZATIONS OF CAPSAICIN TREATED RA AND CONTROL RA AFFERENTS.

	CAPSAICIN TREATED RA			CONTROL RA		
	C	S	C+S	C	S	C+S
N	13	8	21	65	49	114
ML (um)	92±11	80±24	87±11	113±6	55±6	88±5
DV (um)	264±21	147±14	219±19	236±13	127±13	189±11
RC (um)	312±30*	213±38	274±25*	233±11*	174±10	208±8*
VOL(ul) (X10^{-3})	7.7±1.4	3.5±1.9	6.1±1.3	7.0±1.4	1.9±0.5	5.4±0.6

X ± SEM
* = signif. diff. $P<0.05$ level
unpaired t-test.
ML=mediolateral, DV=dorsoventral, RC=rostrocaudal, VOL=volume.
C=complex, S=simple, C+S=terminal
Comparisons made were; C v C, S v S, C+S v C+S


CAPSAICIN
I
II
III
IV
V

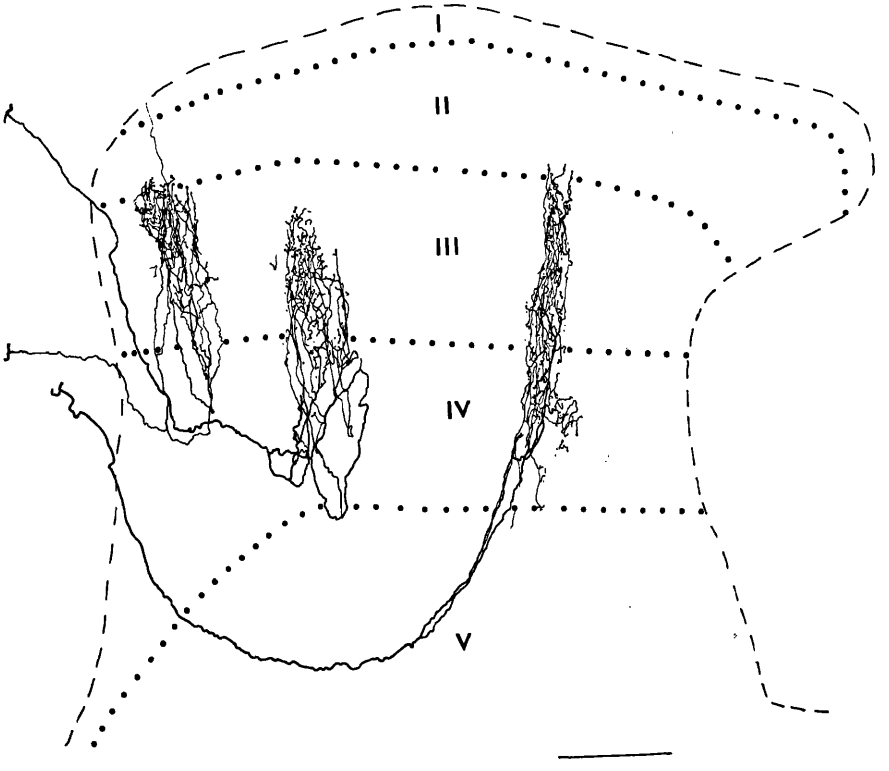
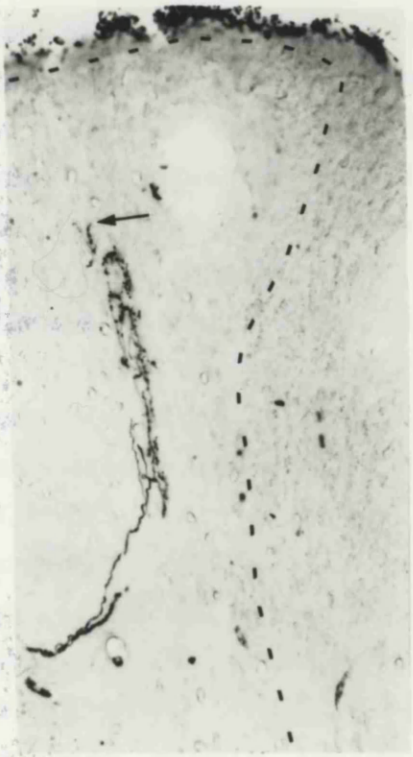

CONTROL
I
II
III
IV
V

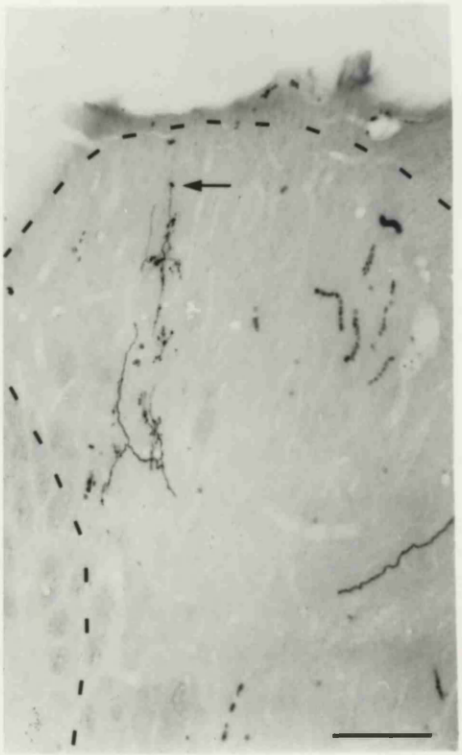
PLATE 8.

Photomicrographs of 50um thick transverse sections of lumbar spinal cord showing a complex terminal arbor from a control and a neonatally capsaicin-treated rat. The dashed line represents the outline of the dorsal horn and the arrows point to the most dorsal bouton in each arbor. Note in the capsaicin arbor the arrow is at a more superficial level in the dorsal horn than in the control. Scale bar. 100um.

CONTROL



CAPSAICIN



collateral arborizations described in detail in section 2.5.2 ie. complex (Plate 8), simple and blind-ending. The proportions of each type were similar for treated and control groups as was the arrangement of collaterals, length of overlapping terminal sheet and intercollateral distance (Table 11). Analysis of the dimensions of terminal arborizations of control and capsaicin HFA's showed that when the dorsoventral length, as measured from most dorsal to ventral bouton, of an arbor was compared, there was a significant difference ($P<0.05$, unpaired t-test) between capsaicin and control rats for lateral leg and foot and toe afferents (which were shown to be significantly different in the mediolateral dimension in section 2.3) while the rostrocaudal and mediolateral dimensions remained similar (Table 13). Capsaicin lateral leg HFA's showed an average 35% increase in arbor length while hindpaw HFA's (foot and toes) increased by an average 20% compared to control data. This increase was seen as a dorsal extension of the terminal branches and boutons into dorsal lamina II (Rexed's lamina IIo) from deeper laminae (Fig 40).

This dorsal extension occurred for simple and complex arborizations but not for blind-ending collaterals. The extension was most apparent for complex arborizations. Figure 41 shows 6 adjacent complex collateral arborizations of a capsaicin HFA in which dorsal extension of terminal branches and boutons had occurred following neonatal capsaicin treatment. Figure 41A-E shows adjacent overlapping complex collaterals which all had branches and boutons within lamina IIo whereas complex arbor F only reached as far as ventral lamina II (Rexeds lamina IIi) indicating that not all the complex arbors entered lamina IIo. Terminal branches and boutons were never found entering lamina IIo in control rats.

In control rats 53/220 (24%) of terminal arbors extended into lamina IIi whereas in capsaicin treated rats 26/65 (40%) reached this level. Furthermore, 20/65 (31%) of arbors in capsaicin treated rats extended terminal

TABLE 13 DIMENSONS OF HFA COMPLEX TERMINAL ARBORIZATIONS.

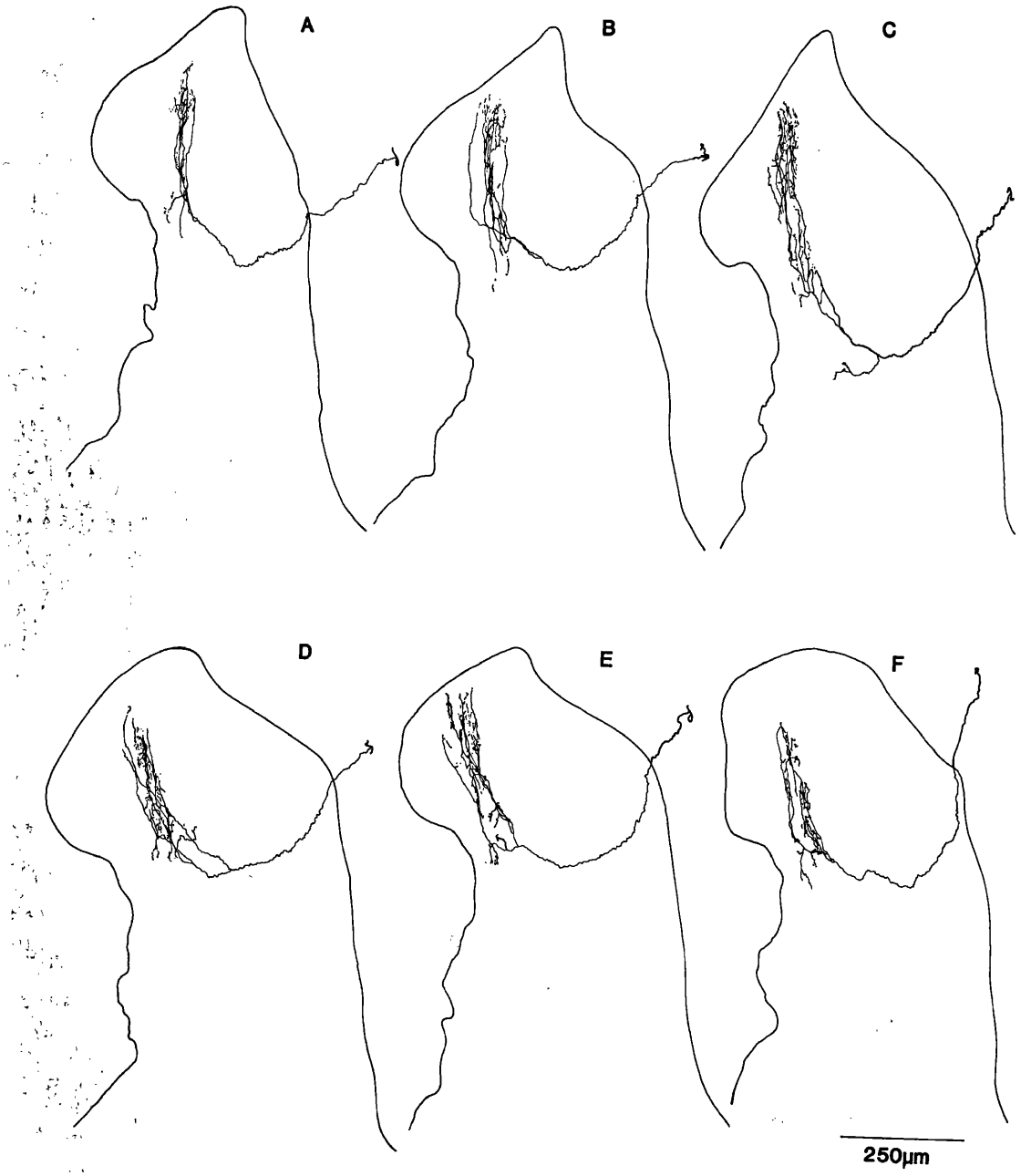
	CAPSAICIN TREATED RATS		CONTROL RATS	
	LEG (N=26)	FOOT & TOES (N=30)	LEG (N=48)	FOOT & TOES (N=42)
Mediolateral (um)	43±2*	53±4*	49±2*	75±1*
Rostrocaudal (um)	298±20	245±26	305±16	242±6
Dorsoventral (um)	173±15	154±11	111±9	123±6

x ± SEM.

* sig. diff. $P<0.05$ level unpaired t-test.

Comparison of capsaicin v control complex arbors.

FIGURE 41: Camera lucida reconstructions of six adjacent complex arborizations from rostral (A) to caudal (F) of a lateral leg HFA in a neonatally capsaicin treated rat. A-E represent collaterals with boutons within dorsal lamina II (II_o) whereas F has boutons in ventral lamina II (II_i).



branches and boutons into lamina IIo but in control animals HFA arbors never extended as far dorsally as this. The remaining terminal arborizations had a dorsal limit of lamina III and below. These represented the majority, 76% (167/220), of control HFA arbors but only 29% (19/65) of capsaicin HFA arbors. From this, it can be seen that 71% of terminal arborizations of capsaicin HFA's were located in lamina II compared to 24% of control HFA's.

Bouton counts from the centre of the overlapping terminal sheet of capsaicin and control HFA's (see methods) revealed that in control HFA's there were 90±12 boutons (n=5 rats) in the area analyzed compared with 115± 10 boutons (n=5, ± SEM) from the same area in capsaicin HFA's. This represents a mean 22% increase in the number of dorsally located boutons in neonatally capsaicin-treated rats. This was not significantly different ($P<0.05$, unpaired t-test).

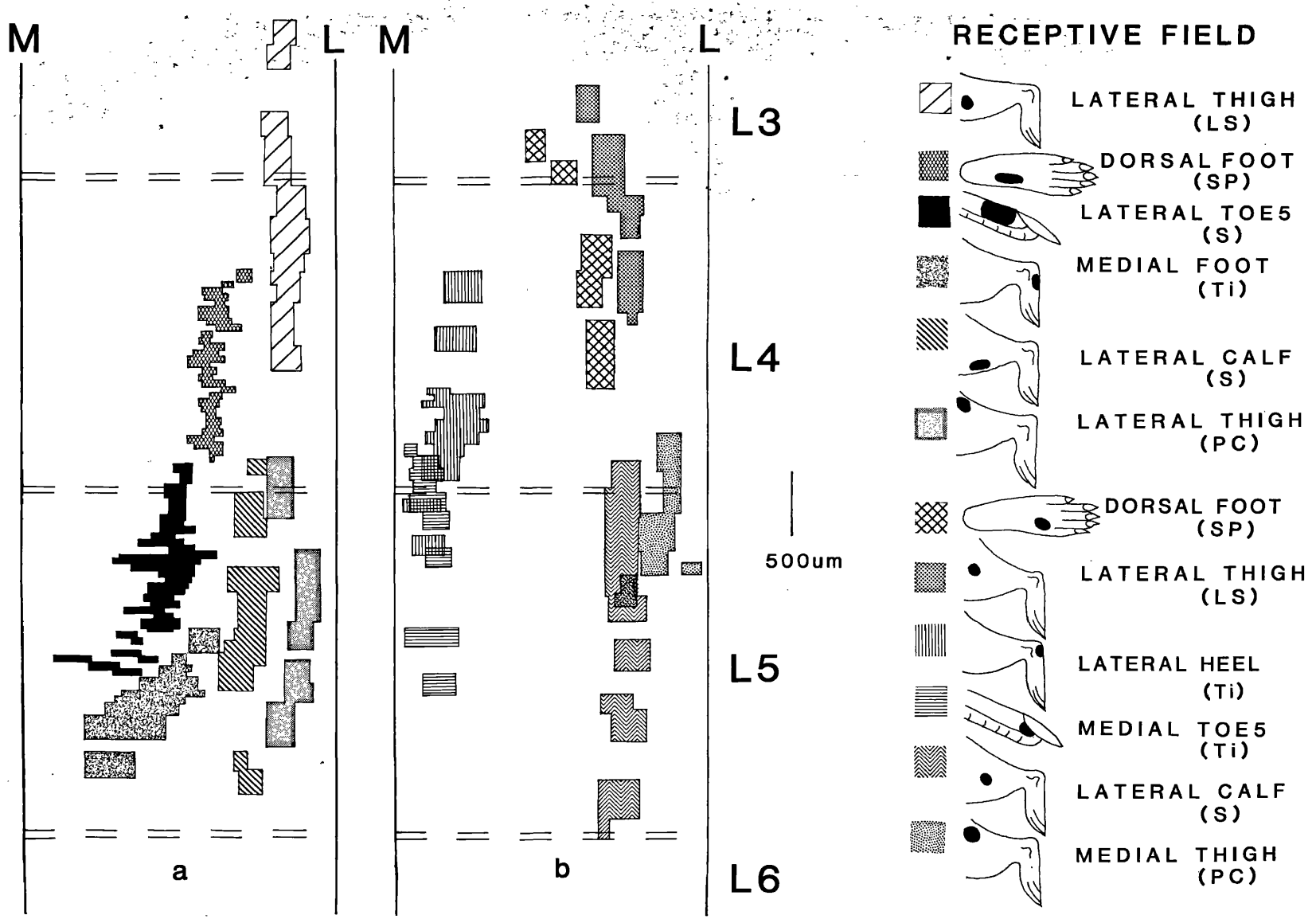
3.3.3 SOMATOTOPIC ORGANIZATION OF CAPSAICIN TREATED AFFERENTS.

The somatotopic organization of control and capsaicin HFA's with comparable peripheral RF's is shown in Fig. 42. It reveals that there was no difference in the somatotopic organization of primary afferents within the dorsal horn of capsaicin-treated and control rats. Each HFA and RA projected to the appropriate central terminal field area within the lumbar enlargement with respect to their peripheral receptive field location (Fig. 14) and occupied the appropriate mediolateral position within the dorsal horn.

3.4 DISCUSSION.

The destruction of up to 95% of unmyelinated primary sensory neurons by neonatal capsaicin removes the normal input from lamina II (the substantia gelatinosa) of the dorsal horn of the spinal cord. The results from the

FIGURE 42: Plan view at the level of lamina II_i of the terminal arborizations in the dorsal horn of HFA's from control (a) and neonatally capsaicin treated (b) rats with receptive fields as shown innervated by the Ti= tibial, S= sural, SP= superficial peroneal, LS= lateral sural, & PC= posterior cutaneous nerves. Abbreviations as in Fig. 15.


M
L
M
L
L3
L4
L5
L6
500um
a
b
RECEPTIVE FIELD
LATERAL THIGH (LS)
DORSAL FOOT (SP)
LATERAL TOE5 (S)
MEDIAL FOOT (Ti)
LATERAL CALF (S)
LATERAL THIGH (PC)
DORSAL FOOT (SP)
LATERAL THIGH (LS)
LATERAL HEEL (Ti)
MEDIAL TOE5 (Ti)
LATERAL CALF (S)
MEDIAL THIGH (PC)

present study show that this area is subsequently invaded by the terminal arborizations of cutaneous HFA's from lamina IIi and below but not by RA afferents.

Previous studies and the results described in section 2.3 using intraaxonal filling of physiologically identified cutaneous mechanoreceptors have shown that they have a distinct morphology and spatial termination pattern in the dorsal horn (Light and Perl, 1979b; Brown, 1981; Sugiura et al., 1986; Shortland et al., 1989a,b). Typically, HFA's arborize within laminae III-IV but some arborizations are found within lamina IIi (Woolf, 1987; Shortland et al., 1989a) while RA's arborize primarily in laminae III-V (Woolf, 1987) with a minority of collateral arborizations sending terminals into ventral lamina II (Shortland et al., 1989b). Studies using HRP bulk labelling techniques (Rethelyi et al., 1986; Nagy and Hunt, 1983) and Golgi staining (Beal and Knight, 1987) have reported invasion by myelinated fibres into the SG following neonatal capsaicin treatment but the fibre type and extent to which they invaded has remained unclear.

Nagy and Hunt (1983) have implicated A-delta or a mixture of A-delta and A-beta fibres as primary invaders while Rethelyi et al., (1986) suggested that HFA's invaded to the lamina IIo-IIi border in the lateral dorsal horn but possibly RA, SA type-II or PC afferents from glabrous skin invaded the medial dorsal horn as far dorsally as the grey-white border, implicating a qualitative difference in the projection from hairy versus non-hairy skin areas. The arborization patterns of invading afferents in Golgi studies led Beal and Knight (1987) to suggest that SA type-I and HFA's but not RA afferents invaded the SG. The results of the present study provide clear evidence that physiologically identified individual HFA's invade from deeper laminae to terminate throughout the mediolateral extent of laminae IIi and IIo of the dorsal horn and in these afferents boutons were found as far dorsally as the lamina I-IIo border but not within lamina I. However, physiologically identified RA afferents innervating the

glabrous skin in capsaicin rats were found not to invade the denervated SG region.

3.4 (i) SHRINKAGE OF THE DORSAL HORN.

When assessing the dorsal sprouting of HFA's in the dorsal horn, one of the possible problems was a retardation of growth of the superifical dorsal horn as a result of neonatal capsaicin treatment. Nagy and Hunt (1983) observed no shrinkage of the superficial layers following analysis of 1um coronal sections of cord stained with toluidine blue. Assessing changes in the dorsoventral depth of the superificial dorsal horn consisting of lamina I and II of Rexed (1952, 1954) was problematic, partly because the boders of these laminae were difficult to define and partly because the existing definitions may not apply following capsaicin treatment. The ventral border of lamina II can normally be determined by the occurrence of transversely cut fibres of lamina III (Ralston and Ralston, 1979; Snyder, 1982; Molander et al., 1984). Such fibres are relatively scarce in lamina II. Also, since these fibres may have sprouted into lamina II as a result of capsaicin treatment, a false impression of shrinkage may have occurred. For the same reason, the method of assessing the dorsoventral extent of the light band that can be seen in unstained clear sections due to light rarefraction of fat rich (myelin) and fat poor (unmyelinated neuropil in laminae II) areas was not employed here. Planimetric measurements of the dorsoventral width of laminae IIi and IIo in Nissl and AChE stained sections were used to assess shrinkage since the lamina IIi-IIo border was relatively easy to see (Molander et al., 1984) and since the AChE band does not change following dorsal rhizotomy (Hunt, 1983). The results showed a mean shrinkage of 8% and 23% in Nissl stained material and 25-27% in the AChE band.

AChE is well known to be associated with primary sensory neurons of vagal and dorsal root sensory ganglia

(Papka et al., 1981) and there is evidence in the chick spinal cord that it may function by hydrolysing SP (Chubb et al., 1980). AChE stain reduction following neonatal capsaicin treatment has been reported in guinea pig heart (Papka et al., 1981) but was unchanged in the sciatic nerve following ligation (McDougal et al., 1983). The reduction seen in the present study may have resulted from the depletion of AChE from intrinsic neurons within the dorsal horn as a result of removal of C-fibre input, or due to shrinkage and cell death of dorsal horn neurons following neonatal capsaicin treatment (Ribero da Silva and Coimbra, 1984; Saporta, 1986; Saporta and Jacobson, 1988) similar to that seen following neonatal peripheral nerve section (Fitzgerald and Shortland, 1988). These results only allowed approximate estimates of the amounts of shrinkage but they indicate that it had occurred and this may confound the results with regard to sprouting. However, the sprouting HFA's were seen to extend more dorsally than could be accounted for by shrinkage alone. Furthermore, the absolute dorsoventral length of the HFA arbors in capsaicin-treated rats was significantly higher ($P<0.05$), and coupled with the increased numbers of boutons in these afferents, these strongly suggest that sprouting of HFA's had occurred.

3.4 (ii) EFFECTS OF CAPSAICIN ON PRIMARY AFFERENT MORPHOLOGY.

The gross morphology of the HFA and RA arborizations was unaffected by neonatal capsaicin treatment. Control and capsaicin-treated HFA's had the classical "flame-shaped" arbors while control and capsaicin-treated RA's terminated in the medial quarter of the dorsal horn similar to those described in detail in the rat (Woolf, 1987; Shortland et al., 1989a,b). Capsaicin HFA's and RA's possessed the three different types of collaterals seen in normal low threshold cutaneous afferents described in detail in section 2.3 and these were present in similar

proportions. The slightly smaller percentages of simple and blind ending collateral arborizations in the capsaicin treated rats (Table 11) may possibly have been due to increased branching and bouton proliferation such that blind-ending collaterals became simple arbors and simple arbors became complex ones. Increased bouton numbers for HFA's treated with capsaicin seemed to confirm this. The mediolateral and rostrocaudal dimensions of the complex arbors of capsaicin HFA's were similar, only the dorsoventral length of the arbors were significantly different ($P<0.05$). Only the rostrocaudal length of RA afferents arborizations were significantly different ($P<0.05$) between control and capsaicin rats. The reason for this is unclear but the small number of RA afferents sampled in capsaicin rats may well affect this result since this significance ($P<0.05$) disappears when the average dimensions /afferent was compared, hence interpretation should be treated with caution. The analysis of HFA dimensions agrees with the results of Beal and Knight (1987). Complex arborizations consistently terminated in lamina IIo although not all complex arbors did. Beal and Knight (1987) found 54% of collateral arborizations invaded lamina II whereas we have found a much greater proportion (71%) perhaps reflecting differences in when the capsaicin was administered (day of birth in this study versus 2 days postnatally (Beal and Knight, 1987)) or differences in methodology.

Terminals were found to extend throughout lamina II across the mediolateral extent of the dorsal horn but never entered lamina I in this study. In this respect the results agree well with those of Nagy and Hunt (1983) and Beal and Knight (1987) but conflict with those of Rethelyi et al., (1986). These latter authors found an expansion of flame-shaped arborizations dorsally in the lateral sector of the dorsal horn into lamina IIi while medially, arborizations of a different type of primary afferent (possibly SA-II, PC or RA) sprouted up to the white/grey border. The earlier administration of capsaicin (PNDO

versus PND2) may have produced a greater denervation allowing invading afferents to terminate more dorsally but this does not explain the mediolateral difference in dorsal termination site observed by Rethelyi et al., (1986) but not by us in this study. Rethelyi et al. (1986) suggested that lamina I and II are possibly missing medially in capsaicin treated rats allowing invasion to the grey-white border. This seems unlikely since the dense AChE stain was seen to extend to the medial border of the dorsal horn in capsaicin treated rats (Plate 7). Rethelyi et al. (1986) used bulk labelling techniques and these label fibres which enter the dorsal horn through the dorsomedial border of the grey matter making it difficult to see terminals inbetween areas where the fibres sweep across through the grey matter. Also, cutting dorsal roots to label fibres (Rethelyi et al.,1986) causes degeneration within the dorsal horn (Kapadia and Lamotte, 1987) which may cause additional problems. It is clear from the present data that single RA fibres that terminate in the medial dorsal horn are normally found lamina IIi in control rats (section 2.5.3, Plate 5) and that the morphology and dimensions of their arborizations was unaffected by neonatal capsaicin, thus arguing against the findings of Rethelyi et al., (1986).

Nagy and Hunt (1983) have shown that SG invasion was predominantly by A-delta fibres whereas this intracellular study shows that invasion was by certain types of low threshold cutaneous A-beta afferents. As A-delta afferents were not filled in this study (A-delta cutaneous afferents have yet to be filled intracellularly in the rat). The possibility that these fibres also sprout into SG cannot be ruled out. It was not possible on the basis of conduction velocity alone to distinguish between low and high threshold A-delta fibres as they overlap considerably (Light and Perl, 1979b) but based a differing termination patterns in the cat and monkey (Light and Perl, 1979b) of the two types of afferent, one may expect that it is the low threshold A-delta afferents

(which are known to terminate in lamina IIi) which may have the opportunity to sprout into the denervated SG rather than the high threshold A-delta fibres which terminate in lamina I and V. However, as lamina I is partially denervated, possible sprouting of high threshold A-delta fibres cannot be ruled out, although these fibres run in a transverse plexus across the mediolateral surface of the superficial dorsal horn (Beal and Bicknell 1981). Doses of 50mg/kg neonatal capsaicin are sufficient to destroy some A-delta fibres (Nagy et al., 1981, 83; Hiura and Sakamoto, 1987b) less than 5um in diameter. Although this phenomenon seems variable and occurs primarily when 85-95% of unmyelinated fibres are destroyed (Lynn, 1984), it raises questions as to which physiological class of A-delta fibre they belong (low or high threshold) and may consequently have implications for the sprouting of surviving A-delta afferents.

3.4 (iii) SOMATOTOPIC ORGANIZATION OF SURVIVING PRIMARY AFFERENTS.

The somatotopic organization of the surviving primary afferent fibres did not appear to have been affected. The results of this study showed that capsaicin HFA's and RA's projected to the correct nerve territory in the lumbar enlargement (Swett and Woolf, 1985) and to the appropriate mediolateral position within the dorsal horn (Shortland et al., 1989a). This agrees with the results of Tattersall et al., (1987) who found no expansion of sural nerve afferents into adjacent nerve territory following neonatal treatment. However, this would be expected since the deafferentation produced here does not produce adjacent vacant synaptic sites, rather it produces vacant synaptic sites above the low threshold afferents, so that the only possible direction for sprouting would be upwards.

3.4 (iv) ELECTROPHYSIOLOGICAL PROPERTIES OF PRIMARY AFFERENTS IN CAPSAICIN TREATED RATS.

The electrophysiological properties, RF sizes and conduction velocities (CV's) of capsaicin and control HFA's and RA's were similar which agrees with the results of Lynn (1984) and Baranowski and Lynn (1985). However, Lynn (1984) did find that capsaicin HFA's had larger RF's compared to control rats but that this was only in male rats. Baranowski and Lynn (1985) did not find any differences in RF sizes, and CV's for G and D saphenous HFA's in capsaicin and control rats. Our results are supported by the findings of Millard and Woolf (unpublished observation) that the peripheral innervation of HFA's, studied using silver staining techniques were unaffected by neonatal capsaicin treatment.

3.4 (v) MECHANISMS OF SPROUTING.

The temporal sequence of development of afferent fibres may explain the HFA invasion of the denervated region after capsaicin treatment. It has been previously shown that flame-shaped arbors are present in lamina III of the dorsal horn at E19 and that they develop in a ventral to dorsal manner (Beal et al., 1988). A-delta and C-fibres enter the dorsal horn on E19.5-E20 (Fitzgerald, 1987) with terminal elaboration occuring in the late fetal and early postnatal period (Pignatelli et al., 1989; Fitzgerald, 1987). Their arrival may act as a block and prevent A-fibres extending more dorsally than lamina IIi. However, if the C-input is removed, as happens with capsaicin treatment, this may allow the A-fibres to grow more dorsally.

There may be factors other than spatial ones which trigger sprouting. Breakdown products of terminal degeneration following treatment may act as a neurotrophic factor attracting HFA arbors dorsally. Another possibility is that sensory neurons require nerve growth

CHAPTER 4: PLASTICITY IN SOMATOSENSORY SYSTEMS.

(B) PERIPHERAL NERVE SECTION.

4.1 GENERAL INTRODUCTION.

The word "plasticity" has been sometimes used in the literature to mean the ability of a damaged axon to regrow to its original terminal site and, hence, has become synonymous with regeneration following CNS injury. Axon damage in the form of a crushed or cut nerve is followed by regeneration and reinnervation in the periphery (Jansco and Kiraly, 1983; Jackson and Diamond, 1984; Nixon et al., 1984; Pomeranz et al., 1984; Shea and Perl, 1985; Brenan, 1986; Sanders and Zimmerman, 1986; Kinnman and Aldskogious, 1986, 1988; Doucette and Diamond, 1987; Kinnman, 1987; Pertovaara, 1987; Brennan et al., 1988; Chiaia et al., 1988; Wiesenfeld-Hallin and Kinnman, 1988) and some central sprouting of the damaged terminals has been observed within the CNS (Molander et al., 1988; Lamotte et al., 1989) but as yet no genuine recovery of function due to regrowth of damaged axons into the CNS has been demonstrated. The environment surrounding the nerve cells, especially glial cells, plays an important role in preventing CNS axon regrowth (Carlstedt et al., 1987; Carldstedt, 1988; Bahr et al., 1989; Wells, 1989). A wider definition of plasticity will be used here, that is alterations in the connections between neurones of the CNS in response to changes in the internal or external environment. This may involve the formation of new connections (Fitzgerald and Woolf, 1987; Wilson et al., 1987; Wilson and Snow 1988b) or alterations in the effectiveness of existing ones (Wall, 1977, 1981, 1986).

Two important aspects of plasticity have to be considered: firstly, the time course over which plastic changes occur ranges from minutes (Wall and Woolf, 1984; Cook et al., 1987; Woolf and King 1988) to days, weeks, months (Basbaum and Wall, 1976; Devor and Wall, 1981a,b;

Wilson and Snow, 1987, 1988a,b); secondly, the age of the animal (neonatal versus adult) affects the response to an experimental manipulation and also the mechanism by which the plasticity occurs. The central change in an immature nervous system appears to be far greater than that of a mature nervous system.

4.1 (i) PLASTICITY IN THE ADULT SOMATOSENSORY SYSTEM.

A large number of electrophysiological studies have been published demonstrating that the somatotopic organization of dorsal horn receptive fields within the somatosensory system can be altered following partial deafferentation. The bulk of these have used the standard paradigm, introduced by Wall and Egger (1971) whereby firstly a 2 dimensional map of the body surface was defined by mapping neuronal receptive fields in a given somatosensory area and then the afferent input to part of the map was deleted by surgical procedures. Early studies (Lui and Chambers, 1958; Basbaum and Wall, 1976; Pubols and Brenowitz, 1981, 1982; Brown et al., 1983; Rodin et al., 1983) used the spared-root preparation while others used peripheral nerve lesions (Dostrovsky et al., 1976; Wall, 1977; Devor and Wall, 1978, 1981a,b; Mendell et al., 1978; Pubols and Goldberger, 1980; Merzenich and Kaas, 1982; Lisney, 1982, 1983; McMahon and Wall, 1983; Sedivec et al., 1983, 1986; Brown et al., 1984; Markus et al., 1984; Pubols, 1984; Rodin and Kruger, 1984; Fitzgerald, 1985; Fitzgerald and Vbrova, 1985; Wilson and Snow, 1986, 1987; Devor et al., 1986; Markus and Pomeranz, 1987; Wilson, 1987).

The observation common to most of the electrophysiological studies was that following deafferentation, the cells lost their normal peripheral RF but in time acquired a new RF in the nearest neighbouring intact part of the somatotopic map. However, some laboratories have failed to find this rearrangement of the somatotopic map (Pubols and Goldberger, 1980; Brenowitz

and Pubols, 1981; Pubols and Brenowitz, 1982; Brown et al., 1983, 1984; Pubols, 1984). Differences between anaesthetic agents, recording electrodes, sampling different classes of DHN's and most importantly mapping methods may all have contributed to the conflicting results (Pubols, 1984). Another possible explanation arose from the study of Wilson (1987) who repeated the experiments of Devor and Wall (1981a,b) and found that although medially situated neurons did not acquire low threshold tactile RF's on proximal skin after chronic denervation they did become responsive to activity from deep receptors and to afferents with large high threshold RF's on the proximal limb. This indicated a time dependent reorganization of peripheral input to DHN's involving high threshold A-delta and C-fibres rather than A-beta afferent fibres, similar to that seen by Sedivec et al. (1983). However, these experiments gave no indication about the mechanism of this rearrangement.

A key question behind the appearance of novel inputs to central cells following deafferentation was whether these inputs were in fact new ie. were from collateral sprouting of surviving intact afferent fibres or whether they resulted from the unmasking of synapses which were normally present but held ineffective by some mechanism(s) (Wall, 1977).

In 1958, Lui and Chambers published results demonstrating sprouting of primary afferent collaterals in the dorsal horn following partial deafferentation by dorsal rhizotomy in the adult and this was supported by some (Goldberger and Murray, 1974, 1978, 1982) but not by others (Kerr, 1972; Rodin et al., 1983; Rodin and Kruger, 1984). Most of the evidence for sprouting in the adult spinal cord has come from light microscopic methods which have demonstrated an increased projection of intact pathways following loss of other pathways. It has to be shown that the lesion induced increase in a projection, which is taken as evidence for sprouting when light microscopy is used, is associated with new synapse

formation by the spared axonal systems. The evidence for this is poor. All studies of physiological and transmitter changes which occur following deafferentation are open to interpretations other than sprouting such as release from inhibition (Brown, 1987). Reorganization of central terminals has also been examined by studying the effects following peripheral nerve section on primary afferent terminal fields using anatomical labelling of the cut nerve and its nearby intact neighbours (Rodin et al., 1983; Rodin and Kruger, 1984; Stelzner and Devor, 1984; Sugimoto and Gobel, 1984; Goldberger and Murray, 1985; Devor et al., 1986; Murray and Goldberger, 1986, 1987; Hallas et al., 1987; Wilson et al., 1987; Arvidsson and Johansson, 1988; Bullit et al., 1988; Molander et al., 1988; Pubols and Bowen, 1988; Rasmusson, 1988; Lamotte et al., 1989). The results here are somewhat equivocal, and there is evidence both for and against rearrangement of central terminal fields. Most of the negative evidence has come from bulk labelled nerve studies with HRP (Rodin et al., 1983; Rodin and Kruger, 1984; Stelzer and Devor, 1984; Hallas et al., 1987; Pubols and Bowen, 1988; Rasmusson, 1988) which have failed to find sprouting or rearrangements of terminal fields of nearby intact nerves that could account for the observed novel RF's seen electrophysiologically.

However, more recent studies have indicated a rearrangement of terminals (Bullit et al., 1988) following anterolateral chordotomy by bulk labelling methods. Sprouting of terminals has been observed in the adult following dorsal rhizotomy has been observed by electron microscopy (Goldberger and Murray, 1985; Murray and Goldberger, 1986, 1987). Other anatomical studies have indicated an expansion of intact terminal fields in the adult spinal cord following peripheral nerve interference (Molander et al., 1988; Lamotte et al., 1989). However, this has necessitated "priming" of intact nerves by previous crush (Molander et al., 1988) or total destruction of a nerve by proteolytic enzymes (Lamotte et

al., 1989) rather than by nerve section. While these new results suggest the adult spinal cord has the capacity for plastic rearrangement following injury, the ability to do so depends on a critical balance between too little and too much deafferentation and on the maturity of the system not only in terms of the ability to sprout but also in terms of neuron vulnerability and to the degree of dendritic and glial response (Lamotte et al., 1989). In conclusion, although there is evidence for anatomical rearrangement of terminals following deafferentation, it cannot be the full explanation for the observed changes in receptive field organization within the dorsal horn.

The alternative to sprouting as the basis of plasticity in the adult dorsal horn has been derived from work by Wall and his colleagues (Merrill and Wall, 1972, 1978; Basbaum and Wall, 1976; Wall and Werman, 1976; Wall, 1977, 1981; Mendell et al., 1987; Pomeranz, 1987). Their suggestion was that the central changes seen were not due to the formation of new connections but to modulations of strength of preexisting but ineffective afferents whose presence could be demonstrated only when they were synchronously activated by, for example, electrical stimulation (Markus et al., 1984; Pubols et al., 1986; Markus and Pomeranz, 1987; Pomeranz, 1987). The lesion induced an alteration of some kind which increased the efficacy to these synapses. These changes may have been the disappearance of presynaptic inhibition, or changes within primary afferent terminals themselves, or postsynaptic changes in the physiology and morphology of neurons (Wall, 1977).

This proposal has received support from anatomical evidence at the intracellular (Meyers and Snow, 1984) and electron microscopic level (Devor et al., 1986). Meyers and Snow (1984) examined the morphology of the projection of single identified primary afferents to somatotopically identified regions of dorsal horn and showed that 42% of afferent collaterals projected to regions where the dorsal horn cells had RF's quite different from that of the

identified afferent. They also showed that at least some of these somatotopically inappropriate collaterals were invaded by action potentials (Meyers and Snow, 1986). Devor et al., (1986) showed by light and electron microscopic studies a population of large diameter axons from thigh afferents that swept through the medial dorsal horn on their way laterally which made axodendritic contacts along the way and as such were good candidates for somatotopically inappropriate connections which could be strengthened following partial deafferentation.

4.1 (ii) PLASTICITY OF THE SOMATOSENSORY SYSTEM IN THE NEONATE.

Peripheral nerve lesions in the neonate cause considerable disruption of the normal somatotopic map and rearrangement of this map by expansion of the surviving intact afferents. This has been demonstrated in the CNS in the spinal cord (Fitzgerald, 1985; Fitzgerald and Vrbova, 1985; Heath et al., 1986; Fitzgerald and Woolf, 1987; McNeill and Hulsebosch, 1987; Smith and Frank, 1988; Wilson et al., 1987; Wilson and Snow, 1988a, b), cuneate nucleus (Kalaska and Pomeranz, 1982); trigeminal nuclei (Jacquin and Rhoades, 1983, 1985, 1986; Rhoades et al., 1983, 1989; Mooney et al., 1987; Renehan et al., 1988) and in the somatosensory cortex (Killackey et al., 1978; Killackey and Shinder, 1981; Chimelli and Scaravilli, 1985; Dawson and Killackey, 1987; Wall et al., 1988). Neonatal nerve section also exerts profound changes on the numbers and size of surviving neurones (Aldskogius and Risling, 1981, 1983; Hulsebosch and Coggeshall., 1981,1983; Trune, 1982a, b; Schmalbruch, 1984, 1987; Yip et al., 1984; Heath et al., 1986; Campbell and Frost, 1987; Fitzgerald and Shortland., 1988; Garraghty et al., 1988; Klein et al., 1988; Himes and Tessler, 1989) resulting in ganglion cell death and central terminal degeneration. In older animals, ganglion cells may survive following nerve section (Devor et al., 1985)

resulting in elimination of fewer central terminals. The net outcome of the removal of a particular afferent input was that the surviving intact afferents expanded into the denervated region (Fitzgerald, 1985; Dawson and Killackey, 1987; Rhoades et al., 1989). This expansion represents a way in which the connectivity of neuronal circuits achieved during development can be modified following altered or removed input. This has been observed as collateral sprouting of intact primary afferents (Campbell and Frost, 1987; Fitzgerald and Woolf, 1987; Garraghty et al., 1988; Snow and Wilson, 1988; Wilson and Snow, 1988b) and a recovery of peptides following initial depletion after nerve injury (McGregor et al., 1984; Fitzgerald and Vrbova, 1985; McNeill and Hulsebosch, 1987; Leah et al., 1988; Rhoades et al., 1988; Enfiejian et al., 1989; Himes and Tessler, 1989; Kniyhar et al., 1989). This expansion is much more profound in the immature animal compared to the adult and the phenomenom of sprouting appears to play an important role in the plasticity seen in the CNS of neonates.

The ability to sprout is critically dependent on the maturity of the CNS (Fitzgerald and Vrbova, 1985; Jacquin and Rhoades, 1985). For example in the rat trigeminal system afferent sprouting can be demonstrated if the lesion is made fetally but not if made neonatally (Rhoades et al., 1989). Evidence is now beginning to emerge that following neonatal nerve section that the somatotopically ineffective projections of primary afferent fibres are stimulated to sprout by proliferation of intact afferent fibres (Fitzgerald and Woolf, 1987; Wilson and Snow, 1988b) and that these sprouts make functional connections in the dorsal horn (Shortland and Fitzgerald in preparation).

In this section, this sprouting was examined in detail using intraaxonal injection of HRP into physiologically characterized primary afferents following hindlimb peripheral nerve section on the day of birth in rats.

4.2 SPROUTING OF LOW THRESHOLD CUTANEOUS MECHANORECEPTORS FOLLOWING NEONATAL PERIPHERAL NERVE SECTION.

Previous studies using bulk labelling methods with HRP have shown sprouting of hindlimb dorsal root afferents after hindlimb preipheral nerve lesion in the neonate, but a failure to sprout in the older animal (Fitzgerald, 1985). A similar observation was made in the kitten trigeminal nucleus (Kerr, 1975). The studies of Fitzgerald (1985) and Fitzgerald and Vbrova (1985) showed an expansion of the HRP labelled saphenous terminal field into the sciatic territories of medial and lateral L3 and rostral L4 following early (before P6) sciatic nerve section. Fluoride resistant acid phosphatase (FRAP) containing saphenous terminals also sprouted into the sciatic terminal zone, restoring FRAP levels in this region. The aim of the present study was to investigate this saphenous field expansion at the single fibre level and to investigate the influence of central and peripheral targets in determining the morphology of the collateral sprouts.

In the earlier section (section 2.3) it was shown that cutaneous primary afferents innervating hindlimb skin in the rat have a distinctive morphology and somatotopic organization. HFA's of the saphenous nerve terminate in the dorsal horn as flame-shaped arborizations running rostrocaudally as an overlapping terminal sheet extending from caudal L2 to rostral L4, while HFA's from the sciatic nerve innervating the hairy skin of the lateral leg and foot and toes were located in the lateral dorsal horn in L3 and extended caudally to the L5/6 border avoiding the medial third of L4 and L5 which was occupied by the central terminals of RA afferents of the paw pads and toes.

Using intraaxonal injection of HRP into saphenous HFA's following neonatal sciatic nerve section and into glabrous RA afferents following neonatal saphenous, sural and superficial peroneal nerve section, the extent and

pattern of the new terminal arborizations has been investigated.

4.3 METHODS.

4.3 (i) NEONATAL NERVE SECTION.

Wistar or Sprague Dawley rat pups of both sexes were removed from the litter on the day of birth (P0) and anaesthetized with ether or by cooling to 4^{0}C on ice. For animals in which the sciatic nerve was to be cut and ligated, the sciatic nerve was exposed on the left hand side by a mid thigh level incision. The muscle layers were parted to reveal the sciatic nerve which was ligated with 5-0 mersilk and then cut distal to the ligation. The incision was then sutured and the pups allowed to recover from the anaesthesia (by warming under a lamp for pups cooled on ice). The posterior cutaneous nerve remained intact in sciatic cut rats.They were then returned to the litter where they grew up and were weaned uneventfully until they reached adulthood. For animals in which the tibial nerve was to be spared, the sural, superficial peroneal and saphenous nerves were ligated and cut on the left hand side. The saphenous nerve was exposed by a medial mid thigh incision and the nerve was ligated, care being taken not to ligate the blood supply which runs adjacent to the nerve, and cut and the wound sutured. To expose the common peroneal, and sural nerves (which are branches of the sciatic nerve) the sciatic nerve was exposed at mid thigh level as for the sciatic cut animals and then the nerve branch points identified and the appropriate nerve branches (which are very difficult to see at this early age) ligated with 5-0 mersilk and cut distally. The wounds were sutured and the animals allowed to recover and then returned to the litter where they grew up uneventfully to adulthood. All surgical proceedures were carried out under aseptic conditions.

4.3 (ii) INTRACELLULAR RECORDING.

Operated rats were checked as adults for peripheral nerve regeneration of the cut nerves. Noxious pinch was applied to the saphenous, sural and common peroneal skin areas to see if a hindpaw withdrawal reflex was elicited in the case of tibial intact rats and to the sciatic nerve territory in the case of saphenous nerve intact rats. The adult rats were then anaesthetized with either urethane (1.5g/Kg) or ether and then their trachea and one carotid artery cannulated. For rats initially anaesthetized with ether anaesthesia was maintained with Althesin (alphaxalone 0.9%, alphadalone 0.3%, Glaxo) given intraarterially in 0.1ml doses until the rat had been decerebrated. Decerebration and preparation for intracellular recording was identical to that described in section 2.2 (i).

4.4 RESULTS.

The saphenous terminal zone is known to be surrounded laterally and caudally by sciatic nerve terminals of the hairy skin of the lateral (LS/S) and dorsal (SP) surface of the leg (H in Fig. 44a) and medially by sciatic nerve terminals from the glabrous skin of the foot (Ti, marked G in Fig.44a). The caudal border of the SA terminal field lies at the mid-L4 segment (Figs. 20, 44a). In L4, the SA arbors never normally extend to the medial edge of the dorsal horn.

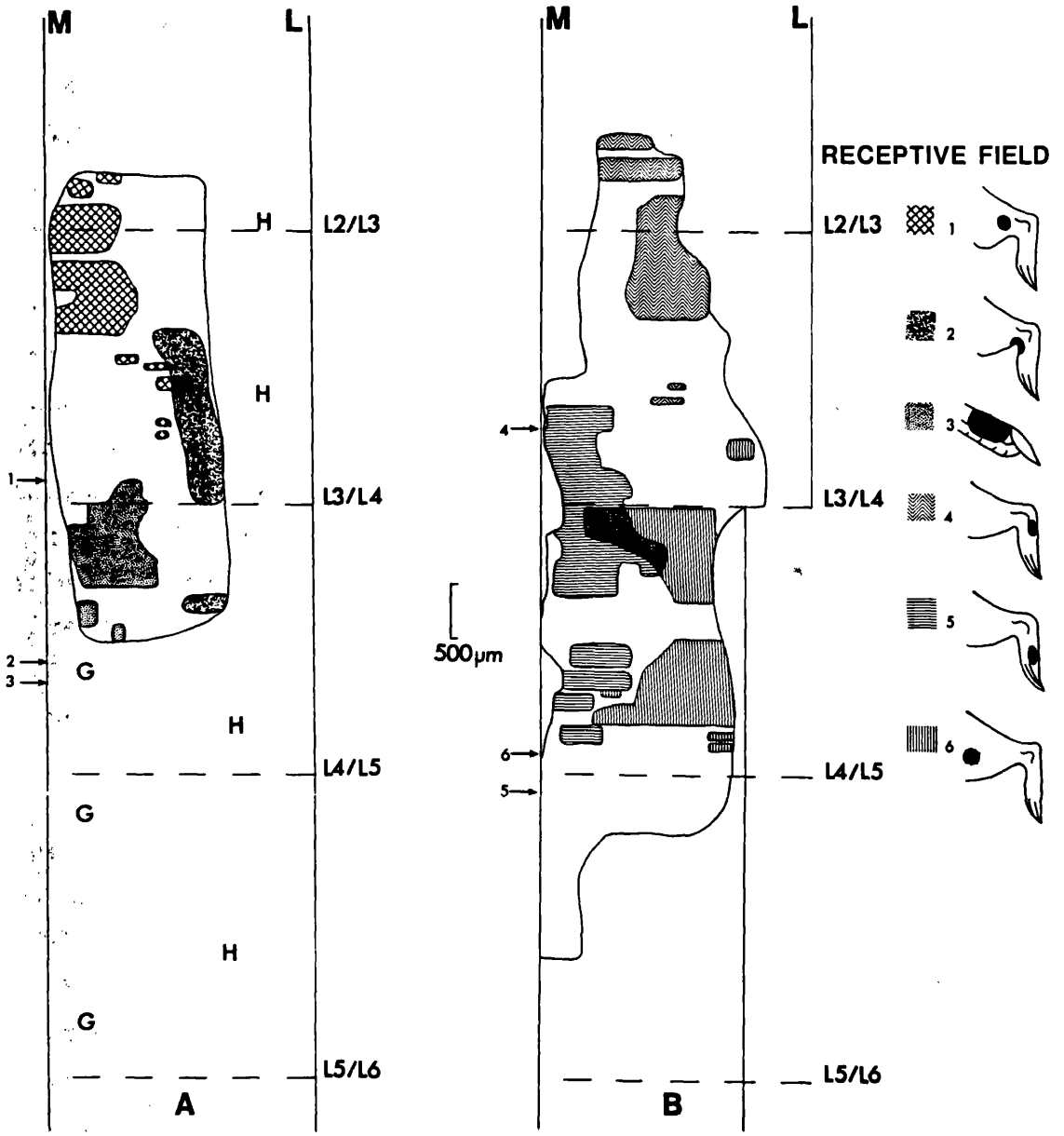
4.4.1 SAPHENOUS NERVE AFFERENT SPROUTS.

Twenty saphenous HFA's with RF's on the medial leg, foot or toes 1,2 were recovered in rats whose sciatic nerve had been cut at birth and shall be termed as "saphenous intact" HFA's. These were compared to the 10 saphenous HFA's whose morphology, dimensions and terminal distribution was described earlier (section 2.3.2 ii). As

can be seen from Fig. 44a, the most caudal extent of normal saphenous HFA terminals was rostral L4. Of the twenty HFA's filled in rats whose sciatic nerve had been cut at birth, fourteen were found to have grown into the area of cord normally occupied by sciatic nerve terminals with densely arborizing collaterals with boutons at least 1000um caudal to mid L4 (Figs. 43 and 44b). Six afferents had terminals located at least 2000um caudal to the normal saphenous terminal area, in mid L5. In four cases these terminals were of the complex variety while in two they were simple arborizations. In the control SA afferents, the dorsal root entry zone (DREZ) was in, or rostral to, the rostral L4 dorsal root while only 1/18 of the SA intact afferents had a DREZ entering caudally to this root.

The apparent trigger for saphenous intact HFA's to sprout was related to the proximity of their central terminals in the dorsal horn to the denervated sciatic terminal zone. Of the 20 saphenous intact HFA's recovered, two were considered as incomplete fills because of the lack of density of staining and scarcity of collaterals. However, those collaterals that were present were abnormal and located in mid-L4. The remaining 18 well filled HFA's were initially compared as a single population to the control SA afferents (Appendix III), but were then split into two groups based on the number of collaterals located in the normal saphenous nerve territory (ie. afferents which had the majority of collaterals restricted to the L3 segment and distant from the sciatic region). Six of the eighteen afferents were found in L3 (the normal SA area) while the other twelve caudally positioned saphenous intact afferents possessed collaterals which terminated in the region normally exclusively occupied by the terminals of sciatic nerve afferents. The control SA afferents were also split into those with the majority of collaterals rostral to the L3/4 (n=7) and caudal to the L3/4 (n=3) so that comparisons of dimensions could be made without biasing the sample

FIGURE 43: A diagrammatic representation of the rostrocaudal extent of a single normal saphenous HFA in the L3 and L4 dorsal horn obtained from a HRP filled afferent (top) and a diagrammatic representation of the rostrocaudal extent of a single saphenous intact HFA that had sprouted into the deafferented sciatic terminal zone (bottom). Note the normal flame shaped arbors in L3 and the bouton containing sprouts in L4. Scale bar 500um.


M
L
H
L2/L3
H
1
L3/L4
2
3
G
H
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G
H
G
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A
M
L
L2/L3
4
L3/L4
500µm
6
5
L4/L5
L5/L6
B
RECEPTIVE FIELD
1
2
3
4
5
6

populations towards the experimental animals (Appendix III).

4.4.1 (i) MORPHOLOGY OF SAPHENOUS COLLATERALS ROSTRAL TO THE L3/4 BORDER IN SCIATIC CUT RATS.

Saphenous intact afferents with the majority of terminals restricted to the L3 segment showed the normal "flame-shaped" arbors (comparison of Figs. 45 and 46). Afferents whose central terminals were located in mid-L3 (Fig.46) and distant from the sciatic region showed no sprouting. Afferents whose terminals were located in caudal-L3, rostral-L4 were slightly different in that although all the collaterals exhibited the flame-shaped arbors, the saphenous intact HFA's had significantly wider complex arborizations than their control counterparts (Figs. 47, 48, Table 15) when individual arbors were compared. However, when the average mediolateral width of the complex arbors /afferent was analyzed (Appendix III, Table 15A) this significance disappears although the arbors were 15% wider. Occasionally some ventrally directed terminal branches with boutons were seen (Fig. 47 D,E; 48B). In all other respects the saphenous HFA's rostral to the L3/4 border were similar to control saphenous HFA's (Table 14).

4.4.1 (ii) MORPHOLOGY OF SAPHENOUS HFA's WITH COLLATERALS CAUDAL TO THE L3/4 BORDER IN SCIATIC CUT RATS.

Equal numbers of saphenous intact HFA's sent sprouts into the glabrous (n=6) and hairy skin (n=6) zones of the sciatic area. The appearance of the sprouted collateral arbor depended whether it lay in the hairy or glabrous part of the sciatic denervated zone (Figs. 49, 50). Collaterals in that part of the L4 segment which normally contained saphenous and sciatic HFA terminals resembled normal flame-shaped arbors but were more widespread mediolaterally with branches outside their normal terminal

FIGURE 45: Camera lucida reconstuctions of six complex arborizations from control saphenous HFA's with receptive fields on the medial leg. Scale bar 250um.

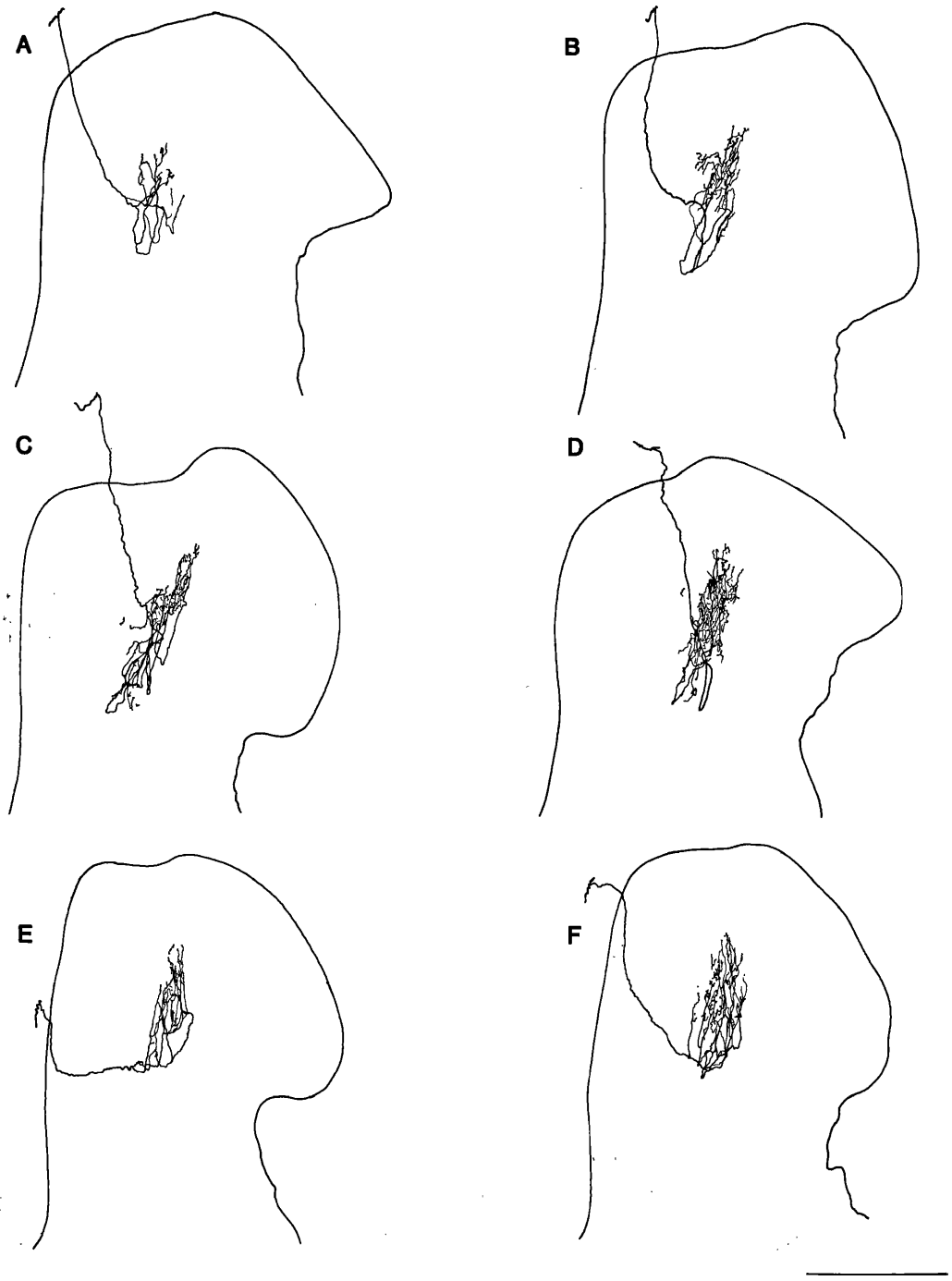
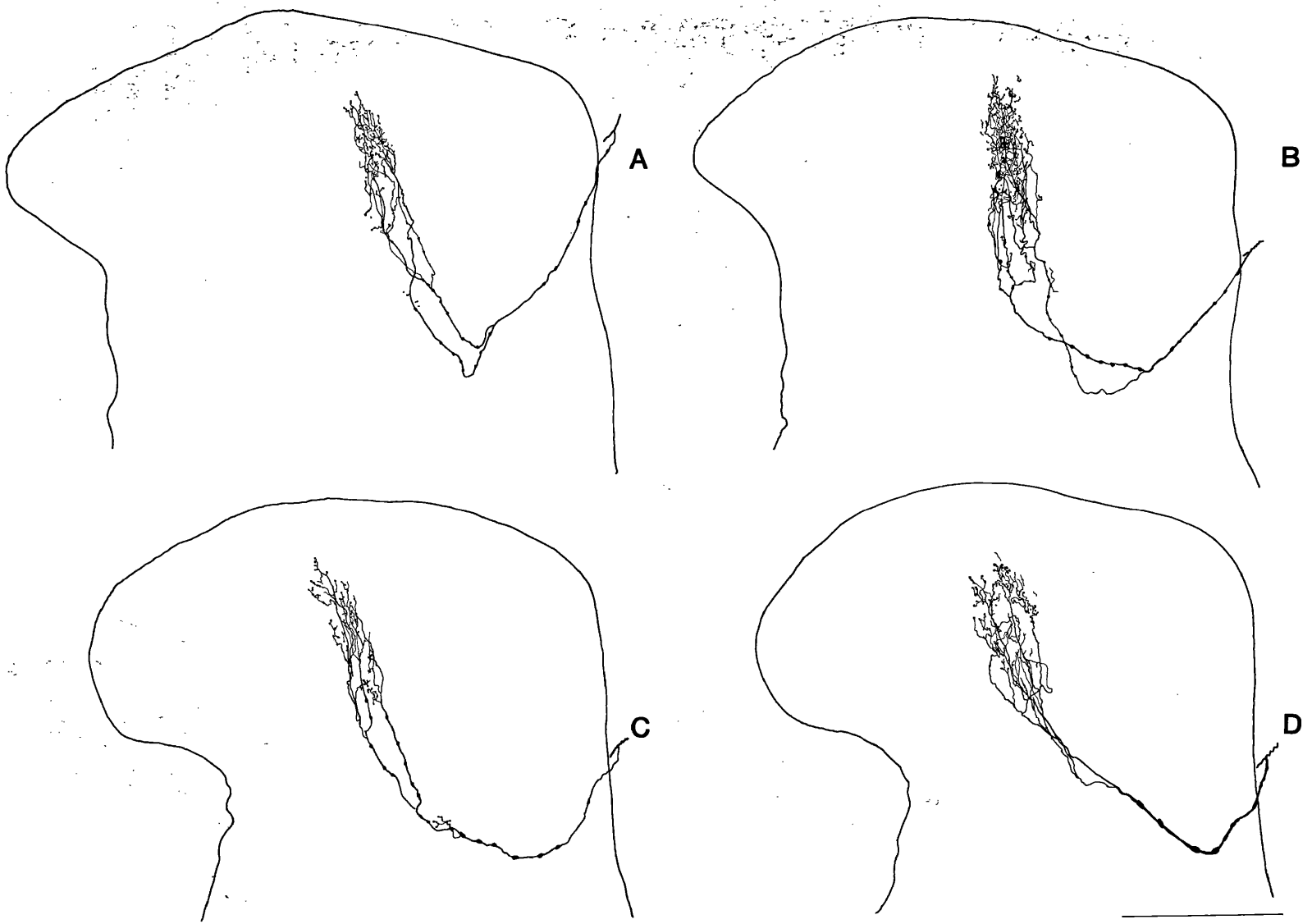


FIGURE 46: Camera lucida reconstructions of four adjacent complex arborizations from rostral (A) to caudal (D) of a HFA from a rat whose sciatic nerve had been cut at birth. The receptive field was located on the dorsal surface of the medial ankle. The central terminals were located in the L3 dorsal horn. Scale bar 250um.


A
B
C
D

sprouting. Afferents whose terminals were located in caudal-L3, rostral-L4 were slightly different in that although all the collaterals exhibited the flame-shaped arbors, the saphenous intact HFA's had significantly wider terminal arborizations than their control counterparts (Figs. 47, 48, Table 14). Occasionally some ventrally directed terminal branches with boutons were seen (Fig. 47 D,E; 48B). In all other respects the saphenous HFA's rostral to the L3/4 border were similar to control saphenous HFA's (Table 15).

4.4.1 (ii) MORPHOLOGY OF SAPHENOUS HFA's WITH COLLATERALS CAUDAL TO THE L3/4 BORDER IN SCIATIC CUT RATS.

Equal numbers of saphenous intact HFA's sent sprouts into the glabrous (n=6) and hairy skin (n=6) zones of the sciatic area. The appearance of the sprouted collateral arbor depended whether it lay in the hairy or glabrous part of the sciatic denervated zone (Figs. 49, 50). Collaterals in that part of the L4 segment which normally contained saphenous and sciatic HFA terminals resembled normal flame-shaped arbors but were more widespread mediolaterally with branches outside their normal terminal zone (Figs. 44, 49, 51). The mediolateral expansion was evident for all the terminal arbors and was significantly different to control saphenous HFA's. Many of the abnormal arborizations also exhibited ventrally directed terminal branches and boutons (Fig. 49C,F,G; 51).
Saphenous afferents which sent collaterals into medial L4 and L5 dorsal horn, which normally receives glabrous afferents (Fig. 44) were extremely abnormal. The complex arborizations were not flame-shaped but had a widely ranging morphology (Figs. 52-54). This observation suggests that the central target tissue influences the pattern of growth of the afferent collaterals. This was examplified by the rostrocaudal distribution of all the collaterals of a saphenous HFA illustrated in Figure 50. The rostral collaterals (Fig. 50A-F) which were located in

FIGURE 47: Camera lucida reconstructions of six complex arborizations from 2 saphenous HFA's from a rat whose sciatic nerve was cut at birth. The receptive fields were located on the medial thigh (A-C) and the medial side of the knee (D-F). The central terminals were located in caudal L3 and rostral L4. Scale bar 250um.

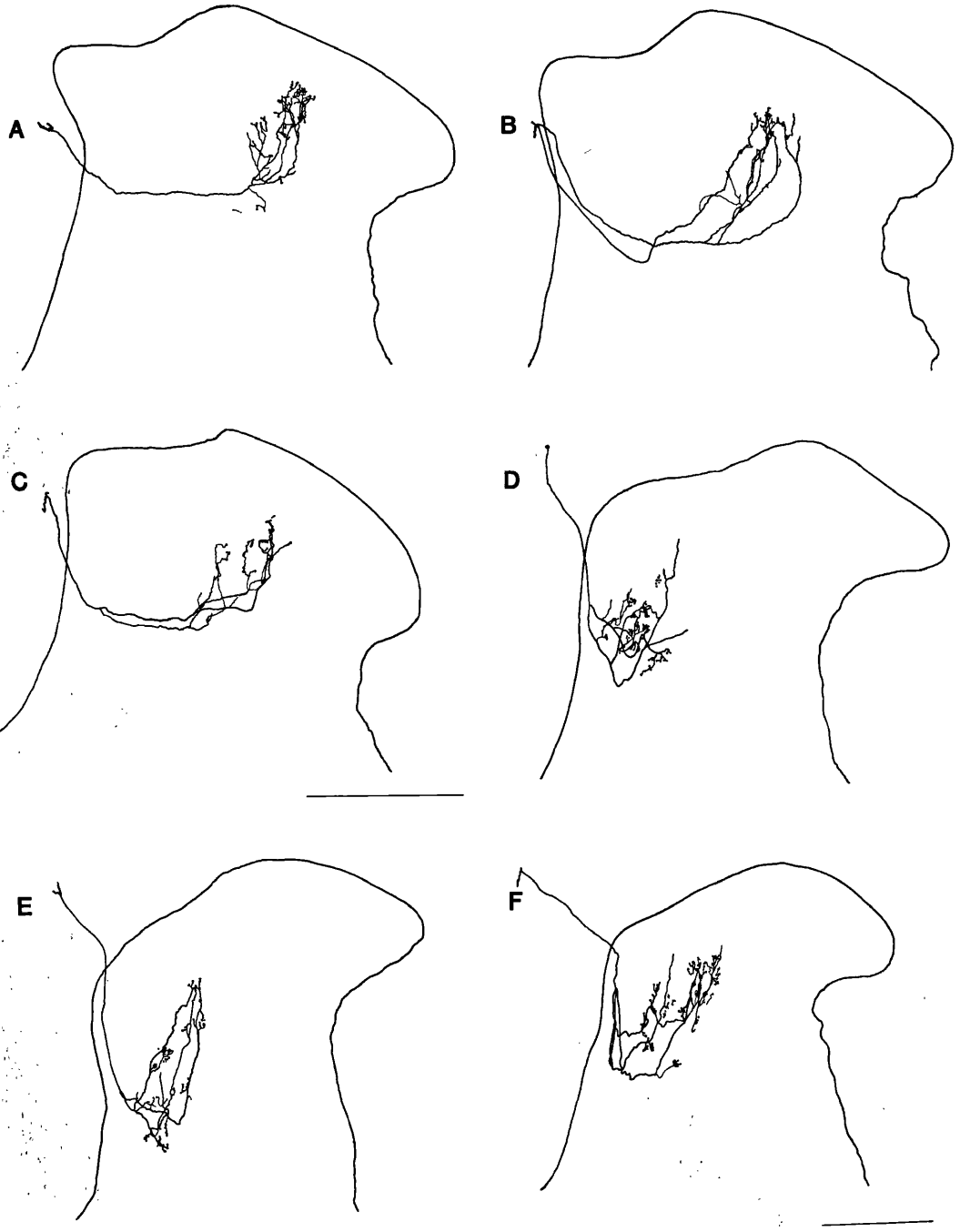


FIGURE 48: Camera lucida reconstructioms of six complex arborizations from rostral (A) to caudal (F) of a saphenous HFA from a rat whose sciatic nerve had been cut at birth. The receptive field was located on the medial side of the foot and the central terminals were located in caudal L3 and rostral L4. Scale bar 250um.

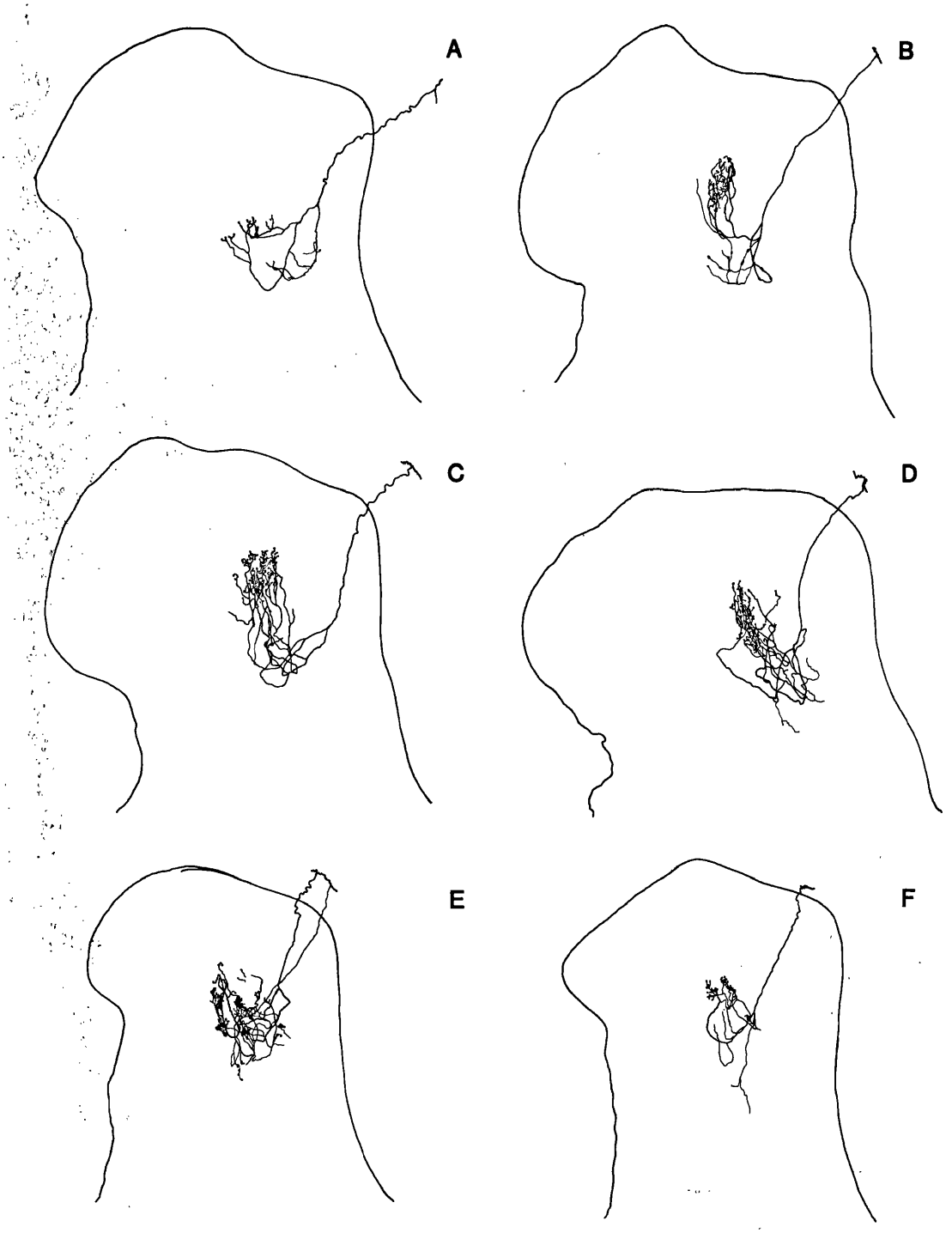


TABLE 14 THE ARRANGEMENT OF COLLATERALS OF EXPERIMENTAL AND CONTROL SAPHENOUS HFA's.

	EXPERIMENTAL		CONTROL
	ROSTRAL TO L3/4	CAUDAL TO L3/4	
No. afferents	6	12	10
Total no. collaterals	46	106	86
No. collaterals/ afferent: total	7.67±1.28	8.83±0.89	8.6±1.00
complex	3.33±0.49 (43.5%)	3.83±0.68 (43.4%)	4.30±0.83 (50.0%)
simple	2.33±0.80 (30.4%)	3.00±0.60 (34.0%)	1.70±0.60 (19.8%)
blind-ending	2.00±0.86 (26.1%)	2.00±0.33 (22.6%)	2.60±0.45 (30.2%)
intercollateral distance (um)	376±36	381±2715	342±26
length from most rostral to caudal collateral (mm)	2.40±0.27	2.97±0.28	2.76±0.27
length of overlapping terminal arbor sheet (mm)	0.95±0.09	0.97±0.28	1.26±0.08
abnormal arbors:	6/34	53/82	0
HFA-like	6	17/53	
RA-like	0	36/53	
projection to lamina IIi	4/34	2/82	

x ± SEM.

TABLE 15 DIMENSIONS OF TERMINAL ARBORIZATIONS OF EXPERIMENTAL AND CONTROL SAPHENOUS HFA's .

	EXPERIMENTAL SAPHENOUS HFA's						CONTROL SAPHENOUS HFA's		
	MAJORITY OF COLLATERALS								
	ROSTRAL TO L3/4			CAUDAL TO L3/4					
	C	S	C+S	C	S	C+S	C	S	C+S
N	20	14	34	46	36	82	43	17	60
ML (um)	116±15^	50±7*	88±11*	146±13^	88±8^*	121±9^*	87±4^	55±5^	78±4^
DV (um)	232±10	86±11	169±22	229±19	135±15	182±13	201±14	113±15	174±12
RC (um)	253±18	129±15	204±16	238±14	163±17	205±11	269±16	129±13	204±9
VOL ($x10^{-3}$)	8.0±2.0	0.7±0.3	4.8±1.3	11.0±2.9	2.1±0.4	6.9±1.6	5.4±0.7	0.9±0.2	4.1±0.6

x±SEM

*,^=sig. diff. P<0.05 level ^ = control SA v experimental SA, * = rostral L3/4 v caudal L3/4

ML=mediolateral, DV=dorsoventral, RC=rostrocaudal,

VOL=volume, C=complex, S=simple, C+S=terminal.

Comparisons made were: C v C, S v S, C+S v C+S

zone (Figs. 44, 49, 51). The mediolateral expansion was evident for all the terminal arbors and was significantly different ($P<0.05$) to control saphenous HFA's. Many of the abnormal arborizations also exhibited ventrally directed terminal branches and boutons (Fig. 49C,F,G; 51). Saphenous afferents which sent collaterals into medial L4 and L5 dorsal horn, which normally receives glabrous afferents (Fig. 44) were extremely abnormal. The complex arborizations were not flame-shaped but had a widely ranging morphology (Figs. 52-54). This observation suggests that the central target tissue influences the pattern of growth of the afferent collaterals. This was examplified by the rostrocaudal distribution of all the collaterals of a saphenous HFA illustrated in Figure 50. The rostral collaterals (Fig. 50A-F) which were located in the normal saphenous nerve territory showed the normal flame-shaped collaterals. However, those collaterals which terminated in the medial L4 and L5 segments (Fig. 50G-L) had lost the flame-shaped morphology and instead had a morphology resembling that of glabrous skin afferents. Another example of this striking change from "HFA-like" collaterals to "RA-like" collaterals is shown in Fig. 52.

4.4.1 (iii) COMPARISONS BETWEEN NORMAL AND SAPHENOUS INTACT HFA's.

Saphenous HFA's whose sciatic nerve had been cut at birth had an average conduction velocity of 26.77 ± 1.53 ms^{-1} (n=32, $\pm$SEM) which was similar to that for normal HFA's ($30.00\pm1.52ms^{-1}$, n=40) supporting the electrophysiological finding that the RF's of single afferents (n=49) were normally sized and lay within the saphenous skin territory. This was further supported by the observation from silver staining of skin HFA terminals that there was no peripheral sprouting of saphenous skin terminals into denervated sciatic skin (Woolf et al., in prep.). The presence or absence of sprouting of an

FIGURE 49: Camera lucida rconstructions of all the collateral arborizations from rostral (A) to caudal (K) of a saphenous HFA that had sprouted into the sciatic terminal region normally occupied by 'hairy' afferents from a rat whose sciatic nerve was cut at birth. The peripheral receptive field was located on the upper medial leg. Note the abnormal flame shaped caudal collaterals. Scale bar 250um.

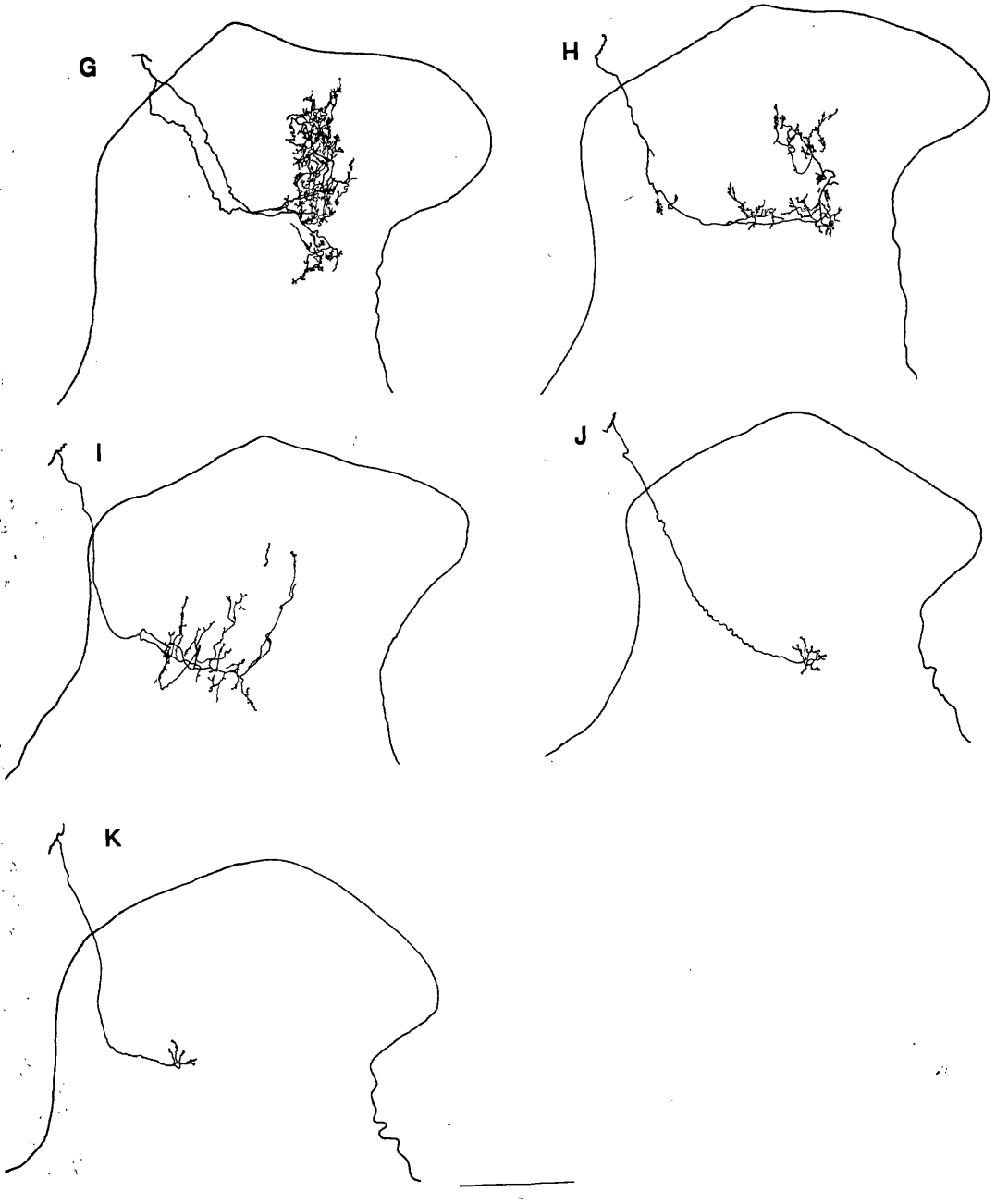

G
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FIGURE 50: Camera lucida reconstructions of all the collateral arborizations from rostral (A) to caudal (L) of a saphenous HFA that had sprouted into the sciatic terminal region normally occupied by glabrous afferents from a rat whose sciatic nerve was cut at birth. Note the loss of flame the shaped morphology of the collaterals in H-L. The peripheral receptive field was located on the medial side of toe 2. Scale bar 250um.

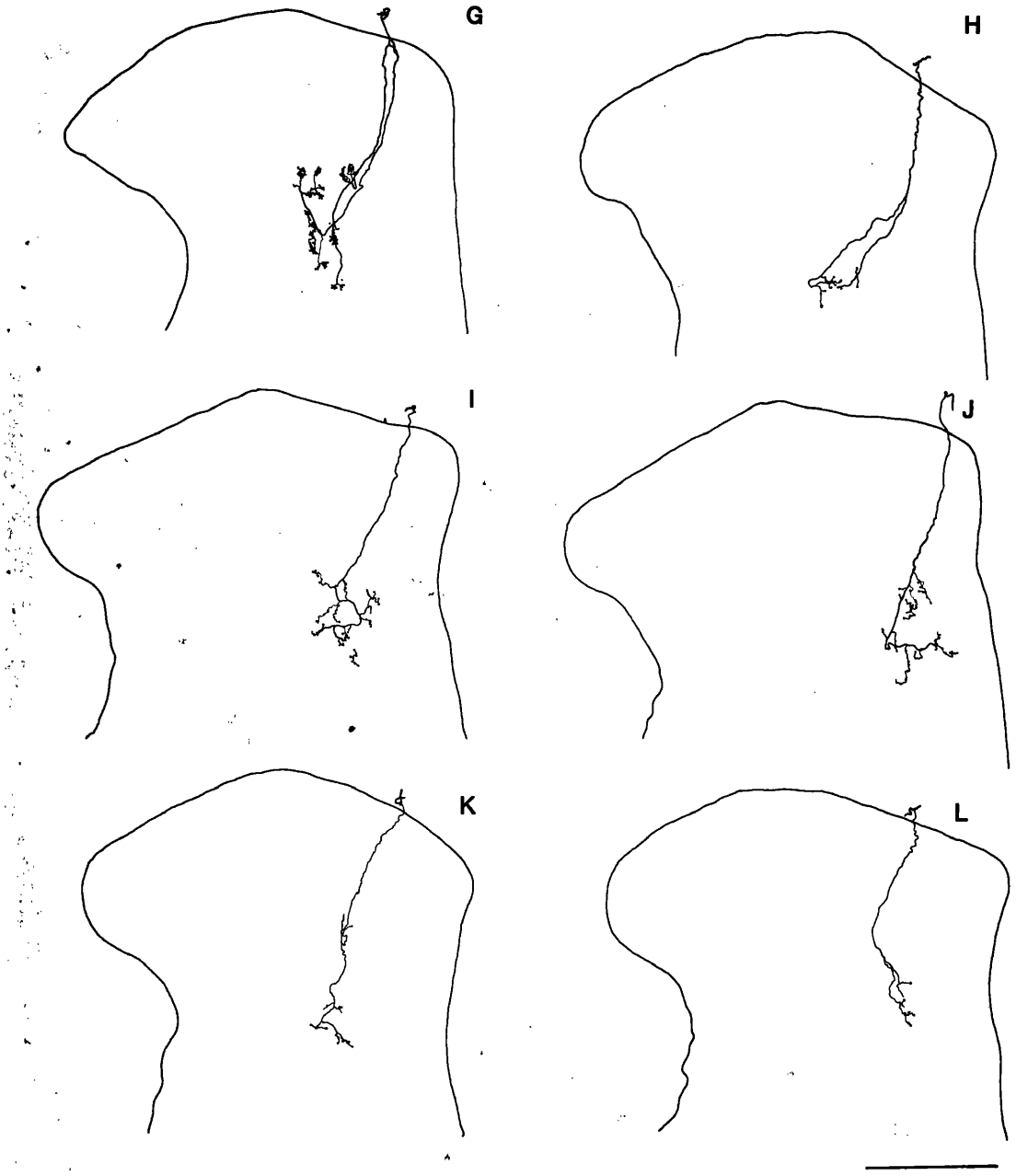
G
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FIGURE 52: Camera lucida reconstructions of six adjacent complex arborizations from rostral (A) to caudal (F) of a saphenous HFA that had sprouted into sciatic 'glabrous' territory from a rat whose sciatic nerve was cut at birth. Note the change of morphology from flame shaped (A-B) to abnormally shaped collaterals (C-F). The peripheral receptive field was located on the medial calf. Scale bar 250um.

FIGURE 53: Camera lucida reconstructions of four adjacent complex arborizations from rostral (A) to caudal (D) of a saphenous HFA that had sprouted into sciatic 'glabrous' territory from a rat whose sciatic nerve was cut at birth. The peripheral receptive field was located on the medial ankle. Scale bar 250um.

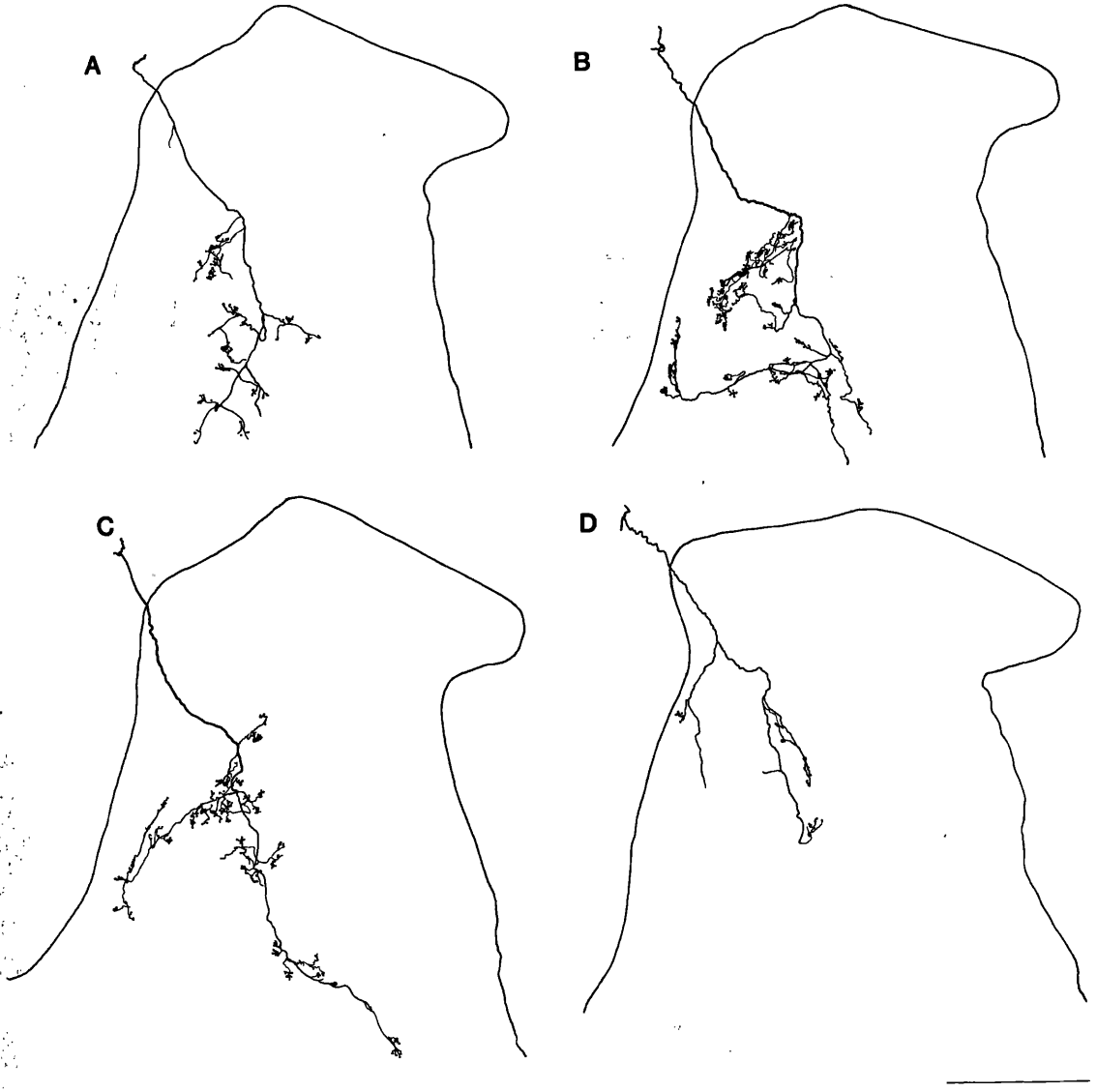
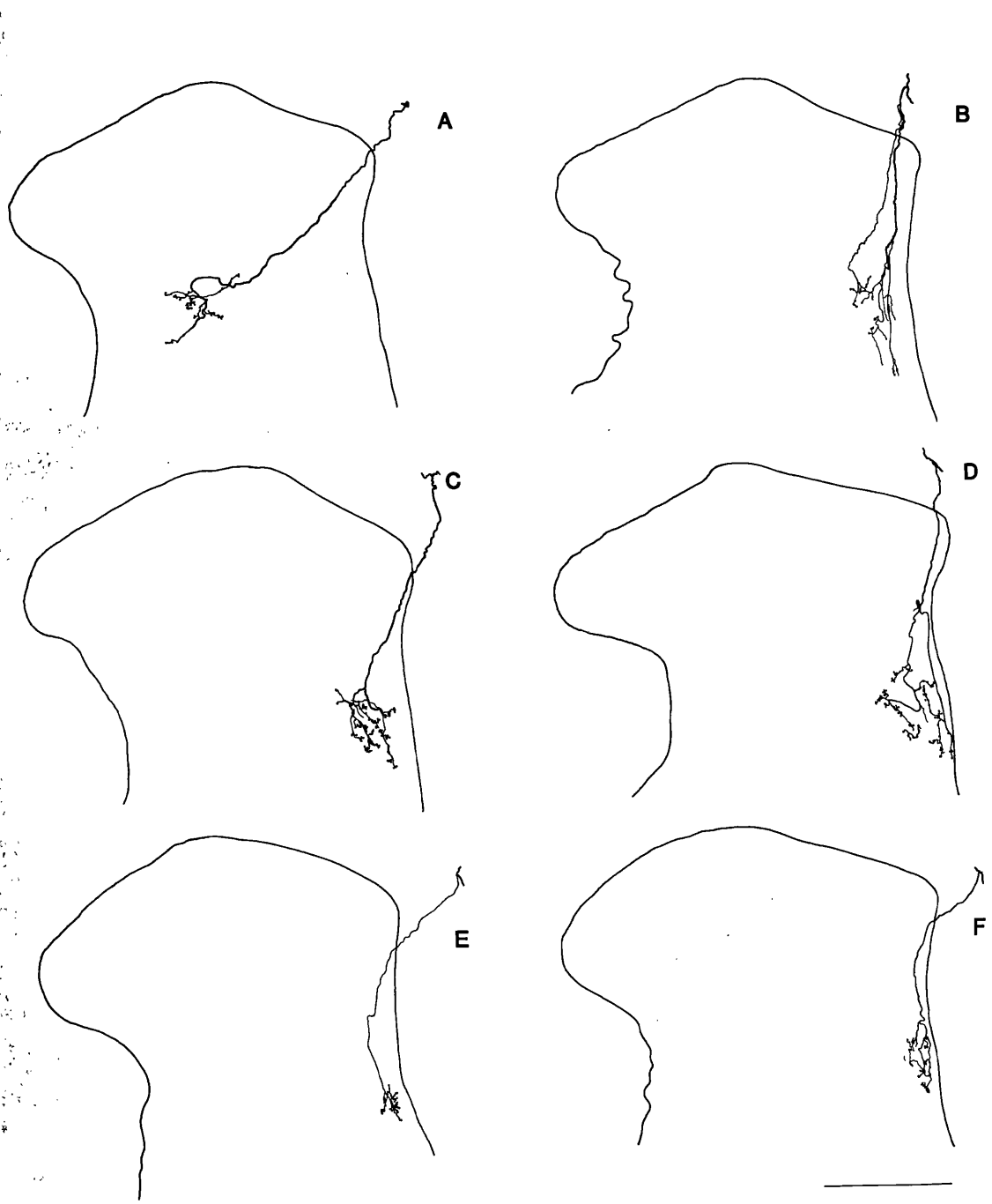


FIGURE 54: Camera lucida reconstructions of six adjacent complex arborizations from rostral (A) to caudal (F) of a saphenous HFA that had sprouted into sciatic 'glabrous' territory from a rat whose sciatic nerve was cut at birth. The peripheral receptive field was located on the lower medial thigh. Scale bar 250um.



afferent was determined by the proximity of the central terminals to the denervated zone in the dorsal horn and not by the position of its peripheral receptive field. The overlap of the central terminals of afferents within a given nerve territory means that the position of the peripheral RF relative to the denervated skin region was not directly linked to the existance of sprouting in the dorsal horn.

Analysis of the dimensions and arrangements of collaterals of control and saphenous intact HFA's revealed several interesting features (Tables 14, 15, Appendix III). Saphenous intact afferents which were found in normal areas of spinal cord (ie. L3 segment) were similar to control saphenous HFA's. They had, on average, slightly fewer numbers of complex, simple and blind-ending collaterals than controls although the percentages of simple arbors were higher and blind-ending collaterals lower than controls. This was reflected in the length of the afferent from most rostral to caudal collateral and length of overlapping arbor. There was a slight increase in the average intercollateral distances between adjacent collaterals and only 18% of terminal arborizations showed any abnormalities, seen as the wider flame-shaped arborizations (Fig. 47).

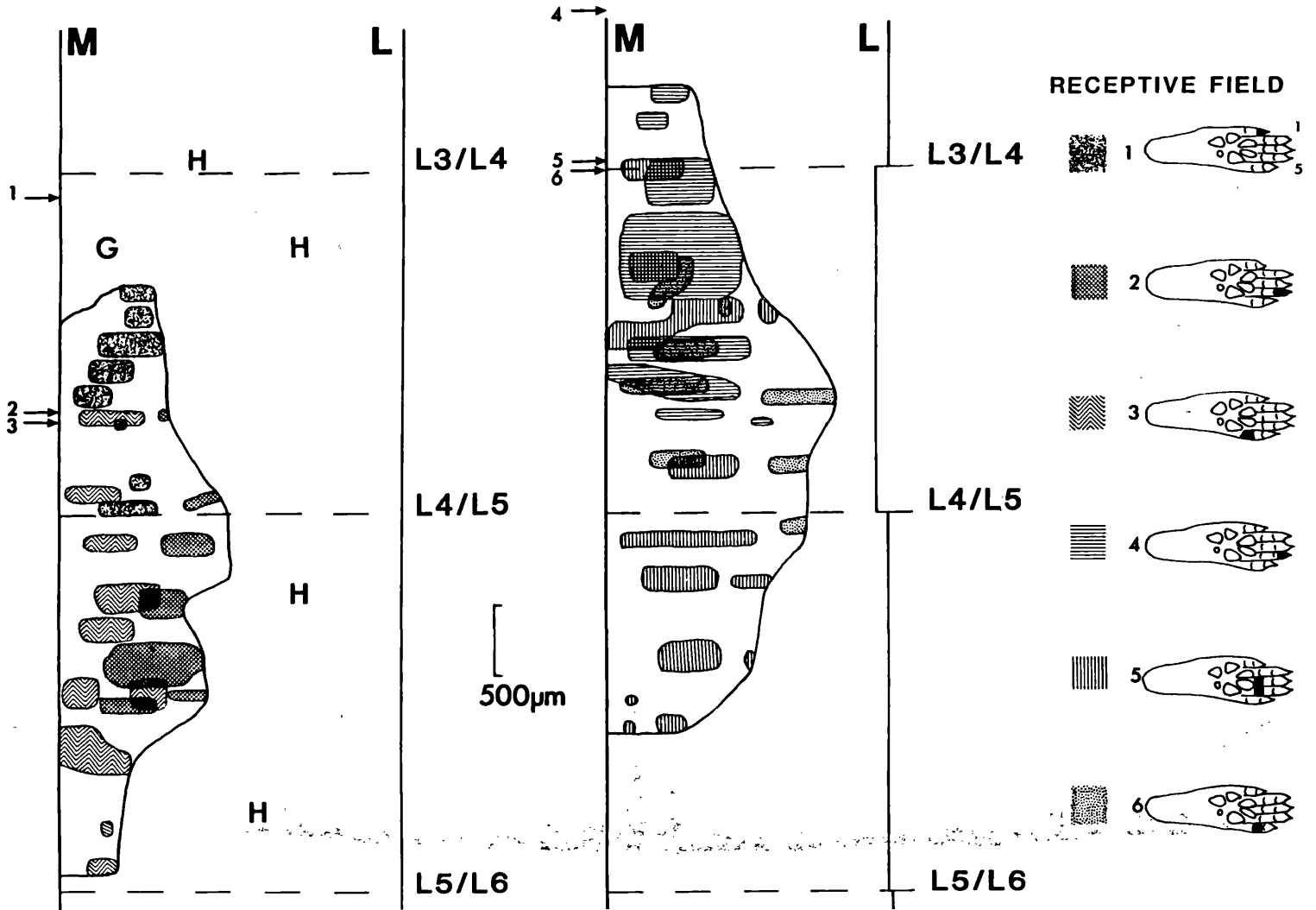
Saphenous intact HFA's with collaterals caudal to the L3/4 border showed similar variations in the percentages of simple and blind-ending collaterals, increased intercollateral distance and length of overlapping terminal sheet when compared to control saphenous HFA's as did the rostrally placed saphenous intact HFA's. Also, caudally placed saphenous intact HFA's showed an increase in the distance from most rostral to caudal collateral when compared to control HFA's. Sixty-five percent (53/82) of the terminal arborizations exhibited abnormal morphology, (17/53) thirty-two percent of these abnormal arbors exhibited a "HFA-like" morphology (Figs. 49, 51) while the remaining (36/53) 68% exhibited a "RA-like" morphology (Fig. 54).

Analysis of the pooled dimensions of terminal arborizations of saphenous intact and control HFA's revealed significant differences ($P<0.05$, unpaired t-test) in the mediolateral widths of complex arbors of sciatic cut afferents with those of control saphenous HFA's (Table 15, Appendix III). In addition, the simple and terminal arbors (complex and simple) of saphenous intact HFA's caudal to L3/4 were significantly larger than those of controls HFA's and saphenous intact HFA's rostral to L3/4. The rostrocaudal and dorsoventral dimensions of all the terminal arborizations of saphenous intact afferents were similar to those of control HFA's. However, when the average dimensions /afferent were compared (Appendix III,Table 15A), then there was only a significant difference ($P<0.05$) between the complex and terminal arborizations of SA intact caudal to the L3/4 border with control SA afferents. Similarly, when the SA intact afferents rostral and caudal to the L3/4 border were treated as a homogeneous population and compared to control SA afferents, then there were no significant differences in any of the dimensions measured although the SA intact afferents were 32% wider (Appendix III). Saphenous intact afferents caudal to L3/4 generally exhibited the largest dimensions for terminal arborizations and this was reflected in the volumes of cord occupied although this was not significantly different compared to control HFA's ($P<0.05$).

4.4.2 TIBIAL NERVE SPROUTS.

The above results could possibly be interpreted as the result of non specific disorganization of sprouted terminals into inappropriate, abnormal targets and not as the result of the central target influencing the pattern of terminal growth. To clarify this, the converse experiment of filling RA afferents in rats whose nerves innervating hairy skin (see methods) had been cut on the day of birth was performed.

FIGURE 55: A plan view at the level of lamina IV of the dorsal horn showing the terminal distribution of bouton-containing collaterals of tibial nerve toe glabrous skin RA mechanoreceptors. A: Control Toe RA afferents (n=8) B: Tibial intact RA afferents (n=7) from rats where only the Ti nerve was left intact as a neonate. Shrinkage of the dorsal horn that occurs following neonatal nerve section was 16-20% in the lumbar segments. Note the significant lateral extension of terminals into areas normally occupied by HFA terminals in L4. The numbered arrows represent the most rostral blind ending collateral of an individual afferent. Abbreviations as in Fig. 44.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500μm
RECEPTIVE FIELD
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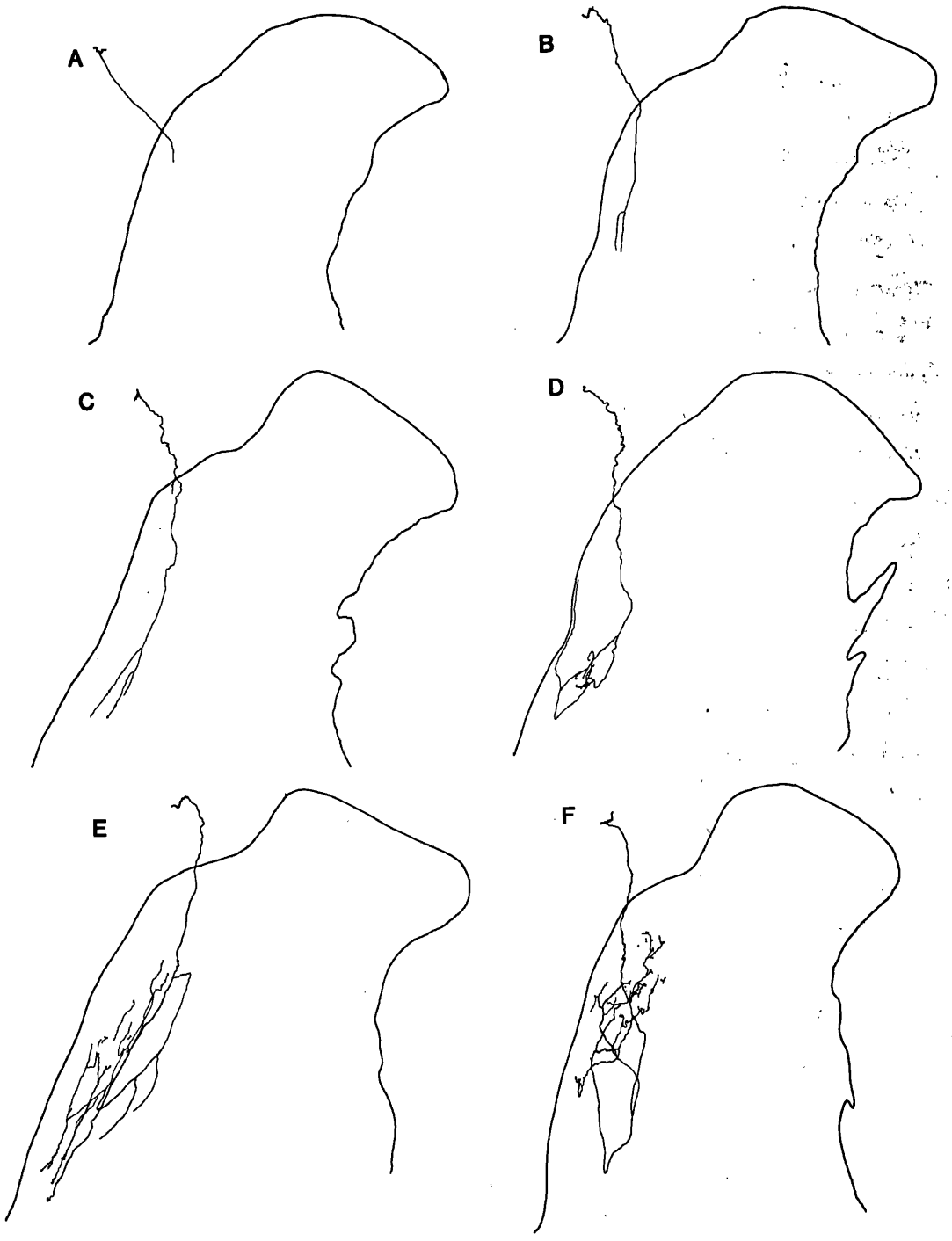
Seven tibial nerve RA afferents (termed "tibial intact") were recovered in rats whose sural, saphenous and superficial peroneal nerves had been cut at birth. Six of the seven afferents had RF's on the toes and five of these showed signs of sprouting. Tibial intact RA's had an average conduction velocity of 37.88±3.51 ms^{-1} (n=10, ±SEM) which was similar to that of control RA's (33.19 ± 1.60 ms^{-1}, n=20, ±SEM) supporting the electrophysiological observations that the RF's of single tibial intact afferents were normally sized and lay within the tibial skin territory. These were compared to the eight toe RA afferents described earlier in section 2.3.3 (iii). Examination of Fig. 55a shows that toe afferent terminals were located from mid-L4 to caudal-L5 segments and occupied the medial quarter of the dorsal horn at L4 and the medial third of the dorsal horn in L5. Laterally they border with the terminal fields of the SP, SU and SA nerves (marked H in Fig. 55a). The total tibial area is larger as it includes paw pad afferents as well as toe afferents and extends more rostrally to the L3/4 border and more caudally to the L5/6 border (Figs. 14, 24). Those tibial intact afferents that showed sprouting had terminals in areas of cord which were adjacent to, or in areas of cord normally devoted to "hairy" afferent input (Fig. 55b).

4.4.2 (i) MORPHOLOGY OF TIBIAL INTACT AFFERENTS.

The complete rostrocaudal extent of the collaterals of a tibial intact RA afferent is shown in Fig 56. The rostral and caudal collaterals (Fig. 56A-E, K, L) were typically those associated with RA toe afferents (see Figs. 29-33 in section 2.3.3) while collaterals F-J show arborizations resembling the flame shapes which are normally associated with HFA's. Other examples of rapidly adapting afferents exhibiting flame-shaped arborizations are shown in Figs. 57-58. Like normal HFA's, the tibial intact RA's that exhibited flame-shaped arbors showed

FIGURE 56: Camera lucida reconstructions of all the collateral arborizations from rostral (A) to caudal (L) of a tibial RA afferent which had sprouted into denervated 'hairy' regions of cord following nerve cut on the day of birth. Some collaterals (F-I) had taken on the appearence of flame shaped arbors typical of HFA's. The peripheral receptive field was located on the glabrous surface of toe 4. Scale bar 250um.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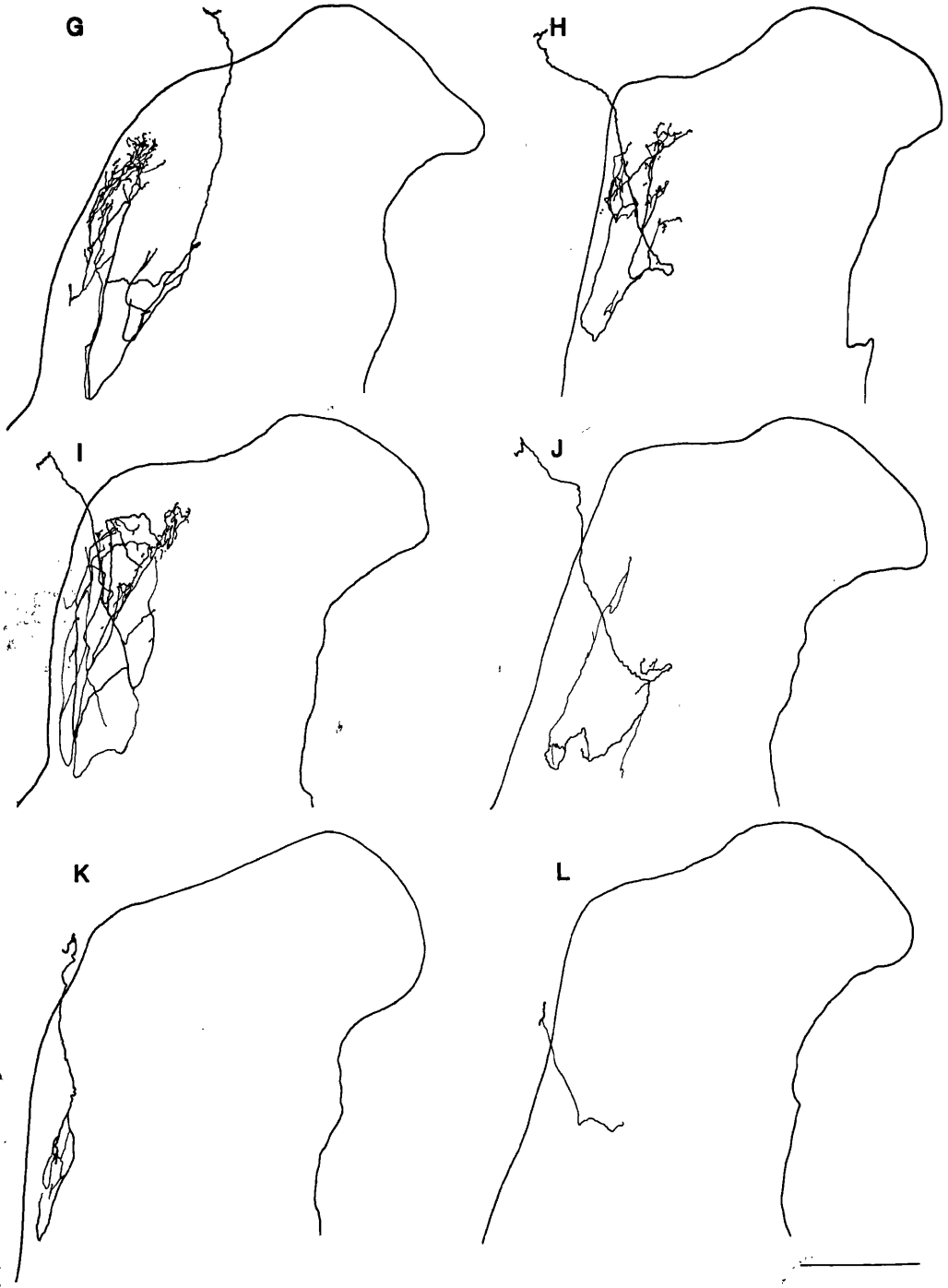
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FIGURE 57: Camera lucida reconstructions of six adjacent terminal arborizations from rostral (A) to caudal (F) of a tibial RA afferent which had sprouted into denervated 'hairy' skin territory following nerve cut on the day of birth. All the collaterals have the flame-shape appearence similar to that of normal HFA's. The peripheral receptive field was on the glabrous surface of toe 5. Scale bar 250um.

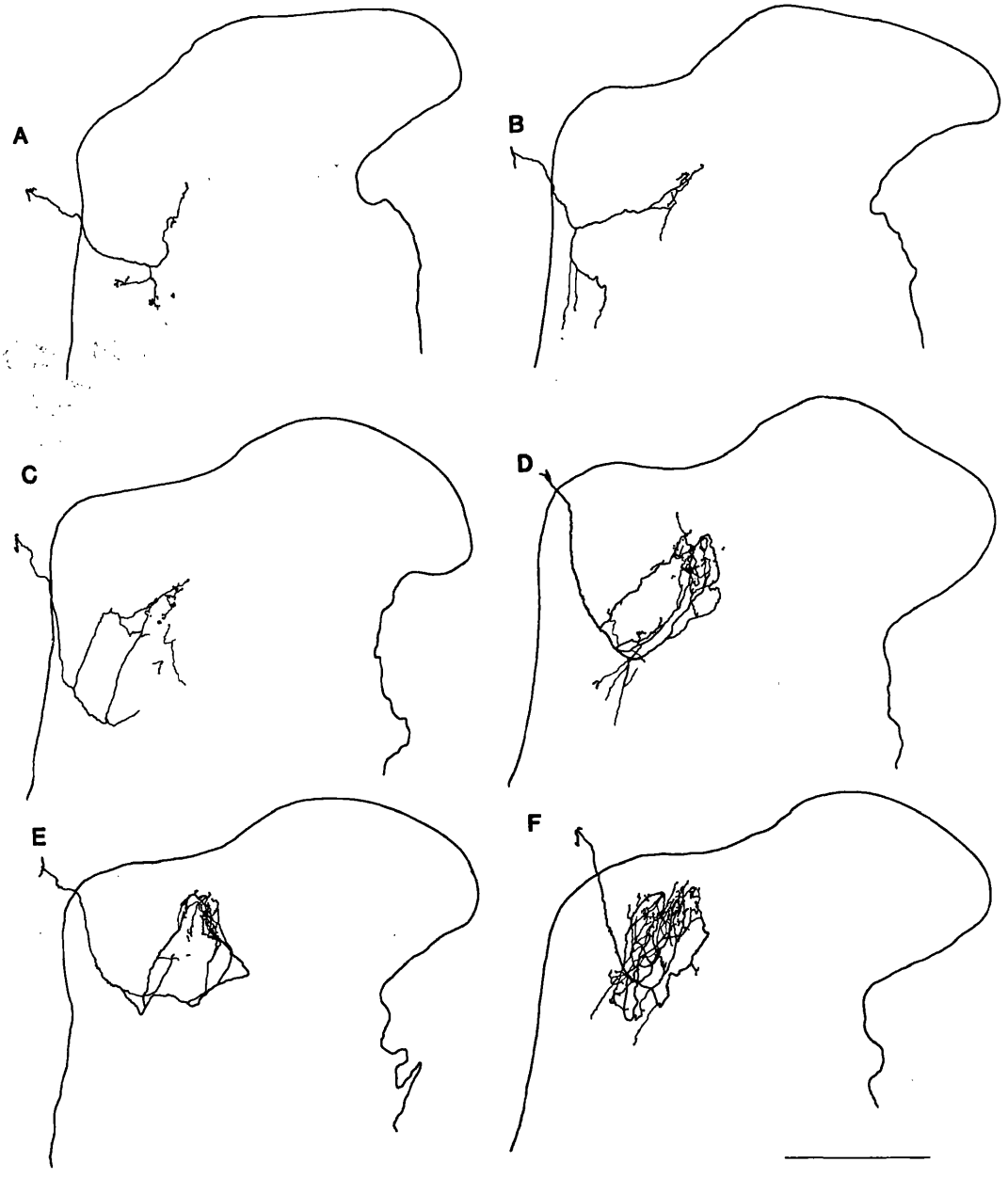
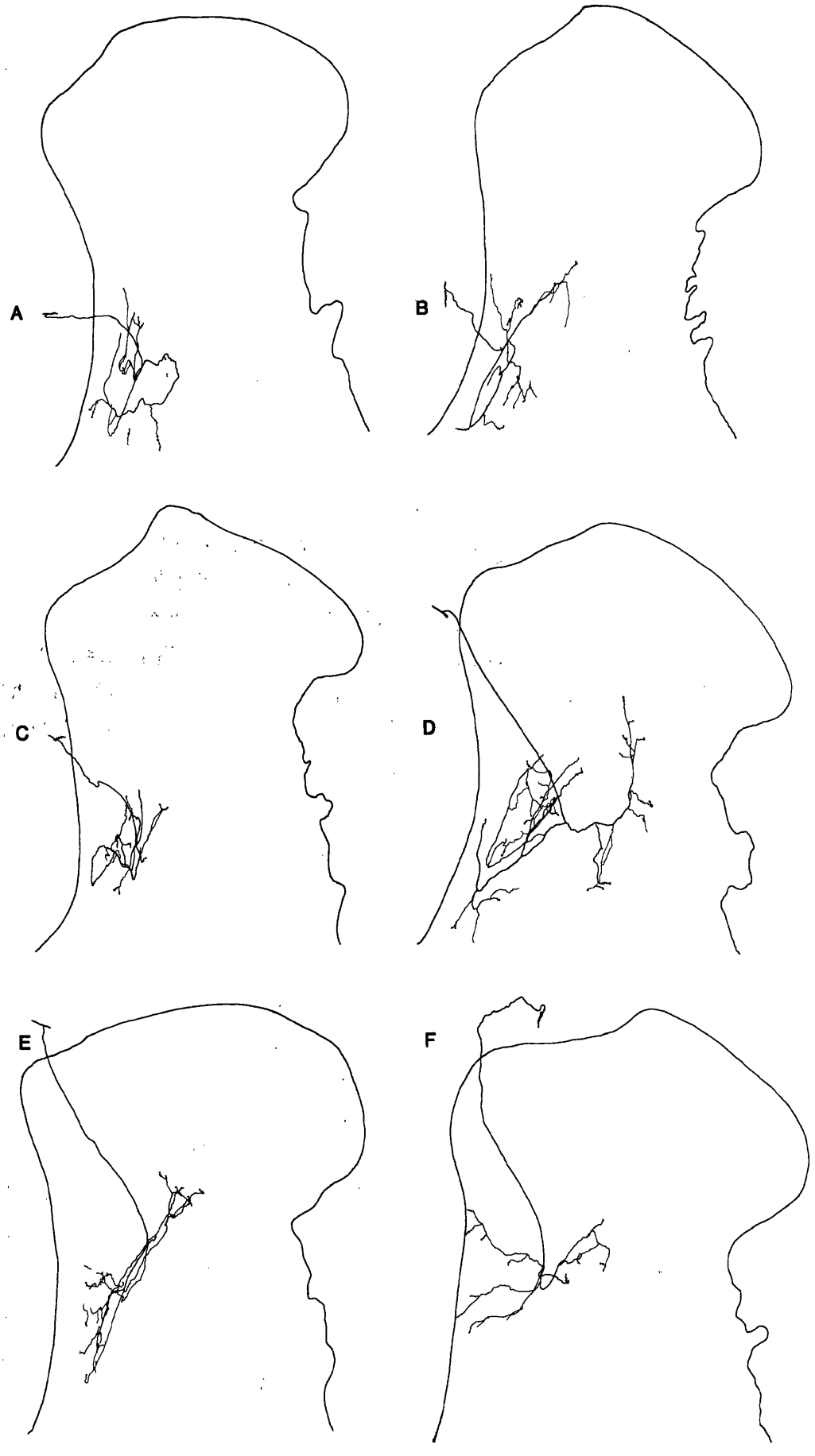


FIGURE 58: Camera lucida reconstructions of six adjacent complex arborizations from rostral (A) to caudal (F) of a tibial RA afferent which had sprouted into denervated 'hairy' skin areas following nerve cut on the day of birth. The collaterals resemble those of glabrous-hairy HFA's. The peripheral receptive field was located on the glabrous surface of toe 5. Scale bar 250um.


A
B
C
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F

overlap between some adjacent collaterals (Figs. 56F-I, 57D-F).

RA afferents which possessed collaterals that had sprouted did not always show a flame-shape. Examples of these types of collaterals showing abnormal RA-type arborizations are seen in Figs. 59-60. The abnormal RA arbors did not appear like normal RA arborizations (Figs. 59A, 60A) but showed a variation of morphology which generally resulted in wider terminal arborizations (Figs. 59B,D,E, 60 B-D).

4.4.2 (ii) COMPARISON OF CONTROL AND TIBIAL INTACT RAPIDLY ADAPTING AFFERENTS.

The above observation was confirmed when analysis of the pooled dimensions of the tibial intact RA afferent arborizations (Table 17) showed a significant difference ($P<0.05$ unpaired t-test) in the mediolateral width of the complex arborizations between control toe RA's and sprouted toe RA afferents. Control RA toe afferent terminal arborizations (complex and simple) were significantly deeper than the tibial intact terminal arborizations, perhaps reflecting mediolateral sprouting of afferent collaterals since the dorsoventral length of the tibial intact arbors was similar to that of control HFA's (Tables 15 and 17). The rostrocaudal dimensions were similar as were the volumes of cord occupied by the arborizations. However, when the average dimensions /afferent were compared then the significant differences disappear ($P<0.05$) but shows that the tibial intact afferents were 30% wider and 28% less deep than control toe RA afferents (Appendix III).

Analysis of the arrangement of collaterals of control toe RA's and tibial intact RA afferents yielded similar percentages of complex, simple and blind-ending collaterals (Table 16). However, the length from most rostral to caudal collateral was on average 500um longer in the tibial intact RA afferents and this was reflected

FIGURE 59: Camera lucida reconstructions of six adjacent compledx arborizations from rostral (A) to caudal (F) of a tibial RA afferent which had sprouted but remained within the tibial nerve area following nerve cut on the day of birth. The morphology of the sprouted arbors resembled those of abnormal RA afferents. The peripheral receptive field was located on the glabrous surface of toes 3 & 4. Scale bar 250um.

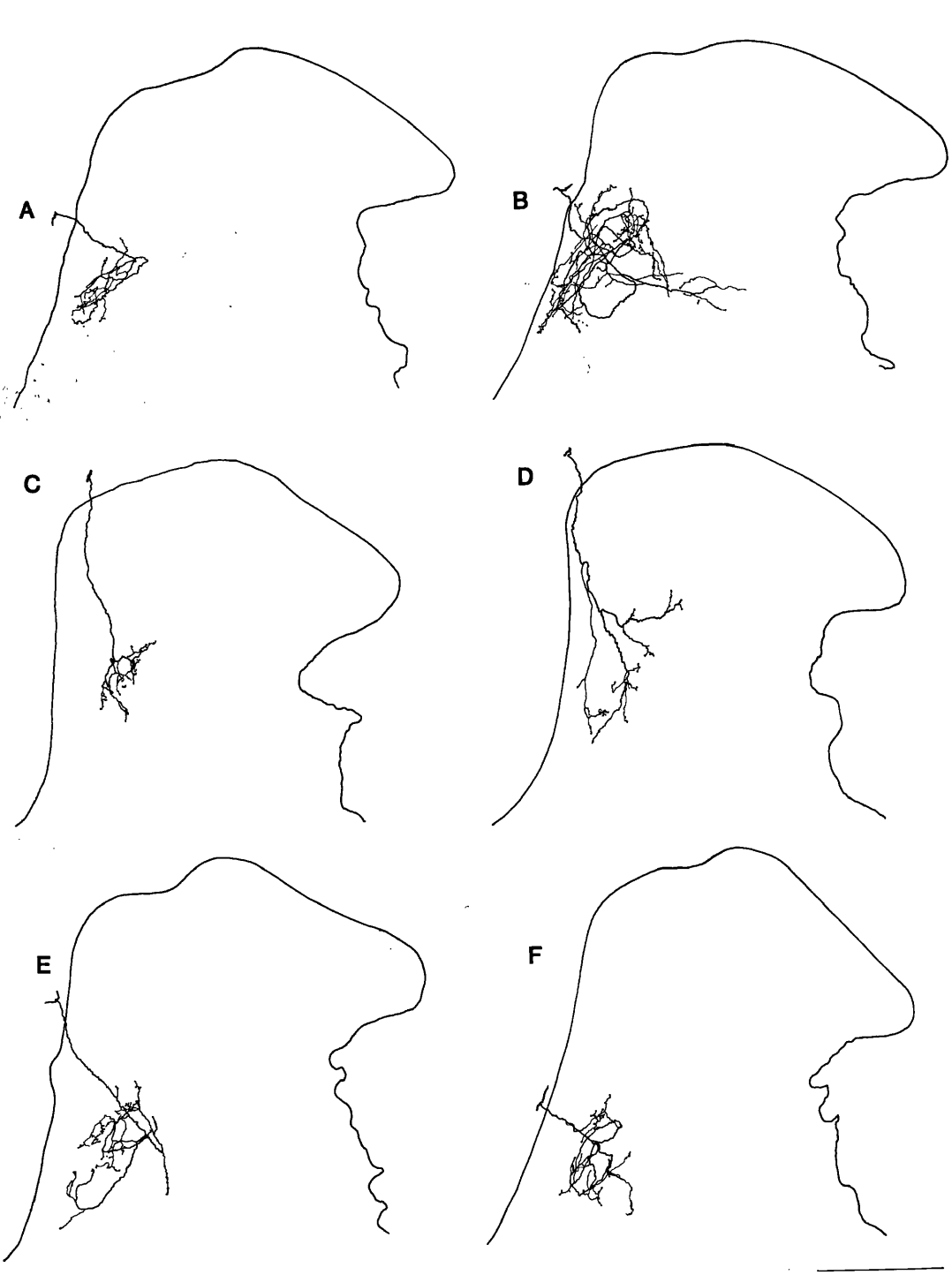

FIGURE 60: Camera lucida reconstructions of four adjacent complex arborizations from rostral (A) to caudal (D) of a tibial RA afferent which possessed abnormal looking RA sprouts (B-D) following nerve cut on the day of birth. The peripheral receptive field was located on the glabrous surface of toe 5. Scale bar 250um.

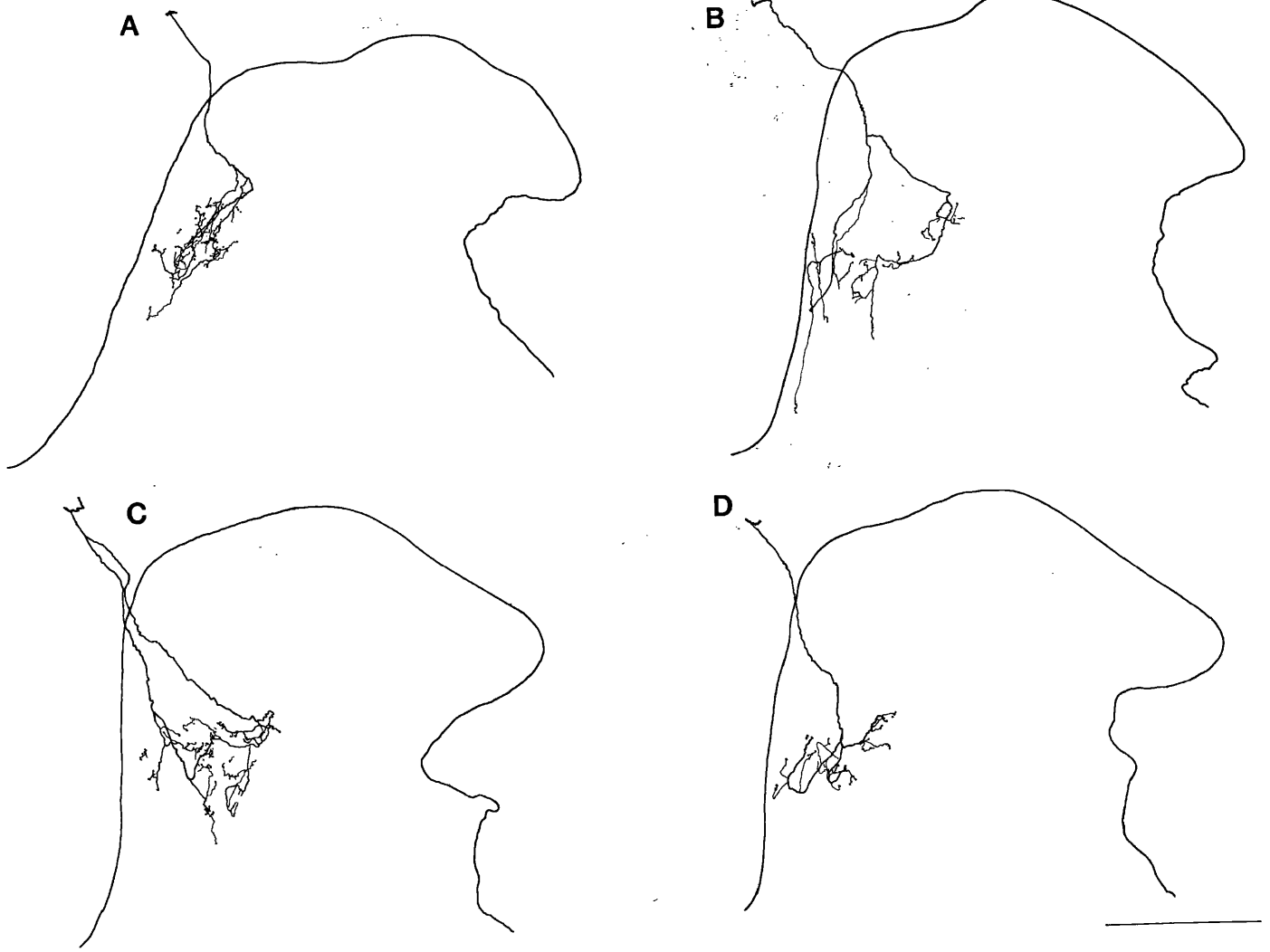
A
B
C
D

TABLE 16

ARRANGEMENT OF THE COLLATERAL BRANCHES OF TIBIAL INTACT AND CONTROL RA AFFERENTS.

	TIBIAL INTACT	CONTROL RA
No. afferents	7	8
Total no. collaterals	67	85
No. collaterals/ afferent: total	9.57±1.53	10.6±0.49
complex	3.43±0.53 (36.0%)	4.1±0.53 (41.2%)
simple	2.86±0.40 (30.0%)	2.9±0.74 (27.0%)
blind-ending	3.28±0.97 (34.0%)	3.4±0.53 (31.8%)
intercollateral distance (um N)	391±23*	307±26*
length from most rostral to caudal collateral (mm)	3.37±0.36	2.89±0.25
projection to II_i	3/36	5/35

x ±SEM

*= signif. diff. $P<0.05$ level
unpaired t-test

N= no. of afferents or no. of collaterals.

TABLE 17 DIMENSIONS OF THE TERMINAL ARBORIZATIONS OF TIBIAL INTACT AND CONTROL RA AFFERENTS.

	TIBIAL INTACT RA			CONTROL TOE RA		
	C	S	C+S	C	S	C+S
N	24	20	44	36	23	59
ML (um)	140±12*	61±8	103±9	99±6*	68±10	87±6
DV (um)	225±21	107±17	177±17*	259±21	157±24	224±17*
RC (um)	218±19	166±15	195±13	210±14	189±13	202±11
VOL(ul) (X10^{-3})	7.7±1.6	1.2±0.3	4.9±1.03	6.7±1.4	3.3±1.2	5.4±1.

X ± SEM
* = signif. diff. P<0.05 level
unpaired t-test.
ML=mediolateral, DV=dorsoventral, RC=rostrocaudal, VOL=volume.
C=complex, S=simple, C+S=terminal

in the significant increase ($P<0.05$ unpaired t-test) in the intercollateral spacing. Of the forty-four recovered terminal arborizations in tibial intact afferents 19/44 (43%) showed a normal RA afferent morphology while 25/44 (57%) exhibited abnormal RA morphology. Of these 25 abnormal arbors, 12/25 (48%) exhibited a flame-shaped morphology (Fig. 57) while the remaining 13/25 (52%) showed an abnormal RA morphology (Fig. 59).

4.5 DISCUSSION.

The results of this study show that cutaneous sensory afferents are capable of extending arbors into distant and inappropriate central target regions, if that region is deafferented and adjacent to the normal terminal zone. The sprouting observed here represents new growth with respect to bouton proliferation, length of axon stained, intercollateral distances and arbor dimensions (Tables 14-17, Appendix III). The growth into inappropriate areas was new rather than simply a failure of withdrawal of excess terminals formed earlier in development, since primary afferents have been shown by bulk labelling methods to grow into specific areas of cord (Honig et al., 1982; Fitzgerald and Swett, 1983; Smith, 1983; Smith and Frank, 1988) so that in the neonatal and adult dorsal horn projections are the same and do not display the exuberant projections seen elsewhere in the developing nervous system (Purves and Lichtman , 1985). Comparison of the bulk labelling of the sciatic nerve in the neonatal (Fitzgerald and Swett, 1983) and adult rat (Swett and Woolf, 1985) shows that the density of stain is much deeper in the dorsal horn compared with that seen in the adult, suggestive of transient overcrowding of terminals within a nerve territory in the dorsal horn. Peripheral nerve section may interfere with the withdrawal of these excess terminals by a mechanism similar to that seen in the peripheral nerve (Jenq et al., 1986) and this may offer an explanation for the increased size of terminal

arbors of saphenous afferents within the normal nerve territory following neonatal sciatic nerve section (Table 15). However, this cannot explain the sprouting of individual collaterals outside the normal saphenous nerve territory.

Nerve section on the day of birth occured at a time when the primary afferents had already grown into the spinal cord and found their central targets (Smith, 1983; Fitzgerald, 1987) although their terminal arborizations are still developing (Beal et al., 1988). Some of the sprouting observed here must of resulted from the expansion of existing arbors to cover wider regions similar to that reported for retinal and trigeminal afferents (Garraghty et al., 1988; Renehan et al., 1989). This is supported by the increase in the dimensions and number of simple arbors and decrease in the number of blind-ending collaterals (Tables 14 and 16). However, sprouting must also have involved new collateral growth of terminal arborizations into regions distant from the normal terminal zone, since saphenous intact collaterals were found upto 1550um in the L5 segment, 2350um distant from the most distal collateral in control saphenous afferents. Comparison of the average number of collaterals per afferent in experimental and control animals indicates that there was no increase. This implies that some more proximal or distal (possibly blind-ending) or intervening collaterals are lost at the expense of new ones. Perhaps primary afferents may be only able to support a certain number of collaterals per stem axon. In support of this, 4/7 tibial sprouted afferents had no rostral blind-ending collaterals while 3/12 caudally sprouting saphenous afferents had no rostral blind-ending collaterals, and 8 of the other 9 only had one rostral blind-ending collateral. Half (5/10) of the control saphenous afferents had more than two rostral blind-ending collaterals and three-quarters (6/8) of the control toe RA afferents commonly had one or more rostral blind-ending collateral. Alternatively, perhaps

inadequate dye filling may have resulted in not all the collaterals being identified. Analysis of saphenous afferents which had collaterals in the L5 segment showed on average of 9.67±1.04 (n=9, ±SEM) collaterals per afferent, higher than that seen in Table 14. The maximum number of collaterals seen on any one control primary afferent was 17 (Shortland, personal observation) and the range seen here was 7-14 for experimental sprouted afferents. As the intercollateral distance for sprouted afferents increased, it is entirely possible that more rostral or caudal collaterals were not stained.

The sprouted collateral terminals did not have the characteristic morphology of normal cutaneous afferent terminals. The saphenous afferent sprouts which terminated in areas of cord normally occupied by glabrous skin afferents resembled glabrous skin afferents but if they sprouted into sciatic territory normally occupied by hairy skin afferents, then they resembled hair follicle afferents albeit abnormal ones. Tibial intact afferent collaterals that had sprouted into hairy skin areas took on the appropriate "flame-shaped" morphology. This was independent of their RF location, physiological receptor type and terminal morphology in the normal, somatotopically appropriate (SA) target region.

Saphenous HFA's that sprouted into sciatic hairy skin territory had abnormal arbors with a wide mediolateral spread and with unusual dorsally or ventrally directed branches but still clearly retained the flame-shaped morphology. Adjacent abnormal arbors overlapped forming wide mediolateral sheets of terminals running rostrocaudally (Fig. 44b) as oppossed to the narrow mediolaterally restricted HFA terminal sheets of the controls. Analysis of the dimensions of these abnormal arborizations revealed that, in rats which had undergone neonatal sciatic nerve section, the complex arbors were significantly wider than control saphenous HFA's (Table 15, Appendix III). In addition, differences were found between saphenous intact HFA's rostral and caudal to the

L3/4 border in the widths of terminal arbors. The increase in the width of rostral L3/4 saphenous complex HFA arbors compared with control saphenous complex arbors indicates that neonatal nerve section may have caused some intraspinal sprouting of afferents within the saphenous nerve territory itself, similar to that seen by McNeill and Hulsebosch (1987).

Saphenous HFA's that sprouted into sciatic glabrous central terminal territory did not have the flame-shape morphology. The arborizations resembled those of RA complex and simple arborizations, being located in the deeper laminae of the medial dorsal horn. Like RA arborizations, adjacent glabrous "sprouted" arbors of saphenous intact HFA's did not overlap but produced a discontinous rostrocaudally running sheet of terminals in which adjacent arbors overlapped in the saphenous nerve territory, but not in the sciatic region (Fig. 44).

The results show that neonatal sciatic denervation induced sprouting of afferents which was primarily in the rostrocaudal direction. These sprouts make functional connections in the dorsal horn (Fitzgerald and Shortland in preparation). Sprouting of the PC nerve and the smaller caudal SA terminal zone (Fig. 14) may also have ocurred. However, all the sprouted SA intact HFA's had normal RF's in the SA nerve territory, root entry zones into the spinal cord in L3 and rostral L4 segments but caudal arborizations which were abnormal implying that it was expansion of afferents from the larger SA terminal field. Only in 1 case did a SA intact afferent enter the cord through the rostral L5 dorsal root, but its peripheral RF was clearly in the SA nerve territory. Furthermore, intraaxonal injection of HRP into HFA's has failed to observe this caudal SA region (Shortland et al. 1989a) and it has not been observed following bulk labelling of the SA nerve with WGA-HRP in the neonate (Fitzgerald, 1985).

A similar rostrocaudal sprouting phenomenon has been observed in the cat (Wilson and Snow 1988b) but on a

smaller scale. Neonatal denervation of a single digit or pair of digits (toes 3,4, Wilson and Snow, 1987, 1988b) caused a rearrangement of SCT cell RF's (Wilson and Snow, 1987, 1988a) and an increased efficacy of somatotopically inappropriate collaterals (SIA) in intact surviving fibres in the adult cat (Snow and Meyers, 1985) . When these fibres were intracellularly stained with HRP, (Wilson and Snow, 1988b) it was observed that half (3/6) of the SIA collaterals were found to give rise to more complex arborizations and bore a higher number of boutons similar to the SA collaterals of normal toe HFA's. Wilson and Snow (1988b) concluded that there was a rostrocaudal reorganization of somatotopy that follows nerve section which involved the local proliferation of the SIA collateral arborizations of cutaneous afferents.

Whereas the direction of sprouting of saphenous afferents was primarily rostrocaudal, sprouting of tibial intact afferents was in the rostrocaudal and/or mediolateral direction. Sprouting in the rostrocaudal direction was supported by the significant increase ($P<0.05$ unpaired t-test) in the intercollateral distance (Table 16) and sprouting also occurred in the mediolateral direction since arborizations were found to occupy up to 80% of the width of the dorsal horn in L4 and rostral L5 and complex arbors were 40% larger than control toe RA afferents.

The morphology of the tibial intact arbors was influenced by the proximity to a denervated territory and to the position of the central terminals within the tibial nerve territory. Afferents located laterally within the tibial nerve territory adjacent to denervated hairy skin territories acquired HFA-like arbors while more medially located tibial intact arbors acquired abnormal RA-type arbors.

The results of the present study demonstrate that the central rather than the peripheral target determines the terminal growth pattern. The postsynaptic neuropil does not just simply influence terminal growth pattern as has

been shown elsewhere in graft (Bregman, 1987; Houle and Reier, 1989) studies, but specifies the precise terminal arborization characteristics of normal primary afferents. Such observations have also been made for retinogeniculate terminals which develop a lemmiscal terminal-like morphology and ultrastructure when induced to terminate in the ventrobasal nucleus of the thalamus instead of the lateral geniculate nucleus (Campbell and Frost, 1987).

The trigger for the induced sprouting was apparently vacant synaptic sites rather than a specific target. Silver staining of saphenous skin terminals failed to find sprouting into denervated sciatic skin (Woolf et al., in preparation) and reinnervation of neonatally denervated skin does not alter the expression of these new inputs (Snow and Wilson, 1988). Neonatal nerve cut causes central terminal degeneration which may release trophic factors such as nerve growth factor or gangliosides which are known to affect the survival of afferent fibres (Hulsebosch et al., 1987; Hulsebosch and Carlton, 1988; Bahr et al., 1989). Absence of glial cells may also provide a permissive environment for sprouting but as glial engulfment of degenerating debris occurs then sprouting may become less likely (Carlstedt, 1988).

The results demonstrated here relate to mechanisms of plasticity rather than of development since the denervation was performed at a time when the primary afferents have already reached their central targets (Smith, 1983; Fitzgerald, 1987) although it is conceivable that similar mechanisms operate during normal development. Whether the central direction of afferent growth is a feature of normal development remains unclear. Primary afferents first enter the cord at embryonic day (E) 15 (Smith 1983; Benowitz et al., 1989) and HFA's are first detected at E19 (Beal et al., 1988) and so would have ample opportunity to influence postsynaptic cell growth or induce "labelling factors" such that new sprouts in that region are forced into a certain type of terminal morphology. Alternatively, the features of the

postsynaptic neuropil (or neuromatrix of Melzack, 1989) that determines the afferents pattern of arborization (its neurosignature) may be intrinsic and present before afferents grow into the cord (Melzack, 1989). What is clear is that the central cues are capable of dominating over peripheral receptor characteristics in determining afferent terminal morphology. Consistent with this is the finding in the cat that hair follicle afferent terminals in the cuneate nucleus have a different morphology from those in the dorsal horn (Fyffe et al., 1986), and in the rat trigeminal subnuclei oralis and interpolaris, vibrissae afferents are different from those in the medullary dorsal horn (subnucleus caudalis, Jacquin and Rhoades, 1985, 1987). This was also seen for the terminals of muscle afferents in Clarkes' nucleus compared to those in the ventral horn (Hongo et al., 1987). The development of terminal morphology would therefore differ from earlier events in primary afferent growth where the collaterals that first enter the spinal cord find their correct termination zone by a process that is influenced by peripheral cues (Smith and Frank, 1987). The combination of peripheral and central influences may act to ensure both the correct general arrangement and the specific morphology of the central terminals of primary sensory neurones.

4.6 SUMMARY.

The present findings indicate that following neonatal nerve section, the surviving intact primary afferents have the ability to sprout into adjacent inappropriate central target regions. The morphology of the sprouted collaterals was appropriate to the new target area rather than to its functional class and was also independent of the receptive field location, providing an example of central rather than peripheral control over afferent growth patterns. Such plasticity may provide some compensation following neonatal injury.

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APPENDIX 1.

STATISTICAL ANALYSIS FORMULA FOR UNPAIRED T-TEST.

USE: to compare 2 means from independent samples.

$$S = \sqrt{\frac{1}{n_1 + n_2 - 2}\left({}_1X^2 - \frac{\leq {}_1X\geq^2}{n_1} + {}_2X^2 - \frac{\leq {}_2X\geq^2}{n_2} \right)}$$

$$t = \frac{X_1 - X_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \qquad \text{degree of freedom} = (n_1 + n_2) - 2$$

where S = standard error of the difference

n_1= number of samples in population 1

n_2= number of samples in population 2

X_1= mean of population 1

X_2= mean of population 2

X_1= sum total of population 1

X_2= sum total op population 2

$\langle X_1 \rangle^2$= sum total of all individual samples squared

$\langle X_2 \rangle^2$= sum total of all individual samples squared

all t-tests were performed at the $P>0.05$ level.

APPENDIX II

Reanalysis of statistical significances ($P<0.05$, unpared t-test) of tables 1-8 by comparing the average dimensions for each collateral type per afferent between different groups of afferents. This has been performed since the initial analysis invovled pooled data for the different collateral types from different afferents and may have given a false impression of statistical differences. As the degrees of freedom in some cases were very high it was reasoned that a more realistic answer would be obtained by comparing samples taking n as the no. of afferents instead of the no. of collaterals.

TABLE 2A AVERAGE DIMENSIONS OF TERMINAL ARBORIZATIONS INNERVATING DIFFERENT REGIONS OF THE RAT HINDLIMB.

Reanalysis of statistical differences between groups by comparing the mean dimensions of the different collateral types per afferent instead of comparing the pooled dimensions of the different collateral types.

	DORSAL FOOT			TOES			LATERAL LEG			MEDIAL LEG		
	C	S	C+S	C	S	C+S	C	S	C+S	C	S	C+S
N	6	6	6	11	11	11	14	14	14	7	7	7
ML (um)	77±10*	35±4	60±8	108±17*	62±13	96±14*	55±2*	38±4*	51±2*	88±8*	56±6*	79±4*
DV (um)	158±37	98±49	145±31	163±20	101±19	147±16	143±17*	72±5	127±15*	210±23*	115±18	193±22
RC (um)	279±42	138±38	232±19	276±30	156±27	259±29	343±32	217±29	317±30	277±42	120±11	251±42

X±SEM
N= no. afferents
* = sig. diff. $P<0.05$ unpaired t-test.
ML= mediolateral, DV= dorsoventral, RC= rostrocaudal
C= complex, S= simple, C+S= terminal arbor.

Comparisons made were; C v C, S v S, C+S v C+S for lateral leg v medial leg, dorsal foot and toes and also for medial leg v dorsal foot, toes and dorsal foot v toes. * represents sig. diff. only for lateral leg v other regions.

LE 4A DIMENSIONS OF TERMINAL ARBORIZATIONS OF HFA's FROM DIFFERENT
'E TERRITORIES.

ıalysis of statistical differences between groups by comparing the
ı dimensions of the different collateral types per afferent instead
:omparing the pooled dimensions of the different collateral types.

'E:	SAPHENOUS		SUPERFICIAL PERONEAL			SURAL		LATERAL SURAL				POSTERIOR CUTANEOUS		
C	S	C+S	C	S	C+S	C	S	C+S	C	S	C+S	C	S	C+S
10	10	10	8	8	8	10	10	10	5	5	5	4	4	4
88±6*	53±5*	79±5*	74±7	42±10	68±5^	97±21	55±13	86±18	55±7*	24±5*	46±5*^	57±4*	58±2	58±2*
216±2*	101±15	195±12*	122±10*	60±12	110±10*	154±21	88±17	137±18*	156±47	78±9	123±39	155±34	74±16	145±35
280±16	130±13	251±14	266±34	120±25	219±18	318±29	196±22	318±41	288±31	221±66	278±35	341±48	200±1	320±52
5.2±0.7*	0.9±0.2	4.1±0.6*	2.9±0.4	0.7±0.4	2.5±0.4	5.1±2.8	1.5±1.1	4.2±2.1	2.9±0.7*	0.4±0.3	2.2±0.5*	3.4±0.6	1.1±0.6	3.0±0.6

EM N= no.afferents.
nediolateral, DV=dorsoventral,RC=rostrocaudal;
olume ($x10^{-3}$ul), C=complex, S=simple C+S=terminal
= sig. diff. P<0.05 level

Comparisons made were: C v C, S v S, C+S v C+S, for each nerve territory.

* = SA v all other nerve territories

^ = SP v all other nerve territories

TABLE 6A DIMENSIONS OF THE TERMINAL ARBORIZATIONS OF RAPIDLY ADAPTING (RA) AFFERENTS.

	PAW PAD RA			TOE RA		
	C	S	C+S	C	S	C+S
N	6	6	6	8	8	8
ML (um)	127±12	40±6*	87±12	100±13	73±13*	88±11
DV (um)	195±21	99±6*	148±20	226±48	148±36*	229±40
RC (um)	266±17	190±29	218±20	214±23	177±23	206±23
VOL(ul) ($X10^{-3}$)	7.3±0.2	0.7±0.5*	4.2±0.6	6.7±1.4	3.3±1.1*	5.4±1.0

X ± SEM N = no. afferents
* = signif. diff. P<0.05 level
unpaired t-test.
ML=mediolateral, DV=dorsoventral, RC=rostrocaudal,
VOL=volume.
C=complex, S=simple, C+S=terminal

TABLE 8A DIMENSIONS OF THE TERMINAL ARBORIZATIONS OF SLOWLY ADAPTING TYPE I (SAI) AFFERENTS.

Reanalysis of statistical differences between groups by comparing the mean dimensions of the different collateral types per afferent instead of comparing the pooled dimensions of the different collateral types.

	SAI HAIRY			SAI GLABROUS		
	C	S	C+S	C	S	C+S
N	2	2	2	4	4	4
ML (um)	163±21	98±36	136±16	207±21	41±8	137±10
DV (um)	184±61	138±24	170±53	272±34	85±20	191±15
RC (um)	276±63	210±29	239±34	201±19	168±21	176±10
VOL(ul) ($X10^{-3}$)	8.9±2.5	2.9±0.5	6.4±1.6	10.7±1.8	0.7±0.1	6.5±1.5

X ± SEM N = no. afferents
ML=mediolateral, DV=dorsoventral, RC=rostrocaudal,
VOL=volume.
C=complex, S=simple, C+S=terminal

APPENDIX III

Reanalysis of statistical significances ($P<0.05$, unpared t-test) of tables 14-17 by comparing the average dimensions for each collateral type per afferent between different groups of afferents. This has been performed since the initial analysis invovled pooled data for the different collateral types from different afferents and may have given a false impression of statistical differences. As the degrees of freedom in some cases were very high it was reasoned that a more realistic answer would be obtained by comparing samples taking n as the no. of afferents instead of the no. of collaterals.

COMPARISON OF THE ARRANGEMENT OF COLLATERALS OF SA INTACT AND CONTROL SA HFA's.

	SA INTACT	CONTROL SA
No. afferents	18	10
Total no. collats.	152	86
Av. no. collats. /afferent o/a	8.44±0.72	8.60±1.00
complex	3.67±0.48 (44%)	4.30±0.39 (50%)
simple	2.78±0.48 (32%)	1.70±0.63 (20%)
blind-ending	2.00±0.34 (24%)	2.60±0.45 (30%)
intercollateral distance (um)	378±22	342±26
Length of overlapping terminal sheet (mm)	0.71±0.11	1.26±0.88
Length from most rostral to caudal collateral (mm)	2.78±0.21	2.76±0.27
Projection to lamina II_i	6/66 (9%)	7/43 (16%)

X±SEM

COMPARISONS OF DIMENSIONS OF TERMINAL ARBORIZATIONS OF SA INTACT AND SA CONTROL HFA's.

(A)	SA INTACT			CONTROL SA		
	C	S	O/A	C	S	O/A
N	66	50	116	43	17	60
ML (um)	137±10*	77±6	110±7*	87±4*	55±5	78±4*
DV (um)	223±15	121±12	178±11	201±14	113±13	174±12
RC (um)	242±11	150±13	204±11	269±16	129±13	229±14

X±SEM
* = sig. diff. P<0.05 unpaired t-test. N= no. arbors.
ML= mediolateral, DV= dorsoventral, RC= rostrocaudal
C= complex, S= simple, C+S= terminal arbor.

(B)	SA INTACT			CONTROL SA		
	C	S	O/A	C	S	O/A
N	18	18	18	10	10	10
ML (um)	136±18	79±12	115±14	88±6	53±5	79±4
DV (um)	225±26	119±15	182±18	216±21	101±10	195±12
RC (um)	253±18	153±18	209±14	280±15	130±13	251±17

X±SEM
N= no. afferents
Note that when N= no. of arborizations (A) then there is a sig. diff. between SA intact and control SA arbors in the ML direction but that when N= no. of afferents (B) this difference is lost.

COMPARISONS OF DIMENSIONS OF SA CONTROL AND SA INTACT HFA's ROSTRAL TO THE L3/4 BORDER.

	CONTROL SA			SA INTACT		
	C	S	C+S	C	S	C+S
N	31	12	43	20	14	34
ML(um)	87±6*	56±8	78±5	116±15*	50±7	88±11
DV(um)	193±16	108±18	169±14	232±10	86±11	169±22
RC(um)	260±19	117±15	220±17	253±18	129±15	204±16

X±SEM; N= no. collaterals.
* = sig. diff. P<0.05 level unpaired t-test.

	CONTROL SA			SA INTACT		
	C	S	C+S	C	S	C+S
N	7	7	7	6	6	6
ML(um)	88±8	56±6	79±4	103±10	54±5	87±12
DV(um)	203±23	115±17	186±20	215±12	86±11	174±20
RC(um)	284±40	120±11	259±41	250±25	149±17	219±18

X±SEM; N= no. afferents.
C= complex, S= simple, C+S= terminal arbor.
ML= mediolateral, DV= dorsoventral, RC= rostrocaudal.

There is only a sig. diff. (P<0.05) when N= no. collaterals and not when N= no. afferents.

COMPARISONS OF DIMENSIONS OF SA CONTROL AND SA INTACT HFA's CAUDAL TO THE L3/4 BORDER.

	CONTROL SA			SA INTACT		
	C	S	C+S	C	S	C+S
N	12	5	17	46	36	82
ML(um)	87±5*	51±7	76±6*	146±13*	88±8	121±9*
DV(um)	224±27	115±29	192±24	229±19	135±15	182±13
RC(um)	288±26	144±35	250±25	238±14	163±17	205±11

X±SEM; N= no. arbors.
* = sig. diff. P<0.05 level unpaired t-test.

	CONTROL SA			SA INTACT		
	C	S	C+S	C	S	C+S
N	3	3	3	12	12	12
ML(um)	89±8	48±6	77±5	153±23	90±16	129±19
DV(um)	229±52	83±41	200±41	230±34	133±20	185±23
RC(um)	287±57	114±47	251±13	254±34	153±3	204±19

X±SEM; N= no. afferents.
C= complex, S= simple, C+S= terminal arbor.
ML= mediolateral, DV= dorsoventral, RC= rostrocaudal.

There is only a sig. diff. (P<0.05) when N= no. collaterals and not when N= no. afferents.

TABLE 15A DIMENSIONS OF TERMINAL ARBORIZATIONS OF CONTROL AND SAPHENOUS INTACT HAIR FOLLICLE AFFERENTS.

Reanalysis of statistical differences between groups by comparing the mean dimensions of the different collateral types per afferent instead of compari the pooled dimensions of the different collateral types.

	CONTROL SA			SA INTACT RL3/4			SA INTACT CL3/4		
	C	S	C+S	C	S	C+S	C	S	C+S
N	10	10	10	6	6	6	12	12	12
ML (um)	88±6*	53±9	79±4*	103±6	54±7	87±5	153±23*	90±16	129±19*
DV (um)	226±26	101±10	195±12	215±13	86±12	174±13	230±34	133±20	185±23
RC (um)	253±18	153±18	209±14	250±11	149±13	219±10	254±27	153±20	204±19

x±SEM N= no. afferents.
ML=mediolateral, DV=dorsoventral, RC=rostrocaudal,
C=complex, S=simple, C+S=terminal.
*= sig. diff. P<0.05 unpaired t-test.

Comparisons made were: C v C S v S, C+S v C+ S

TABLE 15A DIMENSIONS OF TERMINAL ARBORIZATIONS OF CONTROL AND SAPHENOUS INTACT HAIR FOLLICLE AFFERENTS.

Reanalysis of statistical differences between groups by comparing the mean dimensions of the different collateral types per afferent instead of compari the pooled dimensions of the different collateral types.

	CONTROL SA			SA INTACT RL3/4			SA INTACT CL3/4		
	C	S	C+S	C	S	C+S	C	S	C+S
N	10	10	10	6	6	6	12	12	12
ML (um)	88±6*	53±9	79±4*	103±6	54±7	87±5	153±23*	90±16	129±19*
DV (um)	226±26	101±10	195±12	215±13	86±12	174±13	230±34	133±20	185±23
RC (um)	253±18	153±18	209±14	250±11	149±13	219±10	254±27	153±20	204±19

x±SEM N= no. afferents.
ML=mediolateral, DV=dorsoventral, RC=rostrocaudal,
C=complex, S=simple, C+S=terminal.
*= sig. diff. P<0.05 unpaired t-test.

Comparisons made were: C v C S v S, C+S v C+ S

Reanalysis of statistical differences between groups by comparing the mean dimensions of the different collateral types per afferent instead of comparing the pooled dimensions of the different collateral types. Data taken from Table 17.

	TIBIAL INTACT	CONTROL TOE RA
MEAN VALUES	140	59
MEDIOLATERAL WIDTH	141	90
OF COMPLEX ARBORS	153	157
(um)	175	89
	104	104
	77	56
	144	155
		93
MEAN OF THESE	129±13 (n=7)	100±13 (n=8)
MEAN VALUES	126	218
OF DORSOVENTRAL	120	230
LENGTHS OF TERMINAL	152	155
ARBORS (um)	184	109
	204	414
	219	358
	147	244
MEAN OF THESE	165±14 (n=7)	229±40 (n=8)

When the means of the dimensions of the afferents were tested for significance using the unpaired t-test ($P<0.05$) taking n as the no. of afferents neither was found to be significantly different whereas they were when n = no. of collaterals .

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The effect of neonatal peripheral nerve section on the somadendritic growth of sensory projection cells in the rat spinal cord

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Key words: Spinal cord; Dorsal horn; Development; Trophic influence; Primary afferent; Peripheral nerve; Neuronal growth

Sciatic nerve section and ligation on the day of birth results in marked growth retardation of the rat dorsal horn. This transneuronal effect was examined in spinal cord cells that project to the brain by retrograde labelling with HRP from contralateral dorso- and ventrolateral tracts in the thoracic white matter. HRP-impregnated gel pellets were implanted in the tracts for 48–72 h to allow intense somadendritic staining of the projection cells. The results show that cells in rats whose sciatic nerve has been sectioned at birth have a mean somal area that is 40% smaller than controls. Primary dendrites are reduced from a mean of 4.1 per cell to 3.1 per cell and secondary branching is reduced by 75%. The results suggest that there was no actual cell death, only growth retardation. An intact primary afferent input apparently has a strong transneuronal trophic influence on spinal cord sensory cells projecting to the brain.

INTRODUCTION

The role of an intact sensory afferent input in the normal development of central neurons is well established[29]. In the visual, auditory and olfactory systems, cells in the lateral geniculate[25], cochlear nucleus[37] and olfactory bulb[34] display a variety of morphological as well as physiological abnormalities if deprived of their primary sensory input early in development.

In the present study we have investigated the extent of this phenomenon in the somatosensory system. Neonatal sciatic nerve section is already known to have far-reaching effects on the developing nervous system. Nearby dorsal roots sprout into the deafferented region of cord[13] secondary transneuronal degeneration of the corticospinal tract occurs[7] and areas in both spinal cord and cortex[8,37] representing the remaining innervated hindlimb are permanently altered. Such changes represent an extensive plasticity in neonates but also suggest a greater vulnerability to peripheral nerve injury than is present in adults.

Another fundamental change in the spinal cord has been observed following neonatal sciatic nerve section and that is a substantial ipsilateral growth retardation of the lumbar dorsal horn such that in the adult the mediolateral dimensions of the grey matter are up to 40% smaller on the treated side[9,21]. This could be due to reduced growth of dorsal horn cells and their processes or even to cell death of this population, as occurs in motoneurons[36]. The aim of this study was to investigate the growth and survival of individual dorsal horn cells following nerve section and to establish the extent to which these central neurons require an intact afferent input for their normal postnatal growth.

MATERIALS AND METHODS

Experiments were performed on adult Wistar rats of both sexes, weighing 250–350 g. Four experimental rats had undergone unilateral sciatic nerve

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section at birth and 4 rats were used as controls.

Neonatal nerve section

Pups were removed from the litter on the day of birth or up to 24 h postnatally (P0-P1) and anaesthetised by cooling to 5 °C on ice. Under sterile conditions, the sciatic nerve on one side was exposed and cut and ligated. The leg was then sutured and the pups gently rewarmed to room temperature before returning to their mothers. They recovered uneventfully and were allowed to reach adulthood.

HRP pellets

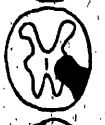
The pellets were made using a modified method from Enevoldson et al.[11]. Agar powder was dissolved in a few ml of distilled water to produce a 3% solution and a small drop of this was placed on a microscope slide warmed to 40 °C adjacent to a small spatula-full of Sigma Type VI HRP (5–10 mg). These were mixed quickly and thoroughly with the aid of two needles, forming a sticky paste which was then separated into small lumps of about 0.5–1 mm in diameter. These were placed on a clean slide and placed in a desiccator for a few hours by which time they had become hard pellets. Such quantities yielded 30–40 pellets of varying size, the larger ones could be broken down to smaller ones. The pellets were stored below 0 °C and under these conditions retained their enzymatic activity for at least 8 months. Before using the pellets, they were allowed to reach room temperature in the container before opening, otherwise condensation in the container made the pellets sticky and unmanageable.

HRP pellet insertion

The animals were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.), supplemented as was necessary. Under sterile conditions one or two vertebral segments were removed at mid-thoracic level to expose the spinal cord. The dura was cut away and a lesion made on the one side of the cord with a hypodermic needle. The position of the lesion varied in dorsoventral extent between animals but involved much of the lateral and ventrolateral white matter (see Table I). The pellet was inserted into the lesioned area and then dorsal columns 2–3 mm caudal to the implantation site were crushed with fine-toothed forceps. This interrupted ascending primary afferent collaterals

TABLE I

The number of cells in L3, L4 and L5 stained with HRP is sho along with their mean somal area (± S.E.) and the exact posit and size of the HRP pellet in the contralateral thoracic white m ter

		No. of cells stained	*Average soma size (μm^2)*	*Pellet position in cord*
Control	A	452	355 ± 10	
	B	410	397 ± 11	
	C	132	361 ± 18	
	D	431	356 ± 6	
Experi-mental	E	156	225 ± 8	
	F	529	171 ± 4	
	G	889	272 ± 5	
	H	440	213 ± 8	

and reduced terminal labelling in the lumbar co Muscle and skin were then sewn up and the anim recovered uneventfully.

After survival periods of 48–72 h the animals w re-anaesthetized and perfused intracardially w normal physiological saline, followed by 2.5% g taraldehyde/1% paraformaldehyde in 0.1 M ph phate buffer and then 20% sucrose in phosphate b fer all at 4 °C.

The sciatic nerve was traced from the sciatic not to its central termination in the cord and the L3, L L5 segment boundaries were identified and mark with pins. The pellet implant site was also identifie The two pieces of cord were removed and stor

overnight at 4 °C in 0.1 M phosphate buffer and 20% sucrose.

Sagittal sections were cut at 20 μm through the lumbar cord and transverse 50 μm sections through the implant region in the thoracic cord. The HRP label was reacted using the TMB procedure. Cells in the lumbar cord retrogradely labelled from thoracic white matter were clearly visible under low power. Those lying within the L3 to L5 segments, identified from the pin marks, which were darkly stained, had well-labelled dendritic processes and with visible nucleoli were drawn under high power (×25) with the aid of a camera lucida. The area of the soma was measured using a computerized drawing pad and the number of primary processes per cell counted.

RESULTS

An HRP pellet in the thoracic lateral white matter resulted in a considerable number of stained cells in the L3–L5 segments in both experimental and control animals. The distribution of these retrogradely labelled cells was the same in both groups and is illustrated in transverse section Fig. 1. The large majority were contralateral to the implantation concentrated in lamine I, and III-V in the dorsal horn and in laminae VII-X in the ventral horn. A few ipsilateral cells were also labelled but these were not analysed. As reported previously with this method, the staining showed up all the primary processes of the cells as well as their soma (Fig. 2).

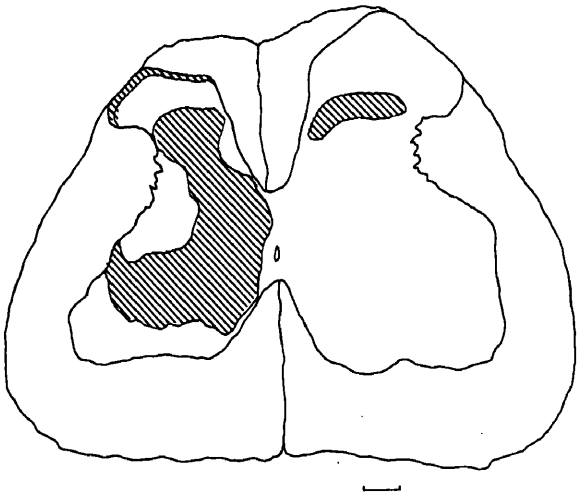


Fig. 1. A diagram of a transverse section through the spinal cord at L4 in a rat whose left sciatic nerve was sectioned at birth. The shaded areas indicate the location of cells retrogradely labelled with HRP placed in the thoracic white matter on the right side (contralateral to the nerve lesion). Scale bar = 200 μm.

Cell soma areas

Well-stained cells in L3, L4 and L5, ipsilateral to the sciatic section and with a prominent nucleolus, were drawn from sagittal sections and the cross-sectional area of the soma measured. The mean somal area for all stained cells measured in 4 control and 4 experimental animals is shown in Table I. The mean values (± S.E.) for the control rats are very consistent ranging from 355 ± 10 to 397 ± 11 μm^2, whereas those in experimental rats were more variable but considerably lower at 171 ± 4 to 272 ± 5 μm^2. This represents a reduction in growth of about 40%.

To obtain a more detailed measure of the effect of neonatal peripheral nerve section on different cell populations, in two control and two experimental rats measurements were restricted to the mid-L4 segment. The cells were divided into: (i) upper dorsal horn neurons, UDH, (those lying in laminae I-IV); (ii) lower dorsal horn neurons, LDH, (lying in lamine V-VI); and (iii) intermediate grey neurons, IGN, (lying in laminae VII and X). The mean somal areas (± S.E.) of these 3 different groups of cells (where *n* is the number of cells measured in each individual animal) are shown in Table II. Direct comparisons can be made between control animal B and experimental animal F and between control C and experimental E since the pellet size and implantation site were comparable within the pairs. Table II shows that the reduction of cell growth following neonatal nerve section is the same in all 3 populations of sensory cells.

Cell processes

Table II also shows the numbers of primary processes and their branches counted from sagittal sections through projection cells in the mid-portion of the L4 spinal cord.

In control animals the mean number (± S.E.) of primary processes was consistent at 3.6 ± 0.4 to 4.5 ± 0.2 per cell, whereas in experimental animals they were rather fewer at 2.6 ± 0.3 to 3.5 ± 0.2. Even more striking was the drop in branching from these primary processes which in control animals ranged from 1.4 ± 0.2 to 2.6 ± 0.3 per cell and in experimental animals was considerably smaller at 0.3 ± 0.1

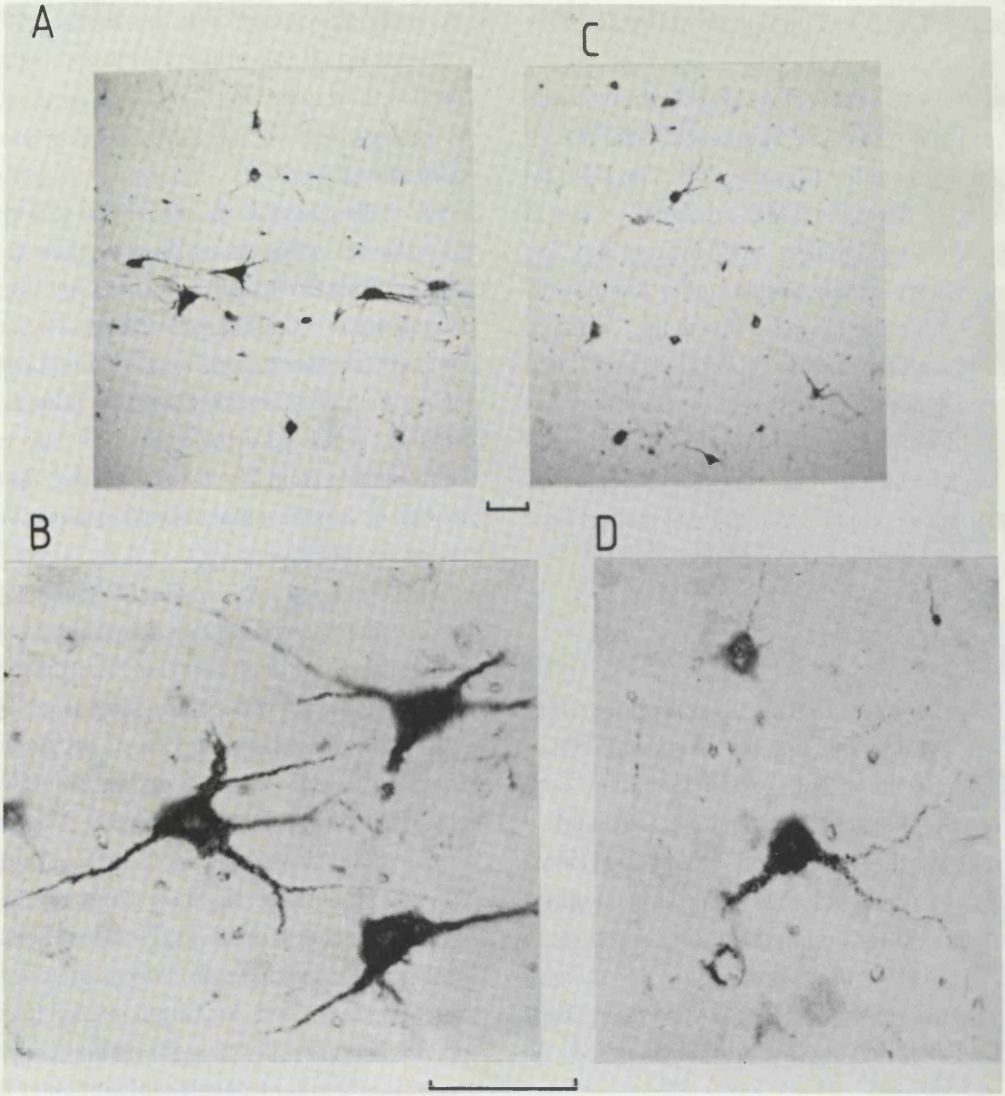


Fig. 2. Photomicrographs of cells in L4 labelled with HRP from contralateral thoracic white matter. A and B are longitudinal 20 μ sections through the L4 cord of control animals, and C and D are sections through L4 cord in rats where the sciatic nerve on that si was sectioned at birth. Dorsal = top; ventral = bottom. Scale bar = 40 μm.

to 0.7 ± 1. These numbers are, of course, limited by the extent of the retrograde filling of the cells with HRP but close examination of the material showed that this was comparable between the two groups.

Cell numbers

Table I shows the total number of cells labelled in the L3, L4 and L5 segments of 4 control and 4 experimental animals. Where pellet size and position are comparable between control and experimental rats, [e.g. between control animal A (n = 452) and experimental animal F (n = 529), and between control animal C (n = 132) and experimental animal E (n = 156], the numbers are in fact slightly higher in the experimental animals. It seems reasonable to conclude from this that little or no cell death has taken place a a result of nerve section.

DISCUSSION

Neonatal sciatic nerve section results in substanti death of dorsal root ganglion (DRG) cells. The ove all percentage lost varies between reports but a pears to be 50–60%[1,2,6,9,25,41]. Those that remain ar abnormal with unusual structural features of somat and processes[1,41]. This early deafferentation has ob vious effects on the normal growth of the hindlimb The hindpaw is considerably smaller than the un affected side and there is ankylosis of the ankl joint[39]. Here we show that there is also clear growt

BLE II

mean (± S.E.) of (a) somal area of labelled cells, (b) number of primary dendritic processes per cell, and (c) number of secondary nches per cell in mid-L4 divided into 3 populations: upper dorsal horn (UDH), lower dorsal horn (LDH) and intermediate grey (IG) text for details)

the number of cells measured in each animal. The results have been divided for comparison between 2 matched pairs of control and erimental rats. The letters B, F, C and E refer to animals in Table I.

	Control (B)	*Exptl. (F)*	*Control (C)*	*Exptl. (E)*
Somal area (mean ± S.E.)				
DH	504 ± 49 (n = 27)	224 ± 20 (n = 28)	469 ± 84 (n = 15)	286 ± 38 (n = 15)
IH	483 ± 36 (n = 28)	230 ± 15 (n = 29)	456 ± 48 (n = 15)	238 ± 25 (n = 16)
	527 ± 37 (n = 34)	224 ± 14 (n = 36)	621 ± 56 (n = 16)	237 ± 26 (n = 17)
No. of primary processes per cell				
DH	3.8 ± 0.2	3.5 ± 0.2	3.6 ± 0.4	2.6 ± 0.2
IH	4.5 ± 0.1	3.3 ± 0.1	3.9 ± 0.3	2.6 ± 0.3
	3.8 ± 0.2	2.8 ± 0.1	3.7 ± 0.2	2.8 ± 0.3
No. of secondary branches per cell				
DH	1.7 ± 0.3	0.6 ± 0.1	2.7 ± 0.6	0.5 ± 0.2
IH	2.4 ± 0.4	0.7 ± 0.1	1.6 ± 0.3	0.5 ± 0.3
	2.6 ± 0.3	0.6 ± 0.1	1.4 ± 0.2	0.3 ± 0.1

ardation in the central nervous system. Spinal d cells in L3, L4 and L5 spinal cord that would rmally receive inputs from the sciatic nerve are nsiderably smaller than in control animals. The an soma size and number of primary dendrites of rsal horn neurons in normal rats agrees well with vious reports[40] but those in experimental animals w dramatically reduced growth in soma size and ndritic branching. We did not observe any signifi- t cell death in the dorsal horn but accurate counts re not attempted. Hamori et al.[25] have presented dence of a small cell loss in n. interpolaris follow- primary trigeminal deafferentation in the neo- e although the overall decrease in volume of this cleus was far greater.

It is not clear how this profound inability to maintain rmal dorsal horn cell growth relates to other con- quences of neonatal sciatic nerve section in the spi- l cord. Saphenous nerve central terminals, for in- nce, grow into the area normally exclusively occu- d by sciatic nerve terminals in the cord[13]. This routing occurs in C fibres in substantia gelatinosa[13] d single A fibre follicle afferents in deeper lami- e[22]. It appears that these aberrant dorsal root ter- nals although functional (Fitzgerald, in prepara- n) are not sufficient to maintain normal growth of e cells in the region.

What factor or factors are missing that result in this central neuronal growth retardation? Clearly maintained separation from the periphery is important, not simply partial deafferentation. Neonatal sciatic nerve crush also results in considerable dorsal root ganglion cell death[6,41] but the regeneration and reconnection with the periphery in the following 7–10 days results in considerably less growth retardation of the dorsal grey matter[21]. The nerve section must take place within the first 10 postnatal days. After this time dorsal horn growth is not affected[21]. This timing is presumably related to developmental events within the spinal cord. Dorsal roots begin to grow into the lumbar cord at E17, beginning with larger A fibres terminating deep in the dorsal horn[35]. Small C fibres begin to grow into substantia gelatinosa on E19.5[15], but both types of fibres undergo considerable terminal growth and arborization over the postnatal period[20,35]. In parallel to this, spinal cord cells are also maturing. By birth, all cells have migrated to their correct positions but considerable dendritic development and synapse formation occurs postnatally[3,33]. This is in a ventrodorsal timetable and cells in substantia gelatinosa are the last to mature[5]. The temporal relationships between spinal cord cell dendritic growth and afferent primary growth is obviously a critical factor in the cells' nor-

mal development. Interestingly, Jackson and Frank[28] have recently shown that motoneuron dendrites

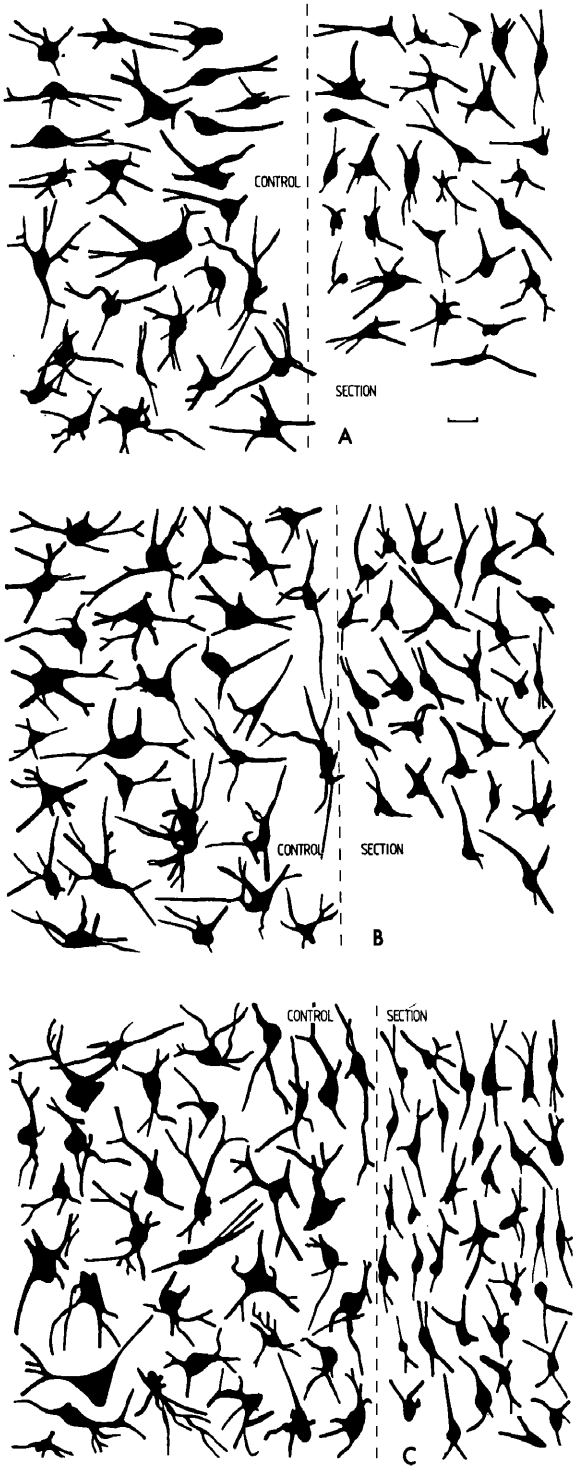


Fig. 3. Camera lucida drawings of cells in L3, L4 and L5 labelled with HRP from contralateral thoracic white matter. In each case cells from control animals are on the left of the dotted line and the equivalent cells in matched experimental animals are on the right. A: upper dorsal horn cells. B: lower dorsal horn cells. C: intermediate grey cells (see text for details). Scale: 20 μm.

grow into an already preformed sensory neuropil muscle afferents and certainly by the time the ela oration of dendrites of substantia gelatinosa cells b gins, sensory C fibre afferents have already gro into position[5,15].

The trophic signals from afferent inputs to growi spinal cord cells could be electrical, chemical or bo Dorsal root ganglion cells show spontaneous activ in the prenatal period but this has died down birth[16]. Primary afferents in the newborn, howev have clear receptive fields and despite low rates firing and a high incidence of habituation on repeat stimulation, would normally provide a substantial put to the neonatal dorsal horn[14]. Furthermore, i well described that cutaneous inputs produce disp portionate amounts of excitation in the spinal corc neonates, far more so than in the adult[10,17,19]. A mented and easily sensitized cutaneous reflexes ha been described in many species and seem to be lated to the long afterdischarges and large recept fields of neonatal dorsal horn cells and the lack of hibition descending from the brain at this time[12] The importance of electrical activity in primary af rent–target interactions during development is c troversial. It is not apparently important for direct growth of primary afferents to their right targets the spinal cord[23], but once they have arrived it mi determine the targets' subsequent growth. Chem factors are also likely to be important[4,31]. The death in DRG following neonatal nerve section be mimicked to some extent by anti-nerve gro factor[41] but it is not known whether spinal cord c are affected under these conditions. It is possible t nerve growth factors transported from the periph also provide a trophic stimulus for spinal cord ce Growth-promoting factors from muscle have b isolated that enhance motoneuron survival in ture[27] but it is not known whether factors in do root ganglion cells are capable of promoting surv of dorsal horn sensory cells transsynaptically.

The results show the importance of intact prim afferent input during the postnatal period. The p found growth retardation of second order cells i follows sciatic nerve section in the neonate is an gous to the effects of deafferentation in other part the somatosensory system[25,30,32] as well as other s sory pathways[24,34,37]. It illustrates the vulnerabilit the developing CNS to peripheral damage.

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Neurosci. Letts. Suppl. 36 (1989)

PERIPHERAL NERVES AND AXONAL PATHOLOGY

THE SOMATOTOPIC ORGANISATION OF SINGLE RAPIDLY-ADAPTING AFFERENT TERMINALS IN THE DORSAL HORN OF THE RAT LUMBAR SPINAL CORD.

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We have studied the morphology and somatotopic pattern of terminals of single hair follicle afferents (HFA's) in the dorsal horn by intracellular filling with HRP.This has shown that while the complex terminals of HFA's are somatotopically organised, there is also considerable overlap of the complex terminals of afferents with non-adjacent peripheral receptive fields (RF's). This overlap was only found however within the central terminations of a given cutaneous nerve,with was no overlap between adjacent nerve territories [1]. Here we report the results of a similar analysis of rapidly adapting mechanoreceptors (RA) innervating the glabrous skin of the hindpaw in urethane-anaesthetized decerebrate rats. These afferent terminals occupy the medial part of the L3-L5 dorsal horn which is devoid of HFA terminals. Terminal boutons were present in laminae II_i-IV and there was occaissional overlap between individual adjacent complex collateral arborizations in an afferent. Within the tibial nerve territory, there was considerable terminal overlap of glabrous afferents with adjacent and non-adjacent peripheral RF's but this overlap did not extend into adjacent nerve territories which contained the terminals of HFA's.

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Morphology and Somatotopic Organization of the Central Terminals of Hindlimb Hair Follicle Afferents in the Rat Lumbar Spinal Cord

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ABSTRACT

The morphology of the central collateral arborizations of 24 A-beta hair follicle afferents (HFAs) innervating different regions of the skin of the hindlimb were studied by the intra-axonal injection of horseradish peroxidase (HRP) in adult rats. A total of 236 collaterals were recovered. These fell into three classes—complex, simple, and blind-ending—based on numbers of boutons and terminal branch patterns. The morphology of the HFA central arbors innervating the lateral and medial leg and dorsum of the foot was flame-shaped. Afferents with receptive fields on the glabrous-hairy skin border consistently had extra terminal branches running ventromedially into laminae IV/V. Differences in the width of terminal arbors were found. HFA terminals innervating the lateral leg formed narrower sheets than those innervating the dorsum of the foot and toes.

The somatotopic organization of the collaterals and terminal arborizations of individual afferents were analyzed both by considering all the collaterals along an axon's rostrocaudal extent and by only examining arbors with boutons (the complex and simple arbors). Thirty-seven percent of blind-ending and 18% of simple collaterals were found to overlap in the rostrocaudal direction with the complex arborizations of afferents whose receptive fields were in a different cutaneous nerve territory. There was no overlap between complex arborizations of afferents from different nerve territories. However, the complex arbors of afferents with receptive fields within a particular nerve territory showed considerable terminal overlap even if they had nonadjacent peripheral receptive fields. The topographical organization of the central terminals of HFAs, forms a coarse somatotopic map of overlapping terminals whereby a particular region of dorsal horn has a maximal, but not exclusive, input from a particular area of skin.

Key words: dorsal horn, somatotopy, horseradish peroxidase

'he cutaneous receptive fields of anatomically adjacent rons in the somatosensory system are organized so that a tinuous, if distorted map of the skin surface is formed at ı level of the neuraxis. Contributing to this at the first e of sensory processing in the CNS is the spatial ngement of the central terminals of cutaneous primary rent neurons in the dorsal horn of the spinal cord. A atotopic organization of the central terminals of cuta- ıs afferents within the spinal cord has been demon- ed by bulk labelling of forelimb (Nyberg and Blomq- '85), thoracic (Ygge and Grant, '83), and hindlimb afferents (Koerber and Brown, '82; Swett and Woolf, '85; Molander and Grant, '85, '86; Woolf and Fitzgerald, '86) by using the transganglionic transport of horseradish peroxidase (HRP). Such peripheral nerve and skin labelling experiments have shown that the afferents that innervate contiguous skin areas have central terminals that occupy contiguous regions of spinal cord in the horizontal plane and that each cutaneous nerve has its own terminal area within

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the dorsal horn with little (Molander and Grant, '85) or no overlap between adjacent nerve territories (Swett and Woolf, '85). This data suggests that the site of the peripheral receptive field of an afferent is encoded by its rostrocaudal and mediolateral position within the dorsal horn.

An alternative approach for studying the central terminals of primary afferents is to use the technique of injecting HRP into single, physiologically characterised cutaneous afferents. The morphology of the collaterals and terminal arborizations of cutaneous low-threshold mechanoreceptor afferents in the cat (Brown, et al., '77; Brown, '81; Meyers and Snow, '84) and rat (Woolf, '87), of low- and high-threshold A-delta cutaneous afferents in the cat and monkey (Light and Perl, '79b), and of C fibre afferent terminals in the guinea pig (Sugiura et al., '86) have been demonstrated. The results from these studies have shown that for each type of afferent there is a distinctive spatial pattern of distribution and morphology of the terminal arborizations in the dorsoventral plane. High-threshold cutaneous A-delta mechanoreceptors distribute their terminal boutons to laminae I and V, while low-threshold A-delta afferents terminate in lamina III with some projections to lamina IIi (Light and Perl, '79b). Cutaneous C fibre terminals are mainly located in laminae I/II (Sugiura et al., '86), whereas low-threshold cutaneous mechanoreceptors in the cat and rat terminate in laminae II_i–IV (Brown et al., '77; Light and Perl, '79a; Brown, '81; Woolf, '87).

There are two superimposed patterns then, in the dorsal horn, one related to somatotopy in the mediolateral and rostrocaudal plane, and one related to receptor type in the dorsoventral plane. The way a given population of single primary afferents is packed together within the dorsal horn to form these two patterns remains unclear. In an individual nerve territory there are many hundreds of afferents which must be packed in such a way that central overlap is inevitable. The aim of the present series of experiments has been to compare the position of the peripheral receptive field of a group of cutaneous afferents with the morphology and position of their central terminal arborizations in the dorsal horn. To do this, single hair follicle afferents innervating different areas of the rat hindlimb have been studied. A preliminary report of the work has been presented (Fitzgerald et al., '88).

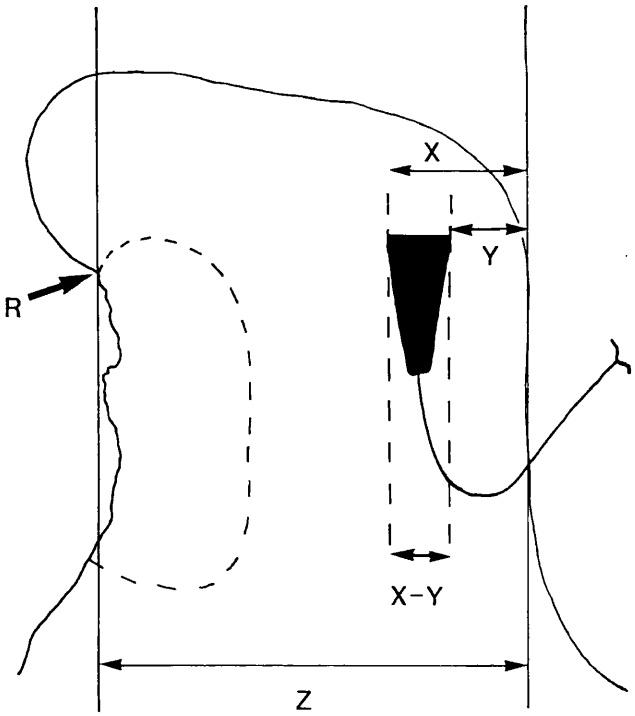


Fig. 1. The normalization of a terminal arbor within the dorsal horn. The two solid vertical lines represent the medial and lateral broders of the dorsal horn. The medial border is taken as the medial edge of the grey matter parallel to the dorsal column, while the lateral border is taken as the border parallel to the medial border at the point where the reticulated area joins the neck of the dorsal horn (R); the distance between the two borders being Z μm. The distance X represents the distance from the medial border to the most lateral bouton in the arbor; the distance Y represents the distance from the medial border to the most medial bouton, X–Y giving the mediolateral width of the arbor and (X–Y)/Z × 100% giving the mediolateral width in the dorsal horn.

MATERIALS AND METHODS

Experiments were performed on 24 Sprague-Dawley rats (200–350 g) of either sex, anaesthetised with urethane (1.5 g · kg^{-1}). The carotid artery and the trachea were cannulated and the animals were decerebrated by aspiration of all the cranial contents rostral to the midbrain. The animals were then paralysed with gallamine and artificially ventilated. Heart rate, rectal temperature, and expired pCO_2 were monitored throughout the experiment and kept within the normal physiological range. The lumbar enlargement was exposed by a dorsal laminectomy, the vertebral column was stabilized by using two spinal and hip clamps, and the spinal cord was supported by a curved metal saddle according to the method described by Woolf and King ('87).

Recordings were made from the dorsal root entry zone and dorsal columns of the lumbar enlargement (segments L3–L5) by using thin-walled glass electrodes filled with 5% HRP in a Tris/KCl buffer (pH 7.7, impedances 20–80 MΩ). Searches were made for rapidly conducting HFAs with receptive fields on the hindlimb. The receptive field properties of these afferents were characterized with the aid of fine camel hair brushes and blunt probes. The conduction velocities were estimated by stimulating the centre of the receptive fields with pin electrodes. HRP was injected into the afferents by using 5 nA, 150 ms depolarising pulses every 200 ms for 2–10 minutes.

The animals were perfused with saline and fixed with 1.25% glutaraldehyde, 1.0% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, a minimum of 2 hours following the HRP iontophoresis into the characterised afferents. Lumbar segments L2–L6 were identified by isolating the dorsal roots and their boundaries were marked by inserting insect pins between adjacent root entry zones; the lumbar spinal cord was then removed and stored overnight at 4°C in 0.1 M phosphate buffer with 20% sucrose (pH 7.4). Serial transverse sections (50 μm) were cut either on a cryostat and dry mounted onto gelatinised slides (1%) or cut on a freezing microtome and stained free floating before mounting onto slides. The slides were incubated in a catechol-p-phenylenediamine mixture (Hanker et al., '77) and camera lucida reconstructions were made from all the transverse sections.

Under darkfield illumination, the lower boundary of the substantia gelatinosa could be clearly identified. This boun-

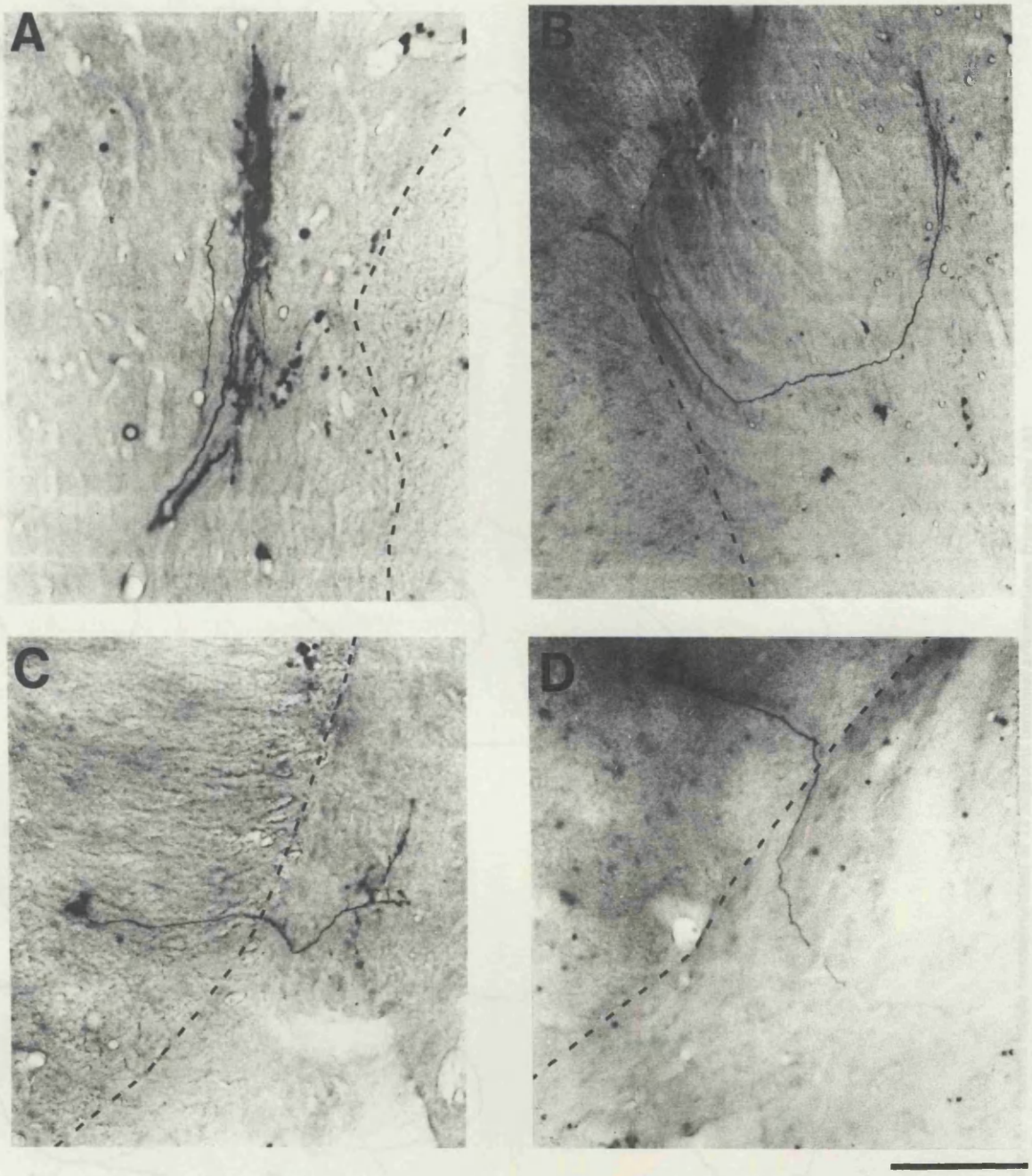


Fig. 2. Photomicrographs of single 50 μm transverse sections of spinal cord showing examples of (**A**) a complex arbor, (**B**) a simple arbor retaining the flame shape, (**C**) a simple arbor located at the rostral end of an axon's rostrocaudal extent, and (**D**) a blind-ending collateral arborization in hair follicle afferents. The dotted lines represents the border between the grey and white matter. Scale bar 100 μm. See text for further details.

dary was noted on tracings, and afferent terminals which extended beyond this boundary into the substantia gelatinosa could then be identified. In all cases those terminals that were located in the substantia gelatinosa were located in the ventral part and for the purpose of this paper this has been termed lamina II_i. No allowance was made for shrinkage.

Construction of plan views of collateral arborizations and somatotopic maps

In order to plot the relative positions of the central terminal arborizations of a population of HFAs with different peripheral receptive fields recorded in different animals and given interanimal variations in the relative sizes of the lum-

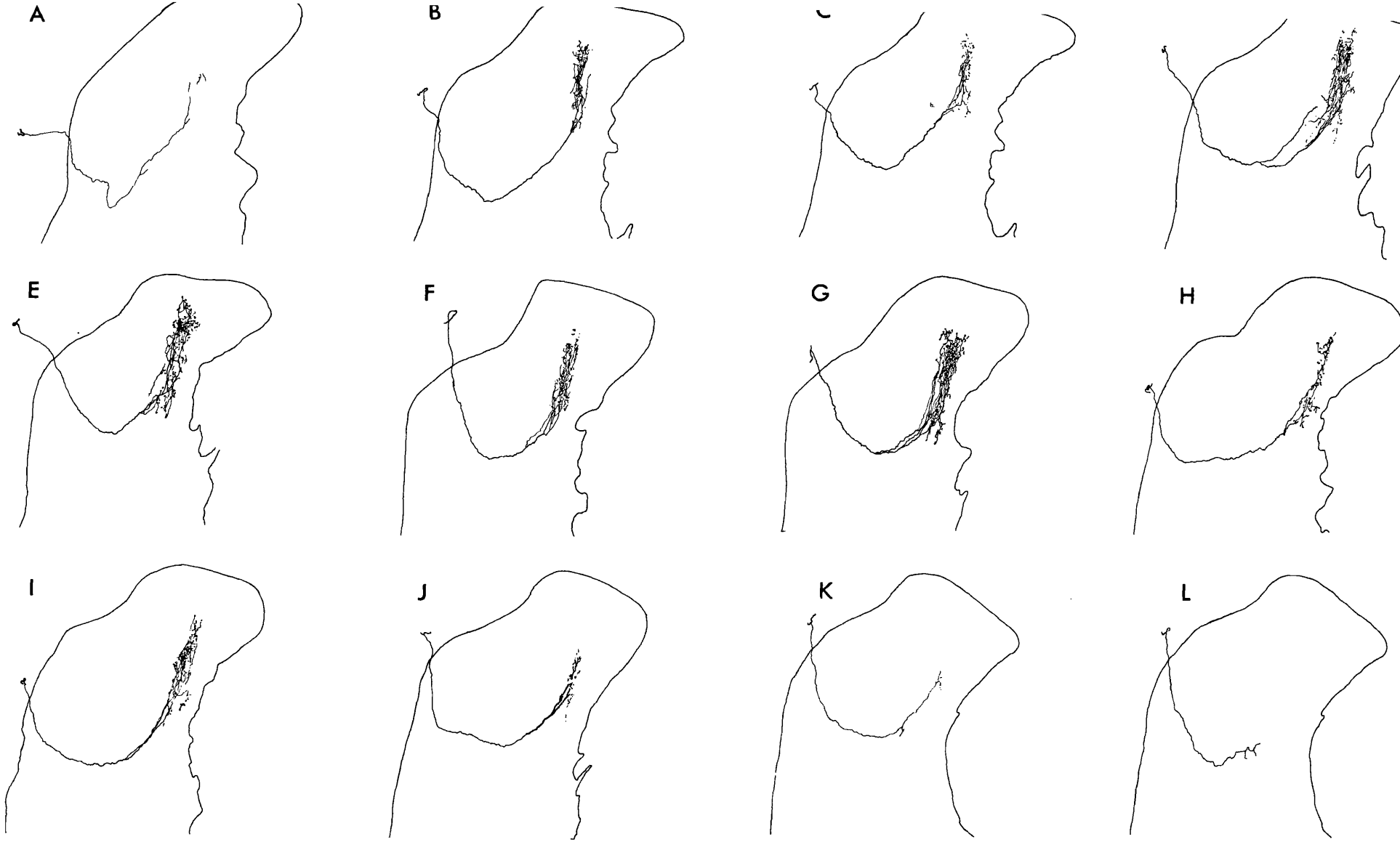
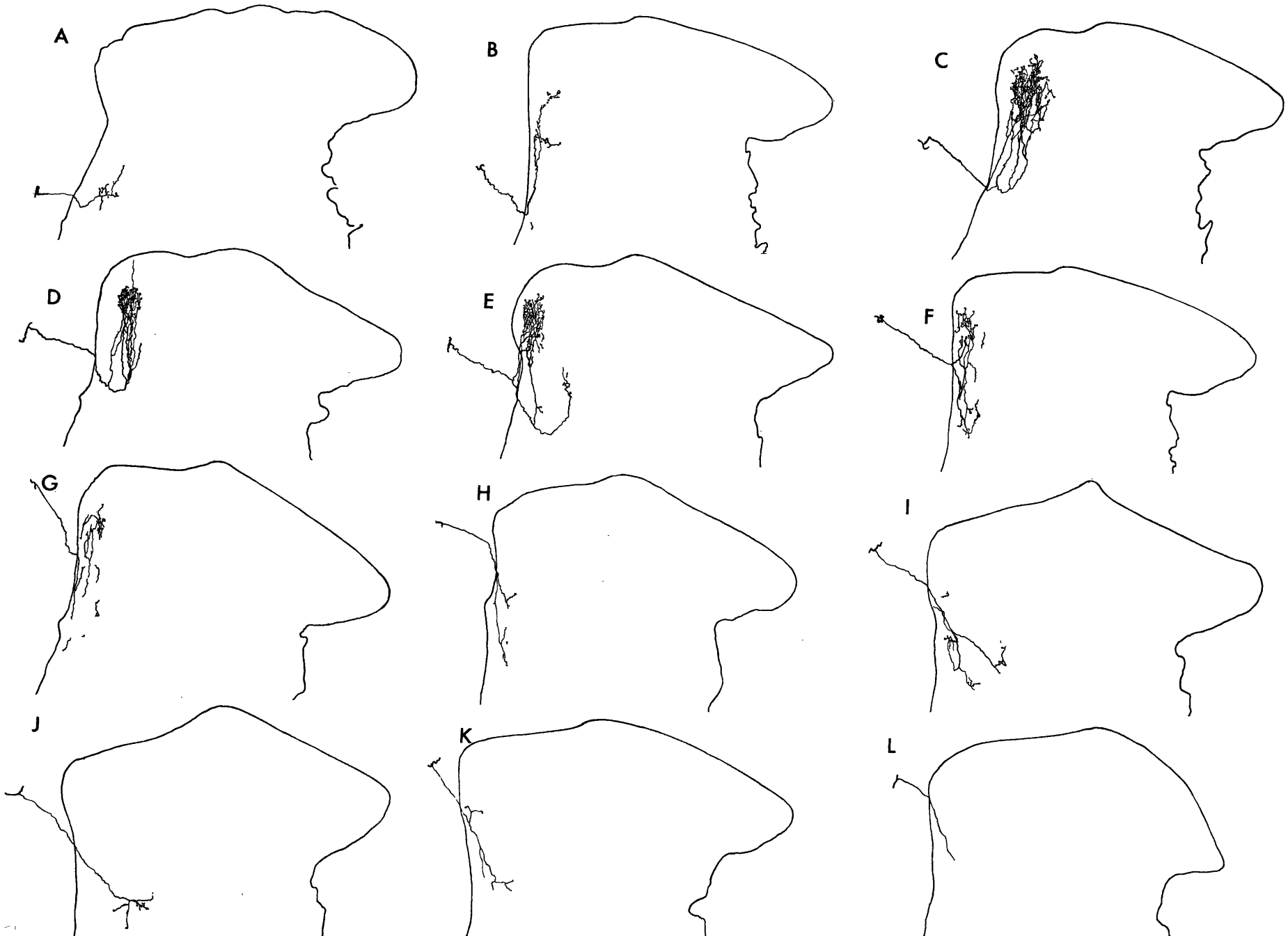


Fig. 3. Camera lucida reconstructions of 12 adjacent terminal arborizations from rostral (A) to caudal (L) of a lateral leg HFA whose receptive field was located on the lateral thigh. Scale bar 250 μm.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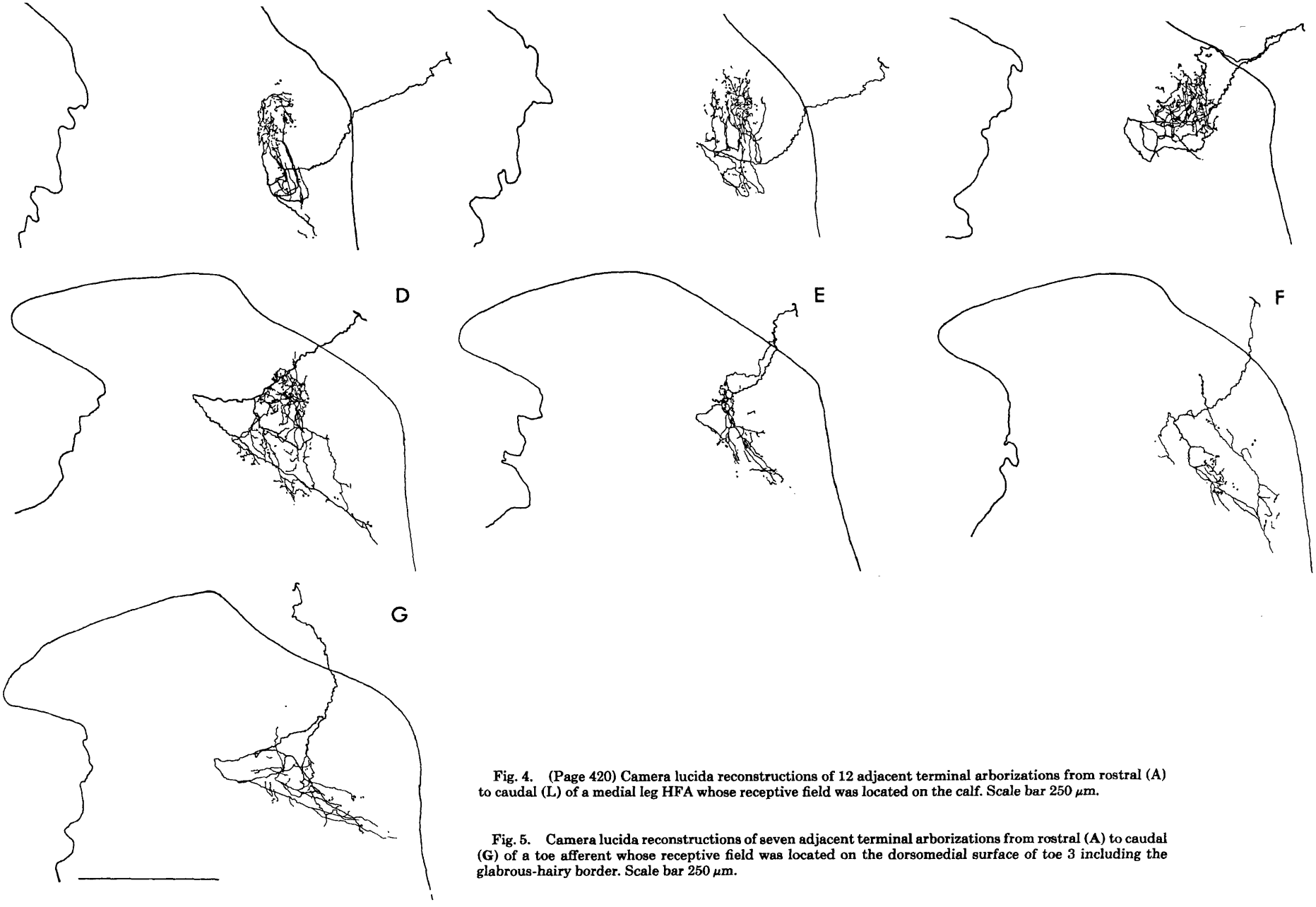


Fig. 4. (Page 420) Camera lucida reconstructions of 12 adjacent terminal arborizations from rostral (A) to caudal (L) of a medial leg HFA whose receptive field was located on the calf. Scale bar 250 μm.

Fig. 5. Camera lucida reconstructions of seven adjacent terminal arborizations from rostral (A) to caudal (G) of a toe afferent whose receptive field was located on the dorsomedial surface of toe 3 including the glabrous-hairy border. Scale bar 250 μm.

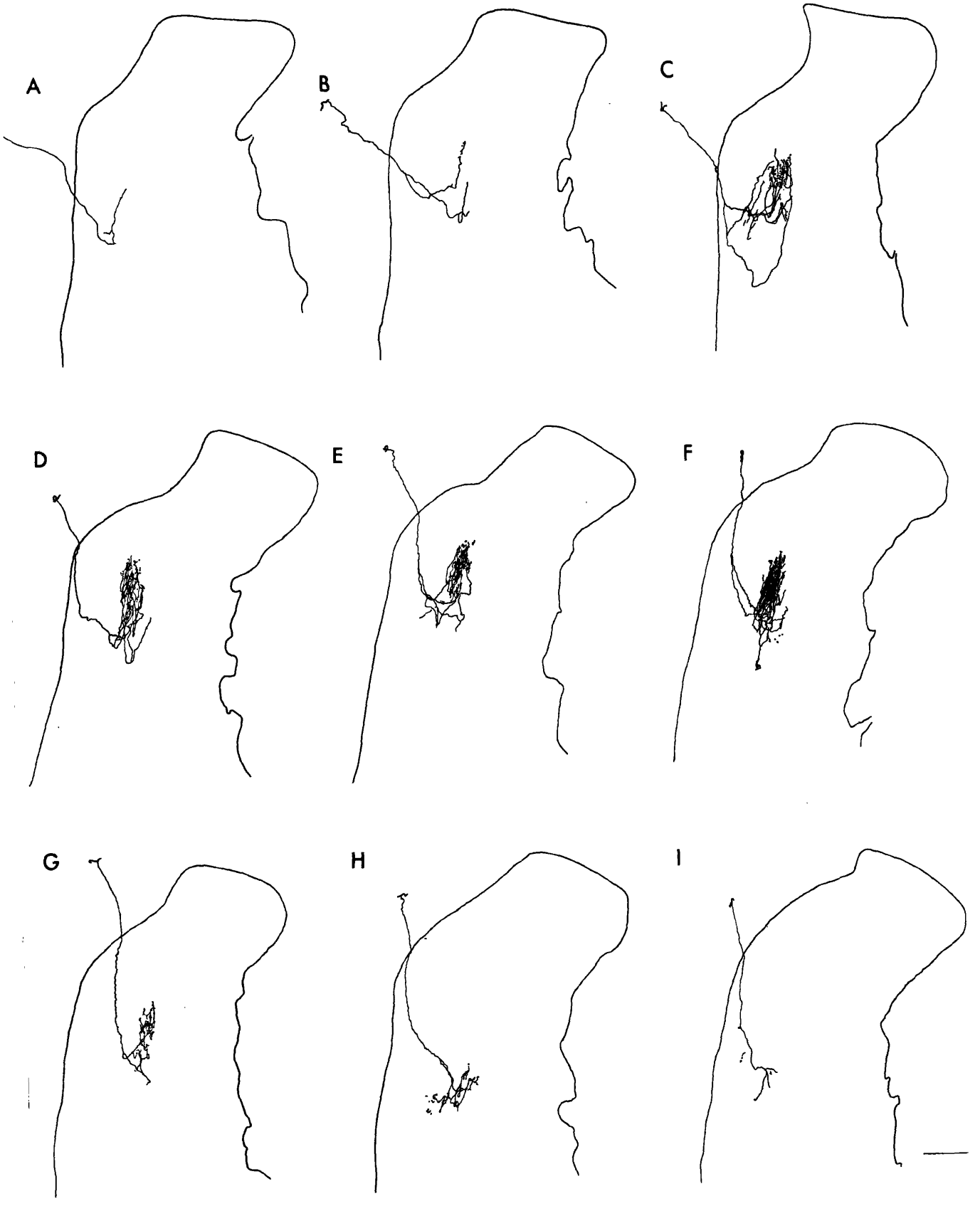


Fig. 6. Camera lucida reconstruction of nine adjacent terminal arborizations from rostral (A) to caudal (I) of an HFA whose receptive field was adjacent to the dorsal toe nail of toe 2. Scale bar 250 μm.

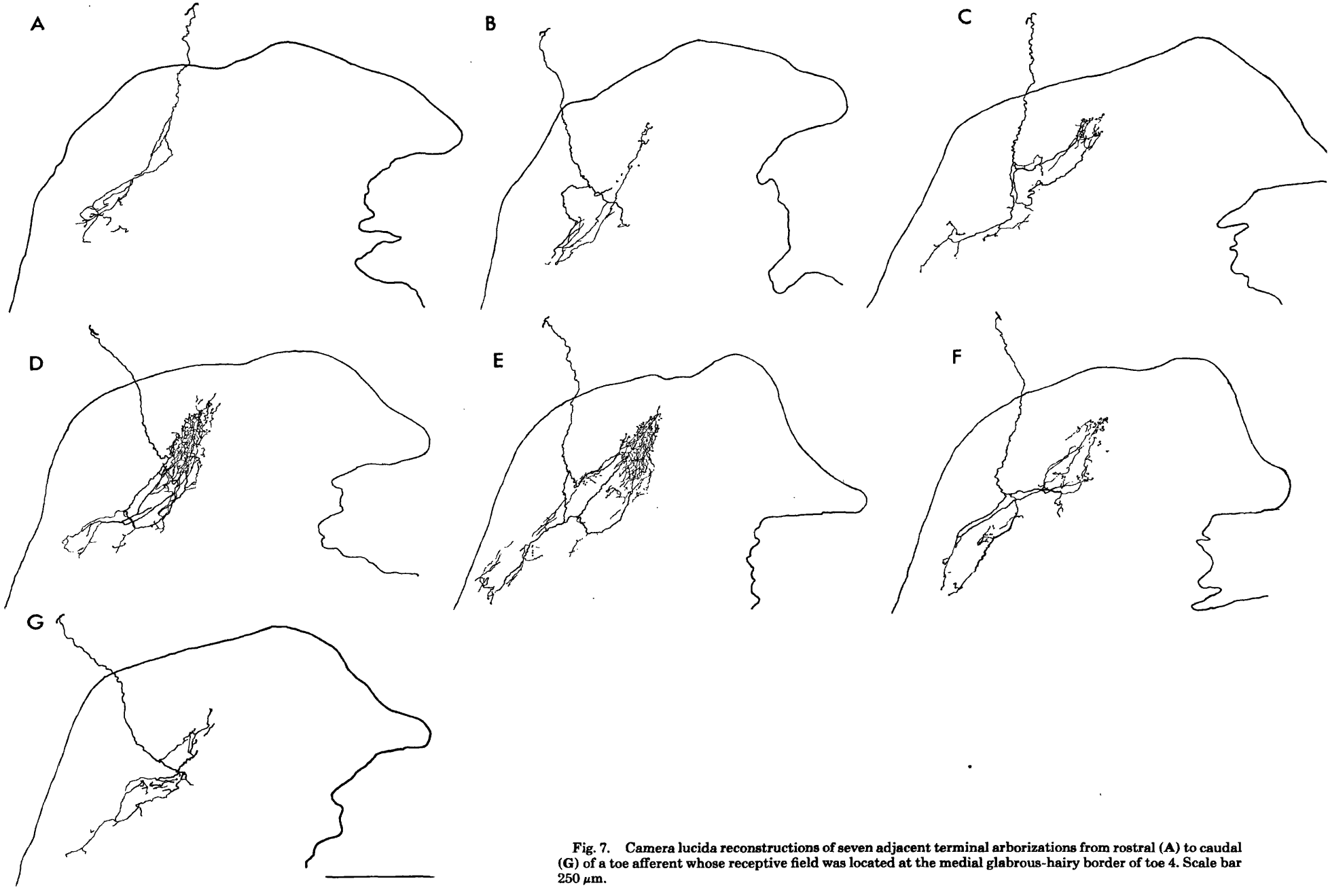


Fig. 7. Camera lucida reconstructions of seven adjacent terminal arborizations from rostral (**A**) to caudal (**G**) of a toe afferent whose receptive field was located at the medial glabrous-hairy border of toe 4. Scale bar 250 μm.

TABLE 1. Regional Differences in Hair Follicle Afferent Central Terminals

	Lateral leg					
ıfferent type	Above knee	Below knee	Medial leg	Dorsal foot	Toes	All types
lo. of afferents	5	5	3	4	7	24
ength of stem axon stained (mm)[1]	4.33 ± 0.59	4.79 ± 0.28	5.20 ± 0.20	3.78 ± 0.34	4.66 ± 0.35	4.50 ± 0.19
lo. collaterals/afferent[1]	10.80 ± 1.74	9.20 ± 1.48	10.60 ± 1.21	6.00 ± 0.60	11.40 ± 0.72	9.83 ± 0.64
ength of afferent from most rostral to most caudal collateral (mm)[1]	3.35 ± 0.59	3.23 ± 0.47	2.72 ± 0.54	2.31 ± 0.55	3.46 ± 0.34	3.11 ± 0.22
ongitudinal length of sheet of complex arbor's (mm)[1]	1.49 ± 0.19	1.46 ± 0.25	1.18 ± 0.05	0.97 ± 0.26	1.28 ± 0.14	1.28 ± 0.04
lo. collaterals per afferent						
Complex[1]	5.80 ± 0.97	4.60 ± 0.60	4.67 ± 0.33	2.50 ± 0.65	6.42 ± 0.68	5.04 ± 0.41
Simple[1]	2.20 ± 0.38	2.00 ± 0.48	3.33 ± 0.88	1.00 ± 0.58	1.29 ± 0.29	1.83 ± 0.24
Blind[1]	2.80 ± 0.80	2.60 ± 1.03	2.67 ± 0.33	2.50 ± 0.96	3.71 ± 0.52	2.96 ± 0.34

= $\bar{x}$ ± SEM.

ɔar segments (Molander and Grant, '85), the dorsal horn was normalised by using the medial border of the dorsal ıorn as an absolute marker for all measurements. The lat-ɛral border was defined for the purpose of these measurements as the border parallel to the medial border at the ɔoint where the reticulated area (R) joins the neck of the lorsal horn (Fig. 1 legend for further details). The location ɔf the collateral arbor could then be located at the same rel-ıtive mediolateral position whatever the size of the cord.

The rostrocaudal position of an afferents central termi-ıals were plotted with respect to the L4/5 pin mark. The engths of the individual lumbar segments in all 24 animals vere measured between adjacent pin marks and the mean engths were: L3, 2,000 ± 20.4 μm; L4, 2,194 ± 86.4 μm; L5, 2,455 ± 89.6 μm ($\bar{x}$ ± SEM), which shows there was only a ımall variation in the size of the segments across the popula-ıion.

RESULTS

The central terminals of 33 HFAs with receptive fields on ifferent parts of the hairy skin of the hindlimb were recov-red after histological processing. In 24 of these, the staining vas sufficiently intense to identify all the collaterals and erminal arborizations and analysis has been confined to hese. Five of these had receptive fields on the upper lateral ɛg, five on the lower lateral leg, three on the medial leg, four n the dorsum of the foot, and seven on the toes. Conduction elocities fell within the range 13–46 ms^{-1} (mean 8.55 ± 7.24 ms^{-1}, SEM). The response properties of the fferents to movement of hairs were essentially similar to hose previously described in the rat (Lynn and Carpenter, 82). Only one out of the 24 afferents responded with a lowly adapting firing pattern in response to sustained hair eflections whereas all the rest only showed a rapidly adap-ing discharge to hair movement.

Receptive fields

The size of the peripheral receptive fields of the different fferents was not uniform. Those on the dorsum of the foot nd toes had receptive fields that were smaller than those ɔcated on the lower and upper leg both medially and later-lly. All afferents innervating the toes and foot had small eceptive fields (1–5 mm in diameter), responding to light rush of the small bristle (guard) hairs. Some afferents had eceptive fields which crossed the glabrous-hairy border of the skin, and in these afferents, light touch of the glabrous portion of the receptive field without any indication of hair deflection elicited a rapidly adapting response. The receptive fields of these afferents tended to be oblong with the long axis parallel to the hairy-glabrous skin border. The receptive fields of the medial and lateral leg afferents were larger (3–15 mm in diameter) and light brush of the guard and tylotrich hairs produced a rapidly adapting discharge. However, two afferents, both with receptive fields on the lateral leg, had receptive fields which were very small, responding only to movement of a single tylotrich hair.

Central terminals

The HFA axons on entering the spinal cord via the dorsal root bifurcated into rostral- and caudal-projecting stem axons, and collaterals were given off at varying distances along the rostrocaudal extent of the stem axons. From all the HFAs studied a total of 236 axon collaterals were recovered. Each afferent had three distinct types of collateral arborizations: complex, simple, and blind-ending (Fig. 2), as described by Woolf ('87). The complex arborizations had extensive third- or higher-order dense networks of terminal axon branches and large numbers of en passant and terminal boutons (Figs. 2A, 3B–G, 4C–E, 5A–D, 6C–F, 7D, E) and showed an overlap between adjacent terminal arbors generating a narrow, mediolaterally restricted sheet of terminal boutons within the dorsal horn. The rostrocaudal distribution of these sheets is readily seen in the plan views of the dorsal horn in Figures 9–13. The rostral and caudal ends of the sheet of overlying complex arbors displayed an abrupt tapering off in the number of boutons over the last 100–150 μm.

Simple collateral arborizations were located rostral and caudal to the complex terminal arbors; they never overlapped with each other and had a reduced terminal branching pattern with fewer boutons (Figs. 2B, C, 3J, K, 4A, B, H–K). The blind-ending axon collaterals had a severely restricted branching pattern and no boutons (Figs. 2D, 3A, L, 4L, 6A, I) and were always located at the most rostral and caudal extremes of an axon's rostrocaudal extent. The number of these types of collaterals per afferent are shown in Table 1. The distribution of these different arbors was independent of the injection site.

Differences in the terminal arborizations of afferents with different peripheral receptive fields. The morphology of the terminal arborizations of typical lateral and medial leg HFAs is illustrated in Figures 3 and 4. These

TABLE 2. Dimensions of Complex HFA Arbors

	Lateral leg				
	Lower	Upper	Medial leg	Dorsal foot	Toes
	23	29	14	10	45
(μm)[1]	49 ± 2.15	48 ± 2.04	77 ± 2.14	74 ± 6.67	75 ± 0.92
(μm)[1]	109 ± 4.80	144 ± 17.46	155 ± 13.90	103 ± 14.39	141 ± 5.66
(μm)[1]	346 ± 29.19	275 ± 14.48	211 ± 7.59	317 ± 33.55	242 ± 10.48
ction to mina II_i (%)	10 (43.48)	17 (58.62)	4 (28.57)	1 (10.0)	12 (26.67)
romedial jection to IV/V (%)	0	0	3 (21.43)	0	17 (37.78)

S.E.M.

erents all have a "U"-shaped curving collateral axon ich ramifies to produce characteristic flame-shaped ar- s identical to those previously described in Golgi (Schei- and Scheibel, '68) and HRP studies (Brown et al., '77; olf, '87). The terminal boutons of the complex arbors e located within laminae II_i–IV but the simple arbors er extended above lamina III. Often the complex arbors ateral leg afferents had a small ventrolateral projection erminal boutons (Fig. 3C–E, G–I) which was not seen in ial leg, dorsal foot, or toe afferents. Flame-shaped rs were also obvious for all the complex and simple col- rals of dorsal foot but not toe afferents.

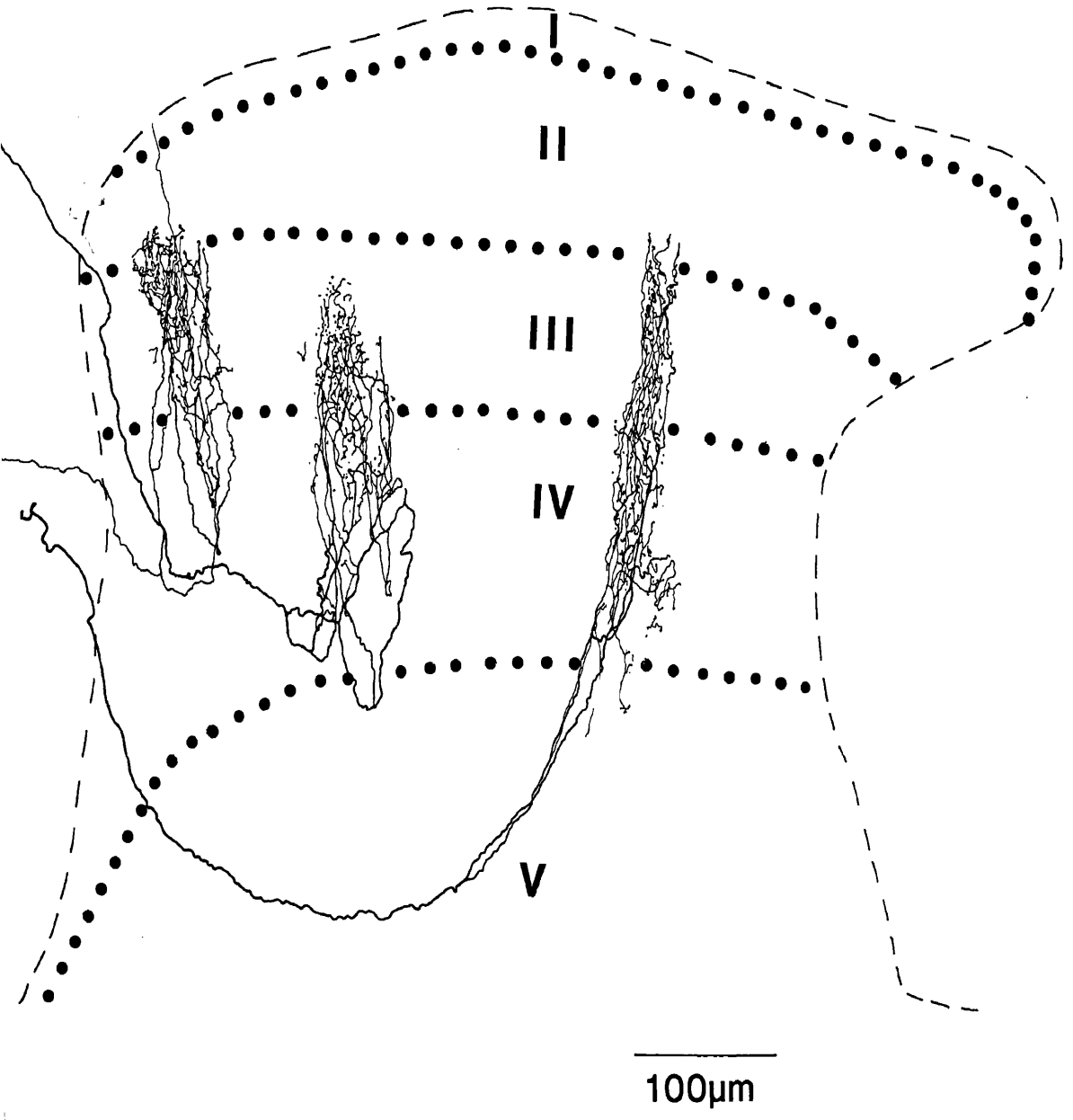
oe HFAs tended to show a considerable variation in the phology of the adjacent collateral arborizations of a sin- afferent. Four of the seven toe afferents had receptive ds only on the hairy skin and two of these had rostral col- rals with a flame-shaped appearance, and their caudal terals had a distorted shape (Fig. 5). The mediolateral tion of the terminal boutons, as for all other HFAs, ained constant throughout the rostrocaudal extent even gh the form changed. The other two toe HFAs with ptive fields only on the hairy skin had terminal arbors the normal flame shape (Fig. 6). Three toe afferents a receptive field that extended from the hairy skin onto glabrous surface and they all showed a unique morpho- al feature common only to this type of afferent: termi- branches that ran ventromedially to arborize in laminae V (Fig. 7). As the axon collateral descends through the ial dorsal horn, it bifurcates sending some branches romedially towards laminae IV/V, while the majority of minal branches terminate dorsolaterally in laminae IIi/ s flame-shaped arbors (Fig. 7C–F). The ventral termi- arbors are relatively simple in structure and have fewer ons than their dorsal counterparts. Adjacent complex terals of the hairy-glabrous skin afferents had overlap- terminals arbors, but this was restricted to the dorsal minal arbors: the ventromedial arbors never overlapped. wever, like the dorsal arbors, the positioning of the ven- edial arbors was in strict mediolateral register.

able 1 shows that there are no differences in the organi- n of collateral axons with receptive fields on the lateral bove or below the level of the knee, each having compa- e numbers of collaterals per afferent, comparable longi- nal length of complex arbor in the cord, and comparable th from most rostral to most caudal collateral. Also, in cases, at least 50% of the collateral arborizations were he complex type. Medial leg afferents have a similar ber of collaterals per afferent and average number of plex arborizations to the lateral leg afferents. However, verage longitudinal length of the sheet of complex ter- al boutons of medial leg afferents was slightly shorter than that of the lateral leg afferents, as was the distance from most rostral to caudal collateral (a feature seen by Brown, '81). Dorsal foot collaterals had the shortest longitudinal distance in the cord and the lowest density of collaterals per afferent. Toe afferents had, on average, the highest number of collaterals per afferent. A general pattern for the complex terminal arbor sheet was that those overlapping sheets which lay in the lateral dorsal horn had the longest length, followed by those in the middle of the dorsal horn (belonging to the toes), while those terminating in the medial dorsal horn had the shortest length (Table 1, Fig. 9). The rostrocaudal length of the complex terminal sheet did not differ between afferents whose receptive field encompassed several hairs and those that innervated a single tylotrich.

Table 2 presents data on the dimensions of the complex arbors from HFAs with different peripheral receptive fields. The most striking observation is the strict mediolateral compression of the terminal arbors of lateral leg afferents compared to hair follicle afferents innervating other areas of the hindlimb ($P < 0.05$, unpaired t-test). Lateral leg afferents which terminate in the lateral part of the dorsal horn are two thirds of the mediolateral width of medial leg, dorsal foot, and toe afferents which terminate in the middle and medial portions of the dorsal horn (Fig. 8).

The somatotopic organization of central terminals. The complete rostrocaudal extent of eight afferents and of their receptive fields is illustrated in Figure 9. When the spatial distribution of all the collaterals of an axon are analysed it is evident that the most rostral and caudal collaterals (which are blind-ending or simple) extend into areas of cord occupied by the complex terminal arbors of afferents with receptive fields in different nerve territories. Of the 71 blind-ending collaterals 26 extended up to 1,500 μm into adjacent nerve territories, while eight of the 44 simple collaterals also overlapped with complex arbors, but they never projected more than 150 μm into the complex terminal area of afferents from a different nerve territory. For example, the three most caudal collaterals of the medial leg (saphenous nerve) afferent extend into the tibial nerve terminal area (Fig. 9a) while the caudal collaterals of the upper leg (lateral sural) afferents are located in regions where lower leg (sural nerve) afferent terminals are found and vice versa (Fig. 9b). Toe afferents also have considerable overlap; the caudal simple collaterals from toe 3 extend into the complex terminal area of toe 5 (Fig. 9b) and those of toe 2 enter the toe 4 complex arbor territory (Fig. 9a) with a reciprocal distribution in the opposite direction.

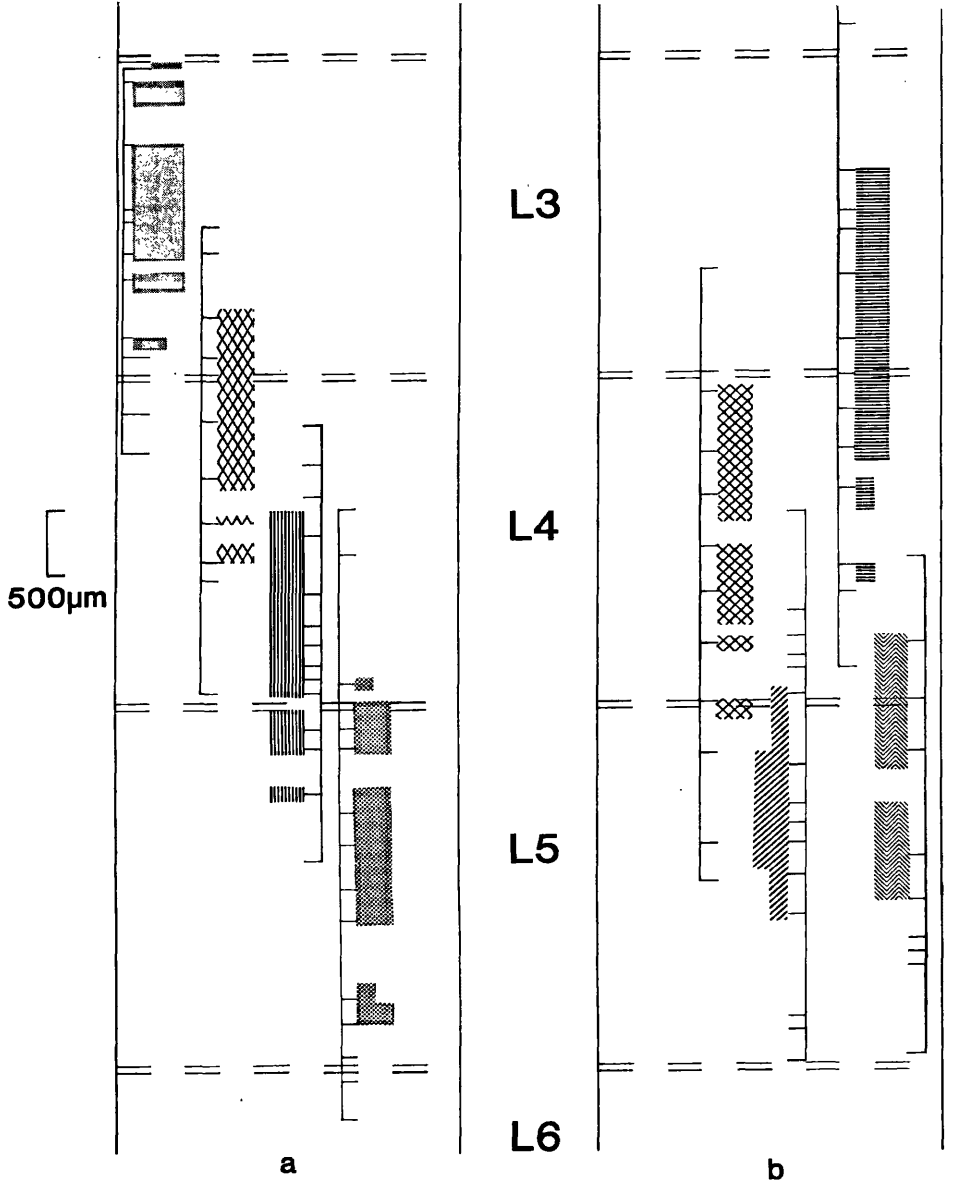
When only complex collaterals are analysed a somatotopic pattern does, however, emerge. A horizontal map of the dorsal horn through lamina III, constructed from all the



. 8. High-magnification reconstructions showing the typical morgy of complex terminal arborizations in a medial leg, dorsal toe, teral leg afferent. The dashed line represents the dorsal horn. The l lines mark the laminar boundaries, which have been derived cytoarchitectonic study by Molander et al. ('84). The most medial arborization is taken from Figure 4D, the middle arborization is from Figure 6D, and the most lateral arbor is from Figure 3I. Note the differences in mediolateral width between the most lateral arbor and the other two arbors.

nts in Figures 3–7, which indicates this somatotopy, is in Figure 10. The complex arbors and 77% of the simbors of afferents with peripheral receptive fields in dif- nerve territories do not overlap; each is restricted to ntral terminal territory of its own particular nerve ed by bulk transganglionic HRP labelling in Swett oolf, '85; and in Woolf and Fitzgerald, '86). For exam- edial leg (saphenous nerve) afferents are located me- in the dorsal horn extending from the lumbar L2/3 to /4 border. Lateral leg (sural nerve) afferents are rep- d in the lateral third of the dorsal horn extending id-L3 to caudal L5, and afferent terminals from the dorsal surface of the foot (superficial peroneal nerve) are located in L4, medial to the L4 lateral leg terminals and lateral to the L4 toe afferent terminals.

Within an individual nerve territory there is, however, considerable overlap between the complex terminal arbors of afferents with nonoverlapping peripheral receptive fields (Figs. 11–13). Afferents with noncontiguous receptive fields on the lateral leg above the knee (Fig. 11) have terminals located from mid-L3 to mid-L4 which all overlap, and a similar overlap is present for afferents with peripheral receptive fields on the lower lateral leg, except that the terminals are located from mid-L4 to caudal L5 (Fig. 12). Taking the lat-



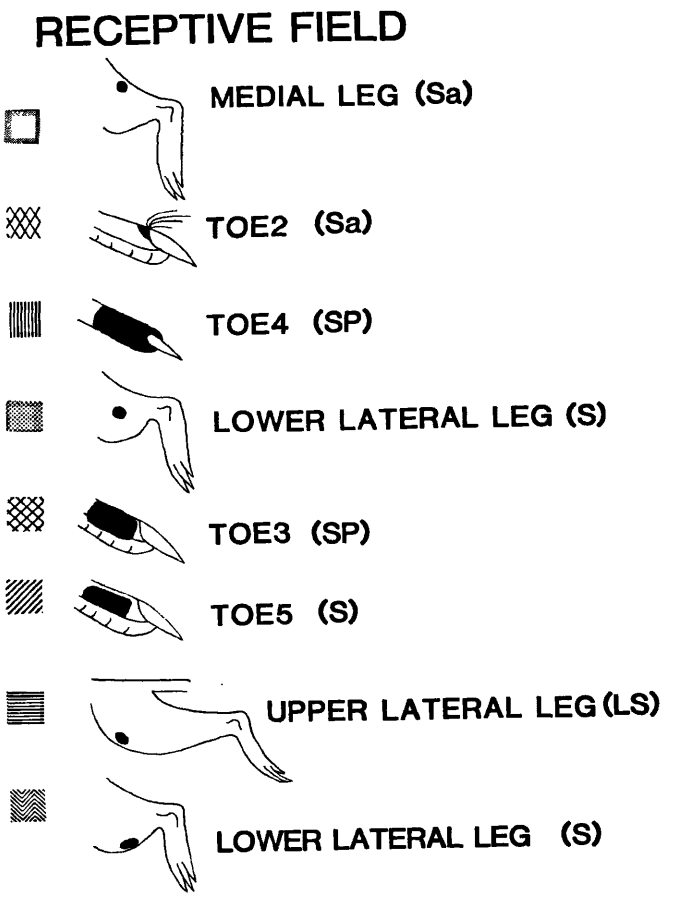


Fig. 9. Plan views through lamina III showing the entire rostrocaudal collateral distributions of eight HFAs with receptive fields innervated by the saphenous (Sa), sural (S), lateral sural (LS), and superficial peroneal (S) nerves. The shading represents complex and simple terminal arbors while the naked lines represent the blind-ending collaterals. L2–L6 represents the lumbar segments; M, the medial grey border; L, the lateral grey border at the neck of the dorsal horn. The horizontal dashed lines represent the border boundaries between segments. The distance to the L5/6 and L3/4 borders from the L4/5 boundary is an average of this distance from all the animals used to reduce interanimal variation in segment size. The afferent terminals have been plotted taking the L4/5 as an absolute boundary for their rostrocaudal extent. The mediolateral width of the arbors has been represented as a percentage of the width of the dorsal horn as described in Figure 1. A and b have been drawn separately for clarity, but if superimposed, overlap between different nerve territories can be seen such that blind-ending collaterals from individual axons end in regions where complex terminal arborization from other afferents occur.

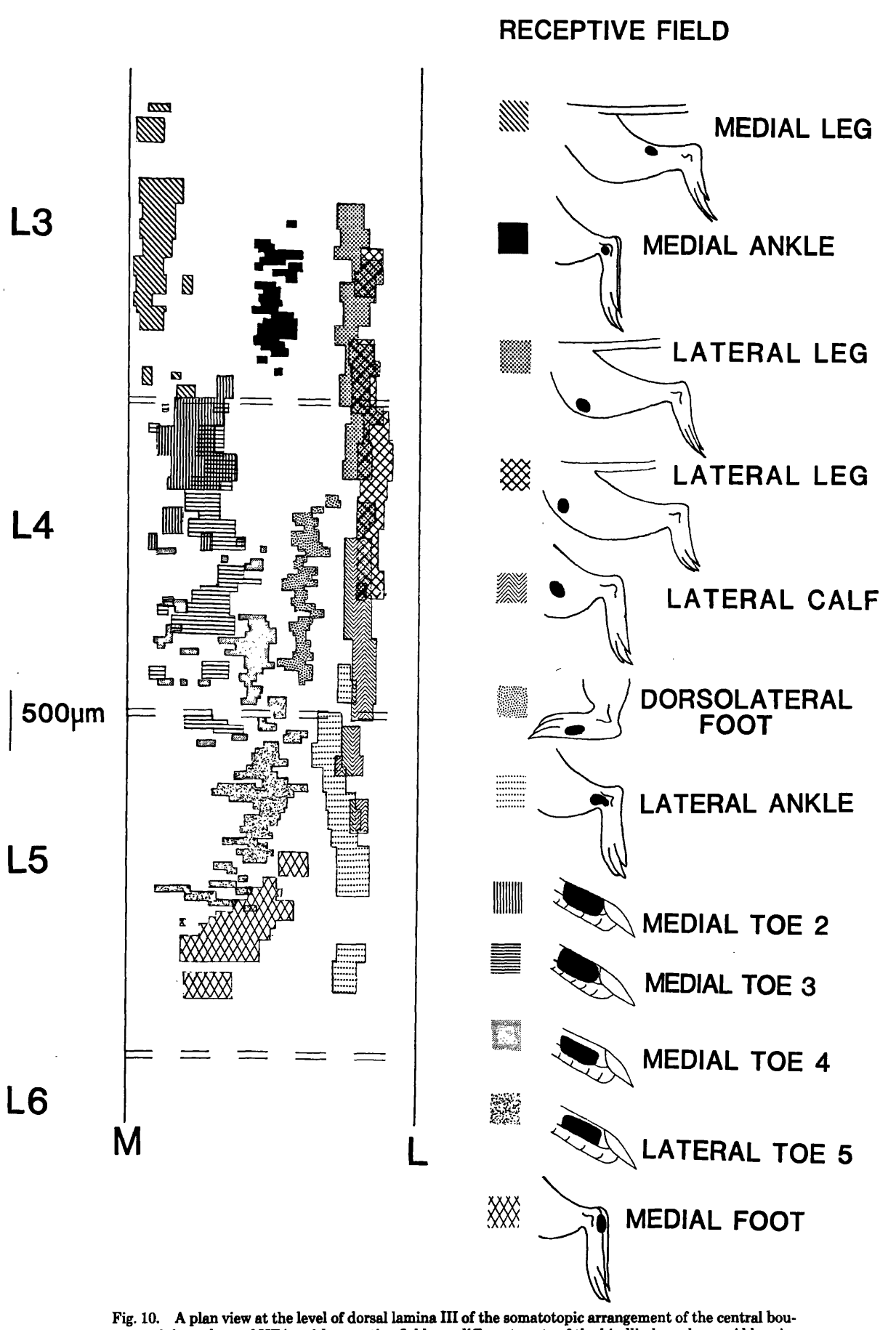


Fig. 10. A plan view at the level of dorsal lamina III of the somatotopic arrangement of the central bouton containing arbors of HFAs with receptive fields on different parts of the hindlimb as shown. Abbreviations as in Figure 9.

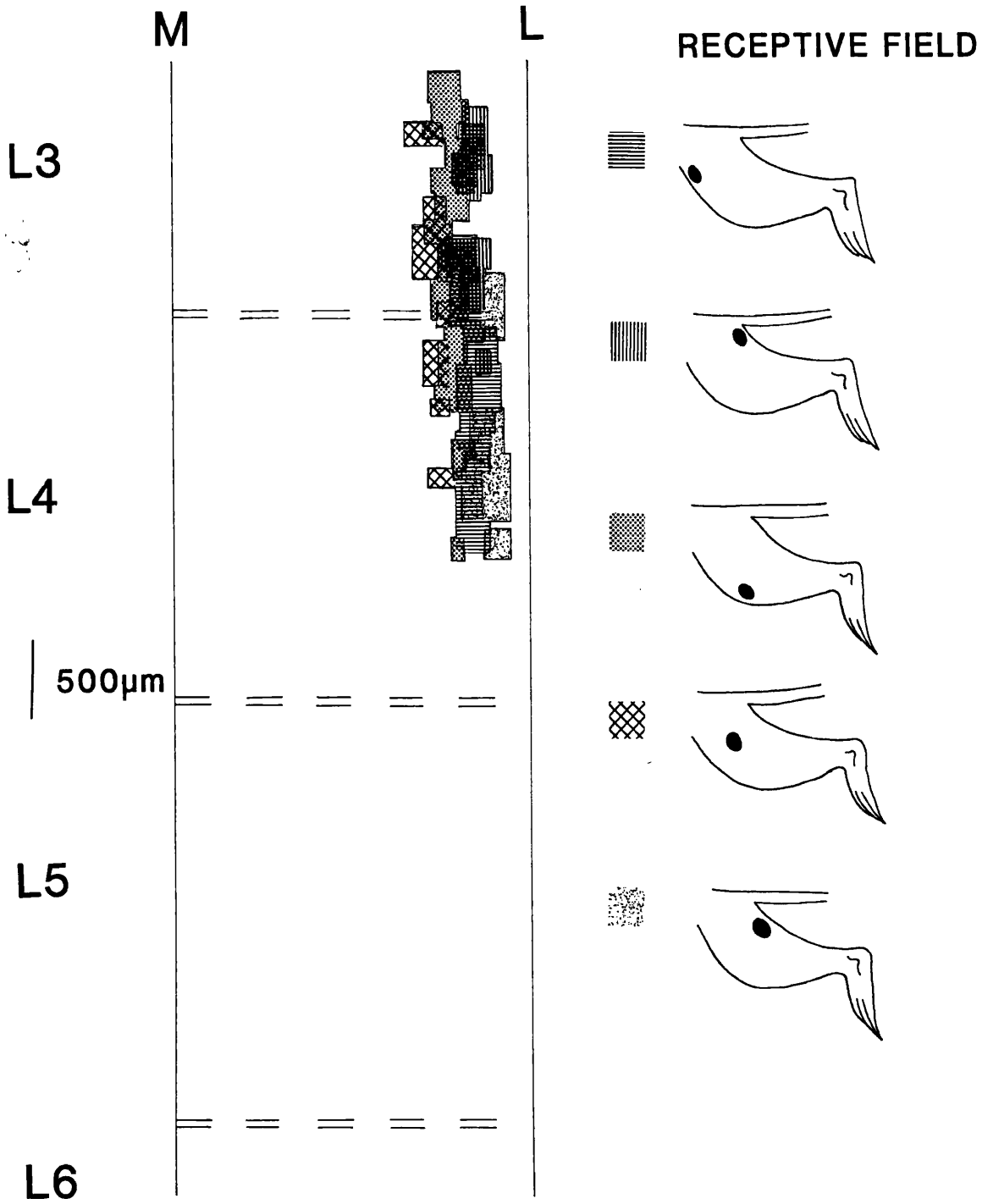


Fig. 11. A plan view showing the somatotopic organization of the terminal arborizations of HFAs with receptive fields on the lateral leg above the level of the knee. Abbreviations as in Figure 9.

ral leg as a whole, there is, however, a spatial somatotopic gradient of the terminals. As one proceeds caudally from L3 to L5 the peripheral receptive fields of the afferents shift from lateral thigh to lateral calf to lateral ankle within the mediolaterally compressed overlapping terminal sheet, although within a given area such as the upper lateral thigh the gradient is not continuous (Fig. 10).

Figure 13 shows the central terminations of afferents from different toes in the dorsal horn. Each toe has its own area of cord into which afferents from that particular toe

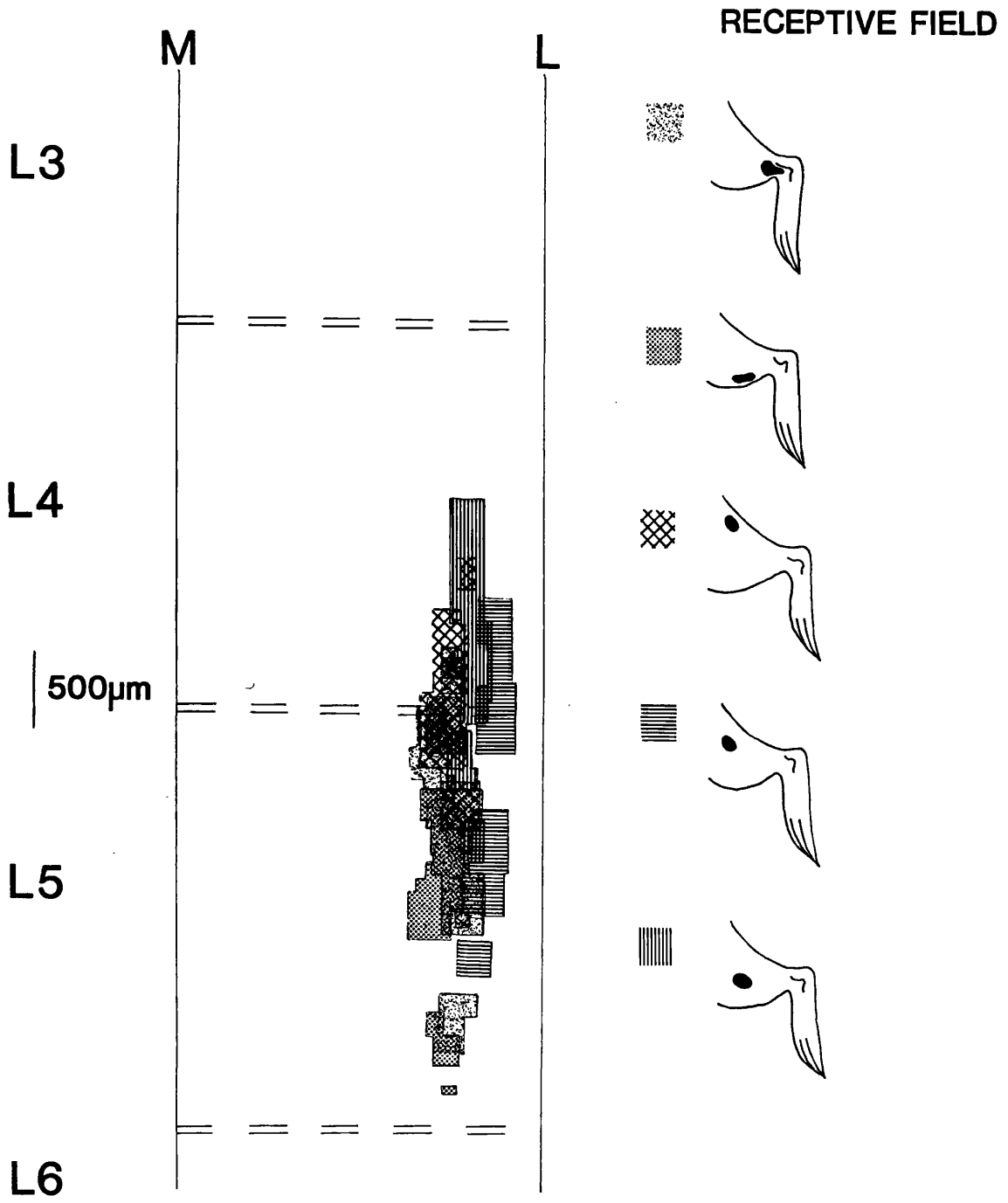


Fig. 12. A plan view showing the somatotopic organization of the terminal arborizations of HFAs with receptive fields on the lateral leg below the level of the knee. Abbreviations as in Figure 9.

terminate. Toes 2–5 are represented progressively more caudally with toe 2 in rostral L4, toe 3 at mid-L4, toe 4 in caudal L4/rostral L5, and toe 5 in mid-L5, with little overlap.

DISCUSSION

The results of the present study confirm in general terms the somatotopy of the lumbar dorsal horn described previously in the rat (Swett and Woolf, '85; Fitzgerald and Woolf, '86; Molander and Grant, '86) and cat (Koerber and Brown, '82) by using bulk labelling techniques. However, reconstructions of individually filled afferent fibres allow for somatotopic mapping within an individual peripheral nerve territory and show up a number of distinctive features in the terminal fields of particular afferents. These include the ventromedial projection to laminae IV/V of afferents with a

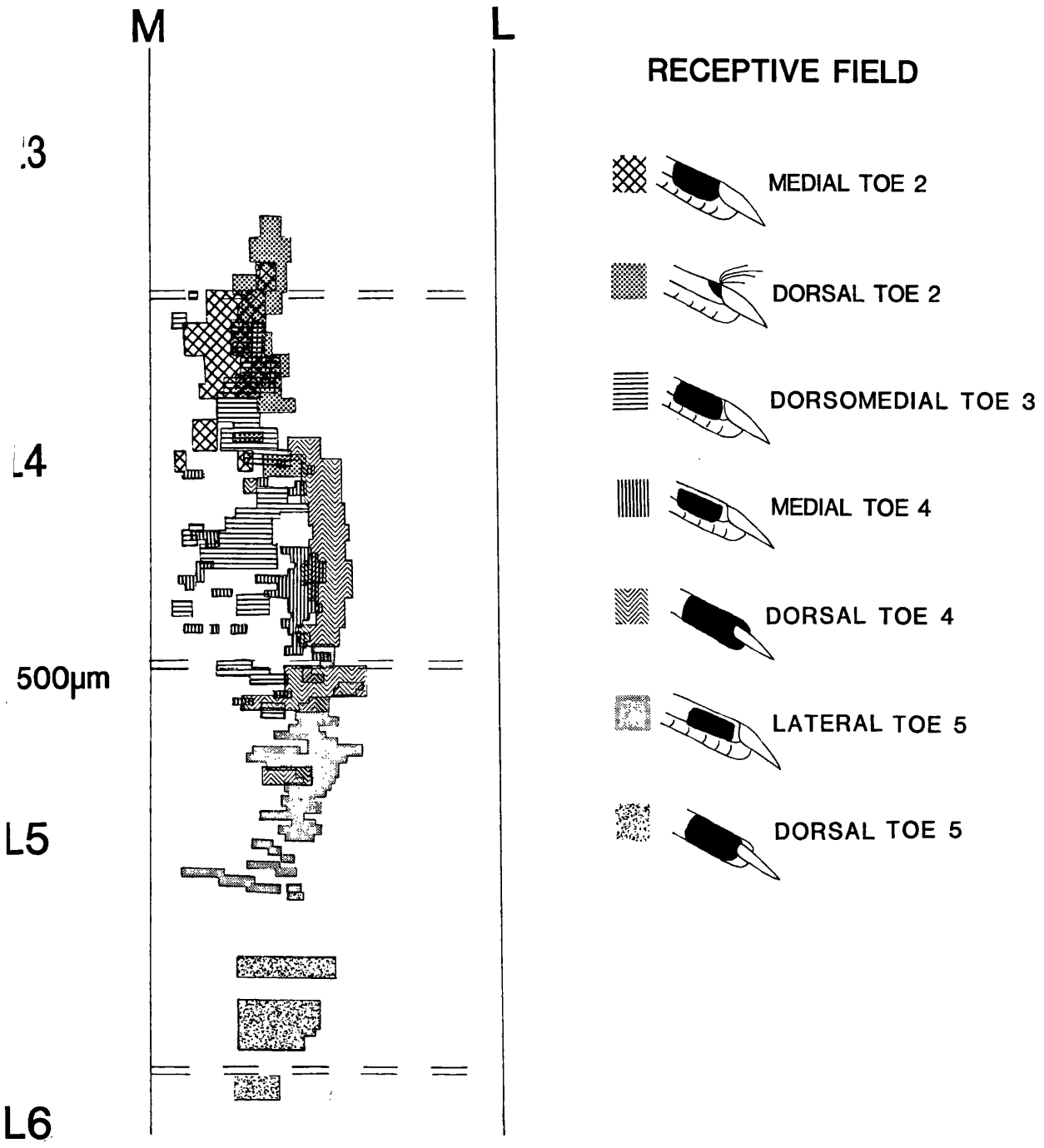


Fig. 13. A plan view showing the somatotopic organization of the terminal arborizations of HFAs with receptive fields on the toes. Abbreviations as in Figure 9.

ceptive field crossing the glabrous-hairy border; the wider ediolateral extent of the arbors of dorsal foot and the toe ferents compared with those of the lateral leg; the central erlap of afferents with nonadjacent receptive fields within single nerve territory and the considerable overlap of the ost rostral and caudal collaterals from an individual affer- at into areas occupied by the complex terminal arbors of her afferents.

Morphology of different types of hair follicle afferents

The morphology of individual HFAs innervating the hindlimb (excluding the foot) has been investigated in detail in the rat (Woolf, '87) and cat (Brown et al., '77; Brown, '81). The results of this study confirm the observation that HFAs innervating the thigh and calf skin form narrow sagittal

sheets of flame-shaped arbors extending from laminae II_i–V in the dorsal horn. Afferents innervating the dorsum of the foot and toes have not previously been studied in the rat, although they have been stained in the cat (Meyers and Snow, '84; Wilson and Snow, '88a,b). They have broader terminal arbors with a more diffuse arrangement of terminal branches than other HFAs, and toe afferents occasionally depart from the flame shape, whereas dorsal foot afferents do not.

Afferents which terminate in the middle and medial areas of the dorsal horn have wider arbors than those which terminate in the lateral part of the dorsal horn (Table 2, Fig. 8). Medial arbors are about 50% wider than the lateral terminal arbors and can occupy up to 20% of the width of the dorsal horn. There are several possible explanations for this. Firstly, there may be more room on the medial side of the dorsal horn for afferents to terminate than laterally. Perhaps during development, afferents grow into the medial dorsal horn first, leaving less space for laterally directed afferents, although it has been shown afferents grow into the cord in a ventrodorsal sequence (Smith, '83). Another explanation could be that a sensory coding function for stimulus discrimination may be employed in the dorsal horn so that those areas of peripheral skin which need to be able to discriminate two closely opposed stimuli can do so within the dorsal horn by having larger central terminal representations. The foot and toes, which have a small external surface area, have a very large central termination area occupying the medial half of the dorsal horn throughout L4/5. A more likely explanation is that the medial side of the dorsal horn contains penetrating muscle, joint (Brown, '81; Hongo et al., '87; Craig et al., '88), and skin afferent fibres from the dorsal columns, and as these pass through the medial dorsal horn, they physically push the arbor apart.

A previously unreported class of afferent that has been studied here is that with a receptive field on the glabrous-hairy border. The complex terminal arbors of these afferents have a split terminal field: a dense, broad dorsal terminal arbor which can occupy up to 15% of the width of the dorsal horn, terminating in laminae IIi/III and a smaller, simpler ventromedial terminal projection near the medial grey matter border in laminae IV/V (Fig. 7C–F). This ventromedial projection terminates in the area which, in L4/5 of the dorsal horn, receives input from glabrous cutaneous receptors (Woolf, '87).

All HFAs, whatever the site of their receptive field, were found in this study to have three types of collateral arborizations—complex, simple, and blind-ending—similar to those previously described by Woolf ('87) and Meyers and Snow ('84). The simple arbors never extended into the ventral substantia gelatinosa, a feature common to complex arbors. Thirty-six percent of the complex arbors projected into lamina II_i with a higher incidence for lateral leg collaterals than for collaterals from other areas of the hindlimb (Table 2). These figures agree with those of Woolf ('87), who found 44% of lateral leg arbors projecting as far as lamina II_i. The complex arbors never extended into lamina II_o (dorsal SG) or lamina I (although one collateral, Fig. 4D, out of all 236, had a terminal branch without boutons that entered lamina I), confirming the observations of Beal et al. ('88) and Woolf ('87).

The possibility that the blind-ending collaterals may reflect inadequate dye filling cannot be excluded, although these were independent of the injection site, and the stem axon could be followed beyond the most caudal or rostral blind collateral, making this unlikely. These blind-ending collaterals at the rostral and caudal extremes of an axon's domain may represent the anatomical substrate for the long-ranging afferents described by Wall and Werman ('76) and are located in somatotopically inappropriate areas of cord as suggested by Meyers et al. ('84). Whether these collaterals have the ability to excite dorsal horn neurons is not certain, but it has recently been shown that they may serve a role in the collateral sprouting that occurs following neonatal peripheral nerve section (Fitzgerald and Woolf, '87; Wilson and Snow, '88a,b) and could represent a potential site for similar sprouting to occur in the adult (Molander et al., '88).

Somatotopic organization of the central terminals of HFAs in the lumbar spinal cord

Minimal overlap is present between the central terminals of primary afferents labelled by applying HRP to cut peripheral nerves (Swett and Woolf, '85; Nyberg and Blomqvist, '85; Molander and Grant, '86). However, when single afferents are labelled as in this study, up to a third of the non-bouton-containing (blind-ending) and 18% of the low-density bouton-containing (simple) collaterals from afferents of a particular nerve overlap with the high-density bouton-containing arbors (complex) of afferents from a different nerve. This discrepancy is possibly due to transganglionically transported HRP accumulating in boutons and not in blind-ending collaterals. Central terminal maps constructed from bulk labelling experiments (Swett and Woolf, '85; Molander and Grant, '86) would then largely be maps of the location of complex and simple arbors rather than of the full extent of all collaterals of an axon.

Within a particular nerve territory, cutaneous afferents with nonadjacent RFs have central terminal fields that overlap to a considerable extent in the rostrocaudal direction. The synaptic density of the terminal arbors of different collaterals may nevertheless contribute to a representation of different skin areas in the dorsal horn. Each afferent's collaterals, although overlapping with other axon's terminals, has a zone of maximal bouton density which falls off relatively gradually as one moves in the longitudinal axis but very rapidly in the transverse axis. These zones of focussed synaptic input differ slightly for different afferents according to the location of their receptive fields. The lateral leg HFAs, for example, show a spatial gradient of their complex arbors, which lie in a narrow sheet in the same narrow mediolateral plane from mid-L3 to caudal L5, because as one moves successively from rostral to caudal there are terminals of afferents from the thigh, calf, and ankle with different but overlapping longitudinally distributed zones of maximal input (Fig. 10).

The central terminal overlap may be related to the pattern of innervation. Hair follicles are innervated by more than one afferent (Millard and Woolf, '88), and a single HFA innervates a number of follicles over a widespread area (Lynn and Carpenter, '82), and that area will be, in turn, innervated by a large number of different afferents, producing a peripheral mosaic of overlapping receptive fields. It is not surprising therefore to find that the central terminals of afferents with different receptive fields overlap to a considerable degree. The relatively large size of the central arbors of individual afferents compared to the area of the dorsal horn devoted to the terminals of individual nerves which consist of many afferents ensures that overlap must occur to

odate all the terminals. The central terminal area related to the peripheral RF area, so the central ter- eas of RFs from a single tylotrich hair or group of re similar. Because the greatest somatotopic gra- in the mediolateral plane it is not surprising that is least in this axis. The width of individual arbors rom 10–20% of the width of the dorsal horn so that maximum of ten nonoverlapping afferents could fit side across the dorsal horn. Because the absolute size ocaudal axis is so much greater than the mediolateral ause the somatotopic gradient in this axis is so much p, it is to be expected that the packing arrangement al terminals is one of overlap in this plane.

CONCLUSIONS

morphology of HFAs may be related to their periph- nervation; flank afferents are different from toe affer- nd these are different from glabrous-hairy afferents. p between different nerves' central territories can be hen one considers all the collaterals of a particular but when only complex collaterals are considered is no central overlap. However, within an individual s central field there is terminal overlap between affer- ith nonadjacent receptive fields. The synaptic bou- f complex arbors do nevertheless form somatotopi- organised longitudinal zones of maximal input. The l distribution of the central terminals of afferents is by insufficiently precise to provide accurate information stimulus location. Such spatial analysis by the CNS depend on the spatial focussing of input in many affer- ogether with the consequent postsynaptic excitatory hibitory interactions on dorsal horn neurons.

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