The cell bodies of origin of sympathetic and Sensory axons in some skin and muscle nerves of the cat hindlimb

ARTICLE *in* THE JOURNAL OF COMPARATIVE NEUROLOGY · MARCH 1983

Impact Factor: 3.23 · DOI: 10.1002/cne.902140202 · Source: PubMed

2 AUTHORS:

Elspeth M Mclachlan
Neuroscience Research Australia
154 PUBLICATIONS **7,260** CITATIONS
SEE PROFILE

Wilfrid Jänig
Christian-Albrechts-Universität zu Kiel
301 PUBLICATIONS **12,191** CITATIONS
SEE PROFILE

THE JOURNAL OF COMPARATIVE NEUROLOGY 214:115-130 (1983)

The Cell Bodies of Origin of Sympathetic and Sensory Axons in Some Skin and Muscle Nerves of the Cat Hindlimb

ELSPETH M. McLACHLAN AND W. JÄNIG
Department of Physiology, Monash University, Clayton, Victoria, 3168 Australia

ABSTRACT

Cell bodies of sensory and sympathetic axons projecting to skin and skeletal muscle of the cat hindlimb have been labeled retrogradely with horseradish peroxidase (HRP) in order to study location, size, and numbers of the somata of these neurons. HRP was applied to the freshly transected axons of nerves supplying hairy skin (superficial peroneal, SP; sural, Su), hairy and hairless skin of the paw (medial plantar, MP), or skeletal muscle (gastrocnemius-soleus, GS). Serial sections of lumbosacral dorsal root and sympathetic ganglia were studied after standard histochemical processing. Additionally, the numbers of myelinated fibers in the same nerves were determined.

All sensory somata and 99.4% of sympathetic cell bodies were located ipsilaterally. Sensory somata were commonly restricted to two adjacent dorsal root ganglia (usually L6-7 for SP, MP; L7-S1 for Su, GS). Although sympathetic somata were more widely distributed rostrocaudally, their maximum frequency always occurred in the segmental ganglia immediately rostral to the sensory outflows, i.e., corresponding to rami communicantes grisei.

Dimensions of sympathetic somata varied little between populations projecting to different tissues and were unimodally distributed. The size distributions of sensory somata were characterized by a peak between 10 and 20 μm radius, similar to sympathetic somata, and a varying smaller number of cells ranging up to 60 μm radius. Each nerve had a characteristic distribution profile of afferent somata. A population of very small cells was only present in GS, while the largest sensory somata in GS and MP were bigger than those in SP and Su.

Numerical analysis of the data disclosed the characteristic composition of both myelinated and unmyelinated fibers in each nerve studied.

Key words: unmyelinated, myelinated, postganglionic, segmental dimensions

Nerves supplying skeletal muscle of the cat hindlimb contain skeletomotor, sensory, and sympathetic postganglionic fibers, while nerves to the skin have only sensory and sympathetic components. The majority of these nerve fibers are unmyelinated. The functions of the myelinated fibers have been extensively investigated, and their numbers in different nerves either counted directly (Boyd and Davey, '68) or estimated by physiological methods (Eccles and Sherrington, '30) with comparable results. The functions of the unmyelinated fibers have been less well analyzed (see Burgess and Perl, '73). In particular, the relative numbers of myelinated and unmyelinated fibers in the nerves supplying different tissues are largely unknown, as is the relative proportion of the unmyelinated population which consists of sympathetic postganglionic fibers. Furthermore, it is not known whether any relationship exists between the location of postganglionic somata in the sympathetic chain and the segmental spinal ganglia in which the afferent somata of a particular organ lie. These quantitative data are of considerable importance to neurophysiologists working in the fields of somatosensory systems and the autonomic nervous system, in order to clarify the significance of electrophysiological data which

Accepted July 28, 1982.

Dr. Jänig's present address is Physiologisches Institut, Universität Kiel, Kiel, Federal Republic of Germany.

0021-9967/83/2142-0115$04.50

are mostly obtained on small populations of peripheral neurons.

We have therefore undertaken a quantitative study of the cell bodies of origin of the sympathetic and sensory axons in nerves projecting to muscle and skin in the hindlimb of the cat. The neurons have been identified by retrograde labeling with horseradish peroxidase (HRP) applied to their transected axons in the periphery. HRP was applied to the nerves supplying medial gastrocnemius, lateral gastrocnemius, and soleus muscles (abbreviated collectively as GS), to the medial plantar nerve (MP) which supplies the plantar surface of the paw (including both hairless and hairy skin), and to the sural (Su) and superficial peroneal (SP) nerves as representative nerves to hairy skin. The distribution of all labeled cell bodies in the lumbosacral sympathetic chain and in the dorsal root ganglia was determined, and the dimensions of these neurons quantified by using a digital image analysis system.

METHODS

Fifteen adult cats of both sexes were used in these experiments. The animals weighed between 3.3 and 4.7 kg with the exception of one young female of 2.1 kg. They were anaesthetized with sodium pentobarbital (35 mg/kg i.p.), and about 4 μl of 20–30% HRP (Sigma Type VI) was applied to the central end of freshly transected hindlimb nerves using a pledget of hemostatic foam and a sleeve of plastic tubing (Method B of Oldfield and McLachlan, '80). Particular care was taken to protect adjacent tissue from contact with the HRP solution by covering it with plastic film, although the location of the application sites usually minimized exposure of other damaged axons to HRP.

Sites of application were:

(1) the nerves to medial gastrocnemius and lateral gastrocnemius-soleus muscles close to their entry into the muscles (GS). These nerves contain about 900 motor fibers (from Boyd and Davey, '68);
(2) the medial plantar nerve (MP) 2 to 3 cm distal to the calcaneum (just proximal to its branching into lateral and medial bundles);
(3) the sural nerve (Su) about 4 cm above the ankle;
(4) the superficial peroneal nerve (SP) near the ankle about 2 cm beyond the most distal peroneal muscle branch.

In cases where the cut nerves were particularly prone to bleeding (especially MP), poor numerical results were obtained unless the bleeding could be prevented by pressure (e.g., with a tourniquet) during HRP application (see Results).

After 48–72 hours, the animals were anesthetized again with sodium pentobarbital (45 mg/kg i.p.) and perfused through the aorta, initially with 500 ml of 0.9% NaCl containing 0.1% albumin and 5,000 units of heparin sodium, and then with at least 1.5 liters of 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. All lumbosacral sympathetic ganglia and dorsal root ganglia (from at least L3-S2 bilaterally), together with the sites of application of HRP, were dissected from the animal, postfixed until the total fixation time was 4–6 hours, and then placed in 0.1 M phosphate buffer containing 30% sucrose for 2–3 days at 4°C. Dorsal root ganglia were marked with indian ink on their dorsal aspect during the dissection, and were usually separated from the ventral roots at L6-S2; no neuron cell bodies were found in any ventral root subsequently examined (see also Webber and Wemett, '66). Serial sections (35 μm) of all ganglia were cut with a freezing microtome and mounted on gelatinized slides before processing for the demonstration of HRP using tetramethylbenzidine (TMB) (de Olmos et al., '78; Mesulam, '78). Sections were dried overnight and counterstained with neutral red before examination using both light and darkfield microscopy.

The anatomy of the sympathetic chains and the origin and destiny of all connecting rami were carefully documented during dissection. Ganglia which were fused bilaterally (present in about 50% of animals at L7 and/or S1) were usually easy to separate under a dissecting microscope before sectioning, or later by tracing the arrangement of their lobes through serial sections.

Myelinated fiber numbers were counted from semithin (0.5 μm) sections of osmicated pieces of some of the labeled nerves. These were generally taken from about 7 to 10 cm central to the point of HRP application in order to avoid the region where the axons had swollen proximal to the nerve lesion.

Quantitative estimations

The total numbers of labeled profiles were counted in darkfield at a magnification of × 100 or × 160, with distinction being made between complete round or ovoid "cells" (in which nuclei could usually be identified) and "offcuts." These were tangential slices of labeled cells in which no nucleus could be detected, and were present in only the superficial few μm depth of any particular section. They were characterized initially by scanning through serial sections in both light and darkfield. Their appearance varied somewhat according to the labeling characteristics of different cells. Small round or ovoid profiles <5–6 μm in diameter, usually containing only a few granules, always proved to be offcuts of cells whose nucleus could be identified in the adjacent section. Some larger, often crescent-shaped, profiles also proved to be offcuts, but similar structures when examined in brightfield were sometimes found to be intact sections through the nucleolus of neurons which contained only a few HRP granules (see Fig. 1). Because of these difficulties in identification, total cell counts are the sum of both "cells" and "offcuts" corrected as described below. In a few cases in which very large numbers of DRG cells were labeled, especially SP, counts were made on every second section and values for the intervening sections extrapolated. Random checks on this procedure revealed error of less than 5%. Numerical counts made on the same material by each of us independently, or by one of us on two occasions, always varied by less than 10%.

For measurement of soma size, sections from ganglia containing the highest numbers of labeled cells in each animal were projected at a magnification of about × 450 with a Leitz Micropromar projection microscope, and cross-sectional areas were determined by using a Zeiss MOP-1 Image Analyser linked with a small computer. There was no difference between area measurements made at this magnification and at × 750. All profiles measured either appeared to contain a nucleus (the nucleolus being obscured in most cases by stained granules), or were complete round or ovoid profiles very densely packed with HRP reaction product. Four hundred profiles containing granules of HRP reaction product were measured from random sections through the thickness of each sympathetic ganglion studied but successively from each of the dorsal and ven-

Abbreviations

GS	nerves to medial gastrocnemius and lateral gastrocnemius-soleus muscles	DRG	dorsal root ganglion
MP	medial plantar nerve	W1 . . . . W15	number of experiment
Su	sural nerve	L1 . . . . L7	lumbar segmental level
SP	superficial peroneal nerve	S1, S2	sacral segmental level
HRP	horseradish peroxidase	L	left
TMB	tetramethyl-benzidine	R	right

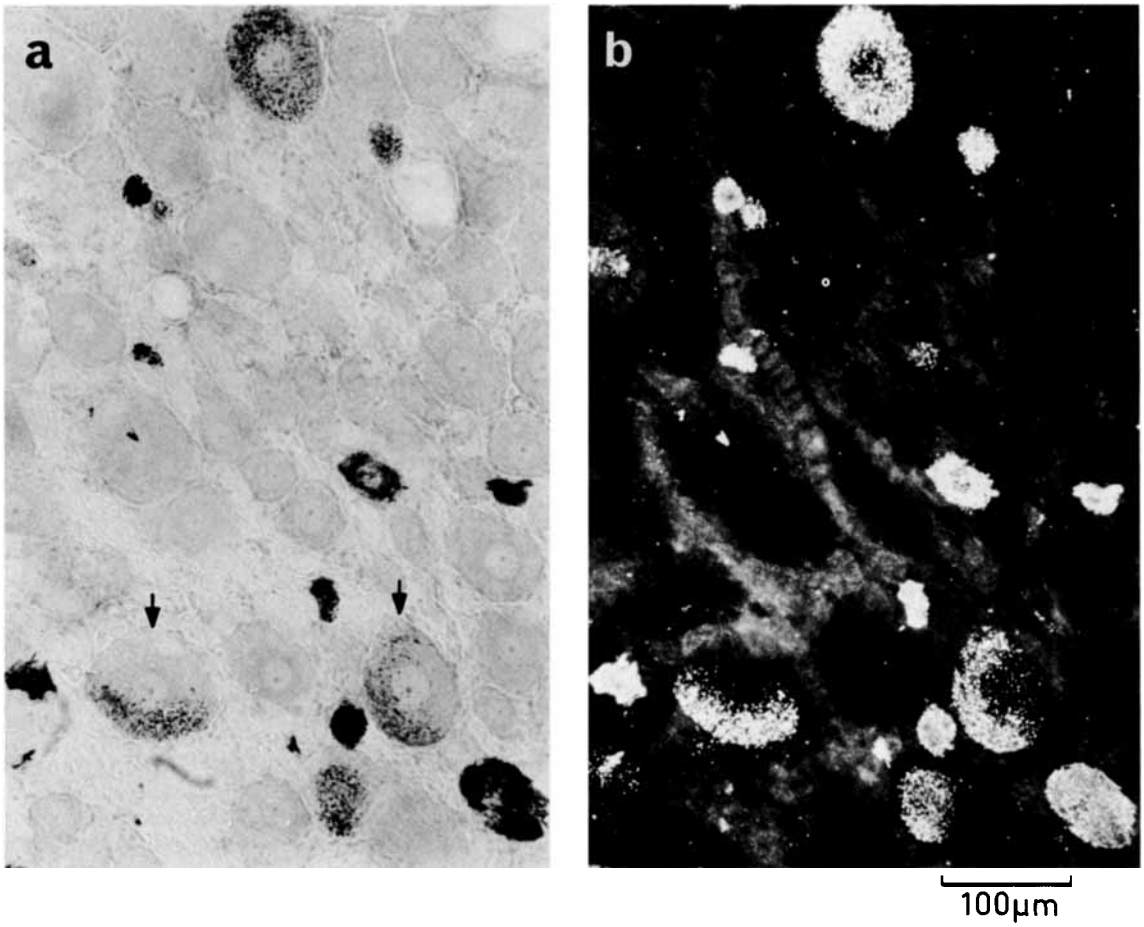

Fig. 1. Bright and darkfield photomicrographs of the same area showing dorsal root ganglion cells near the dorsal surface of L6 ganglion. The eccentric location of HRP granules in some of the larger labeled neurons (arrowed in a) gives the appearance of cell offcuts when viewed in darkfield (b).

tral surfaces of dorsal root ganglia. The most heavily labeled dorsal root ganglion from one example of each experimental group was also sampled at regular intervals from dorsal to ventral surface (total sample of 700–1,000 cells). The data for DRG cells have in most cases been converted to cell radius (by assuming a circular cross section) to simplify their presentation, while sizes of fusiform sympathetic neurons are presented as cross-sectional areas.

Correction for double counting

The extent to which the total cell counts and the area measurements are in error because of double counting of cells in adjacent sections cannot be simply allowed for by a standard correction factor such as that of the Abercrombie ('46), because of several complicating factors related to the HRP technique. The granules of HRP reaction product rarely filled the soma completely so that the practical "cell diameter" was less than the actual one. The extent of filling varied a great deal; in fact, the largest DRG cells which might be expected to appear in several consecutive sections usually contained the lowest density of HRP granules, and these were often present only in a small segment of the soma to one side of the nucleus (Fig. 1). By contrast, if there was overstaining and crystals of reaction product had formed, the likelihood of errors was even greater, and numerical counts could be in error either way (by spurious identification of "offcuts" as "cells," or from crystals of reaction product obscuring the limits of closely juxtaposed cells). On the other hand, the use of brightfield and a

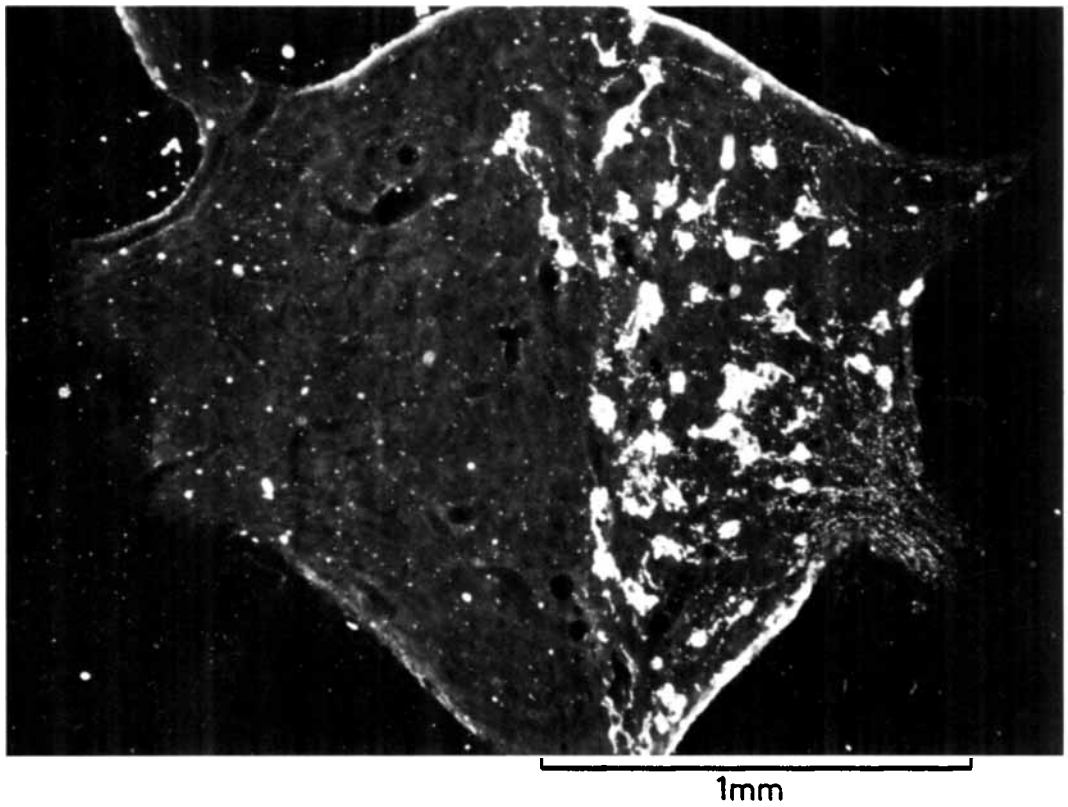

Fig. 2. Fused L7 ganglion from W5, with HRP applied only to the left GS nerves. The labeled neurons are restricted to the left side of the ganglion (on the right in the figure), and their axons can be seen projecting mainly via the more caudal of two grey rami. Caudal direction to top of figure.

higher magnification might be expected to have reduced the incidence of errors during measurement of areas with the MOP analyzer.

We have therefore determined the extent of double counting directly in samples from the most heavily positive parts of sympathetic and sensory ganglia in at least one animal from each experimental series. For each ganglion sampled, projected images of at least five serial sections were traced onto sheets of acetate film, and the outlines of all labeled profiles marked on each. Between 100 and 300 profiles were examined from each sympathetic ganglion studied, and a similar number from both dorsal and ventral regions of each dorsal root ganglion. The percentage of all profiles appearing in adjacent sections was determined, as well as the percentage of "double counting" of cells on which area measurements were made (see earlier).

The occurrence of sympathetic ganglion cells in consecutive sections ranged from 10.5 to 16.2% in different ganglia, the larger errors occurring in preparations with very heavy staining (e.g., most muscle nerve experiments, see Results). Offcuts identified independently but subjectively during cell counting were between 8.5 and 19.6% of all profiles in sympathetic ganglia in different experiments (mean 14.5% ± 1.1%, S.E.M., n = 15). A standard correction of 15% has therefore been applied to the counts of sympathetic neurons presented in Tables 1 and 2.

The situation in the dorsal root was more complex. Between 20 and 25% of profiles labeled from muscle nerves (GS) appeared in two adjacent sections, and a similar conclusion was made for cells in the dorsal parts of ganglia labeled from skin nerves. Labeled cells in the ventral parts of the dorsal root ganglia after application of HRP to skin nerves were generally smaller (see Results), and overlap between sections occurred in only 13.3 to 18.5% of the profiles. The appearance of very large cells in more than two successive sections occurred at a frequency of less than 3%. Offcuts identified independently during counting were 18.9 ± 2.5% (n = 5) for cells labeled from muscle nerves, and 22.3 ± 1.0% (n = 10) for cells labeled from skin nerves. This probably reflects the less uniform staining characteristics of large skin DRG cells; in the latter cases, cells containing sparse granules in one part of the soma were often classified as offcuts during counting under darkfield. A standard correction factor of 20% has been applied to the total numbers of DRG cells presented in Tables 1 and 2.

The profiles selected for cross-sectional area measurement were very rarely measured twice. In sympathetic ganglia, this would have occurred in some densely stained cells in which the nucleus could not be identified; the incidence was 4.1 ± 0.5% (n = 8), which is considerably less than the proportion of cells predicted to appear in more than one section (~ 30%, Abercrombie, '46). The error was

TABLE 1. Total Numbers of HRP-Labeled Cells in Different Experiments[1]

	Exp.	Side	Sympathetic	%	DRG	%	Total
GS:	W5*	L	3,002	65.4	1,586	34.6	4,588
	W7*	L	3,052	59.7	2,062	40.3	5,114
Muscle	W8	L	2,403	70.4	[1,011]	29.6	3,414
	W10	R	3,518	73.7	[1,254]	26.3	4,772
	W15*	L	3,774	68.2	1,760	31.8	5,534
MP:	W10 (medial only)*	L	2,156	46.6	2,474	53.4	4,630
Hairless	W12	R	(395)	44.9	(485)	55.1	(880)
and hairy	W14*	L	5,652	50.4	5,565	49.6	11,217
skin	W14	R	(2,332)	51.1	(2,236)	48.9	(4,568)
	W15*	R	3,701	41.8	5,157	58.2	8,858
Su:	W4	L	1,062	44.3	1,333	55.7	2,395
	W6	L	723	41.4	1,022	58.6	1,745
Hairy	W7*	R	1,336	22.1	4,712	77.9	6,048
skin	W8	R	1,342	30.4	[3,072]	69.6	4,414
	W12*	L	1,794	27.8	4,651	72.2	6,445
SP:	W1	L	(843)	26.2	(2,376)	73.8	(3,219)
	W3	L	1,489	35.2	2,739	64.8	4,228
Hairy	W6	R	1,669	34.9	[3,110]	65.1	4,779
skin	W9*	R	4,611	24.7	14,076	75.3	18,687
	W11*	R	3,090	20.8	11,761	79.2	14,851
	W13*	R	3,516	21.7	12,686	78.3	16,202

[1]Asterisks indicate experiments in which counts are considered to be representative of the total population. Brackets indicate numbers of sensory cells in experiments in which very light staining was noted. Parentheses indicate experiments in which bleeding from the cut nerve diluted the HRP solution during its applications.

only slightly higher in many regions of the dorsal root ganglia (4.0–7.1%) but DRG cells between 3,000 and 5,000 μm^2 would have been counted twice about 20% of the time. This follows from the size of their nuclei and the relatively high density of their staining. However, cells > 5,000 μm^2 were rarely counted twice because the density of staining was usually sufficiently low for the nucleolus to be visible. The cross-sectional area histograms in figures may therefore show about 20% too many cells in the range 2,000 and 5,000 μm^2.

Finally, measurements of the cross-sectional areas of cell profiles in which the nucleolus was clearly visible were made on samples of 400–800 unlabeled cells throughout comparable ganglia, i.e., sympathetic L6 or L7 in six experiments and two or three appropriate DRG from each of three experiments. Although the dimensions of the populations of dorsal root ganglion cells varied at different segmental levels (see e.g., Fig. 6), the histograms of cell dimensions were generally similar to those of HRP-labeled cells in equivalent ganglia and showed comparable minimum cell sizes (see Results). This would not be expected if the measurements of HRP-labeled profiles included more than a small percentage of genuine offcuts.

RESULTS

Numbers of sympathetic and sensory cells labeled

Over the first few experiments it became apparent that the total number of cells that were labeled depended critically on the timing of the HRP application. In one animal (W2) in which there was a delay of about 30 seconds between transection of SP nerve and the application of the HRP solution to it, only 16 sympathetic ganglion cells were labeled (together with some 4,500 DRG cells). We presume this may be a result of rapid sealing over of small-diameter unmyelinated axons. Consequently we ensured in later experiments (from W7 on) that 1–2 µl of HRP solution was in contact with the nerve simultaneously with division of the axons. Using this procedure the total numerical counts of labeled cells became much more consistent (Table 1), although there were still a few experiments in which the density of granules of HRP reaction product was reduced, particularly in DRG cells. In these cases, some DRG cells contained only a few granules (> 10), although these could clearly be identified in darkfield, and were never present in ganglia which did not contain other labeled cells; relatively low total numbers of DRG cells were counted in these cases (see Table 1, brackets). It is not obvious what other technical feature might be responsible for the more variable staining of sensory neurons (see also Oldfield and McLachlan, '77); in cases in which it was noted that bleeding diluted the concentration of HRP during application, both sensory and sympathetic labeling was reduced (see Table 1, parentheses).

Even in preparations in which large numbers of cells were labeled, the density of granules of HRP reaction product was lower in DRG cells than in sympathetic neurons. This was particularly true of the largest DRG cells (see Fig. 1), while small-diameter DRG cells tended to appear quite densely packed with granules of reaction product. The only other notable feature of the staining quality was that the population of sympathetic neurons projecting to skeletal muscle consistently stained more densely than any other group. As a result, overstaining (with the appearance of crystals of reaction product) proved difficult to avoid with reaction times appropriate to demonstrate the labeled cells in other regions. It is tempting to suggest that the degree of labeling may be related to the levels of activity of different neurons; e.g., sympathetic neurons

TABLE 2. Segmental Distribution of Sympathetic and Sensory Neurons[1]

		Segmental level						
	Side	L3	L4	L5	L6	L7	S1	S2
GS Muscle								
W5	L		*0.2*	*1.5*	*69.5*	*24.6*	*4.2*	
			0.2		0.1	50.2	49.6	
W7	L			*0.9*	*21.0*	*71.9*	*6.2*	
			0.05	0.05	0.2	9.1	90.1	0.5
W8	L	*0.1*	*0.6*	*3.5*	*40.7*	*47.1*	*8.0*	
					0.4	24.9	74.6	0.1
W10	R		*0.1*	*1.4*	*76.6*	*17.3*	*4.6*	
					0.2	60.0	39.8	0.1
W15	L		0.1	0.2	83.8	11.7	4.1	0.1
		0.1	0.1			60.3	39.0	0.5
MP-hairless and hairy skin								
W10	L		*1.3*	*39.2*	*51.1*	*8.0*	*0.4*	
(medial branch)					69.5	28.1	2.3	
W12	R			*5.6*	*87.6*	*6.1*	*0.8*	
					36.1	59.4	4.5	
W14	L		*0.4*	*17.7*	*69.7*	*11.9*	*0.4*	
					29.9	62.3	7.7	
W14	R		*0.4*	*11.6*	*60.2*	*26.2*	*1.5*	*0.1*
					23.2	60.7	16.1	
W15	R		*0.1*	*4.2*	*56.8*	*37.9*	*1.0*	
					19.0	75.3	5.7	
Su-hairy skin								
W4	L			*0.8*	*3.3*	*95.9*		
							100	
W6	L	*0.1*	*0.7*	*2.9*	*68.3*	*27.9*		
						62.9	37.1	
W7	R			*0.2*	*1.9*	*97.3*	*0.5*	
							99.9	0.1
W8	R		*0.1*	*0.5*	*8.2*	*91.0*	*0.1*	
						0.5	99.5	
W12	L		*0.3*	*3.3*	*54.0*	*42.4*		
						48.0	52.0	
SP-hairy skin								
W3	L				*56.2*	*43.7*	*0.1*	
					0.1	32.7	67.2	
W6	R	*0.1*	*1.6*		*79.1*	*18.1*	*1.1*	
						63.5	36.5	
W9	R	*0.7*	*2.4*	*33.4*	*63.1*	*0.4*		
				4.5	46.8	48.7		
W11	R	*0.5*	*1.1*	*15.1*	*73.6*	*9.8*		
					19.2	72.4	8.1	
W13	R			*5.1*	*59.2*	*35.7*		
					1.1	75.7	23.2	

[1]Percentage distributions of sympathetic (italics) and sensory (standard type) neurons at different segmental levels. Values underlined are the maxima for each distribution.

to skeletal muscle are largely vasoconstrictor neurones with high resting activity (see Discussion).

Concomitant with the overall improvement in labeling in later experiments, the proportion of labeled sensory neurons rose relative to labeled postganglionic somata (see Table 1). Furthermore, the relative proportions for any one nerve became quite consistent, except in instances in which sparse labeling of DRG cells had been noted (brackets in Table 1). In the remaining cases in which the total number of cells labeled probably approaches the actual number of projecting axons (asterisks in Table 1), the mean percentages of sympathetic and sensory neurons differed characteristically for each nerve examined. Sympathetic neurons represented 64.4 ± 4.3% (S.D.) (GS), 46.3% ± 4.3% (MP), 25.0% ± 4.0% (Su), and 22.4 ± 2.0% (SP) of the labeled populations.

Laterality of sympathetic and sensory projections

In the first five animals, HRP was applied unilaterally to either SP (three cases), Su (one case), or GS (one case). Labeled cells were present only ipsilaterally in dorsal root ganglia and almost exclusively ipsilaterally in sympathetic ganglia (see Fig. 2). A few cells lay in the contralateral sympathetic chain in three of these experiments (one of each type). This occurred only in animals in which anatomical connection between the two sympathetic chains was detected during dissection. As the number of contralateral cells always amounted to less than 0.6% of all labeled cells, HRP was applied on both sides of each animal in subsequent experiments. In most cases different nerves were labeled on each side, except in W14 (bilateral MP) and in three animals in which a neuroma had been produced by transecting the SP nerve 5 months previously (Jänig and McLachlan, unpublished observations). The bilateral segmental representations of both sympathetic and sensory cells were remarkably symmetrical in all four experiments, particularly in view of some irregularities of structure of the lower sympathetic chain (see Table 2).

Segmental distribution of sympathetic and sensory neurons

The percentage distributions of all labeled sympathetic and sensory neurons in ganglia at different segmental levels are shown in Table 2. For both muscle and skin nerves, the peak frequency of postganglionic neurons occurred virtually always in the segmental sympathetic ganglion immediately rostral to the dorsal root ganglion containing the maximum number of labelled sensory neurons. More than 95% of the labelled sympathetic neurons lay in only two adjacent ganglia, with very small numbers present over a few segmental levels along the rostrocaudal axis. Dispersion of sympathetic neurons was more a characteristic of an individual animal rather than of the nerve labeled. For example, sympathetic neurons were widespread in both sides of W6, W8, and W15, but quite restricted in W7.

The mean segmental distributions of sympathetic and sensory neurons labeled from each nerve studied are shown in Figure 3. These graphs tend to obscure the correspondence between labeling in a sympathetic ganglion and the next most caudal dorsal root ganglion because of variable degrees of pre- and postfixation in the animals of each sample. For each nerve, however, sympathetic and sensory somata were distributed in a characteristic pattern between segmental levels.

GS. Labeled neurons in sympathetic chain ganglia occurred in highest numbers in either L6 (three in five experiments) or L7 (two in five experiments). More than 90% of labeled postganglionic somata lay in these two ganglia with progressively smaller numbers in ganglia extending in the extreme cases as far as L2 to S2. Labeled dorsal root ganglion cells were similarly distributed but had peak frequencies at L7 or S1 (see also Burke et al., '77). Only a very few isolated cells were labeled in other dorsal root ganglia, but it was notable that in three of the experiments they lay in ganglia rostral to L5. This did not occur in any of the experiments on skin nerves, and presumably reflects the projection of a few sensory neurons to muscle via the sympathetic chain.

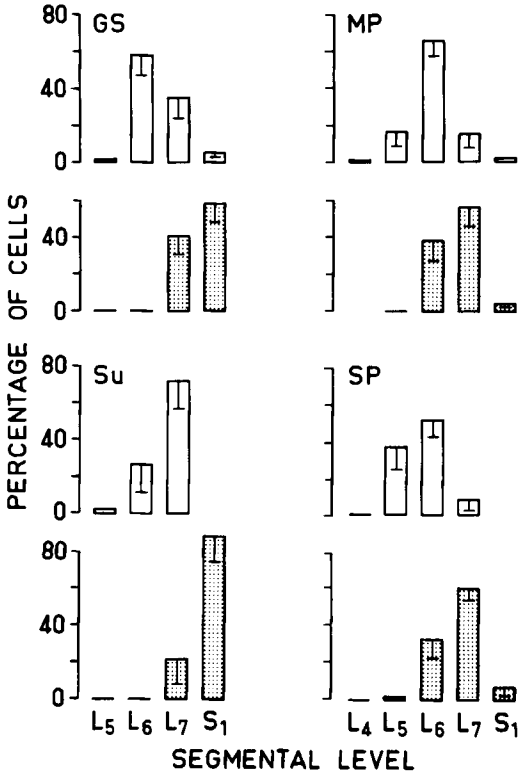

Fig. 3. Mean percentage distributions of sympathetic (white) and sensory (stippled) neurons at different segmental levels for each of the four nerves studied. The bars indicate S.E.M. (n = 5 in each case).

MP. The majority of labeled sympathetic neurons were always located in L6, corresponding to a peak frequency of DRG cells in L7. This was the most consistent projection of those presently under study. The case in which HRP was applied only to the medial branch of the nerve was a little exceptional in that maximum sensory labeling occurred at L6. The entire range of sympathetic ganglia in which labeled cells could be identified was L3 to S2, but labeled sensory neurons were only present in L6 to S1.

Su. Two patterns of distribution of labeled cells were observed: in three cases, > 90% of all cells lay in L7/S1 (sympathetic/sensory); in the remaining cases, most labeled sympathetic and sensory neurons were distributed between two adjacent ganglia extending one segment more rostrally. Thus, DRG cells were restricted to L7 and S1, although small numbers of labeled sympathetic somata extended further rostrocaudally.

SP. More than 90% of labeled sympathetic neurons lay at L5 and L6, corresponding to maximum numbers of labeled sensory neurons in L6 (one in five experiments) or L7 (four in five experiments). Smaller numbers of labeled cells were present over L3 to L7 (sympathetic) and L5 to S1 (sensory).

Localization of sympathetic and sensory neurons within ganglia

No obvious topographical organization of sympathetic neurons could be detected. Labeled sympathetic neurons tended to lie close to their ramus of exit (see Fig. 2), although clustering was not apparent and labeling of even two adjacent neurons occurred only rarely.

The distribution of labeled dorsal root ganglion cells within a ganglion was not remarkable for any preferential grouping of cells of different sizes, apart from the obvious tendency for the largest neurons to lie more dorsally (see below). In GS, labeled neurons of all sizes were scattered throughout the dorsoventral extent of each DRG (see also Burke et al., '77), with the exception of some of the larger cells lying superficially near the dorsal surface (see Fig. 4). The DRG cells labeled from skin nerves had characteristic localizations. The majority of neurons labeled from MP lay ventrally within the DRG; dorsal grouping of the larger neurons was most distinctive for this nerve (Fig. 4). Cells of all sizes labeled from Su were clustered to one side of the DRG, while SP cells were most predominant towards the middle of the ganglion, being particularly sparse just below the dorsal surface (cf., MP).

Dimensions of sympathetic and sensory neurons

The cross-sectional areas of profiles of labeled sympathetic neurons were typical of the whole lumbosacral sympathetic population and virtually independent of the nerve to which the HRP was applied (Fig. 5, Table 3). However, variation in size of sympathetic neurons was evident between animals and was related to the relative sizes of the DRG cells measured (Table 3). Mean cross-sectional areas of labeled sympathetic neurons between sides of the same animal were usually similar (see Table 3, W7, W10, W15). Cross-sectional areas measured in five animals from unlabeled profiles in which the nucleolus was visible were indistinguishable from those of the labeled populations. However, because of the tendency for GS and SP sympa-

TABLE 3. Dimensions of Sympathetic and Sensory Neurons[1]

			Dorsal root ganglion cells		
	Exp.	Level	Dorsal sample	Ventral sample	Sympathetic ganglion cells
GS					
	W5	L6/7	1,725 ± 1,730	1,318 ± 1,304	665 ± 181
	W7	L7/S1	1,599 ± 1,474	1,603 ± 1,539	637 ± 180
	W10	L6/7	1,665 ± 1,667	1,595 ± 1,622	670 ± 206
	W15	L6/7	2,082 ± 2,035	2,219 ± 2,083	673 ± 210
MP					
	W10	L5/6	2,999 ± 2,096	2,140 ± 1,901	656 ± 190
	W14	L6/7	2,507 ± 1,820	1,932 ± 1,620	588 ± 207
	W15	L5/6	2,330 ± 1,935	2,161 ± 1,818	648 ± 217
Su					
	W7	L7/S1	1,321 ± 909	1,235 ± 865	620 ± 210
	W8	L7/S1	1,397 ± 1,164	970 ± 767	586 ± 245
	W12	L6/7	1,564 ± 1,287	1,205 ± 888	615 ± 170
SP					
	W9	L6/7	2,121 ± 1,488	1,590 ± 1,196	660 ± 196
	W11	L6/7	1,887 ± 1,597	1,371 ± 1,182	630 ± 174
	W13	L6/7	2,464 ± 2,022	1,720 ± 1,482	596 ± 211
Unlabeled cells					
	W12	L6/7	2,339 ± 1,703		610 ± 203
		L7/S1	1,743 ± 1,331		625 ± 222
	W14	L6	2,977 ± 1,899		
		L6/7	3,038 ± 1,951		611 ± 196
		S1	1,899 ± 1,572		
	W15	L7	2,316 ± 1,823		
		L7/S1	2,096 ± 1,581		614 ± 195

[1]Values are mean cross-sectional areas in μm^2, ± SD, of profiles of >400 labeled neurons in the ganglia containing the highest numbers of labeled cells, and of >400 unlabeled cells in comparable ganglia. "Level" indicates the sympathetic/sensory segmental level sampled.

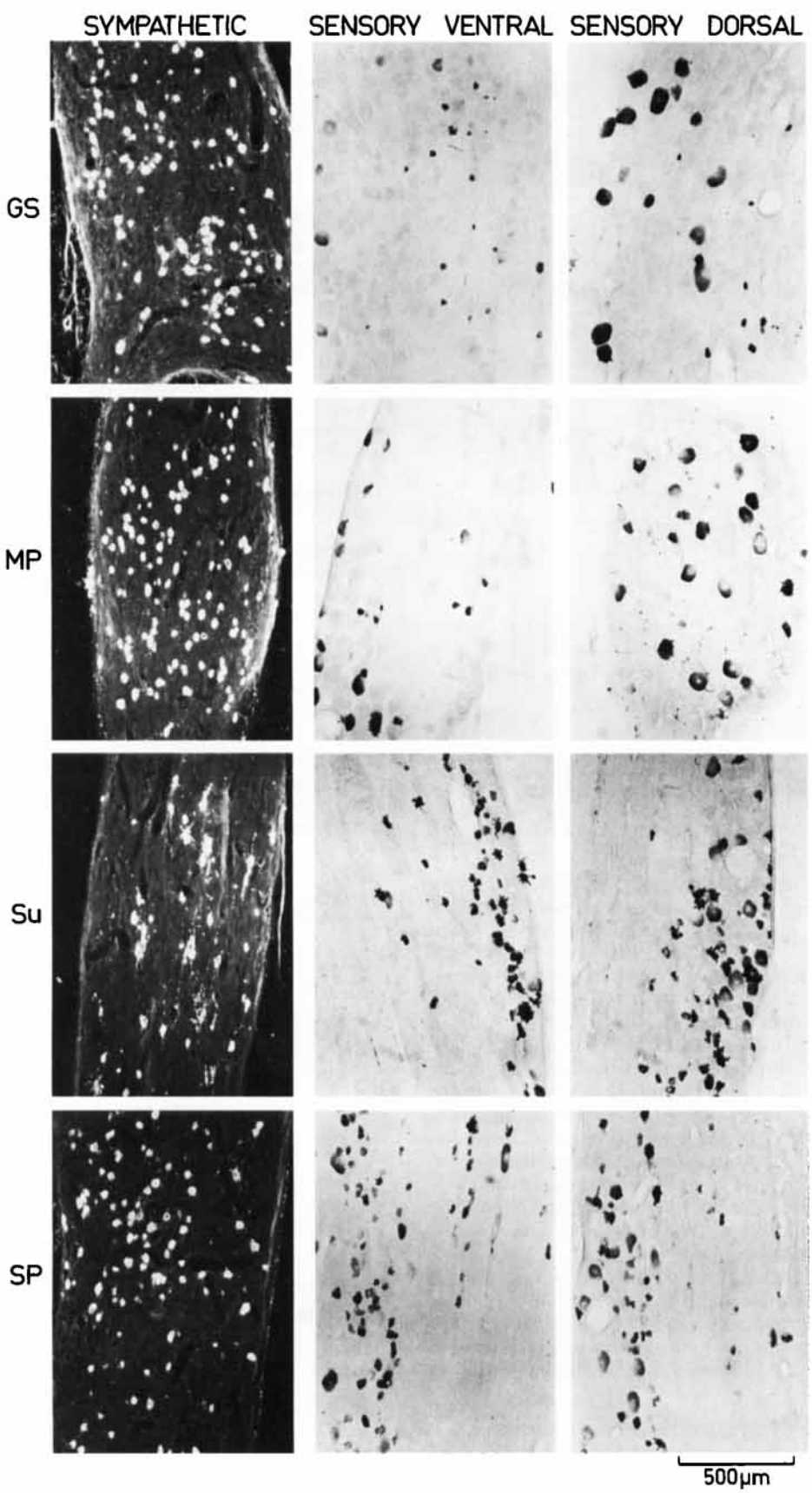

Fig. 4. Sympathetic and sensory neurons labeled with HRP from one experiment typical of each of the nerves studied. Sympathetic neurons photographed in darkfield at the same magnification as DRG cells (brightfield). Calibration applies throughout.

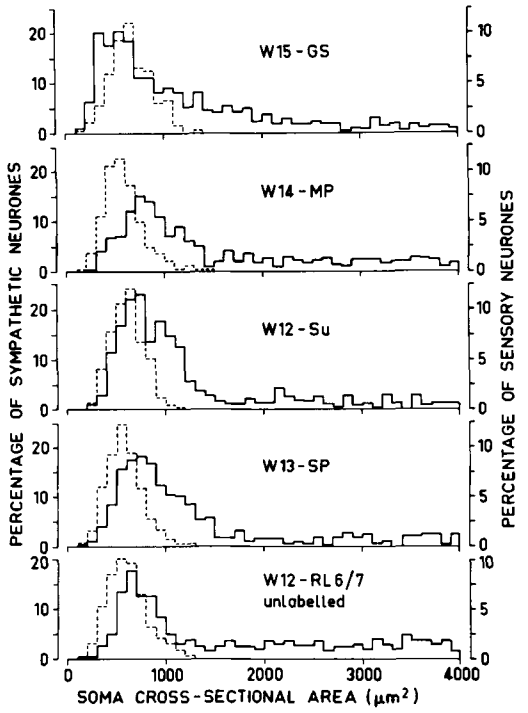

Fig. 5. Frequency histograms of cross sectional areas of profiles of sympathetic (dashed) and sensory (continuous) neurons labeled with HRP from one example of each type of experiment, and of unlabeled neurons measured from profiles containing a nucleolus (bottom panel).

thetic neurons to be overstained, and the failure of HRP granules to fill the soma completely (as in DRG cells, see Methods), variations in area may be obscured. On the other hand, there appeared to be a few more labeled large sympathetic neurons > 1,000 μm^2) in GS and MP than was the case for the other nerves (see Fig. 5).

The dimensions of each population of sympathetic neurons were less than those of the population of smaller sensory neurons labeled from skin nerves, but larger than those of the smallest neurons labeled from GS (Fig. 5). In most other respects, the DRG cells labeled from each nerve had quite different characteristic dimensions and distributions, and these will be described separately. These data are not appropriate for the application of any simple form of statistical analysis, and individual cases have therefore been presented. Some of the differences at least partly relate to the segmental level at which the labeled neurons lay (Table 3, see also Fig. 5).

GS. Labeled sensory neurons ranged from about 180 up to 9,500 μm^2 in cross-sectional area, there being a predominance of groups of larger cells in the dorsal rather than the ventral regions of the DRG (Fig. 4). Particularly notable were large numbers of very small DRG cells present throughout (Fig. 4). As the total number of sensory cells labeled from GS was small (see Table 1), the dorsal and ventral samples included virtually all labeled neurons, so that the anatomical distinction between these regions was not discrete. Consequently, the mean cross-sectional areas (Table 3) of the two samples were similar and the data have been pooled for each ganglion.

There was a systematic difference in the distribution of cell sizes present in ganglia at different segmental levels. This was evident whether the samples came from the same (Fig. 6B) or different (Fig. 6A) animals. A sample including labeled cells from both L7 and S1 DRG (Fig. 6C) showed a combination of the characteristics of each of these segments.

Isolated labeled neurons in "spillover" ganglia (e.g., L6) were > 35 μm in radius and usually lay near the dorsal surface. However, labeled sensory neurons in the upper lumbar DRG were all between 10 and 20 μm in radius, and usually were located ventrally near the larger axon bundles of the DRG.

The mean of populations of GS sensory neurons and that of the entire population of DRG cells at each of these levels are compared in Figure 6D. The cells labeled from GS are distinguished by the population of very small neurons and also by a group of neurons of the largest dimensions present in both L7 and S1.

MP. Cells labeled from MP ranged from about 200 up to 10,000 μm^2 in cross-sectional area so that on average the population was larger than the other groups studied. The mean cross-sectional area was about 2,200 μm^2, due to the higher numbers of larger cells. The clear preferential dorsal location of these larger cells (see Fig. 4) is evident in the differences between the cross-sectional areas of neurons sampled from the dorsal and ventral surfaces (Table 3), although a substantial proportion of large neurons also lay ventrally (Fig. 7A).

Comparison between the data from a population of labeled cells sampled throughout one DRG and the pooled dorsal and ventral samples from the same ganglion (Fig. 7B, upper panel) shows remarkable similarity. The results from the other experiments (Fig. 7B,C) confirmed the characteristically bimodal size distribution of MP cells, as well as showing some variation in cell size with segmental level (cf., GS above). The smaller cells were generally about 16 μm in radius, while the larger group varied about 35–40 μm. The mean overall distribution of sizes of the population of MP cells was very similar to that of the whole DRG (Fig. 7D) although there were slightly more small cells in the labeled groups.

Su. DRG cells labeled from Su ranged from about 250 up to 7,000 μm^2 in cross-sectional area, constituting dimensionally the smallest of the populations examined here. Both dorsal and ventral samples had low mean cross-section areas (Table 3) compared with the other groups, even if allowance is made for the segmental levels represented. The mean cross-sectional area was about 1,400 μm^2 for L7 and 1,200 μm^2 for S1. The major feature of this population was the paucity of larger neurons (i.e., > 40 μm in radius).

Of the Su cells, the larger ones again showed a preferential dorsal location (Figs. 4, 8A), and this also applied to a class of smaller neurons (about 30 μm in radius) which was not evident in the other groups. However, pooled dorsal and ventral samples had a distribution virtually indistinguishable from that of a sample taken at random depths throughout the same DRG; these were relatively consistent in shape despite difference in segmental level (Fig. 8B,C). The distributions were almost unimodal, the bulk of labeled cells being between 12 and 20 μm in radius, with a smaller number skewed to larger sizes. Comparison of the labeled Su cells with the whole DRG population (Fig. 8D) confirms the excess of smaller neurons and the small representation over 22 μm^2 in radius.

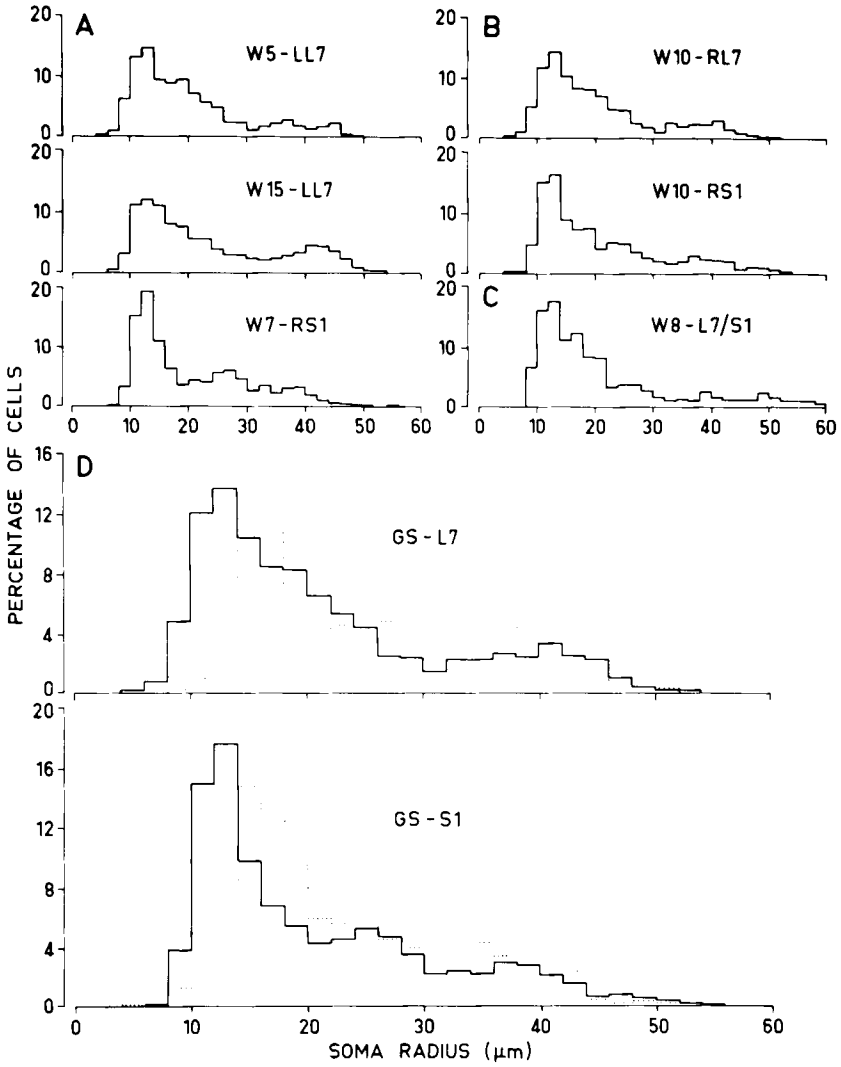

Fig. 6. Frequency histograms of soma radius of DRG cells labeled from GS. A. All labeled neurons from the DRG containing the most labeled cells in each of three experiments. B. All labeled neurons in each of two adjacent DRG from the same experiment. C. All labeled neurons in both L7 and S1 from another experiment. D. Comparison of the distribution of sizes of labeled (continuous) and unlabeled (dotted) neurons at two segmental levels. Each histogram is the mean of three experiments (L7) or two experiments (S1).

SP. Labeled neurons ranged from about 250 up to 8,200 μm^2 in cross-sectional area, and were on average larger than the cells projecting in the other nerve to hairy skin (Su; see Table 3), although the largest cells were not as big as those in MP and GS. Because of the extremely large numbers of sensory cells labeled from SP (see Table 1), the dorsal and ventral samples were quite distinct, and their mean sizes (in Table 3) were significantly different (paired t-test, $P < 0.05$). However, the distributions of sizes for the samples taken throughout the whole of the DRG were virtually identical to those of the ventral samples, the mean cross-sectional area being about 1,600 μm^2. This suggests that the larger SP cells are relatively confined to the dorsal regions of the DRG. The difference between the dorsal and ventral samples was related primarily to those neurons about 35–40 μm in radius (Fig. 9A).

The majority of SP cells (like those of Su) were between 12 and 20 μm in radius, but the SP population contained a greater proportion of larger DRG cells. It seems unlikely that the SP population was larger simply because of the segmental level (L7), as comparison between one Su representation at L7 (Fig. 8C) and those of SP (Fig. 9B) shows correspondence of the peak of smaller cells, with the discrepancy evident only for the larger sizes. The overall distribution for SP lay between that of Su and the bimodal one characteristic of MP, and is compared with that of unlabeled DRG cells in Figure 9C.

Numbers of myelinated fibers in each nerve studied

Semithin sections of nerves taken 1–2 cm proximal to the site of HRP application showed swollen and dark axons in response to the recent lesion. Myelinated fibers were therefore counted from pieces of nerve dissected from 7 to 10 cm proximal to the lesions. Counts for medial gastrocnemius were 675, 694 and lateral gastrocnemius-soleus 863, 821 in two cats; for MP, 2,620, 2,420, and 2,402 (the last two being opposite sides of the same animal); for Su,

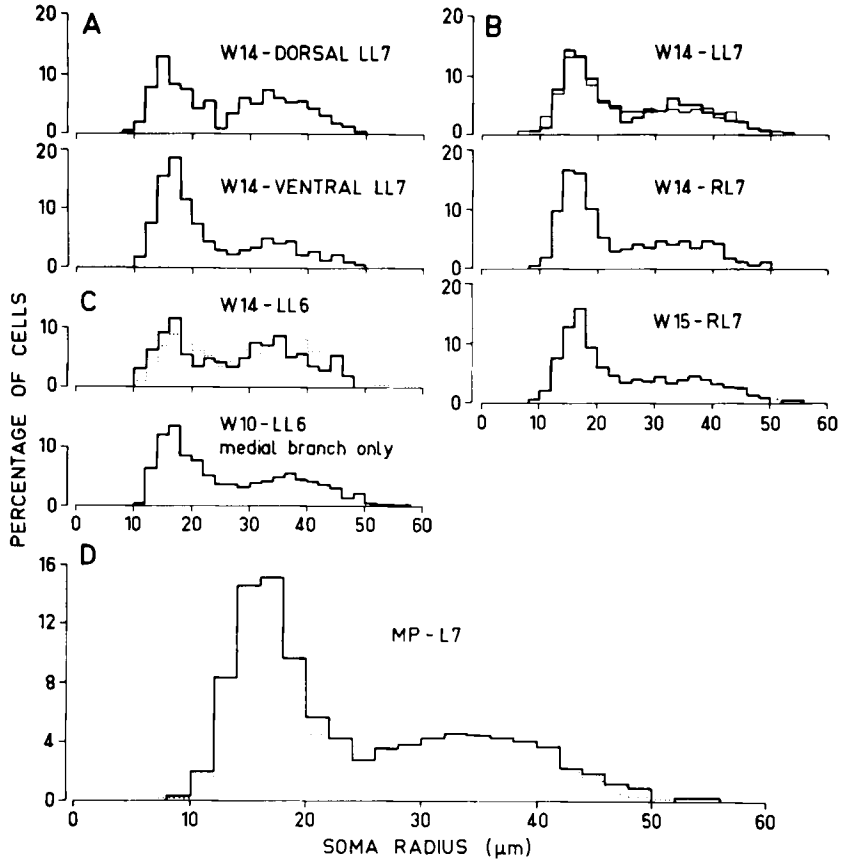

Fig. 7. Frequency histograms of soma radius of DRG cells labelled from MP. A. Distributions of cell size of samples taken from the dorsal and ventral surfaces of one DRG. B. Pooled dorsal and ventral samples from L7 in three experiments, and data from a sample taken throughout the depth of one of these (thinner line, upper panel). C. As B from L6 for two experiments, together with the distribution of sizes of unlabeled neurons in L6 (dotted line). D. comparison of the distribution of sizes labeled (continuous) and unlabeled (dotted) neurons. Each histogram is the mean of three experiments.

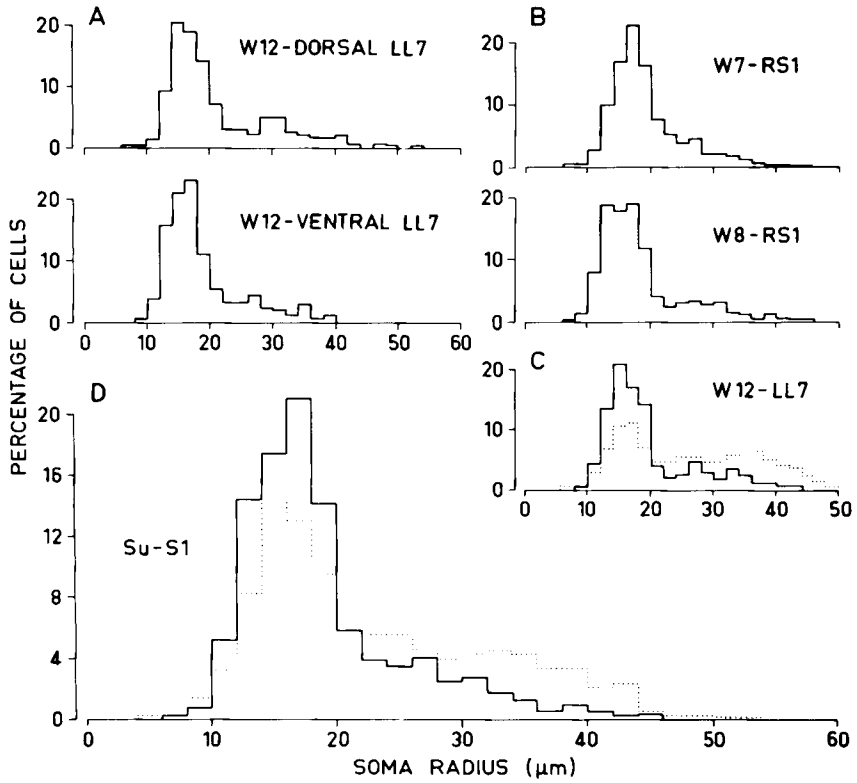

Fig. 8. Frequency histograms of soma radius of DRG cells labelled from Su. A. Dimensions of samples taken from the dorsal and ventral surfaces of one DRG. B. Pooled dorsal and ventral samples from S1, two experiments. C. Dimensions of a sample taken throughout the depth of LL7 (continuous line) and the distribution of sizes of unlabeled neurons in the contralateral ganglion L7 (dotted line). D. comparison of the distribution of sizes of labeled (continuous) and unlabeled (dotted) neurons. Each histogram is the mean of two experiments.

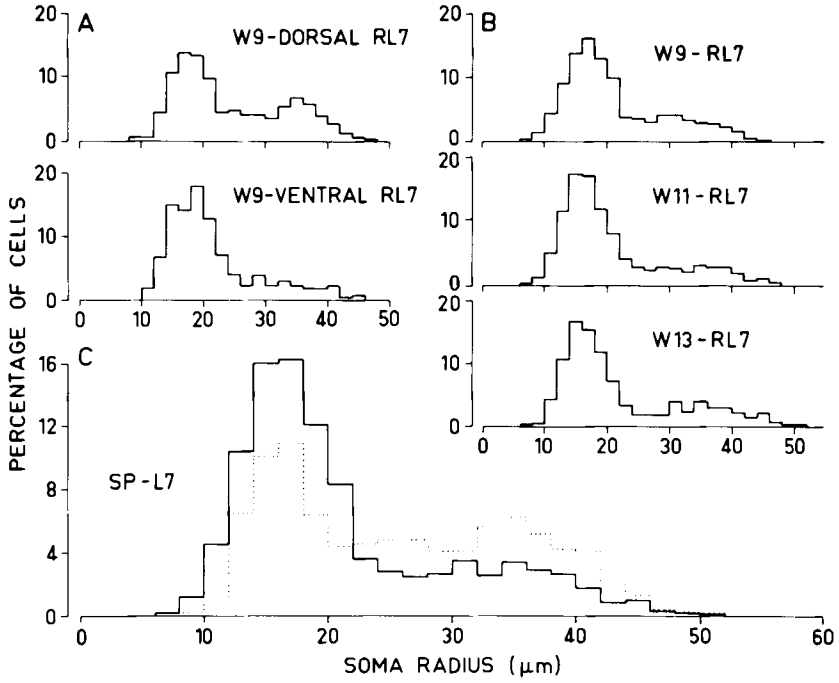

Fig. 9. Frequency histograms of soma radius of DRG cells labeled from SP. A. Dimensions of samples taken from the dorsal and ventral surfaces of one DRG. B. Dimensions of samples taken throughout the depth of each ganglion in three experiments. C. Comparison of the distribution of sizes of labeled (continuous) and unlabeled (dotted) neurons. Each histogram is the mean of three experiments.

796 in one cat, and for SP, 3,964, 3,890, and 3,803 in three cats. These values compare with counts of 760 for MG, 430 for soleus, 2,700 for MP, and 820 for Su reported by Boyd and Davey ('68).

DISCUSSION

The present study yields information on numbers, location, and size of somata of sympathetic postganglionic and sensory afferent axons in four representative nerves to skeletal muscle and skin of the cat hindlimb. It was assumed in this study that all somata of primary afferent fibers lie in the dorsal root ganglia and that all somata of postganglionic neurons lie in ganglia of the sympathetic chain.

Limitations of the HRP technique for quantitative studies

Provided that the HRP solution does not spill over onto damaged axons other than those of the nerve to be labeled, and that the histochemical procedure is standardized for optimal sensitivity, it is possible to label all somata projecting in the nerve (see e.g., Oldfield and McLachlan, '81). However, there are some technical limitations which have to be considered in the interpretation of our results. Those preparations in which the application of HRP was delayed after the nerve was lesioned, or in which the cut nerve bled, yielded lower numbers of stained neurons and these had less dense granules within the somata. However, for each nerve studied, there were several cases in which high and reasonably consistent numbers of labeled cells were obtained (Table 1, asterisks), and we feel that these are likely to approach the real numbers of neurons projecting in each nerve. In addition, we had the impression that afferent somata were often less densely stained than postganglionic somata, possibly reflecting transganglionic transport into the central processes of the afferent neurons. The sizes of postganglionic relative to afferent somata may consequently have been slightly overestimated.

Location of sympathetic and sensory neurons projecting in the same nerve

Labeled sensory cell bodies were found in those spinal ganglia predicted from the literature (Romanes, '51; Bernhard, '53; Gregor and Zimmermann, '72; Mense, personal communication). For all nerves, > 92% of afferent somata were located in two characteristic segmental spinal ganglia. Only in the GS nerve experiments were a few somata found in ganglia L2-L5 rostral to the segmental outflow to the hindlimb. This restricted distribution of afferent neurons indicates that there are only a very few afferent fibers from skeletal muscle, and probably no afferent fibers from skin, which travel through the sympathetic chain from the cat hindlimb.

Sympathetic somata had a somewhat wider rostrocaudal distribution than afferent somata, but the majority (more than 90%) were situated in the ipsilateral paravertebral ganglia corresponding to the spinal ganglia containing the sensory cell bodies from the respective nerve. Thus, the sympathetic outflow to skin and skeletal muscle is nearly as precise in its spatial organization as the peripheral afferent system.

Sympathetic neurons projecting in a given nerve did not show any particular spatial distribution within a paravertebral ganglion except some preferential location near

the gray ramus of exit. Similarly, the somata of the afferent axons exhibited little obvious topographic distribution in individual dorsal root ganglia. Grouping of labeled DRG cells, as observed for chromatolysed cells in the nodose and trigeminal ganglia (see Lieberman, '76), and as expected from the topographical relationship between central processes of DRG cells and their location with the ganglion (Burton and McFarlane, '73), was not a feature in the lumbosacral spinal ganglia. It cannot however be excluded that a more complex spatial organization eluded our simple analysis. On the other hand, there was clearly a preferential association of large somata with the most dorsal parts of the DRG, apparent even in unlabeled material. This has previously been noted in developing human spinal ganglia (McKinniss, '36).

Dimensions of sympathetic and sensory cell bodies

The dimensions of labeled sympathetic postganglionic somata were unimodally distributed and almost identical for each of the four hindlimb nerves, as well as indistinguishable from distributions determined on unlabeled material. In the cases of GS and MP nerves, there was a small proportion of labeled somata which were larger than those of the Su and SP nerves. This may be related to the electrophysiological findings that vasodilator axons to skeletal muscle conduct faster than vasoconstrictor axons (Horeyseck et al., '76), and that sudomotor axons which supply the sweat glands of the hairless skin of the paw pad conduct faster than cutaneous vasoconstrictor axons (Jänig and Kümmel, '77).

The dimensions of labeled cell bodies in the dorsal root ganglia were more complexly distributed, but resembled those of unlabeled cell bodies in ganglia at comparable segmental levels. The overall profile of each distribution was characterized by a peak of small cells between 10 and 20 μm radius, together with varying smaller numbers of cells ranging up to 60 μm radius (see also Aldskogius and Risling, '81). The size range of the peak of small sensory cells was very similar to that of the sympathetic postganglionic somata. However, the population of small sensory neurons projecting in each of the skin nerves included somewhat larger sizes than the population of sympathetic neurons; this feature distinguished them from the equivalent peak in the overall (unlabeled) sensory population. The different size distributions of sympathetic and small sensory neurons projecting to hairy skin (Su, SP) bear a similar relationship to each other as the corresponding distributions of conduction velocities of these types of unmyelinated axons in the same nerves (Blumberg and Jänig, '82). In contrast, the distribution of small sensory neurons projecting in the muscle nerve was distinctly skewed, the peak occurring at sizes smaller than those of the small sensory neurons projecting to skin and even of the postganglionic somata.

Larger sensory somata ranged up to nearly 60 μm in radius, and each nerve again exhibited a characteristic distribution. The nerves to skeletal muscle (GS) and the paw (MP) contained axons with a greater proportion of somata in this range than did the nerves to hairy skin (Su, SP). These latter two examples of the innervation of adjacent areas of hairy skin somewhat surprisingly differed in that the sural neurons very rarely exceeded 35 μm in radius. The extent to which this reflects the different size distributions of the overall populations of sensory neurons in L7 and S1 (see Fig. 7) is hard to assess and may be related to the smaller relative numbers of myelinated axons in the sural nerve (see below).

A notable feature of all distributions of radius of labeled sensory cells was the greater size of the peak of small neurons than was measured in the samples from the unlabeled populations of DRG cells. This might result from some aspects of the methods used. First, it is possible that small neurons were stained in preference to larger ones. Certainly, some of the largest somata contained very few granules (see Fig. 1). This lighter staining could result in an underestimation of the numbers of larger cell bodies. However, measurements made in preparations in which the overall staining of sensory neurons was poor did *not* differ from those made in well-stained preparations; it might be expected that under conditions of light staining proportionally more large cells would be omitted. Second, the discrepancy might be due to the inclusion of significant numbers of densely stained offcuts in which nucleoli could not be seen. Had this been the case, all histograms should have shown at least some measured cells of sizes lower than were present in the unlabeled populations. The histograms for GS (Fig. 6) show this type of skew; however, none of the data from skin nerves does and it is difficult to believe that this occurred by chance. Third, the smallest unlabeled neurons were sometimes hard to identify in lightly counterstained material. This might cause underestimation of the peak of small somata in the overall unlabeled population. However, the extent of the difference between the numbers of labeled and unlabeled small cells seems to depend on the particular nerve labeled so that no systematic error seems likely. It seems more probable that the differences between the size distributions are real.

Numerical analysis of components of skin and muscle nerves

Consideration of the data presented here allows us to make some tentative conclusions concerning the composition of nerves to skin and skeletal muscle of the cat hindlimb. This information has previously only been available for the myelinated fiber components.

If the averages of the highest total numbers of HRP labeled cells in each of the different experiments (see Table 1) are taken as realistic estimates of the absolute numbers of neurons present, and the numbers of myelinated fibers in each nerve are incorporated, we arrive at the numerical able to believeapproximations shown in Table 4A. The assumptions have been made that no sympathetic postganglionic axons are myelinated, and that myelinated axons do not branch between their cell body and the site at which the counts were made several centimeters proximal to their termination. Electrophysiological tests on single axons isolated from Su and SP nerves (Blumberg and Jänig, unpublished) failed to demonstrate branching of either myelinated or unmyelinated fibers within the same nerve or between these two nerves. If branching of peripheral sensory axons does occur (Langford and Coggeshall, '81), it may more commonly involve branching between visceral and somatic nerves (Bahr et al., '81).

The numbers of skeletomotor fibers in MG and soleus nerves (Eccles and Sherrington, '30; Boyd and Davey, '68) match the numbers of motorneurons which are labeled with HRP from these muscles (Burke et al., '77) and our estimates of the myelinated motor and sensory components of GS are virtually identical to those previously de-

TABLE 4. Composition of Muscle and Skin Nerves of the Cat Hindlimb

	GS	MP	Su	SP
A. Numerical estimates				
Skeletomotor (myelinated)	900			
Sensory (myelinated)	700	2,400	800	3,900
Sensory (unmyelinated)	1,100	3,200	3,900	9,000
Sympathetic (unmyelinated)	3,300	4,800	1,600	3,700
Total number of neurons	6,000	10,400	6,300	16,600
B. Relative proportions of different components				
All neurons				
(i) % myelinated	27	23	13	24
(ii) % sensory (all)	30	54	75	78
(iii) % sensory (unmyelinated)	18	31	62	54
(iv) % sympathetic	55	46	25	22
Neurons with unmyelinated axons				
(v) % sympathetic	75	60	29	29
Sensory neurons				
(vi) unmyelinated/ myelinated	1.6	1.3	4.9	2.3
(vii) % myelinated	39	43	17	30

termined (Rexed and Therman, '48). It is however reasonable to believe that the numbers in Table 4A may be underestimates for the technical reasons mentioned earlier, although estimation of the total numbers of fibers in the SP nerve with the electron microscope (Gottschaldt, personal communication) gives values only about 5% higher than ours. It will be necessary to confirm this observation, and to determine the real numbers of fibers in the other nerves by the same method. In particular, our counts for SP may be about 10% too low as the nerves used were contralateral to an established neuroma of the same nerve (Greenman, '13; Mira, '76).

The main interest for the physiologist in the number of fibers in different nerves concerns the relative proportions of different kinds of axons (see Table 4B). For example, the proportion of sensory fibers is higher in all skin nerves than in the muscle nerve (Table 4B (ii)). This is due to the very high numbers of unmyelinated sensory axons (Table 4B (iii)), which arise from low- and high-threshold mechanoreceptors, polymodal nociceptors, and thermal receptors (Bessou and Perl, '69; Bessou et al., '71; Beck et al., '74). Unmyelinated sensory axons from skeletal muscle have been shown to have nociceptive and probably ergoceptive functions (Franz and Mense, '75; Kniffki, Mense and Schmidt, '81).

On the other hand, the relative number of sympathetic (primarily vasoconstrictor) fibers in nerves to hairy skin is very low compared with the nerves to skeletal muscle and to the distal paw which includes hairless skin (Table 4B (iv)). This presumably reflects the importance of these latter tissues in the regulation of peripheral vascular resistance and body temperature, respectively, some fibers of MP nerve being sudomotor (Jänig and Kümmel, '77). The differences are even more pronounced when one considers the relative proportions of unmyelinated axons of sympathetic origin (Table 4B (v)): 29% of both of the nerves which supply only hairy skin, but 75% and 60% of the nerves to skeletal muscle and to the distal paw, respectively. These proportions for Su and SP nerves come very close to the 25 ± 3% sympathetic fibers identified electrophysiologically (Blumberg and Jänig, '82).

It is not obvious how the different dimensions of the sensory neurons measured here are related to the different functional receptors which they innervate. Characteristic myelinated fiber diameter and conduction velocity distributions are reasonably correlated with sensory receptors of different types in many peripheral nerve trunks (see Boyd and Davey, '68; Burgess and Perl, '73; Hunt, '74). However, the size distributions of the larger neurons examined here differ from the characteristic size ranges of the myelinated fiber components; e.g., that of MP nerve to the paw contains some neurons as large as the biggest which innervate skeletal muscle. While this is likely to reflect the innervation of structures specific to hairless skin, the general lack of correspondence would not be so unexpected if the size of the neuron soma is related to the total size of the cell's processes, rather than just the size of the peripheral apparatus (Pannese, '63). In this respect, it can be noted that the Pacinian corpuscles of the paw pad have the largest central extensions present in the dorsal columns (Brown, '68).

Speculations about sensory neurons with unmyelinated axons

If our calculations are correct, unmyelinated sensory axons must outnumber myelinated ones in all of the nerves we have studied. For the cutaneous nerves, there was a remarkably large relative number of unmyelinated sensory axons in the Su nerve with a decreasing proportion in SP and MP nerves. This might conceivably be related to the location of the innervated skin in the hindlimb: The field of innervation of MP is most distal, that of Su most proximal, of the three nerves examined, and the number of structures innervated by myelinated axons increases toward the periphery (see also Ranson and Davenport, '31). The area of skin supplied by each nerve is also different, although this has not been quantified. The MP nerve supplies a small area of the foot with probably the highest innervation density, whereas the more proximal area innervated by Su has probably the lowest innervation density of the areas studied.

The representation of unmyelinated sensory fibers in nerves to hairy skin clearly exceeds that in nerves to skeletal muscle (Table 4B (vi)). This result is similar to that reported by Ranson and Davenport ('31) from light microscope counts on nerves from sympathectomized cat hindlimb. In contrast, it appears that hairless skin is innervated with relatively few unmyelinated sensory axons. This may be related to the large numbers of thin myelinated (group III) fibers which supply the hairless skin of the paw pad (Jänig, '71); these may be activated from high-threshold mechanoreceptors and nociceptors as are some unmyelinated cutaneous sensory axons (Beck et al., '74; see earlier).

From the relative percentages of myelinated and unmyelinated sensory fibers (Table 4B (vii)), we can make one further conclusion. On the reasonable assumption that the larger neurons of each population are those with myelinated axons (Ranson, '12), the size distribution of sensory somata can be subdivided as shown in Figure 10. Despite the wide variation in the proportions of myelinated axons present in each nerve, the resulting division between neurons with myelinated, and those with unmyelinated, axons falls close to the same radius, ~ 22 μm in each case. It seems extremely unlikely that this could occur simply by coincidence, as the total counts of myelinated fiber numbers, the total counts of HRP-labeled neurons, and the neuronal size distributions were each determined inde-

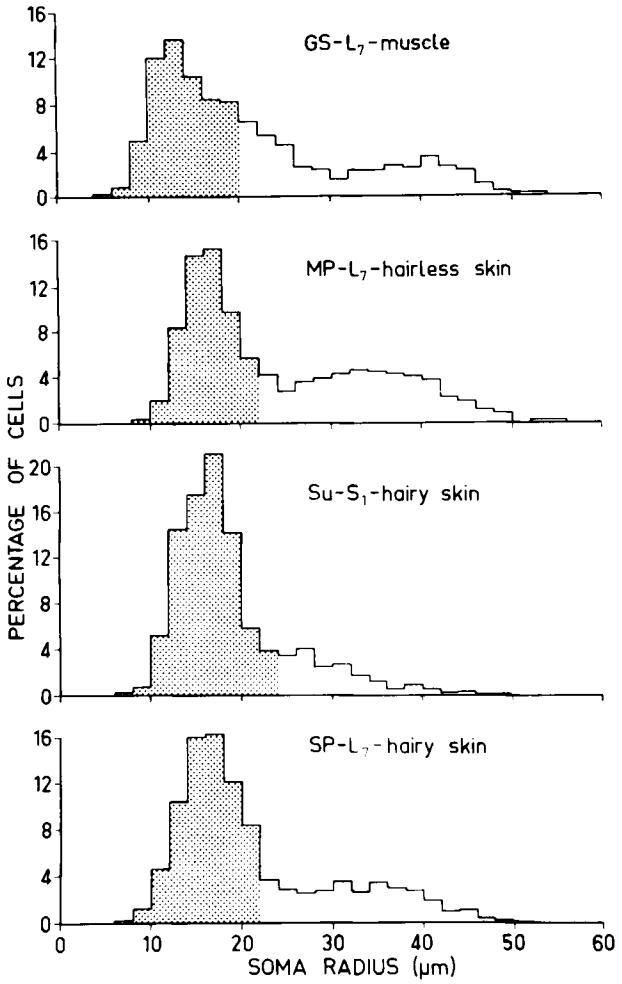

Fig. 10. Comparison of mean sensory neuron soma radius frequency histograms for each nerve showing the probable relative proportions of neurons with unmyelinated axons (shaded). For explanation, see text.

pendently, and these all differed characteristically for each nerve. The peak of small sensory cell bodies must therefore correspond to the population of neurons with unmyelinated axons, at least for the muscle and cutaneous nerves in our experiments. This conclusion directly parallels the recently determined size distributions of small dark and large light neuron types in dorsal root ganglia (Lawson, '79).

ACKNOWLEDGMENTS

We would like to thank Jandri Hoggins for technical assistance, Mike Durham for setting up and programming the data analysis system, and Jill Poynton and Karen Styles for the illustrations. This project was supported by the Deutsche Forschungsgemeinschaft and by the National Heart Foundation of Australia.

LITERATURE CITED

Abercrombie, M. (1946) Estimation of nuclear population from microtome sections. Anat. Rec. *94*:239–247.

Aldskogius, H., and M. Risling (1981) Effects of sciatic neurectomy on neuronal numbers and size distribution in the L7 ganglion of the kitten. Exp. Neurol. *74*:597–604.

Bahr, R., H. Blumberg, and W. Jänig (1981) Do dichotomizing afferent fibers exist which supply visceral organs as well as somatic structures? A contribution to the problem of referred pain. Neurosci. Lett. *24*:25–28.

Beck, P.W., H.O. Handwerker, and M. Zimmermann (1974) Nervous outflow from the cat's foot during noxious radiant heat stimulation. Brain Res. *67*:373–386.

Bernhard, C.G. (1953) The spinal cord potentials in leads from the cord dorsum in relation to the peripheral source of afferent stimulation. Acta Physiol. Scand. 29 (Suppl.) *106*:1–29.

Bessou, P., P.R. Burgess, E.R. Perl, and C.B. Taylor (1971) Dynamic properties of mechanoreceptors with unmyelinated (C) fibers. J. Neurophysiol. *34*:116–131.

Bessou, P., and E.R. Perl (1969) Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. J. Neurophysiol. *32*:1025–1043.

Blumberg, H., and W. Jänig (1982) Changes in unmyelinated fibers including sympathetic postganglionic fibers of a skin nerve after peripheral neuroma formation. J. Auton. Nerv. Syst. (in press).

Boyd, I.A., and M.R. Davey (1968) Composition of Peripheral Nerves. Edinburgh: E. and S. Livingstone Ltd.

Brown, A.G. (1968) Cutaneous afferent fiber collaterals in the dorsal column of the cat. Exp. Brain Res. *5*:293–305.

Burgess, P.R., and E.R. Perl (1973) Cutaneous mechanoreceptors and nociceptors. A. Iggo (ed): Handbook of Sensory Physiology. New York: Springer.

Burke, R.E., P.L. Strick, K. Kanda, C.C. Kim, and B. Walmsley (1977) Anatomy of medial gastrocneumius and soleus motor nuclei in cat spinal cord. J. Neurophysiol. *40*:667–680.

Burton, H., and J.J. McFarlane (1973) The organization of the seventh lumbar spinal ganglion of the cat. J. Comp. Neurol. *149*:215–232.

de Olmos, J., H. Hardy, and L. Heimer (1978) The afferent connections of the main and the accessory olfactory bulb formations in the rat: An experimental HRP-study. J. Comp. Neurol. *181*:213–244.

Eccles, J.C., and C.S. Sherrington (1930) Numbers and contraction values of individual motor units examined in some muscles of the limb. Proc. R. Soc. Lond. (Biol.) *106*:326–357.

Franz, M., and S. Mense (1975) Muscle receptors with Group IV afferent fibres responding to application of bradykinin. Brain Res. *92*:369–383.

Greenman, M.J. (1913) Studies on the regeneration of the peroneal nerve of the albino rat: Number and sectional area of fibres: area relation of axis to sheath. J. Comp. Neurol. *23*:479–513.

Gregor, M., and M. Zimmerman (1972) Characteristics of spinal neurones responding to cutaneous myelinated and unmyelinated fibres. J. Physiol. (Lond.) *221*:555–576.

Horeyseck, G., W. Jänig, F. Kirchner, and V. Thämer (1976) Activation and inhibition of muscle and cutaneous postganglionic neurones to hindlimb during hypothalamically induced vasoconstriction and atropine-sensitive vasodilation. Pflugers Arch. *361*:231–240.

Hunt, C.C. (1974) Muscle receptors. In C.C. Hunt (ed): Handbook of Sensory Physiology. Vol. III/2. Berlin, Heidelberg, New York: Springer, pp. 1–310.

Jänig, W. (1971) The afferent innervation of the central pad of the cat's hind foot. Brain Res. *28*:203–216.

Jänig, W., and H. Kümmel (1977) Functional discrimination of postganglionic neurones to the cat's hindpaw with respect to the skin potentials recorded from the hairless skin. Pflügers Arch. *371*:217–225.

Kniffki, K.-D., S. Mense, and R.F. Schmidt (1981) Muscle receptors with fine afferent fibers which may evoke circulatory reflexes. Circ. Res. *48, Suppl. I*:25–31.

Langford, L.A., and R.E. Coggeshall (1981) Branching of sensory axons in the peripheral nerve of the rat. J. Comp. Neurol. *203*:745–750.

Lawson, S.N. (1979) The postnatal development of large light and small dark neurons in mouse dorsal root ganglia: A statistical analysis of cell numbers and size. J. Neurocytol. *8*:275–294.

Lieberman, A.R. (1976) Sensory ganglia. In D.N. Landon (ed): The Peripheral Nerve. London: Chapman and Hall, pp.188–278.

McKinniss, M.E. (1936) The number of ganglion cells in the dorsal root ganglia of the second and third cervical nerves in human fetuses of various ages. Anat. Rec. *65*:255–259.

Mesulam, M.-M. (1978) Tetramethylbenzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction product with superior sensitivity for visualizing neuronal afferents and efferents. J. Histochem. Cytochem. *26*:106–117.

Mira, J.-C (1976) Etudes quantitative sur la régénération des fibres nerveuses myelinisées. II. Variations du nombre et calibre des fibres régénérées après un écrasement on une section du nerf. Arch. Anat. Microsc. Morphol. Exp. *65*:255–284.

Oldfield, B.J., and E.M. McLachlan (1977) Uptake and retrograde transport of HRP by axons of intact and damaged peripheral nerve trunks. Neurosci. Lett. *6*:135–141.

Oldfield, B.J., and E.M. McLachlan (1980) The segmental origin of preganglionic axons in the upper thoracic rami of the cat. Neurosci. Lett. *18*:11–17.

Oldfield, B.J., and E.M. McLachlan (1981) An analysis of the sympathetic preganglionic neurons projecting from the upper thoracic spinal roots of the cat. J. Comp. Neurol. *196*:329–345.

Pannese, E. (1963) Investigations on the ultrastructural changes in the spinal ganglion neurons in the course of axon regeneration and cell hypertrophy. II. Changes during cell hypertrophy and comparison between the ultrastructure of nerve cells of the same type under different functional conditions. Z. Zellforsch. Mikrosk. Anat. *61*:561–586.

Ranson, S.W. (1912) The structure of the spinal ganglia and of the spinal nerves. J. Comp. Neurol. *22*:159–175.

Ranson, S.W., and H.K. Davenport (1931) Sensory unmyelinated fibers in the spinal nerves. Am. J. Anat. *48*:331–353.

Rexed, B., and P.O. Therman (1948) Calibre spectra of motor and sensory nerve fibres to flexor and extensor muscles. J. Neurophysiol. *11*:133–139.

Romanes, G.J. (1951) The motor cell columns of the lumbosacral spinal cord of the cat. J. Comp. Neurol. *94*:313–363.

Webber, R.H., and A. Wemett (1966) Distribution of fibers from nerve cell bodies in ventral roots of spinal nerves. Acta Anat. (Basel) *65*:579–583.