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Axon Composition of the Proprioceptive PD Nerve During Growth and Regeneration of Lobster Claws

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ABSTRACT A prominent chordotonal organ spanning the propus and dactyl (PD) joint in the chelipeds of the lobster *Homarus americanus* has the somata of its sensory cells embedded in an elastic strand while their axons collectively travel in a nerve to the ganglion. Electron microscopic examination of the PD nerve revealed several large and many small axon profiles denoting few movement-sensitive and many position-sensitive cells. This axon composition typified the nerve in the normally occurring, paired asymmetric crusher and cutter claws, as well as in the experimentally induced, paired symmetric cutter claws. In the asymmetric condition, the crusher nerve had many more axons than the cutter while in the symmetric condition, the numbers were equivalent between the paired cutter claws. In a size-graded series of lobsters with paired asymmetric claws there was an increase in the number and size of axons in keeping with an increase in claw size. In a newly regenerated claw, the PD nerve was a miniature version of its pristine counterpart with smaller and fewer axons. Moreover, in newly regenerated paired asymmetric claws, the crusher nerve did not have a larger number of axons than the cutter, suggesting that PD nerve has not fully differentiated. The distribution of axons according to size was, however, similar to the pristine nerve in that there were few large axons and many small ones, suggesting that the ratio of large, movement-sensitive cells to small, position-sensitive ones in the PD organ is rigidly specified during regeneration and growth.

The body plan of vertebrates and many invertebrates is one of bilateral symmetry in which the right half is a mirror image of the left half. Occasionally, superimposed within this basic plan is structural and functional asymmetry of body parts. One of the better known examples of this bilateral asymmetry is that of handedness either in humans or lobsters. In the case of the lobster *Homarus americanus,* handedness refers to the first pair of thoracic limbs, called chelipeds or claws, which are differentiated into a major and a minor type (Herrick, 1895). The major or crusher claw is stout, molar-toothed, and closes its dactyl against its pollex slowly but with enough force to crack open the shells of bivalves (Scrivener, '71; Govind and Lang, '74). The minor or cutter claw is more slender, incisor-toothed, and closes rapidly enough (~20 msec) to catch free-swimming fish. These differences in closing behavior are due principally to corresponding differences in the fiber composition of the paired closer muscles; the crusher muscle has 100% slow fibers while the cutter muscle has predominantly (90%) fast fibers with a small ventral band of slow (Lang et al., '77; Ogonowski et al., '80).

Bilateral asymmetry of the paired claws is established gradually during primary development (Govind, '84) beginning with the larval stages when the paired claws are small, undifferentiated, and symmetrical in appearance (Herrick, 1895, '11). Sometime during the early juvenile stages the paired claws are determined into a crusher and cutter type based on differences in reflex activity to the paired claws; the side experiencing greater reflex activity becomes the crusher while the opposite side becomes the cutter (Govind and Pearce, '86). In subsequent juvenile stages, the claws gradually differentiate into their respective types. Thus for instance the paired closer muscles which initially consist of a central band of fast fibers sandwiched by slow gradually acquire all slow fibers in the crusher claw and largely fast fibers in the cutter claw (Ogonowski et al., '80). The motor innervation to these muscles also undergoes changes from an initial condition where most muscle fibers are innervated by both fast and slow excitor axons to

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a final condition where most fibers are supplied exclusively by the fast axon in the cutter claw and by both axons in the crusher claw (Costello et al., '81). Neuromuscular synapses of the fast motor axon were seen to differentiate in the degree to which their transmitter output increases with repetitive stimulation, namely facilitation (Lnenicka et al., '88). Thus synaptic facilitation increases markedly in the putative cutter claw but remains unchanged in the putative crusher claw from an initial level in the early juvenile stages.

Sensory innervation to the claws also undergoes changes associated with the development of bilateral asymmetry as revealed by the total number of axons in the claw nerves (Govind and Potter, '87) of which the overwhelming majority are sensory (Bullock and Horridge, '65). The number of sensory axons were similar between the paired claws from the time of hatching to late juvenile stages but became increasingly greater in the crusher claw during adult growth. The identity of the sensory structures associated with these axon profiles in the nerve is not known. In order to address this problem, we selected a prominent proprioceptor in the claw and examined its nerve in an age-graded series of lobsters.

In the event that a cheliped is entrapped or damaged, lobsters have the ability to break off the cheliped at a preformed weak plane at the base of the limb. Autotomy of a cheliped early in the intermolt triggers at the same site the development of an encapsulated limb bud which unfolds into a newly regenerated limb when the animal molts (Bliss, '60). Although smaller in size, the newly regenerated cheliped resembles its predecessor in external morphology and behavior (Herrick, 1895) as well as in specific details such as the fiber composition of the closer muscle (Kent et al., '89) and its motor innervation (unpublished). However, very little is known about sensory structures and their innervation in regenerated chelipeds. The present study attempts to fill in this gap by examining the axon composition of a proprioceptor in newly regenerated chelipeds.

Proprioceptors are sensory receptors designed to monitor internally generated mechanical stimuli. Among arthropods one such proprioceptor is the chordotonal organ in which the sensory endings are carried in a highly specialized structure called a scolopidium (Bullock and Horridge, '65). In this structure the scolopale cells together with associated cells form an envelope around the sensory endings and presumably serve to filter and amplify the mechanical stimuli. Among decapod Crustacea, chordotonal organs are associated with the joint of appendages and are conventionally named after the respective joint, for instance, the chordotonal organ monitoring the most distal limb joint between the propus and dactyl is the PD organ (Alexandrowicz, '58; Whitear, '62). The PD organ consists of an elastic strand spanning the joint, attached to the tendon of the closer muscle in the propus and to the exoskeleton in the dactyl. Each scolopidium within the elastic strand contains the endings of two sensory neurons (Whitear, '62). The somata of these bipolar sensory neurons are located in close proximity to the chordotonal organ whereas the axons travel to the ganglion where they contribute to the neuropil. The sensory axons travel together for some distance as a separate nerve before joining to become part of the main leg nerve.

Physiological recordings from the sensory neurons of PD organ in crabs have established that they are sensitive to length and tension changes in the elastic strand brought about by movement of the dactyl (Wiersma and Boettiger, '59). Hence sensory neurons within the chordotonal organ are broadly differentiated into phasically active movement-sensitive cells and tonically active position-sensitive cells (Hartmann and Boettiger, '67), with the former being generally much larger in axon and soma diameter than the latter. Cross-sections through the PD nerve would therefore provide for the first time an accurate estimate of the total cell number as well as the relative proportion of the two basic cell types, namely the large diameter movement-sensitive cells and the small diameter position-sensitive cells. Since nerve cross-sections can yield such basic information about proprioceptors so readily we decided to use it in our continuing study of the growth and regeneration of asymmetric chelipeds in the lobster *Homarus americanus*.

MATERIALS AND METHODS

The animal used in this study was the lobster *Homarus americanus,* most of which were raised by us at the Marine Biological Laboratory, Woods Hole, Massachusetts. Thus juvenile lobsters ranging in age from one month to five years and in weight from 10 to 300 gm were laboratory-reared specimens whose history was known. Wild lobsters larger than 300 g and with unknown histories were purchased from local suppliers.

In order to study the PD organ in the chelipeds, lobsters were induced to autotomize the cheliped

by a strong pinch at the base of the cheliped. Dissection of the PD organ in the isolated cheliped was done in cool 10–14°C sea water and consisted of first exposing the main leg nerve at the propus-dactyl joint. The dorsal exoskeleton was chipped away around the joint and in the propus the underlying opener muscle and its apodeme was pinned out to expose the main leg nerve. The main leg nerve was followed in the dactyl where its pigmented connective tissue was moved aside to expose the elastic strand of the PD organ which runs in the septum between the opener and closer muscles. From the main leg nerve a small nerve, the PD nerve, may be carefully dissected out and followed along the dorsal distal edge of the apodeme of the closer muscle. Attached to the distal edge of this apodeme is the elastic strand of the PD organ. The bipolar sensory neurons are embedded in this elastic strand with their axons collectively forming the PD nerve. The distal attachment of the elastic strand is in the dactyl at an indentation which is externally demarcated. Exposed in this manner the PD organ may be stained with a dilute (0.25%) solution of methylene blue to facilitate dissection of the PD nerve where necessary. A sizable length of the PD nerve between the elastic strand and the main leg nerve was dissected and pinned out in sylgard lined dishes to which fixatives were added.

Nerves were prepared for electron microscopy by procedures standard to our laboratory (Pearce et al., '86). In brief, the nerves were fixed for 1–2 hr in a 0.15 M cacodylate buffer (pH 7.4) containing 2.5% glutaraldehyde, 0.2% formaldehyde, 2 mM $CaCl_2$, 0.3 M sucrose, and 0.06 M NaCl. Next the tissue was rinsed in several changes of a 0.15 M cacodylate buffer containing 0.3 M sucrose, 0.06 M NaCl, and 2 mM $CaCl_2$ and post fixed for 1 hr in buffered 2% osmium tetroxide. Dehydration in an ascending alcohol series was followed by embedding in an Epon-Araldite mixture. Then cross-sections of the nerve were obtained and placed on Formvar-coated single slot grids. These were double stained with uranyl acetate and lead citrate and examined with a Zeiss or Siemens electron microscope.

In order to be able to count the total number of axon profiles in a cross-section of the PD nerve, a series of overlapping exposures of the entire nerve cross-section was taken at low magnification (×1,800). These were enlarged to ×6,000 as photographic prints which were assembled into a montage, in which the smallest axon could be easily discerned. The diameter of an axon profile was obtained by measuring the long and short axis and calculating the square root of the product of these two measurements (Govind and Pearce, '85). In PD nerves with relatively few (< 100) axon profiles, measurements were made of all axons, whereas in larger nerves samples were taken by drawing between 3–5 transects equidistant apart, along the long axis of the nerve, and measuring all axons along these transects.

Also for each PD nerve counts were made of the total number of axons as well as their distribution into large and small axons. This classification into large and small axons was based on the midpoint of the range of diameter between the smallest and the largest axons. In practice only the diameter of the largest axon was used in obtaining the midpoint since the smallest axons in these PD nerves measured < 0.5 μm.

RESULTS

General structure

The location and gross morphology of the PD organ in the claw of the lobster *Homarus americanus* is shown in Figure 1. The elastic strand runs between the apodeme of the closer muscle and the exoskeleton and has embedded in it nu-

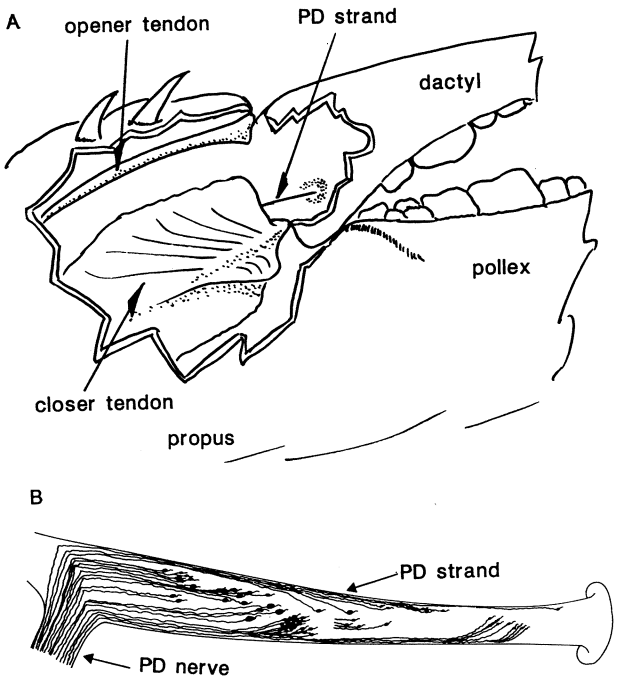

Fig. 1. **A:** Location of the PD strand between the tendon of the closer muscle and the dactyl in a lobster crusher claw. **B:** Distribution of sensory neurons in the PD strand as revealed by staining with methylene blue in a lobster crusher claw.

merous bipolar sensory neurons. The axons of these neurons run together in the proprioceptive nerve which is delineated by an external sheath composed of connective tissue layers interspersed with glial cytoplasm (Fig. 2A,B) similar to that found in crustacean nerves and connectives (Lane and Abbott, '75). The external sheath is considerably thicker in large lobsters than their smaller counterparts. Packed tightly within this envelope are large numbers of axon profiles which are recognized by their generally circular shape, lightly stained cytoplasm, and most distinctly by numerous microtubules that cover the entire cross-sectional face. Many of the axons contain small mitochondria which are distributed around the periphery often forming a characteristic ring in the larger axons.

The small axons are naked in that they are delineated only by their axolemma (Fig. 2C). The larger axons have, in addition to their axolemma, a glial sheath which has a lamellated appearance because of the alternation of glial cytoplasm with connective tissue matrix.

The nerves from the paired asymmetric claws are qualitatively similar. Both have axon profiles that range widely in diameter from < 0.5 μm to 48 μm (Fig. 2). The larger axons appear to occupy most of the cross-sectional area and occur in groups either by themselves or intermingled with smaller axons. Groupings of largely small axons are also seen. In order to determine the distribution of axons according to their diameters, measurements were made of axons along several random transects drawn on the montage. The resulting histogram (Fig. 3) shows the wide range in axon diameters, with most being small in size—for example, approximately 90% of the axons are less than 24 μm in diameter which is the midpoint of the range.

Since the PD nerve carries axons of only two proprioceptive cells (viz. the movement- and position-sensitive cells) and since the somata of this former type are larger than of the latter type, (Hartmann and Boettiger, '67), it is likely that axon composition correspondingly reflects these size differences. Consequently an estimate of the number of each cell type in a PD organ may be made by categorizing the axon profiles as large or small. Because the axon diameter for movement and position sensitive cells are not known, we arbitrarily adopted the midpoint of the range as the dividing boundary; axons with diameters larger or smaller than 25 μm were classed as large or small, respectively. Within each size class we counted the axons and found that the large axons made up 8% and 10% in the cutter and crusher PD nerves (Table 1, last column). As a first approximation, therefore, the movement-sensitive cells constitute a small proportion of the PD organ while the position-sensitive cells constitute the majority.

These counts of the total number of axons also revealed a substantially larger number in the nerve to the crusher claw than in its homologous counterpart to the cutter claw (Table 1, last column). The paired PD nerves are clearly asymmetric in keeping with the rest of the claw.

Growth

The above description of the PD nerve in a 3 kg lobster which we estimated to be 15 years of age based on its weight (Cooper and Uzmann, '80), provided a reference point against which nerves from much younger lobsters could be compared in order to study growth. Thus we selected lobsters of 1, 2, and 5 years of age from our stock of laboratory-reared animals. In lobsters much younger than a year, the PD nerve was difficult to identify unequivocally because of the size of the animal. The PD nerve in the age-graded series of lobsters (Table 1) is qualitatively similar in having a few large axons and numerous small ones, but showed an increase in the total number of axons with age. The increase between the youngest and oldest was approximately 20× for the crusher nerve and 9× for the cutter nerve. Clearly sensory neurons are added to the PD organ during growth of the lobster.

In order to determine the effects of such growth in the two types of cells in the PD nerve, counts of axon profiles in the large and small categories were made and a ratio between these two populations was obtained (Table 1). This showed that the small cells outnumber the large ones 8–11× in the crusher claw and 9–12× in the cutter claw. Moreover, within each claw type, the distribution of large and small axons showed no significant difference (Chi-square test) among the four age classes. The addition of large and small cells during growth, therefore, appears to occur in a relatively fixed ratio. In the growth series of four lobsters the paired claws were distinctly asymmetric, although the asymmetry becomes more marked with age (Herrick, 1895). In each case the crusher PD nerve had a greater number of axons than its cutter counterpart (Table 1) with the differences ranging from 1.3× to 2.5×. Thus the asymmetry in axon numbers is established

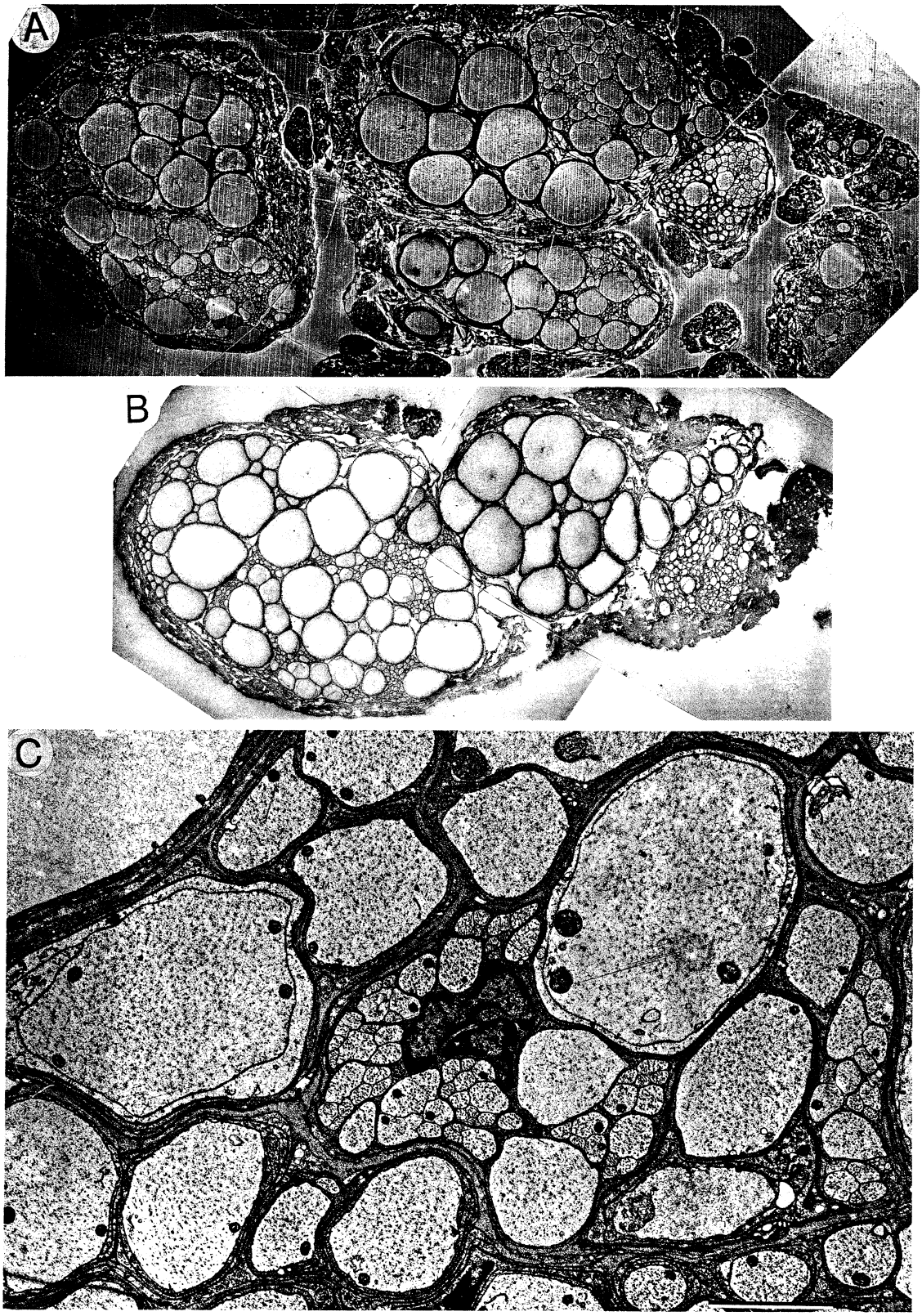

Fig. 2. Cross-section of the PD nerve in the cutter (**A**) and crusher (**B**) claws of a 3 kg lobster showing a wide range of axon profiles. **C:** Higher power view of a representative area of the PD nerve showing characteristic features. Microtubule-lined axons are wrapped with glial tissue to various degrees depending on their size and small axons which are naked. Small mitochondria are seen usually arranged radially in the larger axons. Scale bar = 100 μm (A,B); 2.5 μm (C).

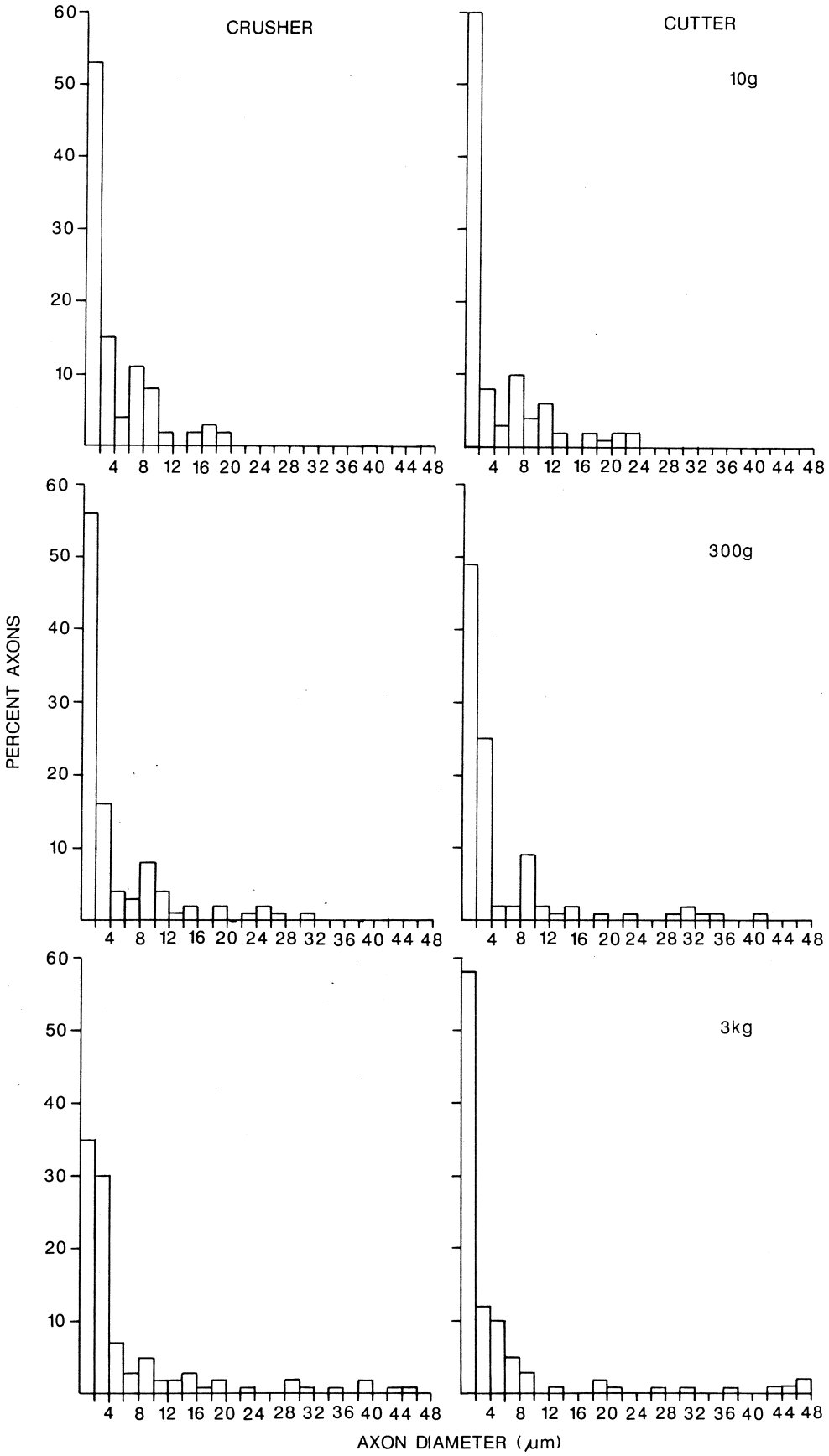
CRUSHER
CUTTER
10g
300g
3kg
PERCENT AXONS
AXON DIAMETER (μm)
60
50
40
30
20
10
4 8 12 16 20 24 28 32 36 40 44 48

Figure 3.

TABLE 1. Total number and number of small and large of axon profiles in the PD nerve of bilaterally asymmetric (crusher and cutter) claws in an age-graded series of lobsters

Age (yr)	Weight (g)	Number of axons: Crusher (cr) Total	Small (s)	Large (l)	s ÷ l	Cutter (ct) Total	Small (s)	Large (l)	s ÷ l	Ratio (cr/ct)
1	10	281	250	31	8.1	214	193	21	9.2	1.3
2	28	543	499	44	10.3	355	320	35	9.1	1.5
5	300	739	676	63	10.7	471	433	38	11.3	1.6
15	2,925	4,677	4,210	467	9.0	1,855	1,707	148	11.6	2.5

in the young juvenile (10 g) lobster and becomes accentuated with growth.

The normal condition of the paired claws in wild lobsters is asymmetry, although occasionally the paired claws are bilaterally symmetric as cutter type claws (Herrick, 1895). In laboratory-reared animals, however, the lack of a grippable substrate in the critical juvenile stages suppresses the development of a crusher claw and results in lobsters with paired cutter claws (Lang et al., '78). We examined one such animal and found the homologous PD nerves to be similar, reflecting the symmetry of the paired claws. Thus the cross-sectional profiles were almost mirror images of each other (Fig. 4) in terms of the distribution of groups of axons. The axon numbers were similar between the paired nerves at 433 and 420 for right and left cutter claws, respectively. Finally, the histograms of axon diameters showed a similar distribution between right and left nerves (Fig. 5). All together the paired PD nerves in this animal were symmetrical and this provided additional if indirect support for the earlier finding that the PD nerves were asymmetric in lobsters with dimorphic claws.

Regeneration

In order to establish the condition of a newly regenerated PD nerve, wild lobsters were used in which one of the paired claws had regenerated within the last molt because it was much smaller than its pristine counterpart. Two lobsters were used, one with a regenerated cutter and the other with a regenerated crusher. The PD nerve in the regenerated claw was substantially smaller than its counterpart in the pristine contralateral claw and the axon profiles were not nearly as spherical (Fig. 6). However, like the pristine nerve, the regenerated one had groups of both large and small axons. Moreover, the regenerated nerve has far fewer axons in total than its pristine counterpart (Table 2). Thus both the size of the nerve and the number of axons comprising it clearly underscore its status as a newly regenerated structure. However, like the pristine nerve, the regenerated one had groups of both large and small axons (Fig. 6) although the size range especially at the upper end is restricted in the regenerated nerve compared to its pristine counterpart (Fig. 7). Using the midpoint of the diameter range as a means of classifying large and small axons, we see that most axons fall into the small category in the regenerated nerve. This is the pattern seen in the contralateral pristine claws as well as in the claws of all the other lobsters listed in Table 1, where there are 8–12 times as many small axons as large ones. In other words the newly regenerated PD nerve shows a distribution of large and small axons which resembles the pristine condition.

The total number of axons in the pristine PD nerve of the wild lobster weighing just over 300 g (Table 2) is approximately 2–3 × greater than that found in a lab-reared lobster of similar weight (Table 1). This difference represents natural variability as well as variability between lab-reared and wild lobsters. However, some adjustment in the weight of the wild lobsters is required as they were weighed with one pristine and one regenerated claw while the lab-reared specimen was weighed with paired pristine claws. Since the paired claws are large and heavily chitinized,

Fig. 3. Distribution of axons according to their diameter in the cutter and crusher PD nerves of a lobster weighing 10 g, 300 g, and 3 kg. Number of axons sampled in the cutter and crusher nerves in each lobster was 48 and 36 (10 g), 82 and 75 (300 g), and 84 and 102 (3 kg).

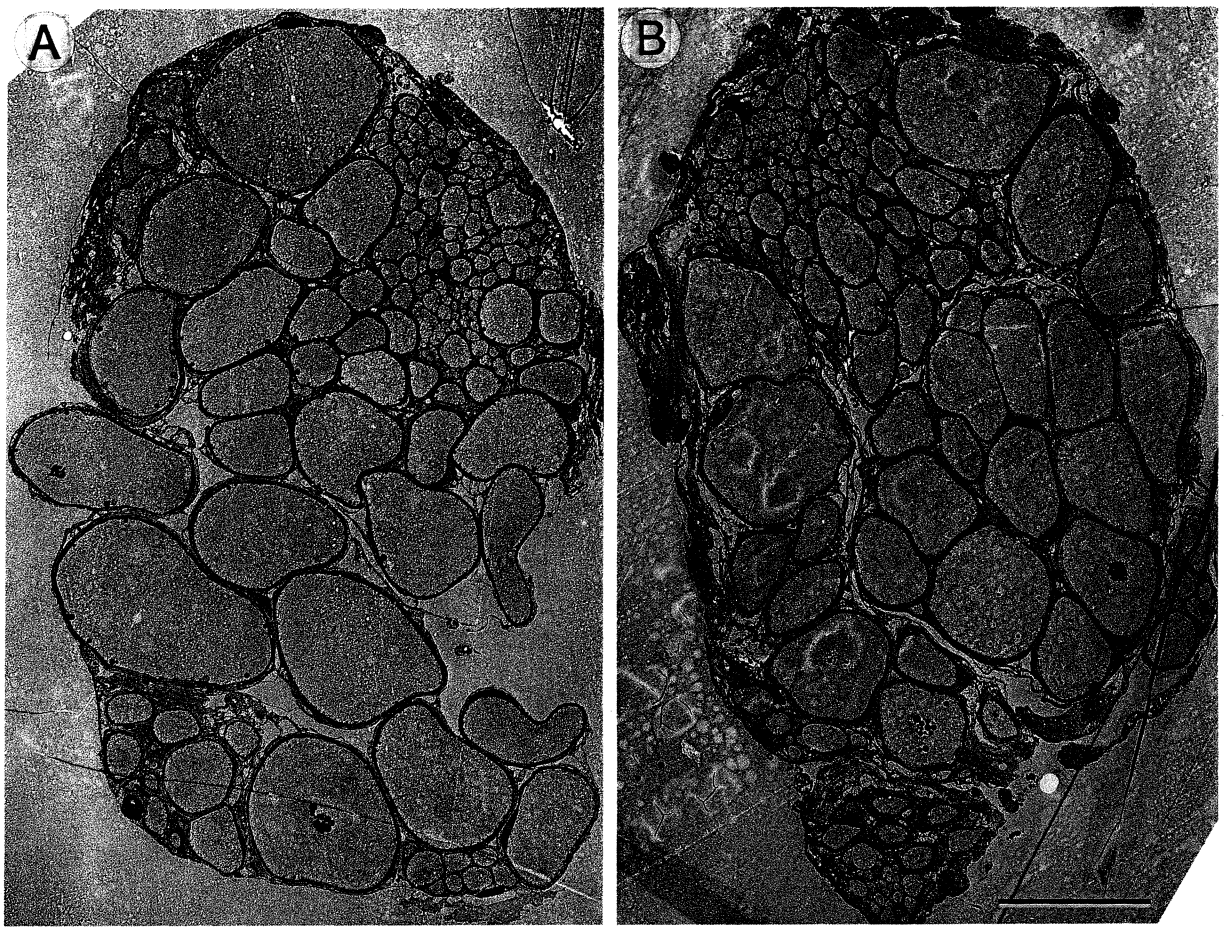

Fig. 4. Cross-section of the PD nerve in the paired (**A,B**) cutter claws of a lobster showing the close similarity in axon composition between the paired nerves. Scale bar = 25 μm.

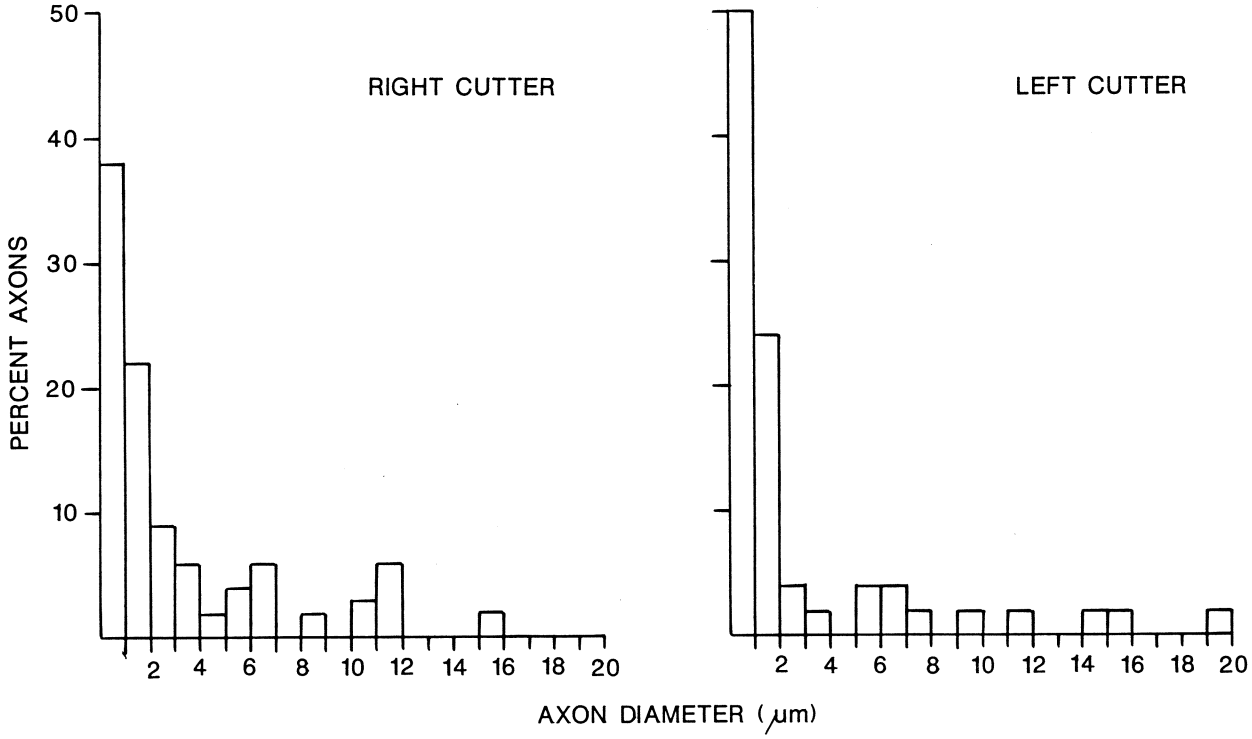

Fig. 5. Distribution of axons according to their diameters in the PD nerves of a lobster with paired cutter claws. Number of axons sampled was 89 and 96 in the right and left nerves.

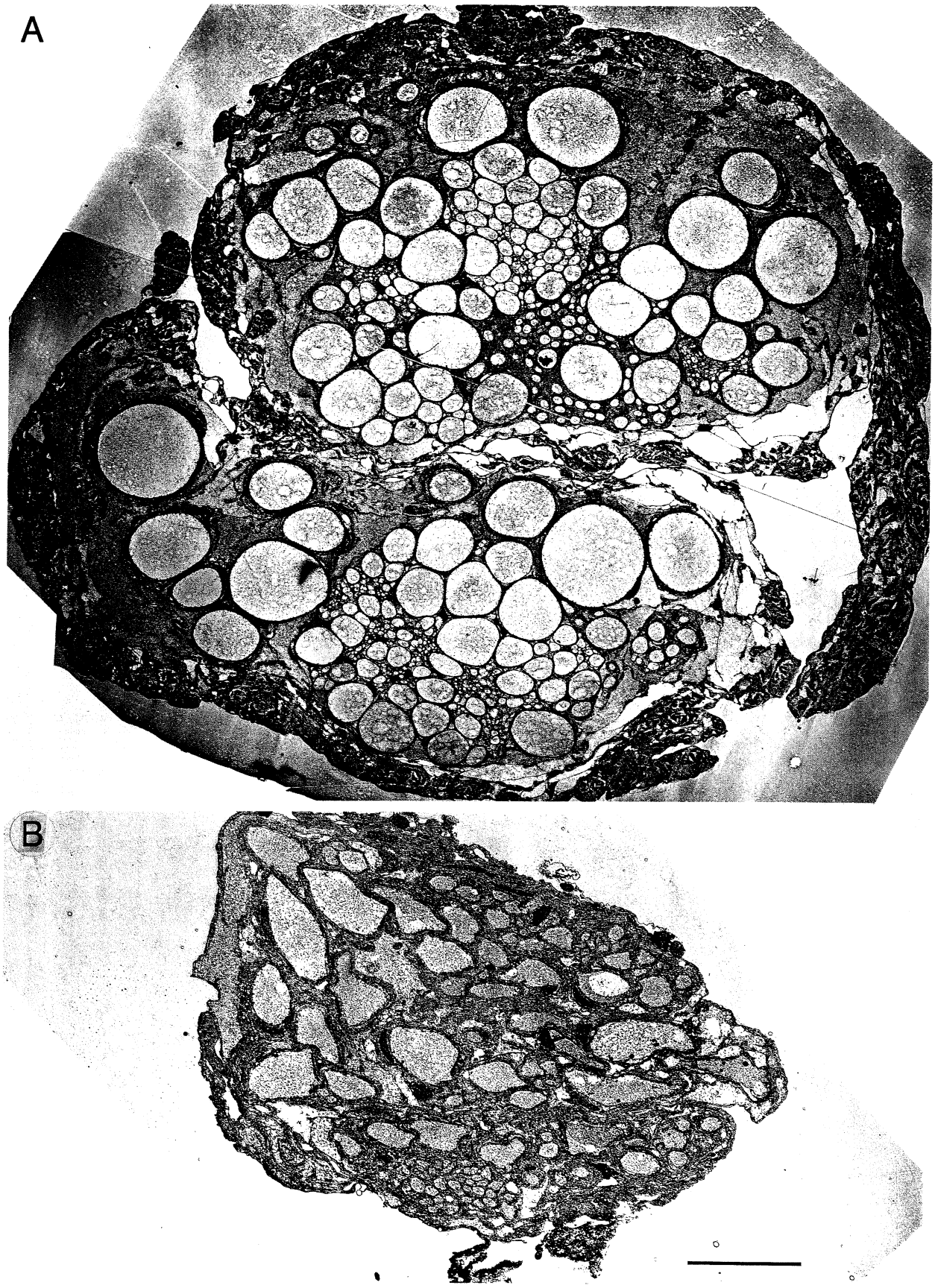

Fig. 6. Cross-section of the PD nerve in a pristine cutter (**A**) and a newly regenerated crusher (**B**) claw of a lobster showing striking differences in size and number of axons between the paired nerves. Scale bar = 50 μm, (A); 15 μm (B).

TABLE 2. Total number and number of large and small axon profiles in the PD nerve of newly regenerated and pristine (parentheses) claws compared to claws in wild and lab-reared lobsters

Weight g	Number of axons							
	Crusher				Cutter			
	Total	Small (s)	Large (l)	s ÷ l	Total	Small (s)	Large (l)	s ÷ l
Wild:								
335	(1,414)	1,276	138	9.3	88	78	10	7.8
318	132	117	15	7.8	(1,717)	1,574	143	11.0
Lab-reared:								
35	46	42	4	10.5	50	46	4	11.5
40	50	44	6	7.3	98	88	10	8.8
	Cutter				Cutter			
27	(260)	239	21	11.4	46	41	5	8.2
34	(200)	182	18	10.1	50	45	5	9.0

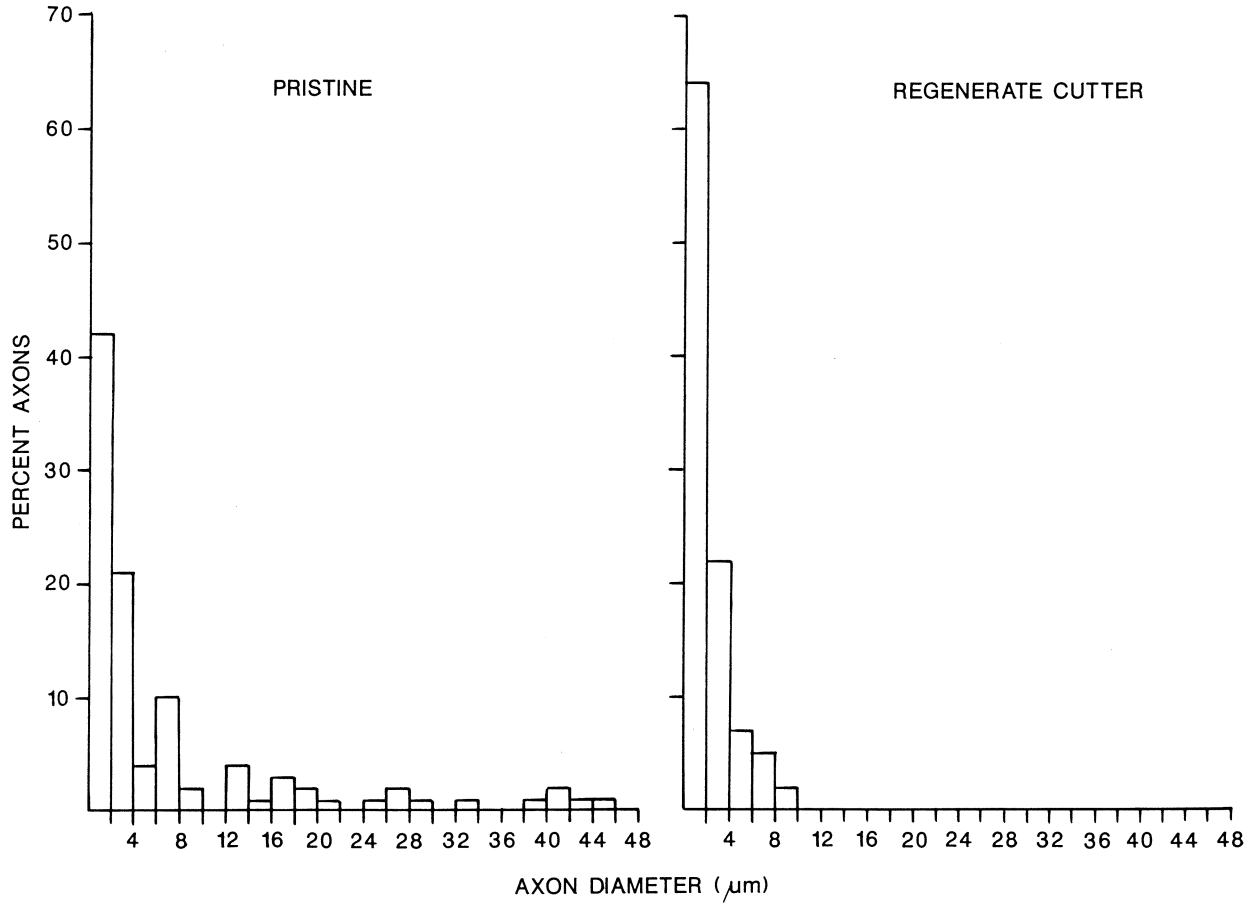

Fig. 7. Distribution of axons according to their diameters in the paired PD nerves of a lobster with a pristine crusher and a regenerate cutter claw. Number of axons sampled in the cutter and crusher was 156 and 89, respectively.

they make up between 30–50% of the weight of the lobster. Consequently the adjusted weight of the wild lobsters in Table 2 would be closer to 400 g and they would therefore be substantially larger than the 300 g counterpart. Now the difference in total number of axons in the PD nerve between wild and lab-reared lobsters would not be nearly as great as seen at first glance.

In order to provide a more valid comparison between regenerated and pristine nerves we examined lab-reared lobsters in which the paired claws were of the same type, namely cutter type. In these symmetrical-clawed lobsters, following removal of one of the claws soon after a molt, a new one regenerated at the next molt. The nerve from this newly regenerated claw was compared to its pristine counterpart (Table 2). As in the earlier example (Fig. 6) the regenerated nerve was smaller and had relatively few axons in total. Indeed the number of axons in the regenerated nerve was a small fraction (18 and 25%) of those in its pristine counterpart, emphasizing its newly regenerated status. Of the total number, however, only a few were large while the majority were small (Table 2), a size distribution not unlike its pristine counterparts.

In the previous experiments the lobsters had regenerated one of the paired claws and we therefore wished to examine the PD nerve when both claws had been regenerated. In two lab-reared lobsters with asymmetric claws, the regenerated PD nerve was small and had few axons in both crusher and cutter claws (Table 2). The size histogram of the axons (Fig. 8) shows a narrow range of diameters particularly at the upper end of the scale. However, these nerves show a typical distribution of a few large and many small axons (Table 2). Thus the PD nerve regenerated in a similar manner when either one or both claws are involved.

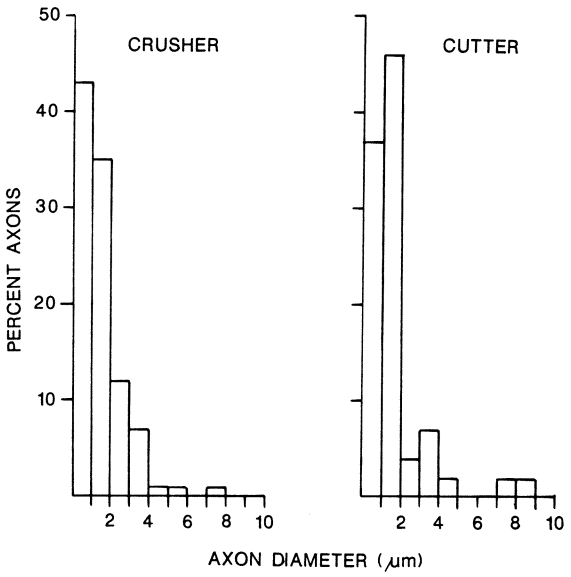

Fig. 8. Distribution of axons according to their diameters in the paired PD nerves of a lobster with newly regenerated asymmetric claws. All 50 and 46 of the axons in the crusher and cutter claws were sampled.

DISCUSSION

Axon composition

Sensory neurons in the PD organ of decapod crustaceans are sensitive to changes in length and tension of the elastic strand and are therefore broadly classified into movement- and position-sensitive cells (Hartmann and Boettiger, '67). The movement-sensitive cells have characteristically larger somata than their position-sensitive counterparts. Estimates of the total number of cells in a PD organ made from methylene blue stained preparations give values of about 100 in adult crabs (Whitear, '62; Mill and Lowe, '73), although how these separate into large and small axons was not considered. The present electron microscopic examination of the PD nerve provides for the first time an accurate estimate of the number of axons emanating from this organ and hence of the number of cells. This deduction is based on the premise that each axon represents a separate cell. The possibility exists, however remote, that sensory axons branch and consequently the number seen in cross-sections of the PD nerve may represent an inflated value. Assuming that each cross-sectional axon profile represents a single cell, the total number ranging from 200 to almost 5,000 in different sized lobsters is high compared to the approximately 100 reported in adult crabs. The difference in number may reflect the different species involved but may also reflect the different methods for estimating numbers. Staining of sensory cells with methylene blue is often capricious especially when the cells are small in size and the possibility exists that small cells on the PD organ may have been missed in previous studies.

In order to provide some perspective, the total number of axons in an individual PD organ may be compared to the total number of sensory axons in the entire cheliped. Information on the latter aspect is available for a number of different sized lobsters (Govind and Potter, '87) and for our purposes it is useful to consider such information in

a 20 g, 680 g, and 4.5 kg lobster as these come closer to the ones examined in the present study. The total number of axons in the dimorphic chelipeds of these three lobsters ranged from 118,000 to 475,000. The PD nerve number which ranged from 400 to 5,000 would therefore constitute 0.3 to 1.0% of the total sensory innervation of the cheliped. Thus the seemingly high number of axons in a single PD organ makes up a very small proportion of the total number to a cheliped.

Bilateral asymmetry in axon numbers between the paired PD nerves is more pronounced in the largest lobster studied compared to the other much smaller ones. Although the sample size does not warrant making any firm conclusions it is worthwhile noting that a similar trend was seen in terms of the total number of sensory axons to the dimorphic chelipeds. A comparative ratio of the numbers between crusher and cutter chelipeds shows an increase from 1 to 2 to 2.6 in a 680 g, 4.5 kg, and 11.3 kg lobster (Govind and Potter, '87). Such differentiation between the paired claws in sensory innervation in general, and in PD nerves in particular, may signify corresponding differences in the behavior of these paired chelipeds.

Since the two principal types of receptor neurons in the PD organ of crabs are distinguished by the size of their soma (Mill and Lowe, '73)—namely the large movement-sensitive and the small position-sensitive cells—the axon diameters in the PD nerve would correspondingly reflect the distribution of receptor types in the PD organ. In the present study of the lobster PD nerve we classified the axons into large and small categories based on the midpoint of the range in their axon diameters. This revealed a typical distribution of a few large axons and many small ones. Indeed, the ratio of large to small axons was constant among all the nerves with small axons outnumbering their larger counterpart by 8–12 times. Moreover, our arbitrary criteria for classifying large and small axons is a reasonably accurate reflection of the axon composition, judging from the fact that over two-thirds of the axons in all the pristine nerves are < 5 μm. Clearly, there is a large population of small axons which presumably represent the position-sensitive neurons in the PD organ. Conversely, the large diameter presumably movement-sensitive cells represent a small ($< 20\%$) fraction of the total number. Such a distribution of the two cell types reflects the relative importance of each in the behavior of the cheliped.

It was therefore surprising to find that the ratio of large to small axons in the PD nerve was similar for both crusher and cutter claws even though they have different behavioral roles. What was less surprising was the asymmetry in the paired PD nerves in the form of a greater total number of axons in the crusher compared to the cutter claw in all four lobsters studied. The greater number of axons in the PD nerve of the crusher claw compared to the cutter claw may reflect the fact that the crusher is a larger, heavier, and more powerful claw than the cutter.

Growth

There is a clear increase in cell numbers with growth in both the crusher and cutter PD nerves. In the present growth series the cell numbers increased from 300 to 6,000 in the crusher and from 200 to 2,000 in the cutter. Moreover, this increase encompassed the entire size range of axons and was not restricted to any one particular size category. Since lobsters lack a terminal molt they continue to grow in mass with each molt, reaching weights of 20 kg over several decades (Cooper and Uzmann, '80). Our results would suggest that the number of axons in the PD organ of the chelipeds continues to increase with growth. This is unlike the situation in the Dungeness crab, *Cancer magister,* in which hyperplasia in the limb PD organ occurs till the 7th instar even though the animal continues to grow in mass till the 13th instar (Hartmann et al., '89). Also in this crab the hyperplasia is confined to the large movement-sensitive neurons and not to the small position-sensitive cells. In the lobster, on the other hand, both cell types show hyperplasia.

Regeneration

The newly regenerated PD nerve is much smaller and has fewer axons than its pristine counterpart and this is in keeping with the fact that the regenerated limb itself is smaller. In a previous study (Govind et al., '88) of lab-reared juvenile lobsters with paired symmetrical cutter claws, a newly regenerated claw was approximately two-thirds the size of its contralateral homolog. On this basis the number of axons in the newly regenerated PD nerve might be expected to reflect this percentage. In the two cases in which lobsters with paired cutter claws were used in the present study, both showed regenerated PD nerves which possessed between 18% to 20% of the total number of axons of the contralateral homologous nerve. Clearly, the PD nerve does not

display the same degree of regeneration as the rest of the limb.

Another aspect in which the regenerated PD nerve does not appear to be differentiated concerns the lack of asymmetry between crusher and cutter claws. Where regeneration involves both claws which had been asymmetric in their original configuration, the PD nerves did not display the typical asymmetry of more axons in the crusher compared to the cutter claw. In this respect regeneration of the PD nerve may mimic events in early development when the paired claws and closer muscles are undifferentiated (Govind, '84).

Although appearing less differentiated in some respects, the regenerated PD nerve closely resembles its original counterpart in the distribution of axons. Thus the regenerate of both crusher and cutter claws shows a typical distribution of a few large and many small axons. This suggests that the ratio of large, movement-sensitive cells to small, position-sensitive ones is rigidly specified during regeneration as well as throughout growth.

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