C-Fiber Structure Varies with Location in Peripheral Nerve

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Abstract. Recent advances in regeneration and pain research have revealed gaps in the understanding of normal C-fiber anatomy. In the rat PNS, C-fiber axons assemble into Remak bundles, but beyond this, features of C-fiber organization are not defined. Systematic sampling and quantitation reveals that Remak bundles exiting from the L5 dorsal root ganglion (DRG) contain large numbers of axons, for example, 56% of unmyelinated axons were in bundles of >20 axons. This is different from distal nerve segments such as the hindpaw plantar nerve where the median number of axons per bundle is 3. The cross-sectional area of unmyelinated axons in dorsal root was homogeneous near the DRG but variability in axonal area increased near the spinal cord (p = 0.00001) and the mean axonal area was unchanged. Unmyelinated axons in peripheral nerve were almost always isolated from one another by Schwann cell processes; however, in dorsal root 7% to 9% of unmyelinated axons were immediately adjacent within pockets containing 2 or more axons. Remak bundles in the distal peripheral nerve clustered with other Remak bundles. We observe that multiple unmyelinated axons are juxtaposed within the C-fiber/Remak bundle and that the close association of afferent axons may have important functional implications.

Key Words: Dorsal root; Peripheral nerve; Primary afferent neuron; Remak bundle; Sensory function; Sympathetic; Unmyelinated axon.

INTRODUCTION

Unmyelinated nerve fibers in the peripheral nervous system are often envisioned as simple but reliable conduits for signals from sensory terminations to the spinal cord. However, a new complexity is emerging. C-fiber axons are now recognized to be diverse in biophysical properties as well as modality and to exhibit complex responses to partial nerve injury (1–3). Recent studies indicate that C-fiber axons of the L4 mixed spinal nerve exhibit novel spontaneous activity after L5 mixed spinal nerve ligation and transection or L5 ventral rhizotomy, procedures that spare L4 unmyelinated axons from direct injury (4, 5). Although the mechanisms by which this spontaneous activity develops are not known, experimental evidence supports a role for peripheral nerve events. Signaling between C-fiber component cells has been demonstrated in that calcium transients arise in Remak Schwann cells in response to action potential propagation in unmyelinated axons and it is likely that multiple signaling pathways exist within the Remak bundle (6–8).

These observations highlight a relative paucity of information about normal C-fiber/Remak bundle organization. Essential unresolved questions include whether multiple neurons contribute axons to a single unmyelinated nerve fiber bundle, whether these bundles typically contain 1 axon or multiple axons, and whether the relationship of axons to Remak Schwann cells changes with location. To address these questions we systematically characterized Remak bundle organization at defined locations in the rat PNS. The findings show important regional differences. This study examines the L5 dorsal root, the sciatic nerve, and specific branches because of regional differences. This study examines the L5 dorsal root, the sciatic nerve, and specific branches because of direct relevance for models of neuropathic pain. The potential pathophysiological implications of the structural complexity of Remak bundles could include understanding how Remak bundle structure influences the electrochemical micro-environment of C-fiber axons and their discriminative capacity, determinants of the response of intact unmyelinated axons to partial nerve injury, and the requirements placed on Remak Schwann cells for successful regeneration.

MATERIALS AND METHODS

Eight- to 9-week-old male Sprague-Dawley rats weighing approximately 250 grams were used in these experiments. A total of 17 animals were studied. The animals were handled using an approved protocol. Brieﬂy, under deep anesthesia (chloral hydrate 400 mg/kg i.p.), cardiac puncture was followed by perfusion with phosphate buffered saline and ﬁxative. Fixation was overnight with 4% paraformaldehyde and 3% glutaraldehyde or with 5% glutaraldehyde in Sorenson’s buffered saline (pH 7.4). The following tissues were collected after dissection (followed by the approximate distance from spinal cord in mm): rostral dorsal root, 0; caudal dorsal root, 30; mid-dorsal root ganglion (DRG), 32.5; mixed spinal nerve, 35; proximal sciatic nerve, 50; plantar nerve, 150. Each anatomical location was evaluated in a minimum of 3 animals.

The fixed samples were dehydrated in preparation for plastic embedding. After 3 rinses with buffer, the tissues underwent a 1-hour incubation with 2% OsO4, 3 buffer rinses, a graded alcohol series of twice for 10 minutes each in 50%, 70%, 80%, 95%, and 100% ethanol; propylene oxide for 5 minutes twice followed by propylene oxide:plastic (1:1) overnight at room temperature (9). Propylene oxide was exhausted from uncapped tubes for 3 hours, followed by immersion in fresh plastic overnight in a vacuum oven at room temperature and embedding in...
fresh plastic on day 3. The plastic was polymerized over 48 hours at 60°C. Plastic components included dodecyl succinic anhydride (DDSA), nadic methyl anhydride (methyl-5-norbornene-2,3-dicarboxylic anhydride, NMA), EMbed 812 (a mixture of bisphenol A/Epichlorohydrin epoxy resin and resin modifier) and 2,4,6-(tri(dimethylaminoethyl)phenol) (DMP-30) (all from EMS, Fort Washington, PA) (10). One-μm-thick sections were stained with toluidine blue (1%). Thin silver sections were mounted on Formvar-coated (EMS, Fort Washington, PA) grids of 50 or 100 mesh and stained with uranyl acetate (2.5% in 50% ethanol) and lead citrate (3%) (11). Tissues were quantitatively evaluated and photographed using a Hitachi 600A electron microscope. Photos were printed by hand and selected images were scanned for digital archiving and montage construction. Contrast and brightness were adjusted for optimal print quality but images are otherwise unaltered.

Unmyelinated axons were distinguished from Schwann cells by several criteria. Compared with Schwann cells, Remak bundle axons have axoplasm that is less electron-dense and an axonal membrane that is more dense than the membrane of Schwann cells. Axons contain longitudinally oriented microtubules and do not form desmosomes. Individual Remak bundles were defined as contained within a continuous distinct basal lamina. Remak bundles were identified at 5,000 times magnification and axons were counted at 50,000 times magnification to ensure consistency. Plantar nerves were assessed in entire cross section; other tissues were systematically sampled and assessed over multiple 50 by 75 μm regions selected for proximity to grid bar intersections. Overall, between 790 and 2,660 axons were counted for each sample evaluated. Clusters of Remak bundles were defined as 2 or more bundles immediately juxtaposed such that a line drawn around them would be a compact space excluding large myelinated axons. Clusters were often near 1 or more small myelinated axons. In cases where 2 small myelinated axons were in close proximity to one another, individual clusters were distinguished by including only those Remak bundles in closest juxtaposition. Axons were defined as being in a poly-axonal pocket when 2 or more axons were immediately adjacent and no extension of Schwann cell cytoplasm separated the 2 axons.

Axonal areas were measured using Bioquant software (R&M Biometrics, Nashville, TN). Electron micrographs were photographed at grid bar intersections using 3,500 times magnification and digitized at 200 dpi. Approximately 100 unmyelinated axons were outlined by hand for each of the nerves studied; this number was sufficient to detect a 2-fold increase in variance at the p = 0.001 level. The distributions of axonal areas were compared using the Student t-test of the mean and an F-statistic for comparison of variance (12). Histograms and basic statistical analyses were carried out using a standard spreadsheet utility.

RESULTS

Characteristics of Unmyelinated Axons and Remak Bundles in the L5 Dorsal Root

At the caudal end of the dorsal root, 2 to 3 mm from the center of the DRG, most Remak bundles contained large numbers of axons (>20 axons). In some instances, several Remak bundles were juxtaposed so that the number of unmyelinated axons in close association could exceed 100 (Fig. 1A). In order to identify differences between the 2 ends of the dorsal root we compared the caudal dorsal root site to the rostral dorsal root at a site within 3 mm of entry into the spinal cord.

In the caudal dorsal root, 56% of the unmyelinated axons were supported in bundles of 20 or more axons; axons in 1-axon bundles were infrequent (1.5%). At this site, the median number of axons/Remak bundle was 9. A typical Remak bundle from the caudal dorsal root is shown in Figure 1B. By comparison, the Remak bundles in the rostral dorsal root contained a smaller number of axons/bundle (median number 5). This was significantly lower compared with the caudal DR (rank sum test, p < 0.01). Examples of Remak bundles in the rostral dorsal root are shown in Figure 1C and D.

The distribution of axons into Remak bundles is shown in Figure 2 for several locations along the nerve axis. The range of axons/bundle was greatest in the caudal dorsal root (Fig. 2B), where the axons/bundle varied from 1 to 50. Near the spinal cord the distribution shifted to the left and Remak bundles containing more than 20 axons were much less common (Fig. 2A). The shapes of these distributions were unusual and could not be characterized as normal, Poisson, or exponential.

Remak bundles of the caudal dorsal root typically contained axons of homogenous size densely packed into Schwann cell profiles. The striking homogeneity of the axons suggested a sense of order, Figure 1B. In contrast, the axons of Remak bundles in the rostral dorsal root appeared less well organized, reflecting greater diversity in axon cross-sectional area (Fig. 1C, D). Quantitative analysis demonstrated that average cross-sectional area of unmyelinated axons was not different in the rostral and caudal dorsal roots (Student t-test, p = 0.28). However, the variability in cross-sectional area was strikingly increased in axons of the rostral dorsal root (F-statistic, p = 0.00001) when compared with the caudal dorsal root.

Associated with the increased variability in axon cross-sectional area in the rostral dorsal root, there were 3 times as many very large (defined as axons with area more than 2 standard deviations above the mean) unmyelinated axons. We used this variability to test the hypothesis that axonal caliber may influence the number of axons in a Remak bundle. The Remak bundles of the rostral dorsal root were evaluated with regard to the number of axons and the cross-sectional area of the largest axon in each bundle. There was a positive correlation between the number of axons in a bundle and the cross-sectional area of the largest axon in that bundle (correlation coefficient = 0.62, p < 0.005). A probabilistic model confirmed that this positive correlation was entirely consistent with the random assortment of axons into Remak bundles without regard to axon caliber. There was a slight excess of large...
caliber axons in single-axon Remak bundles relative to the expectations of the random assortment model.

Remak Bundle Organization in the DRG

Viewed in transverse section at the mid-point of the long axis of the DRG, the DRG consists of both axonal and cellular portions. In the cellular portions, a typical Remak bundle consisted of a single axon ensheathed by Schwann cell cytoplasm with a continuous basal lamina. In the axonal portions there were Remak bundles with multiple axons but in no case more than 15 axons per Remak bundle. Overall, 57% of Remak bundles in the central DRG contained 1 axon, the median number of axons per Remak bundle was 1 and the mean was 2.1.

Features of Remak Bundle Organization in the Sciatic Nerve and Branches

As a population, the Remak bundles of the sciatic nerve contained more axons than did those of the distal branches in the hindpaw (plantar nerves). The median number of axons per Remak bundle was 6 in the sciatic nerve and 3 in the plantar nerves. Representative Remak bundles from the sciatic nerve and plantar nerve are shown in Figure 3A and B. The distribution of axons/Remak bundle in the sciatic and mid-plantar nerve is shown in Figure 2C and D. The Remak bundles of the sciatic nerve contained from 1 to 26 axons while those of the plantar nerve ranged from 1 to 14 axons. Comparison of these distributions showed that the number of axons per Remak bundle in the plantar nerve was lower than in the sciatic nerve (rank sum test, p < 0.01). The shape of the distribution for the proximal sciatic nerve was not normal, exponential or Poisson. Interestingly, the distribution of axons into Remak bundles in the plantar nerve was heavily weighted to a small number of axons per bundle and fit well by a negative exponential distribution (p = 0.0001).

The influence of location on Remak bundle structure is summarized in Figure 4. The number of axons per Remak bundle increased sharply as unmyelinated axons extended outward from the DRG. In both the peripheral nerve and dorsal root, further increases in distance from the DRG were associated with a decrease in the number of axons per Remak bundle. The variability between animals was small; indicating that although the number of axons making up an individual Remak bundle was random, it was highly constrained. Within the central DRG, a large percentage of axons were supported in Remak bundles as single axons but upon exit from the DRG, single-axon Remak bundles were much less frequent, 5% in the mixed spinal nerve and 1.5% in the caudal dorsal root. Further from the DRG, the number of single-axon Remak bundles increased and was 7.2% in the plantar nerve. Even though the plantar nerves harvested in the hindpaw were no more than 1 cm from the afferent nerve terminals, the incidence of single-axon bundles there was less than 10%.

The organization of Remak bundles relative to one another (tertiary structure) varied with location along the nerve axis. In the dorsal root and sciatic nerve, little tertiary structure was apparent except that near the DRG; Remak bundles with many axons were frequently found in juxtaposition (Fig. 1A). In the plantar nerve, a pattern of tertiary organization emerged; Remak bundles often clustered with other Remak bundles. Using quantitative methods we found that the typical Remak bundle cluster consisted of 2 to 6 Remak bundles in association with a small myelinated axon. Not all clusters had an associated small myelinated axon. The distribution of Remak bundles into clusters was evaluated by pooling data from the plantar nerves of several animals (Fig. 5). The number of Remak bundles per cluster was centered about a mean of 4.3 and the size distribution was truncated on the left. This distribution was well modeled by a Poisson distribution with λ = 3.3, χ² = 0.27.

Poly-Axonal Pockets are Found in the Dorsal Root

Most of the unmyelinated axons in the rat PNS were individually ensheathed within Schwann cell cytoplasmic extensions, physically separated from other unmyelinated axons. An exception to this was observed in the dorsal root where about 7% to 9% of axons were immediately adjacent to other axons without an intervening Schwann cell process (Fig. 1A, arrows). We refer to these structures as poly-axonal pockets. Poly-axonal pockets in the dorsal root rarely contained more than 3 axons (<1% of

Fig. 1. A: EM Photomicrograph of the L5 dorsal root near the L5 DRG, stained with uranyl acetate/lead citrate as described in methods. Unmyelinated, small myelinated, and large myelinated axons are seen. Most of the unmyelinated axons are in Remak bundles that contain large numbers of axons. Although most unmyelinated axons are isolated from one another by intervening Schwann cell cytoplasm, there are examples of axons that are immediately adjacent (described in the text as poly-axonal pockets) shown by white arrowheads. B: Remak bundle in the caudal L5 dorsal root. These axons are striking in homogeneity with respect to size and are densely assembled in the bundle. C: The Remak bundles of the rostral dorsal root (near the spinal cord) typically contain smaller numbers of axons. D: This Remak bundle from the rostral dorsal root demonstrates axons with a wide range of cross-sectional areas. Axons in the bundles of this region are more diverse in size compared with the caudal dorsal root, F-test, p = 0.00001. Magnifications: A, B, D, ×3,500; C, ×6,000. Scale bars: A, 5 μm; B–D, 1 μm.
all pockets); poly-axonal pockets with 2 axons were relatively common and occurred throughout the dorsal root (Table). In part because Remak bundles in the dorsal root contained many axons, we found that 28% of the Remak bundles near the DRG contained at least 1 poly-axonal pocket. Poly-axonal pockets were rare in the sciatic nerve.

This work demonstrates that most C-fiber (Remak) bundles in rat support multiple axons and that the organization of unmyelinated nerves in the rat PNS varies with location along the nerve axis. The observation that dozens of axons can be found in a single dorsal root Remak bundle indicates that multiple neurons must contribute axons to that bundle. The currently accepted ratio of centrally directed axons per sensory neuron is 2.3 (15), suggesting that a Remak bundle containing 2 dozen axons could receive axons from as many as 10 neurons. It remains to be established whether the number of axons per neuron is different for small and large DRG neurons, but it is unlikely that small neurons commonly give rise to 20 centrally directed axons.

Our study is the first to definitively show that position along the nerve axis is an important factor influencing the number of axons in each Remak bundle. The amount of variation between animals in our study was small, suggesting that location along the neuraxis establishes fundamental constraints on Remak bundle structure. The factors determining the assembly of axons into Remak bundles are still undefined. Interspecies differences have been observed and Remak bundles in man, as determined distally in the sural nerve, have few axons relative to other vertebrates (16).

**DISCUSSION**

The distributions of axons into Remak bundles are displayed as histograms for selected anatomical locations. The histograms are ordered from top to bottom with respect to distance from the spinal cord: 0, 3, 5 and 15 cm. The vertical axis is number of Remak bundles and the horizontal axis is number of axons per Remak bundle. These distributions show the great variability in the number of axons per bundle and do not match any familiar probability distributions, as described in the text.

**A:** The distribution of axons into Remak bundles for a representative rostral dorsal root near the spinal cord. The distribution appears to follow a monotonically decreasing pattern.

**B:** In the caudal dorsal root, close to the DRG, a substantial portion of unmyelinated axons are bundled in groups of 20 or more.

**C:** Sciatic nerve Remak bundles contained numbers of axons similar to those of the dorsal root near the spinal cord.

**D:** Planter nerve Remak bundles larger than 15 axons in size were not seen.
Fig. 3. EM Photomicrographs of Remak bundles in the sciatic and plantar nerves stained with uranyl acetate/lead citrate.

A: Sciatic nerve Remak bundles may incorporate dozens of axons. All the axons in this figure are enclosed within a single basal lamina, although some division is suggested by the band of collagen fibers running through the bundle. Collagen was much more prominent in the peripheral nerve than in dorsal root. Although some axons are separated by only a very slender extension of Schwann cell cytoplasm, there are no poly-axonal Schwann cell pockets, as seen in the dorsal root in Figure 1A.

B: Remak bundles of the plantar nerve contain fewer axons and single axon-Remak bundles are much more common. Remak bundles are often found clustered in close association with other Remak bundles and with small myelinated axons, described in the text and Figure 5. Magnifications: ×10,000. Scale bars: 1 μm.

As in human sural nerve, Remak bundles of the rat hindpaw form clusters that are identical to the groupings of Remak bundles described by Gasser as typical of skin nerves (17). These clusters are recognized by electrophysiologists who observe that C fibers often have a close physical association with 1 or more Aδ fibers. We found the number of Remak bundles per cluster was distributed as a Poisson function, compatible with the hypothesis that the formation of clusters of Remak bundles is based largely on physical proximity. This is consistent with developmental studies of peripheral nerve which indicate that unmyelinated nerve fibers arise from a persistent core of axons that do not myelinate (18, 19).

### Distribution of Axons into Schwann Cell Pockets in the Dorsal Root

<table>
<thead>
<tr>
<th>Distribution of Axons into Schwann Cell Pockets in the Dorsal Root</th>
<th>Rostral dorsal root (n)</th>
<th>Caudal dorsal root (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 axon/pocket</td>
<td>1069 (93%)</td>
<td>214 (91%)</td>
</tr>
<tr>
<td>2 axons/pocket</td>
<td>72 (6%)</td>
<td>16 (7%)</td>
</tr>
<tr>
<td>3 axons/pocket</td>
<td>12 (1%)</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Percentage poly-axonal*</td>
<td>7.3</td>
<td>9.3</td>
</tr>
</tbody>
</table>

n = Number of axons counted in each category.

* Percentage of poly-axonal axons in 2-axon and 3-axon pockets.

An additional factor that may play a role in determining the number of axons per Remak bundle is axon caliber. We explored this possibility in the dorsal root by measuring axon cross-sectional area (axonal area). It is known that average axonal area and diameter do not vary along the length of the S1 and S2 spinal nerve roots in the rat (20). We confirmed that mean axonal area did not vary along the length of the L5 dorsal root but noted a marked increase in the variation of axonal area in the rostral dorsal root. The reasons for this increased variation in axonal area are not known. Because there was an increased number of very large unmyelinated axons in the dorsal root near the spinal cord, we wanted to re-visit the idea that large axons drive the sorting of axons into smaller bundles, resulting in one-to-one axon-to-Schwann cell association and ultimately myelination (21). In examining the relationship between the cross-sectional area of axons in a Remak bundle and the number of axons in that bundle we found that all the Remak bundles with 2 or more axons contained large caliber axons at a frequency consistent with random assortment. There were Remak bundles with single axons in which the axons were larger than expected, which may represent a pre-myelinating state for the axon-Schwann cell unit.

Important questions arise from the observation that several axons are supported in a single Remak bundle. These include questions about the electrochemical environment of C fibers in the Remak bundle, the response of nociceptors to partial nerve injury, and implications for regeneration. The role of the non-myelinating Schwann cell in defining the electrical environment of the unmyelinated axon is unresolved (17, 22) and recent studies provide direct evidence that Remak Schwann cells are electrochemically responsive to C fiber action potentials (6, 7). The electron microscopic appearance of Remak bundles prompted early concerns that unmyelinated axons were likely to interfere the function of one another (23). Detailed morphological analysis lead to the conclusion that the exchange of axons between Remak bundles was frequent enough to prevent adjacent axons...
The L5 spinal root system extending from spinal cord to hindpaw, indicating location of systematic sampling. Arrows are labeled with distance from the spinal cord in cm as determined by averaging measurements from 3 animals. The sampling locations (from left to right) are referred to in the text as the rostral dorsal root, caudal dorsal root, mid-DRG, mixed spinal nerve, proximal sciatic nerve, and plantar nerve. Figure is not to scale.

Mean and median number of axons per Remak bundle. The vertical axis is the number of axons per bundle and the horizontal axis is distance from the spinal cord in cm. The mean number of axons per Remak bundle (filled circles with standard deviation bars) varied with distance from the primary sensory neuron. The median number of axons (open triangles) paralleled the mean.

The percent of axons in bundles with a single axon is shown as open triangles and is lowest from undergoing supra-threshold voltage changes in response to nearby action potentials. This does not mean that interaction does not occur, but rather that the interaction is not sufficient to induce de novo action potentials along the length of the nerve under normal circumstances. Experimental evidence is actually in favor of limited interaction between C fiber axons. This includes coupling of C fiber action potentials in the periphery, conduction velocity speeding and slowing, as well as the detection of C fiber action potentials with complex morphology, suggesting entrainment of adjacent axons (24) (R. Meyer and S. McMahon, personal communication). It has been shown that the cross-sectional area of a Remak bundle is generally proportional to the number of axons supported by the Schwann cell and that the cross-sectional area of Schwann cell cytoplasm in a Remak bundle is approximately equal to axoplasm area (25, 26). This means that on average each unmyelinated axon is “wrapped” by a thickness of Schwann cell approximately 40% the radius of the axon (the solution to the equation: \[ r_a^2 = (r_a + r_e)^2 - r_e^2 \] where \( r_a \) is the axon radius and \( r_e \) is the thickness of Schwann cell cytoplasm wrapping around the axon). Measurements show that the thickness of Schwann cell cytoplasm between unmyelinated axons is often much less, as little as 50 nanometers, resulting in a distance of 100 nanometers between axons. Knowing that the conductivity of this Schwann cell extension is much higher in the dorsal root near the DRG (1.5%) and highest in the center of the DRG (57%, data not shown). Distance from the spinal cord is marked on the x-axis and corresponds to the specific anatomic locations indicated in panel (A).
than that of myelin and that the Schwann cell is not electrically inert suggests that the multiple axons sharing Remak bundles may not be fully isolated from one another electrically. Beyond this, we find that nearly 10% of axons in the dorsal root are supported in poly-axonal pockets where electrical isolation is impossible. The possibility remains that axonal proximity may influence spatial and modality-specific discrimination of sensory inputs from the C fiber system and that certain pathological states may enhance the interaction of adjacent axons.

Close proximity of unmyelinated axons in a shared Remak bundle may have implications for the response of unmyelinated axons to partial nerve injury. Electrophysiological studies following both ventral rhizotomy and mixed spinal nerve transection have shown increased spontaneous activity in C fibers (4, 5, 27, 28). The spontaneous activity of L4 C-fibers following L5 ventral rhizotomy is particularly interesting because in rat this lesion essentially spares all C-fibers from direct injury. We have demonstrated myelinated axons undergoing Wallerian degeneration closely juxtaposed to C-fibers in peripheral nerves and have proposed that this proximity implicates diffusible factors in the signaling of post-injury spontaneous activity of peripheral origin (4). Following mixed spinal nerve transection, there is an even larger percentage of L4 C-fibers exhibiting spontaneous activity. Possible explanations include the larger number of myelinated axons injured by this lesion or an interaction of unmyelinated axons arising from adjacent sensory ganglia; this is an area of ongoing research (5). In summary, degenerating nerve fibers may release factors that alter the function of neighboring uninjured axons. Because mixtures of intact and degenerating axons are almost universal to neuropathic pain models, it seems likely that post-injury events in the peripheral nerve play a contributory role.

Finally, optimizing the response of Schwann cells after injury and improving C fiber regeneration are important motivations for the study of nerve fiber anatomy. Recovery of distal C fiber function takes place only over a period of months and may be incomplete (29), resulting in abnormal sensory functioning or neuropathic pain (30). There is an emerging appreciation that C fibers are diverse and have a variety of trophic factor requirements (2, 31). Our observation that Remak bundles near the DRG contain large numbers of axons shows that Remak Schwann cells support axons arising from multiple neuronal cell bodies. The idea that multiple small DRG neurons associate with single Remak Schwann cells suggests that following nerve injury, Remak Schwann cells may need to produce a variety of trophic factors to promote successful regeneration.

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