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Nearest-Neighbor Distance of Intermediate Filaments in Axons and Schwann Cells

Distinction Between Axons and Schwann Cell Processes in the Denervated and Reinnervated Peripheral Nerves

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Summary. To distinguish axons from Schwann cell processes in the denervated (Büngner's bands) and reinnervated peripheral nerves, the nearest-neighbor distance of intermediate filaments (NND) was measured in axons and Schwann cells from denervated and subsequent regenerating peripheral nerves. It was revealed that the NND was much larger in regenerating axons (41.9 ± 14.1 nm) than in Schwann cell processes (23.1 ± 7.1 nm in regeneration and 19.7 ± 5.8 nm in denervation).

In addition, the NND was also measured in the normal adult and developing peripheral nerves, and it became clear that in all cases the NND in axons (29.0–41.9 nm) was larger than in Schwann cells (19.7–23.1 nm). Thus, it can be generally considered that the NND is larger in axons than in Schwann cells. This fact can be used for the distinction between axons and Schwann cell processes, when the latter have a profile similar to that of the former as in Büngner's bands and in the regenerating nerves.

Key words: Axon – Schwann cell – Intermediate filament – Nearest-neighbor distance – Büngner's bands

Introduction

During our study on peripheral nerve regeneration, we have often encountered difficulty in distinguishing the regenerating axons from Schwann cell processes in Büngner's bands. Schwann cells in the long-term denervation extend long cytoplasmic processes which intermingle with each other and form Büngner's bands. Such Schwann cell processes often show round contours in cross sections, containing only microtubules and intermediate filaments in the relatively clear cytoplasm. These features are so similar to those of axons that the regenerating axons growing through such a band are hardly distinguishable from the Schwann cell processes.

In an attempt to distinguish the regenerating axons from Schwann cell processes, we measured the NND in axons and Schwann cells under denervated and subsequent regenerating conditions. It was shown that the NND was evidently different between axons and Schwann cell processes. For comparison, the NND was also measured in the normal adult and developing peripheral nerves.

Material and Methods

Mice (ddy) were used in the present study. For the experiment of nerve regeneration, the sciatic nerve was transected, and the proximal and distal stumps were kept so as to be in contact with each other. The animals were allowed to survive for 8 weeks after nerve transection. For the experiment of long-term denervation, the sciatic nerve was transected, removing a segment more than 1 cm long from the distal stump so that the regenerating axons would not re-enter it. The animals were killed 16 weeks after operation. For the observation of developing nerves, the cutaneous nerve bundles in the facial connective tissue were examined in newborn mice. The sciatic nerve of the normal adult mouse was also observed. Animals were fixed by perfusion with modified Karnovsky's fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Tissues were dissected and stored in the same fixative for 5 h. After osmication in 1% osmium tetroxide solution, the specimens were dehydrated through graded alcohol series, and embedded in Epon 812. Ultrathin sections were cut on an LKB Ultrotome and stained with uranyl acetate and lead citrate. The sections were observed in a JEM-100B electron microscope. The NND in axons and in Schwann cells was measured on 50,000-times magnified electron micrographs with a pair of vernier calipers.

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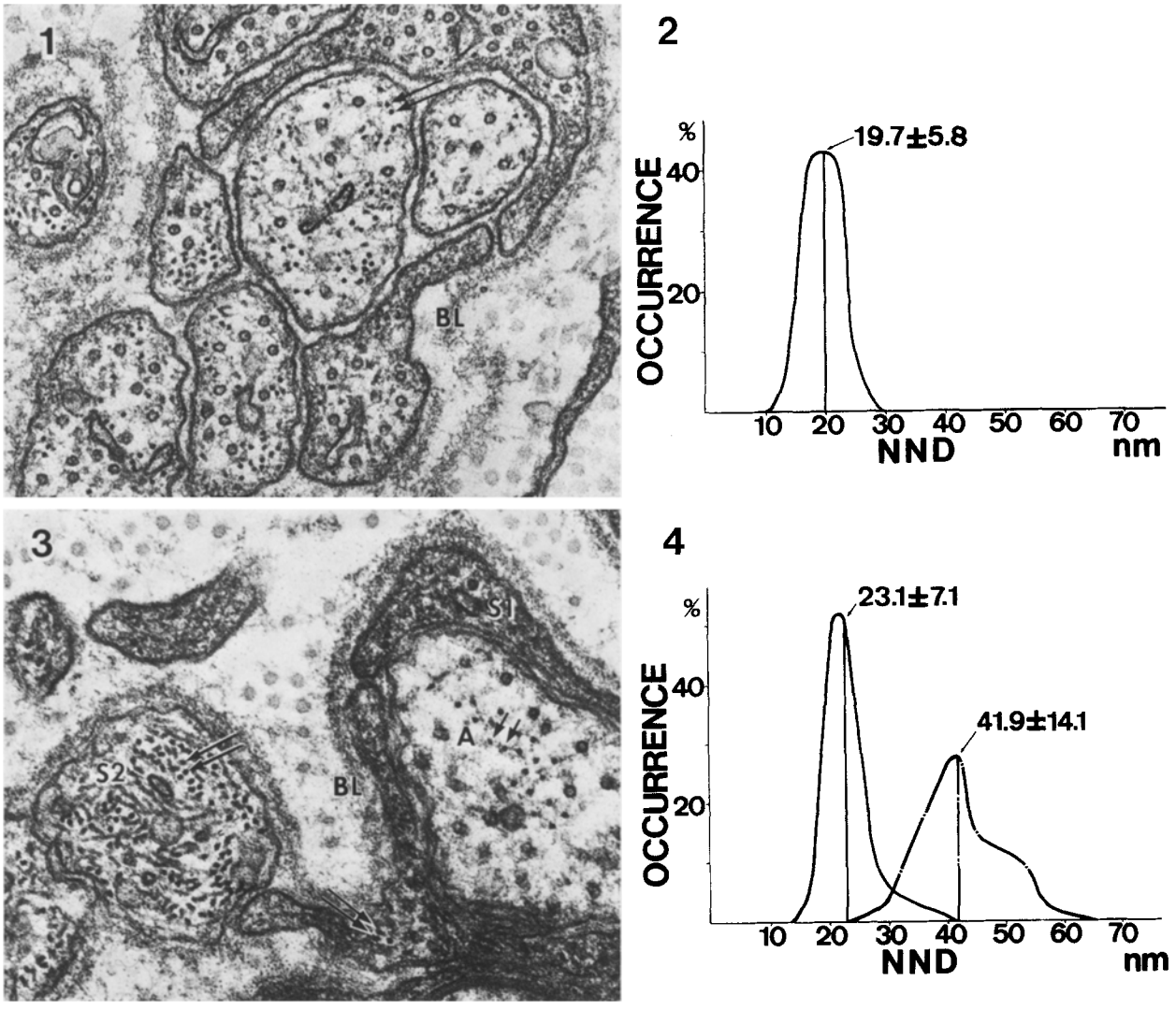


Fig. 1. Büngner's bands (Schwann cell column) in the long-term denervation (16 weeks). Some Schwann cell processes have round contours and relatively clear cytoplasm, containing many longitudinally oriented intermediate filaments (*long arrows*) and microtubules. These Schwann cell processes are quite similar to cross-sectional axons. *BL* basal lamina, × 50,000

Fig. 2. The distribution of NND in Schwann cells in the long-term denervation. The distribution pattern shows normal scatter. Mean value and SD: 19.7 ± 5.8 nm

Fig. 3. Part of the cutaneous nerve through which the axons were allowed to regenerate for 8 weeks. The definite axon (*A*) is surrounded by Schwann cell processes (*S 1*). The neurofilaments in the axon are indicated by *short arrows*. One other Schwann cell process (*S 2*) at the left side is similar in profile to a cross-sectional axon. The intermediate filaments in all of these Schwann cell processes are indicated by *long arrows*. *BL* basal lamina, × 50,000

Fig. 4. The distribution of NND in axons and in Schwann cells obtained from the regeneration materials. A full line indicates the NND in Schwann cells, and a chain line the NND in axons. Mean value and SD: 23.1 ± 7.1 nm in Schwann cells and 41.9 ± 14.1 nm in axons

Results

In the long-term denervation, Büngner's bands (Schwann cell column) were composed of many large and small Schwann cell processes, the cross-section of which varied in profile from round to rectangular (Fig. 1). Some Schwann cell processes extended small finger-like projections (Fig. 1). These Schwann cell processes contained almost exclusively microtubules, intermediate filaments, and occasional smooth endoplasmic reticulum in the relatively electron-lucent cytoplasm. These Schwann cell processes quite resembled in profile the cross-sectional axons as seen in Fig. 1. The feature of such Schwann cell processes was

so close to that of axon that one might often hesitate to identify these as Schwann cell processes. The distribution of NND of every process in Fig. 1 showed the same pattern of normal scatter with the mean value at approximately 20 nm (Fig. 2).

In the regeneration experiment, the regenerating axons were surrounded by Schwann cells (Fig. 3). In this case, the axons were identified as such with confidence as shown in Fig. 3. In addition to these definite axons, there were some cellular processes which resembled the axon in profile (Fig. 3, S2). The distribution of NND in the Schwann cells surrounding axons showed the normal scatter with the mean value at approximately 23 nm (Fig. 4). This pattern of NND distribution in Schwann cells was also the same in the processes marked as "S 2" in Fig. 3, indicating that this process belonged to the Schwann cell. In contrast, the NND in the axon (Fig. 3, A) showed the different pattern of distribution with the mean value at approximately 42 nm (Fig. 4). Thus, the NND in regenerating axons was much larger than that in Schwann cells or Schwann cell processes. The pattern of distribution and the mean value of NND in Schwann cells or Schwann cell processes in Fig. 4 were almost the same as those in the cell processes of the long-term denervation in Fig. 2. Therefore, it can be said that the NND in the Schwann cell processes of the long-term denervation was much smaller than that in the regenerating axon.

The distribution of NND was also examined in the normal myelinated and nonmyelinated nerves (Figs. 5–8). The NND in axons and Schwann cells of these nerves showed the normal scatter distribution. The mean of NND in the myelinated and nonmyelinated axons was approximately 40 nm and 35 nm, respectively (Figs. 6, 8). In myelinated axons, the NNDs in nodal, paranodal, and internodal segments were almost in the same range of distribution. The mean of NND in Schwann cells of myelinated and nonmyelinated nerves was approximately 22 nm and 20 nm, respectively. Thus, in normal peripheral nerves, the NND in axons was also much larger than that in Schwann cells.

In the cutaneous developing nerves examined, almost all the axons were still nonmyelinated at the stage examined. They contained many microtubules, intermediate filaments, and occasional mitochondria in the electron-lucent cytoplasm. Schwann cells surrounded these axons. They had relatively dark cytoplasm and contained a few microtubules and clusters of intermediate filaments (Fig. 9). The distribution of NND in axons and Schwann cells showed almost normal scatter (Fig. 10). The NND was also larger in axons (the mean, 29.0 ± 10.0 nm) than in Schwann cells (the mean, 20.0 ± 11.0 nm).

Thus, the NND in axons was much larger than in Schwann cells or Schwann cell processes in normal, denervated, reinnervated, and developing nerves. This difference was significant ($P < 0.01$, $n = 50$) as examined by Student's t-test.

Accordingly, the NND can be used for the distinction between axons and Schwann cell processes when the latter is difficult to distinguish from the former by mere ultrastructural characteristics. This distinction is especially useful when one must identify regenerating axons running through the Schwann cell column of long-term denervation.

Discussion

Wuerker and Kirkpatrick (1972) reported the regular arrangement of neurofilaments with a constant interfilamentous distance of about 30 nm. The NND has been measured to be about 42–43 nm in axons of cultured spinal ganglion cells (Yamada et al. 1971) and in motor neurons innervating to the musculus lumbricalis of rats (Weiss and Mayr 1971). These values of

Fig. 5. The myelinated axon in the sciatic nerve of adult mouse. The Schwann cell cytoplasm contained some intermediate filaments (*long arrows*), while the axon has numerous neurofilaments (*short arrows*). *BL* basal lamina, × 50,000

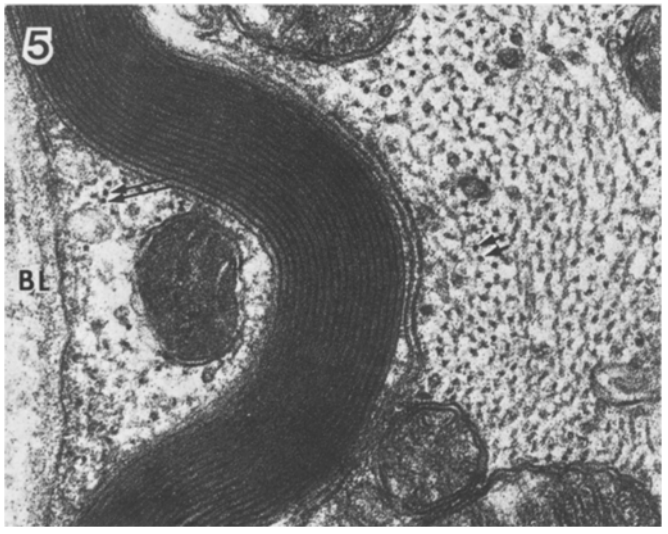
Fig. 6. The distribution of NND in axons (*a chain line*) and in Schwann cells (*a full line*) obtained from myelinated nerves of adult mice. Mean value and SD: 22.1 ± 11.9 nm in Schwann cells and 39.7 ± 10.7 nm in axons

Fig. 7. Nonmyelinated nerves in the sciatic nerve of adult mice. Schwann cell processes surrounding axons contain a small number of intermediate filaments (*long arrows*). One axon contains numerous neurofilaments (*short arrows*). *BL* basal lamina, × 50,000

Fig. 8. The distribution of NND in axons (*a chain line*) and in Schwann cells (*a full line*) obtained from nonmyelinated nerves of adult mice. Mean value and SD: 20.0 ± 5.7 nm in Schwann cells and 35.5 ± 13.0 nm in axons

Fig. 9. Part of a cutaneous nerve of newborn mice. Schwann cell processes surrounding axons contain a few clusters of intermediate filaments (*long arrows*), while one axon possesses neurofilaments (*short arrows*) grouped in the central region. *BL* basal lamina, × 50,000

Fig. 10. The distribution of NND in axons (*a chain line*) and in Schwann cells obtained from cutaneous nerves of newborn mice. Mean value and SD: 20.0 ± 11.0 nm in Schwann cells and 29.0 ± 10.0 nm in axons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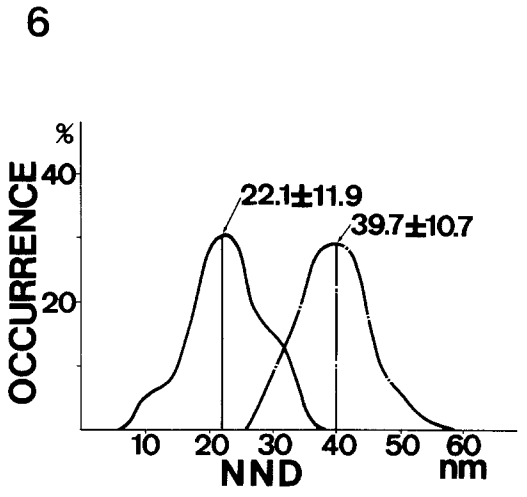
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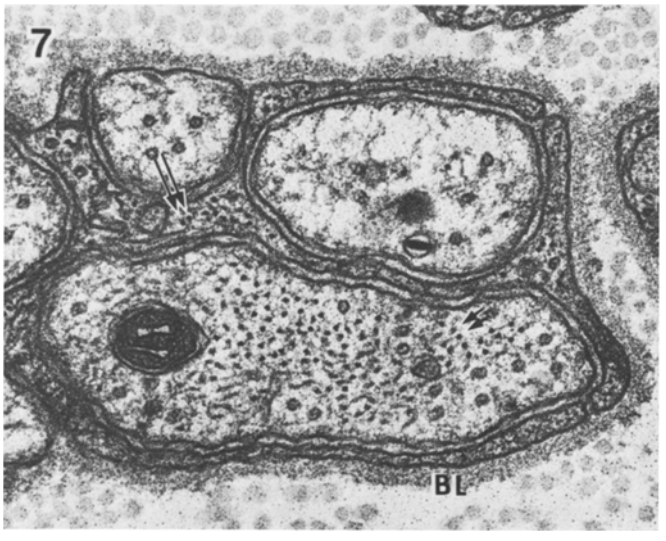
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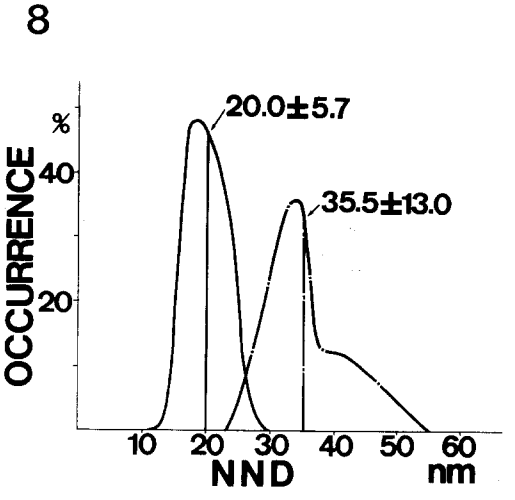
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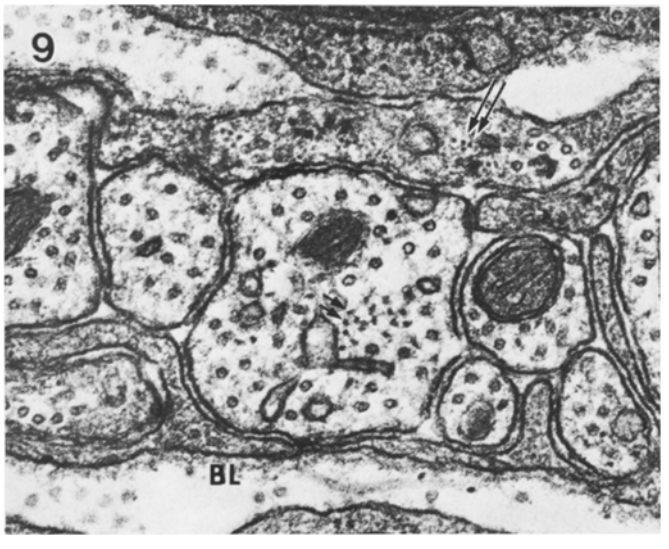
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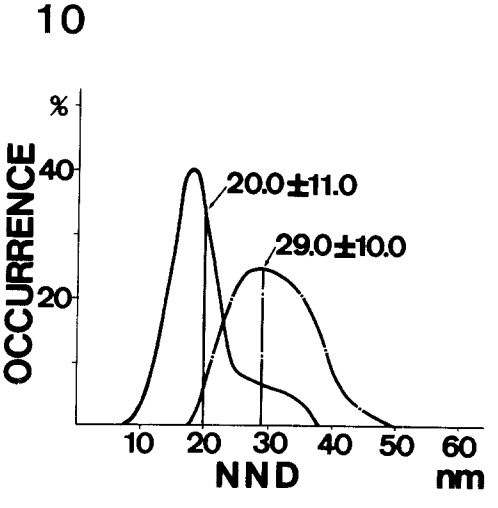
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NND are consistent with those in normal, developing, and regenerating axons obtained in the present study. The study of NND in Schwann cells has not been reported so far. Autilio-Gambetti et al. (1982) have studied immunologically on the constituents of intermediate filaments in the Schwann cells of rabbit sciatic nerves. However, they have made no reference to the NND in Schwann cells.

It has been demonstrated in the present study that NND is significantly different in Schwann cells and in axons whether they are obtained from the normal, developing, denervated, or reinnervated nerves. From these results, it is possible that the axons can be distinguished in terms of NND from axon-like Schwann cell processes as occasionally encountered in the denervated and reinnervated nerves. For example, one might suspect that some aberrant axons had regenerated into the Schwann cell column. This conjecture cannot be simply denied due to the observation that some cell processes in Fig. 1 are very close to axons in profile. However, as shown in Fig. 2, the NND of these cell processes is the same as that of the Schwann cells of other cases, indicating that all these cell processes belong to Schwann cells. If regenerating axons enter these Schwann cell columns, the NND would be useful in distinguishing such axons from the Schwann cell processes.

Some of the upper values of NND in Schwann cells overlap the lower ones of NND in axons (Figs. 4, 6, 8, 10). This fact indicates that one must be careful in the interpretation of NND. One must measure many NNDs to obtain the correct conclusion.

It has been reported recently that the intermediate filaments in Schwann cells are biochemically different from those (neurofilaments) in axons (Shelanski and Liem 1979; Yen and Fields 1981). Such biochemical difference of intermediate filaments might have some relationship to the difference of NND in Schwann cells and in axons.

There is a relatively large fluctuation (from about 30 to 40 nm) in the mean value of NND in axons from different cases. This fluctuation might be ascribed to the minor technical differences in the tissue preparation. However, it is probable that there were some changes in the constituents of neurofilament in each case. No explanation has been offered as to this problem.

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