

The ultrastructure of the lateral spinal nucleus

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SUMMARY

The lateral spinal nucleus (LSN) is located in the dorsal region of the lateral funiculus in the rodent spinal cord. Four to six medium-sized neurons were found in the cross section of the LSN. The neurons were surrounded by the fibers of the lateral funiculus. Small-diameter myelinated fibers and numerous unmyelinated fibers were seen. The unmyelinated fibers were 0.15 to 0.20 μm in diameter; their diameters did not change, and they did not have synapsing en passant widening. Axon terminals in the dorsolateral funiculus (DLF) with various types of synaptic vesicles gathered around the neuronal perikarya as well as followed the straight dendrites and established axosomatic and axodendritic synapses. The most common type of axonal varicosities was the small-sized boutons – 1.0 to 2.0 μm in diameter – loosely filled with round, regular synaptic vesicles (Type I boutons). Dense core vesicles were regularly present in the varicosities. Type I boutons were found alone, but more often in groups. A characteristic synaptic arrangement was seen, in which Type I varicosities completely surrounded a thinner dendrite (rosette-shaped synaptic complexes). Type I boutons made both symmetrical and asymmetrical synaptic contacts. In the rosette-

shaped synaptic complexes, the synapses were invariably of the asymmetrical type.

Axon varicosities densely filled with ovoid synaptic vesicles (Type II boutons) were rarely seen. Axon varicosities of different diameters containing neurosecretory vesicles 130-140 nm in diameter were also found (Type III boutons). No synaptic specializations were found in connections with Type III varicosities. Substance-P labeled axon terminals outlined the dendrites in longitudinally cut sections. At ultrastructural level the SP positive boutons could be classified as Type I varicosities. The dendrites of the LSN neurons frequently coursed towards the pial surface of the spinal cord. Dendrites were found directly below the basal lamina or within the range of the most superficial 1 μm -thick layer of the DLF. Superficially located dendrites often received synapsing axon terminals. Numerous LSN neurons could be labeled with antiserum detecting Type 1a metabotropic glutamate receptors. The labeled dendrites followed a straight course, approached the pial surface, and received numerous synapsing axon terminals. Some of the neurons could be labeled with NK-1 receptor antiserum.

Key words: Lateral spinal nucleus – Synaptology – NK-1 receptor – mGluR – Substance P

INTRODUCTION

The lateral spinal nucleus (LSN) is a special group of neurons in the spinal cord.

(i) It exists only in the rodent spinal cord (Gwyn and Waldron, 1968).

(ii) It is a group of scattered, uniform neurons in the dorsal portion of the lateral funiculus; i.e., outside the spinal grey matter (Gwyn and Waldron, 1968; Réthelyi, 2003a).

(iii) The neurons in the LSN are mainly tract neurons. Their axons course ipsi- or contralaterally and terminate in several brain stem- and diencephalic nuclei. The neurons in the LSN project to the nucleus of the solitary tract (Menetrey and Basbaum, 1987; Esteves et al., 1993; Guan et al., 1998), to the medullary reticular formation (Pechura and Liu, 1986; Nahin, 1987), to the internal lateral parabrachial- (Ding et al., 1995; Feil and Herbert, 1995) and Kölliker-Fuse nuclei (Feil and Herbert, 1995), to the periaqueductal gray (PAG; Pechura and Liu, 1986; Keay et al., 1997; Li et al., 1998), to the thalamus (Battaglia and Rustioni, 1992; Marshall et al., 1996), to the hypothalamus (Burstein et al., 1987, 1990; Li et al., 1997), to the septum (Li et al., 1997), and to the amygdala (Burstein and Potrebic, 1993). Neurons occur with joint projections to the PAG and to the thalamus (Harmann et al., 1988), as well as with simultaneous ascending and descending spinal projections (Verburch and Kuypers, 1987).

(iv) The dendrites of the neurons in the LSN are preferentially oriented in the transverse plane, and they seem to reach the pial surface of the dorsal funiculus (Bresnahan et al., 1984; Réthelyi, 2003a).

(v) A large array of peptides has been detected in the neurons and also in the axon varicosities in the nucleus.

Thus, there are neurons with peptides: vasoactive intestinal peptide (VIP), bombesin, substance P (SP), dynorphin (with axons forming the spinomesencephalic, spinoreticular and spinosolitary tracts – Nahin, 1987; Leah et al., 1988); SP (spinothalamic projection – Battaglia and Rustioni, 1992); calcitonin gene-related peptide (CGRP; Conrath et al., 1989); orphanin FQ/nociceptin (Riedl et al., 1996); neuropeptide FF (Aarnisalo and Panula, 1998); VIP and PHI (Fuji et al., 1985; Sasek et al., 1991).

Axonal arborizations with peptides: SP, dynorphin, met-enkephalin, somatostatin, FMRF-amide (non-peripheral origin, survived

rhizotomy – Cliffer et al., 1988); VIP and PHI (Fuji et al., 1985; Sasek et al., 1991); dopamin beta hydroxylase, serotonin (5-HT), choline acetyltransferase (ChAT), glycin transporter-2, galanin, NPY, neurotensin (NT), SP, somatostatin, enkephalin, glutamic acid decarboxylase (GAD), vesicular glutamate transporter (VGLUT2; Olave and Maxwell, 2004).

Receptor molecules: SP-receptor (neuronal – Olave and Maxwell, 2004; projection to PAG – Li et al., 1998, to the nucleus of the solitary tract – Menetrey and Basbaum, 1987; Esteves et al., 1993; Ding et al., 1995, to the thalamus – Li et al., 1996; Marshall et al., 1996, to the hypothalamus – Li et al., 1997, to the septum – Li et al., 1997, to the parabrachial nucleus – Ding et al., 1995); dopamine D2 receptor (van-Dijken et al., 1996); interferon-gamma receptor (presynaptic; Robertson et al., 1997, Vikman et al., 1998), α_{2c} -adrenergic receptors (Stone et al., 1998; Olave and Maxwell, 2002).

(vi) Cutaneous primary afferent fibers do not terminate in the LSN, although sensory fiber terminations supplying muscles or viscera cannot be excluded. (Giesler et al., 1979; Menetrey et al., 1980).

Neurons issuing ascending axons seem to be activated directly by local spinal interneurons and indirectly by descending impulses (Cliffer et al., 1988; Masson et al., 1991).

In a previous publication, the neuronal structure of the LSN was shown as revealed by Golgi-impregnated specimens prepared in both the transverse and longitudinal planes (Réthelyi, 2003a). Preliminary data about the structure of the white matter surrounding the LSN neurons and about the dendritic trajectory were also reported. Here, a systematic ultrastructural analysis of the LSN is described, using chemical labeling of axons and dendrites.

The results have been presented in abstract form (Réthelyi, 2003b).

MATERIALS AND METHODS

All animal procedures were carried out with the permission of the National Ethical Council supervising animal experiments and according to the Guidelines of Animal Experiments of the Semmelweis University.

Adult Wistar rats of both sexes were used (240–260 g b.w.). The animals were deeply anesthetized with ketamine (Calypsol) com-

bined with xylazine (Primazin; 2:1) and perfused intracardially with a solution containing 4% paraformaldehyde, 1% glutaraldehyde and saturated picric acid, pH 7.4. The fixation was preceded by a quick rinse with saline solution. The spinal cord was carefully dissected and kept in a glutaraldehyde-free perfusion solution for 24 hours at 4°C. The spinal cord was then cut into segments, and 60 µm thick Vibratome sections were prepared in the transverse and sagittal planes from various cervical, thoracic or lumbar segments.

Selected Vibratome sections were processed for preembedding immunohistochemistry. Free-floating sections were kept in a 15% saccharose solution for 1 hour. Saccharose treatment was repeated overnight in a 30% solution followed by repeated freeze-thawing of the sections in liquid nitrogen to enhance the penetration of the reagents. Sections were washed in 0.1 M phosphate buffer (PB; 3x10 min) and treated with 1% sodium borohydride (NaBH₄) for 30 min. After extensive washes in PB, the sections were treated with a 1% H₂O₂ solution to block endogenous peroxidase reaction. This was followed by washing the sections in 0.1 M PB (30 min, RT).

Nonspecific protein binding was blocked by incubation in solution of 10% normal goat serum (NGS; Vector Laboratories, Burlingame, USA) diluted in M PB (2 hours, RT). Some of the sections were incubated in rabbit anti-NK-1 receptor (1:100000, gift from Dr. Z. Puskár) and rabbit anti-substance P (1:60000, gift from Dr. P. Petrusz) primary antibodies for 3 days at 4°C. Some other sections were blocked with 5% bovine serum albumin (BSA, Sigma, St. Louis, USA) in PB for 2 hours (RT) and incubated in mouse monoclonal anti-metabotropic glutamate receptor-1a (mGluR1a; 1:5000, Pannonia Research Park Ltd. Pécs, Hungary) for 3 days at 4°C. The specificity of the mGluR1a serum was described by Kiss et al. (1996). After incubation, the sections were washed in PB (3x10 min) and incubated either with biotinylated anti-rabbit IgG raised in goat or in biotinylated anti-mouse IgG raised in goat (Vector Laboratories, Burlingame, USA) diluted 1:400 in PB overnight at 4°C.

On the following day the sections were washed three times in 0.1 M PB and incubated with the avidin-biotin complex diluted 1:400 in PB (Vectastain ABC Elite kit, Vector Laboratories, Burlingame, USA) for 120 minutes at RT. After washings (3x10 min) in PB

and further in 0.05 M Tris/HCl buffer (TB) pH 7.6 (2x5 min), the tissue-bound peroxidase was visualized with a solution of 0.025-0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, USA.) containing 0.005-0.01% H₂O₂ in TB. The sections were rinsed in TB (2x10 min) and in PB (3x10 min), mounted onto gelatin-coated slides, dehydrated, and coverslipped using DePeX.

Selected Vibratome sections were osmicated in 1% osmium vapor for 30 min, dehydrated, and flat-embedded in Durcupan. One-µm thick plastic sections were cut with a Reichert Ultramicrotome, mounted, and stained with 1% toluidine blue solution. Series of ultrathin sections were prepared with the same ultramicrotome, mounted on single-slot copper grids, contrasted with saturated uranyl acetate and lead citrate, and observed with a JEOL 1200 EMX electron microscope.

RESULTS

Perikarya and dendrites in the LSN

The overall structure of the triangular LSN is apparent in toluidine blue-stained plastic sections cut in the transverse plane (Fig. 1). The straight dorsolateral border of the nucleus is formed by the free surface of the lateral funiculus covered by the pia mater. Also, the convex dorsomedial border of the nucleus is sharp and distinct; it is formed by the convex

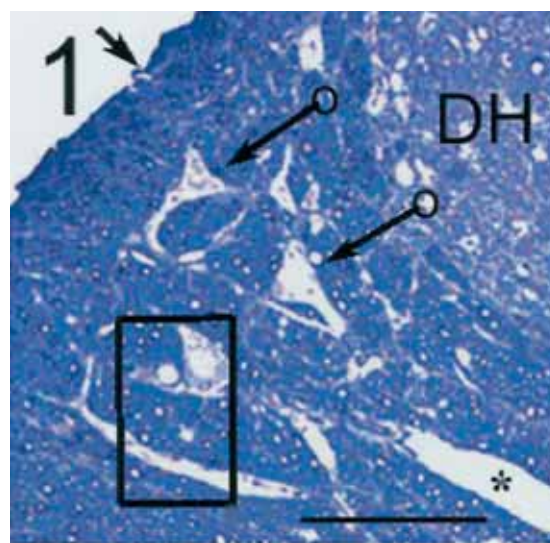


Fig. 1. Toluidine blue-stained plastic section showing the neuronal and fiber composition of the LSN in the cross section of the spinal cord. Arrow points to the free surface of the dorsolateral funiculus. Ringed arrows point to neuronal perikarya. Asterisk indicates a longitudinally cut capillary vessel. The low power electron microscopic structure of the framed rectangular area can be seen in Fig. 5. DH = dorsal horn. Scale bar: 100 µm.

border of the dorsal horn (lamina I). The ventral border of the nucleus is vague in plastic sections, but it is relatively well seen in specimens showing the dendritic arborization of the LSN neurons (Fig. 2).

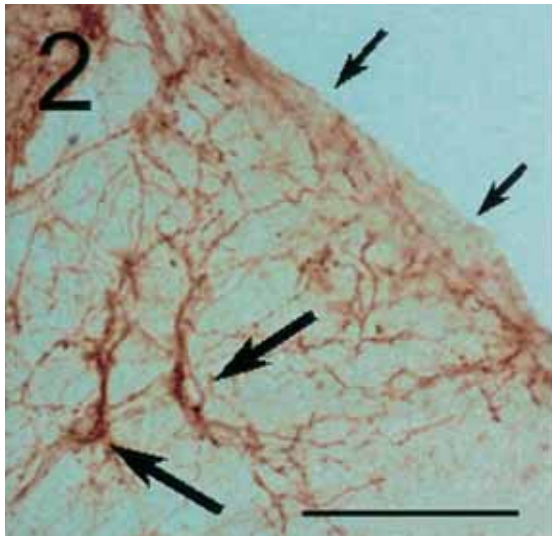


Fig. 2. Vibratome section showing the LSN in the cross section of the spinal cord. Small arrows point to the free surface of the dorsolateral funiculus. Large arrows point to neuronal perikarya immunostained with NK-1 receptor antiserum. Immunostained dendrites from both perikarya arborize in the transverse plane, and the final branches are directed toward the pial surface. Scale bar: 100 μ m.

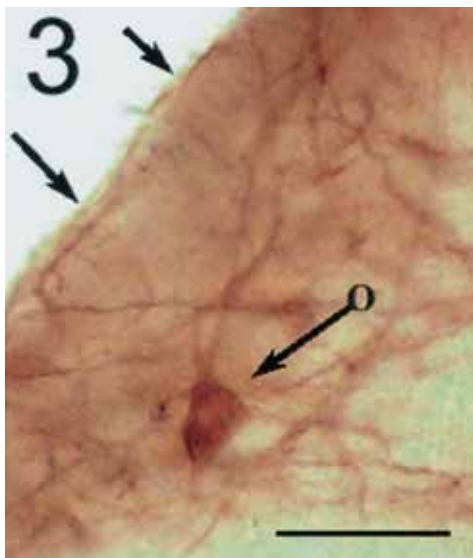


Fig. 3. Vibratome section showing the LSN in a cross section of the spinal cord. Arrows point to the free surface of the dorsolateral funiculus. Ringed arrow points to neuronal perikaryon immunostained with mGluR-receptor antiserum. Long stretches of immunostained dendrites can be followed in the nucleus. Some of them approach the pial surface. Scale bar: 50 μ m.

The components of the LSN as seen in toluidine blue-stained plastic sections are: neuronal perikarya with the proximal trunks of the dendrites, dendrites oriented into the

transverse plane, and fine myelinated axons in cross section (up to 2.5 – 3.0 μ m in diameter). Four to six 14 – 28 μ m large triangular or ovoid perikarya can be seen in the cross section of the LSN in the lumbar segments of the rat spinal cord. Dendrites leaving the perikarya at the tips of the triangular or at the poles of the ovoid perikarya usually leave the plane of the thin plastic sections after a short initial course. Thin-walled capillary vessels often cut through the nucleus in the transverse direction from the surface of the spinal cord towards the grey matter.

Perikarya and the dendritic arborization of neurons in the LSN could be visualized in cross sections of the spinal cord with immunohistochemical reactions using antisera raised against NK-1 receptor (Fig. 2) and metabotropic glutamate receptor-1a (mGluR1; Fig. 3). In both cases, the dendrites originate from the triangular or spindle-shaped perikarya. They follow a straight course and are directed toward the pial surface of the spinal cord. The ventralmost dendrites outlined the ventral border of the LSN. The overwhelming transverse dendritic trajectory was revealed by immunostaining using antisera raised against substance P (SP). The darkly stained SP-positive axon terminals outline the surfaces of the main dendrites of transverse orientation on a longitudinally cut specimen (Fig. 4).

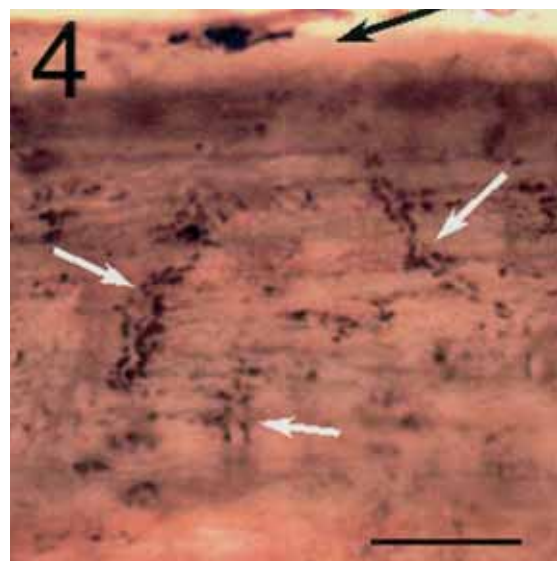


Fig. 4. Vibratome section showing the LSN in longitudinal section of the spinal cord. Black arrow points to the free surface of the dorsolateral funiculus. White arrows point to dendrites in transverse orientation densely outlined with SP-immunostained boutons. Scale bar: 25 μ m.

In low-power electronmicrographs it is possible to visualize perikarya, up to 10 μm thick dendrites cut in various planes, glial cell bodies and a large array of myelinated fibers down to 0.6 μm in diameter (Fig. 5). Clusters of synapsing axon terminals of 1.0 to 2.0 μm in diameter were found on the surface of the perikarya (Fig. 6). Loosely distributed, round synaptic and occasionally dense-core vesicles filled the axon terminals. Less frequently more than a dozen axon terminals were seen aggregating on the surface of a perikaryon (Fig. 7).

Medium-power pictures of dendrites revealed three typical synaptic arrangements. Solitary axon terminals synapse with the dendrites (Fig. 8), or clusters of boutons cover large surfaces of the dendrite (Fig. 9). In most synapses, the axons show the typical vesicular content: loosely organized, round synaptic and scattered dense-core vesicles. The most frequently seen synaptic arrangement is shown in Fig. 10, where 4 to 5 boutons in synapsing position hermetically surround a fine dendritic process.

Dendrites with or without synapsing axon terminals were often found below the pial surface of the lateral funiculus (Figs. 11, 12). The free surface of the lateral funiculus was covered with fine, often interdigitating astrocyte processes covered by a uniform basal lamina and collagen microfibrils. In rare cases, dendrites and axon terminals seemed to approach and interrupt the basal lamina (Fig. 11).

Dendrites heavily laden with synaptic terminals were among those that could be labeled with anti-mGluR serum (Figs. 15, 17). The presynaptic axon terminals contained round regular synaptic vesicles and, occasionally, dense-core vesicles. mGluR-labeled dendrites in cross section completely surrounded by synapsing axon terminals were also found (Fig. 16).

Axon varicosities in the LSN

The most common type of axonal varicosities in the LSN was the small-size boutons – 1.0 to 2.0 μm in diameter – loosely filled with round, regular synaptic vesicles (Type I boutons). Dense-core vesicles regularly occurred in the varicosities. One bouton may form multiple synaptic thickenings with the postsynaptic perikaryon or dendrite (Fig. 8). Type I boutons were found alone, but more often in groups (Fig. 7). A characteristic synaptic arrangement regularly was seen in which Type

I varicosities completely surrounded a thinner dendrite (rosette-shaped synaptic complexes; Figs. 10, 16).

Type I boutons made both symmetrical and asymmetrical synaptic contacts. In the rosette-shaped synaptic complexes the synapses were invariably of the asymmetrical type (Figs. 10, 16). Synapsing Type I varicosities occasionally approached the pial surface of the LF (Fig. 14).

Axon varicosities densely filled with ovoid synaptic vesicles (Type II) were rarely seen. They intermingled with Type I boutons in synaptic aggregates (Fig. 7), but were also found in the most superficial position on the surface of the nucleus (Fig. 13).

Axon varicosities of various diameters containing neurosecretory vesicles of 130–140 nm in diameter were also found in the LSN (Type III; Fig. 18). No synaptic specializations could be found in connections with the Type III varicosities.

Substance-P-labeled axon terminals outlined the dendrites in longitudinally cut sections (Fig. 4). At ultrastructural level, the SP-positive boutons could be classified as Type I varicosities. They contained round synaptic vesicles, dense core vesicles and formed asymmetrical synapses with dendrites (Fig. 19). SP-positive boutons were also found immediately under the free surface of the LF (Fig. 20).

Special neuropil

Characteristic elements of the neuropil are the widespread fields of extremely fine unmyelinated fibers oriented along the axis of the spinal cord (Fig. 21). The diameter of the fibers varies between 0.07 and 0.25 μm . In longitudinal sections, the unmyelinated fibers showed straight, regular contours. No synapsing varicose swellings were seen (Fig. 22). Dense-core vesicles were often found in the unmyelinated fibers. Also, numerous fine unmyelinated fibers showed SP positivity (Fig. 23).

DISCUSSION

Since the neurons of the LSN are distributed in the white matter (DLF), the synapsing components should congregate around the perikarya and along the dendrites. The course of the dendrites will determine the trajectory of the nucleus.

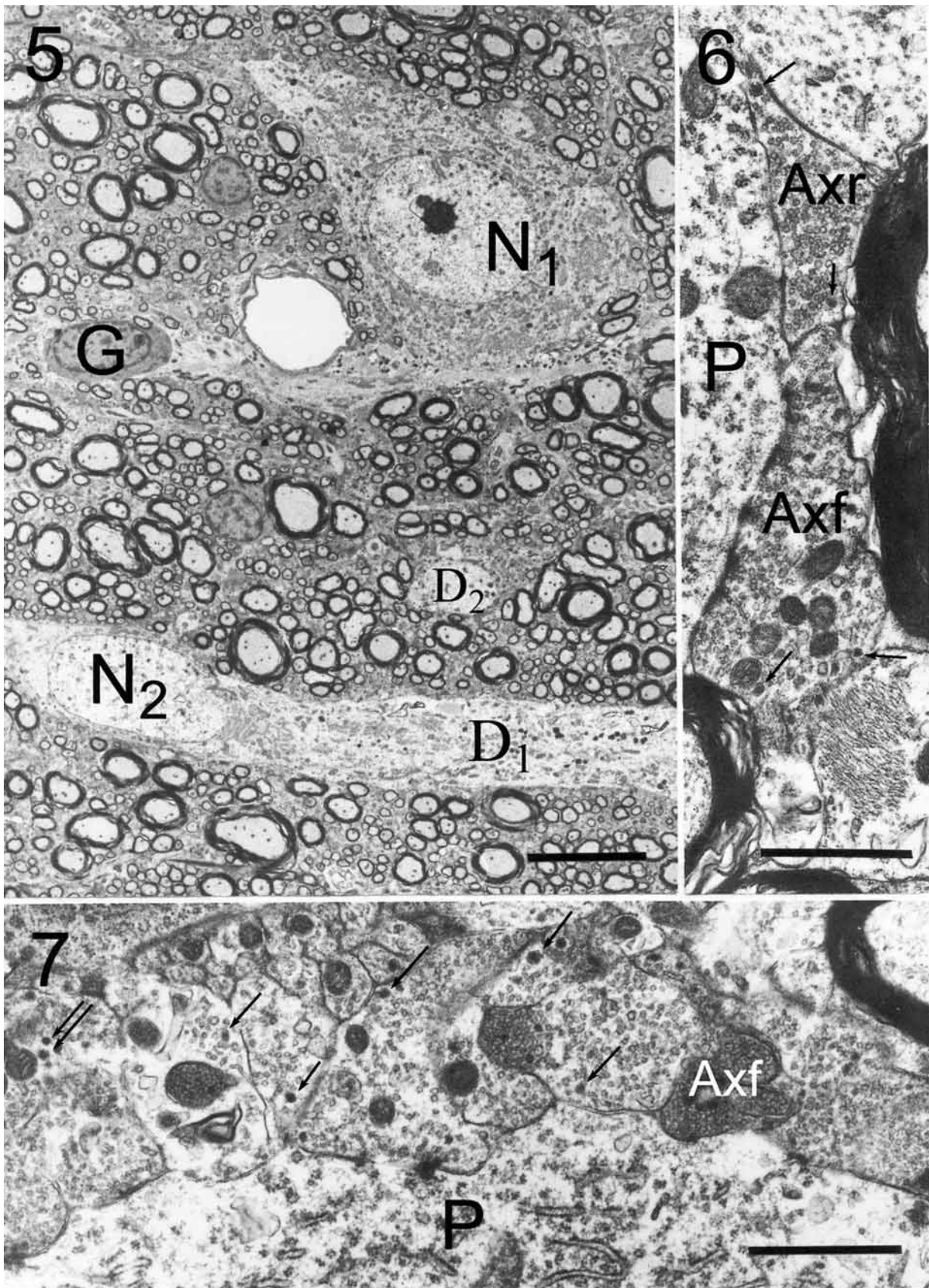


Fig. 5. Low-power electron micrograph showing characteristic elements in the LSN in cross section (same area as seen framed in Fig. 1). The triangular perikaryon with round nucleus (N_1) and nucleolus is seen in the upper half of the figure. An ovoid nucleus indicating the perikaryon of a smaller neuron (N_2) and a relatively thick straight dendrite (D_1) are shown in the lower half of the figure. Another large dendrite can be seen in cross section (D_2). Myelinated fibers of different diameters in cross section indicate that the LSN is located in the white matter (lateral funiculus). G = glial cell. Scale bar: 10 μm .

Fig. 6. Electron micrograph showing two axon enlargements establishing axosomatic synapses. One of the axon enlargements contains round (Axf); the other, flattened synaptic vesicles (Axf). Dense-core vesicles occur in both enlargements (arrows). P = perikaryon. Scale bar: 1 μm .

Fig. 7. Electron micrograph showing axon enlargements around a perikaryon (P). The vast majority of the enlargements contain round synaptic vesicles, whereas few of them are densely filled with flattened synaptic vesicles (Axf). Dense-core vesicles are labeled with arrows. Scale bar: 1 μm .

A former Golgi study indicated that the dendrites of the LSN neurons course longitudinally or are directed medially towards the grey matter and laterally towards the pial surface (Réthelyi, 2003a). The immunolabeled neurons in the present study – NK-1 receptor and mGluR – completed the dendritic architecture of the nucleus. Numerous spoke-like dendrites could be seen in the transverse plane, with frequent bifurcations. The dendrites covered a regular triangular area bordered by the dorsal horn and the pial surface. The third side of the triangle is a horizontal line cutting through the lateral funiculus (LF). The ventralmost dendrites of the stained neurons indicated the indistinct ventral border of the LSN. Dendrites with varicosities that were often found in the Golgi-impregnated specimens could not be seen in the immunostained preparations or in the electron micrographs.

Dendrites were often found immediately under the basal lamina indicating the pial surface of the DLF. Similar observation was made based on light microscopic analysis of the Golgi-impregnated neurons and was reported in a preliminary way earlier (Réthelyi, 2003a). It was also common in the present study to see dendrites with relatively large diameters approaching the pial surface. They sometimes, but not always, had synapsing axon terminals in that peripheral position. A final glial cell layer of a fraction of 1.0 μm usually separated the dendrites from the basal lamina. Occasionally, the glial layer discontinued, and the dendrite almost stuck out of the spinal cord (Figs. 11, 13). mGluR labeled dendrites were also found in subpial position. Further experiments are needed to verify the hypothesis that drugs in the CSF may have a direct action on the dendrites of the LSN (Réthelyi, 2003a; Olave and Maxwell, 2003).

Numerous NK-1 immunostained neurons in the LSN were projection neurons and some of them showed the c-fos reaction upon thermal stimulation (Olave and Maxwell, 2003). The same authors found that NK-1 labeled dendrites could be followed over 100–130 μm in the transverse section, similarly to the dendritic trajectories described in the present study. mGluR-labeled neurons were found in the LSN with extensive dendritic arborizations using mGluR1a and mGluR5 immunosera. Both SP- and VGLUT2-immunoreactive nerve fibers arborize densely in the LSN (Olave and Maxwell, 2004). A straightforward synaptic connection between the appropriate

fibers and the neurons expressing the appropriate receptor is highly probable, and forthcoming investigations should prove their existence and significance.

Axon enlargements occurred in single boutons or in groups of closely attached terminals covering large surfaces of the perikarya and dendrites. The majority of the enlargements contained round synaptic vesicles, numerous dense-core vesicles, and many of them formed asymmetrical synaptic contact. Our observations confirm those of Olave and Maxwell (2004) concerning the high proportion of presumably excitatory synapses in the LSN. After a systematic analysis we were unable to find perikarya or dendrites completely surrounded by axon terminals, as suggested by Bresnahan et al. (1984). Dendrites with only scattered axon terminals were often seen (Fig. 8).

A complex axo-dendritic synaptic arrangement, called the rosette-type of synapse, was repeatedly found. The central element of the rosette is a relatively thin dendrite. Since the dendrites in Golgi material (Réthelyi, 2003a) as well as mGluR-labeled dendrites appeared with a smooth surface, it is unlikely that the central dendrite in the rosette is a dendritic spine or protrusion. It is more probable that 3 to 5 axon terminals with round synaptic vesicles surround the dendrite at its peripheral portion. The synchronized firing of the terminals may cause a spectacular postsynaptic effect.

SP-immunolabeled terminals densely decorate the straight dendrites of the LSN neurons (Fig. 4). This regular distribution of immunolabeled varicosities was confirmed in the ultrastructural analysis. It was common to find SP-labeled synapsing varicosities side by side along a dendrite (Fig. 19).

Axon terminals with flattened synaptic vesicles were rarely seen. They were partly interspersed within groups of axon terminals with round synaptic vesicles, and were partly found alone. Large-sized axon enlargements with flattened synaptic vesicles immediately under the pial surface (Fig. 5) were uncommon.

Occasionally, axon terminals with neurosecretory vesicles with diameter of 130–140 nm were found. Swanson and McKellar (1979) described the axons of the paraventriculospinal tract containing oxytocin and neurophysin and coursing in the DLF in the rat. Apparently, the fibers only travel in that part of the DLF where the neurons of the LSN are

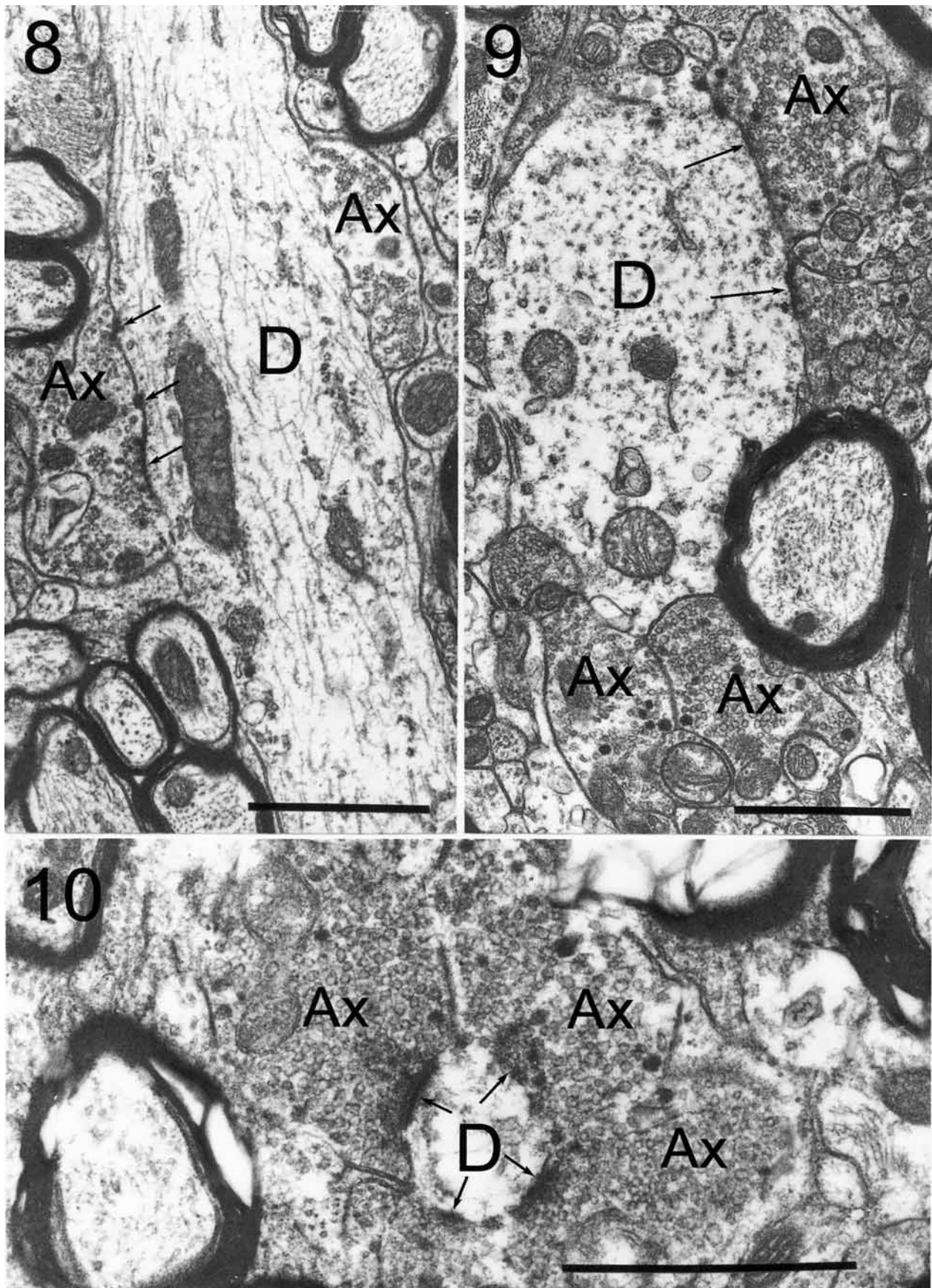


Fig. 8. Electron micrograph showing a dendrite (D) in longitudinal sections. Solitary axon terminals (Ax) are attached to the dendrite. One of them establishes multiple synaptic connections with the dendrite (arrows). Scale bar: 1 μ m.

Fig. 9. Electron micrograph showing a dendrite (D) in cross sections. It is surrounded by several axon terminals (Ax) filled with round synaptic vesicles and dense-core vesicles. Axodendritic synaptic connections are marked by arrows. Scale bar: 1 μ m.

Fig. 10. Electron micrograph showing a small dendrite (D) in cross sections. It is completely surrounded by axon terminals (Ax) containing round synaptic and dense-core vesicles (rosette-type of synaptic arrangement). Asymmetrical axodendritic synapses are labeled by arrows. Scale bar: 1 μ m.

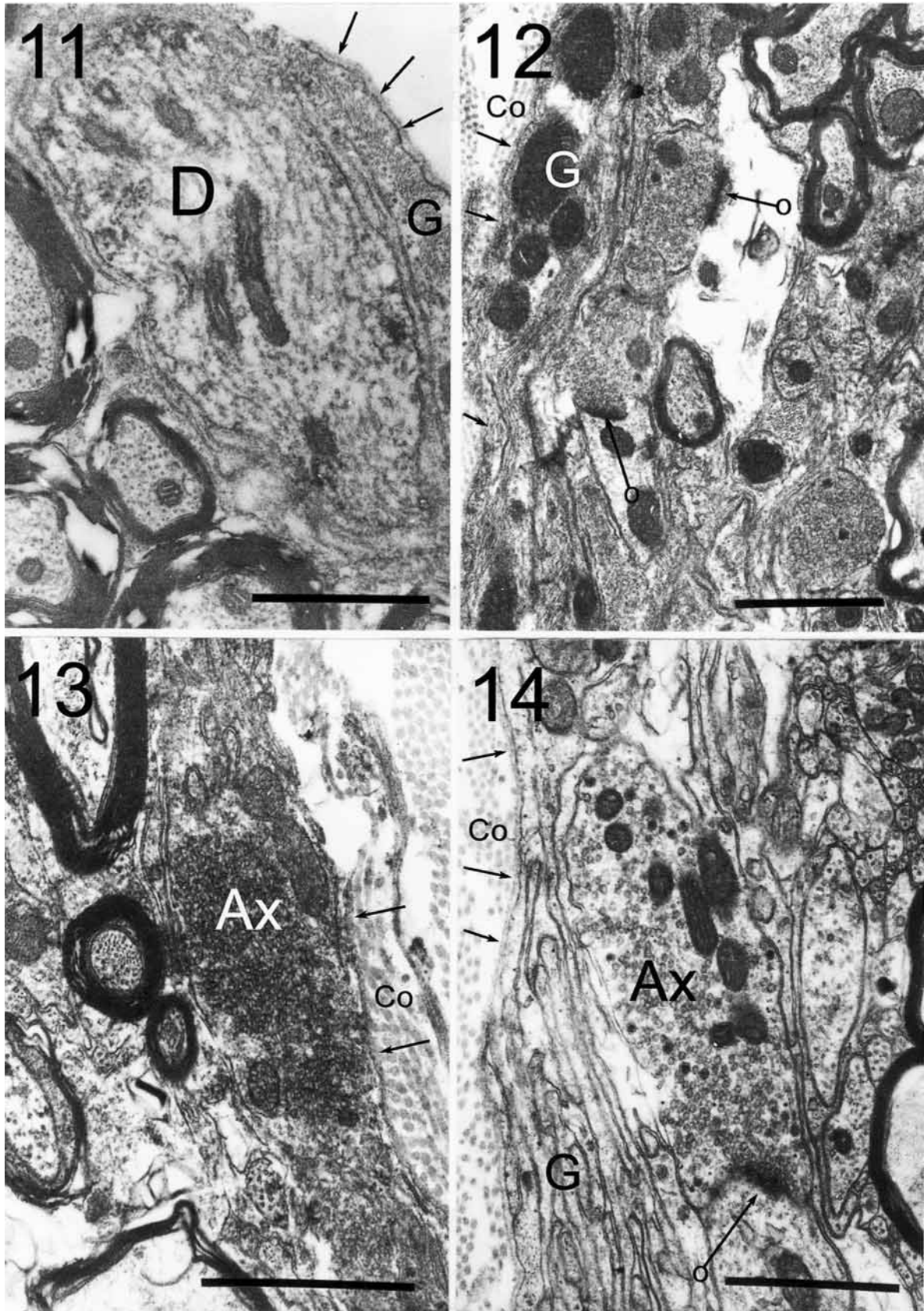


Fig. 11. Electron micrograph showing a thick dendrite (D) immediately below the surface of the spinal cord. Arrows point to the basal lamina that covers the superficial processes of a glial cell (G). Scale bar: 1 μ m.

Fig. 12. Electron micrograph showing asymmetrical axodendritic synapses (ringed arrows) immediately below the surface of the spinal cord enveloped by glial cell processes (G), basal lamina (arrows) and collagen microfibrils (Co) of the pia mater. Scale bar: 1 μ m.

Fig. 13. Electron micrograph showing an axon terminal (Ax) coursing on the surface of the spinal cord. It is densely filled with flattened (ovoid) synaptic vesicles. Arrows label the basal lamina. Co = collagen microfibrils of the pia mater. Scale bar: 1 μ m.

Fig. 14. Electron micrograph showing a synapsing axon terminal (Ax) approaching the surface of the spinal cord. Ringed arrow marks the axodendritic synaptic connection, arrows indicate the basal lamina. G = fine processes of glial cells. Co = collagen microfibrils of the pia mater. Scale bar: 1 μ m.

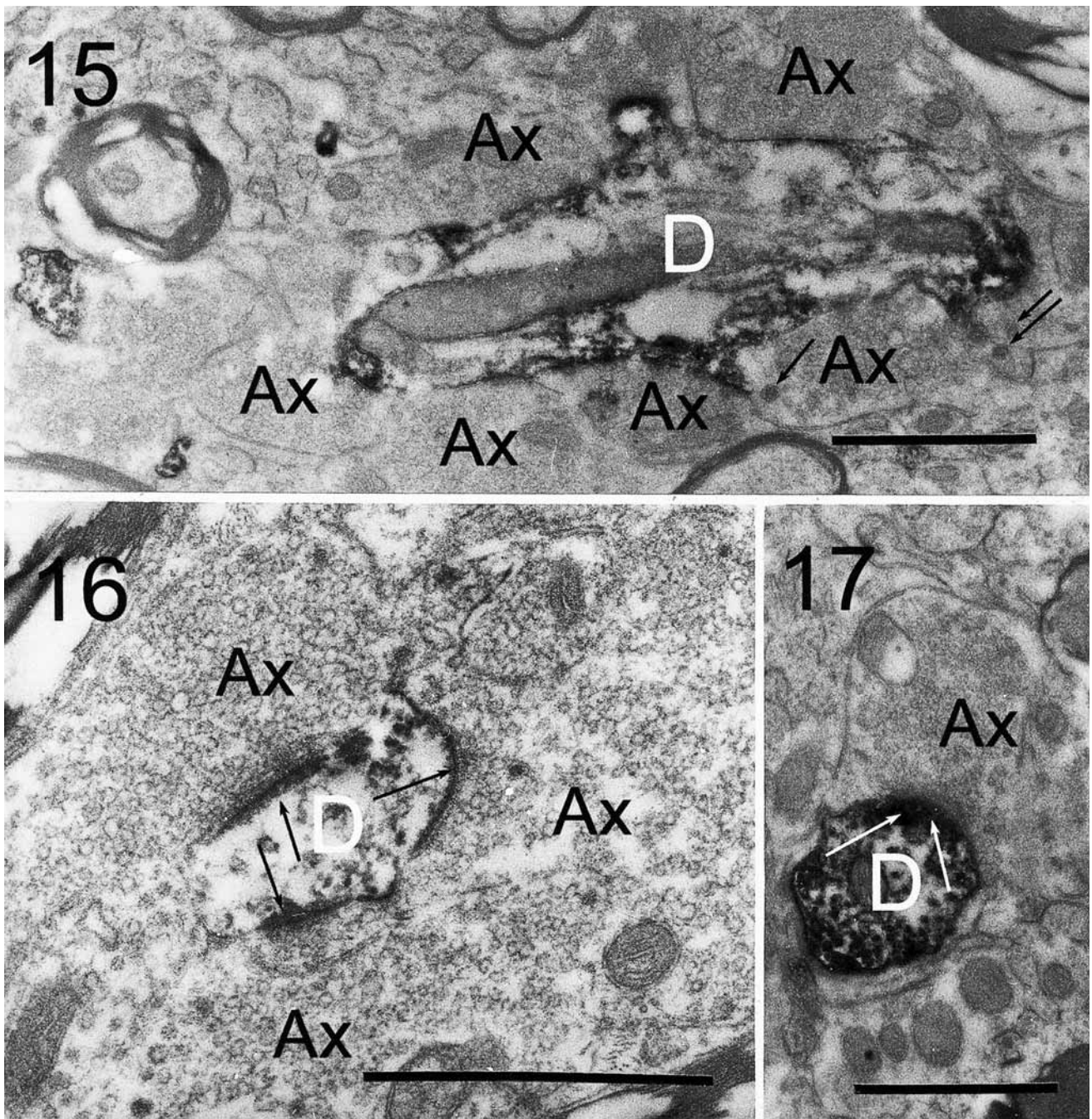


Fig. 15. Electron micrograph showing a longitudinally cut, mGluR labeled dendrite (D). It is surrounded by axon terminals (Ax) containing round synaptic vesicles and dense-core vesicles (arrows). Uncontrasted section. Scale bar: 1 μ m.

Fig. 16. Electron micrograph showing a mGluR-labeled small dendrite (D) in cross section. It is completely surrounded by axon terminals (Ax) filled with synaptic vesicles and dense-core vesicles (rosette-type of synaptic arrangement). Asymmetrical axodendritic synapses are labeled with arrows. Scale bar: 1 μ m.

Fig. 17. Electron micrograph showing an axodendritic synapse in which the postsynaptic dendrite (D) shows the mGluR immunoreaction. Arrows point to the synaptic specialization. Ax = axon terminal. Uncontrasted section. Scale bar: 1 μ m.

located on the way to their final destination in the various nuclei of the spinal grey matter. The en-passant character of the axon enlargements with neurosecretory vesicles is supported by the absence of synaptic specializations. Target-directed immunohistochemical observations should clarify the origin and position of the neurosecretory vesicles containing axon enlargements in the LSN.

No axo-axonic synapse and no glomerular synaptic arrangements were found in the LSN. This negative finding reaffirms the notion that the neuronal organization of the LSN and that in the superficial dorsal horn is different.

A large collection of extremely fine unmyelinated fibers coursing longitudinally in the DLF was reported by Chung and Coggeshall (1983). Identical fine caliber fibers

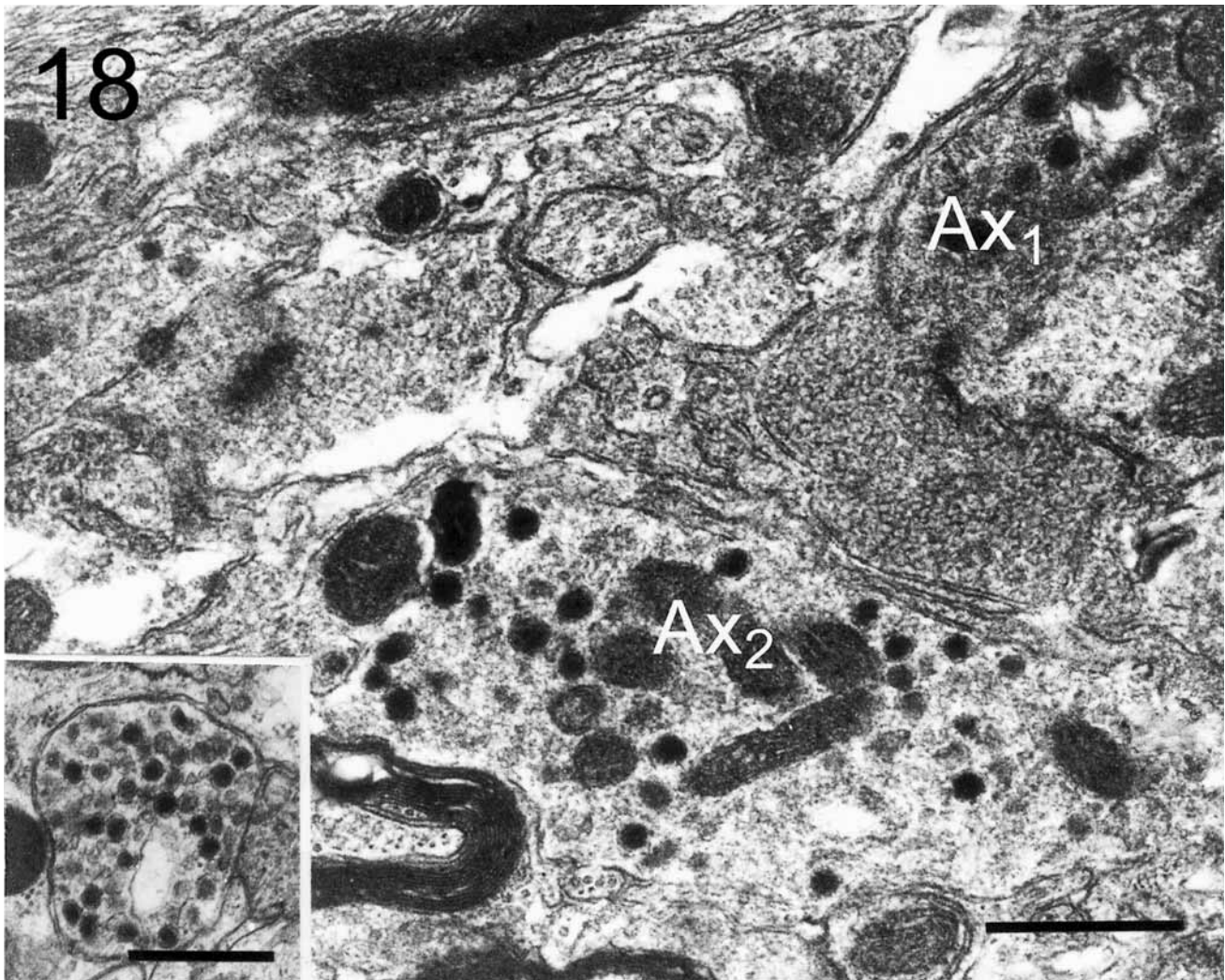


Fig. 18. Electron micrograph showing boutons (Ax_1 and Ax_2) of different diameters containing neurosecretory vesicles. Insert: Medium-sized bouton densely filled with neurosecretory vesicles. Scale bar: 0.5 μ m.

were seen in Golgi specimens over-crossing the transverse dendrites of the LSN neurons (see Figs. 16-17 in Réthelyi, 2003a) and crossing-over type of synaptic connections were suggested between the fine axons and the dendrites. The systematic ultrastructural analysis was unable to verify the above suggestion: no synaptic connections were seen between the fine axons and the dendrites of the LSN neurons. Thus, the fine, unmyelinated fibers are elements of the DLF and not those of the LSN. For comparison, the ventral funiculus contains hardly any unmyelinated fibers and the diameter of the myelinated fibers is greater than those in the DLF (unpublished observation). Thus, in the rat the DLF consists of two intertwined components: the LSN and the fiber tracts of the spinal white matter.

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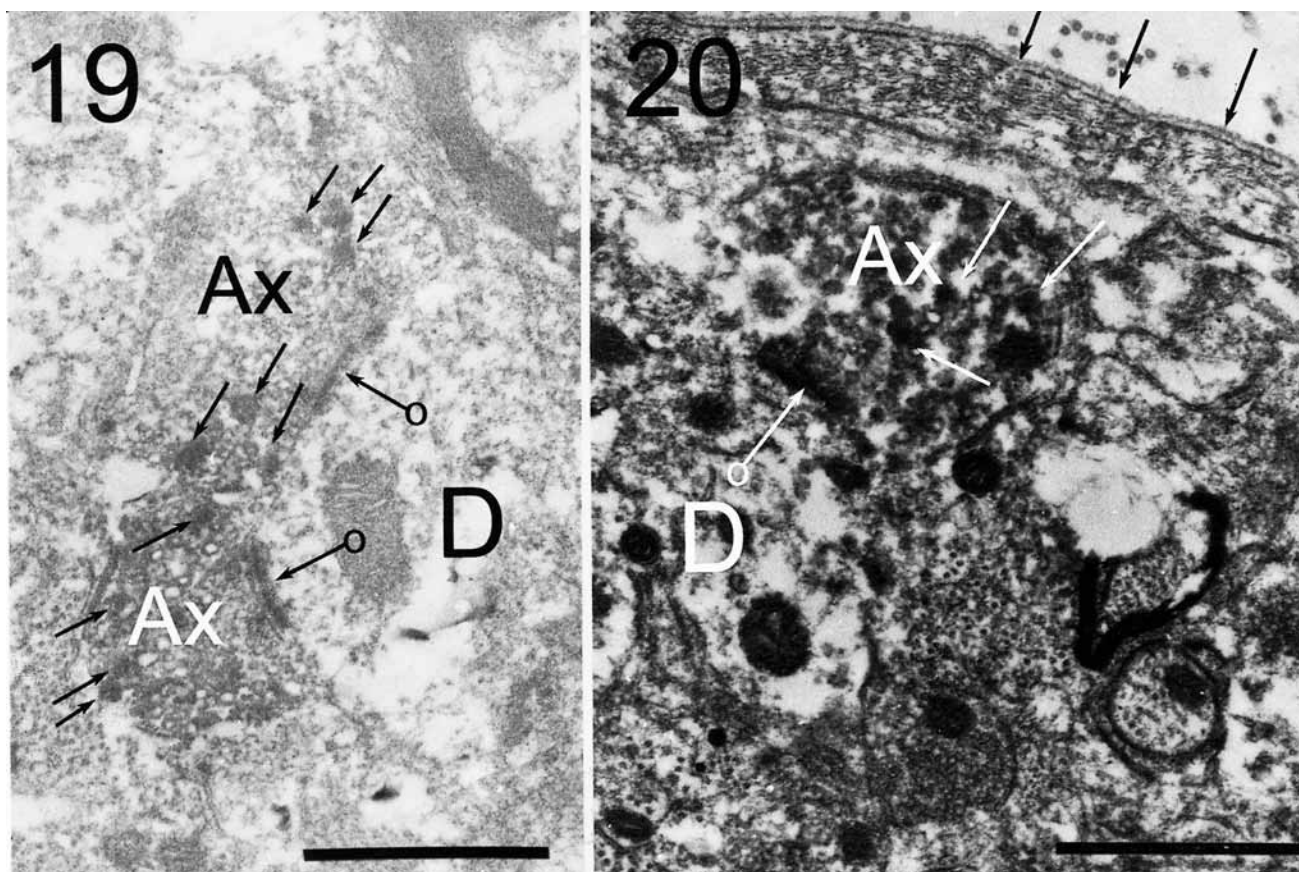


Fig. 19. Electron micrograph showing two SP labeled boutons (Ax) establishing asymmetrical synapses with a large dendrite (D). Both boutons contain round synaptic vesicles and dense-core vesicles (arrows). Ringed arrows indicate asymmetrical synaptic connections. Uncontrasted section. Scale bar: 1 μ m.

Fig. 20. Electron micrograph showing SP-labeled axon enlargement (Ax) in the vicinity of the free surface of the lateral funiculus. Black arrows indicate the basal lamina on the surface of the astrocyte processes; white arrows indicate dense-core vesicles. D = postsynaptic dendrite. Ringed arrow points to the asymmetrical synaptic specialization. Scale bar: 1 μ m.

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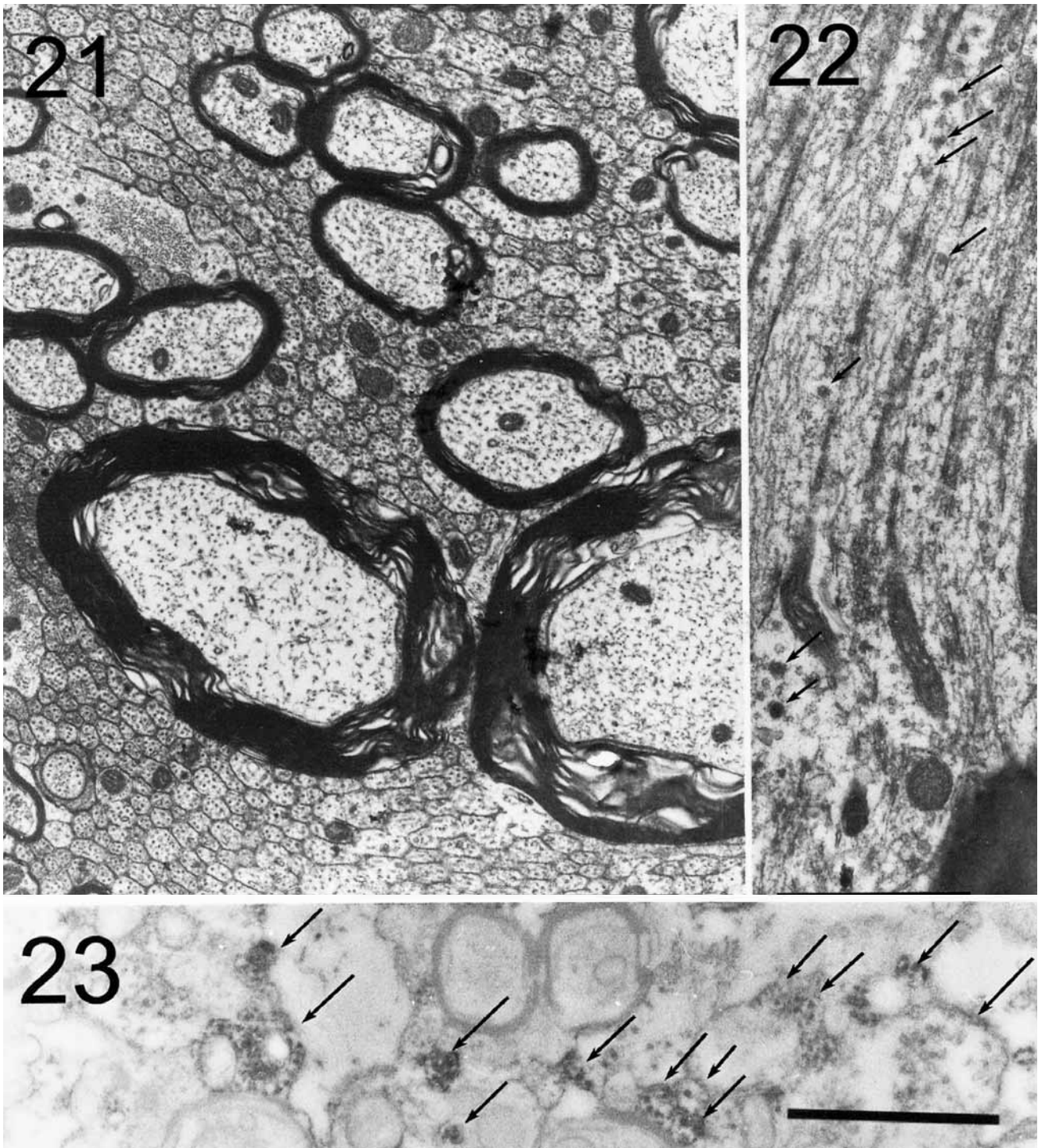


Fig. 21. Electron micrograph showing the fiber components of the LSN. Myelinated fibers of different diameters are surrounded by fields of extremely fine unmyelinated fibers. Scale bar: 1 μ m.

Fig. 22. Electron micrograph showing the bundles of unmyelinated fibers of the LSN in longitudinal section. Unlike the parallel fibers in the cerebellar cortex, these fibers do not possess varicose, synapsing en-passant enlargements. Dense-core vesicles occur frequently in the fibers (arrows). Scale bar: 1 μ m.

Fig. 23. Electron micrograph showing that several of the fine unmyelinated fibers are labeled with SP immunoserum (arrows) in the cross section of the LSN. Uncontrasted section. Scale bar: 1 μ m.

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