Neurons of the lateral spinal nucleus in the rat spinal cord – A Golgi study

Miklós Réthelyi

Department of Anatomy, Histology and Embryology, Semmelweis University, 1094 Budapest, Hungary

SUMMARY

The neurons of the lateral spinal nucleus in the spinal cord of young adult rats were studied in transverse and longitudinal planes using the Golgi-Kopsch method and electron microscope. The perikarya were mainly polygonal or spindle shaped, and measured 20 to 35 μm in the longest diameter. They formed a dense column in the dorsolateral funiculus underneath the pial surface. The dendrites followed three patterns. Several of them turned laterally and approached the surface of the spinal cord. Another group of dendrites ran longitudinally within the column of the perikarya. A third group of dendrites turned medially or ventromedially and coursed towards the reticulated portion of the gray matter. Medium-sized neurons located at the margin of this latter portion of the spinal cord sent some of their straight dendrites into the dorsolateral funiculus. Thus, the dendrites of these two populations of neurons appeared as rungs of a ladder in longitudinally-cut spinal cord specimens. Only the initial portions of the axons of the LSN neurons could be impregnated. They originated with a regular axon hillock from either the perikaryon or from one of the primary dendrites and became unimpregnated after a 20 to 40 μm long course, indicating their myelinated character. Preliminary ultrastructural observations revealed that the laterally directed dendrites of the neurons in the lateral spinal nucleus approached the free surface of the spinal cord and ended immediately underneath the pia mater. Large numbers of fine, unmyelinated fibers were found in the dorsolateral funiculus coursing perpendicular to the laterally and medially oriented dendrites.

Key words: Lateral spinal nucleus – Spinal cord – Rat – Golgi technique

INTRODUCTION

Acetylcholinesterase-positive neurons have been found outside the gray matter, in the dorsolateral funiculus (DLF) along the spinal cord of the rat (Gwyn and Waldron, 1968). The oval, triangular or multipolar neurons are arranged in a loosely organized nucleus (nucleus of the dorsolateral funiculus or lateral spinal nucleus – LSN).

The size of the perikarya of the neurons varies between 15 to 35 μm (Nahin, 1987), although others have reported smaller perikarya (Gwyn and Waldron, 1968). The dendrites of the neurons are directed either laterally towards the pial surface (Bresnahan et al., 1984) or in the opposite direction, i.e. medially, towards the reticulated portion of the gray matter (Menetrey et al., 1982) and only rarely along the long axis of the spinal cord (Gwyn and Waldron, 1968).

The neurons contain at least four types of peptides: VIP, substance P, dynorphin and bombesin (Nahin, 1987; Leah et al., 1988). The VIP containing neurons were multipolar (25-35 μm in diameter) with long dendrites. By contrast, substance P containing neurons were small cells (15-25 μm), while large and small-sized neurons occurred among the dynorphin-positive neurons (15-35 μm).

Submitted: October 24, 2002
Accepted: March 7, 2003

Correspondence to:
Dr. Miklós Réthelyi. Department of Anatomy, Histology and Embryology, Semmelweis University, Tüzoltó utca 58, 1094 Budapest, Hungary.
Phone: 36 1 215 6598; Fax: 36 1 215 5158. E-mail: rethelyi@ana.sote.hu

Experiments using retrograde axoplasmic transport have indicated that the majority if not all of the LSN neurons are tract neurons with ascending axons, because they can be labeled retrogradely from the nucleus accumbens and septum (Burstein and Giesler, 1989), from the thalamus (Leah et al., 1988; Burstein et al., 1990; Verburgh et al., 1990; Li et al., 1996; Marshall et al., 1996), from the hypothalamus (Burstein et al., 1990), from the mesencephalon (Menetrey et al., 1982; Pechura and Liu, 1986; Leah et al., 1988), from the parabrachial nucleus (Ding et al., 1995), and from the nucleus of the solitary tract as well as from the medullary reticular formation (Leah et al., 1988). In addition, LSN neurons in the upper cervical segments can be labeled from the upper thoracic segments (Verburgh et al., 1990). Conversely, anterogradely labeled axons of the LSN neurons located in cervical segments have been detected around the neurons in the intermediolateral nucleus in the middle thoracic segments (Jansen and Loewy, 1997). Some of the LSN neurons could be double labeled from two injection sites in the brain stem and diencephalon, as well as in the brain stem and spinal cord.

Recent studies have revealed that LSN neurons contain the NK-1 receptor (Brown et al., 1995; Ding et al., 1995; Littlewwod et al., 1995; Li et al., 1996; Marshall et al., 1996; Guan et al., 1998; Li et al., 1998) and metabotropic glutamate receptors (Alvarez et al., 2000). The dendrites and perikarya of the LSN neurons are surrounded by nerve fibers containing met-enkephalin, dynorphin 1-8, substance P, somatostatin, FMRF and neuropeptide FF (Ljungdahl et al., 1978; Barber et al., 1979; Hunt et al., 1980; Gibson et al., 1981; Giesler and Elde, 1985; Cliffer et al., 1988; Allard et al., 1991; Li et al., 1997; Aarnisalo and Panula, 1998). Enkephalin and substance P positive boutons synapse with the dendrites and perikarya of the LSN neurons (Bresnahan et al., 1984). In spite of the rich substance P-innervation, whose density decreases after dorsal rhizotomy (Knyihár-Csillik et al., 1990), there is general agreement that primary sensory neurons do not terminate in the LSN (Bresnahan et al., 1984).

Several series of Golgi impregnation of adult rat spinal cord have revealed the distribution of neurons in the LSN and their dendritic trajectory in both transverse and longitudinal sections. Here, the original portion of the axon and a plexus of fine axons in the DLF coursing perpendicular to the dendrites are demonstrated. The results of preliminary ultrastructral observations are presented to complete the light microscopic analysis.

Materials and methods

The spinal cord of young adult rats (Wistar strain, 120-150 g b.w.) was impregnated according to the Golgi-Kopsch procedure (Réthelyi, 1985). The animals were perfused with aldehyde solution containing 4% paraformaldehyde and 0.5% glutaraldehyde in phosphate buffer (0.1 M) at pH 7.4 in deep anesthesia. Pieces of the spinal cord were cut either in the transverse or in the longitudinal plane and kept in 3.6% potassium dichromate solution for 4 days. After repeated rinses in 0.75% silver nitrate solution the spinal cord pieces were kept in the same solution for one day in darkness. Sections of 80 to 120 µm thickness were cut and mounted. Observations were preferentially made on the lower cervical and lumbar segments. Microphotographs were made with a conventional laboratory microscope. High-power drawings were made of individual neurons or groups of them using a drawing tube. The size of the neurons was measured semi automatically with help of a LUCIA (Nikon) image analysis system.

Two adult rats (250 g b.w.) were perfused under deep anesthesia with the aldehyde solution mentioned above. The spinal cord was postfixed in the same solution for 24 hours. Sixty µm thick Vibratome sections were cut from selected cervical, thoracic, lumbar and sacral segments. The sections were osmicated in OsO_4 vapor for 30 min, and flat-embedded in Araldite. Ultrathin sections were prepared with a Reichert ultramicrotome, contrasted with saturated uranyl acetate and lead citrate and observed with Jeol electron microscope.

Results

Shape and location of perikarya and orientation of dendritic trees

The perikarya of the LSN neurons were either spindle-shaped or multipolar, both types of perikarya could be seen in both transverse and longitudinal sections. Superficially located spindle shaped neurons were found parallel with the cord surface (Figs. 1, 3). The primary dendritic trunks emerging from the 20 µm long and 10 µm wide perikarya ran under the pial surface of the cord for a distance of 120 to 140 µm and often gave off short side branches that coursed peripherally towards the pial layer. Other primary dendrites were directed medially or medioventrally (Figs. 4, 6). A small group of partially stained neurons was found about 70 µm from the surface of the spinal cord. The large majority of the dendrites of these neurons ran towards the surface of the spinal cord, resembling the whiskers of a seal (Figs. 2, 5).

In longitudinally-cut spinal cord sections the 20 to 35 µm large multipolar LSN neurons formed a 60 to 70 µm wide column immediately under the cord surface. The dendrites of the neu-

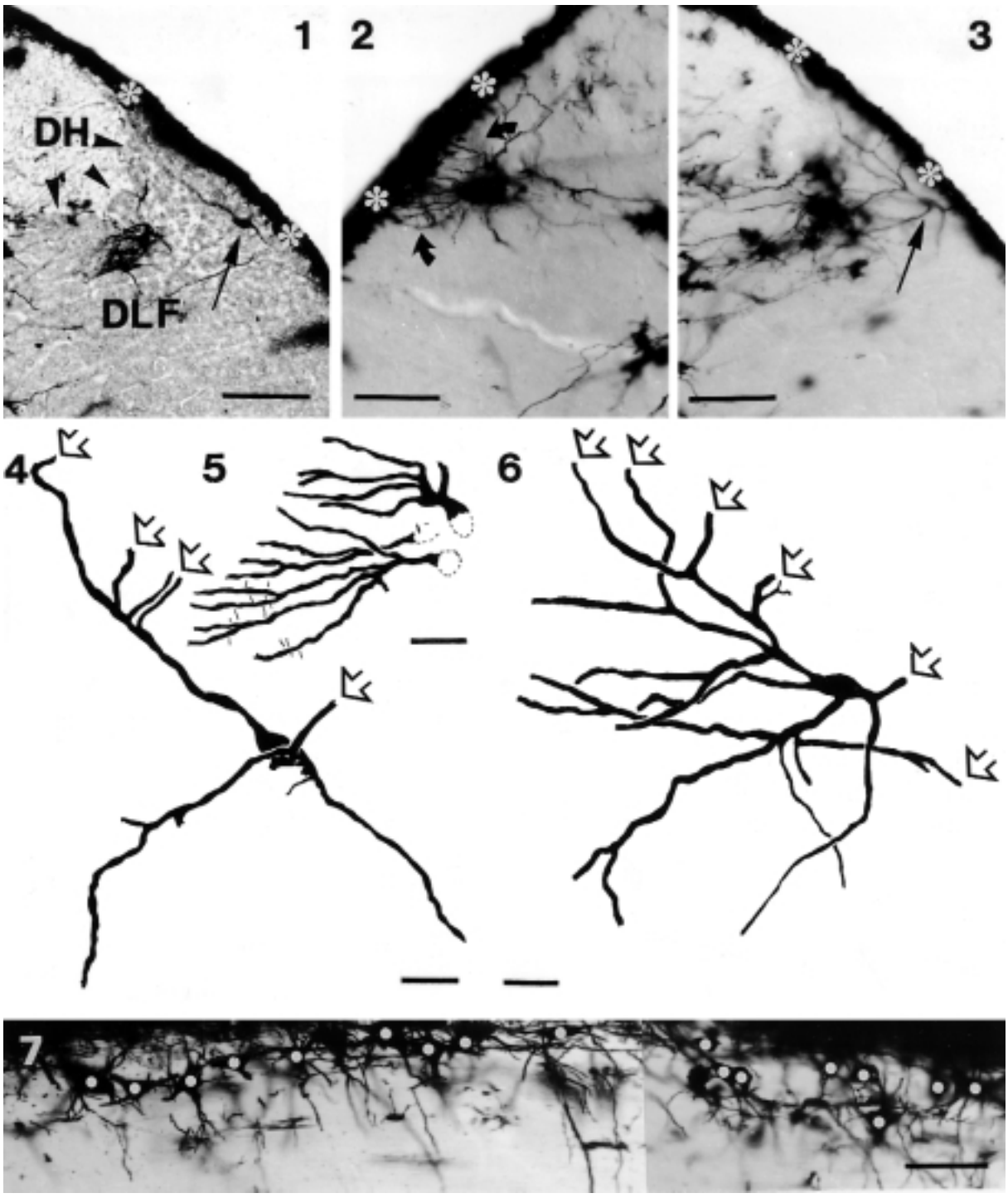

Figure 1.- Microphotograph showing the dorsolateral portion of the lumbar spinal cord in transverse section. The picture was photographed with lowered condensor lens to demonstrate the difference between the gray matter of the dorsal horn (DH) and the white matter of the dorsolateral funiculus (DLF) where the neurons of the LSN are distributed. The contour of the dorsal horn is marked with arrowheads. The arrow points to a spindle-shaped LSN neuron. Asterisks mark the pial surface of the spinal cord. Scale bar: 100 µm. The dendritic distribution of the neuron is shown in Fig. 4.

Figure 2.- Microphotograph showing the dorsolateral portion of the lumbar spinal cord in transverse section. Several dendrites (between curved arrows) of LSN neurons are coursing towards the surface of the spinal cord (marked by asterisks). The parent perikarya of the dendrites are partially blurred by a silver deposit. Scale bar: 100 µm. The camera lucida drawing of the dendrites and perikarya are seen in Fig. 5.

Figure 3.- Microphotograph showing the dorsolateral portion of the lumbar spinal cord in transverse section. The arrow points to a spindle-shaped LSN neuron with extensive dendritic arborization. Asterisks mark the pial surface of the spinal cord. Scale bar: 100 µm. The dendritic arborization of the neuron in shown at higher power in Fig. 6.

Figure 4.- High-power camera lucida drawing of the LSN neuron shown in Fig. 1. Two of the dendritic trunks course parallel with the cord surface. Dendrites directed towards the pial surface of the cord are marked with contour arrows. Scale bar: 25 µm.

Figure 5.- High-power camera lucida drawing of the LSN neurons shown in Fig. 2. The dendrites of probably three LSN neurons (indicated by dotted contours) radiate towards the pial surface of the cord. Fine nerve fibers crossing with the dendrites are also shown. Scale bar: 25 µm.

Figure 6.- High-power camera lucida drawing of the LSN neuron shown in Fig. 3. A large portion of the dendritic arborization is confined to the transverse plane. Dendrites directed towards the pial surface of the cord are marked with contour arrows. Scale bar: 25 µm.

Figure 7.- Photomontage showing the dorsolateral portion of the lumbar spinal cord in longitudinal section. The column of LSN neurons and some of their dendrites can be seen. The pial surface of the spinal cord is at the upper margin of the picture. Neurons are marked with white dots. Scale bar: 100 µm.

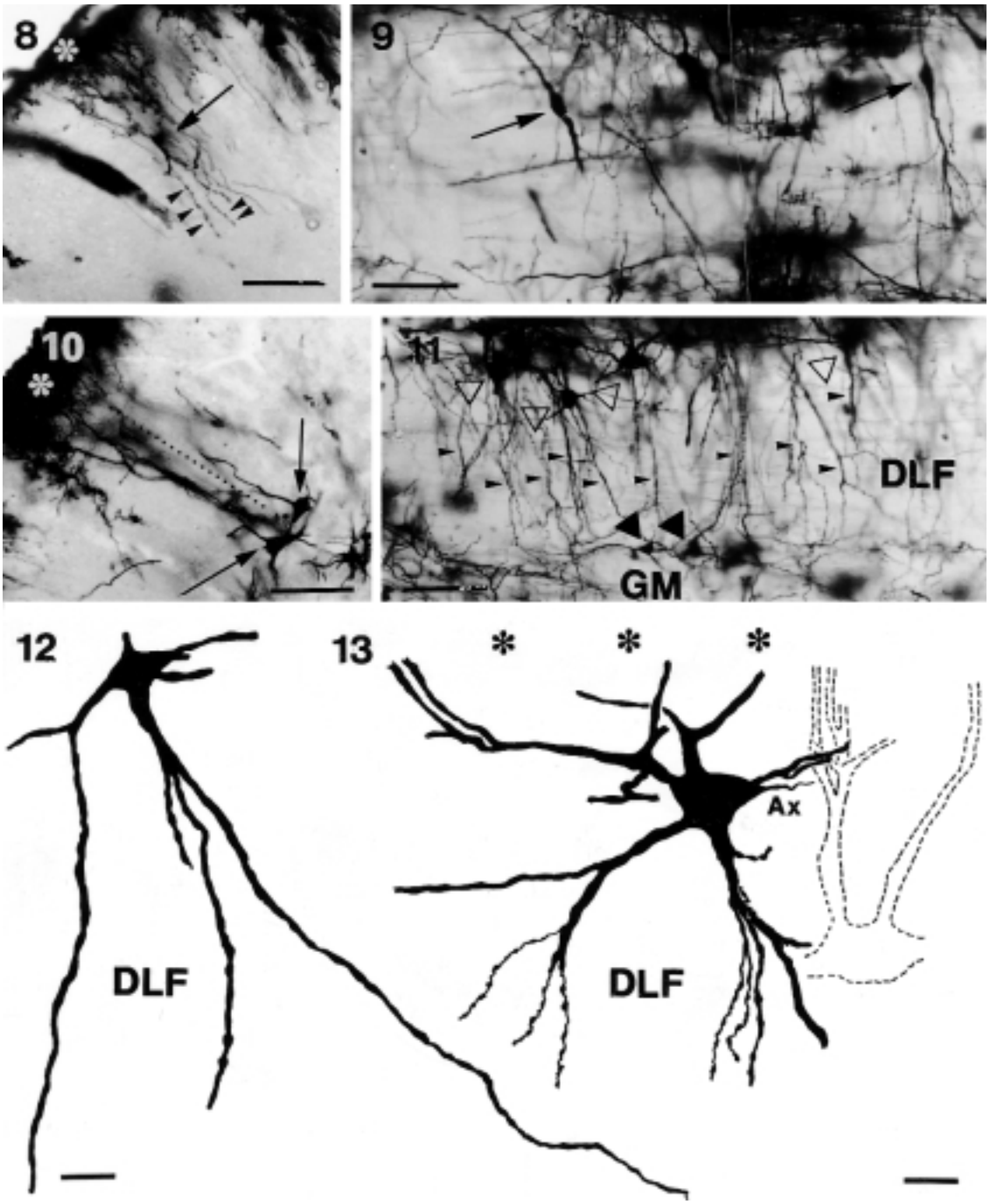

Figure 8.- Microphotograph showing the dorsolateral portion of the lumbar spinal cord in transverse section. The arrow points to a LSN neuron with dendrites coursing in dorsolateral to ventromedial direction. Some of the dendritic branches have a beaded structure (arrowheads). Asterisk marks the pial surface of the spinal cord. Scale bar: 100 µm.

Figure 9.- Photomontage showing the dorsolateral portion of the lumbar spinal cord in a longitudinal section. The pial surface is at the upper margin of the picture. Arrows indicate spindle shaped LSN neurons with transverse dendritic arborizations. Scale bar: 100 µm.

Figure 10.- Microphotograph showing the dorsolateral portion of the lumbar spinal cord in transverse section. Multipolar neurons at the margin of the gray matter in the neck of the dorsal horn (arrows) send their dendrites into the DLF parallel with those of the LSN neurons. Dotted line indicates the plane of the section illustrated in Fig. 11. Asterisk marks the pial surface of the spinal cord. Scale bar: 100 µm.

Figure 11.- Microphotograph showing the dorsolateral portion of the lumbar spinal cord in longitudinal section. The plane of the section corresponds to the joint dendritic trajectory of the LSN neurons and neurons at the margin of the gray matter (tilted plane corresponding to the dotted line in Fig. 10). The upper margin of the picture coincides with the surface of the spinal cord, while the lower margin shows the spinal gray matter (GM) at the level of the neck of the dorsal horn (approx. lamina IV). Multipolar LSN neurons are marked with open triangles, while neurons at the margin of the gray matter are indicated with filled triangles. The straight dendrites of these two groups of neurons coursing medially and laterally, respectively (arrowheads), form a ladder like structure in the dorsolateral funiculus (DLF). Scale bar: 100 µm.

Figure 12.- High-power camera lucida drawing of an LSN neuron in a tilted longitudinal plane (top is lateral). Some of the dendrites are truncated, while others course in medial direction across the dorsolateral funiculus (DLF). Scale bar: 25 µm.

Figure 13.- High-power camera lucida drawing of two LSN neurons in a tilted longitudinal plane (top is lateral). The dendritic arborization of the neuron in full black is quite complete. Some of the dendrites course in medial direction across the dorsolateral funiculus (DLF). The distal portions of these latter often have a beaded structure. Other dendrites course laterally, approaching the pial surface of the cord (asterisks). Ax = the initial segment of the axon of the LSN neuron. - The neuron in dashed contour was only partially visible in the Golgi specimen. In spite of their medial location in the DLF, two of the main dendritic trunks are directed laterally and the finer branches of these dendrites can be traced near the cord surface. Scale bar: 25 µm.

rons were oriented laterally, medially or longitudinally. These latter dendrites were confined to the column of the perikarya (Fig. 7).

Spindle-shaped and multipolar neurons were found on both transverse and longitudinal sections deeper in the DLF, at about 120 to 150 µm from the cord surface. The long axes of the spindle-shaped neurons were oriented obliquely, i.e. from dorsolateral to ventromedial (Figs. 8, 9). The dendrites of the multipolar neurons radiated in the same directions mentioned above, i.e. laterally, ventromedially or longitudinally (Figs. 12, 13).

Several multipolar neurons located along the lateral margin of the gray matter at the junction of the dorsal horn and intermediate zone (reticulated portion) were found to send numerous dendrites preferentially into the DLF (Fig. 10). The dendrites of these latter neurons coursed parallel but in the opposite direction to the medioventrally directed dendrites of the LSN neurons. In fortunate cases when the spinal cord was cut longitudinally in the tilted plane corresponding to the dendritic trajectory, an elongated ladder like structure could be seen in the DLF (Fig. 11). The column of LSN neurons formed the lateral longitudinal member of the ladder, while the medial longitudinal member was built by the neurons located at the margin of the gray matter. The 260-280 µm-long rungs of the ladder were formed by the dendrites of both groups of neurons coursing parallel but with opposite polarity (Fig. 11).

Most of the primary dendrites of the LSN neurons had a smooth surface, while some of them, especially those coursing medially, showed a beaded structure: large, bulbous thickenings were interrupted by thin dendritic portions (Figs. 8, 12, 13).

Initial portion of the axon of the LSN neurons

The initial portion of the axon of the LSN neurons could be seen on both transverse and longitudinal sections. In transverse sections, the stained portion of the axon was 20 to 25 µm long. It originated either from the perikaryon or from a proximal dendrite (Fig. 14). The 2.0-2.5 µm-thick axon hillock tapered gradually and the impregnation of the initial portion ceased after a short straight or wavy course. The ensuing course of the axons remained unimpregnated due to the appearance of the myelin sheath. The axon origin showed a similar arrangement in the longitudinal sections; the initial segment was directed cranially or caudally (Fig. 15).

Assumed axodendritic connections of the LSN neurons

Fine, unmyelinated nerve fibers were occasionally found adjacent to the surface of the

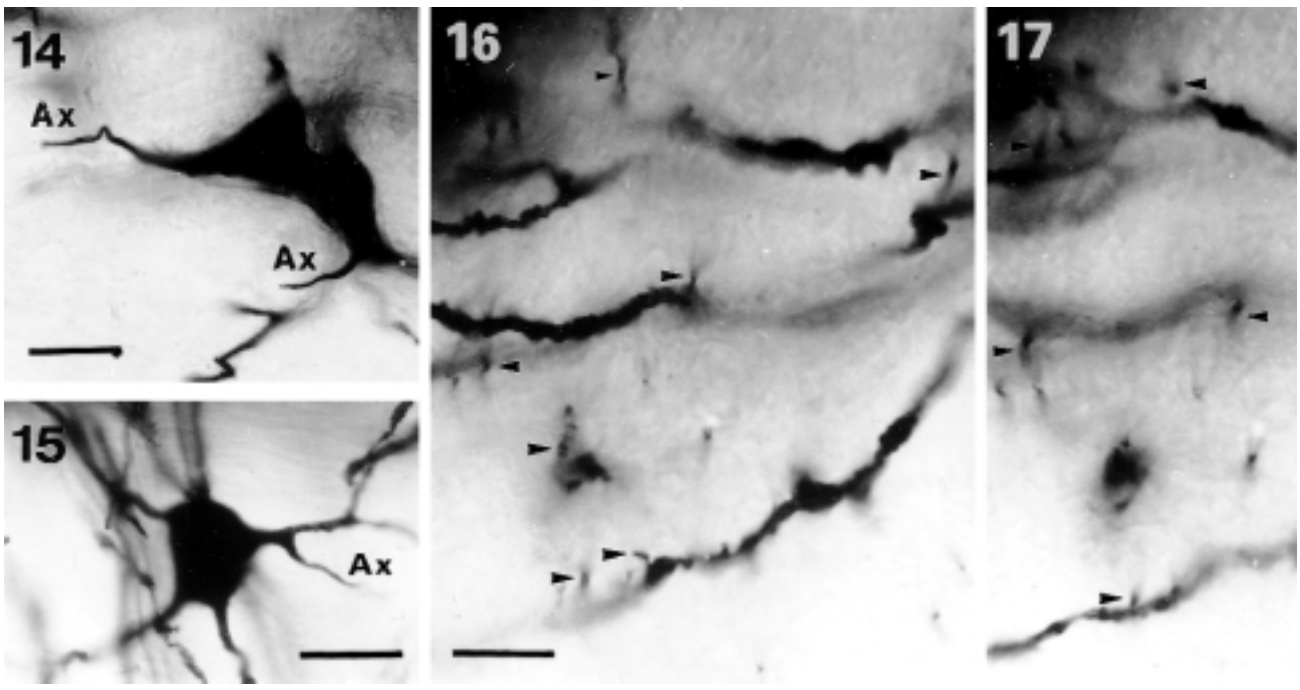

Figure 14.- Higher-power microphotograph showing the perikarya of two LSN neurons. The axons of both neurons (Ax) originate from one of the dendrites with axon-hillocks. The initial axon segment of the lower neuron goes straight in medial direction, while that of the upper neuron shows a small inflection. The silver impregnation of both initial segments stops abruptly due to the beginning of the myelin sheath. Scale bar: 25 µm.

Figure 15.- Higher-power microphotograph of a LSN neurons (second labeled neuron from left in Fig. 11). The axon of the neuron (Ax) originates from one of the main dendrites and the initial segment is directed longitudinally. Impregnation stops abruptly after 22 µm. Scale bar: 25 µm.

Figure 16 and 17.- Higher-power microphotographs of some of the dendrites shown in Figs. 2 and 5. The dendrites are over- and undercrossed by fine axons marked by arrowheads. The two pictures show two nearby focal planes. Scale bar: 10 µm.

spinal cord, coursing in the longitudinal direction. The laterally directed dendrites of some LSN neurons and these fine-caliber nerve fibers were found in identical focal planes under oil immersion lens (Figs. 16, 17). Thus a cross-over type of axodendritic synaptic connections could be expected between the axons and dendrites.

Preliminary ultrastructural observations

Two shortcomings of the light microscopic description were corrected with the results of the preliminary electron microscopic observations. The dense layer of silver precipitate occurring regularly on the surface of the spinal cord prevented the tracing of the laterally oriented dendrites of the LSN neurons until their termination. Low power electron micrographs clearly indicated that numerous dendrites coursed towards the surface and terminated immediately underneath the pial layer (Fig. 18).

Medium-power electron micrographs showed a large number of fine, unmyelinated fibers coursing longitudinally in the LSN (Fig. 19). The thickness of the unmyelinated fibers coincided with that seen in the Golgi specimens (Figs. 16, 17).

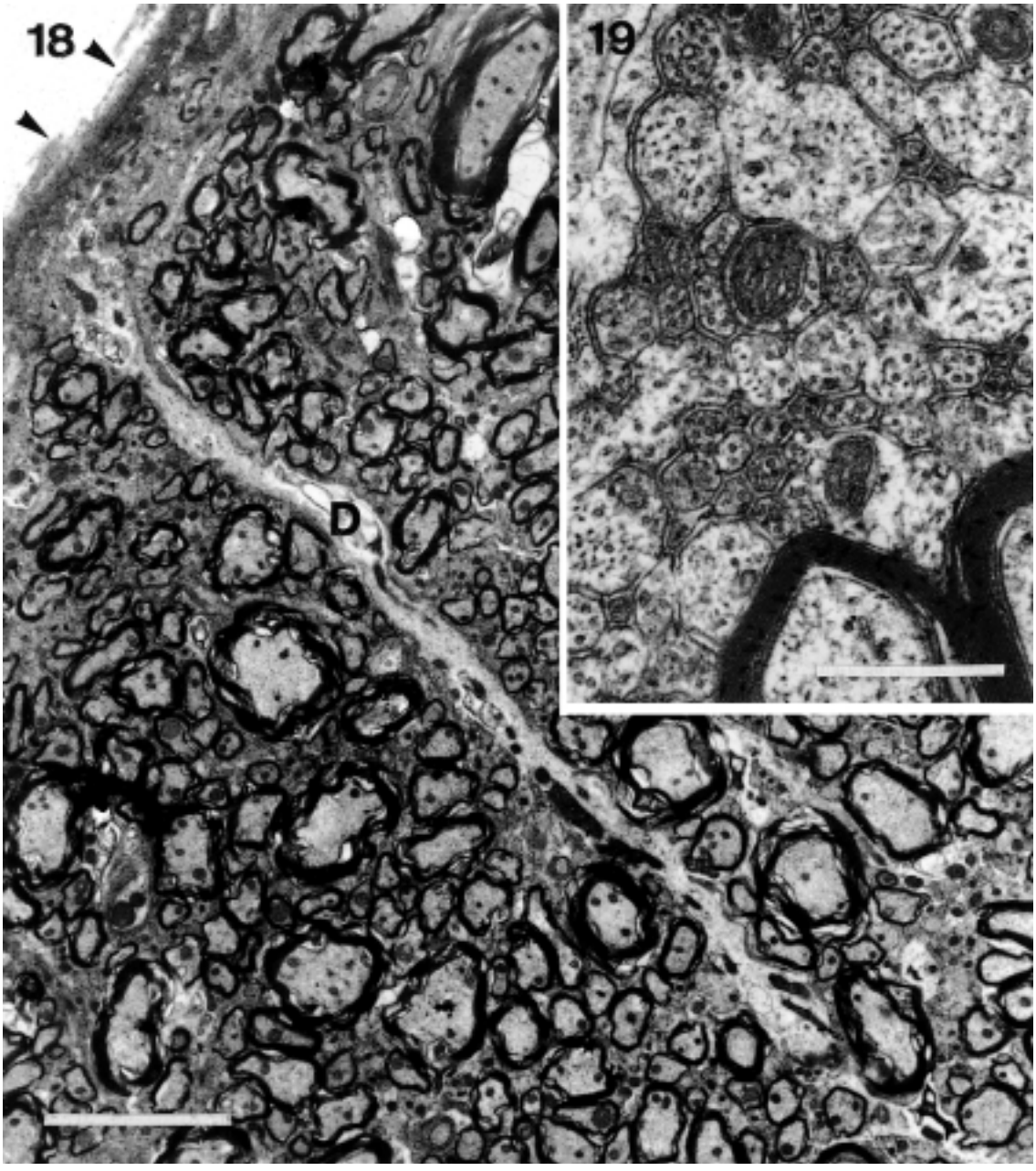

Figure 18.- Low-power electron micrograph showing the straight course of a dendrite (D) of a LSN neuron in the DLF. It terminates immediately underneath the pial surface of the spinal cord (marked by arrowheads). Scale bar: 5 µm.

Figure 19.- Medium-power electron micrograph showing a bundle of fine unmyelinated fibers in cross section in the DLF. Two myelinated fibers are also partially seen in the bottom right corner. Scale bar: 0.5 µm.

Discussion

Observation of Golgi specimens prepared from the spinal cord in two planes – transverse and longitudinal – revealed that the LSN consisted of neurons of characteristic shape, distribution and dendritic arborization. It is important to emphasize that the longitudinal sections must be cut in a tilted plane to incorporate the total course of the medially directed dendrites. Neither the conventional horizontal or the sagittal planes sufficed to show up the whole extent of the dendritic arborization.

The characteristic type of Golgi-impregnated neurons in the LSN was the multipolar neuron. This observation confirms earlier descriptions using metabotropic glutamate receptor immunostaining (Alvarez et al., 2000) and single-cell staining following intracellular recordings (Jiang et al., 1999). Spindle-shaped neurons were also seen on both cutting planes, but they occurred only rarely. It may be concluded that LSN seems to be constructed mainly from a single type of multipolar neurons whose major role is to carry impulses to supraspinal regions.

The LSN neurons formed a continuous column underneath the pial surface of the DLF. Some of the dendrites remained within the column of the perikarya, while others were directed either laterally or medially. A rich array of laterally directed dendrites coursed towards the surface of the spinal cord. Unfortunately, the dense silver precipitate on the surface of the spinal cord, which is an unwanted byproduct of the Golgi technique, prevented analysis of the termination of these dendrites. Electron micrographs revealed, however, that the dendrites approached the free surface of the spinal cord. This arrangement raises the question of whether the LSN neurons could be affected directly, through the latter dendrites, by the components of the cerebrospinal fluid surrounding the spinal cord.

The medially oriented dendrites of the LSN neurons cut across the DLF towards the neck of the dorsal horn (reticulated portion of the gray matter). Interestingly, neurons at the margin of the gray matter opposite to the LSN neurons sent some of their dendrites laterally, into the DLF. A ladder-like dendritic meshwork perpendicular to the longitudinally coursing fiber bundles emerged this way in the DLF. It is very probable that in the rungs of the ladder the dendrites from both sources would be intermixed, forming dendritic corridors across the nerve fibers of the DLF. The intimate topographical relationship between the dendrites of these two relatively distant neuron populations may indicate common synaptic input from the fiber systems terminating in the neuropil. This common synaptic input may explain the well known findings first shown by Menetrey et al. (1982) and later corroborated by practically all retrograde cell labeling studies (Pechura and Liu, 1986; Nahin, 1987; Leah et al., 1988; Burstein and Giesler, 1989; Burstein et al., 1990; Verburgh et al., 1990; Li et al., 1997) to the effect that LSN neurons and the neurons located along the margin of the gray matter project conjointly to several supraspinal locations.

The present observations provided only rudimentary details about the efferent connections of the LSN neurons. The axons of the neurons originated both from the perikaryon, and from one of the stem dendrites with a regular axon hillock. After a course of 20 to 30 μm, the impregnation of the axon gradually disappeared. This regular finding suggests that the axons of the LSN neurons become myelinated soon after their origin, supporting the known fact that LSN neurons are tract neurons. No collateral branches were seen along this short initial axon portion.

In one of the Golgi series, the laterally oriented dendrites of the LSN neurons were over- and/or undercrossed by fine nerve fibers coursing in the DLF. There is a traditional belief – also supported by the present findings – that myelinated fibers cannot be detected by Golgi impregnation. Consequently, nerve fibers that are visible in a Golgi specimen are unmyelinated. A large number of fine, unmyelinated nerve fibers was found in the DLF by Chung and Coggeshall (1983). This latter finding is confirmed in the present paper. The close approximation of the dendrites and the crossing unmyelinated fibers suggest a special synaptic connection of the LSN neurons through the lateral and medial dendritic arborization.

Although the present morphological data may help in appreciating the distribution, shape and dendritic arborization of the neurons in the LSN, they are insufficient to answer other important questions concerning the nucleus. The overlapping dendritic arborization of the neurons located at the margin of the spinal gray matter ("neck cells") and the LSN neurons suggests that the latter group of neurons are "neck cells" displaced into the DLF. A close relationship between these two groups of neurons has been suggested earlier by Bresnahan et al. (1984). A recent electrophysiological analysis of the LSN neurons by Jiang et al. (2000) disclosed several similar features between LSN neurons and deep dorsal horn neurons (e.g. membrane resistance, membrane time constant, action potentials, resting membrane potentials, several features of the afterpotentials). The unique advantage of the white matter location of the LSN neurons may be the contact of the laterally oriented dendritic tree with the pial surface of the spinal cord. Further ultrastructural and single cell studies will be needed to determine the significance of the LSN in spinal cord circuitry.

ACKNOWLEDGEMENTS

The expert histotechnical assistance of Mrs. Ibolya Salamon and the photographic work of M. J. Kiss are appreciated. Supported by a grant from OTKA T 23166.

REFERENCES

AARNISALO AA and PANULA P (1998). Neuropeptide FF in the lateral spinal and lateral cervical nuclei: evidence of contacts on spinothalamic neurons. *Exp Brain Res*, 119: 159-165.

ALLARD M, THEODOSIOS DT, ROUSSELOT P, LOMBARD MC and SIMONNET G (1991). Characterization and localization of a putative morphine-modulating peptide, FLFQPQR-Famide, in the rat spinal cord: biochemical and immunocytochemical studies. *Neuroscience*, 40: 81-92.

ALVAREZ FJ, VILLALBA RM, CARR PA, GRANDES P and SOMOHANO PM (2000). Differential distribution of metabotropic glutamate receptors 1a, 1b, and 5 in the rat spinal cord. *J Comp Neurol*, 422: 464-487.

BARBER RP, VAUGHN JE, SLEMMON PM, SALVATERRA PM, ROBERTS E and LEEMAN SE (1979). The origin, distribution and synaptic relationships of substance P axons in rat spinal cord. *J Comp Neurol*, 184: 331-352.

BRESNAHAN JC, HO RH and BEATTIE MS (1984). A comparison of the ultrastructure of substance P and enkephalin-immunoreactive elements in the nucleus of the dorsal lateral funiculus and laminae I and II of the rat spinal cord. *J Comp Neurol*, 229: 497-511.

BROWN JL, LIU H, MAGGIO JE, VIGNA SR, MANTYH PW and BASBAUM AI (1995). Morphological characterization of substance P receptor-immunoreactive neurons in rat spinal cord and trigeminal nucleus caudalis. *J Comp Neurol*, 356: 327-344.

BURSTEIN R and GIESLER Jr. GJ (1989). Retrograde labeling of neurons in spinal cord that project directly to nucleus accumbens or the septal nuclei in the rat. *Brain Res*, 497: 149-154.

BURSTEIN R, CLIFFER KD and GIESLER Jr, GJ (1990). Cells of origin of the spinohypothalamic tract in the rat. *J Comp Neurol*, 291: 329-344.

CHUNG K and COGGESHALL RE (1983). Numbers of axons in lateral and ventral funiculi of rat sacral spinal cord. *J Comp Neurol*, 214: 72-78.

CLIFFER KD, URCA G, ELDE RP and GIESLER Jr. GJ (1988). Studies of peptidergic input to the lateral spinal nucleus. *Brain Res*, 460: 356-360.

DING YQ, TAKADA M, SHIGEMOTO R and MIZUMO N (1995). Spinoparabrachial tract neurons showing substance P receptor-like immunoreactivity in the lumbar spinal cord of the rat. *Brain Res*, 674: 336-340.

GIBSON SJ, POLAK JM, BLOOM SR and WALL PD (1981). The distribution of nine peptides in rat spinal cord with special emphasis on the substantia gelatinosa and on the area around the central canal (lamina X). *J Comp Neurol*, 201: 65-79.

GIESLER JG and ELDE RP (1985). Immunocytochemical studies of the peptidergic content of fibers and terminals within the lateral spinal and lateral cervical nuclei. *J Neuroscience*, 5: 1833-1841.

GUAN ZL, DING YQ, LI JL and LU BZ (1998). Substance P receptor-expressing neurons in the medullary and spinal dorsal horns projecting to the nucleus of the solitary tract in the rat. *Neurosci Res*, 30: 213-218.

GWYN DG and WALDRON HA (1968). A nucleus in the dorsolateral funiculus of the spinal cord. *Brain Res*, 10: 342-351.

HUNT SP, KELLY JS and EMSON PC (1980). The electron microscopic localization of methionine-enkephalin within the superficial layers (I and II) of the spinal cord. *Neuroscience,* 5: 1871-1890.

JANSEN ASP and LOEWY AD (1997). Neurons lying in the white matter of the upper cervical spinal cord project to the intermediolateral cell column. *Neuroscience*, 77: 889-898.

JIANG MC, LIU L and GEBHART GF (1999). Cellular properties of lateral spinal nucleus neurons in the rat L_6-S_1 spinal cord. *J Neurophysiol*, 81: 3078-3086.

KNYIHÁR-CSILLIK E, TÖRÖK Á and CSILLIK B (1990). Primary afferent origin of substance P-containing axons in the superficial dorsal horn of the rat spinal cord: depletion, regeneration and replenishment of presumed nociceptive central terminals. *J Comp Neurol*, 297: 595-612.

LEAH J, MENETREY D and DE-POMMERY J (1988). Neuropeptides in long ascending spinal tract cells in the rat: evidence for parallel processing of ascending information. *Neuroscience*, 24: 195-207.

LI JL, DING YQ, SHIGEMOTO R and MIZUNO N (1996). Distribution of trigeminothalamic and spinothalamic-tract neurons showing substance P receptor-like immunoreactivity in the rat. *Brain Res*, 719: 207-212.

LI JL, KANEKO T, SHIGEMOTO R and MIZUMO N (1997). Distribution of trigeminohypothalamic and spinohypothalamic tract neurons displaying substance P receptor-like immunoreactivity in the rat. *J Comp Neurol*, 378: 508-521.

LI JL, DING YQ, XIONG KH, LI JS, SHIGEMOTO R and MIZUMO N (1998). Substance P receptor (NK1)-immunoreactive neurons projecting to the periaqueductal gray: distribution in the spinal trigeminal nucleus and the spinal cord of the rat. *Neurosci Res,* 30: 219-225.

LITTLEWOOD NK, TODD AJ, SPIKE RC, WATT C and SHEHAB SAS (1995). The types of neuron in spinal dorsal horn which posses neurokinin-1 receptors. *Neuroscience*, 66: 597-608.

LJUNGDAHL Å, HÖKFELT T and NILSSON G (1978). Distribution of substance P-like immunoreactivity in the central nervous system of the rat – I. Cell bodies and nerve terminals. *Neuroscience*, 3: 861-943.

MARSHALL GE, SHEHAB SAS, SPIKE RC and TODD AJ (1996). Neurokinin-1 receptors on lumbar spinothalamic neurons in the rat. *Neuroscience*, 72: 255-263.

MENETREY D, CHAOUCH A, BINDER D and BESSON JM (1982). The origin of the spinomesencephalic tract in the rat: An anatomical study using the retrograde transport of horseradish peroxidase. *J Comp Neurol*, 206: 193-207.

NAHIN RL (1987). Immunocytochemical identification of long ascending peptidergic neurons contributing to the spinoreticular tract in the rat. *Neuroscience*, 23: 859-869.

PECHURA CM and LIU RPC (1986). Spinal neurons which project to the periaqueductal gray and the medullary reticular formation via axon collaterals: a double-label fluorescence study in the rat. *Brain Res*, 374: 357-361.

RÉTHELYI M (1985). Dendritic arborization and axon trajectory of neurons in the hypothalamic arcuate nucleus of the rat - updated. *Neuroscience*, 16: 323-331.

VERBURGH CA, VOOGD J, KUYPERS HG and STEVENS HP (1990). Propriospinal neurons with ascending collaterals to the dorsal medulla, the thalamus and the tectum: a retrograde fluorescent double-labeling study of the cervical cord of the rat. *Exp Brain Res*, 80: 577-590.