THE HUMAN SPINAL CORD: SUBSTANCE P AND METHIONINE-ENKEPHALIN IMMUNOREACTIVITY¹

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Abstract

The distribution of substance P-like (SPLI) and methionine-enkephalin-like (MELI) immunoreactivity in the thoracic, lumbar, and sacral human spinal cord was studied in sections stained by the indirect antibody peroxidase-antiperoxidase method. The laminar distributions of SPLI and MELI were somewhat similar. Immunoreactive axons and terminal-like processes were found in the marginal zone and substantia gelatinosa, although SPLI was heavier than MELI in Lissauer's tract and the marginal zone. Laterally, both SPLI and MELI extended along the entire dorsal horn border, including laminae IV and V. Isolated, fine fiber bundles also extended deep into the central regions of laminae IV and V. Heavy SPLI and MELI also were found in the intermediolateral column region (IML), while lesser amounts were observed in the intercalatus and intermediomedial (IMM) regions of lamina VII. In lamina IX, SPLI was moderate, and less MELI was found. The distribution of SPLI fibers and terminal-like profiles in the human spinal cord is similar to that in other mammals, whereas that of MELI suggests some differences, particularly in laminae I, II, and III.

Many terminal-like structures were in close apposition to neurons in several laminae. Based upon correlations of the three-dimensional organization of both the labeled terminals and the dendritic trees of the cells, the types of neurons in apposition to SPLI and MELI could be tentatively identified. These may include (1) marginal cells resembling the pyramidal and multipolar types; (2) gelatinosal cells resembling islet and stalked cells; (3) spinothalamic cells in laminae I, V, VI, VII, and VIII; (4) autonomic neurons in the IML and IMM; and (5) motoneurons in lamina IX. In the sacral cord, neurons resembling dorsal band (lamina V) and lateral band (lamina VII) parasympathetic neurons also were outlined by SPLI and MELI, with more SPLI in the region of the lateral band neurons. Often, in many of the laminae, the same neuron was surrounded by both SPLI and MELI. The variety of possible neuronal types supplied by SPLI and MELI processes and the fairly wide distribution of these substances within the spinal cord suggest that they may be involved in a number of spinal functions.

In recent years, a number of peptides have been localized in the nervous system of vertebrates. On the basis of their location in neurons and other physiological evidence, it has been suggested that a variety of peptides may function as neuromodulators or neurotransmitters within the central nervous system (Hokfelt et al., 1980; Elde et al., 1980). However, only a few peptides have as yet been demonstrated in the human central nervous

system. Gramsch and co-workers (1979) demonstrated Met-enkephalin and β -endorphin in the human brain and pituitary with radioimmunoassays on tissue obtained postmortem. In a later study (Gramsch et al., 1980), they showed that β -endorphin and melanocyte-stimulating hormone predominate in the brain, whereas their respective precursors, β -lipotropic hormone and adrenocorticotrophic hormone, predominate in the adenohypophysis of man. Cooper et al. (1980) also have used radioimmunoassays to localize somatostatin, substance P, and neurotensin in human basal ganglia. Immunohistochemically, angiotensin II was shown recently in neural processes of the brain (Quinlan and Phillips, 1981), while substance P and enkephalin were localized in the dorsal tegmental nuclei of the human fetal brain (Pickel et al., 1980) and in the human fetal sympathetic ganglion (Hervonen et al., 1981).

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The spinal cord is an area of the central nervous system endowed with neural structures rich in a variety of neuropeptides. Enkephalin (ENK), substance P (SP), somatostatin, neurotensin, vasopressin, oxytocin, neurophysine, adrenocorticotrophin, thyrotropin, vasoactive intestinal polypeptide, angiotensin II, and cholecystokinin are present in the spinal cord of animals (Hokfelt et al., 1980; Gibson et al., 1981). To date, substance P is the only peptide that has been localized in the human spinal cord. Cuello et al. (1976), in a brief report, described the presence of substance P-like immunoreactivity in axonal processes of the human spinal cord using an immunohistochemistry technique. However, their study did not examine variations in the distribution of SP along the spinal cord. Further, because of the inherent limitation of the immunofluorescence technique (namely, the inability to observe details of the unlabeled background cytology), it is not possible to make detailed correlations of labeled structures to spinal cord organization.

The peptides, substance P and enkephalin, have received much attention in recent years in relation to their role in the perception and modulation of pain. As a part of a study concerned with the position of these peptides in the organization of pain pathways in primates and man, we have studied the distribution of SP and ENK in the human spinal cord using the indirect antibody peroxidase-antiperoxidase immunohistochemical method. Recently, we have reported the presence of both substance P and Met-enkephalin in close apposition to several cell types in the human spinal cord (LaMotte and de Lanerolle, 1981) using this technique. We describe in this paper, in greater detail, the laminar and segmental distribution of substance P-like immunoreactivity and also the distribution of methionine-enkephalin-like immunoreactivity previously undescribed in the human spinal cord. Such descriptions provide not only a basis for making extrapolations from animal studies to human research but also the basis for examining changes that may occur in these neuropeptidergic systems in the course of pathological states. This paper is a fuller description of a previous preliminary communication (de Lanerolle and LaMotte, 1980a).

Materials and Methods

Tissue preparation. The thoracic, lumbar, and sacral spinal cord from three adult male human subjects was removed at autopsy (4 to 12 hr postmortem) and fixed by immersion in picric acid/paraformaldehyde in phosphate buffer solution (Stefanini et al., 1967) for 24 to 48 hr. The tissue then was washed in several changes of a phosphate buffer (Karlsson and Schultz, 1965) and, finally, rinsed in 70% ethyl alcohol to remove any remaining traces of picric acid. Portions of tissue from representative spinal cord regions (T5-10, L3, and S2) were dehydrated, embedded in paraffin wax, and cut into 10- μ m transverse, sagittal, or horizontal sections for staining for light microscopy. Other portions of tissue were cut into 50- to 100- μ m sections on a Vibratome and also immunostained for light microscopy.

Immunochemicals. Antibody to SP (lot 24259, Immuno Nuclear, Stillwater, MN) was produced in rabbit against a carbodi-imide/keyhole limpet hemocyanin conjugate. Antibody to Met-enkephalin (lot 49279, Immuno

Nuclear, Stillwater, MN) also was produced in rabbit against a glutaraldehyde/bovine thyroglobulin conjugate. The link antiserum was goat anti-rabbit serum (lot 12775, Cappel Laboratories, Inc., Cochranville, PA). Peroxidase-rabbit antiperoxidase complex (Accurate Chemical and Scientific Corp., Westbury, NY) was prepared from high avid antibodies.

Immunohistochemical procedure. The procedure adopted was described previously (LaMotte and de Lanerolle, 1981). Paraffin sections were deparaffinized in xylene and then incubated in phosphate-buffered saline (PBS), pH 7.2 (10 to 15 min). The PBS was removed and the sections were incubated in 3% normal goat serum in PBS containing 0.3% Triton X-100 (30 min) and then in anti-substance P or anti-Met-enkephalin in PBS containing 1% normal goat serum and 0.3% Triton X-100 at 4°C (48 hr). The anti-substance P and anti-Met-enkephalin sera were used in dilutions varying from 1:500 to 1:2000, with a dilution of 1:800 being optimal for the human tissue. The primary antibody was replaced by goat anti-rabbit serum (1:20 in PBS) at room temperature (30 min), followed by peroxidase-antiperoxidase (PAP) complex (1:100 in PBS) at room temperature (30 min). After each of the above incubations, the sections were rinsed in two changes of PBS for 5 min each. Following the PBS rinse after the PAP incubation, the sections were incubated in 3,3-diaminobenzidine hydrochloride (DAB; Sigma; 0.05% in 0.05 M Tris buffer, pH 7.6; 15 to 30 min) and then in DAB in ammonium acetate buffer, pH 5.5 (Vacca et al., 1978), and 0.01% hydrogen peroxide (4 to 6 min). The final reaction was stopped by rinsing with distilled water. Some paraffin sections were counterstained with cresyl violet.

Vibratome sections were incubated in the same sequence of reagents as above, with the incubation time in the goat anti-rabbit serum and the PAP complex being 2 hr instead of 30 min. The sections also were washed longer in PBS between incubations (overnight after the primary antiserum and 1 hr after the other incubations). The preincubation in DAB/Tris buffer was omitted, and the final reaction with DAB/hydrogen peroxide was performed with the DAB in 0.05 M Tris buffer, pH 7.6. The stained sections were dehydrated, cleared in xylene, and mounted on microslides with Permount.

Antibody specificity controls were performed on adjacent sections by staining as above, but instead of the primary antibody, we used (1) primary antibody preabsorbed with the specific antigen (substance P or Met-enkephalin, 50 μ g to 1 mg of peptide/ml of antibody solution, 1:800) or (2) antibody pretreated with peptides other than the one under study (somatostatin, neurotensin, and Met-enkephalin or substance P in the substance P and Met-enkephalin immunostaining procedure, respectively, 1 mg of peptide/ml of antibody, 1:800 dilution). As a tissue control, the primary antibody was replaced by PBS with 1% normal goat serum containing 0.3% Triton X-100 (the vehicle for antibody dilution).

Results

Controls

In the antibody specificity control experiments, it was found that preabsorption of the primary antibody with

the specific antigen (substance P or Met-enkephalin as appropriate) completely prevented staining when the preabsorbed antiserum was subsequently applied to the tissue during immunostaining (Fig. 1, *C* and *G*). However, when the primary antibody was preabsorbed with peptides other than the one against which the antibody was raised (somatostatin, neurotensin, substance P, or Met-enkephalin as appropriate) and then used in the staining procedure, staining was produced as if untreated primary antiserum alone was used (Fig. 1, *B* and *F*).

In the tissue control experiment, omission of the primary antiserum from the staining procedure resulted in no staining (Fig. 1, *D* and *H*), indicating that none of the staining observed with the primary antisera was due to nonspecific staining of tissue components.

Immunostaining in Normal Spinal Cord

Three spinal regions were examined in this study—thoracic, lumbar, and sacral. The description that follows applies to all three, but where regional differences were found, they are indicated. In general, the immunoreactive complexes were a granular brown. They appeared as scattered small rings or clubs (terminal-like profiles) or as long beaded fiber-like profiles; these processes often outlined dendritic and neuronal cell bodies (Fig. 3). We did not observe either substance P-like or Met-enkephalin-like immunoreactive complexes within any cell bodies.

Dorsal Horn

Lissauer's tract

In transverse sections of the spinal cord, substance P-like immunoreactive (SPLI) fine diameter axons were seen to enter throughout the width of the dorsal surface of Lissauer's tract (Figs. 1*A* and 2, *A* and *C*). There appeared to be no difference in the lateral or medial distribution of immunoreactive fibers within the tract; however, the density of SPLI fibers increased dorsoventrally. Sagittal sections through Lissauer's tract revealed SPLI axons entering with the bundles of rootlets; some axons extended rostrocaudally, while others descended ventrally into the spinal gray substance.

Little methionine-enkephalin-like immunoreactivity (MELI) was found in this region (Table I; Figs. 1*E* and 2, *B* and *D*), although a few dendrites extending from the superficial dorsal horn into Lissauer's tract were outlined.

Lamina I (marginal zone)

The density of SPLI in lamina I was higher than in any other region of the dorsal horn (Table I; Figs. 1*A* and 2, *A* and *C*). In transverse sections, bundles of SPLI axons leave Lissauer's tract and extend tangentially along the entire medial and lateral border of the dorsal horn. In sagittal sections, the strong rostrocaudal orientation of the SPLI axons is readily apparent. A large proportion of the marginal cells, often of the "Waldeyer" type, are outlined along their soma and both their long rostrocaudally oriented and their tangentially oriented dendrites by SPLI terminals. In some cases, these cells extended a portion of their dendritic arbor into the white matter of the dorsal columns and dorsolateral fasciculus, and these

dendrites also were covered by SPLI processes (Fig. 4, *A* and *B*).

MELI fibers and terminals also were present in this region of the dorsal horn (Figs. 1*E* and 2, *B* and *D*) though they were slightly less abundant than SPLI processes (Table I). Many marginal cells were outlined very distinctly along both their soma and dendritic tree by MELI terminals (Figs. 3*A* and 4, *C* and *D*). In sagittal sections, the MELI fibers were strongly oriented in a rostrocaudal plane. MELI terminals also followed lamina I cell dendrites which extended into the dorsal columns or the dorsal part of the lateral funiculus (DLF) (Fig. 4, *C* and *D*).

Lamina II

Although there was a high density of SPLI fibers and terminals in this area, it was somewhat less than in lamina I (Table I; Figs. 1*A* and 2, *A* and *C*). In lamina II, SPLI terminals aggregate along dendrites which extend dorsoventrally in the lamina II and III region (Fig. 5*A*). Sagittal and horizontal sections revealed an additional strong rostrocaudal orientation of SPLI fibers, again following similarly oriented dendrites. The somas of some small neurons also were outlined by SPLI but less clearly than in lamina I.

The density of MELI was greater in lamina II than in lamina I (Table I; Figs. 1*E* and 2, *B* and *D*). When viewed in transverse sections, the inner part of lamina II stands out as being the region of the superficial dorsal horn containing the most MELI. This immunoreactivity appeared to be confluent, with dorsoventrally oriented dendrites extending through the inner lamina II and lamina III regions, as was the case for SPLI processes (Fig. 5*B*). In sagittal sections, MELI fibers also were oriented rostrocaudally but in a less organized fashion compared to SPLI fibers.

Lamina III

The predominant distribution of SPLI was along dorsoventrally oriented dendrites, with much less SPLI in between these structures (Figs. 2, *A* and *C*, and 5*A*). In sagittal sections, SPLI processes were much less distinct and a rostrocaudal organization was not particularly evident.

In the case of MELI, the close apposition to dorsoventrally oriented dendrites in lamina II continued as these dendrites extended into lamina III (Figs. 2, *B* and *D*, and 5*B*). Again, as with SPLI, little MELI was found in the neuropil interspersed between these dendritic processes.

Lamina IV

This lamina had considerably less SPLI than in the preceding laminae (Table I; Fig. 2, *A* and *C*). There was a faint terminal-like immunoreactivity throughout the lamina, with an occasional cell and its dendrites outlined. There also were small "bundles" of SPLI elements which appeared to be passing from the superficial dorsal horn (laminae I to III) through lamina IV and ventrally into lamina V. These bundles projected as: (1) a distinct lateral bundle which was an extension of the lateral bundle in the marginal zone and which began to break up in lamina IV as it approached the lateral region (reticular area) of lamina V, (2) a smaller medial bundle

which was an extension of the medial bundle of the marginal zone which bordered the dorsal columns and ended in the medial part of laminae V and VI, and (3) occasional bundles which passed through the central region of lamina IV toward lamina V.

MELI also appeared as a fine dusting of terminals throughout lamina IV, and the label in this region was much lighter than in the rest of the dorsal horn (Fig. 2, *B* and *D*). As with SPLI, lateral, medial, and central bundles of MELI fibers passed through lamina IV toward lamina V, but these fibers were much finer and less distinct than the SPLI bundles.

Lamina V

The three bundles of SPLI described above terminated in this lamina (Fig. 2, *A* and *C*). The lateral bundle of SPLI fibers (described above) extended as fine bundles of fibers into the lateral and central regions of lamina V and these were joined by the central bundles descending ventrally from the superficial dorsal horn. A small medial patch of one to three cells (possibly the medial basal nucleus of Cajal) received a light innervation of SPLI from the medially oriented bundle. Many of the cells and dendrites of the reticular nucleus in the lateral part of lamina V were outlined by SPLI terminals (LaMotte and de Lanerolle, 1981), including dendrites which extended into the white matter. In sagittal sections, the lateral reticular region of lamina V appeared as a dense strip of immunoreactivity similar to that of the marginal zone but less organized longitudinally.

The lateral reticular nucleus cells of lamina V also were outlined along their somata and dendrites by MELI terminals (Fig. 3*E*). An occasional cell along the medial border (medial basal nucleus of Cajal) also was outlined by MELI. Very little MELI was seen centrally, which contrasts with the situation for SPLI.

Lamina VI

In the thoracic and lumbar cord, there was almost no SPLI in this lamina (Fig. 1*A* and 2, *A* and *C*). MELI was observed in the lateral edges of this lamina in L3, where the immunoreactivity outlined a few dendrites and cell bodies. In the sacral cord, the somata and dendrites of Stilling's nucleus (lamina VI) were outlined by SPLI, and

there was moderate immunoreactivity in the neuropil (Fig. 6*A*).

MELI terminal-like staining was less apparent; however, some proximal dendrites were outlined (Fig. 6*B*). Further, there was some "streaming" of MELI fibers from lamina V into Stilling's nucleus as was seen for SPLI.

Intermediate Gray (Zona Intermedia; Lamina VII)

Thoracic spinal cord

While the distribution of SPLI was continuous throughout the various regions of lamina VII (the intermediolateralis (IML), intercalatus (IC), and intermediomedialis (IMM) regions), the density of SPLI in the subregions varied. As with SPLI, the distribution of MELI was continuous throughout the subregions of lamina VII (IML, IC, and IMM; Table I).

Nucleus intermediolateralis (IML). The outlines of the somata and dendrites of both the IML pars principalis and IML pars funicularis (Petras and Cummings, 1972) were highlighted by the close apposition of SPLI terminal-like elements (Fig. 7*A*). Approximately 30 to 50% of the cells were so innervated. In the IML pars principalis, both large, round multipolar neurons and medium size spindle-shaped cells (oriented rostrocaudally) were strongly outlined by SPLI (LaMotte and de Lanerolle, 1981). The SPLI was associated mostly with the somata and proximal dendrites of IML cells; there was little immunoreactivity along their rostrocaudally oriented dendritic tree, which extends between the periodically occurring cell nests of the IML (Rethelyi, 1972).

In many cases, the same cell bodies and dendrites outlined by SPLI also were outlined by MELI (Fig. 7*B*; LaMotte and de Lanerolle, 1981). The MELI innervation appeared slightly heavier than the SPLI innervation, and the MELI was more organized longitudinally, as it was apposed to long dendrites extending rostrocaudally between the cell nests.

Nucleus intercalatus (IC). In the IC, an occasional cell or proximal dendrite was lightly outlined by SPLI. These small cells were spindle shaped and oriented transversely.

An occasional cell or dendrite of the IC was outlined by MELI (Fig. 3*B*), with only light immunoreactivity in the neuropil.

Figure 1. Photomicrographs of 10- μ m paraffin sections of the dorsal horn of human thoracic spinal cord. *A*, A section stained by the PAP method using a 1:800 dilution of SP antiserum. Note the heavy immunoreactivity (seen as *black areas*) especially prominent in the superficial layers of the dorsal horn and in Lissauer's tract. Also evident is the prominent streak of SPLI extending along the lateral region of lamina I and into lamina V. *B*, A section adjacent to the one shown in *A* stained with SP antiserum preabsorbed with Met-enkephalin, somatostatin, and neurotensin. The pattern of immunoreactivity is similar to that in *A*. *C*, A section adjacent to *B* stained using SP antiserum preabsorbed with 500 μ g/ml of pure SP. Note the absence of the pattern of immunoreactivity seen in *A* and *B*. *D*, A section adjacent to *C* stained by the PAP method while omitting incubation in the SP antiserum. Immunoreactivity is absent. *E*, A section stained by the PAP method using a 1:800 dilution of Met-enkephalin antiserum. MELI is especially prominent in the superficial layers of the dorsal horn. *F*, A section adjacent to *A* stained as above but using a Met-enkephalin antiserum preabsorbed with substance P, somatostatin, and neurotensin. Note the similarity of the pattern of immunoreactivity to that in *E*. *G*, A section adjacent to *F* stained by the PAP method using Met-enkephalin antiserum preabsorbed with 500 μ g/ml of Met-enkephalin. Note the absence of immunoreactivity. *H*, The next adjacent section stained while omitting the Met-enkephalin antiserum. Immunoreactivity is absent. All of the above sections were counterstained with cresyl violet, and in *C*, *D*, *G*, and *H*, the *dark fine dot-like structures* scattered in the gray substance represent Nissl-stained glial nuclei and small neurons. The *larger* and *slightly darker dots* are red blood corpuscles in blood vessels, which were stained because of their endogenous peroxidase content. There is no specific immunoreactivity in these sections. Bar = 200 μ m.

Figure 2. Photomicrographs of transverse sections through the dorsal horn of the human spinal cord as seen in 100- μ m Vibratome sections. **A**, Lumbar cord stained for SPLI. In each example, immunoreactivity is most intense in *laminae I, II, and III*. Some fibers also extend into the lateral funiculus and dorsal columns (*arrows*). **B**, Lumbar cord stained for MELI. **C**, Sacral cord stained for SPLI. **D**, Sacral cord stained for MELI. The *small dark dots*, particularly clear in the deeper laminae in **B** and **D**, are red blood corpuscles that were stained because of their endogenous peroxidase content. Bar = 200 μ m.

Nucleus intermediomedialis (IMM). Occasional cells were lightly outlined by the apposition of SPLI elements to their surface, and there was some terminal-like immunoreactivity in the surrounding neuropil as well. SPLI was associated with both transversely and rostrocaudally oriented dendrites.

MELI outlined occasional cells, a few heavily. The long rostrocaudally oriented dendrites of IMM cells were innervated by MELI and there was light staining in the neuropil. Sometimes the same cell was outlined by both SPLI and MELI (LaMotte and de Lanerolle, 1981).

Nucleus dorsalis of Clarke (DNC). Less than 5% of the cells were outlined by SPLI and these cells had light soma and moderate dendritic innervation (Table I). The innervated cells were histologically indistinguishable from the unlabeled DNC cells in shape or location. There was no SPLI in the DNC neuropil.

As was the case with SPLI, the MELI outlined less than 5% of DNC cells.

Nucleus cornucommissuralis dorsalis. A small, dense band of SPLI fibers arched over the DNC and appeared to innervate the cornucommissuralis nucleus. A similar band of MELI fibers also was present.

Nucleus commissuralis dorsalis. Approximately 20 to 40% of the small neurons in this area were lightly innervated by SPLI elements, and there were very lightly stained elements in the neuropil. A few SPLI fibers coursed transversely through the commissure. Very little MELI was observed.

Nucleus commissuralis ventralis. SPLI and MELI terminals were very rare in this region at any spinal level.

Lumbar spinal cord

In the lumbar spinal cord as in the thoracic region described above, SPLI and MELI outlined a few somata

TABLE I
Summary of the distribution of SPLI and MELI in the human spinal cord

The number of plus signs denotes a subjective estimate of the relative abundance of immunoreactivity in any region compared to the others, while a minus sign indicates that no immunoreactivity was detected.

Area	SP	ENK
Tract of Lissauer	+++	+
Lamina I (marginal zone) ^a	++++	++
Lamina II (substantia gelatinosa)	+++	+++
Lamina III	++	+
Laminae IV to VI	++	+
Nucleus proprius	+	+
Nucleus reticularis ^a	++	++
Lamina VII (zona intermedia)		
Nucleus dorsalis of Clarke	+	+
Nucleus intermediolateralis ^a	++	++
Nucleus intermediomedialis ^a	+	+
Nucleus cornucommissuralis dorsalis	++	+
Nucleus cornucommissuralis ventralis	—	—
Lamina VIII	—	—
Lamina IX		
Nucleus motorius lateralis ^a	++	+
Nucleus motorius medialis	+	—
Lamina X	—	—

^a Dual innervated cells were detected here (LaMotte and de Lanerolle, 1981).

and dendrites in lamina VII. Along the lateral edge of L3 laminae VII and IX, several cells (which may be spinal border cells) were apposed by SPLI and MELI structures (Fig. 3C) and some cells in the lateral white also were outlined by label. Whereas the dorsal cornucommissuralis region of the lumbar cord contained a few SPLI fibers and terminals, there were almost none in the sacral region. The distribution of MELI in this region of the lumbar cord was similar; only faintly labeled elements were found. There was no MELI in this part of the sacral cord.

Sacral spinal cord

SPLI in lamina VII of the sacral cord was sparse medially, but along the lateral edge, several cells were outlined along their somata and dendrites (Fig. 3F). Lightly stained MELI terminal fields were found in the dorsal part of lamina VII and lateral cells were not outlined.

Ventral Horn

The distribution of SPLI in the ventral horn of the human spinal cord has been described elsewhere (de Lanerolle and LaMotte, 1982). For the purpose of comparison with the pattern of distribution of MELI, the salient features of SPLI distribution are given. In the three spinal regions examined (thoracic, lumbar, and sacral), SPLI profiles were seen to outline some ventral horn neuronal somata and proximal dendrites (Fig. 3D). The immunoreactive profiles were closely apposed to the surface of the cell body and dendrites, with the dendrites often covered by SPLI processes for a considerable distance from the cell body (300 to 400 μ m). The neurons associated with SPLI were multipolar and of the size range of α motoneurons (35 to 80 μ m, maximum diameter in transverse sections). SPLI-innervated neurons were found in all of the major ventral horn cell columns. Less frequently, SPLI fibers were seen in the neuropil in between lamina IX neurons. There was no SPLI in lamina VIII.

In contrast to the distribution of SPLI, there was much less MELI in the ventral horn (Table I). In thoracic sections, a few cells were associated with MELI processes, and in adjacent sections, some of these same cells also were determined to be associated with SPLI (LaMotte and de Lanerolle, 1981). In the lumbar and sacral regions, occasional cells were associated with MELI terminal-like processes. The cells associated with MELI were in the size range of α motoneurons. An extremely small number of MELI fibers and/or terminals also are present in the neuropil of lamina IX.

We have not observed any SPLI or MELI in the region below the central canal (lamina X).

Discussion

Distribution of SPLI and MELI in human and nonhuman mammals

Most studies of the distribution of SPLI in the spinal cord have examined SPLI either in the rat (Seybold and Elde, 1980; Barber et al., 1979; Kishida et al., 1979; Nilsson et al., 1974; Chan-Palay and Palay, 1977; Vacca

Figure 3. Photomicrographs of spinal neurons whose cell bodies (*large arrow*) and dendrites (*small arrows*) are outlined by the indicated immunoreactivity. *A*, MELI: marginal neuron from the thoracic cord (horizontal 10- μ m paraffin section counterstained with cresyl violet). *B*, MELI: neuron in the nucleus intercalatus from the thoracic cord (horizontal 10- μ m paraffin section counterstained with cresyl violet). *C*, SPLI: spinal border neuron from the lumbar cord (transverse 100- μ m Vibratome section). *D*, SPLI: lamina IX neuron (probable motoneuron) from the thoracic cord (horizontal 10- μ m paraffin section). *E*, MELI: lamina V neuron (reticular nucleus) from the lumbar cord (transverse 100- μ m Vibratome section). *F*, SPLI: lateral band neuron (lamina VII) from the sacral cord (transverse 100- μ m Vibratome section). Bar = 50 μ m.

et al., 1980; Ljungdahl et al., 1978; Hokfelt et al., 1975b) or in the cat (Naftchi et al., 1978; Hokfelt et al., 1975a). Cuello et al. (1976) have reported briefly the distribution of SPLI in the human spinal cord. We have tried to expand the data on SPLI by studying the human spinal cord as well as the distribution of SPLI, MELI, and serotonin-like immunoreactivity in the monkey spinal cord (de Lanerolle and LaMotte, 1980a, b; LaMotte and de Lanerolle, 1982).

All of the studies on the rat and cat have described a high density of SPLI fibers and terminals in spinal laminae I and II, with the heaviest concentration being in lamina I. The distribution pattern in these laminae of the human spinal cord is similar. As in the human, SPLI was observed in Lissauer's tract in the rat and cat. SPLI fibers and terminals have been reported in lamina III, where this immunoreactivity was observed either in the dorsal aspect of lamina III (Barber et al., 1979) or as scattered fibers in lamina III. Immunoreactivity in lamina III is described as much less than in laminae I and II, and in this, the rat and cat are also similar to the human, where the distribution of SPLI is along dorsoventrally oriented dendrites. The amount of SPLI in lamina IV in the human was considerably less than in laminae I to III and thus is comparable to that in the rat and cat. In the rat, a few SPLI fibers were seen traversing lamina IV in a dorsoventral direction (Barber et al., 1979), whereas in the cat, the central part of lamina IV is described as almost lacking immunoreactive structures. In the human, however, the limited amount of SPLI fiber and terminal-like processes in lamina IV was distributed in lateral, medial, and central bundles. As in the human, the immunoreactivity seen in rat and cat lamina V appears to be the result of extensions of immunoreactive fibers into it from more superficial laminae. In all of the species, it is heaviest in the lateral reticular region. Further, SPLI fibers and/or terminal-like processes occur in the DLF region of man and animals. SPLI is described in the medial parts of lamina VI in both the rat (Vacca et al., 1980) and cat (Hokfelt et al., 1975a). Vacca et al. (1980) also report SPLI in the lateral parts of lamina VI. In contrast, we saw almost no SPLI in lamina VI of the thoracic and lumbar region of man, whereas in the sacral region, SPLI was evident in Stilling's nucleus of lamina VI.

In the intermediate gray zone (lamina VII), SPLI was seen in the IML, IC, and IMM subregions of the thoracic and upper lumbar spinal cord and also in the sacral autonomic nuclei of sacral cord. Immunoreactivity in this region is reported by Hokfelt et al. (1975a) in the cat and Ljungdahl et al. (1978) and Vacca et al. (1980) in the rat. In particular, the pattern of SPLI described for lamina VII of the rat corresponds closely to that seen by us in man. Of all of the animal studies, only Barber et al. (1979) report SPLI in the nucleus dorsalis of Clarke and nucleus cornucommissuralis dorsalis in the rat as we observed in the human.

In the ventral horn, the subhuman animal studies and our study agree concerning the absence of SPLI in lamina VIII and the presence of SPLI in lamina IX, some of which is in close apposition to large neurons (presumably motoneurons). Whereas rat and cat studies that exam-

ined lamina X report SPLI in this region, particularly ventral to the central canal, we found almost none.

Our findings confirm in essence the distribution pattern of SPLI in the human cord reported earlier by Cuello et al. (1976). We have shown, in addition, strong immunoreactivity in the intermediate zone (lamina VII), especially in the IML. However, these workers do describe some immunoreactivity in regions above and below the central canal as reported in the animal studies. Overall, it would appear that the distribution of SPLI in the various laminae of the spinal cord is quite similar in man and subhuman species. The minor differences could be ascribed to either technical limitations in staining human autopsy tissue or species differences.

In colchicine-treated rats, many SPLI cell bodies have been described in laminae II, IV, and V of the thoracic and lumbar cord and in the thoracic IML (Ljungdahl et al., 1978). Immunoreactive SPLI cells were not seen in any of the above areas in the untreated human cords that we examined.

The distribution of enkephalin-like immunoreactivity (ELI) has been described in the spinal cord of the rat, cat, and monkey. In some of the studies on the rat, the antiserum used was against Leu-enkephalin (Elde et al., 1976—cross-reactivity with Met-enkephalin was not given), while in others, an anti-Met-enkephalin serum was used (Watson et al., 1977—cross-reactivity with Leu-enkephalin was described as low, and serum was preabsorbed with pure Leu-enkephalin before use; Hunt et al., 1980—cross-reactivity with Leu-enkephalin, 0.2%). Simantov et al. (1977) in their study on rats used antibodies raised against Leu-enkephalin and Met-enkephalin, but all antibodies are said to bind both enkephalins. In the study on the cat, an antibody to Leu-enkephalin was used (Glazer and Basbaum, 1981), but its cross-reactivity with Met-enkephalin is not reported, nor was the antibody preabsorbed with Met-enkephalin before use. Hence, its specificity for staining Leu-enkephalin cannot be evaluated. Aronin et al. (1981), studying the monkey spinal cord, also used a Leu-enkephalin antiserum, which is reported to cross-react 0.2% with Met-enkephalin. Our Met-enkephalin antibody does not cross-react with Leu-enkephalin as determined by preabsorption studies using Leu-enkephalin.

Whichever anti-enkephalin antibody was used, all of the studies agree that ELI fibers and terminals are most abundant in laminae I and II and are found in lesser numbers in laminae V, VII, IX, and X. The least amount of ELI is reported in laminae III and IV. The pattern of distribution of MELI that we observed in the human spinal cord agrees with the overall picture in other animals. However, closer examination of the studies on subhuman species suggests that there are various differences in the distribution pattern reported which may be due to species differences, antibody differences, or differences in unreported technical details.

All studies report very heavy ELI in laminae I and II. In the monkey, the greatest density is in lamina I (Aronin et al., 1981). In the human, however, the intensity of MELI was greater in lamina II than in lamina I. This is seen best in lumbar and sacral sections. Within human lamina II, there was more immunoreactivity in the inner

part of lamina II than in the outer part. In the monkey tissue that we examined (LaMotte and de Lanerolle, 1982) using the same Met-enkephalin antibody, the greatest immunoreactivity was in lamina I and the outer part of lamina II; no difference in intensity of immunoreactivity could be discerned between lamina I and outer lamina II. In the rat, using quite a specific Met-enkephalin antiserum, Hunt et al. (1980) found the greatest immunoreactivity in lamina I and outer lamina II as we did in the monkey. Hence, there may be a real difference in the laminar distribution of MELI between man and other animals. In the cat, using an antibody to Leu-enkephalin, Glazer and Basbaum (1981) found the greatest immunoreactivity in lamina I and inner lamina II. The high staining of inner lamina II in the cat resembles the condition of MELI in humans, but an equally high Leu-enkephalin-like immunoreactivity in lamina I and low intensity in outer lamina II contrasts with the condition in humans. These differences may represent species differences or differences in the distribution of Leu- and Met-enkephalin. Whereas some MELI was present in the lateral edge of lamina VI of the human lumbar cord and in Stilling's nucleus of the human sacral cord, only in the monkey (Aronin et al., 1981) have enkephalin-immunoreactive axons been reported in both places previously. Glazer and Basbaum (1981) state that there is no immunoreactivity in Stilling's nucleus in the cat; they also state that the dorsal nucleus of Clarke was devoid of ELI, whereas we observed about 5% of the DNC cells outlined by MELI in the human. Finally, in the central canal region, we could see no MELI in the regions below the central canal in contrast to some nonhuman animal studies.

ELI cell bodies were observed in laminae I, II, III, IV-V border, VII, and X of the cord of colchicine-treated animals (Glazer and Basbaum, 1981; Seybold and Elde, 1980) and in laminae I, II, III, and V of untreated animals (Aronin et al., 1981), but none were seen in the untreated human spinal cords that we examined.

Correspondence of SPLI and MELI with the functional organization of the spinal cord

Certain features of the three-dimensional distribution of the labeled axons in the spinal gray and their associations with certain cell groups allow some speculation about their identity and organization. We also make the assumption that the immunoreactive profiles seen in close apposition to neuronal somata and dendrites may represent immunoreactive axon terminal-like elements. The light microscopic images of these immunoreactive profiles (ring and club shaped) on the somata and dendrites resemble profiles identified as terminals by staining with neurofibrillary stains (Guillery, 1970). Guillery (1970) reviewed the evidence concerning why such formations may reasonably be regarded as axon terminals

and pointed out two important criteria for doing so. Firstly, such profiles are traceable into axons, and secondly, these structures show a preferential distribution around some perikarya and dendrites, as they are closely applied to the cell surface. The immunoreactive profiles that we describe as "terminal-like" meet these criteria. We do realize that the terminal or synaptic identity of a structure can be determined conclusively only on the basis of electron microscopic analysis. However, such an analysis of human tissue is very difficult, because of the poor preservation of ultrastructure in postmortem material. Nevertheless, we have examined, electron microscopically, immunoreactive processes similar to those in human material that are seen in apposition to superficial dorsal horn (LaMotte and de Lanerolle, 1982) and ventral horn (de Lanerolle and LaMotte, 1982) neuronal somata and dendrites in the monkey. These are identifiable as synaptic terminals and form axodendritic, axosomatic, and axoaxonic junctions. Thus, we do not know whether the terminal-like processes identified in human material make direct or trans-synaptic contact with the neuronal somata or dendrites.

The dorsal horn. The cells of the superficial dorsal horn have been described by several authors using Golgi and HRP-filling methods. Our material was either not cell stained or Nissl stained. Thus, it is not possible to determine definitively the cell types which are innervated by SP and ENK. However, certain features of the three-dimensional distribution of the labeled axons and their associations with dorsal horn somata and dendrites allow some speculation about their identity and organization.

Several studies have indicated that many SP axons in the dorsal horn are dorsal root primary afferents (Barber et al., 1979; Hokfelt et al., 1975a, b), since degeneration following rhizotomy depletes dorsal horn SP. We also identified SPLI axons in the dorsal root entry zone and in Lissauer's tract. Recent studies (Chung et al., 1979; LaMotte, 1977) have reported that primary afferents are found in both the medial and lateral halves of Lissauer's tract, contrary to early descriptions which stated that primary afferents are restricted to the medial portion, and that the lateral portion consists of propriospinal axons (Ranson, 1914; Earle, 1952; Szentagothai, 1964). The presence of SPLI in rootlets and in axons in both the medial and lateral regions of the tract is in agreement with the probable origin of some SPLI axons as primary afferents. Likewise, the virtual absence of MELI in Lissauer's tract and the dorsal roots agrees with the past data (Hokfelt et al., 1977) that MELI is not localized in primary afferents. The little that was seen may be terminals bordering dendritic extensions of marginal zone neurons or axonic or dendritic extensions of enkephalin cells in the marginal zone that have been described in other species (Glazer and Basbaum, 1981).

Several studies have now described a heavy termina-

Figure 4. A, Photomicrograph of a marginal neuron in the lateral funiculus border whose cell body (*large arrow*) and dendrites (*small arrows*) are outlined by SPLI (transverse lumbar Vibratome section). Bar = 100 μ m. B, The cell body of the neuron in A showing club- and ring-shaped immunoreactive profiles (*broad arrows*) closely apposing it. An immunoreactive varicose fiber is shown (*narrow arrow*). Bar = 10 μ m. C, A marginal neuron cell body with MELI in apposition to it (*large arrow*). Also seen is a dendrite of another neuron (*narrow arrows*) passing near the cell body and branching extensively. This dendrite also is outlined by MELI (transverse lumbar Vibratome section). Bar = 100 μ m. D, The cell body and dendrite passing near it photographed at a higher magnification to show the immunoreactive profiles apposing them. Bar = 10 μ m.

Figure 6. Photomicrographs of Stilling's nucleus (lamina VI) of the sacral cord stained for (A) SPLI and (B) MELI. Immunoreactive fibers and terminals are indicated by arrows. Bar = 200 μ m.

tion of small diameter myelinated and unmyelinated primary afferents within the marginal zone and substantia gelatinosa and also have demonstrated a lateral bundle of fibers that extends along the marginal zone down to lamina V (Light and Perl, 1979a; LaMotte, 1977; Ralston and Ralston, 1979; Gobel et al., 1981; Morgan et al., 1981). In the monkey, Light and Perl (1979b) have shown high threshold mechanoreceptor afferents to have medial and lateral branches that follow the marginal zone and establish terminal fields there. Further, these afferents extend medial and lateral collaterals which course into lamina V. The distribution of SPLI axons and terminals in the human spinal cord marginal zone and lamina V closely mimics the tracings made of these primary afferents.

The marginal cells which are outlined by SPLI and MELI may include both the pyramidal and the multipolar types described by Gobel (1978a) in the cat. Both of these cell types (especially the pyramidal) appear bipolar in transverse section, but in sagittal or tangential sections, extensive rostrocaudally oriented dendritic trees can be traced for hundreds of micrometers. If it is assumed that similar types of cells are present in lamina I of the human cord, it appears that both SPLI and MELI axons follow a course along the longitudinal axis similar to one that these dendrites would take. Although Gobel (1978a) describes these two cell types as having slightly

different shaped somata and locations in lamina I, we could not distinguish these cells by any preference for immunolabeling, since cells of various shapes throughout lamina I were outlined by immunoreactivity.

All studies of lamina II and III cells describe some cells as having extensive rostrocaudally developed dendritic trees (Gobel, 1978b; Gobel et al., 1980; Beal and Cooper, 1978; Light et al., 1979). The islet cell (Gobel, 1978b; Gobel et al., 1980), which has a dendritic tree that extends rostrocaudally up to 1 mm, is found in lamina II and has an axonal field restricted to lamina II, and on EM inspection, the dendrites have varicosities which form synapses onto other dendrites or terminals. The stalked cell extends dendrites for a shorter distance (500 μ m) rostrocaudally and also sends dendrites dorsoventrally through lamina II and sometimes into laminae III and IV. The stalked cell sends its axon to branch rostrocaudally in lamina I. Other cell types include arboreal and laminar border II-III cells; the border cell also has a rostrocaudally oriented dendritic tree and a similarly oriented axon branching mainly in laminae II and III.

Gobel et al. (1980) state that both islet cells and stalked cells receive considerable primary afferent input. Our data indicate that at least some portion of this input for both cell types is likely to be SP-positive afferents. The longitudinally oriented dendrites of the islet and stalked cells appear to be prime candidates for the identity of

Figure 5. Photomicrograph of the superficial layers (laminae I to III) of the dorsal horn in the sacral spinal cord. A, Distribution of SPLI. B, Distribution of MELI. Examples of dendrites outlined by dark immunoreactive profiles are indicated by arrows. DC, Dorsal columns; Lt, Lissauer's tract. Bar = 100 μ m.

Figure 7. Photomicrographs of the intermediolateral nucleus from adjacent horizontal serial paraffin sections of the thoracic cord stained for (A) SPLI and (B) MELI and counterstained with cresyl violet. The *large arrows* point to identical cell nests in which neuronal somata and dendrites are heavily outlined by immunoreactive processes. Note the greater longitudinal organization of MELI, with the immunoreactivity following the dendritic extensions between cell nests (*small arrows*), whereas SPLI tends to accumulate mostly on neuronal cell bodies and proximal dendrites. *Bar* = 100 μ m.

the longitudinal dendrites that were outlined by SPLI in our studies. Additionally, the dorsoventrally oriented dendrites in laminae II and III are likely to correspond to the cone-shaped dendritic arbors of stalked cells that transect laminae II, III, and IV. Similar patterns are seen in laminae II and III for enkephalin innervation of the longitudinal and dorsoventral dendritic trees, although the intensity of enkephalin innervation was heaviest along the dorsoventral dendrites of laminae II and III, whereas SPLI was heaviest along the longitudinal dendrites of lamina I and outer lamina II.

Hunt et al. (1980) have suggested that enkephalin-positive neurons in lamina II (in the rat) resemble the islet cells of Gobel et al. (1980) and that the vesicle-filled varicosities of islet cell dendrites contain enkephalin; Aronin et al. (1981) found similar enkephalin-positive dendrites in the monkey substantia gelatinosa. Gobel et al. (1980) and Bennett et al. (1980) have described the islet cell as an inhibitory interneuron which may form axoaxonic contacts either onto primary afferent endings in lamina II or onto stalked cells, which they consider to be excitatory interneurons. The proposal of Hunt et al. (1980) that islet cells contain enkephalin could account for the labeled terminals (or varicose dendrites?) found in laminae II and III, to which the vesicle-containing dendrites and the axonal branchings of the islet cell are confined. However, islet cells rarely send branches into lamina I, which also has a very distinctive enkephalin innervation. It is possible that, while islet cells may contribute to lamina II and III MELI innervation, the MELI of lamina I may originate from either descending (supraspinal) sources (Bowker et al., 1981) or from other spinal neurons, including enkephalin-containing cells, within lamina I itself (Glazer and Basbaum, 1981).

Lamina IV is distinct from the superficial dorsal horn in that it receives few direct projections of fine diameter nociceptive afferents. Rather, this area receives intermediate and large diameter dorsal root fibers of mechano- and proprioceptive modalities; correspondingly, the area is sparsely populated by SPLI and MELI processes.

As described above, lamina V receives fine diameter high threshold mechanoreceptor projections in both lateral and medial sites (Light and Perl, 1979b). Likewise, spinothalamic cells have been identified in the primate in these same areas (Willis et al., 1979). The dominant dorsoventral and mediolateral dendritic tree orientation and compressed rostrocaudal spread of these cells (Scheibel and Scheibel, 1968) may account for the extension of both SPLI and MELI along dendrites viewed transversely and the modified longitudinal orientation seen in the sagittal plane.

Spinothalamic neurons. Willis et al. (1979) injected HRP into the ventral posterior lateral thalamic nuclei of monkeys and found labeled cells in the lumbar cord (L5-S1) located throughout lamina I and in the lateral and central parts of lamina V. The spinothalamic cells in these laminae include both high and low threshold neurons. Medial thalamic injections near the central lateral nucleus resulted in labeled cells in the lateral part of lamina VI and scattered throughout laminae VII and VIII. Assuming that the distribution and projection of

spinothalamic cells in the human is similar to that in the monkey, an interesting innervation pattern emerges. Cells in laminae I and V receive a heavy innervation by both SP- and ENK-containing processes; sometimes the very same cell is innervated by both (LaMotte and de Lanerolle, 1981). Lamina VI cells, on the other hand, are innervated by moderate amounts of MELI and little SPLI. Lamina VII had scattered SPLI and MELI, especially laterally. Thus, it would seem that lamina I and V cells projecting to the lateral thalamus may receive a heavier SPLI and MELI innervation than those of laminae VI and VII projecting to the medial thalamus. The observation in monkey studies (Light and Perl, 1979a) that small diameter primary afferents (which include SPLI axons) end heavily in laminae I and V but do not extend into lamina VI of the lumbar cord may account for the different SPLI innervation pattern of cells in these laminae. Additionally, the lateral cells of lamina VII which receive a light SPLI and MELI innervation may represent a more heterogeneous group of cells, including spinothalamic cells (Willis et al., 1979), spinal border (spinocerebellar) cells (Burke et al., 1971), and autonomic cells (Petras and Cummings, 1972).

Similarly, in the sacral cord of the monkey, Willis et al. (1979) found that spinothalamic cells from laminae I, V, and VI (Stilling's nucleus) project to the lateral thalamus; the medial thalamus did not seem to receive projections from the sacral cord. In the human sacral cord of our study, cells of laminae I, V, and VI received substantial SPLI and MELI innervation. Thus, in the sacral cord, a correlation between heavy innervation by SPLI and MELI and the location of spinothalamic cells projecting to the lateral thalamus is again suspected.

Autonomic cell groups. Autonomic neurons in the spinal cord of the cat have been located in lamina VII in the thoracic/upper lumbar region (Petras and Cummings, 1972). In lamina VII, they were located in the IML, IMM, and IC regions, and at the upper lumbar level, a band of cell bodies extended along the outer edge of the ventral horn. Assuming a similar distribution of autonomic neurons in the human cord, it is interesting that we observed SPLI and MELI processes in these same regions of lamina VII in the human. SPLI and MELI seemed to follow the outline of cells and dendrites in the IML, with possibly more innervation of distal dendrites by MELI than by SPLI, whereas somata and proximal dendrites were labeled about equally by both. We observed neurons outlined by SPLI and MELI in the IMM of the human and Bresnahan et al. (1980) also reported SPLI and ELI in this nucleus in the cat. While some of the cells so innervated in the IMM may be autonomic neurons, spinothalamic neurons (Bresnahan et al., 1980) located here are also possible candidates.

Nadelhaft et al. (1980) describe two groups of sacral parasympathetic neurons in the cat. The dorsal band of neurons is approximately located in lamina V and the lateral band of neurons is found along the lateral edge of lamina VII. Cells in these general regions in the human sacral cord often are outlined in SPLI and MELI. The cells outlined in the human cord appear to have somata and dendrites in orientations similar to those described for sacral parasympathetic neurons in the cat, which

supports the view that these cells may be of similar groups.

Further, we observed that, whereas the amount of SPLI and MELI in the dorsal band region was about the same, there appeared to be more SPLI in the region of the lateral band neurons than in the dorsal band. Some of the SPLI in both dorsal and lateral band regions may come from primary afferents which distribute to these regions (Morgan et al., 1981), whereas the additional substance P in the lateral band may be from a supraspinal source. A number of lines of circumstantial evidence support this contention. In the cat, the dorsal band neurons control colon function through local spinal reflexes, whereas the autonomic neurons of the lateral band (where there is more SPLI) control bladder function and are under descending as well as local control (Nadelhaft et al., 1980). Descending projections to this region arise from the raphe pallidus, among other areas (de Groat et al., 1979; Martin et al., 1978), and raphe pallidal neurons which project to the spinal cord have been shown to contain substance P (Bowker et al., 1981). This may be the source of the extra SPLI in the lateral band region.

In the rat, the dorsal commissural nucleus at L1-L2 has been shown to contain sympathetic preganglionic neurons (Hancock and Peveto, 1979); whether this exists in other species is not known. The innervation of neurons in this region in the human does differ from those of the IML. Whereas the IML neurons were heavily outlined by both SPLI and MELI, in the dorsal commissural nucleus, only some small cells were lightly innervated by SPLI and none were innervated by MELI.

Motoneurons. Kawamura et al. (1977a, b) analyzed the size of lamina IX neurons and suggested that neurons with cell body diameters in the range of 33 to 59 μm were probably α motoneurons. In an independent study, Tomlinson et al. (1973) also reported that the majority of α motoneuron cell bodies have diameters in a similar range (45 to 65 μm). In general, neurons having maximum diameters greater than 30 μm are thus presumptive motoneurons in the human cord. All of the cell bodies associated with SPLI observed in this study fall within the category of α motoneurons. This relationship between SPLI processes and lamina IX motoneurons has been reported more fully in a separate paper (de Lanerolle and LaMotte, 1982). Additionally, in the present study, lamina IX neurons also were observed to be apposed by MELI, and these cell bodies were also of the size of α motoneurons. However, fewer cell bodies were associated with MELI than with SPLI. Nevertheless, in some cases, the same motoneuron was found to be associated with both SPLI and MELI (LaMotte and de Lanerolle, 1981).

A review (de Lanerolle and LaMotte, 1982) of animal experiments suggests that the source of much of the SPLI in the ventral horn may be neurons whose cell bodies are located in the caudal brainstem raphe nuclei, whereas much of the SPLI in the dorsal horn is from dorsal root afferents. Likewise, at least some of the MELI neuronal cell bodies projecting to the ventral horn may lie in the same raphe regions as the SPLI neurons (Bowker et al., 1981).

It would appear from the foregoing discussion that, based upon the wide distribution of SPLI and MELI in

the human spinal cord and their relationship to many functional classes of neurons (spinothalamic, autonomic, and motor) these substances may play a role in a wide range of spinal functions by mechanisms that are as yet poorly understood.

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