

The structure of the caudal end of the spinal cord (conus medullaris, filum terminale)

Thesis of the PhD Dissertation

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1. Introduction (background)

The spatial relationship between the spinal cord and the vertebral canal has three variations in the vertebrates (Catala et al., 2000). The spinal cord is as long as the vertebral canal in fish, in amphibian before metamorphosis and in reptiles. The spinal cord fills the entire vertebral canal in birds, but the spinal cord related to the final portion of the tail (pygostyl) has neither ventral nor dorsal roots. The spinal cord of the mammals is shorter than the vertebral canal. The continuation of the spinal cord in the caudal portion of the vertebral canal is called filum terminale (FT). As in the birds, no ventral, no dorsal roots, and no dorsal root ganglia are attached to the FT.

In the three species studied (rat, cat and monkey) the spinal cord ends abruptly. This final portion is the conus medullaris (CM), the tip of which originates the chord-like FT.

The CM contains the coccygeal spinal segments which are connected to the periphery through the coccygeal ventral and dorsal roots. In contrast to the CM, both classical and recent descriptions consider the FT a chord devoid of nervous tissue. The same standpoint is upheld also by the authors of the clinical papers. It is not mentioned in the various descriptions, but the authors are taking as granted the ventral and dorsal root equivalent structures are not attached to the FT.

Human FT consist of intradural and extradural portions, this latter attaches it to the periosteum of the os coccygis (Pinto et al, 2002). Within the dural sac together with lumbar, sacral and coccygeal roots the FT build the cauda equine.

According to the classical thoughts the FT is the result of the disproportionate growth between spinal cord and vertebral canal (Streeter, 1919). More recent descriptions state that the human spinal cord – including the CM – differentiates from the caudal portion of the neural tube. Spinal ganglia develop from the neural crest which differentiates parallel with the neural tube. In contrast, the FT is the product of the tail bud (Nievalstein et al., 1993).

Experiments carried out on the CM of the rat led us to the observation that at the junction between CM and FT neither the central canal nor the white matter discontinue, but could be followed in the FT. Consequently we were not surprised that when noticed

that the gray – in course of a sequence of transformation – continues in the FT. This latter observation necessitated the idea to carry out the systematic neurohistological study of the FT.

In earlier studies glial cells were found in the FT in all animal species studied (frog, rat, cat) and also in the human material. Nerve fibers were described in the FT of the frog and cat, degenerated nerve fibers were found in the human FT. Neurons were detected in the frog FT using both neurophysiological and neurohistological techniques. Neurons between the ependymal cells (liquor contact neurons) were found in the frog and cat. Degenerated neurons were mentioned in the publications describing the structure of the human FT.

The scarcity of the papers reporting the neurohistological structure of the FT is balanced by publications from the clinical literature. The tethered spinal cord syndrome consists of the motor and sensory disturbances on the lower extremities, incontinencia and deformities (scoliosis, enhanced lordosis; Yamada et al., 2001). The stretching as a mechanical factor causes the alteration of the blood supply that induces the malfunctioning of the mitochondria in the neurons of the CM.

2. Aims

The following points were planned to study with the systematic study of the CM and FT:

I. We wanted to describe the transformation of the spinal gray matter at the CM-FT junction. It is known that the spinal gray matter consists of the central core and of the ventral-, dorsal- and lateral horns attached to it (Réthelyi, 1976). We wanted to know if the transformation of the gray matter might be related to the above parcellation.

II. In addition to the application of the classical neurohistological techniques we wanted to describe the chemoarchitecture of the FT in the rat using immunohistochemistry and antibodies characterizing neurons and glial cells.

III. Based on the results of lightmicroscopic observations we wanted to study the ultrastructure of the FT in rat, cat and monkey.

IV. We wanted to detect the neuronal connections between the caudal part of the spinal cord and the FT using axonal tracing techniques (preliminary results).

V. Upon the analysis of the results of the morphological studies we wanted to find out the morphological and functional relationships between the neuron-rich FT and the central nervous system as well as the periphery.

3. Experimental procedures

3.1 Experimental animals

Adult rats (Sprague-Dawley strain, 260-280 g b.w.) and cats of both sexes were used in the studies. The light level- and ultrastructure of the FT was studied in one male monkey (*Macaca mulatto*, a gift from Dr. György Karmos).

3.2 Tissue fixation and sectioning

The animals were transcardially perfused in deep narcosis. The fixation solution was selected upon the requirement of the tissue processing. The fixed spinal cord taken out from the vertebral canal and selected portions – CM and/or FT – were cut into 5 mm pieces. Cross and longitudinal sections of 40 to 60 μm thickness were prepared with Vibratom. Some specimens were stained with 1% toluidine blue, some others were used to demonstrate the chemical features of the neurons using immunosera. The third group of the specimens was processed for electronmicroscopic studies.

3.3 Multiple immunofluorescence staining – confocal microscopy

The alcohol treated specimens were washed and the unspecific binding sites were blocked for 30 min in 3% horse serum (Vector). Then the specimens were incubated for 2 days in various combinations of antibodies at +4°C.

The second incubation was made overnight in cocktails of secondary antibodies labeled with fluorophores at +4°C. Following repeated wash in puffers the specimens were mounted on glass slides and covered with Vectashield (Vector). The specimens were observed with a Bio-Rad Radiance 2100 Rainbow confocal laser microscope.

Quantitative estimation of the number of neurons in the FT was prepared in specimens where the neurons were labeled with neuron-specific nuclear protein (NeuN).

3.4 Electronmicroscopic analysis

Small pieces of tissue blocks were washed in phosphate buffer (0,1 M) and postfixed in 1% osmium tetroxid (Sigma) for 1 hour. After dehydration and propylenoxyd (Sigma) treatment the tissue blocks were infiltrated in ascending series of Durcupan (Fluka) and embedded in Durcupan. Ultrathin sections were cut with a Reichert ultramicrotome and studied with JEOL electron microscope.

3.5 BDA tract tacing

Biotinilated dextranamin (BDA-10000, Sigma) was injected into the sacral segments in 5 adult Sprague-Dawley rats and the transported tracer substance was detected with immunohistochemistry.

3.6 Golgi – Kopsch impregnation

Four young Sprague-Dawley rats (60-80 g b.w.) were transcardially perfused and the spinal cord was impregnated in a potassium dichromate and silver nitrate solutions. Cross sections of 80 to 100 µm thickness were cut from the CM and FT using a Vibrotom. The specimens were mounted on glass slides and viewed as well as photographed with a LUCIA (Nikon) image analysis system.

4. Results

4.1 The morphological transformation of the gray matter at the caudal part of the spinal cord

On the cross section of the cranial portion of the CM the classical parts of the spinal cord can be found: dorsal horn, intermediate zone and the ventral horn. In the dorsal horn the transparent substantia gelatinosa (lamina II) and the denser laminae (lamina III and IV) can be distinguished. The central canal is surrounded by the gray matter of the intermediate zone.

Proceeding in caudal direction, the first step in the morphological transformation of the gray matter is the disappearance of the perikarya of the motoneurons in the ventral horn. In addition, the size of the gray matter is reduced. The dorsal horns are separated from the gray matter around the central canal.

At the final portion of the CM the gray matter consists of the almost completely separated dorsal horns and the intermediate zone around the central canal. The mass of the myelinated fibers and the volume of the substantia gelatinosa are also reduced in size. Finally, following the ventral horns, also the dorsal horns disappear completely. This caudal portion of the spinal cord is the FT.

The gradual reconfiguration and final disappearance of the dorsal horns were studied with immunohistochemistry. After the disappearance of the ventral horns, the dorsal horns are more and more tilted laterally, they dorsal surface is covered only by a narrow rim of the white matter. The osmium treated Vibratom sections demonstrated the superficial, transparent band that was hardly reacted with the osmium, and more ventromedially the dense area that reacted intensively with the osmium. Since the osmium stains the myelin sheath, the transparent superficial band corresponds to the substantia gelatinosa, the deeper territories to laminae III and IV.

The termination pattern of three classes of primary afferent fibers was studied in a specimen similar to the osmium treated Vibratom section using multiple immunohistochemistry. Calcitonin gene-related peptide (CGRP) labeling characterizing one of the classes of fine sensory fibers appeared in the superficial, transparent band of the dorsal horn, while vesicular glutamate transporter 1 (VGLUT1) labeling specific for the thick sensory fibers covered the ventromedial portion of the dorsal horn. Isolated CGRP labeling occurred also within the VGLUT1 labeled field. The joint labeling with CGRP and isolectin B4 (IB4) revealed that also the IB4 positive nerve fibers, representing another class of the fine sensory fibers, arborized in the superficial portion of the dorsal horn, more ventrally and partially overlapping with the CGRP positive nerve fibers. The termination pattern of the three groups of sensory fibers in the CM – fine fibers: CGRP and IB4 positivity, thick fibers: VGLUT1 positivity – identical with that found in the lumbar segments.

In more caudal part of the CM the structure of the dorsal horn showed an unexpected asymmetry in the osmium treated Vibratom sections. The superficial, transparent band (substantia gelatinosa) disappeared in the dorsal horn on one side, whereas it restricted to its lateral portion. The results of the immunohistochemistry showed similar arrangement on a Vibratome section prepared approximately at the same cranio-caudal level. There was no IB4 labeling on one side of the dorsal horn, while

contralaterally the IB4 labeling was shifted to its lateral portion. Also the CGRP labeling showed alterations. CGRP labeled nerve fibers remained in a narrow band on the surface of the dorsal horn on the side where the IB4 was not detected. Similar superficial labeling was found contralaterally in the medial portion of the dorsal horn, while laterally CGRP could be detected in a wide area, partially overlapping with the IB4 labeled nerve fibers. The VGLUT1 labeling spread over the entire dorsal horn on the side without IB4 labeling. Contralaterally VGLUT1 positive nerve fibers reached the surface of the dorsal horn medially, and respected the area where IB4 and CGRP positive fibers concentrated.

The neurons of the dorsal horn were labeled with neuron-specific nuclear protein (NeuN), and a group of neurons that are distributed in a form of a band mainly in the substantia gelatinosa with protein kinase C (PKC γ) labeling (Polgár et al., 1999). Also the PKC γ labeled neurons showed asymmetry, they were distributed jointly with the IB4 labeled nerve fibers.

4.2 Structure of the filum terminale in the rat

4.2.1 Macro- and microscopical relations

The FT is 2,4-2,8 cm long in the rat. At the CM-FT junction its width measures 0,5-0,6 mm which reduces at 5 mm in caudal direction to 0,2-0,3 mm. Our observations were done on the cranial 1 cm section of the FT.

Details occurring on the cross sections of the FT: the narrow gray matter around the central canal and the narrow rim of the white matter. In caudal direction the gray matter disappears, but the myelinated fibers in the white matter could be followed for long distances.

4.2.2 Neurons and glial cells

The structure of the FT can be studied on toluidine blue stained plastic sections. Two larger gray areas are located at both sides of the sagittally oriented central canal (nucleus lateralis; NL), and one smaller gray area is located dorsal to the central canal (nucleus dorsalis; ND). The nuclei are surrounded by the white matter of various thickness consisting of myelinated fibers.

The perikarya of the small size neurons in the gray matter can be unequivocally detected already on the toluidine blue stained plastic sections. On cross sections the perikarya are round or slightly oval in form, the round nucleus shows homogeneous structure, the nuclear membrane often invaginated, the nucleolus round and darkly stained. In longitudinal sections elongated perikarya with elongated nuclei are often seen in addition to the round cells. The perikarya measure 8-15 μm in cross sections, the elongated perikarya may be as long as 35-40 μm .

Immunolabeling with NeuN unambiguously showed that the FT contains a reach network of small, uniform neurons. Similarly to the findings in toluidine blue stained plastic sections, the NeuN labeled neurons were round or slightly oval in cross sections of the FT, but in longitudinal sections also the elongated perikarya occurred. The neurons form groups, often they are adjacent to the ependyma of the central canal.

Together with the identification of the neurons in the immunohistochemical studies, also antibodies detecting glial cells were used. Large number of astrocytes containing glial fibrillary acidic protein (GFAP) was found both in the gray and white matters. The astrocytes processes show radial orientation in cross section, and they are longitudinally oriented, especially in the caudal portions of the FT, in longitudinal sections.

In addition to the GFAP positive astrocytes, oligodendrocytes could be demonstrated using receptor interacting protein (RIP) antibody. RIP labeling was weak in the gray matter and intensive in the white matter, especially dorsolaterally in an area called shoulder region in this study. Dorsally from the shoulder region the white matter, unlike in the spinal cord, discontinues.

4.2.3. Neurochemical characterization of the neurons in the filum terminale

Since it was supposed that the nucleus lateralis of the FT is related to the spinal intermediate zone, immunohistochemical markers used for the identification of the neurons in the intermediate zone was used primarily in the immunohistochemical studies.

One or rarely two nitricoxide-synthase (NOS) positive neurons were found in a cross section of the FT. NOS labeling filled the cytoplasm around the NeuN labeled

nucleus and rarely labeled also proximal dendrites. NOS positive neurons measured in cross sections 5,2 to 11,96 μm (average: 7,96 μm ; n = 25).

Calretinin positive neurons were seen in the FT, one or more calretinin positive neurons were in a single section. They were located often adjacent to the ependyma. Thick calretinin positive nerve fibers were found in the white matter. The size of the perikarya measured 3,50 to 12,87 μm on the transverse sections (average: 7,43 μm ; n = 42). Calretinin positive neurons outnumber the NOS positive neurons with 2 to 1.

Round, choline acetyltransferase (ChAT) positive neurons were found in the nucleus lateralis. The size of the perikarya measured 7.0 to 9,0 μm . Short pieces from the immunostained dendrites were also seen.

The neurokinin 1 receptor (NK-1r) immunopositive neurons were the largest in the FT. The diameter of the perikarya was 12 to 18 μm in cross section. The immunolabeling outlined typically the cell membrane of the perikarya and dendrites. Cranio-caudally oriented dendrites were seen in longitudinal sections. Nk-1r positive fine axon varicosities were found in the gray matter, the axons formed bundles in the shoulder region of the white matter.

Small, round substance P (SP) positive perikarya with rich dendritic arborizations were found in the longitudinal sections.

Based on their location four types of immunostained axon arborizations were seen in the FT.

1. Axon arborizations directed exclusively to the nucleus dorsalis. CGRP immunopositive nerve fibers coursed in longitudinal direction in the nucleus dorsalis.

2. Axon arborizations predominantly in the nucleus lateralis. NOS-, SP-, and NK-1r immunopositive axon arborizations were found in the gray matter of the FT, mainly in the nucleus lateralis. Dense and delicate vesicular glutamate transporter 2 (VGLUT2) positive axon arborizations occurred in the nucleus lateralis. Synaptophysin immunoreaction was used to identify the synapsing axon varicosities. Delicate synaptophysin positive axon terminals covered densely the nucleus lateralis, they were seen also scattered in the nucleus dorsalis. Fine, glycine transporter 2 (GLYT2) positive axon arborizations were seen in the nucleus lateralis.

3. Axon arborizations in all nuclei of the gray matter. Cranio-caudally oriented VGLUT1 positive nerve fibers with occasionally with large size varicosities arborized

both in the nucleus dorsalis and lateralis. ChAT positive axon arborizations occurred in the entire gray matter.

4. Axon arborizations primarily in the shoulder region. A dense arborization of serotonin positive nerve fibers were seen in the shoulder region, they occur scattered also in the nucleus lateralis. Also enkephalin positive nerve fibers coursed in the shoulder region and seemed to terminate in the nucleus lateralis. Calretinin and NK-1r positive nerve fibers create a dense bundle in the shoulder region.

4.2.4 Co-localization, tight connections

The VGLUT2 and synaptophysin immunoreaction showed an almost complete co-localization in the nucleus lateralis. Serotonin positive varicosities showed partially co-localization with ChAT immunoreaction in the shoulder region. SP and enkephalin double labeled varicosities were often seen in the shoulder region and in the nucleus lateralis.

SP positive varicosities were located often direct on the surface of the NK-1r labeled perikarya and dendrites. In addition, GLYT2 positive delicate varicosities were found on the surface of NK-1r labeled perikarya.

4.2.5 Quantitative results

Approximately 75 to 85 NeuN labeled perikarya could be counted on a Vibratom section in the cranial portion of the FT. More caudally the same figure dropped to 20 to 25 perikarya. About 10% of the perikarya could be seen only in two optical sections 4 μ m apart from each other. Fifty-three percent of the perikarya could be found in at least three, 26% of the perikarya at least on four and 11% of them could be seen in five optical sections separated with 4 μ m distance from each other. Out of these raw data we concluded that approximately 37000 neurons are in a 5 mm long portion of the FT at its cranial portion. More distally, the number of the neurons in the same volume drops to approximately 15000.

4.2.6 Ultrastructure

The electron microscopic picture of the nucleus lateralis of the FT shows the ependyma. Lateral from the ependyma the perikarya of the small size neurons can be seen. More laterally the cross sections of the myelinated nerve fibers occur.

The lumen of the canalis centralis is slit like between the ependyma, it is filled with the cilia originating from the free surface of the ependymal cells. Perikarya of neurons are often attached directly to the basal surface of the ependyma. Neurons with dendrites extending into the lumen of the canalis centralis between the ependymal cells were also seen (liquor contact neurons).

Similarly to the lightmicroscopical findings, the 8-15 μm large neurons showed round or slightly oval shape on cross sections of the FT. The round nucleus in the perikarya contained the densely stained nucleolus. In longitudinal sections the perikarya showed oval shape, the nuclei within the perikarya were also of oval shape.

Synapses were classified in standard manner based partly upon the postsynaptic component (axosomatic and axodendritic synapses) and partly synaptic vesicles found in the axon terminals.

Axosomatic synapses were rarely found. In the presynaptic component the ovoid synaptic vesicles grouped next to the membrane thickening. Axodendritic synapses were frequently seen. The presynaptic terminals of 1 to 2 μm size established synapses equally well with postsynaptic dendrites of the same and much smaller size. It is conceivable that the dendrites consist of thicker and thinner portions and synapses occur also on the thin dendritic portions.

Synaptic arrangement consisting of 2 to 4 axon terminals surrounding and synapsing with a thicker dendrite – called synapse in wreath - was a characteristic feature in the FT. Synaptic vesicles of various shape occurred in the axon terminals. Axoaxonic synapses have not been seen.

Synapses were rarely seen in the nucleus dorsalis. Large quantity of exceptionally fine nerve fibers with cranio-caudal orientation was a characteristic finding in the nucleus dorsalis.

The periphery of the FT consists of longitudinally oriented myelinated fibers of various diameters.

Among the unusual ultrastructural findings were the liquor contact neurons and axon arborizations within the lumen of the canalis centralis. Surprisingly, axon varicosities attached to each other, but not necessarily to the ependyma were found in the canalis centralis. In addition to the unusual and non-conventional location, the axon terminals excelled with a rich content of dense core vesicles of 90-100 μm in diameter.

4.3 The structure of the filum terminale in cat

The transition at CM-FT junction in kittens is similar to that found in the rat. Toluidin blue stained plastic sections show the layer of the ependyma. The clear difference between the gray and white matter seen in the FT of the rat was somewhat ambiguous in the cat FT. Namely, myelinated nerve fibers occur only infrequently in the cat FT.

The neurons in the FT of the cat are small size neurons, but they occur less frequently in the cat than in the rat.

The processes of GFAP positive astrocytes showed different distribution pattern in cat than in the rat. In the FT the GFAP positive astrocyte processes formed a dense network encompassing the entire width of the FT.

NeuN labeled nerve cells were distributed predominantly around the ependyma. SP positive varicose nerve fibers coursed in the dorsal portion of the FT.

On the electron microscopic picture few myelinated fibers and large number of exceedingly thin unmyelinated nerve fibers can be seen. Similar arrangement of nerve fibers was found in the nucleus dorsalis of the rat.

Axosomatic synapses were rarely seen, however, axodendritic synapses could be easily found. Similarly to the rat, the synaptic connections between a large axon terminal and a much smaller dendrite occurred also in the cat. It was a conspicuous difference with respect to the findings in the rat that the astrocyte processes were filled with fibrils in the cat.

4.4 The structure of the filum terminale in the monkey

The characteristic features of the transition from the CM to the FT in the monkey were similar to those found in the rat and in the cat.

In the cranial portion of the FT the myelinated nerve fibers could well be seen in peripheral location. More caudally the nervous tissue is organized in spheres of 50 to 80 μm in diameter that are loosely connected to the basal surface of the ependyma. The free surface of the ependyma is decorated by cilia and microvilli. Perikarya of neurons, glial cells and numerous myelinated nerve fibers occur in the spheres. The size and the structure of the perikarya is identical to those found in the rat. Synapses in wreath occurred frequently, in which a dendrite was surrounded by several presynaptic terminals.

4.5 Connections of the filum terminale

The connections of the FT were studied by injection of BDA into the sacral segments of the spinal cord in rats.

The site of the injection was the intermediate zone. Adjacent to the injection site labeled neurons with long dendrites were found, axons were labeled in the anterior funiculus. Few retrogradely labeled neurons were found in the CM. In the cross section of the FT BDA labeled small structures were seen. Since these labeled structures could be followed in 15 to 20 $1\ \mu\text{m}$ thick optical sections (maximum 27) we suggested that they were the sections of the fine caliber, descending fibers

5. Discussion and conclusions

In this chapter the neuronal transformation in the caudal spinal cord, findings obtained in the rat FT will be discussed. Findings in the three animal species will be compared, and we will try to look into the future.

Neuronal transformation in the caudal spinal cord

The neural transformation of the spinal gray matter in the CM corroborates the view about the gross architecture of the spinal gray obtained from Golgi impregnated material.

The alterations in termination fields and disappearance of various classes of nerve fibers in the dorsal horn confirm earlier conclusions that terminal field of the peripheral myelinated fibers is more extensive both in cranial and caudal directions than that of the

fine, unmyelinated nerve fibers. Accordingly, only myelinated peripheral nerve fibers should terminate in the most caudal portion of the CM. The unmyelinated fibers terminate more cranially. One may conclude from this arrangement that the dorsal horn (laminae I-IV) consists of two large groups of neurons: the neurons of one of the groups are related to the substantia gelatinosa, the neurons of the other group is unrelated to the substantia gelatinosa.

The filum terminale is highly ordered nervous tissue

The neuronal and glial structure of the FT was studied using conventional and modern neurohistological techniques in the rat, cat and monkey. In all three species the axis of the FT is the canalis centralis. Highly structured nervous tissue has been found around the central canal in all three species.

In all three species the neurons were of small and middle size (8-15 μm in diameter) in the transverse sections of the FT. The neuronal character of the cells was proven with the immunostaining with the antibody raised against neuronal nuclear proteins (NeuN). Longitudinal sections of the FT and the serial optical cross sections revealed that the shape of a substantial portion of the neurons in the rat is ellipsoid. The longitudinal dimension of these neurons may reach 35 to 40 μm .

The longitudinally oriented myelinated nerve fibers were found in all three species.

In all three species the FT - consisting of nervous tissue - is the contradictory continuation of the spinal cord. The essence of the contradiction is that in spite of the nervous tissue character, the FT is not in direct connection with the periphery. It is well known that the direct sensory and motor connection with the periphery is the *sine qua non* character of the spinal cord.

The neuronal structure of the filum terminale is not identical in the species studied

In the cranial portion of the FT in the rat the neurons occurred densely and formed three nuclei. The myelinated nerve fibers at the surface of the FT reproduced the funiculi of the spinal cord. The discrepancy with respect the spinal cord appeared in the absence of the dorsal funiculus, and the white matter U shaped.

In the nucleus lateralis of the rat numerous axodendritic synapses were found, axosomatic synapses occurred rarely. The characteristic form of the axodendritic synapse was the synapse in wreath: several presynaptic axon varicosities, located in a ring, established synapses with a dendrite.

The neurons occurred rarely and predominantly in the vicinity of the ependyma in the cat, groups of neurons (nuclei) cannot be identified. Myelinated fibers were only occasionally found in the cat. In spite of this the structural difference between the gray and the white matters was obvious.

The nervous tissue was arranged in globular packages in the monkey FT, an arrangement that has never been seen in the two other species. Synapses in wreath occurred also in the monkey, 6 to 8 presynaptic axon varicosities around a large dendrite often could be seen.

In spite of the fact that the FT consisted of ordered nervous tissue in all three species, the neuronal structure showed significant differences between the species. Although the structure of the FT in the cat and monkey was not studied as comprehensively as in the rat, it might be assumed that due to the dissimilarities in the structure the functional significance of the FT – which is for the time being not clarified at all – is different in each species. Otherwise, the same neuronal function must have been presented with different neuronal structure.

The chemoarchitecture of the filum terminale

The structure of the glial cells in the FT was studied with two antibodies. The processes GFAP positive glial cells form a three dimensional, loosely woven network in the rat. With the same antibody a dense array of fine processes was found in the complete thickness of the FT in the rat. Fine threads of immunostained material were found between the ependymal cells. The difference in the astrocytes between rat and cat, the ultrastructural equivalent of which was also found further support structural differences in the FT as described and emphasized in the previous section.

The topographical arrangement of the oligodendrocytes was studied in the rat. Confirming the expectations, rich immunolabeling was seen in the white matter rich in myelinated nerve fibers. The immunostaining of the oligodendrocytes called our

attention to the U shape of the white matter. This was all the more conspicuous, because at the upper tips of the U the immunostaining was especially intensive.

The antibodies or the characterization of the neurons in the FT were selected upon the premise that the nucleus lateralis is the continuation of the intermediate zone of the CM. That is the reason why antibodies labeling NOS, calretinin, ChAT, NK-1r and SP were used in this study.

The ChAT and NOS containing neurons of the dorsal horn are inhibitory neurons. Also the neurons labeled with calretinin are mostly inhibitory. Glycin containing neurons are the prototypes of the inhibitory neurons both in the dorsal and ventral horns. The supposedly inhibitory neurons of the FT are balanced by the excitatory NK-1r immunoreactive neurons and the rich axon arborization labeled with VGLUT2 that is of excitatory nature, too.

The results of the immunohistochemical characterization of the neurons in the FT of the cat did not yield sufficient basis for interspecies comparison. NOS and calretinin immunolabeled neurons occurred also in the FT of the cat.

The varicosities of at least seven axon arborizations were found in the nucleus lateralis of the rat. NOS, SP and ChAT labeled varicosities are supposed to belong to the axons of the local neurons. VGLUT1 positive varicosities may be the descending collaterals of the thick primary afferent fibers of the coccygeal segments. It probable that also the VGLUT2 and GLYT2 positive varicosities belong to local neurons, although no labeled perikarya have been found in the FT. Serotonin and enkephalin containing varicosities may represent the descending fibers.

The chemoarchitecture of both the neuronal perikarya and the axon arborizations seems to indicate that the intermediate zone of the CM and the nucleus lateralis of the FT generate multiple interconnected neuronal networks, reminiscent of the reticular formation. As a working hypothesis we assume that the afferent fibers from the dorsolateral funiculus of the CM to the FT form a concentrated bundle in the shoulder region. Based on their size, rich dendritic arborization and their axons that course in the shoulder region, the NK-1r immunolabeled neurons may represent the efferent neurons in the FT. The other neuron labeled with various antibodies may function as interneurons of the networks.

The fine structure of the filum terminale

The ultrastructural studies confirmed the lightmicroscopical findings and revealed further details about the organization of the neuropil. Axon terminals synapse mainly with the dendrites. In addition to the synapses in which 1 axon established synapse with 1 dendrite, several axons and one dendrite synaptic configuration was also found. These latter synapses were called synapse in wreath. The presynaptic terminals contained round or ovoid synaptic vesicles, similar to those found elsewhere in the central nervous system. Dense-core vesicles were also seen in the axon terminals. Longitudinally cut sections showed that the presynaptic axon terminals were distributed in clusters also along the length of the dendrites. It was strange to see axon terminals of conventional size synapsing with a thin dendrite. Such synaptic arrangements were seen both in the FT of the rat and the cat. Large bundles of extremely fine axons found in the nucleus dorsalis of the rat and in the FT of the cat occur in also in the spinal cord.

Synapses typical to central nervous system were seen between the axon terminals and the neurons of the FT. Since the source and the termination pattern of the axons are unknown for the time being, it would be premature to devise functioning neuronal circles. To do this, the light- and electron microscopic study of functionally characterized and intracellularly labeled neurons would be a great contribution.

The connections of the filum terminale

The tract tracing studies let us presume the existence both ascending and descending connections between the spinal cord and the FT. The dorsolateral portion of the white matter, the area called shoulder region, seems to be the gate for both the ascending and descending immunostained axons. Just because of the location of the axons is the sign why we think that efferent neurons of the FT should be looked for among the NK-1r and perhaps the calretinin containing neurons. In addition to the shoulder region, the axons coursing in the ventral and lateral funiculi of the FT might belong to ascending or descending fiber tracts.

Since the results presented in the thesis revealed that the FT of the rat consists of organized nervous tissue, the extension of the morphological studies with tract tracing techniques are indispensable and inescapable. It can be foreseen reciprocal neuronal

connections between the FT and the spinal cord made by morphologically and neurochemically different fiber tracts.

Perspectives

After having seen the results obtained in three species and matching in many details it would be important to see the structure of the human FT. The classical view has been supported with up to date technique showing that the human FT is constructed by longitudinally oriented Type I collagen fibers and transversally oriented Type III collagen fibers. Large amount of elastic fibers were also seen in the human FT. Considering all these that transection of the FT, as the treatment of the “tight filum terminale syndrome” seems to be a routine surgical intervention.

In contrast, Choi et al. (1992) emphasized that the human FT is not a bundle consisting of connective tissue and blood vessels, but it contains nerve cells, and it may play an important role in the normal and pathological function of the spinal cord. Cummings and George (2003) concluded their results in which they compared human CM and FT using immunohistochemistry that the FT is not a fibrovascular tag.

The investigation of the neuronal and glial architecture of the human FT and tract-tracing studies in animal experiments are the two most urgent research topics.

Since the beginning of our studies the popularity of the FT, especially in stem cell related studies, has been increasing. Continuously dividing stem cells in the central nervous system are distributed along the ependymal layer of the lateral ventricles in the subventricular zone. Cells differentiating to neurons, astrocytes and oligodendrocytes were found around the third and the fourth ventricles, and also in the spinal cord, around the canalis centralis (Weiss et al., 1996). Recently, cells from adult human FT maintained under in vitro circumstances differentiated to the three prototypes of the nervous tissue: neurons, astrocytes and oligodendrocytes (Varghese et al., 2008).

We put forward that the entire FT – because of its size - is par excellence a subventricular organ. In this thesis we tried to build the foundation of molecular biological and genetical studies to further elucidate the function of the FT.

6. List of personal publications

Original papers

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