Tapering of Human Nerve Fibres

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Abstract. To determine the tapering of human nerve fibres, rostral and caudal root pieces of cauda equina nerve roots were removed and nerve fibre diameter distributions were constructed for 4 myelin sheath thickness ranges for the two sites, and compared with each other. The reduction of the group diameter in the different α-motoneuron groups was 0.2 % *per* 13 cm. Accounting for systematic errors, there may be even less tapering. An identified single nerve fibre showed no tapering. Further, there is indication that γ-motoneurons, preganglionic sympathetic and parasympathetic fibres and skin afferents also reduce their fibre diameter by 0.2 % *per* 13 cm or less. Consequently, a nerve fibre with a diameter of 10 μm would be reduced to approximately 9.8 μm at 1m from the cell soma. Preganglionic parasympathetic fibres were found to be represented in roots S1 to S5. At similar distances from the spinal cord, the mean diameter of ventral root α_1-motoneuron (FF) axons increased from the thoracic towards the lumbo-sacral region before decreasing again in the lower sacral region. Usually no α_1-motoneuron axons were found in S5 roots. The diameter distribution of unmyelinated nerve fibres of a ventral S5 root showed three peaks at 0.25, 0.95 and 1.2 μm. The unmyelinated fibres with diameters around 0.25 μm may represent parasympathetic fibres. In six selected areas of the ventral S5 root, 6.6 times more unmyelinated nerve fibres than myelinated fibres were found on the average.

Key words: Human — Nerve fibres — Tapering — Autonomic fibres — Root representation

Introduction

A research project is in progress, to treate central nervous system (CNS) lesions by first analysing human CNS functions under physiologic conditions and following CNS injury (Schalow 1991a,b, 1992, 1993a,b,c; Schalow et al. 1995a,b), and then

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to develop treatments for the patients through a functional reorganisation (and structural repair) of neuronal networks of the CNS in the way that the changed self-organisation of the patient's CNS give rise to natural physiologic functions again (Schalow and Zäch 2000). The partly lost relative phase and frequency coordination of the timed firing of the neurons in the lesioned human CNS is relearned (Schalow 2005) with the newly developed coordination dynamics therapy. A partial repair of the injured or malfunctioning human CNS by Schalow coordination dynamics therapy has been achieved (Schalow 2002a,b, 2003; Schalow et al. 2004).

The organisation and reorganisation of CNS networks can be better understood if one knows what natural impulse patterns run along what afferent fibre kinds to give rise to certain network organisations, measured by the natural output patterns running along somatic and autonomic efferent fibres. The identification of the neuron type can be improved when knowing the tapering of human nerve fibres.

By recording single-nerve fibre action potentials from thin long lower sacral nerve roots of the cauda equina, regulation mechanisms of the spinal cord and partly also of higher centres can be extracted from the simultaneous natural impulse patterns of afferents and functionally related efferents. These sacral nerve roots are ideal for simultaneous recording of single-nerve fibre action potentials of several afferents and efferents with wire electrodes, because of the mixing of afferents and efferents in the lower sacral nerve roots (Schalow 1991a) and because of the missing epi- and perineurium, due to which single-nerve fibre action potential currents are shunt. The nerve roots are only ensheathed by a thin layer of cells (Fig. 2A,B). Because of the ascensus of the spinal cord, the human caudal spinal canal is only filled with long nerve roots (Fig. 1A) (cauda equina), and offers a unique anatomical situation which is generally of interest: there is plenty of space in the spinal canal for performing surgery and to implant devices, e.g. electrical stimulators; the visibility is good (Fig. 1A) and the nerve roots are ideal for recording electrical signals and for electrical stimulation.

The only problem concerns identification. For example, we need to know from what kind of nerve fibres the recording is from, and what kind of nerve fibre groups have to be cut and which one to save for electrical stimulation in deafferentation surgery when performing a nerve anastomosis (Schalow 1992; Schalow and Barth 1992). We need to know the representation of certain functions in the cauda equina nerve roots like continence or locomotion, and we need a solid classification scheme of the peripheral nervous system (PNS) to be able to identify nerve fibre groups. A precise classification scheme for the human PNS has partly been developed by characterising a nerve fibre group by its group conduction velocity and its group nerve fibre diameter (Schalow 1991a), and partly by the natural impulse patterns of single fibres (Schalow et al. 1995b). The existing classification schemes for the PNS for animals, developed by Erlanger and Gasser (1937), Gasser and Grundfest (1939), Hursh (1939), Lloyd (1943), Lloyd and Chang (1948), Hunt (1954) do not apply to man and are too inaccurate for advanced human neurophysiology and modern microneurosurgery.

To identify nerve fibre groups in the cauda equina nerve roots and to improve the classification scheme of the human PNS, we need to compare the nerve fibre diameter distributions of peripheral nerves of a known nerve fibre group composition (e.g. skin, muscle or autonomic nerves) (Schalow 1992; Schalow et al. 1992) with the distributions of the cauda equina nerve roots. Such a comparison, however, is only justified if there is no (or only little) tapering of nerve fibres, which means that the nerve fibres have to have the same (or nearly the same) diameter both close to and distant from the cell soma. The tapering of nerve fibres is especially of interest in human neurophysiology since afferent and efferent nerve fibres can exceed 1 m in length.

The morphometric measurements indicate, that there is only very little tapering of human nerve fibres. Consequently it seems justified to compare nerve fibre diameter distributions of peripheral nerves with those of the nerve roots. Secondly, the classification scheme of the human PNS holds close to the CNS as well as at a distance from it, and thirdly, conduction velocities (saltatoric conduction) measured in the cauda equina can be compared with those measured from peripheral nerves. By teased fibre dissections it was shown earlier (Fig. 10C of Schalow 1989) that in human sacral nerve roots the internodel length L is related to the nerve fibre diameter (d, including myelin sheath) by $L/d = 100$. In frequency distribution histograms of internodal lengths therefore also grouping of nerve fibres can be observed (Fig. 10B of Schalow 1989).

Since with branching nerve fibres reduce in diameter (and internodel length), tapering (not due to branching) can only be measured in preparations where there is no or only seldom branching of nerve fibres over longer distances (several cm). Since peripheral nerves as nerve roots consist of many nerve fibre groups with different nerve group conduction velocities (Schalow and Zäch 1994, compound action potentials) and nerve group diameters, a peripheral nerve (or a nerve root) has not a certain conduction velocity. May be that the group conduction velocity of the fastest conducting group can be taken as the nerve conduction velocity.

No evidence of gradual tapering of nerve fibres were found in human lumbosacral spinal nerves (Liguori et al. 1992). In rat dorsal root ganglion cells, the peripheral myelinated axons have a 30 % thicker axon diameter than the central myelinated axons (Suh et al. 1984).

No comparable study of tapering of human or animal nerve fibres could be identified. No values with respect to tapering of nerve fibres could be found in the literature on animal neurophysiology concerning conduction velocities and fibre diameters.

Materials and Methods

Nerve root measurements were performed in 4 cadavers with no known neurological disease. The individuals were 50 to 76 years of age (male cadavers 1 to 2 days old); data were also obtained for one paraplegic patient (para 5) during

surgery for urinary bladder deafferentation and implantation of an electrical bladder stimulator (to improve the urinary bladder function and in this way to save the kidneys). The measurements were in accordance with the Helsinki Declaration of 1975.

Following laminectomy in the cadavers, the lower spinal cord with the cauda equina nerve roots and the covering dura were removed. After the opening of the dura, the medulla and the cauda equina nerve roots were mounted with needles on cork in a chamber 22.5 × 9 × 2 cm, in some similarity to Fig. 1B (the roots were aligned with the filum terminale), and fixed for 20 h in 4% glutaraldehyde in cacodylate buffer. Under a dissecting microscope, thin long root pieces were freed over a distance as long as possible between the spinal cord and the dura rootlets. Root pieces of 1 to 2 cm length were removed close to and distant from the spinal cord (Fig. 1) and postfixed in 1% OsO_4 for 4 h, and dehydrated and embedded in Araldite according to standard techniques. Pictures of semi-thin sections (approximately 1 μm thick) stained with thionin-acridine orange, were taken under a light microscope (magnification ×1000) (Fig. 2A,B; upper parts). Thin sections were made of the nerve root of the paraplegic patient for electron microscopy to perform morphometry of unmyelinated fibres (Fig. 6). Nerve fibre diameters (including myelin sheath) $\varnothing = 1/2\ (\varnothing_1 + \varnothing_2)$ ($\varnothing_1$ and $\varnothing_2$ are the larger and the smaller diameter of non-round shaped fibres, respectively) and the mean myelin sheath thickness d were measured by hand. A correction of 8% for shrinkage was allowed (Dyck et al. 1980). Four classes of myelin sheath thickness have been distinguished for the myelinated nerve fibres: $0.25\ \mu\text{m} \leq d < 0.8$; $0.8 \leq d < 1.3$; $1.3 \leq d < 1.8$; $1.8 \leq d < 2.3\ \mu$m. For each range of myelin sheath thickness, a diameter distribution histogram was constructed (Fig. 2 A,B; lower parts). In fibres damaged due to autolysis (split myelin sheath), the myelin sheath thickness was measured at the most preserved part. Very strongly damaged fibres were not considered when only a part of the cross-section could be measured, but were considered when the entire cross-section could be analysed. Because of the preference of the author of even to odd values, neighbouring histogram classes were partly pooled to easier compare nerve fibre diameter changes from rostral to caudal. Too poorly fixed and therefore too poorly stained roots (mostly because of advanced autolysis) were not considered. Even though the whole cauda equina of each individual was fixed at once, the fixation was still sometimes slightly different rostrally and caudally. Also, the osmotic pressure and the desiccation sometimes differed between rostral and caudal parts. Probably, more cerebral fluid was left sticking in the rostral cauda equina nerve roots, where the roots are more numerous (Fig. 1A,B). The morphometry was performed by hand. Computer-assisted morphometry can more precisely measure mean nerve fibre diameters of nicely stained and fixed fibres not damaged by autolysis. Since, however, computer programs cannot handle artifacts and altered nerve fibres sufficiently well, such programs will give worse results.

Results

Measuring procedure

To measure the tapering of human nerve fibres, the spinal cords were removed together with the cauda equina nerve roots from 4 cadavers (Fig. 1), fixed, and the nerve roots were then dissected to a maximum extent under the dissecting microscope in a chamber filled with the fixative. Especially the thin sacral roots were of interest because they are long and thin and can be expected to allow performing morphometry of the whole root cross-sections over a long distance between the rostral and the caudal root parts. After freeing a root from the other roots, samples of rostral and caudal roots were taken, post-fixed, embedded, stained, photographed, and the mean diameter and myelin sheath thickness were measured and nerve fibre diameter distribution histograms were constructed (Fig. 2A,B). The nerve fibre diameter distributions for the rostral and the caudal part were compared quantitatively, to find out whether human nerve fibres do or don't taper. The data of all analysed roots are summarised in Table 1.

For thicker roots (see vS3 root, Fig. 2A), the fibres were only measured in a part of the cross-sections and only nerve fibre diameter distributions were compared for the different myelin sheath thickness d ranges. The different α-motoneuron groups were mainly compared for the myelin sheath thickness range $1.8 \leq d < 2.3$ μm. Since there was often a considerable reorganisation and fasciculation of the roots on their way caudally (compare nerve root cross-sections in Fig. 2A and B), the same root cross-section area could not be identified safely rostrally and caudally. Only comparable areas were chosen for the analyses. In 5 cases, the root cross-sections could be measured completely and the rostral and caudal distributions were compared.

Reduction of the mean diameter of α-motoneurons in completely and partially measured roots

The reduction of the mean diameter of α-motoneurons was mainly measured for the different roots in the diameter range from 8 to 16 μm (including the individual measurements of the 3 α-motoneuron groups). The mean diameter of the α-motoneurons of the 10 measured cases dropped by –2.3 to 3.8 % between the cord and 13 cm caudally on the roots (Table 1). A mean value of tapering of 0.6 % *per* 13 cm was obtained for the α-motoneurons (and afferent fibres in one case) from the reductions of the mean diameters.

Reduction of the mean diameter in completely measured roots

In the roots which could not be measured completely, slight differences sometimes appeared in the composition of α_1-, α_2-, and α_3-motoneurons in the chosen areas of the cross-sections. More reliable tapering values are therefore obtained from Table 1 by only selecting cases in which the whole root or the fascicle have been analysed rostrally and caudally.

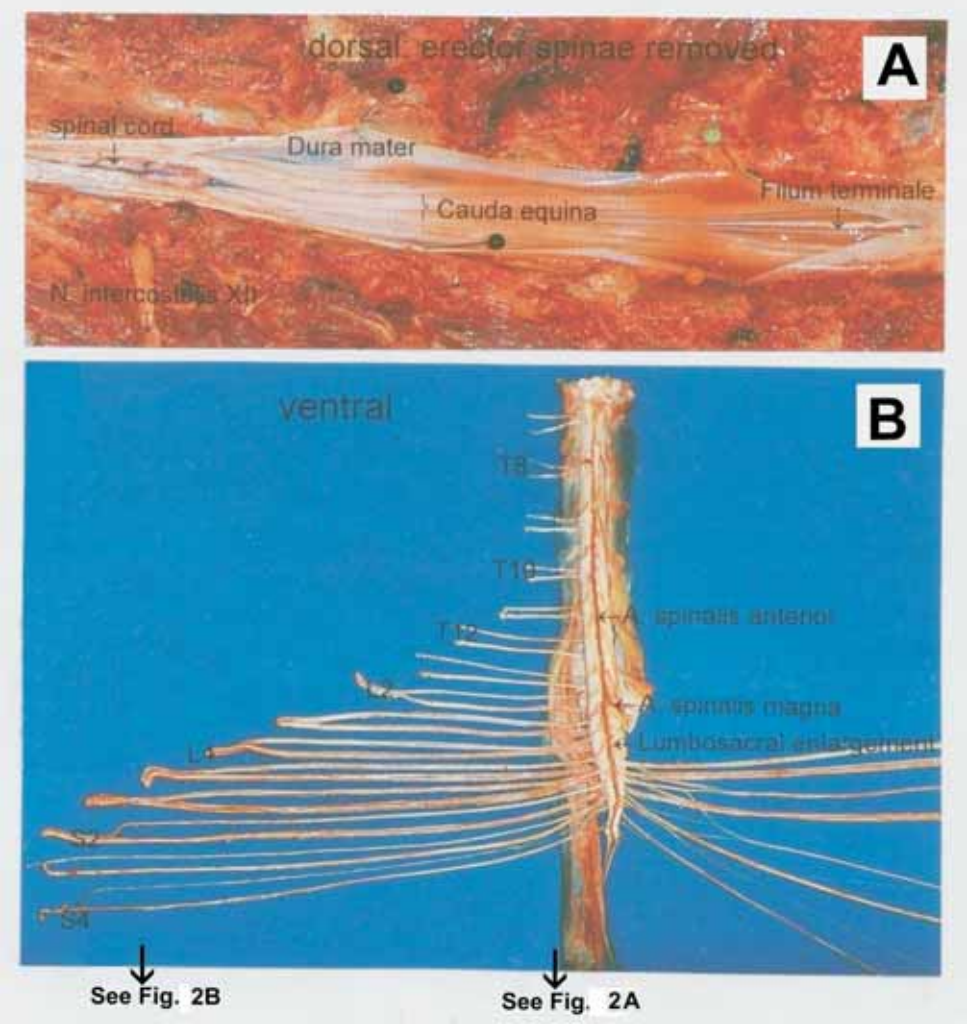

Figure 1. Schematic layout for the measurements of the tapering in human nerve fibres. After the opening of the spinal canal (A) and removing of the lower spinal cord with the cauda equina nerve roots and the dura mater (B), rostral and caudal nerve root samples of 1 to 2 cm length were removed from identified dissected and fixed nerve roots (mounting in some similarity to B).

In Fig. 3, the nerve fibre diameter distributions are shown for a ventral (v) S4 root for the 4 myelin sheath thickness ranges, measured close to and at a distance from the spinal cord. The distributions for the different myelin sheath thickness ranges were very similar, apart from the distribution for the myelin sheath thickness range $0.25 \leq d < 0.8$ μm. It seems that at 3 cm from the spinal cord there is a

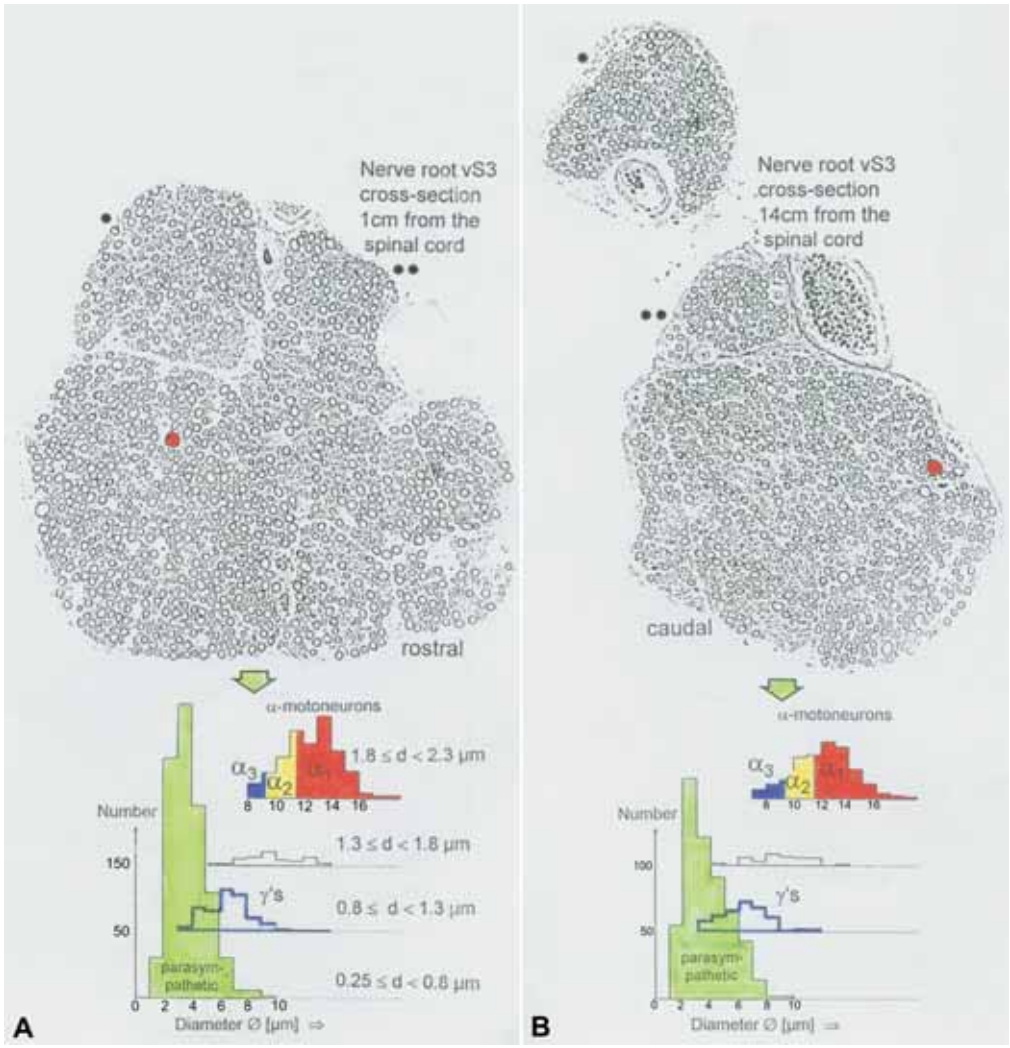

Figure 2. A. Rostral; **B.** Caudal. Comparison of the nerve fibre diameter distributions. After embedding, cutting and staining (thionin acridine-orange), the cross-sections were photographed (magnification $\times 1000$), and mean nerve fibre diameter and myelin sheath thickness were measured of the whole cross-section or parts of it. Nerve fibre diameter distribution histograms (with nerve fibre group characterisations (α's and γ's) from earlier measurements (Schalow 1991a; Schalow et al. 1995a, 1996a)) from the rostral and caudal cross-sections for the 4 myelin sheath thickness ranges d and the corresponding rostral and caudal distributions were compared with respect to tapering. Probable fasciculation of the vS3 root is marked by one or two stars. An identified single fibre in the rostral and caudal cross-sections is marked by red. If only a few ventral root afferents are present in the vS3 root, then the fibre diameter distribution of the myelin sheath thickness range $0.25 \leq d < 0.8$ μm represents mainly parasympathetic fibres.

Table 1. Reduction of the mean diameter of α-motoneurons (diameter range 8 to 16 μm; myelin sheath thickness d range 1.8 to 2.3 μm) from rostral (distance from the spinal cord 1 to 3 cm) to caudal (distance from the spinal cord 12 to 16 cm) site of the cauda equina nerve roots (Fig. 1). Sympathetic (symp.) or parasympathetic (para.) fibres – presence of symp. or para. fibres in the nerve root; vS3, ventral S3 root; dS5, dorsal S5 root; Para 5, paraplegic patient 5; α-mot, α-motoneurons; prox., proximal; γ's, γ-motoneurons. Number of α-mot (in the entirely or partly measured root cross-sections) rostral $\rightarrow$ caudal – α-mot in the diameter range 8 to 16 μm. Column 4 indicates the presense of symp. or para. fibres in the nerve root or nerve root fascicle

Case	Root	Part measured	Symp. or para. fibres	Reduction of mean diameter		Number of α-mot	Special notes
				rostral $\rightarrow$ caudal distance from cord	%	rostral $\rightarrow$ caudal	
1	vT12	whole spectrum	symp.	3 cm –			
1	vS1	whole spectrum	para.	3 cm –			
1	vS3	whole spectrum	para.	12.0 μm $\rightarrow$ 11.7 μm 1 cm 14 cm	2.5	282 $\rightarrow$ 205	
1	vS3	single fibre		23.5 μm $\rightarrow$ 23.5 μm 1 cm 14 cm	0		identified by thin myelin sheath and 2 blood vessels
1	vS4	whole crosssection, only α-mot	para.	11.4 μm $\rightarrow$ 11.4 μm 1 cm 13 cm	0	37 $\rightarrow$ 30	more fibres prox., root connections should exist
2	vL1	whole spectrum	symp.	1 cm –			
2	vL5	whole spectrum	–	12.0 μm – 1 cm –			
2	vS4	whole spectrum of whole root	para.	11.5 μm $\rightarrow$ 11.3 μm 3 cm 15 cm	1.7	174 $\rightarrow$ 175	73 % more fibres rostrally for $0.25 \leq d < 0.8$ μm
3	vL1	whole spectrum	symp.	3 cm –			
3	vL5	whole spectrum	–	13.2 μm $\rightarrow$ 12.7 μm 1 cm 13 cm	3.8	129 $\rightarrow$ 119	
3	vS1	whole spectrum	para.	2 cm –			
3	vS4	whole spectrum of whole root	para.	11.7 μm $\rightarrow$ 11.7 μm 1 cm 16 cm	0	27 $\rightarrow$ 52	root fascicle of 20 μm measured
3	vS4	whole crosssection, only α-mot	para.	12.3 μm $\rightarrow$ 12.3 μm 1 cm 11 cm	0	23 $\rightarrow$ 15	
4	vL5	whole spectrum	–	12.8 μm $\rightarrow$ 13.1 μm 1 cm 12 cm	2.3	163 $\rightarrow$ 159	no tapering for γ's
4	vL5	α-mot spectrum	–	16 cm			
4	dS5	whole root, α-mot	/	13.4 μm $\rightarrow$ 13.5 μm 1 cm 16 cm	–0.7	26 $\rightarrow$ 22	
Para 5	S5	whole spectrum, whole fascicle S5	para.	8.3 μm $\leftrightarrow$ 8.2 μm 3 cm 6 cm $7 \leq d < 11$ μm	1.2	70 $\rightarrow$ 70	

thin myelinated fibre population (fibre diameter ≈2 to 2.5 μm), which leaves, on its way to the caudal parts, the root *via* root interconnection (see below).

From the 5 cases, for which the whole cross-sections could be measured (in Table 1 marked by the thick frame), a mean tapering of 0.2 % *per* 13 cm of α-motoneurons was obtained. If one considers that, on average, distal parts of the roots showed a stronger desiccation in places where the cauda equina consisted of fewer thinner roots (Fig. 1A, right part), then the tapering of the nerve fibres may even be smaller than 0.2 %.

No tapering of an identified single α-motoneuron axon

In one case (vS3 root), it was possible to identify a single α-motoneuron axon rostrally and caudally by its size (the thickest fibre), its thin myelin sheath thickness (1.8 μm) and its close location to two thin blood vessels (marked by red in Fig. 2A,B). The measured mean diameter was 23.5 μm both rostrally and caudally, and showed therefore no tapering. The single fibre measurement makes it likely that there may be very little or no tapering at all of nerve fibres if there is no branching of nerve fibres in between the sites where the measured root samples were taken.

Tapering of afferents, γ-motoneurons and preganglionic parasympathetic and sympathetic fibres

The rostral and caudal nerve-fibre diameter distributions of the same root (Fig. 3) and other cases seem to indicate, that the γ-motoneurons and the preganglionic parasympathetic fibres do taper only little or not at all.

The tapering of the preganglionic sympathetic fibres, probably leaving the spinal cord *via* the TH10 to L2 roots, is more difficult to measure since these roots are much thicker than the lower sacral roots in which the parasympathetic fibres are contained.

Since, however, the preganglionic sympathetic and parasympathetic root fibres had very similar nerve fibre diameter distributions rostrally and caudally (Fig. 4), it seems that also the preganglionic sympathetic fibres (contained even more caudally in the ramus albeus), taper only very little or not at all. The much thinner and unmyelinated postganglionic sympathetic fibres (contained in the ramus griseus) have not been measured here.

Representation of preganglionic parasympathetic fibres in nerve roots S1 to S5

The measurements summarized in Table 1 indicate that the S1 roots also contained a substantial number of preganglionic parasympathetic fibres. The S1 root was identified by counting from the 12th intercostal nerve (subcostalis) and by the fact that the vS1 root is the last thick root caudally (Schalow et al. 1996a), (Fig. 1B). Parasympathetic fibres were found earlier in thin S5 roots. Since S5 roots are very thin (much thinner than the S4 root (Fig. 5 of Schalow et al. 1996a), their fibre numbers are not as important as those of the S1 root.

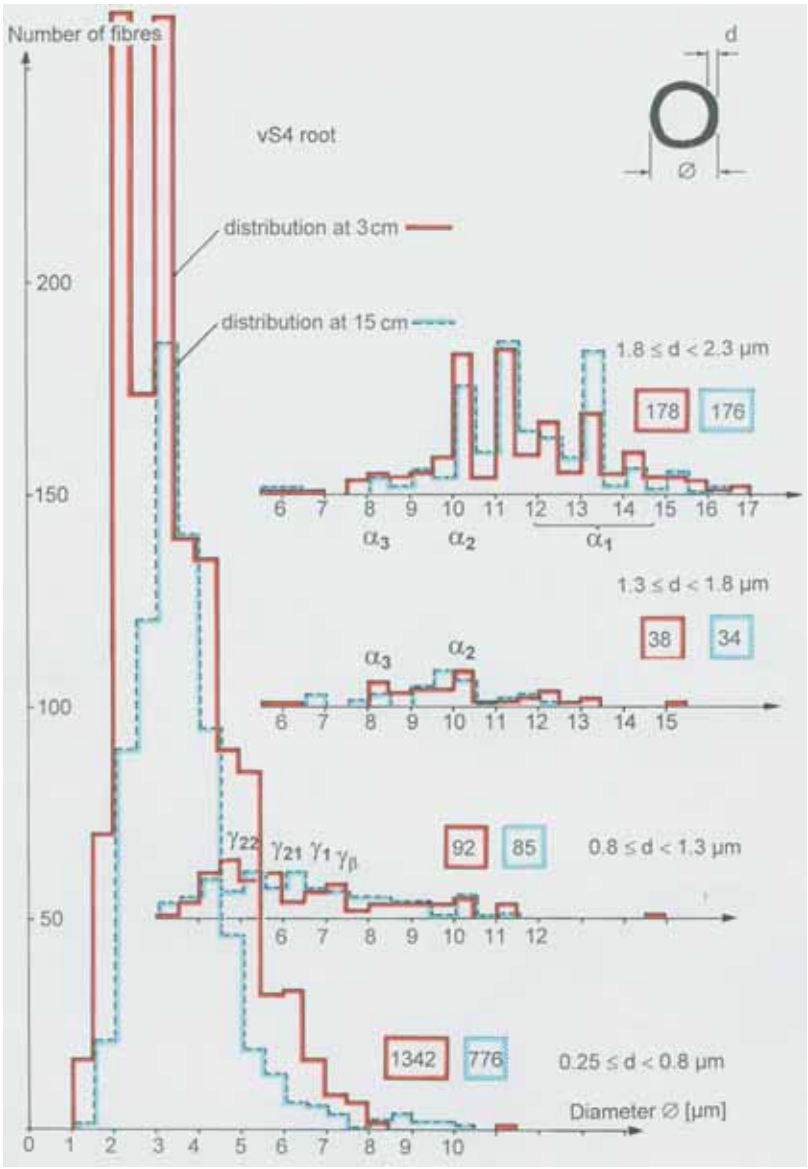

Figure 3. Nerve fibre diameter distributions for 4 myelin sheath thickness ranges d of the rostral (solid line) and the caudal (dashed line) cross-section of a vS4 root. Nerve fibre groups (characterised in earlier publications (Schalow 1991a; Schalow et al. 1995a, 1996a) by group conduction velocities and group nerve fibre diameters) are partly marked at the corresponding distribution ranges or peaks. Figures in the solid and dashed line rectangles give fibre numbers from the fibre distributions of certain myelin sheath thickness ranges rostrally and caudally. α_1, α_2 and α_3 – α_1- (FF), α_2- (FR) and α_3-motoneurons (S); γ_1 – dynamic γ-motoneuron; γ_{21}, γ_{22} – static γ-motoneurons.

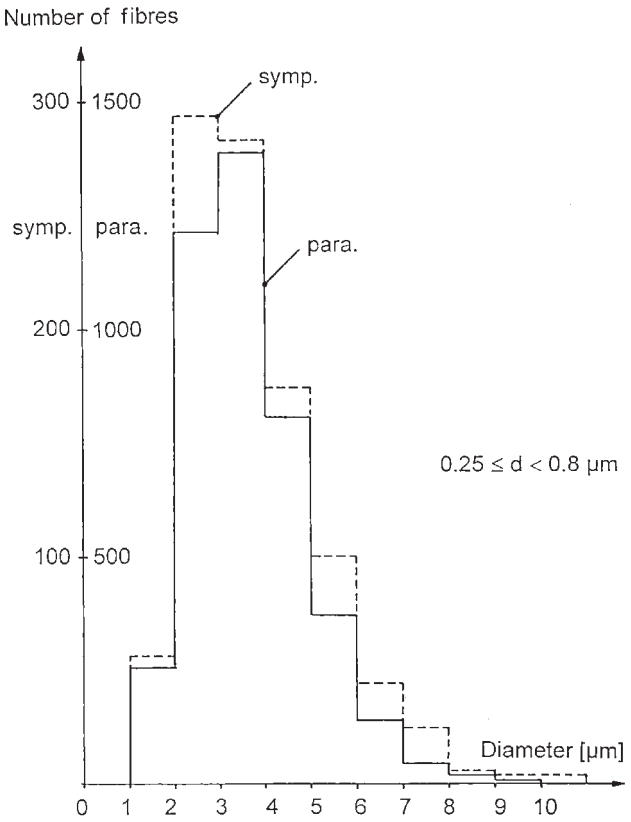

Figure 4. The sum of sympathetic (dashed line) and parasympathetic (solid line) efferent nerve-fibre diameter distributions of several roots. Myelin sheath thickness d between 0.25 and 0.8 μm. Note that the sympathetic and parasympathetic distributions are very similar. sympathicus (symp.): vL1, vT12 (3 cases); parasympathicus (para.): vS1, vS3, vS4 (7 cases).

Root inter-connections

As shown in Fig. 1B (right lower part), there are root inter-connections among the dorsal and among the ventral roots close to the spinal cord. More caudally at the nerve rootlets from the dura, there are also root inter-connections between the whole roots. Actually, the roots or root fascicles were dissected in between these two root connections. When dissecting the nerve roots, arachnoidea connections were cut, which kept the roots together.

But since a root fascicle of 20 μm diameter (with 14 myelinated nerve fibres) has been found here and since sometimes the roots had different nerve fibre group populations rostrally and caudally (for different numbers of α-motoneurons rostrally and caudally, see Table 1), there is indication that some of the arachnoidea

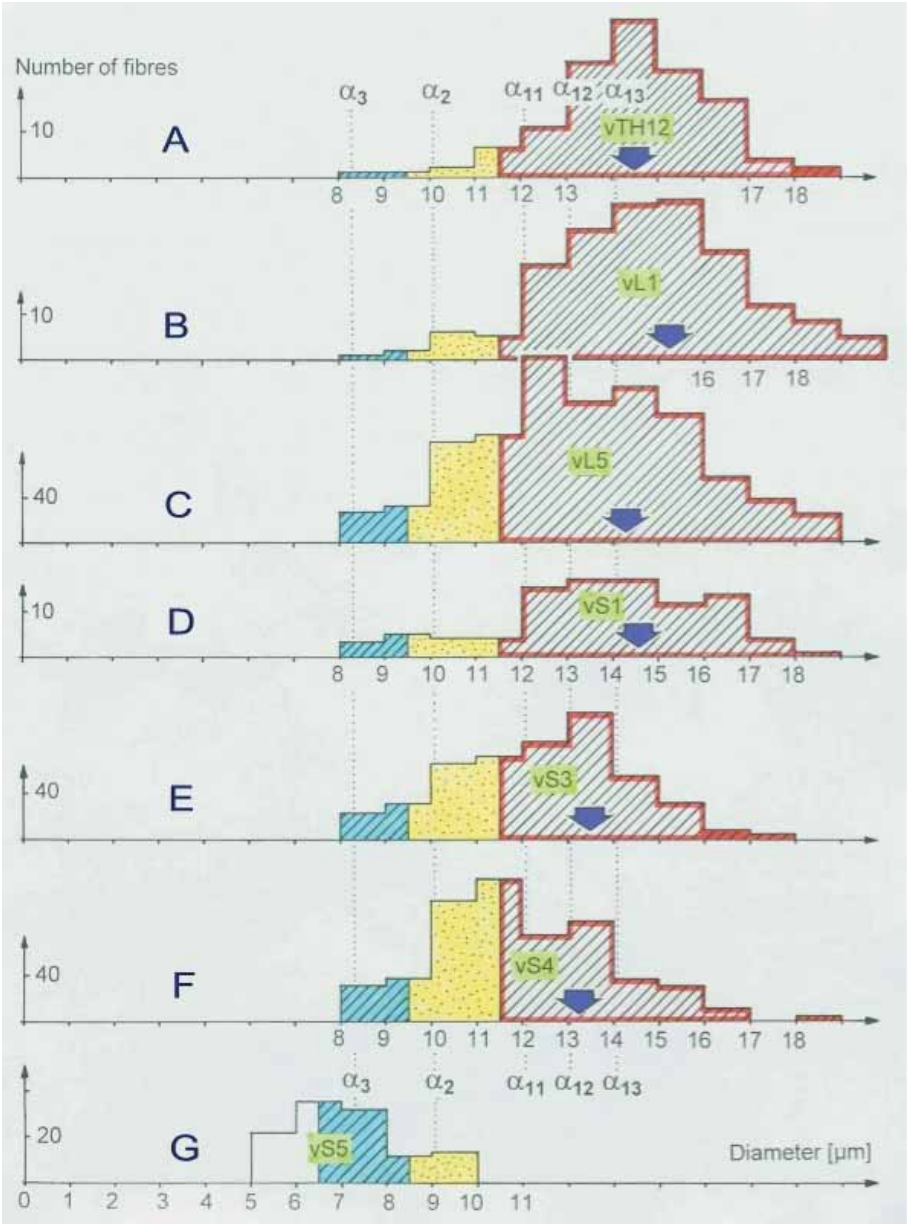

Figure 5. α-motoneuron type representation in nerve roots (A–G). Composition of sub-motoneuron groups in a number of diameter distributions of ventral roots in the myelin sheath thickness range $1.8 \leq d < 2.3$ μm. α_1, α_2, α_3 – α_1- (FF), α_2- (FR), α_3-motoneurons (S); α_{11}, α_{12}, α_{13} – possible subgroups of α_1-motoneurons.

cross-connections were really nerve root connections with an interchange of nerve fibres, which gave rise to a regrouping of the nerve groups in the roots; this is known to take frequently place in peripheral nerves.

Representation of differently sized α_1-motoneuron axons at different spinal cord levels

Fig. 5 shows that the mean of the α_1-motoneuron axon diameter distributions (indicated by an arrow) increase from the vTH12 root towards the lumbar roots, before decreasing again in the lower sacral roots. In the vS5 root, there were no α_1-motoneuron axons at all; this agreed with the electrophysiologic identification, when recording single-nerve fibre action potentials from undissected nerve roots during surgery. In the vL1 root, there were only a few α_2 and α_3-motoneuron axons contained. Even though these measurements were obtained from an arbitrarily selected set of cross-sectional areas, and the fibre distributions could vary in other sets, these measurements still reflect somehow the different distributions of differently sized α_1-motoneuron axons at different spinal cord levels.

Unmyelinated nerve fibres in a ventral sacral S5 root

In the cross-sections of the vS5 root of the paraplegic patient (24 years of age) unmyelinated nerve fibres were observed (Fig. 6A). Because of the existence of ventral root afferents in the lower sacral nerve roots (Schalow 1991a,b), the finding of unmyelinated fibres was to be expected.

Since in different cross-sections there seemed to be sometimes mainly thick or mainly thin unmyelinated fibres, mean diameter of unmyelinated fibres were measured, and diameter distributions were constructed (Fig. 6B). The distribution in one area of unmyelinated fibres showed three peaks (solid line distribution) at 0.25, 0.95 and 1.2 μm. In another part of the cross-section with mainly thick unmyelinated fibres (dotted line) the distribution showed only peaks at 0.95 and 1.2 μm. Because of the 3 distribution peaks at least 3 groups of unmyelinated nerve fibres seem to exist in the lower sacral nerve roots, which may belong to the somatic and parasympathetic nervous system divisions.

Numbers of unmyelinated nerve fibres in relation to myelinated fibres

In 6 areas of the vS5 root of the paraplegic patient, the number of unmyelinated fibres was measured. The fibre number varied quite much according to whether more thin fibres with a group diameter around 0.25 μm were contained or more thickly unmyelinated fibres were contained with probable group diameters of 0.95 or 1.2 μm. In the chosen areas (approx. 23×17 μm), the number of unmyelinated fibres ranged from 42 to 173, and the number of myelinated fibres from 10 to 16. The relation between unmyelinated and myelinated fibres ranged from 3.5 to 14; the mean ratio was 6.6. The ratio between unmyelinated and myelinated fibres depended therefore strongly on the number of thin myelinated fibres contributing to it.

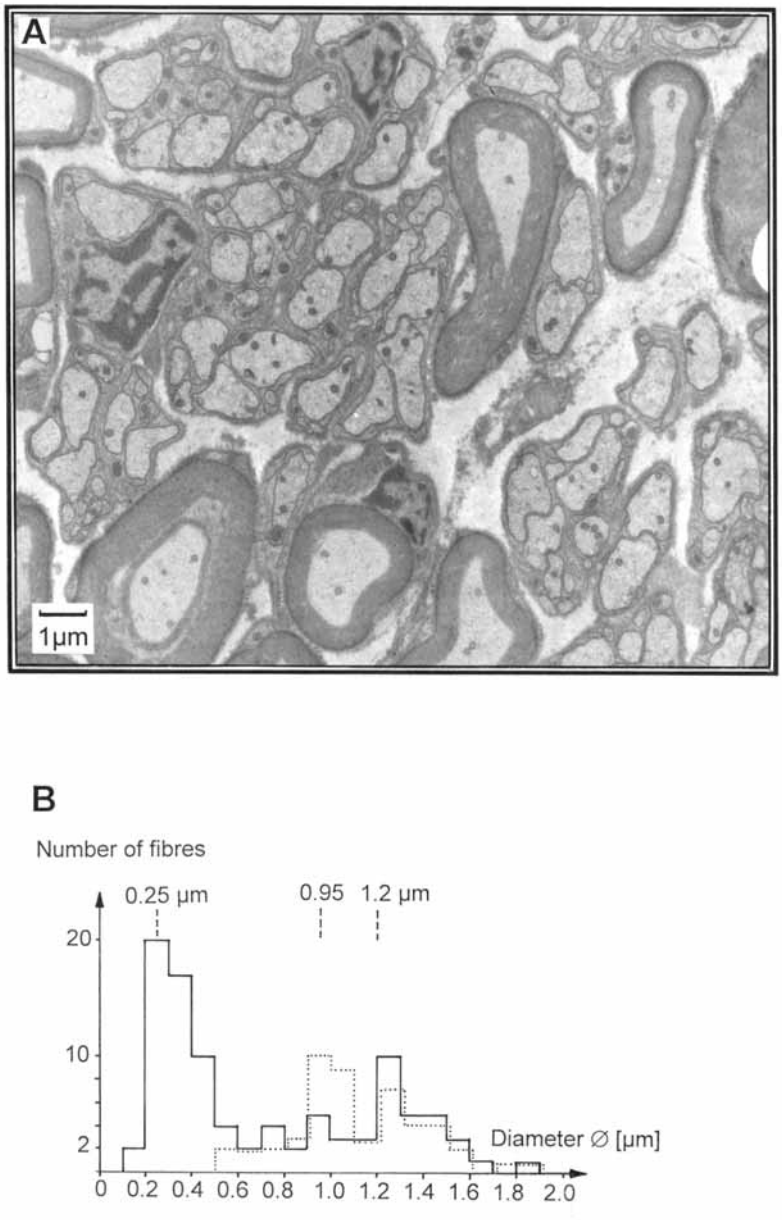

Figure 6. A. Section of a ventral sacral S5 root of a 24 years old female paraplegic patient Thin and thick unmyelinated nerve fibres can be seen besides myelinated nerve fibres. **B.** Nerve fibre diameter distributions of unmyelinated nerve fibres. The solid line distribution was taken from an area which the section in A was a part of. The dotted line distribution stems from an area, in which mainly only thick myelinated fibres were present. Note that the peak at 0.25 μm is missing in the distribution marked by the dotted line.

Discussion

Tapering of nerve fibres

A tapering of nerve fibres (including myelin sheath) by 0.2 % *per* 13 cm was identified by the present measurements, when the whole cross-sections of nerve roots were measured. Concerning the systematic error due to the mostly better fixation rostrally as a result of a slightly different osmotic pressure and different autolysis, and taking into account that an identified single fibre showed no tapering, it is safe to conclude that the tapering of nerve fibres is 0.2 % *per* every 13 cm of length or smaller. Morphometric work is in progress to show that the phrenic nerve fibres ($0.8\ \mu\text{m} \leq d$) reduce their diameter by 0.2 % *per* every 10 cm of length. There is indication for human nerve fibres showing a slight tapering tendency.

The conclusion that nerve fibres only show little tapering is in accordance with an earlier comparison of morphometric measurements on skin nerve branches of intercostals and a coxygeal nerve root (Schalow 1992), through which mainly skin nerve fibres run to the coxygeal dermatome. The nerve fibre diameter distributions of the nerves and the root were very similar, even though the coxygeal root piece was taken approx. 10 cm away from the ganglion and the spinal cord, whereas the skin nerve branch sample of the intercostal was taken approx. 25 cm away from the ganglion.

In Table 1, the dorsal (d) S5 root of case 4 shows a negative tapering value of 0.7 %. This negative value does not mean that afferent fibres get thicker the further away they are measured from the ganglion. One explanation for this distal bigger diameter is that nerve fibre diameters (including myelin sheath) vary from one internode to the next (Fig. 10A of Schalow 1989).

By taking the rostral root sample at a distance of 1 to 3 cm from the spinal cord, the central-peripheral transition zone (Carlstedt 1977) was not touched.

The analysed fascicle of the S5 root of paraplegic patient was most likely a ventral one, since a dorsal root fascicle would have thicker fibres, at least from the T1 (Pacinian corpuscle) skin afferents. The distance of approx. 3 cm between the rostral and the caudal (no safe identification of rostral and caudal because of surgery conditions) cross-sections is too small in comparison to the variation of the morphometry due to the random error, even though as expected, the quality of the root cross-sections from the patient was better than those from the cadavers (no autolysis). Long distances are needed to measure tapering.

Root inter-connections and branching of the cauda equina nerve root fibres

When these measurements were designed it was thought that there only are root interconnections close to the spinal cord (very rostrally, Fig. 1B) and distant to the spinal cord close to the ganglion. There should be no nerve fibre exchange between the different rather free roots in between the two boundaries: the dorsal and the ventral roots each stick together when leaving the spinal cord, whereas shortly before entering the ganglion, dorsal and ventral root fibres get combined. It was

found here directly that some of the arachnoidea connections were actually really root connections with a transposition of nerve fibres. Indirectly, transposition of nerve fibres between the roots was found because sometimes more fibres were found rostrally (and caudally) in completely measured roots. More fibres caudally can be explained by branching, but more fibres rostrally can only be explained by a nerve fibre transposition from one root to another one (or blindly ending nerve fibres).

Since often the number of α-motoneurons was very similar rostrally and caudally, it seems that there was no numerous branching of fibres in the cauda equina nerve roots.

As could be expected, a mild peripheral neuropathy was observed in these cadavers according to the age of the individuals: axonal degeneration and regeneration was suggested by circularly arranged groups of thinly myelinated nerve fibres.

Some fibres may be expected to end blindly in the roots. Also, some loops may be performed by a few nerve fibres. Even though there most likely were many minor variations (including mixing of afferents and efferents in dorsal and ventral roots), it still turns out that there is nearly no tapering of nerve fibres. The quality of the measurements might be improved, especially with respect to a better morphologic identification of the different nerve fibre group distributions (identification of groups), by measurements performed in the cauda equina nerve roots and certain nerves from brain-dead humans. However, such samples are extremely difficult to get.

Unmyelinated nerve fibres in lower sacral nerve roots

In the S5 root of the paraplegic patient, peaks at 0.25, 0.95 and 1.2 μm were observed in the distributions of the unmyelinated fibres. In the sural nerve (with mainly only skin afferent fibres) the unmyelinated fibres had diameters from 0.2 to 2.8 μm in some similarity to the values observed in this work. The peak value for the sural nerve ranged from 0.6 to 1.4 μm (page 72 of Dyck and Thomas 1993). The pronounced peak at 0.25 μm measured in this work may therefore be correlated to afferent or efferent parasympathetic nerve fibres.

The number of unmyelinated fibres in the selected areas ranged from 100,000 to 400,000 fibres *per* mm^2. In the sural nerve values of 19,000 to 65,000 unmyelinated fibres *per* mm^2 were found (page 72 of Dyck and Thomas 1993); the values found here are most likely higher because of the existence of additional thin parasympathetic unmyelinated fibres, and because selected areas of unmyelinated fibres were taken (values taken from whole roots could be smaller).

The number of unmyelinated fibres observed in the present work was approx. 6.6 times higher than that of the myelinated fibres. In the sural nerve, the number of unmyelinated fibres was approx. 5 times higher than the number of myelinated fibres (Dyck and Thomas 1993).

If the unmyelinated fibres with a group diameter of 0.25 μm were specific for parasympathetic fibres, then the morphometry of unmyelinated fibres could be used to identify parasympathetic pathways.

Consequences of little tapering

Since nerve fibres taper only very little, also the conduction velocities vary only little if there is no branching and change of membrane properties along the fibres. The characterisation of nerve fibre groups by a group conduction velocity and a group nerve fibre diameter can therefore be used to identify nerve fibre groups and neuron types.

Another consequence of the very little tapering of nerve fibres is that natural impulse patterns of efferents in nerve roots can be compared with the natural impulse patterns of single motor unit action potentials (MUAPs) of muscles as the impulse patterns will not change essentially along the way to the periphery. An improvement of the electromyography (EMG) in general and an improvement of the identification of natural impulse patterns of single MUAPs in multiunit EMG recordings seem possible by simultaneous recording of single-nerve fibre action potentials from nerve roots and single MUAPs from muscles innervated by the motoneurons recorded from (in the nerve roots), or simply by using the known natural impulse patterns obtained previously from motoneurons for the identification of single motor unit firings (Schalow et al. 1996b; Schalow 2005).

Identification of dynamic and static γ-motoneurons (and α-motoneurons)

In the human cauda equina, where a nice alignment of nerve fibres was expected, quite a lot of variability could be encountered. In peripheral nerves, more changes in the regrouping of nerve fibres and branching have to be expected. In CNS research, there is much discussion whether the branching of the reticular-spinal pathways already occurs in the reticular formation (Scheibel and Scheibel 1958; Waltzer and Martin 1984) or during their course in the spinal cord (Peacock and Wolstencroft 1976; Martin et al. 1981).

In man, α- and γ-motoneuron groups were identified by the group conduction velocities, and partly by group nerve fibre diameters and impulse patterns. Even though the conduction velocities were measured over small distances (10 mm), the relative group conduction velocities were very reliable, because the conduction velocities of all nerve fibre groups were measured simultaneously and were calibrated by the calibration relation; the secondary muscle spindle afferents conducted with the same velocity as the α_2-motoneurons, and the calibration relation was temperature independent (Schalow et al. 1996a), even though the conduction velocities themselves were strongly temperature-dependent. Sufficient characterisation of nerve fibre types is a principal problem when nerve fibres have to be identified safely for the understanding of neuronal network self-organization or the clarification of peripheral receptor properties of muscle spindles. Since group conduction velocity distributions overlap, γ- (and α-) motoneurons have to be identified additionally by their functions.

In measurements in man, the groups of α- and γ-motoneurons were identified by the speed and the dynamics to respond to natural stimulations such as touch,

pin-prick and catheter pulling, and the conduction velocity (Schalow 1993a,b,c). The characteristics of the static (γ_2) and the dynamic (γ_1) γ-motoneurons are not the same as in animal research. In human research, the γ-motoneurons were therefore characterised by the CNS properties, namely by the cell soma and in what neuronal networks the motoneuron cells are integrated in, whereas in animal research they were characterised by the periphery, namely how do primary muscle spindle afferents respond to repetitive 75 Hz stimulation (not always 75 Hz) of the static and dynamic γ-motoneurons in parallel with a ramp stretch (Boyd 1980).

To fulfil the needs of the human research and the clinical demands, we need a multiple group characterisation of γ-motoneurons, which can be used in animal research and in the clinical setting. For α-motoneurons, this has partly been achieved. α_2-motoneurons for example, fire for continuous high activation at about 6.7 Hz, have a group conduction velocity of 50 m/s, a group nerve fibre diameter of 10.2 μm and innervate FR-type muscle fibres which are fast oxidative glycolytic fibres (Schalow et al. 1995b). For dynamic and static γ-motoneurons, such a multiple characterisation is not known in humans. In human measurements, three conduction velocity peaks of γ-motoneurons (γ_1, γ_{21}, γ_{22}) seem to exist. The γ_1-motoneurons respond faster to stimulation than the γ_2-motoneurons (Schalow 1993a,b,c). It has to be shown whether the human γ_1-motoneurons (identified centrally) actually correspond to the dynamic γ-motoneurons as identified in animal research (identified in the periphery), and whether the γ_2-motoneurons correspond to the static γ-motoneurons. The notion will be helpful that there is no tapering of nerve fibres.

First electrophysiologic measurements from single human muscle spindles in the periphery have indicated that the human muscle spindles may function similarly as do cat spindles, even though the human muscle spindles seem to be more complex (Gladden 1986), and human muscle spindles may be quite different in various parts of the body.

Nerve conduction velocities

Since there seems to be no tapering in human nerve fibres, group conduction velocities obtained with the single-nerve fibre action potential recording method can be compared with the conduction velocities of the components of compound action potentials. For small conduction distances, this comparison has been started (Schalow and Zäch 1994). Nerve conduction velocities (Campbell et al. 1981) are frequently used in the clinics. They are not clearly defined, since a nerve contains different kinds of nerve fibres with different conduction velocities.

Identification of muscle spindle afferents

Primary and secondary muscle spindle afferents (Gladden 1986, 1992) have been distinguished from each other in animal research by their responses to a ramp stretch. Primaries respond stronger to stretch and show an instantaneous frequency rise. The secondaries show only an initial burst and frequency changes as if they

were smoothed by a condenser (Boyd 1980). The conduction velocities of the primary and secondary spindle afferents are clearly different. If spindle afferents from several muscles are pooled, the conduction velocity ranges start overlapping, and if velocities are even pooled from different animals the velocities from primaries and secondaries overlap more strongly.

In the recordings from the human lower sacral roots, most spindle afferents are probably secondaries, because they conduct in the secondary range, they are projecting polysynaptically onto α-motoneurons and they drive premotor spinal oscillators with phase relations of the broad peak type (page 61 of Schalow and Zäch 1996). The possibility cannot be excluded that the primaries in continence muscles have different properties. Probably, a few primaries do innervate the spindles of the pelvic floor and the musculus flexor hallucis brevis (*via* S4 (!) and more rostral roots). The secondary muscle spindle afferents conduct with a group conduction velocity of 50 m/s at 36 ℃ (group nerve fibre diameter 12.1 μm) and the primaries with a group conduction velocity of 60 m/s (group fibre diameter 13.2 μm), as identified by the group conduction velocities (Schalow et al. 1995a) and the conduction velocities of components of compound action potentials (Schalow and Zäch 1994). Since the conduction velocity ranges of primaries and secondaries overlap, further criteria are needed for a safe identification. In one measurement, a primary spindle afferent fibre could safely functionally be identified by its monosynaptic drive of an oscillatory firing α_1-motoneuron (FF; Schalow and Zäch 1996).

Research strategies

To obtain knowledge on the intricate functions and regulations of the somatic and autonomic nervous system divisions, a precise classification of the human peripheral nerve fibre groups is needed to identify safely what natural impulse patterns are conducted along what fibre types into the CNS for self-organisation. The fact that there is only very little or no tapering in human nerve fibres will help in the further characterisation and identification of nerve fibres.

It has been argued that the organisation of the human CNS is mainly determined by the integrated properties of the neurons when activated integratively in the neuronal networks (Kelso 1995) rather than by the properties of single isolated neurons. This may be so. But to understand specific CNS organisation in relation to behaviour (Schalow and Zäch 2000), we also have to measure the specific coordinated afferent input patterns with respect to time and space in many identified neurons (Schalow 1992), giving rise to specific CNS organisation.

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