Spatial Pattern of Nerve Fiber Abnormality Indicative of Pathologic Mechanisms

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Estimates of the number, density, and size distribution of myelinated fibers at selected levels of roots, spinal tracts, and sampled levels of peripheral nerves may be used in the detection and characterization of alterations of motor, sensory, and autonomic neurons and their axons with development, aging and disease. Use of imaging techniques, now available, increases the reliability, versatility, and speed of such analysis. In this study, the authors evaluated the spatial pattern of fibers in sampled frames and contour areas of transverse sections of nerve fascicles, utilizing, the coefficient of variation and index of dispersion (ID), the latter extensively employed by plant ecologists. The ID was used for recognization of increased, normal, or decreased variability of density within fascicles, between fascicles, and between nerves in health and in various experimental neuropathies. In addition, various morphometric measurements were made in transverse sections at defined levels along the hind limb nerves of rats in acute and chronic ischemia, after rhizotomy and in galactose neuropathy. These stereomorphometric studies, emphasizing the number, size, shape, and spatial pattern of fibers, revealed differences among experimental neuropathies and may be found to be helpful in the characaterization and prediction of pathologic mechanisms in neuropathies of unknown cause. Specifically, these approaches could be used for study of whether fiber loss in human diabetic neuropathy is multifocal and determination of the levels of such losses. (Am J Pathol 1984, 117:225–238)

EARLY ATTEMPTS to enumerate and size myelinated fibers (MF) in nerves and tracts produced quite variable results. A notable exception was the work of Erlanger and Gasser,[1] who related peaks of diameter histograms of fibers to components of the compound action potential, demonstrating that there were functional and morphologic classes and that conduction velocity could be related to diameter. With the introduction of improved fixatives, thinner sections, electron microscopy, improved sampling, and statistical approaches and computer techniques and imaging, it has become possible to determine the number, size, and shape of fibers (F) and of their subcellular components and organelles in transverse sections of peripheral nerves or fiber tracts with greatly improved precision and reliability.[2–4]

Morphometric approaches have also been extensively employed in the study of pathologic abnormalities of nerve.[5] In a typical experiment, the morphometric results, as derived from a sampled level of nerve from a group of animals (or of man) with a similar condition, are statistically compared with those from control sections. The summated areas of fascicles (transverse fascicular area, TFA) may be used in the detection of congenital maldevelopment,[6] hypertrophy,[7] or edema.[8,9] The number of fibers (MF or UF) per nerve, or F/sq mm, may be increased (as from sprouting) or decreased (as from degeneration). Diameter histograms, drawn to reflect numbers of F/nerve or F/sq mm may indicate congenital absence, acquired decrease or increase; absence, decrease, or increase of a subclass, and hypertrophy or atrophy of fibers. Such hypertrophy or atrophy may be suggested by an alteration in the range, the median diameter, and the position of the peaks in the diameter histogram. Regression lines relating axonal area to myelin area, myelin thickness, or myelin spiral length may provide evidence of hypertrophy or atrophy of axons. An altered shape of F profiles can be evaluated by determining percentages of certain fiber

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shapes or by calculating the index of circularity (IC = $\pi D/P$; where D = diameter and P = perimeter). An increase or decrease of axonal organelles may be studied by expressing their number per square micron of axon area or preferably per fiber. The size of the fiber may be expressed as area or as the diameter of a circle of equivalent area. When testing for atrophy, the number of organelles, eg, neurofilaments per fiber, may be regressed on myelin spiral length, if one assumes the latter measurement to be the best measurement of the fibers' former size. In health, the number and diameter histogram of fibers in a transverse section may be representative of a considerable distance of the nerve. In disease, however, single transverse sections may not be representative of pathologic alterations along the length of nerves. Three-dimensional morphologic reconstruction of peripheral motor, sensory, or autonomic neurons by serial section is generally not possible in mammals mainly because cells are too long and ramify too extensively. Reconstruction for short distances, however, is possible and can be performed on serial or skip semithin and thin sections. In simpler organisms, computer reconstruction of individual neurons and assemblies of neurons in serial sections has made it possible to study the size and shape of the soma and the branching pattern and size relationships of dendrites.[10,11] As a second approach, longitudinal sections may be used for recognition of alterations along the length of nerve fibers. For technical reasons, however, the study of a series of consecutive internodes of the same fiber is not possible. In the third approach, the examination of teased fibers, prepared in glycerin, may be used for assessment of many consecutive internodes of the same fiber. Such preparations have been used for recognition of alterations of internode diameter and length, myelin thickness, the presence and frequency of myelin wrinkling, segmental demyelination, remyelination, myelin reduplication, axonal degeneration and regeneration, and sprouting.[5] A modification of this approach, in which teased fibers are embedded into epoxy, allows for examination of transverse sections at defined points under light and electron microscopy.[12,13]

Because all of these approaches (serial reconstruction, longitudinal sections, and teased fibers) only evaluate morphologic changes for short distances (a cm or two), and because peripheral neurons are very long (approaching 1–2 m in man), sampling at specified levels is necessary for delineation of three-dimensional pathologic alterations.[14]

In this study, we have morphometrically evaluated sampled levels along the nerves of the hind limb of rats with experimental neuropathies to recognize stereomorphometric patterns which could be helpful in predicting pathologic mechanisms in human neuropathic diseases of unknown cause. The models differed in respect to whether fiber loss occurred and to the level and distribution of such loss. The objective was first to assess variability of MF density among sampled frames and between outer and inner contour areas in transverse sections of nerve with the use of the coefficient of variation (CV) and the index of dispersion (ID). Additionally, it is then possible to evaluate variability, with these approaches, among various levels along the length of nerves and among nerves with various conditions of development and disease. The study illustrates the use of these approaches and the advantages and limitations of the CV and the ID for these purposes. Models of neuropathy revealed different spatial patterns of fiber pattern, loss, and regeneration.

Materials and Methods

Models of Neuropathy

Lewis rats, weighing approximately 250 g, were used. The sciatic nerve, with attached tibial and peroneal nerves to the level of the knee, was removed after perfusion (rhizotomy) or after *in situ* (acute and chronic ischemia, lead, galactose, and control) fixation with 4% glutaraldehyde in 0.025 M cacodylate buffer for approximately 15 minutes. The nerves were then immersed in 2.5% glutaraldehyde in 0.025 M cacodylate buffer for another 8 hours. After washing, nerve collars were cut at the levels shown in Figure 1, dehydrated, infiltrated, and embedded in epoxy. Transverse 0.75 μ semithin sections were cut and stained with paraphenylenediamine.

Rhizotomy

Under sodium pentobarbital anesthesia, a laminectomy was performed and the L5, L6, and L7 ventral (VSR) and dorsal roots (DSR) were cut. This causes degeneration of peripheral nerve axons derived from VSR but not, in 30 days, of axons derived from spinal ganglion.

Acute Ischemia

Polystyrene microspheres, 5.6×10^6, 15 μ in diameter, were injected into the vascular supply of the sciatic, tibial, and perineal nerves for selective occlusion of capillaries. This procedure reproducibly produced regions of ischemic damage, beginning in the central fascicular regions of the sciatic nerve in the thigh and extending more diffusely into the tibial and perineal nerves.[15]

Chronic Ischemia

Nerves damaged by microsphere embolization, as described above, were examined 6 weeks later. At this time

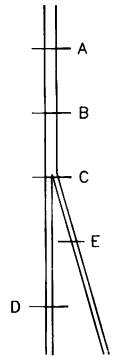

Figure 1—The levels of sciatic (*A*, *B*, and *C*), tibial (*C* and *D*) and peroneal (*E*) nerves morphometrically evaluated.

the central fascicular regions contained many small regenerating fibers.

Lead Neuropathy

Four percent lead carbonate was mixed into their pellets and fed to the rats for 6 months.

Galactose Neuropathy

Forty percent galactose was mixed into the pellets and fed to the animals for 8 months.

Morphometric Approaches

Semithin (0.75 μ), carefully orientated, transverse nerve sections were stained with paraphenylenediamine and evaluated with the use of our Imaging System for Nerve Morphometry (ISNM). At low magnification and using a digitizing tablet, we traced the inner edge of the perineurium of each fascicle. The areas of fascicles were summated to provide the transverse fascicular area (TFA). At a high magnification (eg, $\times 2000$), fascicular areas were sequentially traversed, in an x and y direction, under an ocular frame. The area examined corresponded to the frame digitized and displayed on video. The numbering of the frames began on the left-hand side of the upper horizontal row of frames of a fascicle. Systematic sampling of frames was achieved as shown in Figure 2. The first frame to be analyzed was chosen at random. Imaging was used to identify MF in the sampled frames, to border the inside of myelin, and to measure perimeter and average thickness of myelin. From these measurements we derived the following for each fiber: for axons, area, perimeter, diameter (of a circle of equivalent area), and index of circularity ($IC = \pi D/P$); for myelin, inner and outer perimeters, area and thickness; and for MF, area and diameter (of a circle of equivalent area). The ratio of πD to P is a measure of circularity which will equal unity for a circle and less than unity for a noncircular profile such as an ellipse and will converge to 0 as the minor axis for the ellipse converges to 0. We stored this information for each MF by frame, by nerve, by rat, and by experimental model on a computer disk. From these stored values, we determined for MF the number of MF per frame, the number of MF per fascicle, the number of MF per square millimeter and the number of MF per nerve, and the CV and ID as described below. Histograms of axons (area or diameter), myelin (area or thickness), and MF (area or diameter of equivalent area) for fascicles per square millimeter or per nerve were then plotted. Using healthy nerve, we were able to identify the diameter position of troughs between peaks and to determine the number of large (LMF) and small (SMF) MF.

Morphometric and Statistical Approaches Used to Assess Fiber Pattern

Measurements which might be used in an assessment of fiber distribution or pattern would include the distance to the nearest fiber (FD), the shortest distance to the perineurium (SPD) or to another structure, the num-

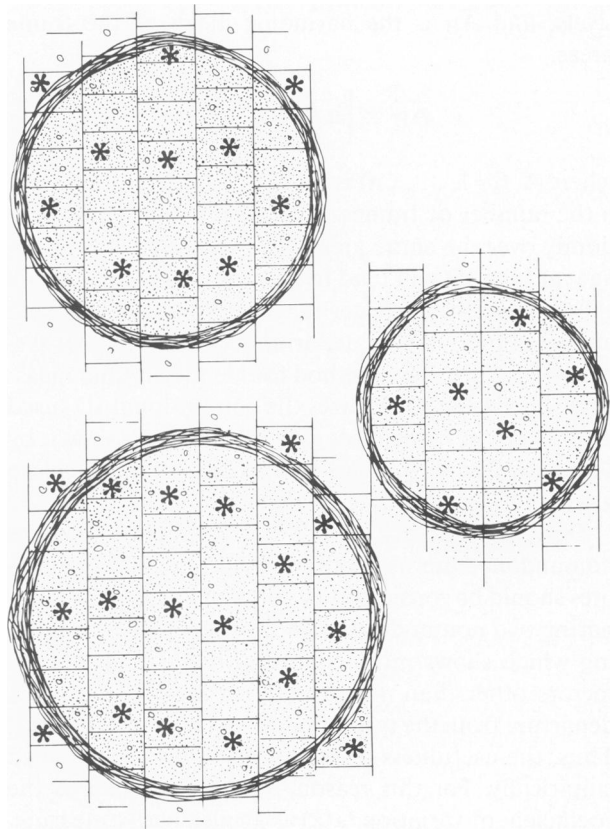

Figure 2—Diagrammatic representation of systematically sampled frames (1 in 3) in an x and y traverse of the fascicular area of a nerve beneath the ocular frame of a microscope. The frames which were morphometrically assessed are indicated by an *asterisk*.

ber of MF per frame or MF per square millimeter, and the density of fibers in outer to inner contour areas of fascicles. An outline, equidistant to the inner edge of the perineurium, was generated by computer program so that 50% of the fascicular area lay outside and 50% lay inside of it.

To recognize focal or multifocal fiber density decreases or increases, systematic sampling of frames was performed. We assumed frame size to be an important variable in this type of study. If frames are too large, multiple fields of a fascicle cannot be surveyed and variability of density within fascicles cannot be estimated. If too small, and an extreme example, a frame would have one or no fibers. For most nerves, the use of an ×63 objective and a final magnification of approximately ×2000 appeared to be suitable for the purposes of determining the number, size, shape, and spatial pattern of MF.

Statistical Indices

Let x_i represent the fiber density in the ith frame (i = 1, . . . , number of frames). The index of dispersion is defined by ID = $\bar{A}_H s^2/\bar{x}$, where $\bar{x}$ and s are the mean and standard deviation of the densities, respectively, and $\bar{A}_H$ is the harmonic mean of the frame areas.

$$\bar{A}_H = \left[n \sum_{i=1}^{n} \left(\frac{1}{A_i} \right) \right]^{-1}$$

where A_i (i=1, . . . , n) is the area of the ith frame and n the number of frames. If fibers are distributed randomly over the entire area, $\bar{A}_H s^2$ will equal x on average and the ID will tend to be near 1. Conversely, the ID will tend to be greater than 1 if fibers are clustered and less than 1 if they are arranged in a uniform, systematic pattern. This method for identifying and measuring clustering generalizes the conventional ID (used extensively in plant ecology) and has been shown by Perry and Mead[16] to have good sensitivity in detecting a wide range of clustering patterns.

Despite the ability of the ID to detect and measure nonrandom patterns, there are reasons why other measures should be considered as well. For example, in comparing two nonrandom patterns, the criteria for judging which shows more clustering might be based on factors other than departures from randomness (eg, departure from the type of pattern expected in health). Thus, the usefulness of any measure must be verified empirically. For this reason, we have considered the coefficient of variation ($s/\bar{x}$) as an alternative measure.

The differences between the ID and CV are illustrated with some idealized hypothetical examples in which frame areas all equal 1 and nerves and number of frames are very large, so that observed values of $\bar{x}$ and s may be taken to be the true values in the nerve. First, we suppose that fibers are randomly distributed in the nerve with x = 4 and s = 2. In ths case the CV = 0.5 and the ID = 1.

Next, suppose that fibers are randomly distributed in a nerve of equal size, but the density of fibers is increased 100-fold, so that $\bar{x}$ = 400. (Note that this is not the same as multiplying the number of fibers in each frame by 100, because this would introduce a clustered, nonrandom arrangement.) To visualize this, consider again the extreme case where, although fibers are distributed randomly, frame sizes are so small (or fibers so sparse) that each frame contains either 0 or 1 fiber. If the number of fibers in each frame is then multiplied by a very large number, a clear pattern of clustering emerges. Because the ID must equal 1, s^2 = 400 and the CV 20/400 = 0.05. Although the fibers are distributed randomly in both cases, the CV would indicate a marked reduction in clustering. Similarly, when density is decreased, the CV will indicate increased clustering.

At first glance, these examples would suggest that the CV should not be used to measure clustering. However, despite the limitations within the context of random distributions, the CV may retain some usefulness for comparative purposes when fiber distribution is known to be nonrandom. To illustrate, suppose that in health fibers tended to be distributed in such a way that s = $\bar{x}$. In this case, the CV = $s/\bar{x}$ may be a more meaningful measure of clustering. Because for many phenomena the standard deviation is directly proportional to the mean, we have computed both the ID and the CV in our evaluations.

We observe that if the underlying distribution of fibers is random, both the ID and the CV are unaffected by changes in area observed, number of frames observed, or the magnification used. In terms of the first example, the ID and the CV will still equal 1 and 0.5, respectively. In practice, of course, the ID and CV are subject to random fluctuation. This random variation may be reduced for both indices by increasing the number of frames sampled.

If one still assumes a random distribution, increasing the area will also reduce the random fluctuations in the CV but not the ID. On the other hand, if the distributions are nonrandom, increasing the area will cause the ID to increase (enhancing our ability to detect any departure from randomness), while the CV will be unchanged, providing frames are sampled from homogeneous regions, ie, providing that the areas do not become too large for detection of clustering, as discussed previously. This phenomenon may also be illustrated in terms of the first example. Suppose that the

Table 1—Acute Ischemia

Group	TFA	Number per nerve			Median diameter (μ)	Range (μ)		CV between frames			IV between frames		
		MF	LMF	SMF		Minimum	Maximum	MF	LMF	SMF	MF	LMF	SMF
Upper sciatic (A)													
Control (n = 4)													
Mean	0.632	10,687	7733	2954	7.14	1.24	14.32	17.40	15.50	37.23	2.20	1.32	3.03
SD	0.142	2490	1618	909	0.25	0.10	1.19	1.63	4.00	15.23	0.43	0.67	1.76
Disease (n = 4)													
Mean	0.771	11,715	7978	3737	6.91	1.29	12.33	16.75	15.85	35.63	1.80	1.13	2.77
SD	0.061	691	862	218	0.40	0.10	1.48	2.43	3.23	10.49	0.53	0.44	1.80
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Lower sciatic (C)													
Control (n = 4)													
Mean	0.431	7924	5556	2368	6.58	1.13	12.16	13.95	11.35	31.85	1.48	0.75	2.21
SD	0.091	406	541	241	0.59	0.11	1.27	1.53	2.65	6.58	0.15	0.28	0.50
Disease (n = 4)													
Mean	0.663	7353	5130	2223	6.52	1.26	13.07	34.78	31.53	58.17	5.61	3.64	4.32
SD	0.123	988	607	414	0.04	0.32	1.83	4.55	3.43	13.20	0.64	.32	1.64
P	0.02 < *P* < 0.025	NS	NS	NS	NS	NS	NS	< 0.001	< 0.001	0.01 < *P* < 0.02	< 0.001	< 0.001	0.025 < *P* < 0.05
Lower sciatic (tibial only)													
Control (n = 4)													
Mean	0.275	4753	3516	1237	6.92	1.23	12.08	19.05	16.73	40.03	2.48	1.44	2.79
SD	0.064	361	460	140	0.72	0.10	1.10	4.50	4.34	15.99	0.70	0.58	1.41
Disease (n = 4)													
Mean	0.463	3447	2642	805	7.13	1.58	13.10	59.50	57.00	89.40	10.96	7.68	10.12
SD	0.099	488	306	191	0.05	0.25	1.83	10.96	8.01	31.48	3.36	1.69	3.46
P	0.01 < *P* < 0.02	0.005 < *P* < 0.01	0.01 < *P* < 0.02	0.01 < *P* < 0.02	NS	0.025 < *P* < 0.05	NS	0.001 < *P* < 0.005	< 0.001	0.025 < *P* < 0.05	0.001 < *P* < 0.005	< 0.001	NS
Tibial (D)													
Control (n = 4)													
Mean	0.147	3003	2234	768	6.71	1.11	11.50	9.75	8.80	33.63	0.82	0.48	2.58
SD	0.029	292	313	74	0.49	0.10	0.92	3.54	1.51	16.23	0.48	0.17	1.68
Disease (n = 4)													
Mean	0.202	1796	1322	474	6.45	1.33	10.42	41.18	42.45	57.40	6.54	5.15	3.35
SD	0.380	399	355	70	0.36	0.15	1.04	10.78	13.21	18.29	3.56	3.05	1.94
P	NS	0.001 < *P* < 0.005	0.005 < *P* < 0.01	0.001 < *P* < 0.005	NS	NS	NS	0.001 < *P* < 0.005	< 0.001	NS	0.01 < *P* < 0.02	0.02 < *P* < 0.025	NS

area sampled in each frame were multiplied by 4 and the density held constant, thus introducing clustering. To visualize this, consider again the extreme example of two frames of equal area in which the first contains 1 fiber and the second contains none, providing no evidence of clustering. If both areas are enlarged 1000-fold and density is held constant, we will now have 1000 fibers in one frame and none in the other – clear evidence of clustering. Since the density of each frame is unchanged, the CV will still equal 0.5, but the ID is now equal to 4.

Results

Acute Ischemia

In Table 1 we give morphometric values which were derived from transverse sections of upper sciatic nerve (A), lower sciatic nerve (C), the tibial division of C, and the tibial nerve below the knee (D) as diagramatically shown in Figure 1.

Considering morphometric results among different nerves of healthy rats, differences were found, as expected, for TFA, number of MF per nerve, number of LMF per nerve, number of SMF per nerve and per square millimeter, median MF diameter, and MF range of diameters. Most of these differences were statistically significant.

Statistically significant differences in the variability of MF densities between frames, as measured by the CV and ID, were found between some of the nerves. Without exception, the CV and ID were always highest for SMF and lowest for LMF. Using the ID, the variability of frame densities for large fibers varied between 0.48 and 1.32, whereas for small fibers it varied between 2.21 and 3.03. These results indicate that in healthy rat nerves, large fibers approach a random distribution, with some differences between levels, while small fibers tend to be clustered.

If acute ischemic nerves are now considered, striking differences are found (Table 1). At level A, no statistically significant differences in morphometric parameters were encountered between ischemic and normal nerves. At level C, striking morphometric differences were found between the whole and the tibial division. Considering the whole nerve, statistically significant increases in transverse fascicular area (TFA) but not in the number of MF per nerve were obtained. The increase in TFA was relatively greater for the tibial nerve than for the whole nerve. A highly significant decrease of MF per nerve (both large and small MF) was found when the tibial nerve was considered alone.

Considering the variability of MF densities between frames, both the CV and the ID were greatly increased for both the whole nerve and for the tibial nerve alone. For the tibial nerve, the CV was 3.1, 3.4, and 2.2 and the ID was 5.3, 4.4, and 3.6 times greater in disease than in control nerves for MF, LMF, and SMF, respectively. For the whole distal sciatic nerve and for the tibial nerve, the greatest increase in the CV and the ID was for LMF, followed by MF and then by SMF.

At level D, the number of MF per nerve (both large and small MF) was significantly decreased from normal. The CV was 4.2, 4.8, and 1.7 and the ID was 8.0, 10.7, and 1.3 times greater than for controls for MF, LMF, and SMF, respectively. In order to display these changes graphically, we computed the mean value for each measured characteristic at each site on the diseased

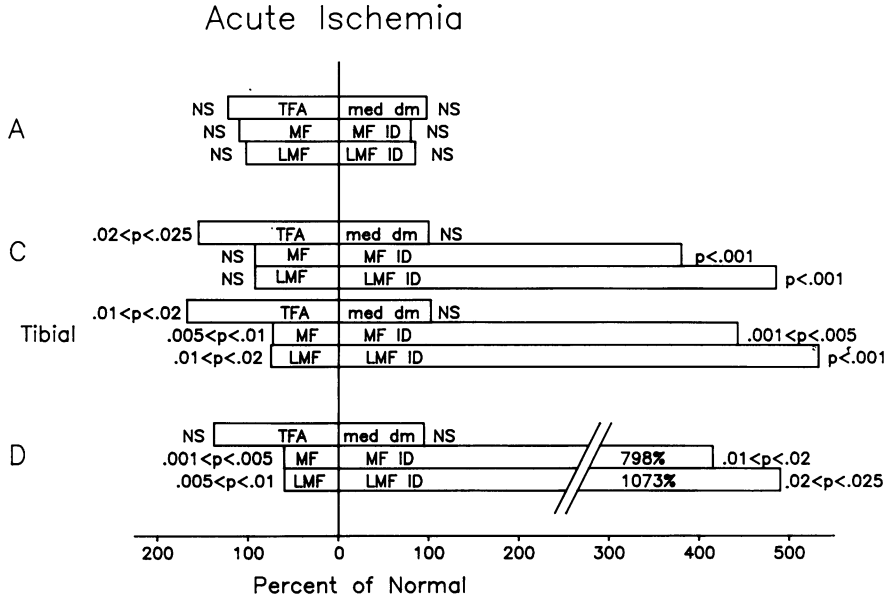

Figure 3 – The morphometric and MF density variation in upper sciatic (*A*), distal sciatic (*C*), tibial at *C* and tibial at *D* nerves of a rat with acute ischemia, as described in the text. *TFA*, transfascicular area; MF (myelinated fiber), MF number per square millimeter; *LMF*, LMF number per square millimeter; *med dm*, median diameter of MF; *MF ID*, MF index of dispersion; *LMF ID*, large MF ID.

Table 2—Tibial (Mid) Acute Ischemia

Group	TFA	Number per square milliliter			Median diameter (μ)	Range (μ)		CV between frames			ID between frames		
		MF	LMF	SMF		Minimum	Maximum	MF	LMF	SMF	MF	LMF	SMF
Control (n = 4)													
Outside													
Mean	—	17,785	13,025	4760	7.03	1.41	11.63	19.83	15.60	40.90	2.69	1.47	3.23
SD	—	2890	2037	1323	0.70	0.20	1.02	1.88	7.58	18.22	0.66	1.23	2.44
Center													
Mean	—	18,495	13,647	4848	6.84	1.43	11.66	15.10	9.63	40.13	2.08	0.67	3.68
SD	—	2598	1488	1550	0.55	0.27	1.08	4.22	3.14	15.23	1.04	0.37	1.90
P	—	NS	NS	NS	NS	NS	NS	$0.025 < P < 0.05$	NS	NS	NS	NS	NS
Acute ischemia (n = 4)													
Outside													
Mean	—	11,134	7949	2686	7.16	2.01	11.55	33.48	34.13	51.43	4.95	4.08	2.34
SD	—	1723	1019	1592	0.76	0.35	1.56	9.01	8.80	14.74	3.05	2.36	0.62
Center													
Mean		4927	3268	1660	6.14	1.93	12.27	53.05	52.50	61.54	6.90	4.85	2.80
SD	—	2580	1628	1026	0.45	0.36	1.89	17.82	22.51	10.36	4.86	3.78	1.38
P		$0.001 < P < 0.005$	$0.005 < P < 0.01$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Outside control vs outside acute ischemia		$0.01 < P < 0.02$	$0.02 < P < 0.025$	$0.025 < P < 0.05$	NS	NS	NS	NS	NS	NS	NS	NS	NS

nerves, then expressed this as a percentage of the mean observed in the controls. The results for acute ischemia are shown in Figure 3. To illustrate, the mean TFA of disease nerves at level A was slightly greater than (ie, greater than 100% of) the mean value observed in controls. Similarly, the means of all measured characteristics were nearly equal to the control means at level A. By comparison, the ID was many times larger in disease nerves at level D.

The stereomorphometric abnormalities observed in acute ischemia are graphed in Figure 3. A significant increase in TFA occurred in the distal sciatic nerve. A similar, but not significant, increase was also observed in the tibial division. The number of MF per nerve was decreased to statistically significant levels only for the tibial division of the distal sciatic nerve and the tibial nerve below the knee. The median MF diameter was not decreased at any level.

Consideration of Table 2 indicates that a comparison of morphometric results between the 50% outer and inner contour areas of fascicles at selected levels of nerve provides additional information regarding the fiber pattern. A striking reduction in density of MF and LMF, but not of SMF, for the tibial division of the distal sciatic nerve was found for the inner, as compared with the outer, contour area. A statistically significant reduction of MF was also found when we compared outer contour areas of ischemia nerves with those of control nerves. No statistically significant difference in the CV or the ID could be shown between outer and inner contour areas.

Chronic Ischemia

Neuropathologic examination of nerves harvested 6 weeks after microsphere embolization revealed that the central fascicular cores, which at 1 week had shown a decreased number of degenerating fibers, now contained many small-diameter MF. The morphometric values are presented in Table 3 and Figure 4. The only statistically significant abnormalities observed were a decrease in the median diameter of MF at levels A and D and an increase in the number of SMF per nerve and smaller and larger MF at level C. Whereas the CV and ID for MF and LMF, in particular, were increased in the more distal disease nerves, the levels did not reach statistical significance because the number of nerves evaluated was small.

Rhizotomy

We chose the rhizotomy model to produce degeneration of ventral root MF in peripheral nerve, hoping to simulate the effect of fiber degeneration in motor neu-

Table 3—Chronic Ischemia

Group	TFA	Number per nerve			Median diameter (μ)	Range (μ)		CV between frames			ID between frames		
		MF	LMF	SMF		Minimum	Maximum	MF	LMF	SMF	MF	LMF	SMF
Upper sciatic (A)													
Control (n = 4)													
Mean	0.632	10,687	7733	2954	7.14	1.24	14.32	17.40	15.50	37.23	2.20	1.32	3.03
SD	0.142	2490	1618	909	0.25	0.10	1.19	1.63	4.00	15.23	0.43	0.67	1.76
Disease (n = 3)													
Mean	0.703	14,189	9373	4815	6.25	1.27	13.00	15.70	12.40	31.10	2.19	1.90	2.75
SD	0.066	3032	961	2105	0.48	0.11	0.89	3.50	3.30	2.70	1.27	0.47	1.29
P	NS	NS	NS	NS	$0.02 < P < 0.025$	NS	NS	NS	NS	NS	NS	NS	NS
Lower sciatic (C)													
Control (n = 4)													
Mean	0.431	7924	5556	2368	6.58	1.13	12.16	13.95	11.35	31.85	1.48	0.75	2.21
SD	0.091	406	541	241	0.59	0.11	1.27	1.53	2.65	6.58	0.15	0.28	0.50
Disease (n = 3)													
Mean	0.431	8528	4797	3731	5.49	0.92	12.36	14.70	19.80	43.83	1.92	1.65	6.62
SD	0.026	476	1407	941	0.87	0.08	.45	4.02	11.80	10.73	1.18	1.54	5.09
P	NS	NS	NS	$0.025 < P < 0.05$	NS	$0.005 < P < 0.01$	$0.025 < P < 0.05$	NS	NS	NS	NS	NS	NS
Tibial (D)													
Control (n = 4)													
Mean	0.147	3003	2234	768	6.71	1.11	11.50	9.75	8.80	33.63	0.82	0.48	2.58
SD	0.029	292	313	74	0.49	0.10	0.92	3.54	1.51	16.23	0.48	0.17	1.68
Disease (n = 4)													
Mean	0.168	3331	1654	1676	5.26	1.13	11.17	11.98	24.63	40.40	1.40	2.08	6.25
SD	0.032	393	475	868	0.94	0.08	0.87	7.49	15.74	7.97	1.59	1.48	3.01
P	NS	NS	NS	NS	$0.025 < P < 0.05$	NS	NS	NS	NS	NS	NS	NS	NS

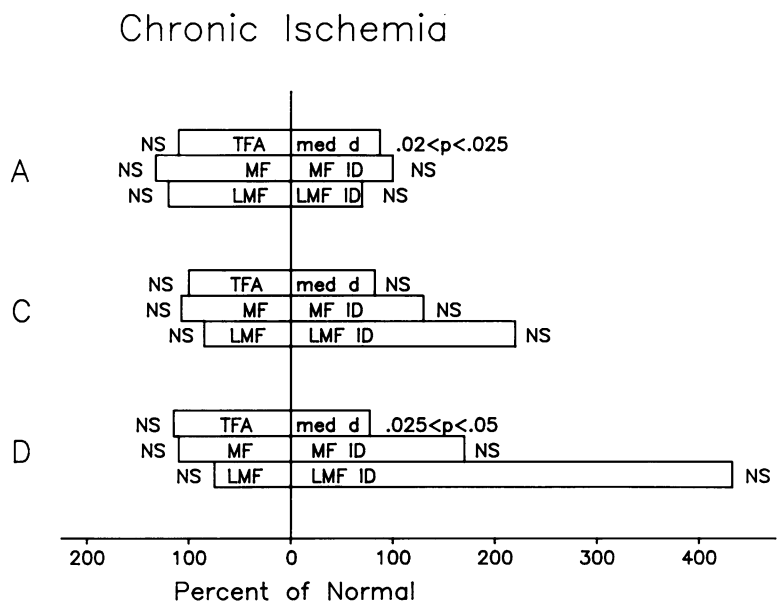

Figure 4—The morphometric and MF density variation in nerves of rat in chronic ischemia, as described in the text. For abbreviations, see Figure 3.

ron disease. The results are given in Table 4 and Figure 5. The TFA was significantly increased at levels C and D. The number of MF and LMF per nerve was significantly decreased at level A. The CV of MF and LMF densities was significantly elevated at levels C and D. The ID values were significantly elevated at level C but did not reach significance at level D.

Galactose

The galactose model was chosen because it is known to produce nerve edema, an increase of TFA, and a low rate of fiber degeneration. Only peroneal nerves were studied (Table 5). The TFA was significantly increased, but the number of MF per nerve was unaffected. The number of SMF was significantly increased and was reflected in an alteration of the fiber spectrum: the median diameter was smaller. The CV for LMF was significantly raised. Although the ID was slightly increased for both MF and LMF, statistical significance was not attained.

In some sections, the edema appeared to have affected the outer more than the inner contour area. The higher average values of the CV and ID may reflect this variability in density.

Lead

Lead was chosen because it produces a demyelinating neuropathy with nerve edema and little or no fiber loss. Nerve level A was used for the controls and level B for lead. Differences may therefore be attributed either to the level of the nerve evaluated and/or to disease. The statistical decrease in TFA at level B must reflect a difference in sampling level, because it is known that more distal nerves are smaller and that lead causes nerve swelling and not shrinkage (Table 6 and Figure 7). There are significantly less MF and LMF per nerve in disease (B) than in controls (A). Both the CV and the ID for MF, LMF, and SMF densities are respectively less for disease than for controls. Assuming it to be due to disease, and in contrast to earlier examples, this would imply a more regularly arranged pattern than in controls.

Discussion

Morphometric studies of nerves or fiber tracts have been shown to be of value for the study of neuropathologic abnormalities. It is possible to recognize 1) alterations in nerve bundle size due to maldevelopment, edema or infiltration; 2) increase (eg, sprouting) or decrease (failure of development or degeneration) in the number of fibers; 3) failure of development or preferential degeneration of classes of neurons (axons); 4) alteration in size and shape; 5) increase or decrease in number of size and/or alteration in the proportion of classes of nuclei; 6) autoradiographic abnormalities; and 7) ultrastructural abnormalities.

The use of morphometric approaches in neuropathologic study of the peripheral nervous system has recently been reviewed.[17] Selective absence or degeneration of populations of neurons (axons) could be related to specific clinical deficits and to a selective decrease or absence of a peak(s) of the compound action potential of nerve.[18,19]

Table 4—Rhizotomy

Group	TFA	Number per nerve			Median diameter (μ)	Range (μ)		CV between frames			ID between frames		
		MF	LMF	SMF		Minimum	Maximum	MF	LMF	SMF	MF	LMF	SMF
Upper sciatic (A)													
Mean	0.632	10,687	7733	2954	7.14	1.24	14.32	17.40	15.50	37.23	2.20	1.32	3.03
SD	0.142	2490	1618	909	0.25	0.10	1.19	1.63	4.00	15.23	0.43	0.67	1.76
Disease (n = 4)													
Mean	0.647	6866	4848	2019	7.11	1.64	13.08	27.75	26.65	47.68	3.30	2.15	2.75
SD	0.079	1253	673	630	0.38	0.24	1.25	10.79	10.83	16.80	1.65	1.12	0.99
P	NS	$0.025 < P < 0.05$	$0.01 < P < 0.02$	NS	NS	$0.02 < P < 0.025$	NS	NS	NS	NS	NS	NS	NS
Lower sciatic (C)													
Control (n = 4)													
Mean	0.431	7924	5556	2368	6.58	1.13	12.16	13.95	11.35	31.85	1.48	0.75	2.21
SD	0.091	406	541	241	0.59	0.11	1.27	1.53	2.65	6.58	0.15	0.28	0.50
Disease (n = 4)													
Mean	0.697	6686	5001	1686	7.33	1.46	13.57	27.75	27.05	52.25	3.01	2.23	2.53
SD	0.070	1201	817	460	0.42	0.20	1.46	6.38	5.30	16.63	0.57	0.54	0.65
P	$0.001 < P < 0.005$	NS	NS	$0.025 < P < 0.05$	NS	NS	NS	$0.005 < P < 0.01$	$0.001 < P < 0.005$	NS	$0.001 < P < 0.005$	$0.001 < P < 0.005$	NS
Tibial (D)													
Control (n = 4)													
Mean	0.147	3003	2234	768	6.71	1.11	11.50	9.75	8.80	33.63	0.82	0.48	2.58
SD	0.029	292	313	74	0.49	0.10	0.92	3.54	1.51	16.23	0.48	0.17	1.68
Disease (n = 4)													
Mean	0.275	3029	2346	683	7.57	1.49	12.93	18.30	14.48	47.05	1.65	0.81	2.64
SD	0.057	306	332	58	0.48	0.49	0.96	2.53	3.30	15.33	0.59	0.39	1.68
P	$0.005 < P < 0.01$	NS	NS	NS	$0.025 < P < 0.05$	NS	NS	$0.005 < P < 0.01$	$0.02 < P < 0.025$	NS	NS	NS	NS

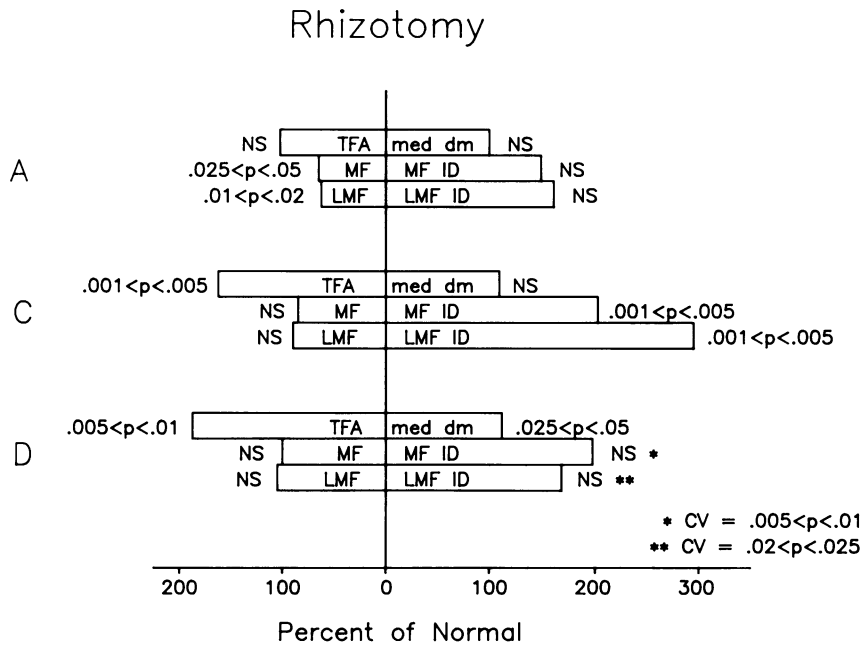

Figure 5—The morphometric and MF density variation in nerves of a rat after rhizotomy, as described in the text. For abbreviations, see Figure 3.

Morphometric studies of sampled levels along the length of peripheral nerves in disease and in controls allow the observer to make extrapolations about the distribution of pathologic abnormality in the third dimension.[14] To reconstruct abnormalities of motor neurons, the number and size of the soma in motor neuron columns of spinal cord segments and of MF in a ventral root and a distal muscle nerve were determined. Striking deficits of alpha and gamma motor neurons could be demonstrated in motor neuron disease (alpha) and in dysautonomia (gamma). For peripheral sensory neurons, the sampled sites included various levels of fasciculus gracilis, dorsal root, spinal ganglia, and sural nerve. Characteristic abnormalities of classes of axons were found in various hereditary sensory neuropathies, amyloidosis, and Fabray's disease. For sympathetic neurons, the interomediolateral nucleus, ventral spinal root, gray and white rami, and paravertebral sympathetic ganglia were assessed. Abnormalities were documented for familial dysautonomia and Shy-Drager syndrome. Use of these approaches in patients with neuropathic disease has revealed the class and proximal to distal level of involvement of neurons.

In this study, we extended these morphometric studies to a consideration of spatial pattern of fibers in transverse sections of nerve. Focal loss of fibers is encountered in ischemic neuropathy, sarcoidosis, leprosy, and primary nerve tumors. In many metabolic and inherited neuropathies, focal fiber loss has not been recognized, but they have not been studied by the approaches introduced here. The idea that fiber loss is focal or multifocal in some neuropathies, while in others it is not, was the basis for this research. The approaches used here could be extended to a study of the fascicular nerve vessels, nuclei, or other structures.

We have used the idea of spatial pattern to describe the distribution of fibers within and between fascicles of nerves as plant ecologists use the term to describe the distribution of plants within quadrats.[16] We have also made use of their ID to test for the presence of a pattern or of random distribution. Similarly, we employ data based on counts from sampled quadrats (frames) and have extended their idea to the use of outer and inner contour areas of the fascicle. Usually, ecologists use quadrats of the same size. This cannot be done with nerves. Some of the frames we sampled contained

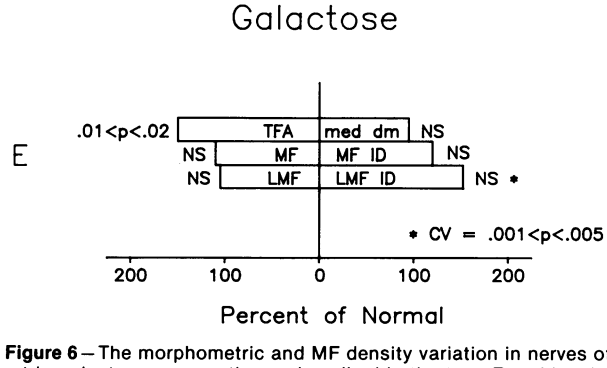

Figure 6—The morphometric and MF density variation in nerves of rat in galactose neuropathy as described in the text. For abbreviations, see Figure 3.

Table 5—Galactose

Group	TFA	Number per nerve			Median diameter (μ)	Range (μ)		CV between frames			ID between frames		
		MF	LMF	SMF		Minimum	Maximum	MF	LMF	SMF	MF	LMF	SMF
Peroneal (E)													
Control (n = 6)													
Mean	0.187	2011	1275	736	7.96	1.49	14.40	22.90	16.93	44.12	2.19	0.78	2.95
SD	0.023	71	91	44	0.32	0.26	0.98	4.31	2.81	8.56	0.75	0.34	0.76
Disease (n = 5)													
Mean	0.280	2225	1331	894	7.58	1.57	15.00	27.62	24.30	46.42	2.63	1.19	3.00
SD	0.077	262	178	159	0.61	0.20	1.23	6.32	3.21	10.85	1.07	0.34	1.15
P	$0.01 < P < 0.02$	NS	NS	$0.025 < P < 0.05$	NS	NS	NS	NS	$0.001 < P < 0.005$	NS	NS	NS	NS

Table 6—Lead

Group	TFA	Number per nerve			Median diameter (μ)	Range (μ)		CV between frames			ID between frames		
		MF	LMF	SMF		Minimum	Maximum	MF	LMF	SMF	MF	LMF	SMF
Upper sciatic (A)													
Mean	0.632	10687	7733	2954	7.14	1.24	14.32	17.40	15.50	37.23	2.20	1.32	3.03
SD	0.142	2490	1618	909	0.25	0.10	1.19	1.63	4.00	15.23	0.43	0.67	1.76
Upper sciatic (B)													
Diease (n = 6)													
Mean	0.493	8196	5414	2782	6.14	1.20	15.30	16.22	13.38	34.02	1.86	0.88	2.75
SD	0.041	490	220	417	0.37	0.15	2.28	0.99	2.91	5.94	0.34	0.41	0.88
P	$0.025 < P < 0.05$	$0.025 < P < 0.05$	$0.005 < P < 0.01$	NS	$0.001 < P < 0.005$	NS	NS	NS	NS	NS	NS	NS	NS

* As compared with upper sciatic nerve A.

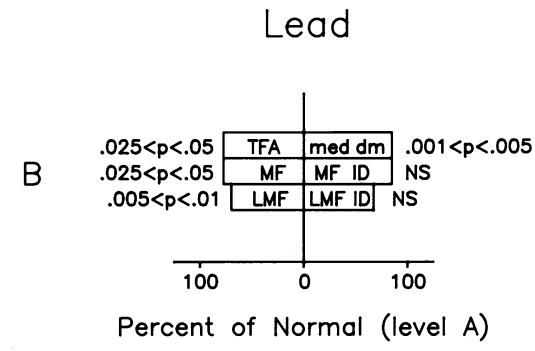

Figure 7—The morphometric and MF density variation in nerves of a rat with lead neuropathy as described in the text. For abbreviations, see Figure 3.

less than a full frame of fascicular area. The harmonic mean of frame size was used in calculating the ID.

Three-dimensional patterns of fiber degeneration or loss emerge from these studies. In acute ischemic neuropathy from microsphere embolization, no abnormality was demonstrated in proximal sciatic nerve, whereas at more distal levels of the same nerve, loss of fibers and excessive variability of MF densities (indicated by a markedly elevated CV and ID) have been demonstrated. At proximal levels of ischemic injury, the density of MF (also of LMF) was higher in outer than in inner contour areas of fascicles. In chronic ischemia (the same lesion as in the acute variety but tissue taken 6 weeks after embolization), similar but less severe changes were demonstrated. One difference between the acute and chronic variety was that proximal MF of the chronic variety exhibited a lower median diameter. Although this could reflect a biologic difference unrelated to the model, it is likely to be a real atrophy of proximal stump fibers after disease transection. Such atrophy takes weeks to develop and therefore is found only in chronic ischemia. Such atrophy of stump fibers with concomitant reduction of maximum nerve conduction has previously been reported.[17,20,21] When rhizotomy is used as a model of ventral root MF loss, important differences between this pattern and that of ischemia can be shown. The principal difference was that the number of MF per nerve was reduced even at the upper sciatic nerve level in rhizotomy. The tibial nerve below the knee did not show a decrease in MF, however, probably because the sacral roots were not cut. The ID was not as high as in ischemia, but at certain levels it was elevated. Although not tested, one might expect a different pattern of fiber loss in outer and inner contour areas in ischemia as compared with rhizotomy. Central fascicular degeneration has been reported in diabetic cranial third nerve palsy,[22] in necrotizing angiopathic neuropathy,[23] and in experimental ischemic neuropathy.[24,25]

No fiber loss was demonstrated in galactose neuropathy, differing from acute and chronic ischemia and rhizotomy. Similar to them, however, it showed an increase in the CV and ID, but not as large an increase. In marked contrast to what is found in ischemia, density is decreased in outer, as compared with inner, contour areas.[15]

Finally, no increase in the variability of fiber density was demonstrated in lead neuropathy.

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