

Aspects of the Macro and Microscopic Anatomy of the Sciatic Nerve in Wistar Rats

Aspectos de la Anatomía Macro y Microscópica del Nervio Ciático en Ratas Wistar

Carla Adelino Suaid^{*}; Ana Paula Santos^{**}; Fernanda de Oliveira Yamane^{***};
Valéria Paula Sassoli Fazan^{****} & Amilton Antunes Barreira^{*****}

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SUMMARY: The present study seeks to systematize morphological and morphometrical parameters and brings new data on the main branch of the lumbosacral plexus – i.e., sciatic nerve – in Wistar rats aged four and seven weeks. Sixteen female were divided into two groups, namely animals aged four weeks, and animals aged seven weeks. The specimens were studied at proximal and distal segments of the right hind limb sciatic nerves. Semi-thin transverse sections (0.25 µm thickness) were stained with 1 % toluidine blue, and the morphometric analysis was processed through the KS 400 software. Except for the number of fascicles and fascicular diameter, no differences were found between the proximal and distal segments. We observed differences when morphometric values were compared between 4- and 7- week old animals, with some exceptions (number of fascicles and myelinated fibers, and capillary area and number). The macroscopic data disagree with a previous description of the sciatic nerve being composed by two fascicles. Instead, sciatic nerve's only fascicle trifurcates or quadrifurcates at the distal third of the thigh. The total capillary area and density were calculated, and these are the first referential data for the sciatic nerve. Histograms of myelinated fiber and axons considering the animal ages were built. The results presented here are important because experimental studies, mainly studies on nerve regenerations require comparison with normal reliable data.

KEY WORDS: Rat sciatic nerve; Myelinated nerve fibers; Capillaries; Anatomy and histology; Animal experimental use.

INTRODUCTION

The sciatic nerve of rats is the nerve that is most commonly employed in experimental studies involving nerve injury, regeneration, and models of peripheral nervous system diseases. Most of the time, macroscopic and/or microscopic data on the sciatic nerves refers to the control groups of these experimental studies, and so it is still necessary to systematize these data through a specific study. The morphology of the sciatic nerve in rats has interested neuroscientists for many decades and, despite an extensive publication in the last decade (Prodanov & Feirabend, 2007), information on normal sciatic nerve data in rats is deserves more clarification.

There are important differences between the disorders of the brachial plexus and those of the lumbosacral plexus with respect not only to the nerves but also to the adjacent connective tissue. Investigators preference to experimentally study the lumbosacral plexus might be explained by the

predisposition to diabetic neuropathies, which is the most common disease of the lumbosacral plexus roots in men, and by the diagnostic difficulties faced due to location of the roots (Stevens, 1984). The length of the sciatic nerve and its branches puts them at risk of being the first nerves to be committed by the “dying back” neuropathies. Moreover, these nerves are less susceptible to traumas that would interfere with the experimental results. Taken together, all these factors have led investigators to develop experimental models of neuropathies using nerves of the hind limbs of rats.

Experimental models of peripheral neuropathies and regeneration studies use morphoquantitative methods. Multiple approaches have been used: hormonal treatment (Voinesco *et al.*, 1998), guide tubes (Hernández-Cortés *et al.*, 2014), and conservative therapies (Dias *et al.*, 2015). Alterations in the sciatic nerve due to experimental diabetic

^{*} University of Ribeirão Preto (UNAERP), Ribeirão Preto, Brazil.

^{**} Federal University of Jequitinhonha and Mucuri Valleys (UFVJM), Diamantina, Brazil.

^{***} Central South University of Mines, Varginha, Brazil.

^{****} Department of Surgery and Anatomy, Medical School of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, Brazil.

^{*****} Department of Neuroscience and Behavior, Medical School of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, Brazil.

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neuropathy have also been extensively investigated (Yagihashi, 1993; Sugimoto & Yagihashi, 1996).

The present study aims to investigate and systematize the morphoquantitative parameters of the sciatic nerves, including the number of fascicles, nerve area, minimum diameter of fascicles, number and density of myelinated fibers, minimum diameter of the myelinated fiber and respective axons, myelin sheath area, fiber distribution histograms, relation between axon diameter and myelin area, and capillary number and area. A comparison between proximal and distal segments, and also between rats aged 4 and 7 weeks was performed, using highly sophisticated computer morphometry.

MATERIAL AND METHOD

Sixteen female Wistar rats were divided into two groups: animals aged four weeks weighing between 96 and 137 g (n= 8), and animals aged seven weeks with weights varying from 214 to 271 g (n= 8). The animals were anesthetized with sodium pentobarbital (Nembutal, 40 mg/kg, i.p.) and perfused with a 0.05 M phosphate-buffered saline solution, pH 7.4 (1 mL/g) followed by a 3.0 % glutaraldehyde solution in 0.07 M cacodylate buffer, pH 7.2 (1.5 mL/g) through the left ventricle, using a perfusion pump (Aga 2 intel pump). All experimental procedures adhered to The Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (Copyright© 1996 by the National Academy of Sciences) and were approved by the Institutional Ethics Committee

for Animal Research (CETEA – Comitê de Ética em Experimentação Animal). Every effort was made in order to minimize the number of animals used.

After careful anatomical dissection, the sciatic nerves were totally exposed, and proximal (immediately after passing through the major sciatic foramen) and distal (at popliteal fossa, immediately before the terminal branching) segments were removed for histological processing (Fig. 1).

After removal, the nerves were kept in the fixative solution. They were then prepared, cut, stained, and observed as described previously (Santos et al., 2007). The nerves were kept in the fixative solution for an additional 48 h and processed for epoxy resin embedding (Poly/Bed 812; Polysciences, Warrington, PA). After careful positioning of the nerve fragments in the embedding molds, transverse sections were cut at 0.25 mm, stained with 1 % toluidine blue, and observed by means of an Axiophot photomicroscope (Carl Zeiss, Jena, Germany).

For the morphometric study, the images were acquired via a digital camera (TK-1270, JVC, Victor Company of Japan, Ltd, Tokyo, Japan) and analyzed with an IBM/PC. The transverse sectional areas of the fascicles were manually obtained, and the number of fascicles were counted. The luminal area and the number of capillaries were also obtained. The endoneurial area occupied by the capillaries was calculated. For the study of myelinated fibers, the endoneurial space was observed using an optical set with immersion oil lens (100 X), optovar (1.6 X), and camera (0.5 X), as well as an 8 X computerized magnification, which provided images with good resolution for the morphometric approach. The whole fascicle transverse sections were fully scanned using an automatic, motorized microscope stage (Carl Zeiss, Jena, Germany), without overlap of the microscopic fields. Thirty percent of such microscopic fields were randomly studied in proximal and distal segments of the sciatic nerve. Morphometric parameters for the myelinated fibers were obtained, the total number of myelinated fibers present in each microscopic field was identified by visual inspection and counted. The numerical density of the myelinated fibers was calculated. The minimal diameter of the myelinated fibers was automatically measured by means of an image analysis software (KS 400, Kontron 2.0, Eching bei München, Germany). Only fibers of circular shape were measured. Both the axonal minimal diameter and the total fiber minimal diameter were measured, and the ratio between the two diameters, the so-called g-ratio (a measure of the degree of myelination), was obtained. The myelin sheath area was

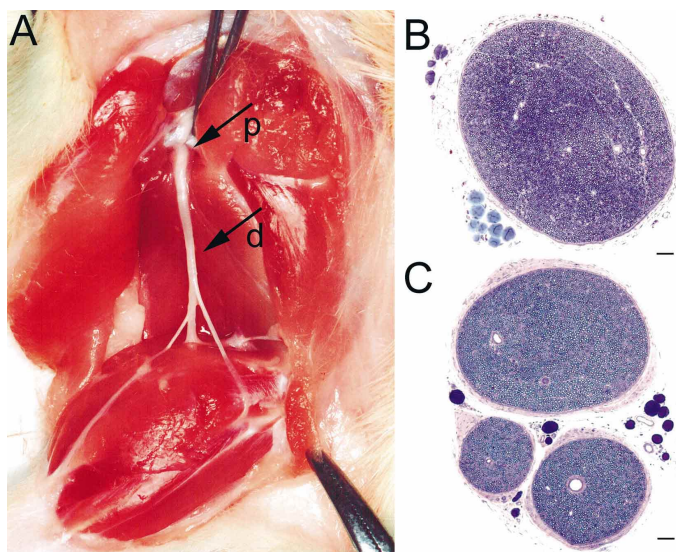


Fig. 1. Removal sites of the proximal (p) and distal (d) segments of the sciatic nerve of a female Wistar rat, aged 7 weeks (A). Photomicrographs of transverse semi-thin sections of the proximal (B) and distal (C) segments (Toluidine blue stained. Bars= 100 μ m).

automatically calculated for each measured myelinated fiber. Histograms of the distribution of the myelinated fiber and axon populations separated into 0.5 µm diameter class intervals were constructed. Vertical lines pointing down from the base line were included from left to right, to show the first percentile of the diameter range, the position of the median (line in bold), and the 99th percentile range of the diameter. A regression analysis was generated to determine the relationship between the diameter of the axon and that of the myelinated fiber.

Morphometric data are represented as mean ± standard deviation of the mean (SDM). Data were compared between the two groups (rats aged four or seven weeks) using the Mann-Whitney, non-parametric test, as well as between segments within the same group (proximal and distal segments) using the Wilcoxon non-parametric test for paired samples. Differences were considered significant at p ≤ 0.05.

RESULTS

Fascicle. Morphometric data of the sciatic nerves of animals aged 4 and 7 weeks are listed in Table I. Proximal segments presented one fascicle only, while distal segments showed 3 to 4 fascicles (Fig. 1). No significant differences in the internal fascicular area were observed when comparisons

were made between proximal and distal segments from animals of the same age. Nevertheless, the fascicular area was significantly larger in animals aged 7 weeks compared to those aged 4 weeks. There was a significant reduction in the fascicle diameter from proximal to distal grade in both groups. There was also a significant difference between ages for the proximal segments in this parameter.

For animals aged 4 weeks, the total fascicular area occupied by the capillaries was 0.7 % and 3.7 % on proximal and distal segments, respectively, with a significant difference between them. In the case of animals aged 7 weeks, this percentage was 0.6 % and 1 % for the proximal and distal segments, respectively.

Myelinated fibers (Table II). Number and density of the myelinated fibers were similar when proximal and distal segments from animals of the same age were compared. The number of myelinated fibers in the two groups did not differ, while the fiber density was smaller in animals aged 7 weeks, compared to those aged 4 weeks. The myelinated fibers, as well as the diameter of their respective axons and the myelin sheaths were similar in the proximal and distal segments from animals of the same age. Nevertheless, comparisons between animals aged 4 and 7 weeks revealed a significant increase in the fiber caliber (fiber and axon minimum diameter and myelin sheath) of the 7- week-old animals.

Table I. Morphometric data for proximal and distal segments of rats aged 4 and 7 weeks old. Data expressed as mean ± SDM.

		4 weeks old		7 weeks old	
		Proximal	Distal	Proximal	Distal
Nerve	Fascicle number	1±0*	3±0.40	1±0*	3±0.51
	Area (mm ²)	0.32±0.03	0.31±0.03	0.41±0.03#	0.44±0.03#
	Diameter (µm)	579.61±48.15*	301.35±27.27	636.77±18.81*#	336.66±38.15
Capillary	Number	38±19	44±7	33±18	34±13
	Area (µm ²)	2303±1776*	11447±15621	2433±2387	4205±3200

* Indicates a significant difference between nerve segments (proximal vs. distal)

Indicates a significant difference between the same segment for the two age groups (4- vs.7- week old)

Differences were considered significant at p ≤ 0.05.

Table II. Morphometric data for proximal and distal segments of rats ages 4 and 7 weeks old.

		4 weeks old		7 weeks old	
		Proximal	Distal	Proximal	Distal
Myelinated fibers	Number	2730±303♣	2478±244♣	2610±137♣	2619±228♣
	Density (fibers/mm ²)	27689±2067	28520±2203	21714±1682#	22964±2185#
	Minimal diameter (µm)	4.29±0.32	4.30±0.14	5.04±0.12#	5.16±0.15#
	Myelin area (µm ²)	14.01±2.55	13.75±1.33	18.09±1.88#	18.60±2.24#
Axon	Minimal diameter (µm)	2.23±0.19	2.27±0.17	2.89±0.19#	2.93±0.23#

Data expressed as mean ± SDM.

* Indicates a significant difference between nerve segments (proximal vs. distal).

Indicates a significant difference between the same segment for the two age groups (4- vs.7- week old).

♣Represents 30 % of the total number of myelinated fibers.

Differences were considered significant at p ≤ 0.05

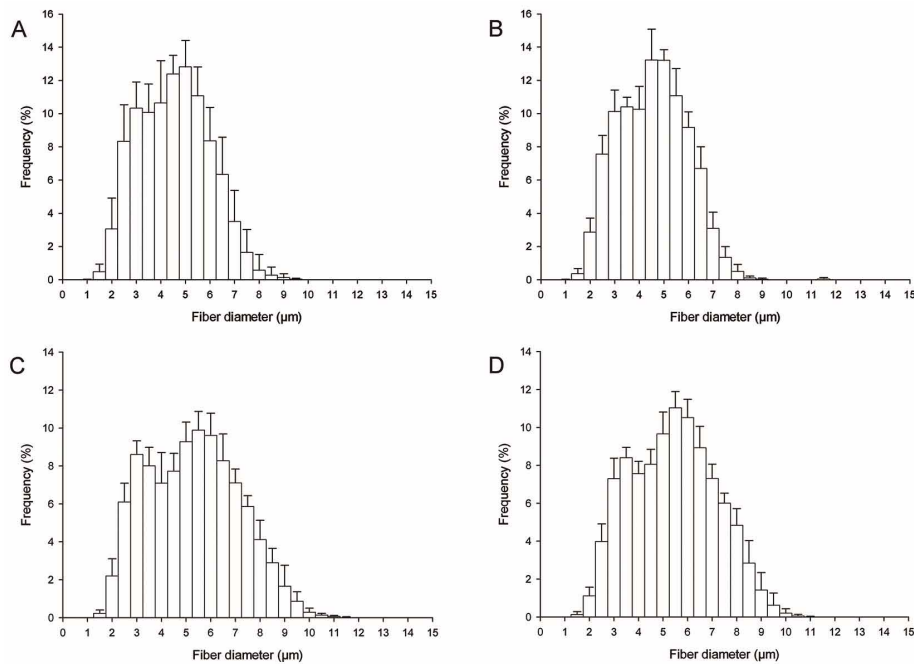


Fig. 2. Histograms showing the distribution of the diameters of myelinated fibers of the proximal (A and C) and distal (B and D) segments of the sciatic nerve of female Wistar rat aged 4 weeks (A and B) and 7 weeks (C and D). Each column and vertical bar represents mean \pm SD.

Frequency distribution histograms of the minimum fiber diameter and the respective axons were similar when the proximal and distal segments from the same group and also between groups were compared. Myelinated fiber and axon distribution was unimodal with a discrete asymmetry, with a shift of the distribution to the right (Figs. 2 and 3). As for the linear regression analysis obtained by the least-squares method, correlation coefficients were 0.85 and 0.84 for the proximal and distal segments of 4-week old rats, respectively. For rats aged 7-weeks data correlation coefficients of 0.91 for both segments (Fig. 4).

DISCUSSION

The sciatic nerve is the largest lumbosacral plexus branch and is the most widely investigated nerve among those comprising the rat peripheral nervous system. The present study still adds to the literature a confirmation on the macro and new data on the microscopic anatomy of this nerve in rats.

Macroscopic anatomy.

Anatomically, the sciatic nerve in rats is formed by L4, L5, and L6 spinal roots that join to form the nerve on the lesser pelvis, in Sprague-Dawley rats was checked that the components of sciatic nerve vary from L3 to L6, however, the major components are L4 and L5 (Asato *et al.*, 2000). It is separated from the pudendal nerve by the superior gluteal artery. Together, they run through the deep groove between the dorsal border of the ischium and the root of the tail, as far as

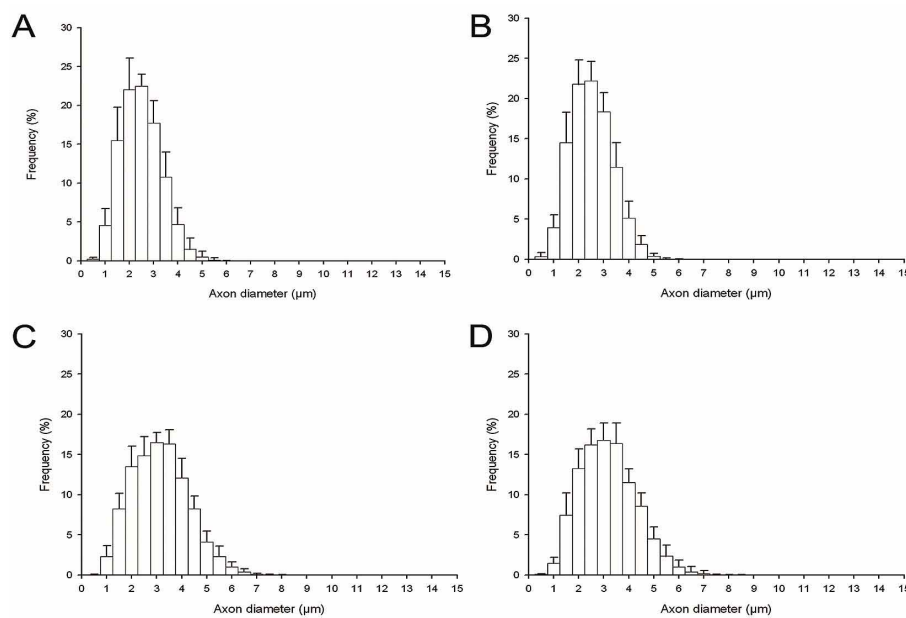


Fig. 3. Histograms showing the axon diameter distribution of the proximal (A and C) and distal (B and D) segments of the sciatic nerve of female Wistar rat aged 4 weeks (A and B) and 7 weeks (C and D). Each column and vertical bar represents mean \pm SD.

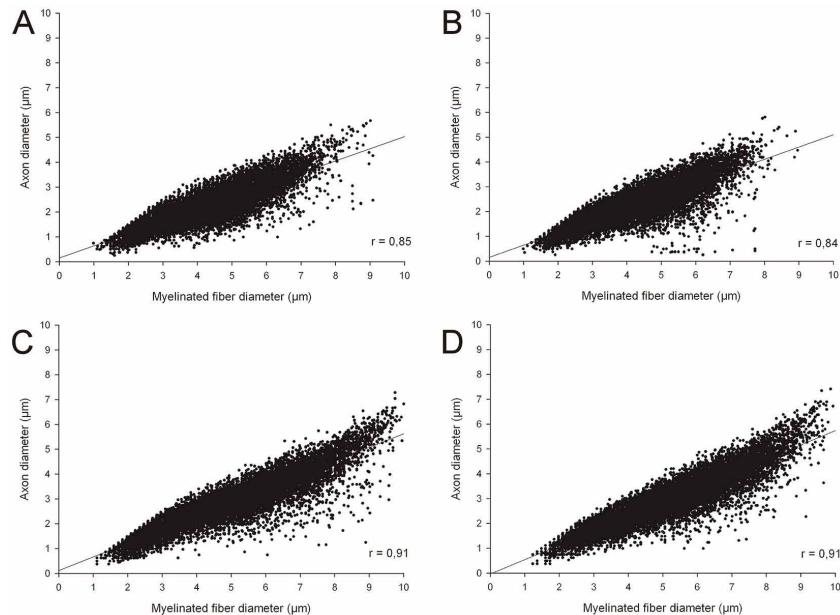


Fig. 4. Regression analysis of the relationship between axon diameter and myelinated fiber diameter in the proximal (A and C) and distal (B and D) segments of the sciatic nerve of female Wistar rats aged 4 weeks (A and B) and 7 weeks (C and D).

the caudal extent of the sciatic notch, where the sciatic nerve enters the thigh. Then, it takes a descending path as a single fascicle (Greene, 1963), above the obturator externus, quadratus femoris, biceps femoris, and adductor magnus muscles, giving off collateral branches to the ischiotibial muscles (proximal portion of the sciatic nerve). After innervating these muscles, it penetrates the popliteal fossa and bifurcates, giving off tibial (medial) and fibular (lateral) branches.

It was stated that the sciatic nerve of the rat is constituted, from its origin, by two separated fascicles – the tibial and fibular nerves (Greene). Later, it was described that the proximal portion of this nerve is constituted by a single fascicle (Schmalbruch, 1986), as we have also shown in the present study.

Macroscopically, since its origin from the greater sciatic foramen the rat sciatic nerve is constituted by a single fascicle that follows a caudal path toward the popliteal fossa (distal region), where it branches into three divisions: tibial, fibular, and sural nerves. As in the case of the present study, a single fascicle on the proximal segment and three fascicles distally were observed. These gross observations are confirmed by light microscopy carried out in the present study. Some of the distal sciatic segments present four fascicles, which have also been described by (Schmalbruch; Prodanov & Feirabend, 2007), as a cutaneous branch.

Microscopic anatomy. For the discussion about the morphometric data, we have separated the data for the nerve variables from those related to the fiber variables. The nerve variables include the nerve area, the vessels area, the fascicles area, as well as the minimal fascicular diameters and the number and density of the fibers. The fibers variables are the minimal diameter of the fiber and axon, the myelin sheath area, and the G ratio. The minimal diameter was chosen in our study because it is the most reliable and represents the nerve caliber and their fibers in the transversal sections, taking in to account that this kind of section can be oval or irregular (Fazan & Lachat, 1997).

A variation in the fascicular area, diameter, and number of fibers in different segments of the same nerve can be due to the fascicular ramification during their course. The ramification of the fascicles can result in a difference

between 4 % and 30 % of the total area when the proximal and distal segments of the sural nerve in men are compared (Behse *et al.*, 1974). Nevertheless, when the variation of the diameter and the area of the same nerve occur among groups of different ages, such a difference is believed to be due to nerve growth, which follows animal growth (Jacobs & Love, 1985). The same reasoning cannot be applied to the number of fibers because the latter does not change with age in normal animals.

Analysis of the histograms of myelinated fiber diameter distribution indicates a progressive elevation in the diameter and respective axons with age, which is sufficient to alter the pattern of distribution of those fibers (Fazan & Lachat). Older ages had a direct relation with the myelinated fibers in Wistar rats (Sharma *et al.*, 1980). Immediately after birth there is an abrupt enlargement of that diameter. Subsequently, it continues enlarging, but in a lesser proportion. The maximum diameter is reached at the age of 9 to 12 months. This enlargement is also directly associated with an enhancement in nerve conduction velocity.

In man, the relation between the axonal diameter and the respective fiber diameter, the G ratio, is important for theoretical extrapolations related to the conduction velocity. G-ratio values between 0.6 and 0.7 would be ideal for the maximum conduction velocity (Rushton, 1951).

Minimal fascicular area and diameter and capillaries.

There are no differences between fascicular areas of proximal and distal segments in animals of the same age, suggesting that the sciatic nerve does not give major branches throughout its descending pathway on the thigh (Prodanov & Feirabend). The mean values for the fascicular areas in animals aged 7 weeks are similar to those reported for older adult Sprague-Dawley rats (Nukada, 1988). Small variations in the observed values might be due to differences in the animals' age used by different authors. The statistical differences between animals aged 4 and 7 weeks observed, by us, which showed that the nerve of 7-week old animal is larger, are due to the fact that during this period of life there is intense animal growth, which reaches a plateau at about six months of age (Sharma *et al.*; Jeronimo *et al.*, 2005). The same observations have been made for the sciatic nerve fascicular growth in Wistar rats aged 12 and 20 months (Yagihashi *et al.*), and 12, 14, 16, and 18 weeks (Nukada). Similar reports have also been made by other authors. The progressive increase in fascicular area, along with animal growth is in accordance to our observations.

No references on the minimum fascicular diameter of the rats' sciatic nerve, embedded in Epon 812 and sectioned transversally are available in the literature. In the present study, we have shown that there are differences between proximal and distal segments of animals with the same age. The fact that there is only one fascicle proximally and that there are at least three fascicles distally corroborates the differences in the average minimum fascicle diameter between segments. We have also observed an increase in the fascicle diameter with age, as observed for the fascicular area.

Among the several studied nerve segments, the endoneural area occupied by the capillaries did not exceed 3.7%. In order to calculate the density of myelinated fibers, there are descriptions about subtraction of the capillary area from the total endoneural area in other nerves (Kerns & Lucchinetti, 1992; Wesselmann *et al.*, 1994), but not in the sciatic nerve. However, these authors have not mentioned the values of capillary area for the studied nerves. In the present study, the density of the myelinated fiber was obtained without subtraction of the values of capillary area. Nevertheless, morphoquantitative analysis of the capillaries is important for understanding the vascular diseases of nerves, such as the diabetic neuropathy. It is important to mention that there is an increase in the endoneural area occupied by the capillaries from proximal to distal gradient in both groups studied here. This finding is in accordance to previous observations made for the vagus nerves (Schiavoni & Fazan, 2006) and might be an indication that proximal segments are more susceptible to ischemia. Another study on the sciatic nerve of rats (Wadhvani *et al.*, 1991), performed with a histological technique very

similar to the one used in our study showed no differences in the mean number of blood vessels per nerve cross-section or in the percent ratios of surface areas between ages 3 and 11 months, and between proximal (sciatic) and distal (tibial branch) segments. Nevertheless, we must take into account that the sciatic nerve is long enough to be studied in different segments, and that it branches to give origin to the tibial, fibular, and sural nerves (Jeronimo *et al.*). The latter are considered isolated anatomical structures, long enough to be studied as separate nerves as well. In this way, it is difficult to compare data obtained in the sciatic nerve with those achieved with one of its branches and to consider them as proximal and distal segments of the same nerve. The data on capillary area and density, referred for the first time in the present study, are important since angiogenesis could be a clue to explaining the regenerative effect of mononuclear bone marrow cells on sciatic nerve regeneration (Pereira-Lopes *et al.*, 2006).

Myelinated fibers and axons. The number of myelinated fibers in the proximal and distal segments was similar, and there were no difference in these values in terms of age. The absence of significant branching of the sciatic nerve (Schmalbruch) associated with a constant number of nerve fibers during development (Jeronimo *et al.*) explains our findings. Also, the absence of significant difference in the number of fibers between proximal and distal segments in animals of the same age has been documented for the sciatic and other nerves (Dyck *et al.*, 1984; Jeronimo *et al.*; Santos *et al.*). The numbers presented in this section are similar to those reported for Wistar rats (Schmalbruch; Yagihashi *et al.*; Voinesco *et al.*; Prodanov & Feirabend); and for Lewis and Sprague-Dawley rats (Dyck *et al.*; Mackinnon *et al.*, 1991).

We describe similar values for the density of myelinated fibers in the proximal and distal segments of animals of the same age, in agreement with previous reports (Sharma *et al.*). However, our data show that animals aged 7 weeks have smaller average values compared to animals aged 4 weeks. This inverse relation is expected once the fascicle area increases with age, while the number myelinated fibers remain constant. Reduced fiber density with aging has also been described (Sharma *et al.*; Jeronimo *et al.*).

Analyzing the fiber density values reported in studies using Wistar rats, we have not found any similarities between our data and those of other authors (Yagihashi *et al.*; Sugimoto & Yagihashi), taking into account the different ages of the studied animals. When a comparison among the data of those authors is made, there are no similarities either. The same observation has been made for studies using Sprague-Dawley rats (Mackinnon *et al.*). Probably, these differences are related to the different animals ages used for the experiments, and also to differences in the employed histological and

morphometric methods. The degree of specimen shrinking depends particularly on the fixing solution, the buffer osmolarity, and dehydration, and it varies from 10 to 24 % (median: 19 %) of the total endoneurial transversal area. Consequently, the number of myelinated fibers is the most reliable morphometric parameter (Behse *et al.*).

The minimum diameters of myelinated fibers and their respective axons do not reveal significant differences between segments. These findings are similar to those obtained for Lewis rats (Dyck *et al.*). Comparison of the data obtained by us for animals aged 4 and 7 weeks, shows a significant increase in the diameters of myelinated fibers (a shift to the right), with animal growth. This has also been observed for rats aged 4 and 28 months (Voinesco *et al.*). The same observations about fiber and axon diameters are applicable to the myelin sheath. Comparing animals aged 12 and 20 months, there is an increase in the size of the myelin sheath with animal growth (Yagihashi *et al.*).

Frequency distribution histograms for myelinated fibers and their respective axons with unimodal shape seems to be characteristic of the sciatic nerve in young animals. Comparing histograms obtained for 4- week and 7- week-old animals; there is a shift to the right, indicating an increase in the diameters of fibers with animal growth, which should happen as a way to maintain the ideal conduction velocity of the fibers. We were not able to find previous published data on the histograms of sciatic nerve axon diameters for rats aged 4 and 7 weeks as shown here.

The present data might be used as a reference for comparisons with studies that investigate pathological alterations or regeneration of the sciatic nerve, mainly those on the experimental models of diseases that affect the peripheral nervous system, which are important for a better elucidation of the pathological mechanisms underlying the human peripheral neuropathies.

The present macroscopic data disagree with a previous description of the sciatic nerve being constituted by two fascicles. Instead, its only fascicle trifurcates or quadrifurcates at the distal third of the thigh, as seen by two other groups. This paper brings data on the sciatic nerve minimal fascicular diameter in two levels and ages that have not been previously reported. The total area occupied by the capillaries and capillaries density were calculated, and these are the first referential data for the sciatic nerves mainly for studies on nerve regeneration preceded by enhancement of angiogenesis. Histograms of myelinated fiber axons for rats aged 4 and 7 weeks are presented for the first time. The data reported here for Wistar rats overlap the same data published for the Sprague-Dawley, Lewis, Fischer 344 rats strain

concerning the nerve area, fascicles, number of fibers, diameter of myelinated fibers, and G ratio, indicating that the genetic differences among those strains do not interfere with the morphology and morphometry of sciatic nerve.

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RESUMEN: El objetivo fue sistematizar los parámetros morfológicos y morfométricos y traer nuevos datos sobre el ramo principal del plexo lumbosacro - es decir nervio ciático - en ratas Wistar de 4 a 7 semanas. Dieciséis ratas fueron divididas en dos grupos, con 4 y 7 semanas de edad. Las muestras estudiadas fueron los segmentos proximal y distal del nervio ciático derecho. Secciones delgadas (espesor 0,25 mm) fueron teñidas con azul de toluidina al 1 % y el análisis morfométrico se llevó a cabo utilizando el programa KS 400. Excepto para el número de fascículos y diámetro fascicular, no se encontraron diferencias entre los segmentos proximal y distal. Fueron observadas diferencias cuando se compararon los valores morfométricos entre animales de 4 y 7 semanas, con algunas excepciones (número de fascículos y fibras mielinizadas, área y número de capilares). Los datos macroscópicos no están de acuerdo con la descripción anterior del nervio ciático siendo compuesto por dos fascículos. En cambio, sólo trifurcación o cuadrifurcación fueron encontrados en el tercio distal del muslo. El área total capilar y la densidad fueron calculadas y estos constituyen los primeros datos de referencia para el nervio ciático. Se construyeron histogramas de fibras mielínicas y axones, teniendo en cuenta las edades de los animales. Los resultados presentados aquí son importantes porque los estudios experimentales, en especial aquellos sobre la regeneración nerviosa, requieren comparación con datos confiables normales.

PALABRAS CLAVE: Nervio ciático; Fibras nerviosas mielinizadas; Capilares; Anatomía e histología; Uso de animales experimentales.

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Correspondence to:

Amilton Antunes Barreira
Department of Neuroscience and Behavior
Medical School of Ribeirão Preto
University of São Paulo (USP)
Av. Bandeirantes 3900, CEP: 14049-900
Ribeirão Preto
São Paulo
BRASIL

Email: aabarrei@fmrp.usp.br

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