ISSN- 0102-9010

MORPHOMETRIC ANALYSIS OF MYELINATED AXONS DURING MATURATION IN THE *FASCICULUS GRACILIS* AND *FASCICULUS CUNEATUS* OF RATS. A TRANSMISSION ELECTRON MICROSCOPY STUDY

João Roberto Da Mata, Simone Maria Teixeira Sabóia-Morais and José Oscar Rodrigues Morais

Department of Morphology, Institute of Biological Science, Federal University of Goiás, Goiânia, GO, Brazil.

ABSTRACT

The process of myelination is an important event in the maturation of the nervous system. In this study, the *fasciculus gracilis* and *fasciculus cuneatus* in the first and sixth cervical segments of the spinal cord of a mutant strain of Wistar rats, were studied by electron microscopy in order to determine the perimeter of the axons, the thickness of the myelin sheath, and the diameter and perimeter of the nerve fibers. At birth, the *fasciculus gracilis* and *fasciculus cuneatus* had unmyelinated fibers, but by the fifth day after birth myelination was in progress. The mean perimeter of nerve fibers was significantly greater ($p < 0.05$) in the *fasciculus cuneatus* compared to the *fasciculus gracilis* at 15, 20 and 120 days of age. The diameter of the fibers, perimeter of the axons and thickness of the myelin sheath were more developed in the *fasciculus cuneatus* than in the *fasciculus gracilis* at all ages studied. The axons were relatively thinner 15-20 days after birth than at 20-120 days after birth. The myelination of the *fasciculi gracilis* and *cuneatus* in the spinal cord of this mutant strain, agreed with previous reports indicating that the mutation, which causes the rats to have bare skin, had no significant effect on myelination in the anatomical areas studied.

Key words: Axons, *fasciculi cuneatus* and *gracilis*, morphometry, myelination, transmission electron microscopy

INTRODUCTION

During development, the axons of the central nervous system (CNS) are surrounded by a myelin sheath made up of the cytoplasmic processes of oligodendrocytes. Axon myelination is an important process in the development of the nervous system, and causes rapid neuron development. Morphological studies have shown that most of the main tract of the CNS is not myelinated at birth, thus indicating that the nervous system is not fully functional at this age [2,17]. The degree of myelin sheath development is considered to be an index of the maturation reached by neurons and depends on several factors [1,4-7,11,12]. Rapid action potential conductance over great distances is essential for appropriate neuronal activity. Schwab and Schnell [14] reported that myelination was required for quick conduction of nerve impulses, and is therefore of considerable functional importance because it allows the saltatory conduction of impulses [10,15].

In this work, we measured the perimeter and diameter of nerve fibers, the perimeter and diameter of axons and the thickness of the myelin sheath in the first and sixth cervical segments of the *fasciculi gracilis* and *cuneatus* of the spinal cord of a mutant strain of Wistar rats, from birth to 120 days of age.

Correspondence to: Dr. João Roberto Da Mata
Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Goiás (UFG), CEP 74001-970, Goiânia, GO, Brasil. Tel.: (55) (62) 5211489, Fax: (55) (62) 5211190, E-mail: jrdamata@bol.com.br

MATERIAL AND METHODS

Eighteen specimens of a mutant strain of Wistar rats, three animals per age (0, 3, 15, 20, 30 and 120 days), were used in the present study. The animals were born from normal rats undergone spontaneous mutation characterized phenotypically as naked skin. The rats were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg) and perfused with heparinized saline solution via the left ventricle of the heart for 10 min, followed by fixation with a modified Karnovsky solution consisting of 3% glutaraldehyde and 3% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 for 20 min.

The soft tissue of neck region was removed and an opening was made in the cervical vertebrae**.** The cervical nerves were identified with a stereomicroscope and the spinal cord then dissected, sectioned through the first and sixth cervical segments and immersed in Karnovsky fixative for 24 h at 4°C. This was followed by postfixation in 2% osmium tetroxide (1 h), dehydration in an acetone series and embedding in Spurr resin (EMS – Ft., Washington, USA). Ultra-thin sections (60–90 nm) were obtained with a MT2B ultramicrotome and the sections were stained with uranyl acetate and lead citrate and examined with either a Jeol CX 100 or a Jeol CX 100 II transmission electron microscope operated at 80 kV.

Ten electronmicrographs of the *fasciculi* were analyzed for each age. The parameters measured were: a) the diameter of the nerve fibers, b) the perimeter of the nerve fibers, c) the perimeter of the axons, and d) the thickness of the myelin sheath. For each age interval, the best-oriented section was selected based on the circularity of the fibers, as described by Han *et al.* [3]. A total of 100 fibers were examined. Only myelinated fibers located entirely within each electron micrograph were used. The perimeters were measured by tracing the fibers and axons of interest using a planimeter. Fiber and axon diameters were determined using the formula $D=P/\pi$, where D is the fiber or axon diameter and P is the fiber or axon perimeter. The thickness of the myelin sheath membranes was calculated from the measurements made in 10 electronmiographs. The score of the mean perimeter fibers were distributed in classes [9]. The results were expressed as the mean ± standard deviation, as appropriate. Student's t-test was used for statistical analysis, with $p<0.05$ considered significant.

RESULTS

On day zero (day of birth), the *fasciculi gracilis* and *cuneatus* have unmyelinated or showed few myelinated fibers with a small axon perimeter and thin myelin sheath (Fig. 1). Three days after birth, there were still few myelinated fibers.

On the 15th day after birth, the average perimeter of nerve fibers in the *fasciculi gracilis* was 5.50 ± 3.09 μm, with most fibers having a perimeter between 3.3 and 6.1 μm (Table 1). The average perimeter of nerve fibers in the *fasciculus cuneatus* was 7.43 ± 3.63 μm, with most fibers having a perimeter between 6.10 and 8.90 μm (Table 1). The axons showed different diameters, and axons with a wider diameter had a thicker myelin sheath (Fig. 2).

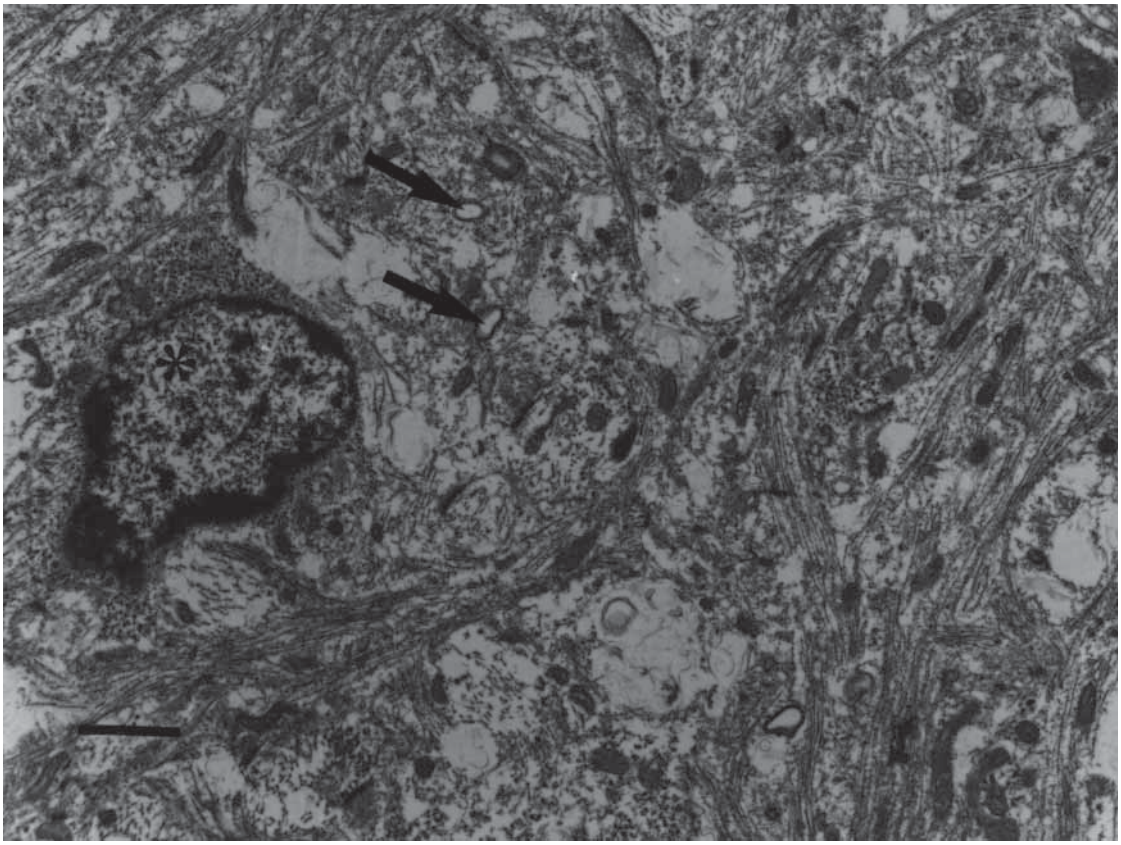

Figure 1. Electronmicrograph of the rat spinal cord dorsal *funiculus* on the day of birth. An astrocyte (*) and some small diameter fibers (arrows) with a thin myelin sheath can be seen, but there are few fully myelinated fibers. Bar = 1 μm.

Table 1. Distribution in class and by *fasciculus* of myelinated fibers in relation to the mean perimeter of nerve fibers (axon + myelin sheath), of the *fasciculus gracilis* and *cuneatus* in the first cervical segment of the spinal cord of rats 15 to 120 days old.

Age (days)	mpf ± SD (µm)		mdf (µm)		mpa (µm)		mts (µm)		Classes/class limits (µm)	% of nerve fibers by classes and *fasciculi*	
	Gra	Cun	Gra	Cun	Gra	Cun	Gra	Cun		Gra	Cun
15	5.50 ± 3.09	7.43 ± 3.63	1.48	1.73	3.51	4.01	0.24	0.29	1 (0.5 – 3.3)	28	12
									2 (3.3 – 6.1)	52	24
									3 (6.1 – 8.9)	8	38
									4 (8.9 – 11.8)	5	18
									5 (11.8 – 14.6)	4	2
									6 (14.6 – 17.4)	2	2
									7 (17.4 – 20.3)	1	4
20	5.90 ± 3.57	7.49 ± 3.97	1.55	2.10	3.59	4.20	0.26	0.32	1 (0.5 – 3.3)	20	10
									2 (3.3 – 6.1)	41	19
									3 (6.1 – 8.9)	27	30
									4 (8.9 – 11.8)	6	25
									5 (11.8 – 14.6)	3	9
									6 (14.6 – 17.4)	2	4
									7 (17.4 – 20.3)	1	3
30	6.52 ± 4.01	8.95 ± 4.04	1.88	2.60	4.36	5.21	0.31	0.39	1 (0.5 – 3.3)	10	6
									2 (3.3 – 6.1)	36	10
									3 (6.1 – 8.9)	32	20
									4 (8.9 – 11.8)	10	36
									5 (11.8 – 14.6)	4	16
									6 (14.6 – 17.4)	4	6
									7 (17.4 – 20.3)	4	6
120	10.67 ± 3.38	14.64 ± 5.20	3.40	4.66	6.28	9.42	1.21	1.99	1 (0.5 – 3.3)	2	2
									2 (3.3 – 6.1)	8	4
									3 (6.1 – 8.9)	34	10
									4 (8.9 – 11.8)	28	10
									5 (11.8 – 14.6)	19	16
									6 (14.6 – 17.4)	4	26
									7 (17.4 – 20.3)	5	18
									8 (20.3 – 23.2)	-	14

A total of 100 nerve fibers were examined per age. mpf - mean perimeter of nerve fibers (axon + myelin sheath), mdf mean diameter of nerve fibers (axon + myelin sheath), mpa - mean perimeter of axon, mts - mean thickness of myelin sheath, Gra - *fasciculus gracilis,* Cun - *fasciculus cuneatus.* The mean perimeter of nerve fibers of the *fasciculis gracilis* and *cuneatus* did not show significant difference between *fasciculi* only at 30 days old mutant strain rats. (t-test, $p < 0.05$).
(–) Note that class intervals are up to but do not include the upper limit.

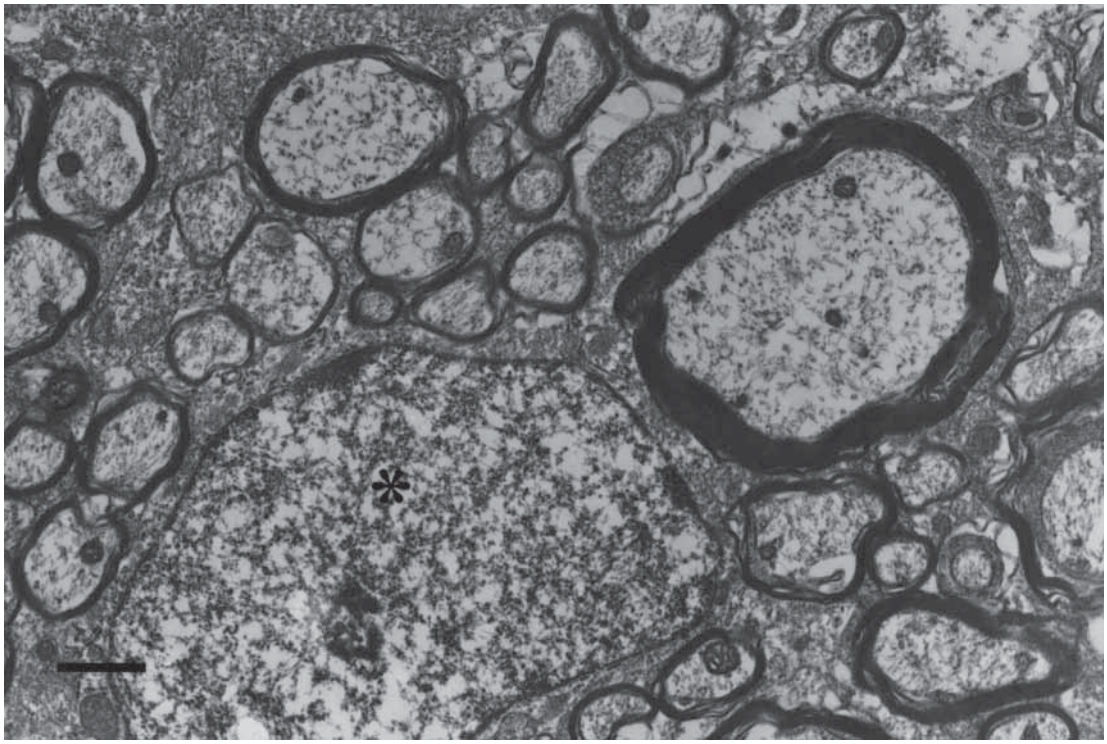

Figure 2. Electronmicrograph of the rat dorsal *funiculu*s at 15 days of age. Note the oligodendrocyte (*) and myelinated fibers of different diameters. Bar = 2 μm.

At 20 days of age, the fibers in the *fasciculus gracilis* had an average perimeter of 5.90 ± 3.57 μm and the second class had a larger number of fibers. In the *fasciculus cuneatus* the nerve fibers were concentrated in the third class and the average perimeter was 7.49 ± 3.97 μm (Table 1).

On the 30th day after birth, there was a greater number of nerve fibers in the second class *fasciculus gracilis,* the overall average perimeter being of 6.52 ± 4.01 μm. In the *fasciculus cuneatus,* the fourth class showed the highest concentration of fibers; the average perimeter of all fibers was 8.95 ± 4.04 μm (Table 1).

By 120 days of age, the nerve fibers of the *fasciculus gracilis* were most common in the third class and the average perimeter was 10.67 ± 3.38 μm. In the *fasciculus cuneatus*, fibers were not common in the sixth class fibers, with the overall average perimeter being 14.64 ± 5.20 μm (Table 1).

The mean perimeter of nerve fibers in the *fasciculus cuneatus* increased with age ($p<0.05$), when compared with the *fasciculus gracilis* at 15, 20 and 120 days of age.

A small growth in axon thickness was observed between 15 and 20 days in both *fasciculi* when compared with the growth at 5-day interval from 20 to 120 days of age (Table 2).

Table 2. Mean growth of axon thickness (μm) in the *fasciculus gracilis* and *fasciculus cuneatus* in the first cervical segment of rat spinal cord, at five day intervals from 15 to 120 days after birth.

Fasciculi	Age (days)	Overall growth (μm)	Mean growth every 5 days
Gracilis	15 – 20	0.08	0.08
	21 – 30	0.77	0.38
	31 – 120	1.92	0.10
Cuneatus	15 – 20	0.19	0.19
	21 – 30	1.01	0.50
	31 – 120	4.14	0.23

DISCUSSION

Our results show that myelination of the *fasciculus gracilis* and *fasciculus cuneatus* begins during gestation and continues after birth. A few myelinated axons were present at birth, but

myelination was completed only during the postnatal period. The thickness of the myelin sheath increased with axon diameter, in contrast to results reported by Towe and Harding [16] who observed no relationship between myelin sheath thickness and axon diameter. Myelin sheaths first appeared on axons with wider diameter in both *fasciculi,* whereas in adult rats these sheaths occurred in axons of increasingly smaller diameter, since axons measuring 0.2 μm had a myelin sheath. Our results, showed that when myelination begans in both *fasciculi* of the spinal cord, the axons were not myelinated at the same time, and myelination started in thicker unmyelinated axons and progressed to thinner axons, in agreement with data reported by Matthews and Duncan [8].

A marked increase in the number of myelinated axons was observed from 1 to 15 days of age; however, the increase in thickness was not measured during this period. Between 15 and 20 days of age, a smaller rate of axonal growth was observed and, from 20 to 120 days of age the average growth during each 5-day period was higher, as also reported by Noppeney [9].

The *fasciculus cuneatus* showed an early, rapid development when compared with the *fasciculus gracilis* because the fibers of the latter were thinner than those of the *fasciculus cuneatus* at each stage of postnatal development, and the rapid increase in nerve fiber thickness observed after 20 days of age was more marked in the *fasciculus cuneatus* than in the *fasciculus gracilis*. These observations show that the *fasciculus cuneatus* has fibers of wider diameter than the *fasciculus gracilis* throughout neural development in these rats. As a result, the epicritic sensitivity of the anterior extremities occurs earlier than in the posterior extremities. Fibers with moderate myelination therefore have a lower conductance when compared to fibers with a thick myelin sheath.

At birth, myelination activity was weak in the *fasciculus gracilis,* with no myelinated fibers being observed in some areas, and only a few fibers in others, as previously reported [2]. At 15 days of age, the largest number of fibers present in the *fasciculus cuneatus* belonged to the third class, with 38% of myelinated axons showing a perimeter ranging from 6.1 to 8.9 μm.

Comparison of the results obtained here at 15, 20, and 120 days of age with those reported by Schultes [13] revealed a greater average in the latter study. According to Krigman and Hogan [6] and Robain and Ponsot [11], factors such as nutritional status, body weight and size of the specimen can influence axonal development. Thus, from 15-30 days of age, the largest number of fibers belonged to the second and third classes (6.1 to 8.9 μm), and at 120 days of age, the addition of one more class to the *fasciculus cuneatus* was necessary i.e., an eighth class (20.3 to 23.2 μm) containing 14% of the axons.

The change in fiber distribution among classes was smaller in the *fasciculus gracilis* because the perimeter of the axons was smaller than in the *fasciculus cuneatus*, as also reported by Noppeney [9]. In the *fasciculus gracilis,* the third size class (6.1-8.9 μm) had the most fibers (34% of axons) after 120 days. Indeed, the proportion of fibers in this size interval increased progressively with age, with the greatest increase occurring in first 20 days after birth.

In conclusion, the *fasciculus cuneatus* develops earlier than the *fasciculus gracilis*, and has a larger number of nerve fibers with greater axon perimeters and diameters. The mutation which produced a skin naked in the rats apparently did not affect the myelination of the *fasciculi gracili* and *cuneatus*.

ACKNOWLEDGMENTS

The authors thank the Laboratório de Microscopia Eletrônica (UnB) and the Departamento de Histologia (ICB-USP) for the use of their equipment. This work was supported by Fundação de Apoio à Pesquisa da Universidade Federal de Goiás (FUNAPE).

REFERENCES

1. Carratu MR, Cagiano R, Desantis S, Labate M, Tattoli M, Trabace L, Cuomo V (2000) Prenatal exposure to low levels of carbon monoxide alters sciatic nerve myelination in rat offprings. *Life Sci.* **67**, 1759-1772.
2. Da Mata JR, Morais JOR, Sabóia-Morais SMT (1997) Quantitative analysis of the myelinated axons in the *fasciculus gracilis* and *fasciculus cuneatus*. Light microscopy study. *Rev. Chil. Anat.* **15**, 65-70.
3. Hahn AF, Chang Y, Webster HD (1987) Development of myelinated nerve fibers in the sixth cranial nerve of the rat: a quantitative electron microscope study. *J. Comp. Neurol*. **260**, 491-500.

4. Hanlo PW, Gooskens RH, van Schooneveld M, Tulleken CA, van der Knaap MS, Faber JA, Willemse J (1997) The effect of intracranial pressure on myelination and the relationship with neurodevelopment and infantile hydrocephalus. *Dev. Med. Child Neurol.* **39,** 286-291.
5. Knobler LR, Stempak JG, Laurencin M (1974) Oligodendroglial ensheathment of axons during myelination in the developing rat central nervous system. A serial sections electron microscopic study. *J. Ultrastruct. Res.* **49,** 34-49.
6. Krigman MR, Hogan EL (1976) Undernutrition in the developing rat: effect upon myelination. *Brain* **107**, 239-255.
7. Lehman DM, Hale DE, Cody JT, Harrison JM, Leach RJ (1999) Molecular, morphometric and functional analyses demonstrate that the growth hormone deficient little mouse is not hypomyelinated. *Brain Res. Dev. Brain Res.* **116**, 191-199.
8. Matthews MA, Duncan D (1971) A quantitative study of morphological changes accompanying the initiation and progress of myelin production in the dorsal funiculus of the rat spinal cord. *J. Comp. Neurol.* **142**, 1-22.
9. Noppeney R (1991) Quantitative analysis of postnatal myelin sheath development in the dorsal funiculus of rat. *Acta Anat.* **140**, 48-59.
10. Remahl S, Hildebrand C (1990) Relation between axons and oligodendroglial cells during initial myelination. II. The individual axon. *J. Neurocytol.* **19,** 883-889.
11. Robain O, Ponsot G (1978) Effects of undernutrition on glial maturation. *Brain* **149**, 379-397.
12. Sarah A, Luse MD (1956) Formation of myelin in the central nervous system of mice and rats, as studied with the electron microscope. *J. Biophys. Biochem. Cytol.* **2,** 777-784.
13. Schultes G (1991) Quantitative analysis of the postnatal axon growth in the dorsal funiculus of rats. A*cta Anat.* **140**, 85-96.
14. Schwab ME, Schnell L (1989) Region-specific appearance of myelin constituents in the developing rat spinal cord. *J. Neurocytol.* **18,** 161-169.
15. Stampfli R (1954) Saltatory conduction in the nerve. *Physiol. Rev.* **34**, 101-112.
16. Towe AL, Harding GW (1985) Pattern of myelination in the pyramidal tract of the rat. *Exp. Neurol.* **89**, 284-288.
17. van der Knaap MS, Valk J, Bakker CJ, Schooneveld M, Faber JA, Willemse J, Gooskens RH (1991) Myelination as an expression of the functional maturity of the brain. *Dev. Med. Child. Neurol*. **33**, 849-857.

Received: May 21, 2001
Accepted: September 17, 2001