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Quantitative Assessment of a Peripheral Nerve in Chronic Constriction Injury Model of Neuropathic Pain

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Neuropathic pain is a result from damage to the nervous system caused by many diverse processes, rather than stimulation of pain receptors. Chronic constriction injury model (CCI) is a classical model of neuropathic pain based on a loose ligation of the rat sciatic nerve. Here this animal model is used to derive quantitative data. Image analyzer with a motorized stage was applied to analyze the very large light microscopy images from nerve tranverse sections. Three weeks after CCI the mean axon size of the injured nerve appears almost the same as in intact nerve. The sphericity and the mean myelin width of the damaged nerve also do not show significant differences as compared to the intact ones. However, the mean axon density in an injured nerve is diminished to 64% of the value from the intact nerve. The connection of these data with pain pathogenesis is discussed.

Key words: CCI, sciatic nerve, axon, myelin, density.

Introduction

Neuropathic pain is defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system [4]. Neuropathic pain results from damage to the nervous system due to many diverse processes, rather than stimulation of pain receptors. Notwithstanding the steady efforts in many laboratories to unravel the mechanisms underlying the existence of neuropathic pain, they are still unclear. Therefore different animal models are continuously developed to study the pathophysiology of this type of pain [1, 5]. Chronic constriction injury (CCI) model [1] of neuropathic pain is used to investigate the underlying mechanisms of pain associated with damage to the peripheral nervous system and can spur development of novel therapy approaches.

CCI model was proposed in 1988 by Bennett and Xie and is based on a loose ligation of a peripheral nerve that in the rat produces hyperalgesia and allodynia in the sciatic distribution of one hindlimb [1]. Morphological analysis of the damaged nerve has shown that the intact myelinated nerve fibers were reduced and the few surviving myelinated fibers were in the small to medium size range [6]. In the recently proposed mouse model of neuropathic cancer pain the severity of damage to the myelinated fibers was considerably less expressed and the pain characteristics are somewhat different [5].

All these data suggest that the size and density of axons in the damaged nerve could be of importance for the pathogenesis and manifestation of neuropathic pain. Therefore, the aim of the present investigation was to study the light microscopic morphology of sciatic nerve in CCI model of neuropathic pain quantitatively by means of computer assisted image analyzer of high resolution multiple images (Figs. 1-3).

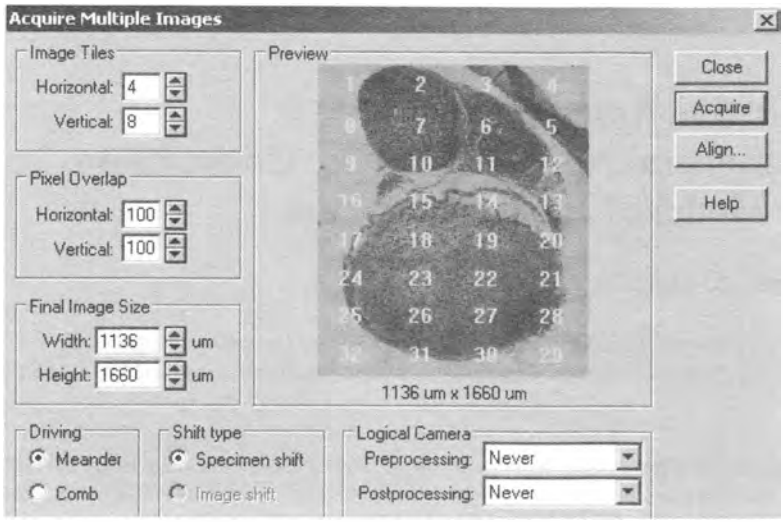


Fig. 1. "Print Screen" from the desktop of the Olympus image analyzer presents the division of a large image of sciatic nerve in 32 individual images

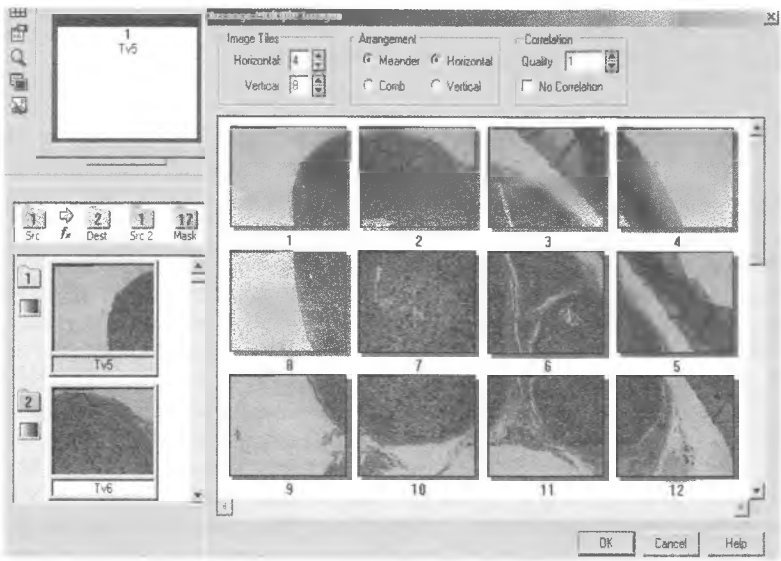


Fig. 2. "Print Screen" from the desktop of the Olympus image analyzer presents at higher magnification 12 from the total 32 individual images

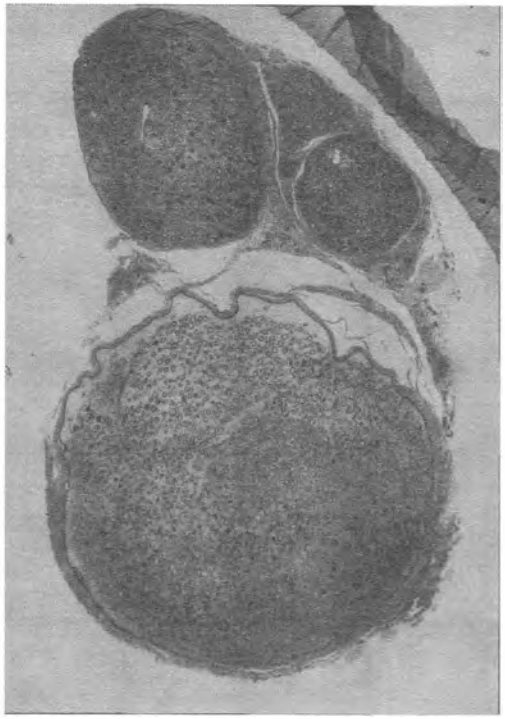


Fig. 3. "Print Screen" from the desktop of the Olympus image analyzer presents the total final image after image alignment

Materials and Methods

Adult female Wistar albino rats (200-250 g) were used in this study. Animals were provided ad libitum access to food and water until the day of death. The experimental protocols were approved by the Ethics Committee of the Medical University of Sofia.

CCI of the sciatic nerve of the rats was induced over the right hind limb (Bennett and Xie, 1988) [1]. Control animals were sham operated. All animals were operated under general anesthesia (thiopental 40 mg/kg, i.p.). The rats were allowed to recover and survive. During the postoperative period the nociceptive thresholds were determined by paw pressure, hot plate, plantar heat, dynamic plantar and incapacitance analgesia tests.

Three weeks after CCI, 3 animals per group were anesthetized with sodium pentobarbital (40 mg/kg). The rats were perfused intracardially with half-strength Karnovsky solution (2% paraformaldehyde and 2.5% glutaraldehyde) in 0,1M phosphate buffer pH 7,4 for 20 min. Small tissue samples of the sciatic nerve distal to the ligatures were post-fixed for several hours in the same fixative at 4°C. They were then rinsed in buffer and post-fixed with 1% osmium tetroxide for 1 hour. Following a second wash the tissue pieces were dehydrated in graded ethanols and embedded in durcupan. Semithin transverse sections of the sciatic nerve were cut on a Reichert-Jung ultramicrotome, stained with toluidine blue and photographed (×40 objective) in an Olympus image analyzer. It was equipped with automatic stage unit and image analysis system AnalySIS. The results were statistically evaluated using the Student's t-test. $P < 0,05$ was considered significant.

Results

Under the light microscope the transverse sections of the loosely ligated and the control (intact) sciatic nerves show very different images (Figs. 4a, b). The characteristic view of an intact sciatic nerve comprising of many fascicles separated from one another by thin laminae of connective tissue is not present in a CCI nerve. The connective tissue appears as a common background in which the individual axons are distributed. Distal to the ligature the exposed axonal profiles are obviously fewer than the ones in the in-

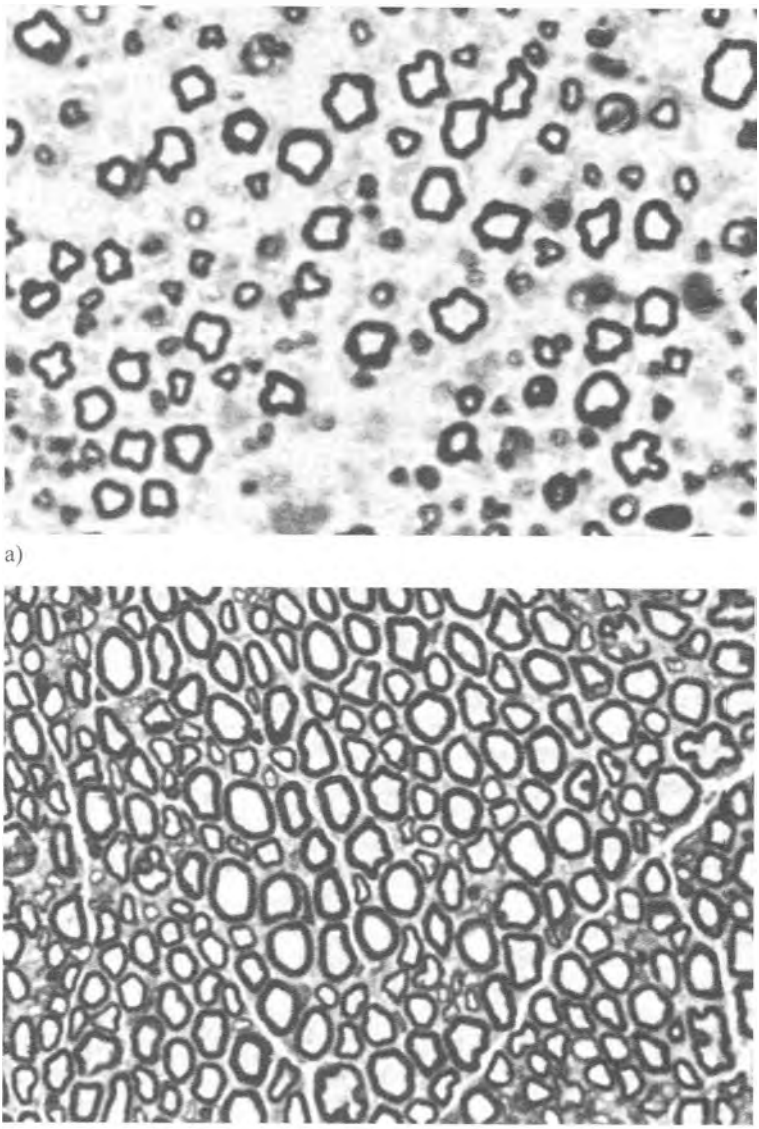
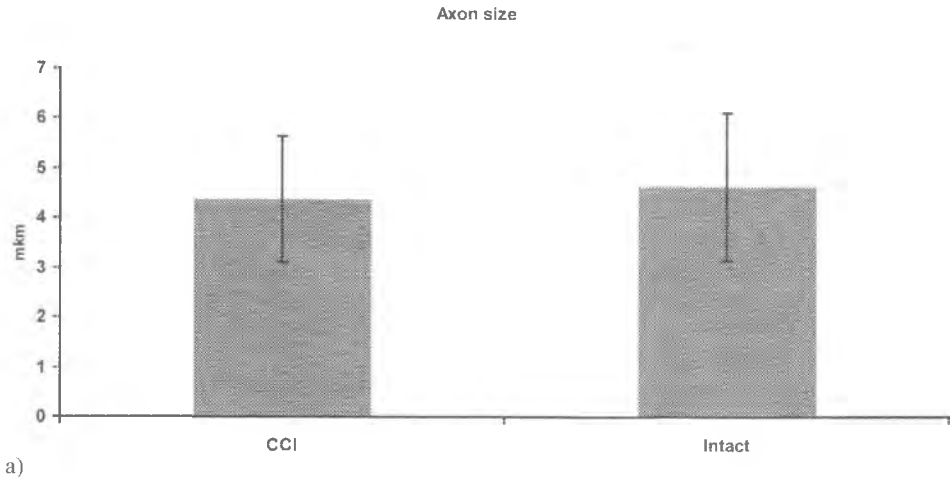
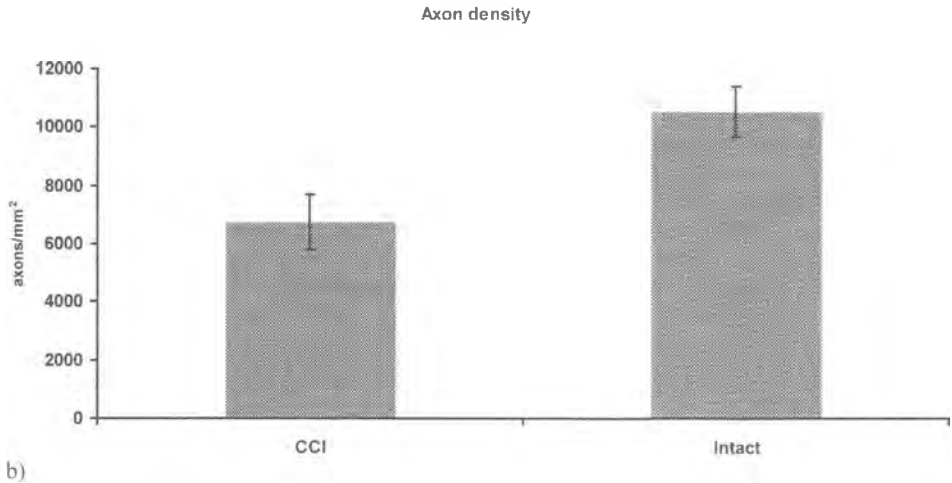


Fig. 4. Light micrographs of transverse sections of sciatic nerve of female rat: a) CCI, b) Intact rat. × 400



a)



b)

Fig. 5. Quantitative parameters of axons in the sciatic nerve after CCI and in intact rat: a) Mean axon size, b) Mean axon density

tact nerve. They are distantly located from the preserved neighboring ones as compared with the relatively tightly packed axons in the control sciatic nerve. Moreover, the contours of the axonal profiles in the CCI nerve are indented and more irregular whereas the intact nerves display approximately oval profiles. The myelin sheath of the axons in the injured nerve is unevenly thick along their circumference. Additionally, all myelin sheaths in a CCI nerve appear to be almost equally thick regardless of the caliber of the axon they ensheath. Therefore, in cases with very small axon profiles the impression is that they comprise of only myelin sheath. Some of these are very pale. On the contrary, in an intact sciatic nerve the thickness of the myelin sheath is dependent on the axon caliber – larger axons have usually thicker sheaths and vice versa.

The quantitative analysis of the images exposed gives additional information. The mean axon sizes in CCI and intact sciatic nerves are very close (Fig. 5a). The sphericity

of the axon profiles and the mean width of the myelin sheaths do not display significant differences as well (data not shown). However, the axon density in CCI sciatic nerve – 6743 ± 965 axons/mm^2 is significantly diminished as compared to the density in intact nerve – 10542 ± 874 axons/mm^2, i.e. the difference is very significant at $p<0,01$ (Fig. 5b).

Discussion

CCI is a peripheral neuropathic pain model that is caused by an injury to the peripheral nervous system and refractory to available conventional treatment [3]. The last circumstance makes the neuropathic pain a serious clinical problem. Therefore, any information derived by using the CCI model could add to elucidating the yet unknown pathogenesis of this problematic pain.

The results of the present study are very interesting. Here for the first time the density of the axons in a sciatic nerve in CCI model of neuropathic pain is quantitatively determined. This is carried out by using a motorized stage with automatic stage control unit. This progressive approach makes it possible to analyze simultaneously the entire area of nerve transverse sections, which in rat sciatic nerve are very large. Using this approach the transverse section is divided in smaller individual images with their subsequent multiple image alignment. The resulting overview image shows a high resolution previously not possible. Our results indicate that three weeks after the CCI the axon density in the sciatic nerve is 64 % of the respective value in intact rats. This is in accordance with the fact reported by others that the pathogenesis of the extended hyperalgesia following chronic constrictive nerve injury is temporally linked with Wallerian-like degeneration [6]. It should be pointed out that the persistence of the usual mean axon size value after CCI means that not the large [2] but also the small axons are affected by the loss of myelinated nerve fibers. This fact could be connected with the pathogenesis of neuropathic pain and therefore it deserves more attention in future investigations.

The lack of significant difference between the mean myelin width values in CCI and intact rats must also be born in mind. Whereas in the intact nerve the mean myelin width is derived from myelin sheaths with very different widths, in CCI nerve it results from sheaths with nearly the same thickness. This is very characteristic and therefore, may be also of importance for generating the neuropathic pain. Additional studies are needed to solve this problem.

References

1. Bennett, G. J, Y. K. Xie. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. – Pain, **33**, 1988, 87-107.
2. Gabay, E, M. Tal. Pain behavior and nerve electrophysiology in the CCI model of neuropathic pain. – Pain, **110**, 2004, 354-360.
3. Gilron, I, C. P. N. Watson, C. M. Cahill, D. E. Moulin. Neuropathic pain: a practical guide for the clinician. – CMAJ, **175**, 2006, 265-275.
4. Merskey, H, N. Bogduk. Classification of chronic pain: description of chronic pain syndromes and definitions of pain terms. 2nd edn., Seattle, IASP Press, 1994, p. 212.
5. Shimoyama, M., K. Tanaka, F. Hasue, N. Shimoyama. A mouse model of neuropathic cancer pain. – Pain, **99**, 2002, 167-174.
6. Sommer, C., A. Lalonde, H. M. Heckman, M. Rodriguez, R. R. Myers. Quantitative neuropathology of a focal nerve injury causing hyperalgesia. – J. Neuropathol. Exp. Neurol., **54**, 1995, 635-643.