Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 17
Sofia • 2011

Effect of Gender and Sex Hormones on Chronically Injured Nerve as a Model of Neuropathic Pain

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Gender differences in pain perception are caused by differential modulating effects of estrogens and androgens. The aim of this study was to disclose any possible relation between the microscopical changes and the sex following chronic constriction injury (CCI) of the sciatic nerve. CCI model of neuropathic pain was induced in male and female Wistar rats (200-250 g) three weeks after gonadectomy. The animals were randomly assigned into groups: (1) ovariectomized females; (2) ovariectomized, estradiol treated (0.5 mg/kg, p.o. in 11 doses for 21 days) females; (3) gonadally intact males and (4) castrated males. Light microscopy and computer assisted amage analysis were applied to reveal the morphological changes. The axonal and myelin injuries are more heavily expressed in castrated males than in ovariectomized females. However, ovariectomized and estrogen treated females show greater changes than gonadally intact males. Results show clearly that there are sex differences in the morphological changes after CCI.

Key words: chronic constriction injury, androgens, estrogens, axons, sciatic nerve.

Introduction

Pain perception is characterized by sex differences according to its differential modulation by estrogens and androgens with females typically presenting higher sensitivity to noxious stimuli and higher incidence of various painful conditions [3-5]. Gonadal hormones are thought as one of the most important factors causing the gender differences in response to pain and analgesia [1, 5]. Such differences suggest that gonadal steroid hormones – estradiol and testosterone could modulate drug induced analgesia. A large number of experimental and clinical investigations imply the role of estrogens in pain sensitivity, endogenous pain modulation and analgesia [11, 14, 16]. Depending on the stimuli, lower pain thresholds have been reported in female animals compared to males, and variability in pain perception is established across menstrual cycle in women [7, 15]. Androgens are also discussed as possible modulators of nociception [2]. However, little is known about influence of sex hormones on the microscopic structure of peripheral nerves affected by neuropathic pain.

The role of sex hormones in neuropathic pain remains elusive. According to some investigators, gender and hormonal status play a key role in experimental neuropathic pain [9]. Simultaneously, there are no corresponding morphological studies on animal models for neuropathic pain. However, some other researchers suggest that sex or sex hormones can influence the nerve injury and/or subsequent recovery [12, 17].

Therefore, the aim of this investigation was to study differences in the light microscopic structure of the sciatic nerve following chronic constriction injury (CCI), a rodent model of neuropathic pain, in relation to gender and sex hormones.

Material and Methods

Adult male and female Wistar rats (200-250 g) were raised under standard laboratory conditions with food and water available ad libitum. All experimental protocols were approved by the Ethics Committee of the Medical University of Sofia. Some of the animals were gonadectomised under anesthesia with ketamine (50 mg/kg, i.p.) and nembutal (12 mg/kg, i.p.). A single dose of gentamycin (8 mg/kg, i.m.) was applied postoperatively.

CCI of the sciatic nerve was induced three weeks after gonadectomy through the following procedure. The animals were anesthetized (ketamine, 50 mg/kg, i.p. and nembutal 12 mg/kg, i.p.). The right sciatic nerve was exposed at mid-thigh level through a small incision, and one-third to one-half of the nerve thickness was loosely ligated with 2 silk threads. The wound was closed with muscle and skin suture. The rats were allowed to survive and were divided into following treatment groups: (1) ovariectomized females, (2) ovariectomized, 17-β-estradiol treated females (0.5 mg/kg, 11 s.c. injections through 21 days), (3) gonadally intact males, (4) castrated males. The nociceptive thresholds were determined by paw pressure, hot plate, plantar heat, dynamic plantar and incapacitance analgesia tests.

Thirty days 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 (x40 objective) in an Olympus image analyzer, which was equipped with automatic stage unit and image analysis system AnalySIS. The obtained data were evaluated by the one-way analysis of variance (ANOVA), followed by two-tail P value or Dunnett's multiple comparison test; $p < 0.05$ was considered significant.

Results

Under the light microscope the transverse sections of the CCI sciatic nerves of rats from the four experimental groups show different images (Figs. 1-4). The nerve sections from ovariectomized rats show reduced number of mainly small to medium sized axonal profiles with relatively thickened myelin sheaths. The sections from ovariectomized and estradiol treated rats display almost the same images with the obvious distinction that the axonal profiles persisted are fewer in number and smaller as compared to the former

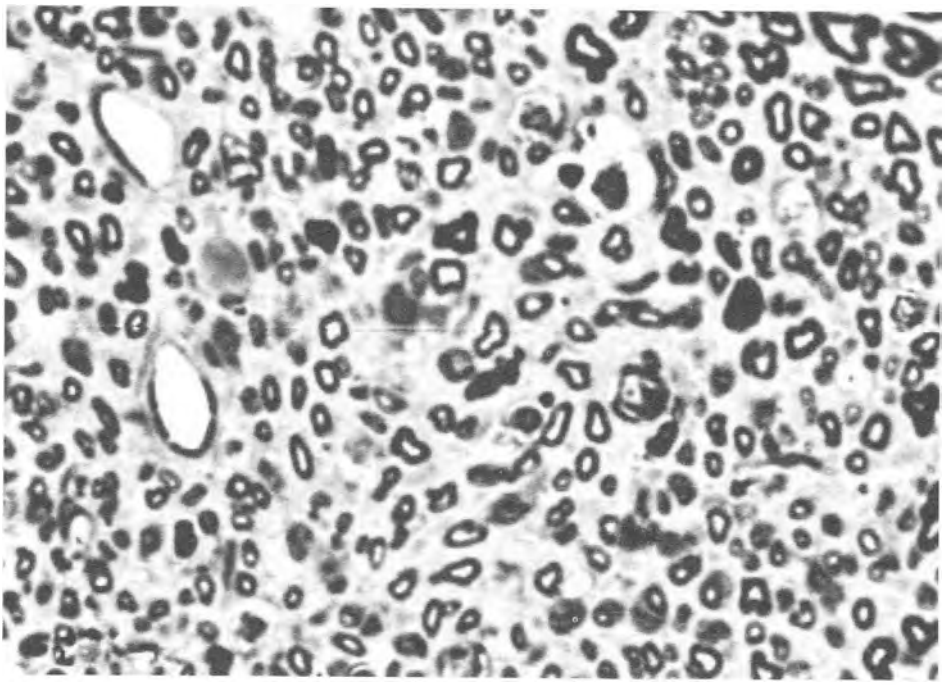


Fig. 1. CCI of sciatic nerve, ovariectomized rat. Light micrograph of transverse section. × 400

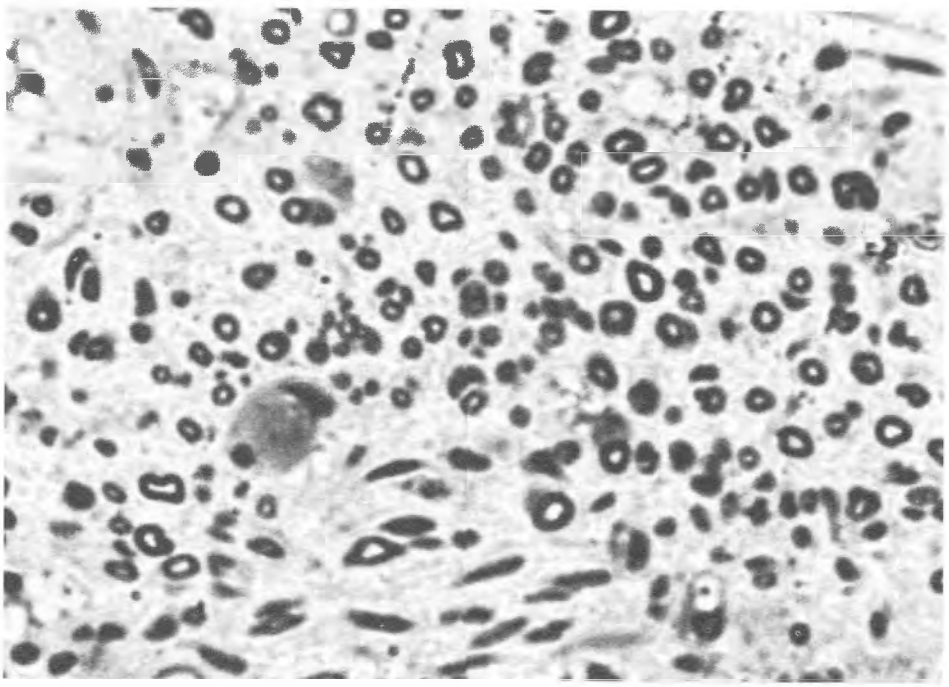


Fig. 2. CCI of sciatic nerve, ovariectomized and estradiol treated rat. Light micrograph of transverse section. × 400

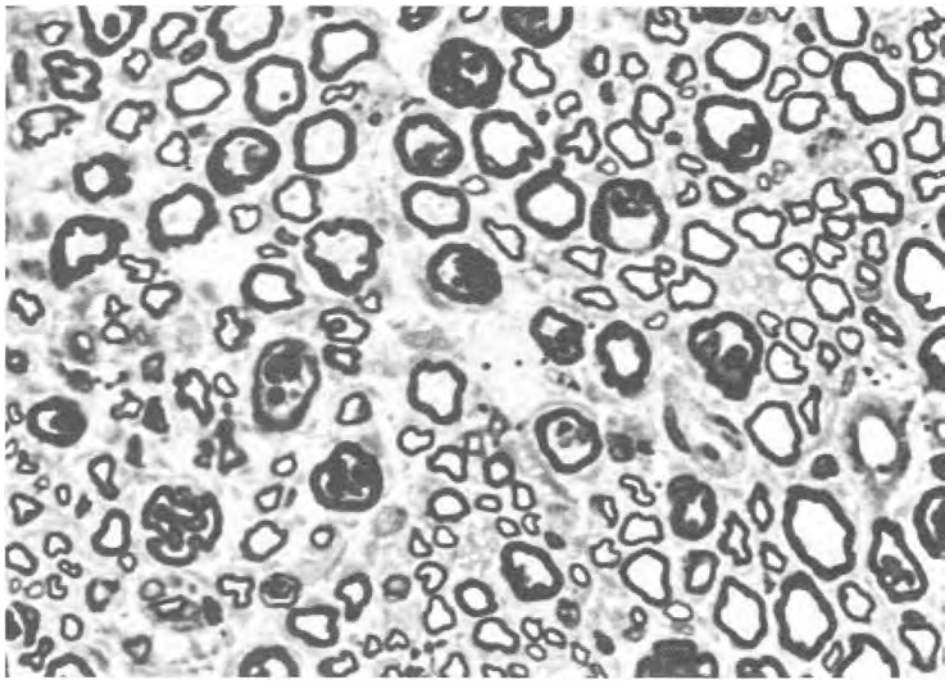


Fig. 3. CCI of sciatic nerve, intact male rat. Light micrograph of transverse section. × 400

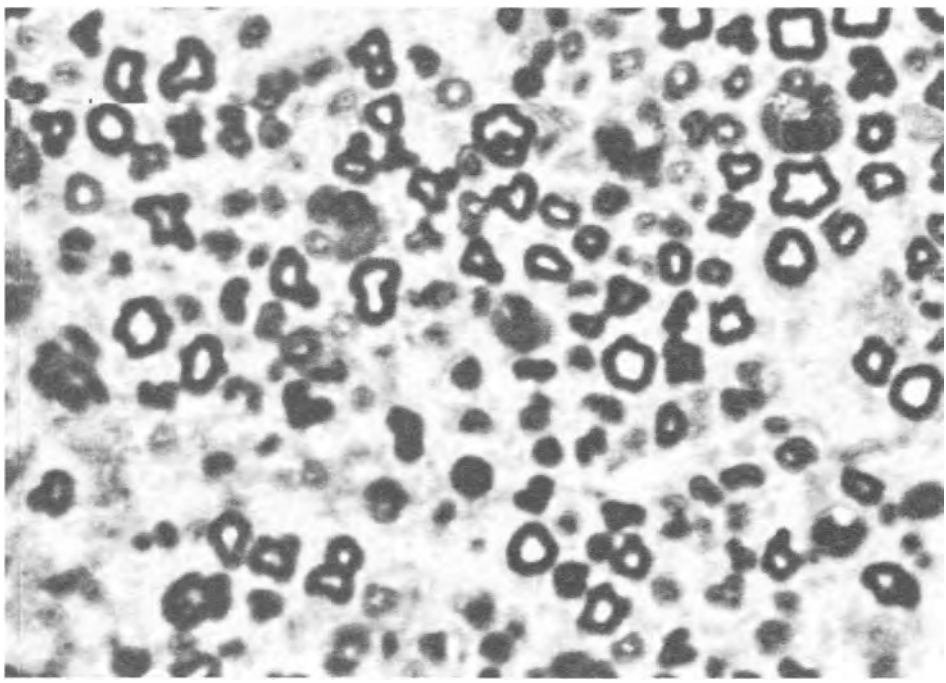
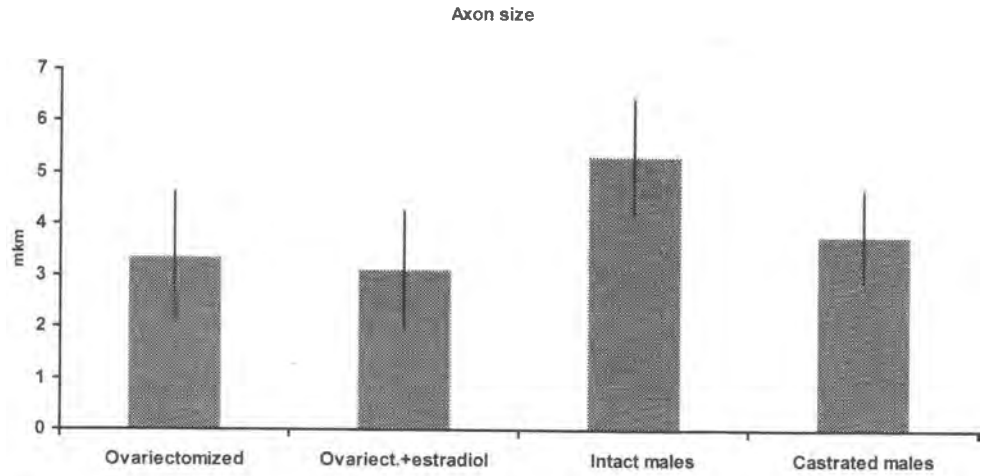
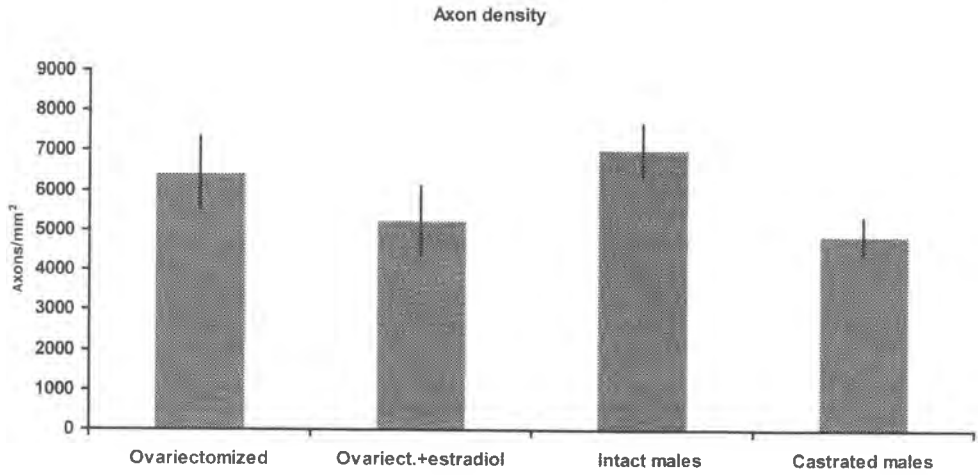


Fig. 4. CCI of sciatic nerve, castrated male rat. Light micrograph of transverse section. × 400



a)



b)

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

group. The sciatic nerve sections from intact male rats expose many and relatively large axon profiles. They also show some signs of deformation but it is comparatively light and the myelin sheats are not thickened. The nerve sections from castrated rats show reduced number of mainly small axonal profiles, showing signs of severe deformation.

The quantitative image analysis indicates that the mean axon size in the group of intact male rats is larger than the means from all other groups, although this difference is not significant (Fig. 5a). The mean axon density values of the four groups investigated differ from one to another with the greatest value for the group of intact male rats (Fig. 5b). These differences are again not statistically significant.

Discussion

A considerable body of evidence has been collected indicating that sex-related differences exist in nociception and gonadal steroids influence the analgesic response in animals and humans [10].

The effects of estrogens on pain perception remain controversial [e.g. 13]. About the effect of androgens on pain, the literature data are not so plentiful. Experimental and clinical evidence exists for an analgesic effect of testosterone [2, 6].

The reported here morphological data are of special interest. In the present study for the first time light microscopic changes in the CCI model of neuropathic pain are described separately in different experimental groups of males and females. These changes show clear sex differences. The axonal and myelin destruction are more expressed in castrated males than in ovariectomized females. Surprisingly, the destructive changes are lightly more severe in ovariectomized and estradiol treated animals than in solely ovariectomized rats. This could mean that the artificially applied estradiol cannot replace and is not fully identical with its naturally produced hormone in the animal. The ovariectomized and estrogen treated females display greater injuries than the gonadally intact males. Taken together all these findings outline the stronger action of testosterone as compared to estrogens in relation to the microscopic changes in the CCI model of neuropathic pain. This fact corresponds with the protective role of testosterone on the nerve injures [8].

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