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Morphometric Properties of the Myenteric Ganglia in the Rat Colorectal Region

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The aim of the present study was to determine the sectional area of ganglia and the sectional area of neuronal perikarya in the proximal colon, distal colon and rectum of the rat. As a result of the conducted morphometric studies, we found that ganglia have similar sectional size in the three regions of the large intestine. In the proximal colon, 42% of the ganglia had sectional area sizes in the range of 1000 μm^2 to 2000 μm^2, whereas in the distal colon (32%) and rectum (21%), ganglia with sizes in the field of 500 μm^2 to 1500 μm^2 were most frequent. In the proximal colon and rectum, neuronal perikarya with a smaller sectional area (an average value of 50 μm^2) were represented more frequently at 52% and 48%, respectively. Neuronal bodies with an average value of 100 μm^2 occur in the distal colon with the highest frequency of 39%.

Key words: myenteric plexus, morphometry, rat, colon, rectum

Introduction

The enteric nervous system (ENS) is a web of neurons embedded in the wall of the gastrointestinal tract [1]. Multiple functions of the ENS related to gastrointestinal

motility, gastric acid secretion, blood flow, nutrient uptake, and interaction with the intestinal immune and endocrine systems have been described in the literature [2]. An essential part of the enteric nervous system is Auerbach's plexus (Myenteric plexus). This myenteric plexus exists between the longitudinal and circular layers of the extrinsic muscle in the intestinal tract [5]. The ganglia that make up the myenteric plexus have been described in the literature being of various sizes and to be interconnected by nerve fibers [4] and have been shown to have properties that are very similar to those of the central nervous system. These properties include the presence of interneurons, glia, extracellular space, synaptic neuropil, multiple synaptic connections and neurotransmitters. The morphology of the ganglia in the plexus myentericus varies in length, width, and area. It has been shown that there is a difference in the morphology of the ganglia at different levels of the large intestine [6]. The average area of neuronal perikarya in different regions of the large intestine can vary widely [3].

The present study aimed to determine at the light microscopic level the sectional area of myenteric ganglia and the sectional area of neuronal perikarya in the rat proximal colon, distal colon and rectum. 2D images of thin paraffin sections from the three regions of interest were examined and compared.

Material and Methods

The scientific studies were carried out on six adult (3-month-old) male Wistar rats with an average weight 250-280 g, delivered from the vivarium of the Faculty of Medicine at Trakia University – Stara Zagora. The animals were housed under an artificial 12-h light/dark cycle and at a temperature of 22°C. Water and food pellets were supplied *ad libitum*. The experiments in this study were approved by the Research Ethics Committee at the Medical Faculty of Trakia University and the Commission for Ethical Treatment of Animals at the Bulgarian Food Safety Agency. All the experiments were carried out in full agreement with the Directive 2010/63/EU on the protection of animals used for scientific purposes. For the purposes of morphometric analyses all rats were anesthetized with 87 mg ketamine/kg of body weight and 13 mg xylazine/kg after simultaneous intraperitoneal injection and transcardially perfused first with cold 0.05 M phosphate buffered saline (PBS) and after that cold 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB), pH 7.36. Three tissue segments were collected from the proximal colon, distal colon, and rectum. The tissue samples were postfixed in the same fixative for 24 h and then were first washed in tap water, followed by distilled water, dehydrated, and embedded in paraffin. Paraffin blocks were cut into 6 μm tissue sections and mounted on chrome-gelatinized glass slides and then processed for hematoxylin and eosin staining. The slides were examined and photographed with a research microscope Leica DM1000 equipped with a digital camera Leica DFC 290 and the images were processed with Adobe Photoshop CC software.

The graphic analyzing software ImageJ (National Institutes of Health, Bethesda, MD, USA) was used to perform the morphometric analysis of the ganglions and the neurons. As ganglion, we defined an element of the plexus containing at least one nucleated nerve cell profile according to the described methodology of Gabella and Trigg [3]. The cross-sectional area of ganglia and neurons was determined using the ImageJ program by precisely delineating neuronal bodies and well-distinguished ganglion boundaries.

Statistical analysis was performed by GraphPad Prism®6 software (San Diego, CA, USA) and Kruskal-Wallis One Way Analysis of Variance followed by post-hoc pairwise multiple comparison Dunn tests. Statistically significant differences were considered if p-values were <0.05.

Results

In the present study, we measured the sectional area of the ganglia and the area of the neuronal perikarya that form these ganglia in the myenteric plexus of the colorectal region. Transverse and tangential sections of the proximal colon, distal colon and rectum were analyzed. The ganglia of the myenteric plexus were clearly visible and delineated with the classic histological stain hematoxylin and eosin, which allowed us to better define the shape, nucleus and type of the nerve cell (**Fig. 1**). The non-parametric Kruskal–Wallis test to investigate the sectional area of the ganglia with Dunn's multiple analysis was used to compare medians (Med) between the three segments of the large intestine [in the proximal colon (Med = 1701 μm^2), distal colon

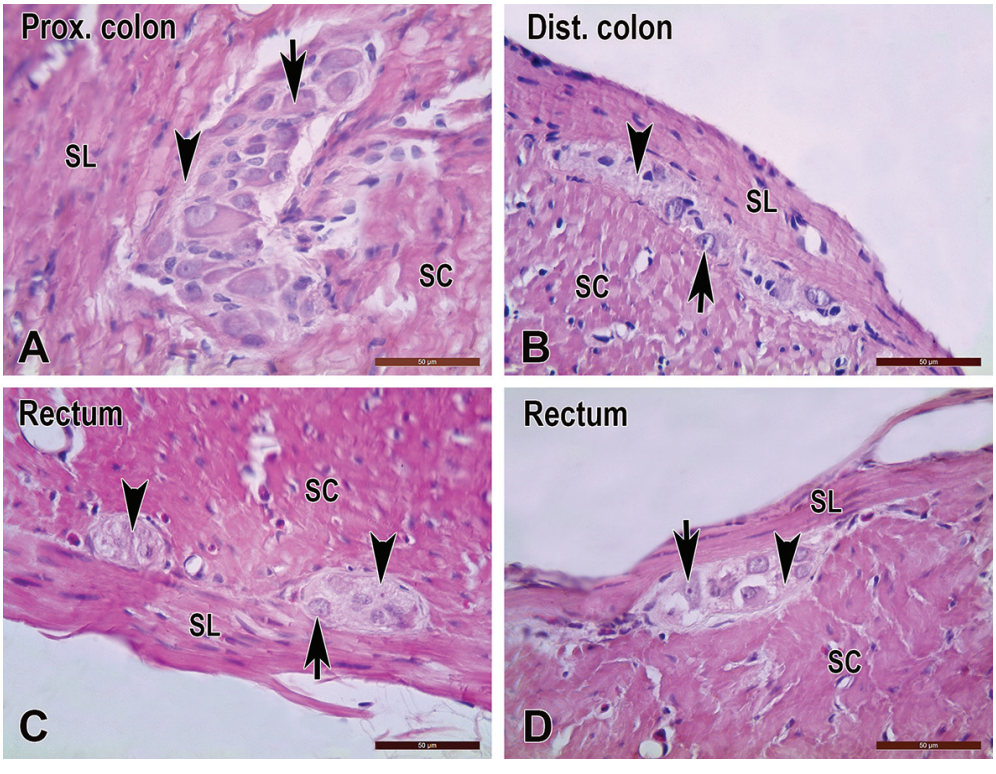

Fig. 1. Hematoxylin and eosin stained sections visualizing the myenteric ganglia and the neuronal perikarya in the proximal colon **(A)**, distal colon **(B)**, and rectum **(C, D)**. Arrows indicate neurons, while arrowheads indicate ganglia. Scale bars: 50 μm.

(Med = 1399 μm²) and rectum (Med = 1893 μm²)]. The sectional area of the ganglia in the three regions of the large intestine did not differ statistically significantly, H(2) = 1.738, p = 0.4194 (**Fig. 2A**). Ganglia with an average cross-sectional area of 1500 μm² (in the range of 1000 μm² to 2000 μm²) occur in the proximal colon with the highest frequency of 42% (**Fig. 2B**). In the distal colon and rectum, ganglia with a smaller cross-sectional area size (an average value of 1000 μm² and the range of 500 μm² to 1500 μm²) were represented more frequently at 32% and 21%, respectively (**Fig. 2B**).

Morphological examinations of the perikarya had also been carried out. We have examined more than 138 – neuronal cell bodies (perikarya) from ganglions of all three segments. The perikarya were well-defined, and in the ganglions that we measured, all of the neuronal bodies were nucleated and easily distinguished from the glial cells. A Kruskal-Wallis test showed statistically significant differences in the cross-sectional area of neuronal perikarya size in the three examined areas of the colorectal region, H(2) = 16.75, p = 0.0002. The medians in all three study regions were different and

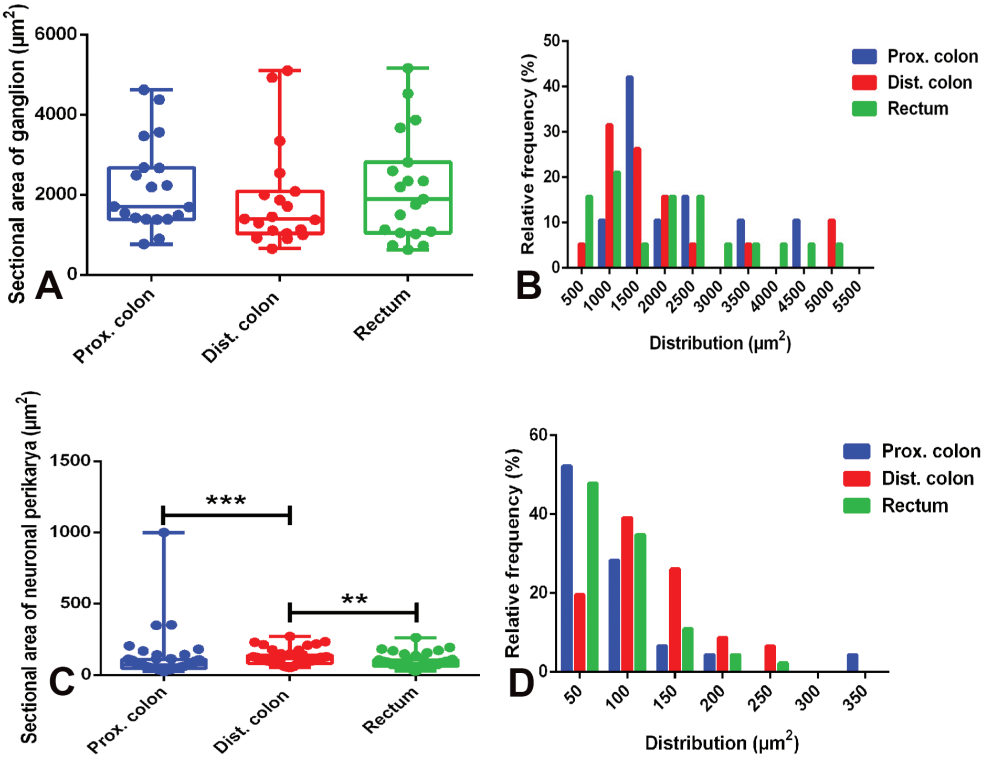

Fig. 2. Box plot diagrams showing statistical comparison of the sectional area of ganglions **(A)** and the sectional area of neuronal perikarya **(C)** in proximal colon (boxes outlined in blue), distal colon (boxes outlined in red) and rectum (boxes outlined in green) in the rat. The data are presented as box plots, where the line within the box represents the median and the boxes represent the second and third quartiles (25–75%). Individual points are the corresponding values. The data compared using the Kruskal-Wallis test, where p** < 0.01; p*** < 0.001. Histograms showing the relative frequency in percent of a distribution of values for the cross-sectional area of ganglia **(B)** and cross-sectional area of neuronal perikarya **(D)**.

as follows: proximal colon (Med = 72.25 μm^2), distal colon (Med = 116.99 μm^2) and rectum (Med = 80.749 μm^2) (**Fig. 2C**). A post hoc pairwise multiple comparison test using Dunn's test showed significant sectional neuronal perikarya size differences between the proximal colon and distal colon, $p < 0.001$ and between the distal colon and rectum, $p < 0.01$ (**Fig. 2C**). In the proximal colon and rectum, neuronal perikarya with a smaller sectional area (an average value of 50 μm^2) were represented more frequently at 52% and 48%, respectively. Neuronal bodies with an average value of 100 μm^2 occur in the distal colon with the highest frequency of 39% (**Fig. 2D**).

Discussion

In this study, we attempt to verify previous data regarding the size of the ganglia and the sectional area of the neurons that make up Auerbach's plexus in the rat colorectal region. Our results showed a slight morphological difference in the sectional area of the ganglia forming the myenteric plexus in the rat's proximal colon, distal colon and rectum, but these differences were not statistically significant. We did not obtain sufficient evidence that a specific pattern of increase or decrease in the size of the ganglia from the upper to the lower parts of the large intestine. This gives us reason to agree with the conclusions drawn by Elżbieta Nowak and her colleagues [6].

The most common size of Auerbach's plexus neuronal bodies in the colon and rectum is between 200 and 400 μm^2 [7]. In the guinea pig myenteric plexus, the average sectional area of neuronal perikarya varies between 300 μm^2 per colon and from 157 μm^2 to 278 μm^2 for the rectum [3]. In our study, however, the mean size of the neuronal bodies we examined in the colon and rectum was generally smaller than that reported by other authors.

The method we used to conduct our morphometric analysis could be criticized. Cross section method was preferred because it was less time-consuming, easier to prepare and better for obtaining more data. Whole mount preparation could have given better opportunities to get more relevant data for the ganglions at the different levels of the large intestine. The sections we mainly used were tangential as they were known to give better accuracy of the size of neuronal cells and ganglions [3]. It had been determined [3] that the tangential sections were visualizing the ganglion much better and would be given better opportunities to examine the neurons, the neuronal nuclei and the glial cells.

Conclusions

The detailed examination provides new scientific data for the overall size difference of the ganglions and neuronal perikarya at the different levels of the large intestine.

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