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Morphological Characteristics and Cytoarchitecture of the Myenteric Ganglia in the Rat Proximal Colon

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The myenteric plexus, which consists of interconnected ganglia, has been a subject of interest since its discovery in the late nineteenth century. In our study we focus on the morphology of these ganglia, when observed on routine sections through the rat intestinal wall of a randomly chosen segment, in this case the proximal colon. After applying routine histological methods, we found myenteric neurons of variable shape and size, interspersed between enteric glial cells and nerve fibers. Although the exact morphological type cannot be surely determined, the presence or absence of short and broad neuronal processes may lead the examiner to a conclusion about the major morphological group, which it belongs to. In conclusion, the sections are still one of the simplest and, therefore, frequently used methods of examination of the enteric nervous system, because they provide basic, but valuable information about the cytoarchitecture of the enteric ganglia.

Key words: enteric neurons, myenteric plexus, morphology, cytoarchitecture.

Introduction

The myenteric plexus (plexus of Auerbach) is one of the two major components of the enteric nervous system and consists of variably sized ganglia, interconnected by nerve fiber bundles (inter-ganglionic strands). The structure of the plexus has been a subject of intense scientific interest since its first description by Auerbach in the distant 1864. The most widely accepted classification of its constituent neurons is that of Dogiel, who subdivided them into three morphological classes, now called Dogiel-type-1, 2 and 3 [2]. Other classification schemes have also been proposed [5], as well as extensions of the one of Dogiel [1]. However, even though it is oversimplified, the original classification is still widely used, which is mostly due to the extensive research in the area, which has proven that there is a strict correlation between the structural type and the electrophysiological and neurochemical properties of the neurons [3].

Over time, Dogiel-type-1 and 2 morphological subtypes have become major checkpoints during primary investigation of unexamined material, perhaps due to their

distinct outlook and opposing functions. Dogiel-type-1 neurons have a single long process (an axon) and several short and broad processes (lamellar dendrites), emerging from the soma [2]. Functionally, these neurons serve as inhibitory and excitatory motor neurons for the circular and longitudinal musculature and also as interneurons. The Dogiel-type-2 neurons are perhaps the best example of correlation between morphology and function and, therefore, there is hardly any disagreement between researchers regarding their nature. These neurons are often regarded structurally as "multiaxonal", i.e. their neuronal somata have oval, smooth contours and give rise to several long processes, all of which functionally are proven to be axons. The role they play in enteric circuitry has also well established. In particular, these belong to the so-called intrinsic primary afferent neurons (IPAN), a major sensory component of the enteric reflex circuitry.

However, despite their structural and functional differences, most of the enteric neurons share a common morphological feature: their bodies and processes are more or less flattened in a plane that is parallel to the intestinal external surface, i.e. to the interface between the two muscle layers [4]. Therefore, for specific scientific purposes, the most beneficial methodology includes whole-mount preparations or at least tangential (oblique) sections through the enteric muscle layers, so as to observe the larger neuronal surface or cross-section, respectively. The contours of this surface are the main factor, which the morphological classification of the enteric neurons is based on.

Nevertheless, the sections, either cross- or longitudinal, through the organs of the digestive tract remain the first and sometimes only method for visualization of a variety of structures, both for scientific purposes and student education. Therefore, in this article we have aimed to describe the appearance of the myenteric ganglia on cross- and longitudinal sections of a specific enteric segment (in this particular case the proximal colon), using several of the most common staining techniques.

Material and methods

In our study we used 20 adult (3-month-old) Wistar rats of both sexes with average weight (200-300 g). The entire array of experiments was carried out at the Medical University of Sofia in accordance with its ethical principles for the care and use of laboratory animals. We performed a routine transcardial perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.2-7.4. This neutral buffered fixative penetrates rapidly and does not cause excessive tissue shrinking or distortion of the cellular structure. After perfusion, we collected specimens from the region of the proximal colon, each measuring approximately 10 mm, which were postfixed in the same fixative overnight, at 4°C. Thereafter, the tissue blocks were washed in tap water and then in distilled water, dehydrated with alcohol series, embedded in paraffin and cut into7 μm and 10 μm (for the impregnation technique) thickcross- and longitudinal sections. Then we applied standard protocols of hematoxylin and eosin (H&E) staining, Nissl staining and the Gomori method of silver nitrate impregnation.

Results

In most cases, the cross-sections reveal myenteric ganglia that appear as small clusters of cells, populated only by a few neurons with unimpressive dimensions (**Fig. 1A**). Neurons with larger sectional surface were very rare. On the longitudinal sections, the myenteric plexus acquires the form of larger clusters of neurons, occupying a cleft-like space, lying between bundles of the circular and longitudinal muscle layers (**Fig. 1B,**

C and D). The shape of the ganglia appears to be variable, including circular, oval, egg-shaped and polygonal shapes. The same is also valid for the number of neurons per ganglion and the shape of their bodies. The nerve cell count ranges from five to more than twenty per ganglion. The contours of the perikarya vary largely; we registered rounded, oval, fusiform and stellate shapes. The differences that the neurons show, when applying the different staining techniques, are few, but readily noticeable.

Whenever the cut engages neuronal nuclei, with the routine H&E staining they appear to be quite euchromatic, slightly eccentrically placed and with vesicular appearance. Should the nucleolus be also present, it usually lies centrally (**Fig. 1B**). A regular finding in the vicinity of the neuronal bodies are the nuclei of the supporting enteric glial cells. They are usually oval or fusiform, intensely heterochromatic, and relatively small in size, especially in comparison to the neuronal ones. The cellular boundaries of those cells are poorly demarcated and their cytosol is practically indistinguishable, due to their poor staining capabilities and the cellular interposition. The intercellular spaces are occupied by nerve fibers, which give it a reticular appearance.

With the Nissl staining, the findings are slightly different, because the cresyl-violet has high affinity to neurons only (**Fig. 1C**). There, we can still observe the aforementioned neuronal shapes and nucleoli, but the nucleus itself remains relatively unstained. The latter is not true for the glial cells, as their nuclei are still readily recognizable, whereas the rest of the cellular structure is lost.

When applying the silver impregnation technique, determining the exact neuronal borders is not always easy, because there is significant interposition of cellular structures (**Fig. 1D**). Nevertheless, there is significantly more reduction of silver ions in the cytosol of the neurons, when compared with the glial cells. Combined with their overall bigger size, this is usually enough to distinguish the two cellular types. The nerve fibers are not stained with this technique, so the ganglionic intercellular spaces appear to be empty.

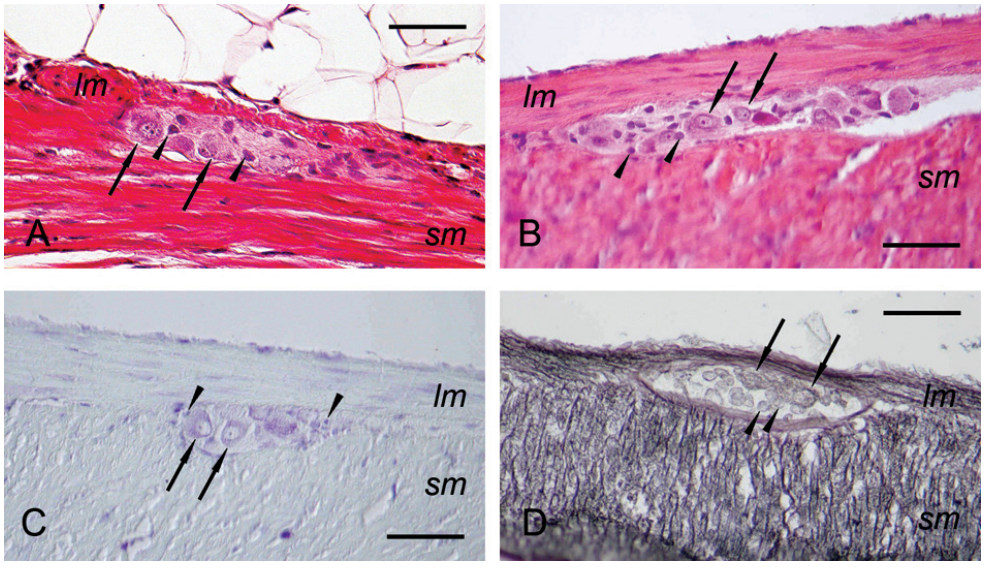

Fig.1. Demonstration of myenteric ganglia in the rat proximal colon on sections through the smooth muscle layer. Note the variable shape of the neuronal perikarya. lm – longitudinal muscle; cm – circular muscle; arrows – perikarya of myenteric neurons; arrowheads – nuclei of enteric glial cells. Scale bar: 50μm. (A) Cross section, H&E; (B) Longitudinal section, H&E; (C) Longitudinal section, Nissl staining; (D) Longitudinal section, Gomori silver nitrate impregnation.

Discussion

The sections through the intestinal wall reveal significant, but sometimes insufficient information about the exact neuronal morphology. This is especially true for cross-sections, in which the plane of the cut passes quite unfavorably through the myenteric ganglia. In those sections the ganglia appear to be falsely small and the neurons quite difficult to be distinguished. Naturally, this is due to the fact, that the longer axis of the myenteric ganglia is parallel to the longitudinal axis of the gut. Moreover, for similar reasons the sectional surface of the cells is severely reduced. That is why, in terms of tissue sections, a much more beneficial approach is the series of longitudinal sections, which is the main substrate of our current study.

As mentioned previously, the shape and size of the ganglia appears to be variable. This is hardly a surprise because, naturally, they would depend on the extent to which the ganglia are affected by the cut. Moreover, the ganglia are prone to be more or less deformed, depending on the state of intestinal distension. In our study, we have put all the specimen under the same conditions (appr. 10% stretching), but when evaluating results from different sources, this factor should be taken into consideration. This factor is also valid when attempting to calculate the nerve cell count and density. Consequently, even in adjacent ganglia the cell count is greatly variable, as reported above.

In terms of neuronal morphology, it is only sometimes possible to determine the exact morphological subtype, according to the universal classification of Dogiel. Should the general shape of the neuronal soma be close to that of a multipolar neuron, one may be inclined to accept that this is a motor neuron, innervating the smooth muscle (since they have been proven to express Dogiel-type-1 morphology). Conversely, the IPANs, which in most cases show Dogiel-type-2 morphology, are expected to present themselves with oval perikarya, devoid of any broader cellular processes. However, without confirmation via specific staining methods (e.g. immunohistochemical reaction), solid conclusions regarding the morphologic affiliation should be avoided, because findings on sections might be misleading.

Conclusions

The myenteric ganglia are a common occurrence on cross- and longitudinal sections through the enteric wall, regardless of the examiner's intentions, educational or scientific. Despite providing only partial information about the neuronal morphology, these sections remain one of the simplest and most frequent means of tissue observation and, therefore, correct interpretations of the structures found is crucial. Thus, this study is simply done to emphasize on the basic checkpoints, needed for successful evaluation of looked for or random findings in the myenteric ganglia.

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