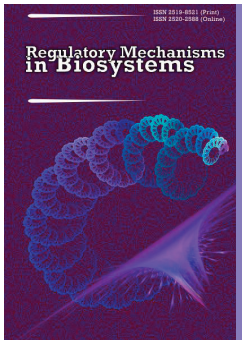

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2020, 11(4), 501–505
doi: 10.15421/022076

Morphology of spinal ganglia of different segmentary levels in the domestic dog

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Article info

Received 04.10.2020
Received in revised form 15.11.2020
Accepted 16.11.2020

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Horalskyi, L. P., Kolesnik, N. L., Sokulskiy, I. M., Tsekhmistrenko, S. I., Dunaievska, O. F., & Goralska, I. Y. (2020). Morphology of spinal ganglia of different segmentary levels in the domestic dog. Regulatory Mechanisms in Biosystems, 11(4), 501–505. doi:10.15421/022076

The spinal ganglia, which perform the function of the first link on the afferent impulses' way from the receptors to the central nervous system, recognize internal and external irritations, and are the first to transform them into a nervous impulse. As the representatives of the peripheral nervous system, they are some of the main objects of the studies in contemporary neuromorphology. Based on the results of anatomic, neurohistological, histochemical, morphometric and statistical methods of the studies, we conducted a complex survey, revealing the morphology of spinal ganglia of different segmental levels in the domestic dog. In particular, we determined the differences in the microscopic structure and morphometric parameters of cervical, thoracic, lumbar and sacral spinal ganglia and the ganglia of the cervical and lumbar enlargements in mature domestic dogs. The study showed that the spinal ganglia of domestic dogs can have different skeletotopy, different shape and sizes due to their species peculiarity. Also, the surveyed animals, according to the results of our studies, had the cervical and thoracic spinal ganglia of oval, while the lumbar and sacral – spindle-like shapes. According to the results of morphometry, the area of the spinal ganglia in lengthwise section differed: the smallest area belonged to the thoracic, the largest to the sacral spinal ganglia. The density of neuronal arrangement per 0.1 mm^2 of the area of the spinal ganglia correlated with their sizes: the highest parameter was identified for the thoracic spinal ganglia, the lowest – for the sacral. The conducted studies revealed that histo- and cyto-structure of the spinal ganglia is characteristic of notable differentiation of the nervous cells of small sizes. Therefore, we differentiated neurons of the spinal ganglia into large, medium and small. The highest quantity of large neurons was found in the sacral ganglia, and largest amount of medium-sized neurons – in the ganglia of the lumbar enlargement. In other ganglia, small neurons dominated. Correspondingly, different nuclear-cytoplasmic ratio in these neurons was determined, indicating different extent of morphofunctional condition of nervous cells. We determined content of localization and separation of nucleic acids in histostructure of the spinal cord at the tissue and cellular levels.

Keywords: peripheral nervous system; spinal ganglion; morphometric parameters; neuron; dog.

Introduction

Despite the significant success and achievements of the domestic and foreign neuromorphology and large amount of studies on the structure of the nervous system (Hirose & Jacobson, 1979; Lazriev, 2001; Goral's'kyi et al., 2018), in-detail study and morphometric assessment of histo- and cyto-structures of the spinal ganglia remains a relevant issue in neurology (Vehnovskaja, 1988; Kobayashi et al., 2010; Khokhlova et al., 2017), as they are the primary afferent nervous centers of intermediate position between the central and peripheral nervous systems. The spinal ganglia have a great functional importance in reflector functions of the organism of animals, which is related to the entrance of information from both external and internal environment through them (Hanani, 2005; Safonova & Kovalenko, 2006; Goralskiy, 2018).

The most thorough studies were carried out on the spinal ganglia of the laboratory animals, in the norm (Pannese et al., 1972; Nazarchuk, 2009; Sobol' & Gapanovich, 2015), and in the conditions of various types of experimental damage (Topp, 2000; Safonova & Kovalenko, 2006; Hamzianpour et al., 2015; Yun et al., 2020) and diseases in the organism (Li, 2020). However, insufficiently studied aspects, according to the analysis of neuromorphologic studies, are peculiarities of microscopical structure of the spinal ganglia of small animals, including the domestic dog. Furthermore, the literature contains controversial data on neuron organization of the spinal ganglia in the the normal condition and regarding age (Rubinow & Juraska, 2009). Also, such research concerning the structure of the spinal ganglia of dogs has been seen to be fragmentary in character. Therefore, most scientists studied the lumbar ganglia, possibly on account of participation of ganglia in the innervation of the sciatic nerve (Tandrup, 1993; Schionning & Larsen, 1997). Some works analyzed the thoracic spinal ganglia (Larnicol, 1988; Nazarchuk, 2009, 2010), though even these reports contain controversies about the structural organization of the spinal ganglia, their cellular composition, neuron organization, etc. Therefore, the objectives of our study were mophofunctional characteristic and determining morphometric parameters of the spinal ganglia of different parts of the spinal cord of the dog in the comparative aspect. For this purpose, we selected bilateral spinal ganglia: cervical, thoracic, lumbar, sacral, and also the ganglia of cervical and lumbar enlargements to study their morphofunctional characteristic of clinically healthy dogs, as norm parameters for the differential diagnostics of diseases affecting the nervous system.

Materials and methods

Morphological studies of the spinal ganglia were performed on the material selected from 6 shepherd dogs. Before the selection of animals for the study, we conducted their clinical examination using the generally accepted methods, including: visual examination, determining body weight of the animals, frequency of their pulse, respiratory tracts, thermometry (Levchenko et al., 2004). The animals selected for morphological studies were subjected to euthanasia according to the International Con-

vention for Using Laboratory Animals as of 1985. During the studies, we also followed the main rules of laboratory practice GLP (1981), provisions "The main ethical principals of the experiments on animals" adopted by the I National Congress of Bioethics (Kyiv, 2001) and requirements to the "Rules of conducting works on use of experimental animals" approved by the Decree of the Ministry of Healthcare of Ukraine No 281 "On the measures for further improvement of the organizational forms of work using experimental animals" as of 1 November 2000.

The study was performed at the Department of Anatomy and Histology of the Veterinary Medicine Faculty of the Polissya National University. The objects of the study were the cervical, thoracic, lumbar, sacral ganglia and the ganglia of the cervical and lumbar enlargements in clinically healthy mature shepherd dogs of 40–47 cm withers height, and body weight of 26–41 kg.

The material for neurohistological and histochemical surveys of the cervical spinal ganglia was taken from the level of the 3rd and 6th cervical neurosegments of the spinal cord (C_3 and C_6); thoracic ganglia – level of the 5th neurosegment (Th_5); lumbar – level of the 3rd and 6th neurosegments (L_3 and L_6), sacral spinal ganglia – at the level of the 2nd sacral neurosegment (S_2).

In the study we used anatomical, neurohistological, histochemical, morphometric and statistical methods.

The corpses of the animals were skinned, removing the muscles, the head, the limbs, the thoracic and abdominal walls from the vertebral column. Then, the vertebral column with the spinal cord was fixated first in 5% aqueous solution of neutral formalin over 5 days, and then in 10% solution for 10 days. The dissection was started by removing the soft tissues of the back and tail and the dorsal surface of the neck. Afterwards, using the Luer forceps, the vertebral arches were removed together with the transverse and spinous processes, opening access to the spinal cord and its nerves. In the spinal cord we determined the boundaries of its parts and the skeletopy of the spinal ganglia. Then, we extracted the spinal cord with its filum terminale and the nerve roots from the vertebral column canal.

For the histological surveys, the extracted spinal ganglia were fixated in 12% aqueous solution of neutral formalin with subsequent embedding of the material into paraffin. Paraffin sections were prepared using sledge microtome MS-10. The sections did not exceed thickness of 10 μm. The samples were stained according to Nissl, using hematoxylin and eosin. Separate histosections were subjected to impregnation with silver nitrate according to Ramón y Cajal and Bilshovsky-Gross. Morphometric surveys of the structural elements of the spinal ganglia were undertaken in light microscopy in accordance with the recommendations proposed in the book by Horalsky et al. (2015).

Nucleic acids were detected using Einarson method, separate detections of DNA and RNA were carried out using Brachet method, and total proteins were determined according to Shust.

To prepare histochemical preparations, we implemented the methods described in text books on histochemistry. All histological surveys were accompanied by the necessary control, confirming their specificity.

To study the histochemical preparations, we determined not only localization, but the intensity of reactions of determining DNA, RNA and proteins. Staining reaction was evaluated as weak (+), moderate (++) and highly- (+++) intense.

The digital parameters of the results were analyzed using variation-statistical methods, calculating the arithmetic mean (x), mean arithmetic error (m), correlation coefficient (r), and mean squared error (δ). Reliability of the difference between arithmetic mean of two variation rows was determined using the reliability criterion (t_d) according to Student-Fisher (Avtandilov, 1990; Horalskyi, 2015). The difference between two values was considered reliable at $P < 0.05$.

Results

The spinal ganglia of domestic dog are located in the area of the intervertebral foramina. They are the thickenings of the dorsal roots of the spinal ganglia which ramify from the spinal cord. According to the parts of the vertebral column, the spinal ganglia are divided into cervical (C_1–C_7), thoracic (Th_1–Th_{13}), lumbar (L_1–L_7), sacral (S_1–S_3) and tail (Ca_1–Ca_5) ganglia. Furthermore, we distinguished the corresponding spinal ganglia which ramify from the cervical and lumbar enlargements of the spinal cord, formed as a result of load on the thoracic and pelvic limbs which they innervate. According to our observations, the borders of the localization of the spinal ganglia in relation to the vertebral column vary. Thus, the cervical ganglia are located in the intervertebral foramina, while the thoracic, lumbar, sacral and the ganglia of the enlargements are located outside of them, being somewhat more cranial from them, which in our opinion is a species peculiarity.

The shape of the spinal ganglia of domestic dog, according to our observations, differs as well. The cervical ganglia and the ganglia of the corresponding enlargement were distinctive of having oval shape, while the thoracic ganglia were ovoid. The lumbar and sacral ganglia of the lumbar enlargements had spindle-like shape.

The spinal ganglia of domestic dog differ also by their sizes, as indicated in the area of their length-wise sections. Therefore, the largest sizes belonged to the sacral spinal ganglia (8.95 ± 0.20 mm^2), and the smallest to thoracic ganglia (2.52 ± 0.50 mm^2). The area of the length-wise sections of the cervical ganglia accounted for 3.83 ± 0.52 mm^2, thoracic – 3.47 ± 0.26 mm^2, cervical enlargement – 5.16 ± 0.20 mm^2 and lumbar enlargement – 5.61 ± 0.30 mm^2.

At the tissue level, on the outside the spinal ganglia are covered by notable connective-tissue capsule. The characteristic morphological feature of the spinal ganglia is structured arrangement of the perikarya and the nerve processes. Perikarya are localized on the periphery under the capsule as a strip, whereas nerve process – mostly in the central part of the ganglion (Fig. 1). Their perikarya were round in shape. Thickness of the connective-tissue capsule of the spinal ganglia correlated with the area of their length-wise section, seen largest in the sacral ganglia (96.5 ± 0.8 μm), and smallest in the thoracic ones (59.3 ± 0.6 μm).

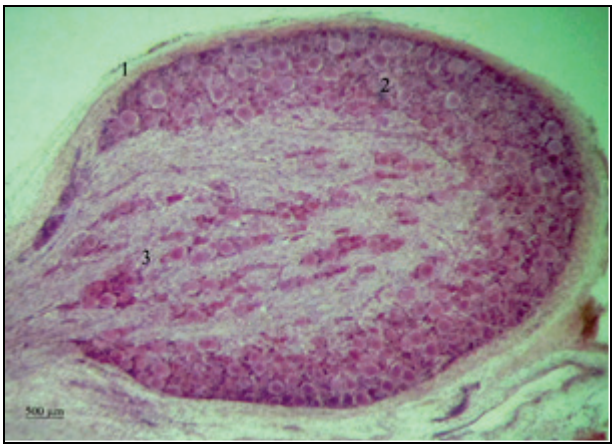

Fig. 1. The length-wise section of the spinal ganglion of the cervical enlargement in domestic dog: *1* – the connective-tissue capsule; *2* – nervous cells; *3* – nerve fibers; staining using hematoxylin and eosin

The main morphofunctional elements of the spinal ganglia are the pseudounipolar neurons, each with a big rounded body, in the center of which, rarer acentric, a large bright nucleus is located, having one or two nucleoli (Fig. 2). Perikarya of neurons of the spinal ganglia are surrounded by neuroglia cells which are adjacent to the perikarya of neurons known to form some sort of mantle (Fig. 3).

During total impregnation of the spinal ganglia of dogs with silver nitrate according to Ramón y Cajal, there were observed different intensities of impregnation of neurons, which does not depend on the size of cell's body. The histosections were observed to have dark neurons with large bright nucleus, bright neurons and cells of average level of impregnation (Fig. 4). Perikarya of neurons of the spinal ganglia of different parts of the spinal cord varied in volume (Table 1). The highest values of these measurements were determined for separate spinal ganglia, and also the ganglia of the cervical and lumbar enlargements. Therefore, the mean parameter of the volume of perikarya of nerve cells accounted for $119{,}408 \pm 4{,}816$ μm³ in the sacral spinal ganglia, $76{,}011 \pm 4{,}335$ μm³ in the cervical enlargements of the spinal cord, and $97{,}386 \pm 3{,}857$ μm³ in the lumbar enlargement. Neurons of the spinal ganglia also had different volume of nuclei (Table 1). This parameter was highest for the neurons of the sacral

ganglia (2,824 ± 118 μm^3), and lowest for the cervical ones (1,248 ± 46 μm^3). Nuclear-cytoplasmic ratio of neurons is the indicator of their functional activity. Therefore, according to our data, this indicator was lowest in neurons of the spinal ganglia of the lumbar enlargement (0.037 ± 0.002) and sacral ganglions (0.038 ± 0.003).

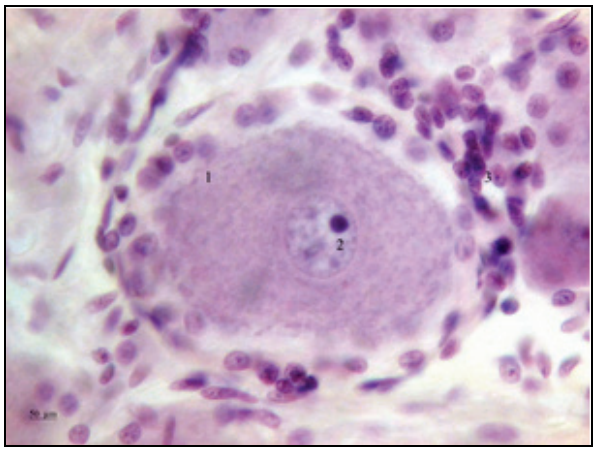

Fig. 2. Fragment of microscopical structure of the cervical spinal ganglion of domestic dog: *1* – cytoplasm of neuron; *2* – nucleus and nucleolus; *3* – nuclei of glial cells; hematoxylin and eosin

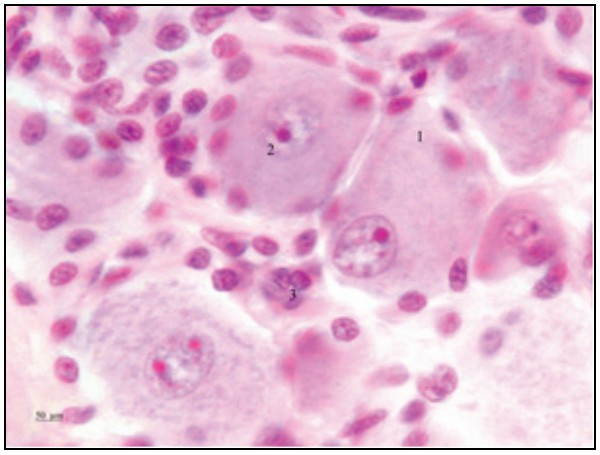

Fig. 3. Fragment of microscopical structure of the thoracic spinal ganglion of domestic dog: *1* – cytoplasm of neuron; *2* – nucleus and nucleolus; *3* – nuclei of glial cells; hematoxylin and eosin

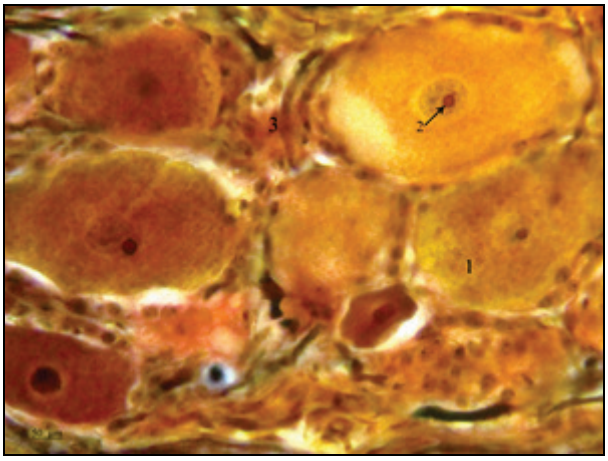

Fig. 4. Fragment of microscopic structure of the thoracic spinal ganglion of domestic dog: *1* – cytoplasm of neuron; *2* – nucleus and nucleolus; *3* – nuclei of glial cells; Ramón y Cajal

The nuclear-cytoplasmic ratio was somewhat higher in neurons of the lumbar ganglia and the ganglia of the cervical enlargement, and the highest in neurons of the cervical and thoracic ganglia (0.054 ± 0.004 and 0.055 ± 0.008) (Table 1).

Table 1

Volume of perikarya, nuclei and nuclear-cytoplasmic ratio of neurons of the spinal ganglia of domestic dog (x ± m, n = 6)

Spinal ganglia	Volume of cells, μm^3	Volume of cell's nucleus, μm^3	Nuclear-cytoplasmic ratio
Cervical	61,455 ± 3163	1,248 ± 46	0.054 ± 0.004
Cervical enlargement	76,011 ± 4335***	1,752 ± 65***	0.047 ± 0.002
Thoracic	43,862 ± 2614***	1,383 ± 35***	0.055 ±0.008
Lumbar	65,774 ± 3794***	1,506 ± 52*	0.042 ± 0.002*
Lumbar enlargement	97,386 ± 3857***	2,077 ± 63***	0.037 ± 0.002*
Sacral	119,409 ± 4817***	2,824 ± 118***	0.038 ± 0.003

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001 in relation to the spinal ganglia of the previous part.

According to the morphological parameters, depending on the volumes of neuronal perikarya among cells of the spinal ganglion, we distinguished three main groups of cells: large, medium and small (Fig. 5).

Thus, in the neuron composition of the surveyed spinal ganglia, the prevailing population was small nerve cells. It was especially expressed in the thoracic spinal ganglia in which this indicator in the percent ratio reached 72.9 ± 0.5%. However, neuron populations of the lumbar enlargement and the sacral part were significantly different: largest populations of cells belonged respectively to medium-seized (44.6 ± 0.5%) and large (47.4 ± 0.9%) cells (Fig. 5).

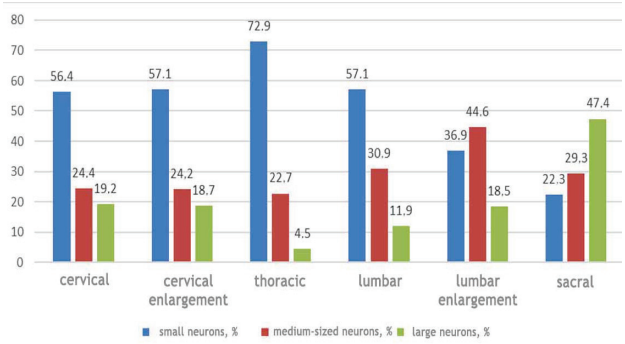

Fig. 5. Parameters of the content of neurons in the spinal ganglia of domestic dog (%, n = 6)

Identification of density of location of nerve cells per 0.1 mm^2 of the area of the ganglia revealed that the greatest one was in the thoracic ganglia (34.2 ± 0.7), and the smallest – in the sacral ganglia (20.8 ± 0.6). We also observed different quantities of the neuroglia cells which were densely adjacent to perikarya of neurons of the spinal ganglia. The highest number of them (38.2 ± 0.8) was observed around the perikarya of the ganglia of the lumbar enlargement, and the lowest number (17.7 ± 0.1) – near perikarya of the cervical ganglia.

Cytoplasm of neurons of the spinal ganglia contained perspicuous "boulders" of Nissl substance, indicating high degree of the development of protein-synthesizing apparatus in them. The sizes of the "boulders" of Nissl substance and density of their arrangement in the neuroplasm of the neuronal perikarya differed. The most dense arrangement of the Nissl substance "boulders", according to our observations, was located in neuron spinal ganglia of the enlargements of the spinal cord, which also suggests their significant functional activity (Fig. 6).

During the histochemical surveys of the spinal ganglia of different parts of the spinal cord of the domestic dog, we determined peculiarities of localization and the content of nucleic acids and total protein in neurons of the spinal ganglia. As it is known, DNA and RNA are nucleic acids. Highest intensity of histochemical reaction to the total detection of nucleic acids was found in neurons and glial cells of the spinal ganglia. The highest intensity of this reaction was manifested in their nuclei (+++). This was especially noted for neurons of the spinal ganglia of the cervical and the lumbar enlargements of the spinal cord (Fig. 7).

Total protein located in nucleus and neuroplama of neurons of the spinal ganglia. Highest intensity of histochemical reaction and its detection, similarly to histological reaction to the content of nucleic acids was seen in neurons of the spinal ganglia ramifying from the enlargements of the spinal cord (Fig. 8).

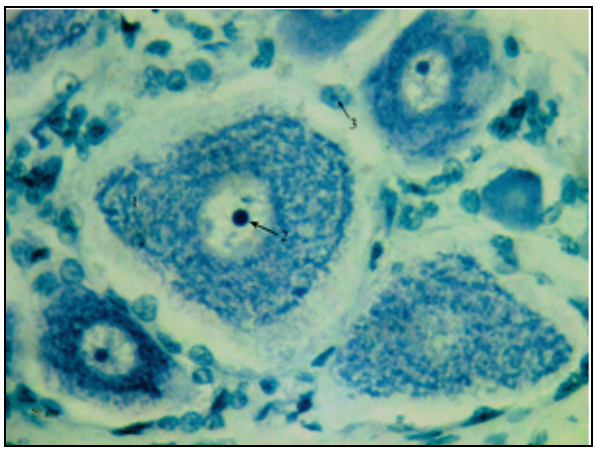

Fig. 6. Distribution of Nissl substance in the cells of the spinal ganglion of the cervical enlargement of domestic dog: *1* – "boulders" of Nissl substance in neuroplasm; *2* – nucleus and nucleolus; *3* – nuclei of glial cells; Nissl

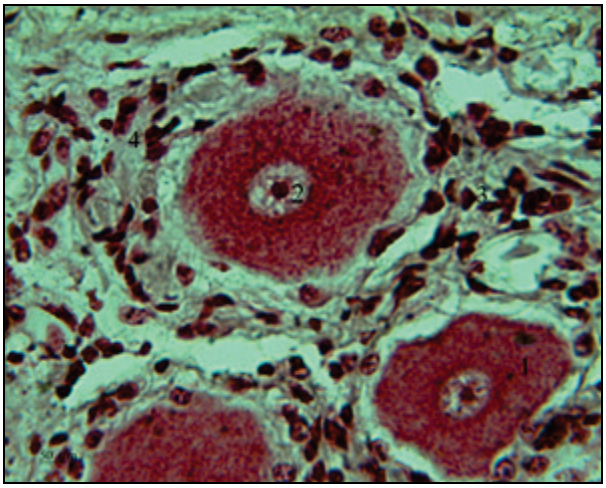

Fig. 7. Distribution of nucleic acids in neurons of spinal ganglion of the lumbar enlargement of domestic dog: *1* – "boulders" of nucleic acids in neuroplasm; *2* – nucleus and nucleolus; *3* – nuclei of glial cells; *4* – mantle membrane; Brachet

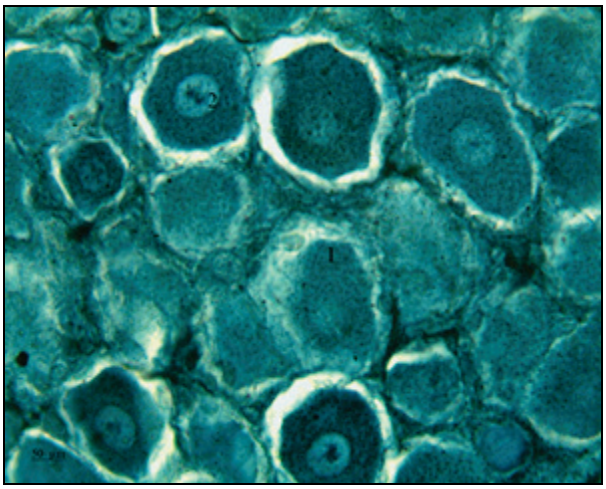

Fig. 8. Distribution of total protein in the cervical spinal ganglion of domestic dog: *1* – neuroplasm, *2* – nucleus and nucleolus; Shust

Discussion

The data on morphofunctional characteristics and morphometric analysis of the spinal ganglia of different parts of the spinal cord are fragmented. Furthermore, the available literature (Pannese et al., 1972; Zherebcov, 1991; Nazarchuk, 2010) contains significant differences in morphometric parameters of populations of neurons, which could be explained, in our opinion, first of all by different morphological criteria the authors used to calculate various cell parameters. Also, one should take into account the difference in methods and objects of the survey, especially the species, breed, age peculiarities of animals, their living conditions, etc. (Bailey, 1901). Also, the differences in morphological criteria should be considered, in spite of different segmentary levels of the spinal ganglia, which in turn also have inter-segment peculiarities, as confirmed by our studies.

Among the literature sources we analyzed, the results of our studies' regarding the area of the length-wise sections of the spinal ganglia correlate with the data of Nazarchuk (2010), who described these parameters of the area of the thoracic spinal ganglia of domestic dog. Our data on the content of neurons of different sizes in the spinal ganglia also coincide with the results of the survey (Nazarchuk, 2009; Nazarchuk, 2010) which revealed that the largest share of small neurons is present in the thoracic spinal ganglia of domestic dog. At the same time, these results contradict the results of other surveys (Lobko et al., 2000) indicating that the thoracic spinal ganglia in the dog are represented by the greatest population of neurons being medium-sized (50.5%).

As we know, the spinal ganglia are formed of the fibery connective tissue, neurons and neuroglia. Cells of the latter form the mantle adjacent to the neurons of perikarya, and also form the membrane of the nerve fibers (Polak, 1965; Citkowitz & Holtzman, 1973). Furthermore, neurons of the spinal ganglia, which according to the functional peculiarities are sensitive (Vekhnovskaya, 1988; Nazarchuk, 2010; Tongtako, 2017), have axons which form the dorsal root of the spinal nerve (Kikuchi, 1994), while the dendrites are included in the content of the nerve itself (Sharpey-Schafer, 1881; Bahar, 2006; Kobayashi et al., 2010).

Moreover, on the basis of the analysis of morphometric data of various populations of nerve cells of the spinal ganglia of different segmentary levels, we should note the heterochrony of the parameters of volume of cell perikarya, their nuclei and therefore nuclear-cytoplasmic ratio. In our opinion, such changes occur due to the fact that the spinal ganglia of different location level innervate different structures – act as receptors of the skin, the movement apparatus, while the other ganglia act as receptors of the internal organs, etc, i.e. the structures of different levels of morpho-functional activity (Rubinow & Juraska, 2009; Nazarchuk, 2010; Stepanchuk, 2020). Therefore, morphometric analysis of the populations in the spinal ganglia revealed high parameters of the volumes of neuronal perikarya and nuclei in the segments of the cervical and lumbar enlargements which take part in the innervation of accordingly the muscles of thoracic and pelvic limbs. It should be noted that they were greatest in the neurons of the sacral spine ganglia, indicating their functional activity.

The main morphometric parameter of the level of metabolism and cell differentiation in different conditions of their existence characterizes the parameter of nuclear-cytoplasmic ratio, using which one may evaluate the level of morphological and functional maturity of species of animals and their age aspects (Ermolin, 2006). Thus, nuclear-cytoplasmic ratio of neurons, which is the indicator of their functional activity, according to our data, was the lowest for neurons in the spinal ganglia of the lumbar enlargement and the sacral ganglion, and the highest for neurons of the cervical ganglia. This, in our opinion, is associated with different area and volume of the objects of sensitive innervation of corresponding spinal nerves.

An important aspect of the morphofunctional activity of the nervous system of animals is neuronal density, since such parameter depicts the dynamics of the development of the brain and is a morphological indicator of the physiological and pathological changes in the nervous system (Schröder, 2020). Because the density of arrangement of neurons per 0.1 mm^2 of the area of the ganglia, according to the results of our studies, is highest in the thoracic ganglia and lowest in the sacral ganglia, in our opinion this parameter is directly proportionally related to the size of the spinal ganglia themselves (area of the length-wise section).

It has to be noted that neurons are distinctive for significantly high level of metabolic processes, especially regarding the nucleic and protein metabolisms (Russo et al., 2010). High level of protein synthesis suggests presence of Nissl substance in neurons. Thus, in the process of differentiation of Nissl substance, there is observed increase in the concentration of nucleic acids and intensity of protein synthesis, underlying the progressive growth of the nucleus, cytoplasm and the processes. Our analysis of histopreparations for detection and separation of nucleic acids and proteins

revealed the pattern of their distribution in the structures of the spinal ganglia of the domestic dog. Therefore, the results of the surveys we conducted using the histochemical methods, identifying the largest areas of the localization of nucleic acids and total protein in the nerve cells, confirm the results of the previous studies (Goralsky et al., 2018) concerning the functional activity of neurons being expressed the most in the cervical spinal ganglia and the ganglia of the lumbar enlargement.

Conclusions

The spinal ganglias of mature domestic dogs have various skeletolotopy, shape and sizes, determined by their species peculiarity. The cervical spinal ganglia are located in the intervertebral foramina, and all the others – outside of them and in position slightly cranial from them. The cervical ganglia are oval in shape, the thoracic – ovoid, lumbar and sacral – spindle-like. Different shape of the spinal ganglia in domestic dog, in our opinion, is also a species peculiarity.

The largest sizes were characteristic for the sacral, the smallest for the thoracic ganglia. The density of location of neurons per 0.1 mm^2 of area of the spinal ganglia depends on their sizes. This parameter was greatest (34.19 ± 0.70) for the thoracic ganglia and smallest in the sacral ganglia (20.78 ± 0.60).

Neurons of the ganglia were large, medium-sized, and small. Large neurons were seen in the largest amount in the sacral ganglia (47.4 ± 0.9%), and medium-sized ones in the ganglia of the lumbar enlargement (44.6 ± 0.5%). In other ganglia, small neurons dominated (57.1–72.9%). The number of glial cells which form the mantle around each neuronal perikaryon in the ganglia varied. The largest amount of them was seen in the ganglia of the lumbar enlargement (38.2 ± 0.8), and the lowest number of them – in the cervical ganglia (17.7 ± 0.1).

Also, morphometric analysis of populations of the spinal ganglia revealed the highest parameters of the volumes of neuronal perikarya and neuronal nuclei in the segments of the cervical and lumbar enlargements.

The nuclear-cytoplasmic ratio of neurons of the spinal ganglia, which characterizes their functional activity, varied among the populations. Therefore, the highest, according to our data, was the nuclear-cytoplasmic ratio of neurons in the spinal ganglia of the lumbar enlargement and the sacral ganglia, while the lowest – neurons of the cervical ganglia. The highest intensity of histochemical reactions for the detection of nucleic acids and total protein manifested in neurons of the cervical spine ganglia and ganglia which ramify from the lumbar enlargement of the spinal cord. The same neurons contained the largest amount of Nissl substance.

The conducted survey is a part of Scientific-Research Work "Development, morphology and histochemistry in animals in the normal and pathological conditions", Ministry of Education and Science of Ukraine, state registration number 0120U100796.

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