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Specific features of the morphology of the spinal nodes of homeothermal vertebrate animals in the comparative and anatomical series

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Abstract

Scientific studies that reflect the evolutionary morphology of the nervous system, namely the spinal nodes of homoeothermic vertebrates, allow us to identify specific patterns, trends, and criteria that relate to the structural organization of the organ at the population and cellular levels of their organization. The evolutionary direction of research into the macro- and microstructure of spinal cord nodes provides an opportunity to investigate the patterns of formation of optimal relationships of their structural components: nerve and glial cells, capillaries concerning the level of development of the organism, and their motor activity. The article contains the results of the morphometric characteristics of the spinal nodes of warm-blooded vertebrates in a comparative aspect: class Aves – Birds (Gallus gallus, forma domestica L., 1758 – domestic chicken); class Mammalia – Mammals (Oryctolagus cuniculus L., 1758 – European krill, Canis lupus familiaris L., 1758 – domestic dog, Sus scrofa, forma domestica L., 1758 – domestic pig, Bos taurus taurus L., 1758 – domestic bull). When performing this work, complex research methods were used: anatomical, neurohistological, morphometric, and statistical. The morphological study of the structural components of the organs of the nervous system was studied on histological sections by the method of light microscopy following the requirements of the international principles of the "European Convention for the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes" (Strasbourg, 1986), a decision of the First National Congress on Bioethics (Kyiv, 2001), Law of Ukraine No. 692 "On the Protection of Animals from Cruel Treatment" (3447-IV) dated February 21, 2006. For histological examination, the newly selected material was immediately fixed in a 10% aqueous neutral formalin solution, with a fixation period of 30 days, followed by step-by-step embedding in paraffin. Using a sled microtome, histological sections with a thickness of 6–10 μm were obtained. The general histological structure and microstructural changes in histological preparations were studied under a light microscope MC (Micros Austria) at magnifications from 70 to 600 times. Photomicrographs of histological preparations were carried out using a CAM V200 video camera mounted in a Micros MC microscope. The work aims to conduct a complex morphofunctional study of spinal nodes in a comparative anatomical series of representatives of higher vertebrates. It was investigated that in phylogenesis, a particular structural and morphofunctional rearrangement of the central and peripheral nervous system organs, namely the spinal nodes, takes place. They differ in shape and size. It was established that the neurocytic organization of the spinal cord nodes of all species of animals studied is characterized by the presence of large, medium, and small nerve cells. Adaptation to various living conditions of animals was formed based on morphometric indicators (the volume of neurocytes and their nuclei), their morphological structures, the density of nerve cells per 0.1 mm^2, the number of glial cells per 0.1 mm^2, an indicator of nuclear-cytoplasmic ratio, degree of polymorphism chromatophilia. Nissl staining of histopreparations of spinal nodes of homoothermic animals showed that the neuroplasm of nerve cells contains well-defined depths of basophilic substance, compared with lower animals, as evidence of a higher degree of development in nerve cells of the protein-synthesizing apparatus. According to the method of impregnation of spinal cord nodes with silver nitrate in all studied animals, a different intensity of coloring of nerve cells is revealed: dark, light-dark, light, which is related to the peculiarities of species and age neuromorphology, the morphofunctional state of the nervous system and the type of higher nervous activity. The obtained morpho-functional features of the organs of the nervous system are essential not only for evolutionary and comparative morphology but also for developing issues of physiology, pathology, and treatment of peripheral nervous system diseases.

Keywords: evolutionary anatomy; micromorphology; organs of the nervous system; morphological studies; basophilic substance; nuclear-cytoplasmic relationship.

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1. Introduction

The nervous system is a highly complex structural and functional system of the body, which plays the most important role in the regulation of all processes occurring in it (Karpovskiy et al., 2016; Ladle & Hippenmeyer, 2023). It ensures interconnection and coordinated work of tissues, organs, and their systems, thanks to which the animal and human body functions as a single entity (Grechukha & Otych, 2020). In addition, with the help of the nervous system, the body communicates with the external environment (Zhurenko et al., 2018). Its activity is the basis of the processes of adaptation, behavior, feelings, and other mental processes that allow higher vertebrates and humans not only to learn about the environment but also to actively change it (Dehtyarenko, 2018). The integrating system allows animals to perceive external and internal stimuli and quickly react to them (Garman, 2011; Shnurenko et al., 2020).

The cellular organization of nervous tissue and the nervous system as a whole is a crucial established experimental fact already within the last hundred years (Gage, 2015). However, the final ordering of ideas about the types and properties of nerve cells, their structures and connections, evolutionary development, the creation of a peculiar periodic system of neurocytes, their interconnections, and the density of glial cells, continues to be relevant for evolutionary morphology and neurophysiology.

The nervous system of living organisms in the process of evolution has come a long way from a set of primitive reflexes in the simplest to a complex system of information analysis and synthesis in higher vertebrates (Budd, 2015). At the same time, the restructuring of neural networks took place as a result of the formation of new sensory connections (Monk, & Paulin, 2014). In the course of evolution, an even more complex behavior emerged, characteristic only of higher mammals and humans. This was associated with further complications of the senses, motor activity, and nervous and muscular systems.

The primary type of behavior of lower and higher vertebrates consists of elementary nervous reactions called unconditioned reflexes. These are innate reactions to vital stimuli (Holland et al., 2015; Holland, 2016). Such reactions have been preserved and are essential for the behavior of higher animals, including humans. Such reactions are controlled by the lower, oldest parts of the nervous system (Popele & Bosco, 2003). In vertebrates, such a department is the spinal cord with spinal nodes (Horalskyi et al., 2020; Sokulskyi et al., 2021).

The nervous system, together with the integrating organs, controls the life processes of the organism as a whole and its parts, in particular, the motor activity of animals (Wisniewski, 1983). Control, regulation, and coordination of the morpho-functional states of the body are carried out by neurohumoral means (Grechukha & Otych, 2020). The nervous system ensures the relationship between the body and the environment (Sokulskyi et al., 2021). In terms of adaptive and compensatory transformations of the structural components of organs and their systems in the conditions of the transition from an aquatic to a terrestrial environment, in the macroevolutionary plan, the study of the nervous system of vertebrates is of considerable interest: domestic chicken, European krill, domestic dog, domestic pig, domestic bull.

For a long time, spinal cord nodes (SCN) have been particularly interested in neuromorphology (Starchenko et al., 2018; Horalskyi et al., 2020), which are afferent structures of reflex arcs and are the basis of primary centers on the way to transmit sensory information to the central system (Ernsberger, 2009), providing appropriate reactions to the action of specific factors (Medici & Shortland, 2015).

One of the primary manifestations of the functioning of the nervous system is afferent impulse (Snider & Silos-Santiago, 1996; De Moraes et al., 2017). Therefore, the study of spinal nodes in a comparative anatomical series of vertebrates allows us to follow the historical process of their morphofunctional formation, which reflects the degree and nature of the animal's motor activity, habitat, and lifestyle. At the same time, the study of sensory neurons, which are the initial link of the reflex arc, will allow the establishment of the relationship between the organism and the environment, as well as the patterns of combining segments and different parts of the organism into a single system (Meltzer et al., 2021; Ribeiro & Xapelli, 2021).

Attempts by scientists to find out the etiology and pathogenesis of diseases of the nervous system require in-depth knowledge of the structure of the regulatory systems of the entire organism. Peculiarities of spinal cord nodes' morphology and chemical architecture are still poorly understood, especially in domestic animals. Evolutionary development, species, age of vertebrates, type and functional state of the neuron, phase of neurogenesis, degree of reflex activity, and localization in the body determine these features. However, these data are very important not only for age-related morphology but also for developing issues of physiology, pathology, and treatment of peripheral nervous system diseases. Therefore, a critical moment is the morphological study of the macro- and micromorphology of the organ, and the rearrangements of their structures in the evolutionary series of vertebrates, which served as the goal and task of our research.

2. Materials and methods

Neurohistological studies were carried out in the conditions of the pathomorphology laboratory of the Department of Normal and Pathological Morphology, Hygiene, and Expertise of the Polissia National University. The conducted research is part of the scientific subject of the corresponding department on the topic “Development, morphology and histochemistry of animal organs in normal and pathological conditions”, state registration number 0120U100796.

The object of the study was the bilateral spinal cord nodes of the thoracic section of vertebrates (n = 6), which belonged to two classes of the subtype class Aves – Birds – Gallus gallus, forma domestica L., 1758 – domestic chicken; class Mammalia – Mammals – Oryctolagus cuniculus L., 1758 – European krill, Canis lupus familiaris L., 1758 – domestic dog, Sus scrofa, forma domestica L., 1758 – domestic pig, Bos taurus taurus L., 1758 – domestic bull. Following the tasks set for the study of the histomorphology of spinal cord nodes in the comparative-anatomical series, studies were conducted, which were based on the use of a complex of anatomical, neurohistological, morphological, and statistical research methods (Horalskyi et al., 2019).

For histological examination, pieces of nervous system organ material were fixed in a 10 % aqueous solution of neutral formalin and Carnois fluid, dehydrated in alcohols of increasing strength, and embedded in melted paraffin. Histological sections with a thickness of 6–10 μm were made from paraffin blocks on a sled microtome MC-2. To study the general histological structure of the spinal cord nodes, the state of their histo- and cytostructures, and perform morphometry, serial paraffin sections were made with their subsequent staining, after deparaffinization, with hematoxylin and eosin.

Processing of the results of the study of histological sections in the amount of 38 histopreparations was carried out using a light microscope "Micros" (Austria) with a digital camera at a magnification of × 100–600, according to the recommendations for morphometric studies in the author's modification (Horalskyi et al., 2019).

Counting the number of nerve cells and gliocytes of spinal cord nodes was performed on the cross-sectional area of the sections using a morphometric grid on a conventional unit of area (based on 0.1 mm^2) in 45 fields of view for each animal.

The cytostructure of spinal cord nodes, their shape and cellular typing of nerve cells, nerve fibers, and the characteristics of the neurofibrillary apparatus were studied on histopreparations impregnated with silver nitrate according to Ramon-y-Cajal and Bilshovsky-Gros.

To determine the localization and distribution of the basophilic substance in the neuroplasm, the study of glial cells, the tissue preparations were stained with toluidine blue - according to the Nissl method. To obtain and compare the quantitative characteristics of the structural organization of the spinal cord nodes of the studied animals in the species aspect, morphometric research methods were used (Horalskyi et al., 2019). Statistical processing of digital data was carried out according to the Montsevichyute-Eringene method. The Student determined the value of the probability criterion and the level of significance (P) (Horalskyi et al., 2019).

During the research, the basic rules of good laboratory practice GLP (1981) and the provisions of the "General Ethical Principles of Animal Experiments" adopted by the First National Congress of Bioethics (Kyiv, 2001) were followed. The entire experimental part of the study was carried out following the requirements of the international principles of the "European Convention for the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes" (Strasbourg, 1986), "Rules for Conducting Works Using Experimental Animals", approved by Order of the Ministry of Health No. 281 dated November 1, 2000 "On measures to further improve organizational forms of work with the use of experimental animals" and the corresponding Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV dated February 21, 2006, Kyiv) (Nichiporuk et al., 2022).

3. Results and discussion

The behavior of a living organism as a complete system of functionally adapted and coordinated body movements necessary for the implementation of biologically essential functions in its formation and development in vertebrates reveals regularities, a correct understanding, and an evolutionary explanation that is possible only with a comprehensive study of both the physiological mechanism of phenomena and morphological studies.

A topical issue of animal neuromorphology is the study of spinal cord nodes, which play the role of primary centers on the way to transmit sensory information from receptors to the central nervous system, ensuring appropriate reactions to the action of certain factors (Hanani, 2005; Kirkpatrick, 2014).

Morpho-functional features and chemical architecture of spinal nodes remain poorly understood. The type and age of vertebrates determine them, the degree of reflex activity, the type and functional state of nerve cells, the phase of neurogenesis, and the degree of chromatophilia. Such data are very important not only for neuromorphology but also for developing issues of physiology, pathology, and treatment of peripheral nervous system diseases.

From the results of our research and publications (Gilmore, 1972; Nazarchuk, 2008) was established that the neurocytes, which are part of the nervous, sensory nodes of animals and humans, are heterogeneous in their structure and function (Pannese, 1960; Vitko, 2016). Modern morphological methods of research have made it possible to establish that the neurons of sensitive nodes differ in the size and shape of the perikaryon, the size of the nucleus, nucleolus, and the ultrastructural organization of structural components (Krastev, 2007; Krastev et al., 2007). Analyzing literary sources, it was established that during the life of an animal and a person, the number of nerve cells that make up nerve nodes changes (Bortolami et al., 1990; Morrison & Hof, 1997). There is information about age-related degeneration of nerve cells of sensitive nodes in humans and experimental animals (Dayan, 1971; Vukojevic et al., 2016). At the same time, in the available literature, insufficient attention is paid to the dynamics of changes in the qualitative composition of neurocytes in the spinal cord nodes of animals during development.

Birds are among the most numerous classes of higher vertebrates regarding species. Birds are homoeothermic organisms that received the ability to fly due to the transformation of their front limbs into wings. The adaptation of birds to flight greatly impacted the structure of the body and vital systems of the bird's body. In the evolutionary plan, significant changes have occurred, particularly in the structure of integrating organs. In birds, the nervous system reaches a significantly high degree of development and differentiation (Kang, 2021). Representatives of the bird class have several biological features: the development of the embryo outside the mother's body, physiological precociousness, rapid growth, high body temperature (+40–42 °C), the peculiarity of the structure of the skin and its derivatives. Therefore, the histocytoarchitectonics of the spinal nodes in chickens is significantly different from that in poikilothermic animals and is characterized by a higher level of their development.

Spinal nodes of chickens have a similar structural organization characteristic of sensor nodes. They are located in the intervertebral openings and appear like small thickenings, are oval, and are located on the dorsal root of the spinal nerves. The area of nodes is 0.5 ± 0.04 mm^2. From the outside, the nodes are covered with a well-defined connective tissue capsule (15.2 ± 0.84 μm), from which numerous trabeculae extend into the parenchyma of the organ.

From the well-developed connective tissue membrane, which covers the spinal nodes, numerous partitions penetrate the thickness of the organ, between which groups of nerve cells are located (Fig. 1). A variable feature in birds, in contrast to cold-blooded animals, is the presence of a well-defined mantle around neurocytes, which consists of perineuronal glial cells, nerve fibers and layers of connective tissue.

In contrast to the spinal cord nodes of lower vertebrates (river carp, pond frogs), in chickens, the differentiation of neurocytes, which are divided into small, medium, and large, is already clearly defined, which is confirmed by the morphological studies of the authors (Nazarchuk, 2008). They are more rounded with a centrally located core (Fig. 2). Such research results obtained by us on these issues are consistent with the data (Huang et al., 2021; Zdora, et al., 2022), according to this, depending on the diameter, the neurons of the spinal nodes are classified into three classes: small ones, the diameter of which is 29–50 microns, and medium ones from 51 to 79 microns. and large (from 80 to 129 microns).

The nervous system of warm-blooded animals is complicated. It has a formed brain and spinal cord, spinal cord nodes, and somatic nerves departing from them. Birds have already developed conditioned reflexes. Individual experience in the life of these animals plays a significant role in the evolutionary restructuring of the spinal nodes.

In representatives of higher vertebrates, birds, and mammals, as is known from the literature (Severtsov, 1990; Khorooshi et al., 2001) and according to the results of our research, there is a significant similarity in the histological structure of the spinal nodes. However, we have established that the variability of the sizes of nerve cells in the spinal cord nodes of birds fluctuates within much narrower limits than in the nodes of mammals. The density of placement of neurocytes and glial cells is much higher. Thus, the average volume of neurocytes in the spinal cord of chickens is 1.8 times smaller than that of representatives of the class of Mammals, and the density of their placement is 2.2 times greater. That is, the density of their placement compensates for the smaller volume of neurocytes. The number of glial cells in the spinal cord nodes of the domestic chicken exceeds the number in the spinal cord nodes of mammals by only 1.2 times (Fig. 3).

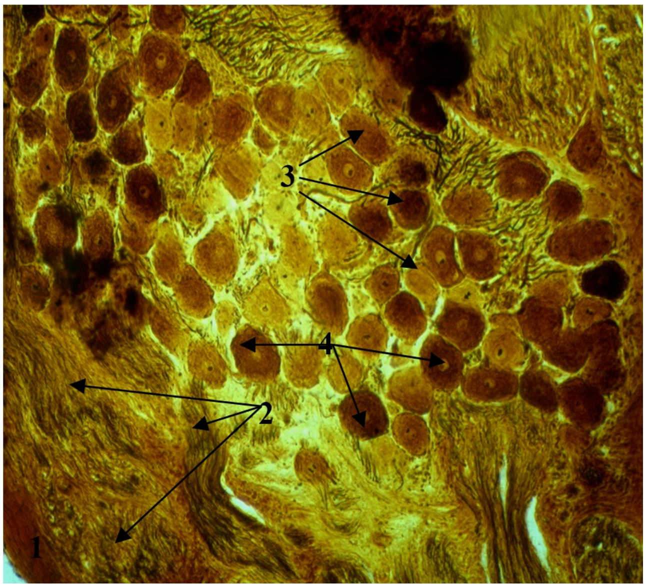

Fig. 1. Fragment of the histological structure of the spinal node of a domestic chicken: 1 – connective tissue capsule of the node; 2 – nerve fibers; 3 – groups of neurocytes; 4 – nuclei of neurocytes. Bilshovsky-Gros. × 280

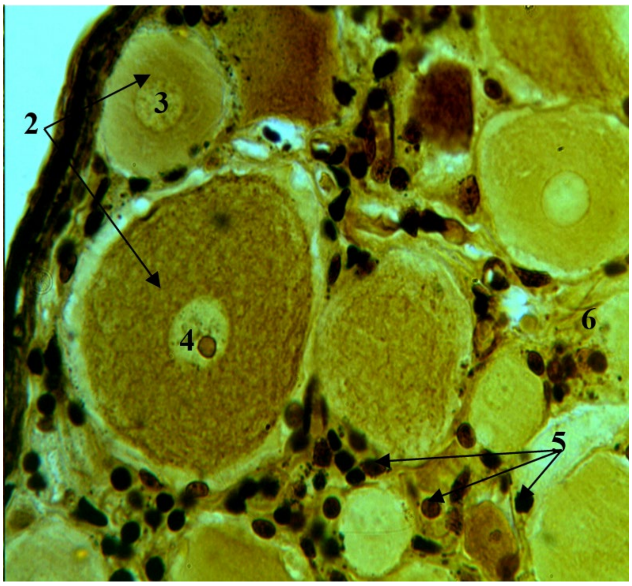

Fig. 2. Fragment of the histological structure of the spinal node of a domestic chicken: 1 – connective tissue capsule of the node; 2 – nerve cell; 3 – the nucleus of a nerve cell; 4 – cell nucleus; 5 – nuclei of neurocytes; 6 – nerve fibers. Bilshovsky-Gros. × 400

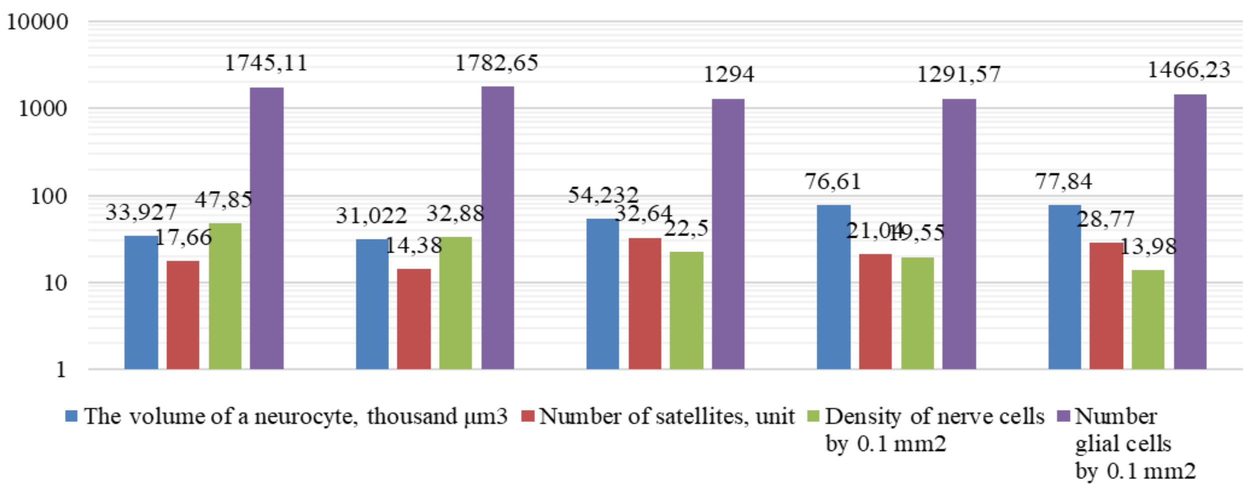

Fig. 3. Morphometric indicators of spinal nodes in a comparative anatomical series of studied vertebrates

Normochromic cells predominate over hyperchromic cells in the spinal nodes of the domestic chicken, and their neuroplasm contains well-defined pockets of fine-grained chromatophilic substance. There are also neurocytes with localization of chromatophilic substances on the periphery of the neuroplasm. The nuclear chromatin is clearly expressed and evenly fills the karyoplasm. The nuclei of glial cells were stained most intensively.

Representatives of the Mammal class are characterized by complex behavior – complex conditioned and unconditioned reflexes. At the same time, the level of development of the nervous system as a whole and the spinal cord, particularly in different species of mammals, is not the same and depends on the conditions of existence.

Thus, the spinal cord nodes of the studied animals of the class Mammals (European krill, domestic dog, domestic pig, domestic bull) are located outside the intervertebral foramina. However, the dorsal and ventral roots of the domestic pig and cattle's spinal ganglia are formed by several unrelated nerve fibers. The spinal nodes of the European rabbit and domestic dog are rounded, while those of the pig and cow are spindle-shaped and flattened in the dorsoventral direction.

Elastic and collagen fibers of different spatial orientations form the connective tissue capsule of the spinal nodes. A unique morphological feature of the spinal nodes of higher vertebrates is the orderly arrangement of nerve cells and their processes. The neurocytes are located on the periphery of the spinal nodes under the capsule as rollers. In the thickness of the organ, nerve cells are grouped between bundles of nerve fibers, where a significant number of hemomicrocirculatory vessels are found. The spinal nodes of cattle are characterized by their lobulation.

During a review histological study of the spinal cord nodes of mammals on histological preparations – total impregnation with silver nitrate according to the Ramon-y-Cajal method, light nerve cells of small sizes were found, which are placed in small groups, next to which there are significant, more intensively impregnated neurocytes (Fig. 4). A mantle shell is visible around the latter, which consists of satellite cells, nerve fibers and layers of connective tissue (Fig. 5). It was established that the presence of large, medium and small neurocytes characterizes the neuronal organization of the spinal nodes.

The neurocytes of the spinal nodes of rabbits have a rounded shape with precise contours of the cytoplasm. They are placed in groups under the connective tissue capsule and singly between nerve fibers in the thickness of the organ (Fig. 4). During the total impregnation of the spinal nodes with silver nitrate according to the Ramon-y-Cajal method, light nerve cells of small sizes are found, which are placed in small groups, next to which there are significant, more intensively impregnated neurocytes (Fig. 5). Around the latter, a mantle shell is visible, which consists of satellite cells, nerve fibers and layers of connective tissue.

When staining the histological sections of the spinal nodes of mammals with hematoxylin and eosin, particularly in sexually mature dogs, a coarse granularity of the cytoplasm of large nerve cells was revealed. The most intensively adsorbed dyes were the nuclei of glial cells and the nuclei of neurocytes. Small-sized neurons were stained more intensively than large ones, with the noticeable fine-grained granularity of the neuroplasm (Fig. 6). The neurocytes of the spinal nodes of domestic bulls are round with a large light nucleus, which is located mainly in the center or shifted to one of the poles of the nerve cell body. Such cells have a well-defined mantle shell, represented by satellite cells and fibrous components of nervous tissue (Fig. 7).

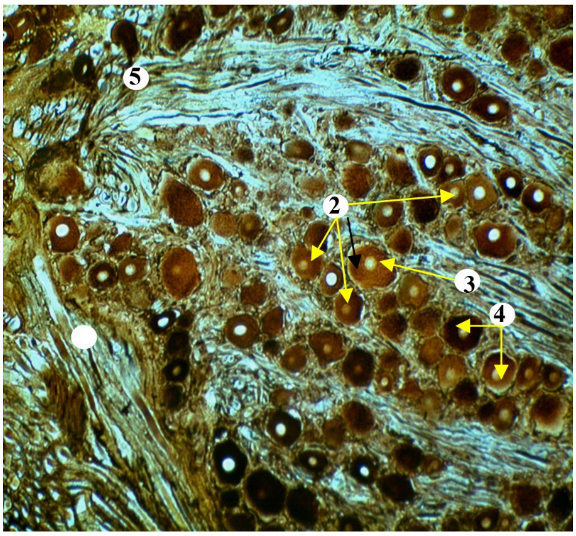

Fig. 4. A fragment of the histological structure of the spinal node of the European rabbit. 1 – nerve fibers; 2 – groups of nerve cells; 3 – cell neuroplasm; 4 – nuclei of neurocytes; 5 – nerve fibers. Ramon y Cajal. × 120

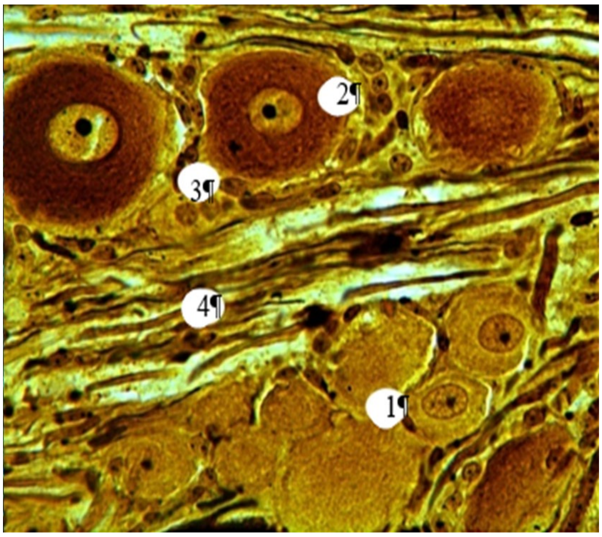

Fig. 5. A fragment of the histological structure of the spinal node of the European rabbit: 1 – bright neurocytes; 2 – dark neurocytes; 3 – nuclei of mantle gliocytes; 4 – nerve fibers. Ramon y Cajal. × 280.

As the phylogenetic age of animals increases, the number of nerve cells per unit area of the SCN decreases (Table 1). The low density of their placement in the studied representatives of the Mammal class with high locomotor function is primarily due to a high level of differentiation of cellular components and an increase in their functional role. The number of gliocytes ranges from 1291.57 ± 34.56 in a domestic pig to 1782.65 ± 41.81 in a European rabbit (Table 1).

The neurocytes of the spinal nodes of mammals have different sizes in the comparative anatomical series of the studied vertebrates. The neuronal population is characterized by high heterogeneity and an extensive range of variants.

The neuroglial supply of the neurocyte is also complicated. The analysis obtained from morphological studies shows that the number of satellites is the largest in the domestic dog, as the most active representative of the Mammal class, and is 32.64 ± 1.96 (Table 1).

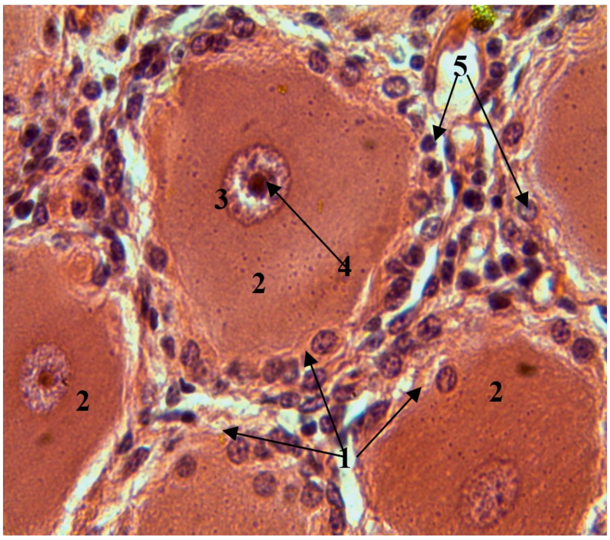

Fig. 6. A fragment of the histological structure of the spinal node of a domestic dog: 1 – neuron body; 2 – neurocyte cytoplasm; 2 – cell nucleus; 4 – cell nucleus; 5 – nuclei of glial cells. Hematoxylin and eosin. × 640

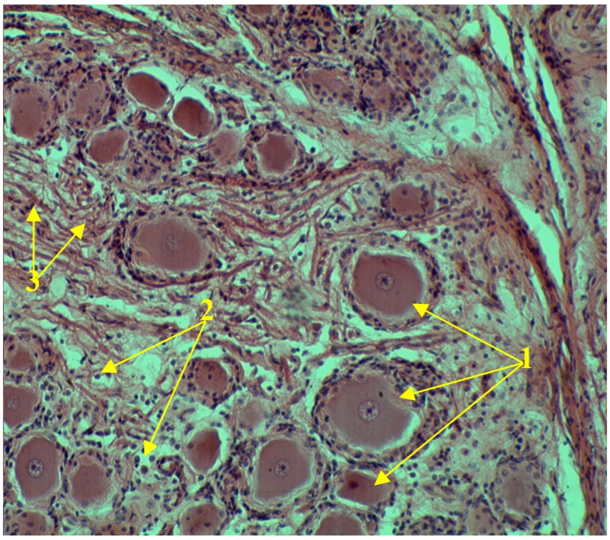

Fig. 7. A fragment of the histological structure of the spinal node of a domestic bull: 1 – neurocytes; 2 – nuclei of glial cells; 3 – nerve fibers. Hematoxylin and eosin. × 280

Table 1
Morphometric indicators of spinal nodes in a comparative anatomical series of studied vertebrates of the Mammal class (M ± m, n = 6)

Animal species	The volume of a neurocyte, thousand μm³	Nucleus volume, μm³	Nuclear-cytoplasmic ratio	Number of satellites, unit	Density of nerve cells by 0.1 mm²	Number glial cells by 0.1 mm²
European rabbit	31.022 ± 7.215	1429.6 ± 352.77	0.072 ± 0.002	14.38 ± 1.32	32.88 ± 1.61	1782.65 ± 41.81
Domestic dog	54.232 ± 7.007*	1720.77 ± 180.75	0.069 ± 0.005	32.64 ± 1.96***	22.5 ± 1.59***	1294.05 ± 34.44***
Domestic pig	76.61 ± 1.21*	2559.15 ± 162.75**	0.072 ± 0.01	21.04 ± 1.55***	19.55 ± 1.02	1291.57 ± 34.56
Domestic bull	77.84 ± 9.28	2452.4 ± 181.07	0.043 ± 0.003*	28.77 ± 1.91*	13.98 ± 1.27**	1466.23 ± 49.84*

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001 in relation to the previous animal representative

According to the results of morphometric studies, it was established that the average value of the density of nerve cells in the spinal nodes of a domestic dog is low and is 22.5 ± 1.59 units, which is 1.5 times less than that of the European rabbit.

The average number of glial cells per unit area of the dog's spinal nodes was 1294.05 ± 34.44 units, 1.4 times less than in the rabbit. But due to the larger neurocytes in the dog, the average number of mantle cells around one nerve cell was 32.64 ± 1.96 units, which is 2.3 times more compared to this figure in the European rabbit (Table 1).

According to the results of morphometric studies, it was established that the density of placement of neurocytes in a domestic pig is 19.55 ± 1.02, and glial cells are 1291.57 ± 34.56 units.

The average number of domestic pig satellite cells around one neurocyte was 21.04 ± 1.55 units. Small neurocytes are equipped with a more significant number of satellite cells per unit area.

According to the results of morphometric studies, the spinal nodes of domestic bulls had a lower density of neuron distribution compared to the nodes of pigs, amounting to 13.98 ± 1.27 units per unit area (0.1 mm²), which is 1.4 times lower than the average values of the compared group. Our research has shown that the number of glial cells per unit area of bull spinal nodes is 1.1 times greater than that of pigs (Table 1).

The mantle of neurocytes in domestic bulls is represented by more satellite cells than in pigs. Thus, the average number of satellites around one nerve cell in a bull was 28.77 ± 1.91, while in a pig, it was 21.04 ± 1.55 units (Table 1).

Among the complex morphological research methods, a special place belongs to morphometrics. This method is widely used in modern neuromorphology (Larnicol et al., 1998). Its high accuracy for assessing the structural and functional state of the animal organism at the organ, tissue, and cellular levels in normal conditions, at various stages of development, and under pathology has been proven.

The average indicators of the volume of the perikaryon of a neuron in a domestic dog were 54.232 ± 7.007 thousand μm³, 1.7 times larger than the indicator of the same name in a rabbit. The larger size of their nuclei also characterizes the neurocytes of the spinal nodes of domestic dogs. Thus, the average value of the nucleus volume in a dog is 1720.77 ± 180.75 μm³, and in a rabbit, it is 1429.64 ± 352.77 μm³, respectively. As a result, the average nuclear-cytoplasmic ratio of both species of the studied animals was almost approximate (Table 1).

The average volume of the nucleus of nerve cells in a domestic pig is 2559.15 ± 162.75 μm^3, which is 1.5 times larger than in a dog. Accordingly, the width of the distribution of values for this indicator is much broader in pigs than in dogs and ranges from 701.97 to 7643.7 μm^3 (Table 1).

The average volume of the perikaryon of the nerve cells of the spinal nodes of domestic bulls is 77.84 ± 9.28 thousand μm3, which is close to that of pig nodes. The indicators of the volume of the nucleus of neurocytes of spinal cord nodes of a bull and a pig have close values of 2452.4 ± 181.07 and 2559.15 ± 162.74 μm^3, respectively. Based on the average indicators of the volume of the perikaryon of nerve cells and their nuclei, it was established that the average nuclear-cytoplasmic ratio in a pig is 1.7 times higher than in a bull (Table 1).

It is a well-known fact that the size of neurons depends on the taxonomic status of the mammalian level (the higher the species in the systematic relationship, the larger the volume of the nerve cell body), as well as on the size and weight of the animal's body (Liebeskind et al., 2016; Kverková et al., 2022).

Our morphometric analysis of neurocytes of spinal cord nodes in experimental vertebrate animals confirmed the point of view regarding the dependence between linear parameters of nerve cells, taxonomic status, and body size and weight of the animal within the class. Thus, representatives of cattle had the most considerable body weight among the animals we studied – 590.49 ± 15.64 kg. According to the results of morphometric studies, in the spinal nodes of such animals, the average volume of neurocytes was the highest (77.84 ± 9.28 thousand μm3). Still, it did not significantly differ from that in domestic pigs (76.61 ± 1.21 thousand μm^3), whose body mass is almost 1.8 times less than that of cattle (Table 1).

The leading morphometric indicator of the level of metabolism and differentiation of cells under different conditions of their existence is the nuclear-cytoplasmic ratio, thanks to which it is possible to assess the level of morphofunctional maturity in species and age aspects (Svahn et al., 2018; Moore et al., 2019).

Therefore, in a series of studies of representatives of vertebrate animals, the nerve cells of the spinal cord nodes of mammals have the largest sizes in the comparative anatomical series of studied vertebrates and, accordingly, the smallest nuclear-cytoplasmic ratio, the indicator of which tends to decrease during the phylogeny of vertebrate animals (Table 1). This is argumentative evidence of a higher level of morphofunctional maturity of neurocytes in representatives of the class of Birds and Mammals.

There are several signs, according to which it is possible to judge the morphological maturation of neurocytes, namely the appearance and accumulation of a basophilic substance, the content and placement of chromatin in the nucleus, the ratio of the sizes of the perikaryon and the nucleus, the formation and subsequent reduction of nucleoli, the development of axons and dendrites, as well as their branching (Stoyanova, 2004; Tongtako et al., 2017).

On the histopreparations of nerve cells of the spinal cord nodes of rabbits, the basophilic substance is unevenly distributed in the neuroplasm in the form of grain-like pits of small sizes. In some cells, Nissl's substance is concentrated on the periphery of the neuroplasm. The nuclei of neurocytes, the nucleolus, and nuclear chromatin are visible in fine granularity. The nuclei of glial cells are stained most intensively (Fig. 8).

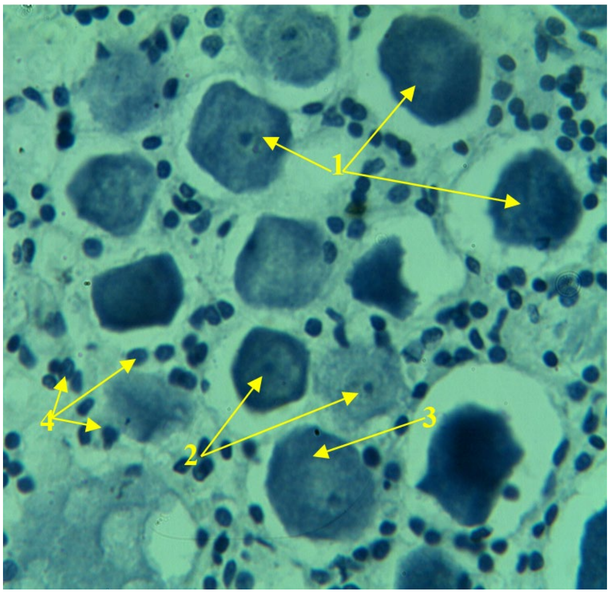

Fig. 8. Fragment of the histological structure of the spinal node of the European rabbit: 1 – cytoplasm of a neurocyte; 2 – nucleus and nucleolus; 3 – cytoplasmic chromatophilic substance; 3 – nuclei of glial cells. Nissl. × 280

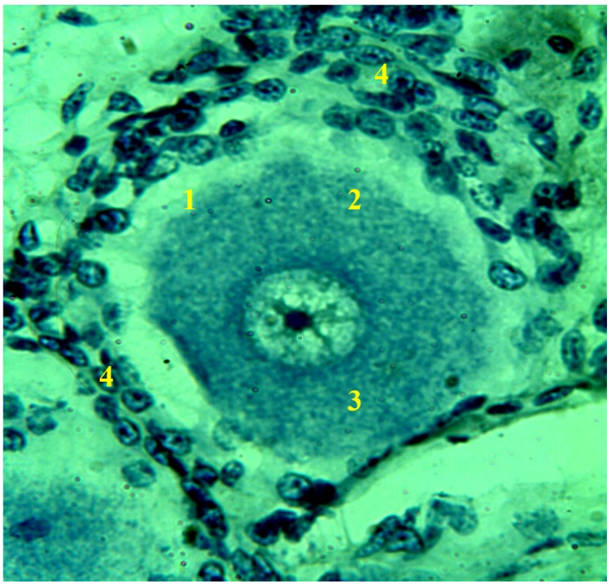

Fig. 9. A fragment of the histological structure of the spinal node of a domestic bull: 1 – neurocyte body; 2 – neurocyte cytoplasm; 3 – cytoplasmic chromatophilic substance; 3 – nuclei of glial cells. Nissl. × 640

Neurocytes of the spinal nodes of domestic bulls, the cytoplasm of the nerve cells contains well-defined and evenly spaced pits of fine or coarse-grained basophilic substance. The nuclei of neurocytes and glial cells were most intensely stained. In these structures, the depths of the chromatophilic substance are coarser-grained and do not have a precise, ordered arrangement (Fig. 9).

Thus, the most characteristic variable feature in the organization of the spinal cord nodes of warm-blooded animals in the process of evolution is the structural rearrangement of their histo- and cytoarchitectonics, which is manifested by an increase in their area, a more straightforward expression of the differentiation of the neuronal composition, an increase in the number and size of neurocytes, etc. In addition, a morphological feature of the spinal nodes of higher vertebrates is the orderly arrangement of nerve cells and their processes: neurocytes are located on the periphery under the capsule, and nerve processes – are mainly in the central part of the node. Moreover, the nerve fibers of the cells are more developed and branch out in the thickness of the spinal cord nodes of mammals.

4. Conclusions

Our research from the point of view of assessing the plasticity of the spinal cord nodes of vertebrate animals of different levels of structural organization, with different ecological and functional organization, which differ in the degree of motor activity, the environment allowed us to identify specific patterns, trends, and criteria that relate to the structural organization of the spinal cord nodes on a macro- and microscopic level. levels of their organization. Spinal nodes in the comparative anatomical series of vertebrates differ in topography and shape: in warm-blooded animals (chickens, rabbits, dogs), they are dorsoventrally flattened; in pigs and cattle, they are irregularly rounded.

Different sizes of nerve cells were found in the phylogenetic series of experimental vertebrates. At the same time, the density of the distribution of neurocytes in the cell population of spinal cord nodes of vertebrate animals decreases in historical development. A comparison of the histomorphology and morphometric parameters of the spinal cord nodes in different representatives of vertebrates is reflected within a separate class of animals, clearly revealing reliable differences in the cytometric parameters of neuronal populations, which can be considered idioadaptation – a progressive phylogenetic development accompanied by specific changes in the structure of the nervous system of research animals; as a result their adaptation to the specific conditions of being in a particular habitat.

Perspectives of further research. Further research is devoted to studying histochemical studies of spinal nodes in a comparative anatomical series of homoeothermic vertebrates.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Bortolami, R., Lucchi, M. L., Callegari, E., Barazzoni, A. M., Costerbosa, G. L., & Scapolo, P. A. (1990). Simultaneous cell death in the trigeminal ganglion and in ganglion neurons present in the oculomotor nerve of the bovine fetus. *Journal of anatomy*, 169, 103–113.
[Abstract] [Google Scholar]

Budd, G. E. (2015). Early animal evolution and the origins of nervous systems. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1684), 20150037.
[Crossref] [Google Scholar]

Dayan, A. D. (1971). Comparative neuropathology of ageing. Studies on the brains of 47 species of vertebrates. *Brain : a journal of neurology*, 94(1), 31–42.
[Crossref] [Google Scholar]

De Moraes, E. R., Kushmerick, C., & Naves, L. A. (2017). Morphological and functional diversity of first-order somatosensory neurons. *Biophysical reviews,* 9(5), 847–856.
[Crossref] [Google Scholar]

Dehtyarenko, T. V. (2018). Ontology of Determining the Main Properties of the Human Nervous System in the Concept of Developing the Problem of Individuality. *Ukrainskyi zhurnal medytsyny, biolohii ta sportu*, 3(5), 266–274 (in Ukrainian).
[Crossref] [Google Scholar]

Ernsberger, U. (2009). Role of neurotrophin signalling in the differentiation of neurons from dorsal root ganglia and sympathetic ganglia. *Cell and tissue research*, 336(3), 349–384.
[Crossref] [Google Scholar]

Gage, F. H. (2015). Neuroscience: The Study of the Nervous System & Its Functions. *Daedalus*, 144(1), 5–9.
[Crossref] [Google Scholar]

Garman, R. H. (2011). Histology of the central nervous system. *Toxicologic pathology*, 39(1), 22–35.
[Crossref] [Google Scholar]

Gilmore, S. A. (1972). Spinal nerve root degeneration in aging laboratory rats: a light microscopic study. *The Anatomical record,* 174(2), 251–257.
[Crossref] [Google Scholar]

Grechukha, V., & Otych, D. (2020). The influence of neuroplasticityof the nervous system on the development of personality in adolescence. *Scientific Journal of National Pedagogical Dragomanov University. Series 12. Psychological Sciences*, 11(55), 248–256.
[Crossref] [Google Scholar]

Hanani, M. (2005). Satellite glial cells in sensory ganglia: from form to function. Brain research. *Brain research reviews,* 48(3), 457–476.
[Crossref] [Google Scholar]

Holland, N. D. (2016). Nervous systems and scenarios for the invertebrate-to-vertebrate transition. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 371(1685), 20150047.
[Crossref] [Google Scholar]

Holland, N. D., Holland, L. Z., & Holland, P. W. (2015). Scenarios for the making of vertebrates. *Nature*, 520(7548), 450–455.
[Crossref] [Google Scholar]

Horalskyi, L. P., Khomych, V. T., & Kononskyi, O. I. (2019). *Fundamentals of histological technique and morphofunctional research methods in normal and pathology*. Zhytomyr: Polissia (in Ukrainian).
[Google Scholar]

Horalskyi, L. P., Kolesnik, N. L., Sokulskiy, I. M., Tsekhmistrenko, S. I., Dunaievska, O. F., & Goralska, I. Y. (2020). Morphology of spinal ganglion of different segmentary levels of domestic dog. *Regulatory Mechanisms in Biosystems,* 11(4), 501–505.
[Crossref] [Google Scholar]

Huang, B., Zdora, I., de Buhr, N., Lehmbecker, A., Baumgärtner, W., & Leitzen, E. (2021). Phenotypical peculiarities and species-specific differences of canine and murine satellite glial cells of spinal ganglia. *Journal of cellular and molecular medicine,* 25(14), 6909–6924.
[Crossref] [Google Scholar]

Kang, S. W. (2021). Central Nervous System Associated With Light Perception and Physiological Responses of Birds. *Frontiers in physiology*, 12, 723454.
[Crossref] [Google Scholar]

Karpovskiy, V. I., Zhurenko, O. V., Trokoz, V. O., Postoy, R. V., Sysyuk, Y. O., Kravchenko–Dovha, Y. V., & Landarenko, L. S. (2016). Cortico–autonomic relations in the regulation of physiological functions in cows. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences*, 18(1), 64–68 (in Ukrainian).
[Abstract] [Google Scholar]

Khorooshi, M., Hansen, B. F., & Kelling, J. (2001). Prenatal Localization of the dorsal root ganglion in different segments of the normal human vertebral column. *Spine*, 26(1), 1–5.
[Crossref] [Google Scholar]

Kirkpatrick, J. P. (2014). Spinal Cord and Peripheral Nervous System. *Springer: Verlag Berlin Heidelberg*, 22–48.
[Crossref] [Google Scholar]

Krastev, D. (2007). Electronmicroscopical investigation of the small neurons in trigeminal ganglion. *Journal of IMAB-Annual Proceeding*,14(1), 27–29.
[Article] [Google Scholar]

Krastev, D., Palof, D., Hinova-Palova, A., & Apostolov, W. (2007). Light - microscopic structure of trigeminal ganglion in humans. *Journal of IMAB –Annual Proceeding,* 14(1), 111–115.
[Article] [Google Scholar]

Kverková, K., Marhounová, L., Polonyiová, A., Kocourek, M., Zhang, Y., Olkowicz, S., Straková, B., Pavelková, Z., Vodička, R., Frynta, D., & Němec, P. (2022). The evolution of brain neuron numbers in amniotes. *Proceedings of the National Academy of Sciences of the United States of America*, 119(11), e2121624119.
[Crossref] [Google Scholar]

Ladle, D. R., & Hippenmeyer, S. (2023). Loss of ETV1/ER81 in motor neurons leads to reduced monosynaptic inputs from proprioceptive sensory neurons. *Journal of neurophysiology*, 129, 501–512.
[Crossref] [Google Scholar]

Larnicol, N., Rose, D., & Duron, B. (1988). Morphometrical study of the cat thoracic dorsal root ganglion cells in relation to muscular and cutaneous afferent innervation. *Neuroscience research*, 6(2), 149–161.
[Crossref] [Google Scholar]

Liebeskind, B. J., Hillis, D. M., Zakon, H. H., & Hofmann, H. A. (2016). Complex Homology and the Evolution of Nervous Systems. *Trends in ecology & evolution*, 31(2), 127–135.
[Abstract] [Google Scholar]

Medici, T., & Shortland, P. J. (2015). Effects of peripheral nerve injury on parvalbumin expression in adult rat dorsal root ganglion neurons. *BMC neuroscience*, 16, 93.
[Crossref] [Google Scholar]

Meltzer, S., Santiago, C., Sharma, N., & Ginty, D. D. (2021). The cellular and molecular basis of somatosensory neuron development. *Neuron,* 109(23), 3736–3757.
[Crossref] [Google Scholar]

Monk, T., & Paulin, M. G. (2014). Predation and the origin of neurones. *Brain, behavior and evolution,* 84(4), 246–261.
[Crossref] [Google Scholar]

Moore, M. J, Sebastian, J. A, & Kolios, M. C. (2019). Determination of cell nucleus-to-cytoplasmic ratio using imaging flow cytometry and a combined ultrasound and photoacoustic technique: a comparison study. *Journal of Biomedical Optics*, 24(10), 1–10.
[Crossref] [Google Scholar]

Morrison, J. H., & Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science (New York, N.Y.)*, 278(5337), 412–419.
[Crossref] [Google Scholar]

Nazarchuk, G. O. (2008). Morphological and morphometric characteristics of spinal nodes of chickens in the postnatal period of ontogenesis. *Bulletin of DAU*, 1(21), 113–118 (in Ukrainian).
[Google Scholar]

Nazarchuk, G. O. (2008). Morphology and histochemistry of spinal cord nodes of pigs. *Scientific Messenger LNUVMB. Series: Veterinary sciences*, 3(38), 149–153.
[Google Scholar]

Nichiporuk, S., Radzykhovskyi, M., & Gutyj, B. (2022). Overview: eutanasia and methods of antanasia of animals. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Veterinary sciences*, 24(105), 141–148.
[Crossref] [Google Scholar]

Pannese, E. (1960). Observations on the morphology, submicroscopic structure and biological properties of satellite cells (s.c.) in sensory ganglia of mammals. *Zeitschrift fur Zellforschung und mikroskopische Anatomie (Vienna, Austria : 1948),* 52, 567–597.
[Crossref] [Google Scholar]

Popele, R, & Bosco, G. (2003). Sophisticated spinal contributions to motor control. *Trends Neurosci*, 26(5), 269–276.
[Crossref] [Google Scholar]

Ribeiro, F. F., & Xapelli, S. (2021). An Overview of Adult Neurogenesis. *Advances in experimental medicine and biology,* 1331, 77–94.
[Crossref] [Google Scholar]

Severtsov, A. S. (1990). Intraspecific diversity as a cause of evolutionary stability. *Journal of general biology*, 51(5), 579–589.
[Google Scholar]

Shnurenko, E., Studenok, A., Karpovskiy, V., Trokoz, V., & Postoi, R. (2020). Influence of tone of autonomous nervous system on growth intensity in chickens. *Scientific Horizons*, 07(92), 14–18.
[Crossref] [Google Scholar]

Snider, W. D., & Silos-Santiago, I. (1996). Dorsal root ganglion neurons require functional neurotrophin receptors for survival during development. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 351(1338), 395–403.
[Crossref] [Google Scholar]

Sokulskyi, I. M., Goralskyi, L. P., Kolesnik, N. L., Dunaievska, O. F., & Radzikhovsky, N. L. (2021). Histostructure of the gray matter of the spinal cord in cattle (Bos Taurus). *Ukrainian Journal of Veterinary and Agricultural Sciences*, 2021, 4(3), 11–15.
[Crossref] [Google Scholar]

Starchenko, I. I., Nikiforov, A. H., Cherniak, V. V., Prylutskyi, O. K., & Bilokon, S. O. (2018). Structural features of the human spinal ganglion capsule at the intrauterine stage of development. *Herald of problems of biology and medicine,* 4, 1(146), 233–236 (in Ukrainian).
[Abstract] [Google Scholar]

Stoyanova, I. I. (2004). Gamma-aminobutiric acid immunostaining in trigeminal, nodose and spinal ganglia of the cat. *Acta histochemica*, 106(4), 309–314.
[Crossref] [Google Scholar]

Svahn, A. J., Don, E. K., Badrock, A. P., Cole, N. J., Graeber, M. B., Yerbury, J. J., Chung, R., & Morsch, M. (2018). Nucleo-cytoplasmic transport of TDP-43 studied in real time: impaired microglia function leads to axonal spreading of TDP-43 in degenerating motor neurons. *Acta neuropathologica*, 136(3), 445–459.
[Crossref] [Google Scholar]

Tongtako, W., Lehmbecker, A., Wang, Y., Hahn, K., Baumgärtner, W., & Gerhauser, I. (2017). Canine dorsal root ganglia satellite glial cells represent an exceptional cell population with astrocytic and oligodendrocytic properties. *Scientific reports*, 7(1), 1–15.
[Crossref] [Google Scholar]

Vitko, Yu. N. (2016). Morphometric characteristic of human trigeminal ganglion neurocytes at different stages of intrauterine growth. *Bulletin of problems biology and medicine,* 1(128), 368–371.
[Abstract] [Google Scholar]

Vukojevic, K., Filipovic, N., Tica Sedlar, I., Restovic, I., Bocina, I., Pintaric, I., & Saraga-Babic, M. (2016). Neuronal differentiation in the developing human spinal ganglia. *Anatomical record*, 299(8), 1060–1072.
[Crossref] [Google Scholar]

Wisniewski, H. M. (1983). Difference in the morphology of Wallerian degeneration in the central nervous system (CNS) and peripheral nervous system (PNS) and its effect on regeneration. *Birth defects original article series*, 19(4), 389–395.
[Abstract] [Google Scholar]

Zdora, I., Jubran, L., Allnoch, L., Hansmann, F., Baumgärtner, W., & Leitzen, E. (2022). Morphological and phenotypical characteristics of porcine satellite glial cells of the dorsal root ganglia. *Frontiers in neuroanatomy,* 16, 1015281.
[Crossref] [Google Scholar]

Zhurenko, O. V., Karpovskiy, V. I., Danchuk, O. V., & Kravchenko-Dovga, Yu. V. (2018). The content of calcium and phosphorus in the blood of cows with a different tonus of the autonomic nervous system. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies*, 20(92), 8–12.
[Crossref] [Google Scholar]