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THE FEATURES OF THE STRUCTURAL ORGANIZATION OF SPINAL GANGLIA IN A SUBPHYLUM OF VERTEBTATES

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The Features of the Structural Organization of Spinal Ganglia in a Subphylum of Vertebrates. Goralskiy, L. P., Guralska, S. V., Kolesnik, N. L., Sokulskiy, I. M. — The paper presents the results of research and studied the morphology of spinal ganglia in representatives of different classes of vertebrates: bony fish, amphibians, reptiles, birds and mammals, differing by level of organization, locomotion, habitat. It is revealed that certain structural and morphofunctional restructuring of spinal ganglia is carried out in the process of phylogenesis. Adaptation to different conditions of existence was accompanied by a change in the number of parameters of the spinal ganglia: density and size of neurocytes, increasing of the total number of gliocytes and perineuronal glial cells, polymorphism of neurocytes to the degree of chromatophily.

Key words: morphology, neurocytes chromatophily, cario-cytoplasmatic relation.

Introduction

Among the integrative systems of the human body and animals to ensure its integrity and unity with the environment, the nervous system is the most efficient and evolutionary advanced.

One of the main manifestations of the functioning of the nervous system is afferent impulsation. Therefore, the study of the spinal ganglia (SG) in a comparative-anatomical series of vertebates allows to trace the historical process of their morphofunctional development, reflects the degree and characrter of the motor activity of the animal, habitat and way of life. The study of sensitive neurons, which are the initial link of the reflex arc, will allow to find out *interaction between* living *organism and* the environment as well as the patterns of integration of different body parts into a single system (Zhangbayev, 1987).

However, the study of structural-functional transformations of the spinal ganglia in the process of their historical development gives the opportunity to reveal the mechanisms of plasticity of the nervous system. Evaluation of the level of morphological and cytochemical rearrangement of their structure in the evolutionary line of vertebrates was the aim of our research.

The text is given in the authors' redaction.

Materials and methods of researching

The work was performed at the department of anatomy and histology, faculty of veterinary medicine of Zhytomyr National Agroecological University. In total 128 specimens of 8 species belonging to 5 classes of the subphylus Vertebrate were studied: class Osteichtyes — bony fish (*Cyprinus carpio, forma domestica* L., 1758 — common carp, carp); class Amphibia — the edible frog (*Rana lessonae*, 1882); class Reptilia — the sand lizard (*Lacerta agilis exigua*, 1758); class Aves — the domestic hen (*Gallus gallus, forma domestica*, 1758); class Mammalia — the rabbit european (*Oryctolagus cuniculus* L., 1758), domestic dog (*Canis lupus familiaris* L., 1758), domestic pig (*Sus scrofa, forma domestica* L., 1758), domestic bull (*Bos taurus taurus* L., 1758).

Material was collected during summer-autumn period, mature animals were selected. Assessment of sexual maturity was determined by the weight of the animals (Zapadnyuk, 1971).

The object of the study was bilateral SG of thoracic, fixed in 10 % neutral formalin solution and in the Carnoy's solution, followed by fast infiltration with paraffin. To study the general characteristics of the SG, their structures and for morphometric studies conducting, serial paraffin sections were prepared, followed by staining with hematoxylin and eosin. Cytoarchitectonic of SG, shape and cell typing of neurons, status of neurofibrillar apparatus, and the nature of offshoots were studied on preparations impregnated with silver nitrate by Bilshovskiy-Gros and Ramon-Kahal. To identify basophile substances histopreparats were stained by toluidine blue by Nissl staining *method*. To obtain and compare of quantitative characteristics of the structural organization of the studied SG morphometric methods of research used (Goralskiy et al., 2011).

Results of research

Spinal ganglia of representatives of cold-blooded animals. In bony fish, in the process of evolutionary development, there is a progressive development of the nervous system: the anterior part of the brain and the cerebellum increases - structures which provide coordination of movements. The nervous system of amphibians having the arbitrarily stereotyped locomotion, in general, is structurally similar to fish. The transition of vertebrates from aquatic to terrestrial way of life requires certain changes of the nervous system. In particular, the development of thalamocortical system in reptiles can lead to the formation of new pathways in the nervous system. In this case the nervous system in reptiles in comparison with the nervous system of amphibians is complicated, due to their more active lifestyle.

The improvement of the morphological structure of the SG which serve as the first link in the path of afferent impulses from receptors to the central nervous system is together with the increasing complexity of the nervous system in representatives of cold-blooded animals, as a result of modific variability that is associated with changes in the phenotype of the organism, and in most cases are adaptive in nature.

So in carp SG have fusiform and are located outside of the intervertebral holes. At the pond frog and the sand lizard, on the contrary, SG placed at a maximum close to the spinal cord, on its dorsal roots and are rounded. SG externally covered by connective tissue capsule, which has a different degree of development in different representatives of the studied cold-blooded animals. Connective tissue capsule in carp poorly expressed, its thickness is equal to 4.89 ± 0.03 μm. In pond frog thickness of a connective tissue capsule increased 4.7 times in comparison with carp ($p < 0.001$), and the equal of 23.2 ± 3.2 μm. In sand lizard, the thickness of the capsule of SG is 15.87 ± 1.28 μm, which is 1.5 times less than that of pond frog ($p < 0.05$). The SG area of pond frog (0,32 ± 0,02 mm^2) significantly ($p < 0.001$) reduced, in comparison with carp (1,04 ± 0,06 mm^2), which is obviously associated with the stereotypical locomodation of pond frog, as well as the fact that their conditioned-reflex activity, motor activity, and orientation on land limited.

On histopreparats are shown that neurocytes of carp SG posted disoderly. In form they resemble little differentiated neurocytes or neuroblasts (fig. 1). These cells have incorrectly rounded shape with an offset eccentric churchtime large bright core. On histopreparats in such cells one process that is a strand of cytoplasm clearly shows. The process at a slight distance from the body of the neuron has a T-shaped branching that indicates the classicism of pseudounipolar forms of neurons of SG (fig. 1).

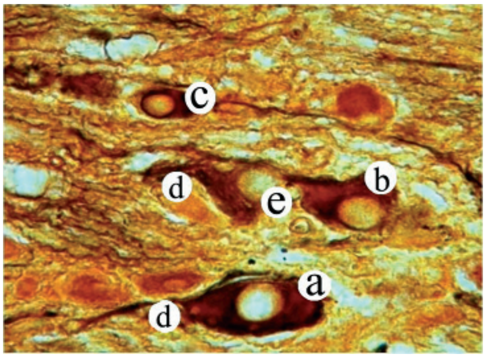

Fig. 1. Microscopical structure of spinal ganglion of carp: a — large neurocyte; b — middle neyrocyte; c — small neurocytes; d — process of neurocyte; e — the core. Ramon-Kahal. ×320.

The eccentric location of the nucleus was observed in neurocytes of pond frog (fig. 2). Unlike fish nerve cells of SG of frogs already have a rounded shape, and their density per unit area of SG significantly ($p < 0.01$) greater than that of carp, respectively, 58.22 ± 1.38 and at 49.14 ± 2.38 cells. The highest density of neurotics in the SG detected in the sand lizard (62.35 ± 5.01 cells).

The results of neurohistological research also found that the neurons of SG of researched cold-blooded animals characterized by a certain degree of polymorphism relative to the degree of chromatopily: against the prevailing majority of normochromic nerve cells in the pond frog and the sand lizard the same number of hyperchromic and hypochromic cells was observed. The presence of hypochromic cells and a very rare hyperchromatic in the carp is more typically. Basophile substance in such neurons placed on the periphery of the cytoplasm in the form of small grains ans in the nucleus — several larger. Chromatophilic substance of gliocytes cores gives them more glencastle figure that it is better expressed in SG of sand lizard. The highest stage of protein-sintesed apparatus development is characteristic for SG neurocytes of sand lizard, compared to the previous studied vertebrates.

It should be noted that a characteristic feature of SG of carp is a large number of glial elements (2813,75 ± 245,18), whereas in the SG of pond frog density of gliocytes is less than 3.6 ($p < 0.001$), and a sand lizard — 1.9 times.

The average volume of spinal ganglia neurocytes, by contrast, was greater in representatives of the class Amphybians, then in animals of the class of bony fish and the lowest in representatives of the class reptiles. According to the results of morphological studies, the largest size had neurocytes of pond frog (28,67 ± 4,09 thousand μm^3), medium — in carp (7.94 ± 0.63 thousand μm^3), and small size — in the sand lizard (6,59 ±

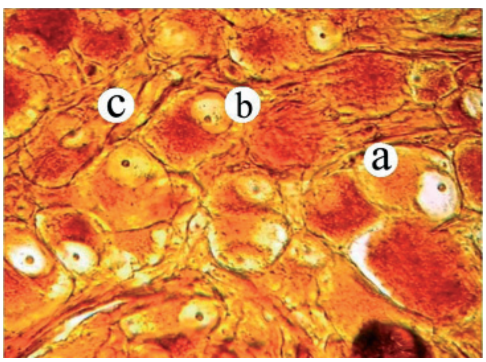

Fig. 2. Fragment of microscopical structure of spinal ganglion of pond frog: a— nervous cell; b — the core and kernel; c — nervous fibers. Bilshovskiy-Gros. ×128.

1,41 thousand μm³). Structural factor of nuclear-cytoplasmic attitude — fNCA is largest in carp (0.21 ± 0.03) and the smallest in lizard.

Thus, in SG of cold-blooded animals, which are at the same level of phylogenetic development, but differing by physical activity, there are significant differences in their structural relationships. So, in SG microarchitectonic of cold-blooded animals marked morphological variability of the most characteristic signs is occured, which is manifested by changing of the shape of the SG, their size, volume of neurocytes and their glial support, which is accompanied by a high degree of homogeneity of the neuronal populations and displays their stable functioning in a particular habitat. Moreover, in the population of SG neurons of studied representatives of cold-blooded animals there is a certain degree of polymorphism relative to the extent of chromatophily. So, amid the prevailing majority of normochromic nerve cells in the pond frog and the sand lizard was observed the same number of hyperchromic and hypochromic cells. While in the carp more typical is the presence of hypochromic cells with small presence — hyperchromic.

Spinal ganglia of representatives of warm-blooded animals. The nervous system of warm-blooded animals is more complicated. It has the formed brain and spinal cord, spinal ganglia and the nerves extending from them. Birds have already developed reflexes. Individual experience in the lives of these animals plays a significant role in the evolutionary restructuring of SG.

In particular, the spinal ganglia of domestic hen, unlike cold-blooded animals are placed in the intervertebral holes at the connection level of the dorsal and ventral roots. They are oval in shape, their area is 0.5 ± 0.04 mm². SG of hen are covered with a pronounced connective tissue capsule (15.2 ± 0.84 μm), which is inside the parenchymal depart numerous trabecules. It is found that the structural and metabolic complexes of domestic hen SG are typical for receptor neurons. Unlike SG researched cold-blooded animals, in the hens differentiation of nerve cells in large, medium and small clearly expressed. Neurocytes have more rounded shape with a central located nucleus, around which the mantle shell are clearly expressed (fig. 3). The latter is represented by perineuronal glial cells, nerve fibers and layers of connective tissue. In domestic hen SG normochromic cells prevail over hyperchromic cells, and their neuroplasma contains distinct clumps of basophile substances of small grain. Nervous cells with localization of basophilic substance on the periphery of the neuroplasm occur. Nuclear chromatin is well pronounced, evenly fills carioplasma. The cores of gliocytes stain most intensively.

At results of morphometric studies, the density of nerve cells in SG of domestic hen, compared with poykiloterms animals is less ($p < 0.05$), due to the complexity of branching of neurofiber component of SG, large size of neurocytes (33.93 ± 3.47 thousand μm³), the increase in the number of gliocytes (1745.11 ± 75.3) and of satellite cells (17.66 ± 1.53) ($p < 0.001$). Neurocytes of domestic hen SG are characterized by low index of fNCA (0.05 ± 0.02), which is a testament to their high morphological activity.

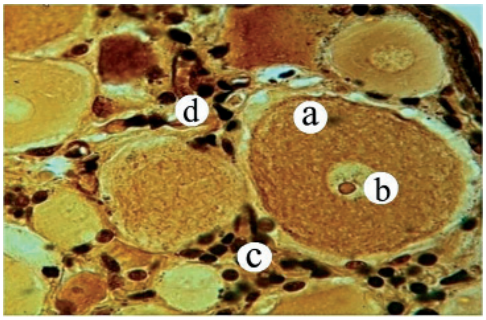

Fig. 3. Fragment of microscopical structure of spinal ganglion of domestic hen: a — neurocyte; b — nucleus and nucleolus; c — cores of gliocytes; d — nerval fibers. Bilshovskiy-Gros. ×400.

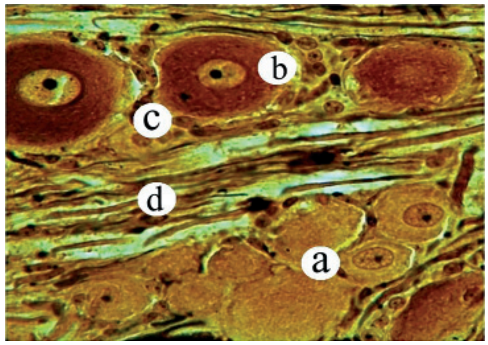

Fig. 4. Fragment of microscopic structure of spinal ganglion of european rabbit: a — light neurocytes; b — dark neurocytes; c — cores of mantle gliocytes; d — nerval fibers. Ramon-Kahal. ×280.

For members of the class of mammals complex behavior is characterized — complex conditional and unconditional reflexes. At the same time, the level of development of the nerval system in general and SG in particular differs in different species of mammals depending upon conditions of existence.

So, SG in studied representatives of the class Mammals are located outside the intervertebral foramen. At the same time, the dorsal and ventral roots of home pig and *Bos taurus* are represented by several bundles of nerve fibers that are not connected. Spinal ganglion of the european rabbit and the domestic dog is rounded, and in pig and *Bos taurus* — fusiform, tapered to dorsoventral direction. Connective tissue capsule is formed by collagen and elastic fibers of different spatial orientation. A characteristic morphological feature of SG of higher vertebrates is an ordered placement of neurocytes and their processes. Nerve cells are localized at the periphery of the SG, under the capsule, in the form of beads and in the bulk of the organ — grouply, between the bundles of nerve fibers, where numerous vessels of hemomicrocirculative channel also met. For domestic bull SG their segmentation is characterized.

With total impregnation of the spinal ganglia with silver nitrous oxide by the Ramon-Cahal method, light nerve cells of small size are located in small groups, along with which large, more intensely impregnated neurons are present (fig. 4). The mantle shell, which consists of satellite cells, nerve fibers and connective tissue layers, is clearly revealed around the latter (fig. 5, 6).

In number of studies of representatives of vertebrates from fish to mammals the number of nerve cells per unit of SG area decreases. Their low density in the studied representatives of the class of mammals with a high locomotor function, primarily due to the high level of

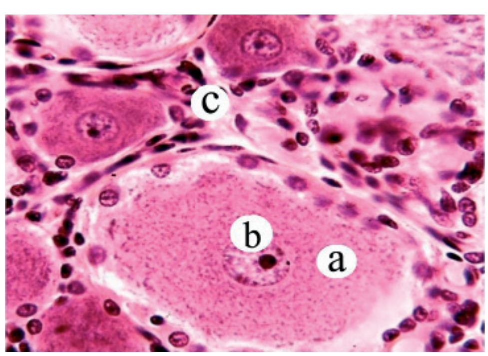

Fig. 5. Fragment of microscopic structure of spinal ganglion of domestic dog: a — cytoplasma of neurocyte; b — nucleus and nucleolus; c — cores of glial cells. Hematoxilin and eosin. ×640.

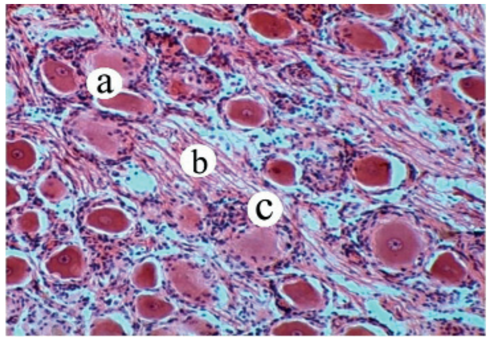

Fig. 6. Fragment of microscopic structure of spinal ganglion of domestic bull: a — neurocytes; b — cores if glial cells; c — nerval fibers. Hematoxilin and eosin. ×120.

differentiation of the cellular components and increase their functional roles. The number of gliocytes ranges from 1291,57 in the domestic pig to 1782,65 in european rabbit. Neuroglial ensure of neurocyte also complicates. In particular, the number of satellites is the largest of the domestic dog it is 32.64 ± 1.96. Neurocytes of mammals SG have the greatest dimensions in the comparative-anatomical series of the studied vertebrates, and therefore fNCA is less, whose index in the process of phylogenesis of vertebrates tends to decrease, which is evidence of the high level of morphological and functional maturity of neurotics in the representatives of the class of Mammals.

Thus, the most characteristic symptom of changing in the organization of the SG in warm-blooded animals in the process of evolution is restructuring their histological and cytoarchitectonic that increasing their size, a more clear expression of the differentiation of neural structure, an increase in the number and size of neurocytes etc. However, the morphological characteristic of SG of higher vertebrates is an ordered placement of neurocytes and their processes: the first is localized in the periphery under the capsule, the latter mainly in the middle part of the node. Nerval fibers are more developed and branched in the thickness of the SG of mammals, in comparison with the corresponding organ of lower vertebrates.

Discussion

Our research from the position of estimates of the SG plasticity of vertebrate animals of different levels of structural organization, with different ecological and functional organization, which differ in the degree of physical activity, the habitat has allowed to identify certain patterns, trends and criteria that relate to the structural organization of SG at the cellular and population and cellular levels of organization.

Improving of the morphology of the SG, their morphological variability in phylogenetic row of vertebrates is evident in the restructuring of the cerebrospinal ganglia, their quantitative characteristics, depending on the conditions of existence of animals in the environment. So, the largest size of neurocytes in poykilotermal animals were noted in the pond frog, medium — in carp, small size — in sand lizard, which indicates the specialization of neurons in a representative of the class of reptiles, as according to Yu. Geinisman (1974), the presence of the small size of nerve cells is a testament not primitive, and their specific expertise, which leads to the most economical ways of executing of functions with minimal costs of morphogenic material. Structural factor nuclear-cytoplasmic relations — fNCR was the largest in carp (0.2 1 ± 0.03) and the smallest in sand lizard (0.13 ± 0.03). In addition, in SG of cold-blooded, instead of the unipolar sensitive neurons, which are characteristic for SG invertebrates are pseudounipolar with T-shaped apophysis pronounced, resulting in greater speed of conduction of excitation without the involvement of the cell body.

By works of row national (Goralskiy at al., 2016; Kovaleva, 1985) and foreign (Pannese et al., 1999; Khorooshi et al., 2001; Rubinow, Juraska, 2009) neurohistologists it was determined, that neural tissue is characterized by marked heterogenity of cellular composition, meanwhile, glial cells greatly predominate quantitatively over the nervous, occupy the entire volume of the nervous tissue between blood vessels and neurons. According to our data, the density of glial cells per unit of area is highest in spinal ganglia of river carp in comparison with other representatives of vertebrates, and the lowest — the pond frogs. From the perspective of some researchers, this is due to morphological rearrangements of the structures of the nervous system in adaptation to the new environment of stay (Savelyev, 2006; Severtsov, 1990).

The nervous system of birds achieves a high degree of development and differentiation (Vorobyova, 1986). Representatives of the class of birds have a number of biological characteristics: rapid growth, physiological maturity, relatively high body temperature (+40–42 °C), the development of the embryo outside the mother's body, the uniqueness of the structure of the skin and its derivatives. And so histoarchitectonic of spinal ganglia in chickens differs from one in poykilotermal animals and is characterized by a high level of development. Unlike from SG of pond frog and river carp, in the chickens differentiation of nerve cells in large, medium and small clearly expressed. They are more rounded in shape with a central located nucleus. From a well-developed connective tissue sheath that covers the SG, in the thickness of the body many walls penetrate between which groups are the nerve cells. Mutable signs in birds, unlike of cold-blooded animals, is the presence distinct mantle shell around neurocytes, which consists of perineuronal glial cells, nerval fibers and layers of connective tissue.

In the representatives of the higher vertebrates (birds and mammals) are known from literature (Severtsov, 1990; Khorooshi, 2001) and the results of our research, there is substantial similarity in the histological structure of the SG. However, we found that variability in size of nerve cells of birds SG varies within a much narrower range than in the mammals SG, and the density of neurocytes and glial cells placement is much higher. Thus, the average volume of SG neurocytes in hens less in 1.8 times than that of the class of Mammals, and their density is 2.2 times more. Thus a smaller volume of neurocytes compensates density of their placement. The number of glial cells in the domestic hens SG exceeds, respectively, the number of mammals SG only in 1.2 times.

It is an accepted fact that the sizes of the neurons depend on the taxonomic status of the mammals level (higher in taxonomically, the greater the volume of the body nerve cells), as well as the size and weight of the animal. Our researches in this direction allowed to confirm the view on the dependence between the linear parameters of the nerve cells, taxonomy status and body size and weight of the animal within a class. Thus, the greatest body mass (590.49 ± of 15.64 kg) among the studied animals were representatives of the *Bos taurus*. Results of morphometric studies in SG of *Bos taurus* the average volume of neurocytes was high, but it is not significantly different from that in a pig, body weight is almost 1.8 times less than in *Bos taurus*. This dependency is missing when comparing representatives of different vertebrate classes. So, the chicken is significantly higher in phylogenetic row of vertebrates than the pond frog and is much superior to the latter in body size. Meanwhile, the average volume of neurocytes in the pond frog SG only in 1.2 times less than the same figure for domestic hen.

The main morphometric indicator of the level of metabolism and cell differentiation under different conditions of their existence is a nuclear-cytoplasmatic attitude, through which you can assess the level of morphological and functional maturity in the species and age aspects (Ermolin, 2005). According to our findings SG neurocites in lower vertebrates had the highest rate of fNCA. In the process of historical development of animals, this indicator tends to decrease, which is evidence of the high level of morphological and functional maturity of neurocytes in the representatives of the classes of Birds and

Mammals, animals with high locomotor function, first and foremost, we associate with a high level of differentiation of the cellular components in the SG and increasing their functional role.

Conclusions

Spinal ganglia in a comparative-anatomical series of vertebrates differ in topography and shape: in cold-blooded (river carp) — fusiform, rounded — in pond frog and sand lizard; in warm-blooded animals (domestic chicken, rabbit and domestic dog) dorsoventrally tapered, in the pig and cattle irregular rounded shape.

In phylogenetic row of vertebrates different sizes of neurocytes bodies determined: the smallest volume of nerve cells in the spinal ganglia are in cold-blooded animals; the largest — in warm-blooded representatives of Mammals and Birds. The density of distribution of neurocytes in cell population of spinal ganglia of vertebrates in the process of historical development is reduced.

Comparison of histomorphology and morphometric parameters of the spinal ganglia in different representatives of vertebrate animals indicates an increase in the level of their organization (in the series from fish to mammals). However, within each class significant differences in the morphometric parameters of neuronal populations that can be considered deadaptation — adaptations of vertebrates to the specific conditions of their stay in a particular environment clearly observed.

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