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FEATURES OF HISTOMORPHOLOGY OF THE PITUITARY GLAND, SPINAL CORD AND CEREBRAL IN CATTLE

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The anatomical, morphological, neuro-histological and morphometric research methods were used for outlining the features of the histological structure of the pituitary gland, spinal cord and cerebellum of cattle in the article. For histological and neurohistological examinations, pieces of material were fixed in a 12 % aqueous solution of neutral formalin, followed by paraffin filling, after which serial sections were made, which were stained with hematoxylin and eosin. Impregnation with silver nitrate was also performed according to the Bilshovskym-Gross method. The general histological structure (histo- and cytostructure) of organs in histological specimens was studied under a light microscope. This investigation with domestic animals was guided by the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Based on morphometric studies, different thicknesses of the pituitary and cerebellar cortex of the cattle were established. The quantitative characteristics of the neural composition and the ratio of nerve cell populations in the structure of the gray matter of the spinal cord of cattle are given, which indicating a pronounced differentiation of nerve cells that have different shapes and sizes and, accordingly, different nuclear-cytoplasmic ratios.

Key words: *microscopic structure, histostructure, morphological researches, morphometry, histoarchitectonics, nerve cells, pituitary gland, cerebellum, spinal cord, domestic animal.*

Formulation of the problem. With intensive animal farming, a necessity has arisen for in-depth investigation of the structure of all systems of the animal body [1]. Therefore, an urgent problem in biology, human and veterinary medicine today is the study of development, growth and formation of the structural organization of the animal body [2]. Knowledge of the parameters of the structural features of organs and tissues in domestic animals in species and comparative aspects are important prerequisites for this.

The priority in solving this problem is a comprehensive study of the nervous and endocrine systems of domestic animals.

It is known that the main manifestation of life is metabolism, which directly depends on environmental conditions and changes with it. Such changes occur with the participation of integrating systems of the body: nervous and endocrine [3, 4, 5]. The pituitary gland belongs to the endocrine system. This is the main endocrine gland, which with its hormones affects the incretory function of peripheral endocrine glands [6].

The basis for maintaining a dynamic balance between the environment and the organism is the interaction of heredity, environment and natural selection, causing the emergence of numerical diversity of variations in the manifestation of physiological, biochemical, morphological characteristics. The nervous system, influencing the formation of the adaptive response, itself undergoes significant changes [7, 8]. In addition to the brain, cranial nodes and nerves,that includes the spinal cord and spinal nodes, are important objects of experimental research and therapeutic manipulations in animals. Their importance in the regulation of vital functions and the complexity of the structure has led to a significant amount of research on evolution, individual development, morphology, the topography of these organs in normal and pathological, as well as the relationship with other organs and tissues [9].

Particular attention to the study of the nervous system is due to its various functions and properties: perception and conduction of nerve impulses, transformation, generation and storage of various types of energy and environmental information, as well as its ability to excitation, inhibition, and processes of synthetic and analytical order, functions, etc. [10].

One of the main conditions for the functioning of the nervous system is the reflex coordination of muscle contractions, which is responsible for maintaining the balance of the body and controls all types of motor activity [11]. This is the function performed by the cerebellum. It is an organ of adaptation of the organism to changes in the basic properties of animal weight and inertia, maintaining muscle tone, posture and balance. The degree of development of the cerebellum has a direct relationship to environmental conditions and the movement of animals in the environment [12]. The interaction of the cerebellum with other parts of the central nervous system allows this part of the brain to provide accurate and coordinated body movements in different external conditions.

Therefore, our work aimed to find out the features of morphology and morphometric characteristics of the corresponding integrating organs in cattle.

Analysis of recent research and publications. To date, many studies of the nervous system in vertebrates have been published [13, 14]. However, today many questions of the histophysiology of the pituitary gland, cerebellum and spinal cord remain unresolved, there is no consensus on the functional significance of different types of nerve cells and their interneural connections, not quite clear morphology of age-related changes in adaptive-compensatory processes. All this obliges researchers to carry out a comprehensive study of the endocrine and nervous systems as one of the most important integrated systems in the body.

Materials and methods of research. The research was conducted at the Department of Anatomy and Histology, Faculty of Veterinary Medicine, Polissya National University. The study is part of the research work of the Department of Anatomy and Histology "Marker signs of the development of immunogenesis and nervous system of vertebrates in onto- and phylogeny", № state registration 0120U102370.

The material for histological examination was the pituitary gland, spinal cord and cerebellum of clinically healthy cattle (n = 8).

The anatomical, histological, neurohistological methods of research were used in the work, which allowed to establish changes at different levels of structural organization of the studied organs of cattle.

The research followed the basic rules of good laboratory practice GLP (1981), the provisions of the "General ethical principles of animal experiments", adopted by the First National Congress of Bioethics (Kyiv, 2001). The entire experimental part of the study was conducted following the requirements of the international principles of the "European Convention for the Protection of Vertebrate Animals Used in Experimental and Other Scientific Purposes" (Strasbourg, 1986), "Rules for Working with Experimental Animals" 281 of November 1, 2000 "On measures to further improve the organizational forms of work with the use of experimental animals" and the relevant law "On protection of animals from cruel treatment" (№ 3447-IV of 21.02.2006, Kyiv).

For histological examination, pieces of material were subjected to fixation in 10–12% solution of neutral formalin as well as Carnois liquid, followed by pouring the material into paraffin according to the schemes described in the manual of L.P. Horalsky, V.T. Khomycha, O.I. Kononsky [15]. Histological sections were made on a sliding microtome MS-2. Their thickness did not exceed 6-10 μm.

To study the morphology of cells and tissues and to perform morphometric studies of the pituitary, spinal cord and cerebellum, serial sections were stained with hematoxylin and eosin and Van Gieson 's. The basophilic substance in neurons was studied on sections stained with toluidine blue according to Nissl. The cytoarchitectonics of the cerebellum, spinal cord, and the state of the neurofibrillary apparatus were studied on preparations impregnated with silver nitrate by Ramón-y-Cajal and Bilshovskym-Gross [15, 16].

Microphotography of histological sections was performed using a CAM V-200 camcorder mounted in a Micros MC-50 microscope.

Research results. *The pituitary gland* in cattle has the shape of a round body, which is located in the pituitary fossa of the Turkish seat (sella turcica) of the cuneiform bone and is connected to the diencephalon by a funnel. It has a grey-red color, dense consistency. It is covered with a connective tissue capsule, which in the area of the pituitary fossa fuses with the dura mater.

Analysis of morphometric parameters shows that the absolute mass of the pituitary gland of mature cattle is 4,45 ± 0,18 g, relative – 0,001 ± 0,07 %.

In the review of histological examination of the pituitary gland on histopreparations stained with hematoxylin and eosin, the main part is distinguished - the adenohypophysis and neurohypophysis - less pronounced (Fig. 1).

The adenohypophysis consists of three particles that have different sizes. The largest is the anterior lobe (Fig. 1), slightly smaller - the rear, and between them - the intermediate lobe. The pituitary gland of cattle is represented by three main cell types: acidophiles, basophils and chromophobic cells. The last cells occupy most of the area of epithelial strands. The diameter of such cells averages 47,55 ± 0,44 µm. Basophilic cells are mostly round or oval, with large nuclei, unevenly distributed throughout the parenchyma. Their cytoplasm is basophilic, chromatin is finely granulated. Acidophilic cells have a polygonal shape.

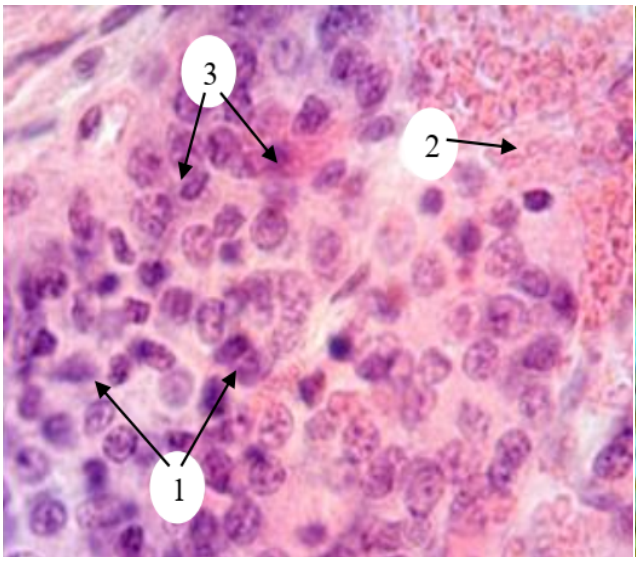

Fig. 1. A fragment of the microscopic structure of the anterior pituitary gland of cattle: 1 – acidophilic cells; 2 – layers of connective tissue stroma; 3 – basophilic cells. Ehrlich's hematoxylin and eosin. ×220

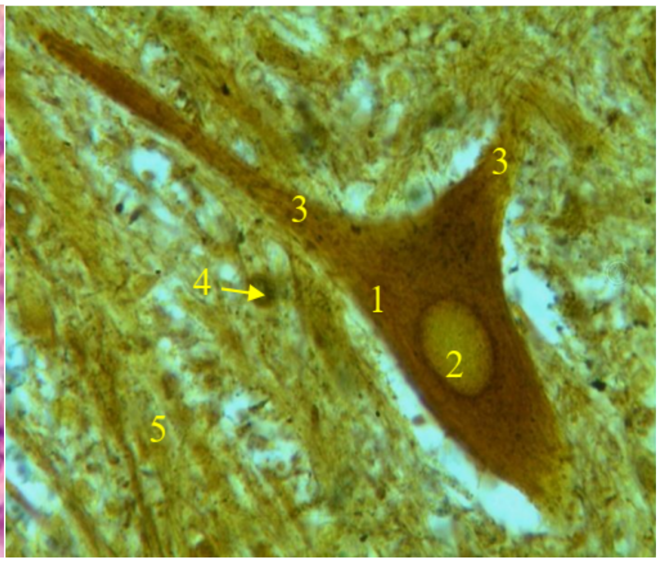

Fig. 2. A fragment of the microscopic structure of the gray matter of the spinal cord of cattle: 1 – nerve cell; 2 – nucleus; 3 – processes of a nerve cell; 4 – nuclei of glial cells; 5 – gray matter. Bilshovskym-Gross. ×400.

Morphometric studies have shown that in cattle the pituitary gland has a different area and a different percentage: the largest area of the pituitary gland is occupied by the anterior lobe – 96,7 ± 0,7 mm^2 (65,0 ± 0,15 %), then the rear – 18,54 ± 0,3 mm^2 (19,9 ± 0,5 %), and intermediate – 15,3 ± 0,24 mm^2 (15,8 ± 0,25 %).

The pituitary cavities, which do not have a characteristic and organ-specific location, contain colloid. When staining histosection with hematoxylin and eosin, the depths of the colloid, which are located in the wells of the anterior and posterior lobes, have mainly basophilic or slightly eosinophilic properties.

The cross section of the spinal cord of cattle has a transverse-oval shape. On the cross section of the gray matter of the spinal cord, we note white matter that is localized on the periphery and gray – in the center of the spinal cord. The latter is represented by dorsal, ventral and lateral horns. The gray matter of the spinal cord consists of nerve cells, which in turn are grouped into nuclei, the location of which mainly corresponds to the segmental part of the spinal cord [17].

Morphometric studies showed that the cross-sectional area of the thoracic spinal cord is 73,45 ± 0,84 mm^2. The area of gray matter occupies 9,74 ± 0,13% (7,16 ± 0,14 mm^2) of the brain area, the area of white – 90,25 ± 0,13 % (66,28 ± 9,74 mm^2). The ratio of gray to white matter in cattle is 9,74 ± 0,13 %.

It is well known that groups of nerve cells with the same functional value form the nuclei of the gray matter of the spinal cord. According to the results of our studies, in the gray matter of the spinal cord of cattle, we identified the following cell nuclei: Clarke's nucleus, the dorsal horn's nucleus, lateral and medial intermediate nuclei, lateral and medial ventral nuclei.

Nerve multipolar cells of the gray matter of the spinal cord have a variety of shapes (spindle-shaped, triangular, oval) with a pronounced perikaryon, the volume of which they differentiate into small, medium and large. Contoured nuclei are found in the neuroplasm of cells (Fig. 2). In large nerve cells, they are mostly round shape, less often – oval, mostly in the center of the cells, less often – eccentrically. In elongated neurons, the nuclei have an oval shape and are located eccentrically. Most nuclei have a well-defined nucleolus, which is located in the center of the karyoplasm.

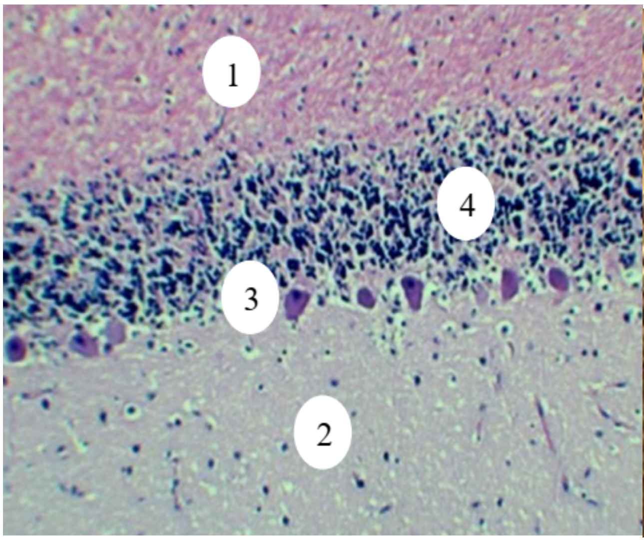

Fig. 3. Fragment of the microscopic structure of the cerebellar cortex of cattle: 1 – white matter; 2 – molecular layer; 3 – ganglion layer; 4 – granular layer. Hematoxylin and eosin. × 120.

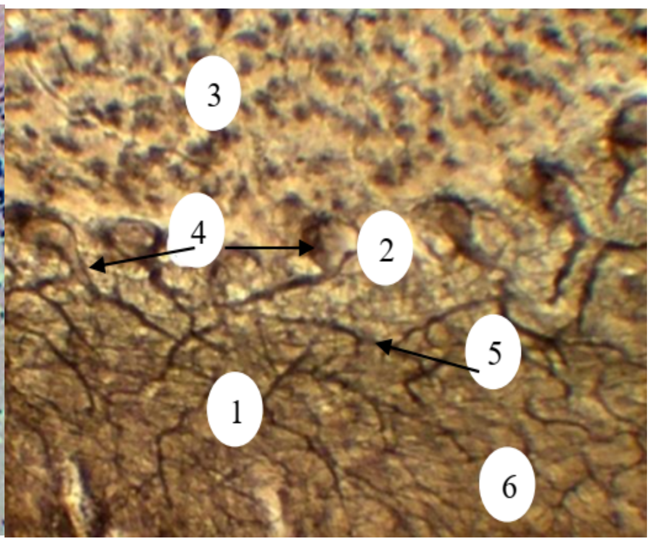

Fig. 4. Fragment of the microscopic structure of the cerebellar cortex of cattle: 1 – molecular layer; 2 – ganglion layer; 3 – granular layer; 4 – Purkinje cells (pear-shaped); 5 – dendrites of Purkinje cells; 6 – branching of neurites of basket cells around the perikaryon of Purkinje cells in the form of "baskets". Ramón-y-Cajal × 280.

Glial cells of gray matter are located near multipolar large neurons, which form groups of 3-4 gliocytes, which are most often located near the processes.

The cytopopulation of neurons in the volume of their perikaryon varies within different limits: the most detected small nerve cells (47,91 ± 0,32 %) of their total number, have a volume ranging from 757 μm^3 to 6222 μm^3. The next place is occupied by average neurons (33,70 ± 0,46 %), the volume of which is from 7892 μm^3 to 22723 mm^3. The smallest cells are found to be the smallest (18,37 ± 0,50 %), with a volume ranging from 25297 μm^3 to 76629 μm^3.

The cerebellum is a typical suprasegmental structure whose afferent and efferent connections begin and end in other parts of the brain. It affects the functions of some autonomous centers, but its main role – is to ensure coordinated motor activity, overcoming the motility of the two main properties of mass – gravity and inertia.

The cerebellum in cattle is located under the occipital region of the cerebral hemispheres in the posterior cranial fossa. In the cerebellum, the lateral lobes (hemispheres) are clearly defined, between which is the middle narrow part – the worm. The cerebellum has three pairs of legs: anterior, middle and posterior. The forelegs connect it to the midbrain, the middle legs to the cerebral pons, and the hind legs to the medulla oblongata. At the anterior edge of the cerebellum is the anterior lobe, which covers the adjacent part of the brainstem, at the posterior, there is a narrower posterior lobe that separates the hemispheres from each other.

The surface of the cerebellum is collected in numerous folded lobes and gyrus, separated by furrows. The main part of it is elongated in cattle, and the anterior cavity is wider than the posterior part. The cerebellar hemispheres are divided by clear slits into leaves.

According to the results of our organometric studies, it is relatively large in cattle and relatively short, wide and high in linear measurements. Its absolute weight is 72,59 ± 0,94 g, relative – 0,02 ± 0,002 %, length is 42,1 ± 0,36 mm, width – 55,3 ± 0,41, height – 43,5 ± 0,44 mm.

Microscopically, the cerebellum consists of gray and white substances. The surface of the cerebellum is covered with a layer of gray matter, which forms the cerebellar cortex and forms narrow convolutions - the leaves of the cerebellum. The leaves are separated from each other by furrows. Each convolution of the cerebellum is a thin layer of white matter covered with bark in which the outer (molecular), ganglionic and deepest (granular) layers of different thicknesses are distinguished (Fig. 3).

As a result of our morphometric studies, different thicknesses of the cerebellar cortex were found in cattle. Thus, the largest thickness of the cerebellar cortex is inherent in its molecular layer – 413,01 ± 10,84 μm (53,2 %), it is slightly smaller in the granular – 313,60 ± 13,84 μm (40,4%) and the smallest in the ganglionic – 49,03 ± 1,94 μm (6,32 %). The total thickness of the cerebellar cortex in cattle is 775,64 ± 26,62 μm.

The molecular layer of the cerebellar cortex is the most superficial. In cattle, it contains small neurons – basket-shaped and stellate.

The ganglion layer of the cerebellar cortex is represented by extremely large Purkinje cells, placed in the middle layer in a row at a small distance from each other (Fig. 4). From the apex of the perikaryon of these cells, 2 – 3 dendrites depart into the molecular layer, which, branching bush-like in the plane of the gyrus, pass through the entire thickness of the molecular layer.

Conclusions.

1. Characteristic differences in the histostructure of the spinal cord (percentage of gray matter to white) of the thoracic part of cattle are manifested by a pronounced differentiation of nerve cells that have different shapes and sizes, and accordingly different nuclear-cytoplasmic ratio, which depends on the morphofunctional state of nerve cells and department neurosegment.

2. According to the results of research, the cross-sectional area and shape of the spinal cord is 73,45 ± 0,84 mm². The ratio of gray and white matter in cattle is 9,74 ± 0,13 %.

3. The total thickness of the cerebellar cortex in cattle is 775,64 ± 26,62 μm. It is formed by the corresponding layers (molecular, ganglionic, granular) and is characterized by different populations of neurons, which have a conditional relationship between the level of morphofunctional state of nervous and innervated structures.

4. The area of the pituitary lobes of cattle is different: the largest area is occupied by the anterior part – 96,7 ± 0,7 mm² (65,0 ± 0,15 %), then the rear – 18,54 ± 0,3 mm² (19,9 ± 0,5 %) and intermediate – 15,3 ± 0,24 mm² (15,8 ± 0,25 %).

Research prospects. Further morphological studies are planned to focus the study of the histostructure of the nervous and endocrine systems in other representatives of domestic animal species.

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ОСОБЛИВОСТІ ГІСТОМОРФОЛОГІЇ ГІПОФІЗА, СПИННОГО МОЗКУ ТА МОЗОЧКА ВЕЛИКОЇ РОГАТОЇ ХУДОБИ

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Радзиховский М., Васєйкіна Ю.

У статті за використання анатомічних, морфологічних, нейро–гістологічних та морфометричних методів досліджень викладено особливості гістологічної будови гіпофіза, спинного мозку і мозочка великої рогатої худоби. Для гістологічного та нейрогістологічного досліджень шматочки матеріалу фіксували в 12 %-му водному розчині нейтрального формаліну з наступною заливкою в парафін, після чого виготовляли серійні зрізи, які фарбували гематоксиліном та еозином. Також проводили імпрегнацію азотнокислим сріблом за методом Більшовського-Грос. Загальну гістологічну будову (гісто- та цитоструктуру) органів в гістологічних препаратах вивчали під світловим мікроскопом. Під час даної роботи з свійськими тваринами керувалися «Європейською конвенцією із захисту хребетних тварин, які використовуються в експериментальних та інших наукових цілях» (Страсбург, 1986 р.).

На основі морфометричних досліджень встановлено різну товщину шарів гіпофіза та кори мозочка великої рогатої худоби. Наведена кількісна характеристика нейронного складу та співвідношення популяцій нервових клітин в структурі сірої речовини спинного мозку великої

рогатої худоби, що свідчать про виражену диференціацію нервових клітин, які мають різну форму та розміри і відповідно різне ядерно–цитоплазматичне відношення.

Ключові слова: *мікроскопічна будова, гістоструктура, морфологічні дослідження, морфометрія, гістоархітектоніка, нервові клітини, гіпофіз, мозочок, спинний мозок, свійська тваринна.*

ОСОБЕННОСТИ ГИСТОМОРФОЛОГИИ ГИПОФИЗА, СПИННОГО МОЗГА И МОЗЖЕЧКА КРУПНОГО РОГАТОГО СКОТА

Горальский Л., Сокульский И., Колесник Н., Дунаевская О.,
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В статье при использовании анатомических, морфологических, нейрогистологических и морфометрических методов исследований изложены особенности гистологического строения гипофиза, спинного мозга и мозжечка крупного рогатого скота. Для нейрогистологичесского исследования кусочки материала фиксировали в 12%-ном водном растворе нейтрального формалина с последующей заливкой в парафин, после чего изготавливали серийные срезы, которые окрашивали гематоксилином и эозином. Также проводили импрегнацию азотнокислым серебром по методу Бильшовського-Грос. Общее гистологическое строение (гисто- и цитоструктуру) органов в гистологических препаратах изучали под световым микроскопом. Во время данной работы с домашними животными руководствовались «Европейской конвенцией по защите позвоночных животных, используемых в экспериментальных и других научных целях» (Страсбург, 1986 г.).

На основе морфометрических исследований установлено разную толщину слоев гипофиза и коры мозжечка крупного рогатого скота. Приведенная количественная характеристика нейронного состава и соотношения популяций нервных клеток в структуре серого вещества спинного мозга крупного рогатого скота, свидетельствуют о выраженной дифференциации нервных клеток, которые имеют различную форму и размеры и соответственно разное ядерно-цитоплазматическое отношение.

Ключевые слова: *микроскопическое строение, гистоструктура, морфологические исследования, морфометрия, гистоархитектоника, нервные клетки, гипофиз, мозжечок, спинной мозг, домашнее животное.*