Short Communication

Ultrastructural organization of endothelial cells of the cerebellar cortex capillaries of the domestic bull

Authors:

Prusakov Alexey Viktorovich [a], Vladimir Sergeevich Ponamarev [b]

[a] Department of internal diseases of animals, Federal State Budgetary Educational Institution of Higher Education "Saint Petersburg State University of Veterinary Medicine", Russia

[b] Department of pharmacology and toxicology, Federal State Budgetary Educational Institution of Higher Education "Saint Petersburg State University of Veterinary Medicine", Russia

Corresponding author: Vladimir Sergeevich Ponamarev

Postal address: Russia, Leningrad Region, Vsevolozhsk District, Murino, Mendeleyev Boulevard, 3, 188677

University/organization email address: nir@spbguvm.ru

ORCID ID: https://orcid.org/0000-0002-6852-3110

Tel. number: +7(921)363-00-56

Keywords: brain, cerebellar cortex, histohematic barriers, blood-brain barrier, homeostasis

Abstract

The goal of the study was to establish the features of the ultrastructural organization of endothelial cells in the cerebellar cortex capillaries of a domestic bull. The study of the ultrastructural organization of endotheliocytes in the cerebellar cortex capillaries of domestic bulls (*Bos taurus*) is of significant interest both for fundamental science and for applied aspects of veterinary medicine and neurobiology. Understanding the ultrastructural organization of capillary endothelial cells in the cerebellar cortex allows better understanding of the microcirculation and metabolism mechanisms in this important part of the brain. This, in turn, may contribute to the development of new methods for diagnostics and treatment. Tissue fragments from the cerebellar cortex of a domestic bull, prepared using standard methods, were studied using electron microscopy. When writing the work, the terminology was given in accordance with the International Histological Nomenclature. It has been

established that the endotheliocytes of the cerebellar cortex capillaries of the domestic bull are the primary link to the blood-brain barrier. Forming the lumen of the capillary, they contact each other with tight junctions and lie on a continuous basal membrane. The continuity of the latter and the presence of tight junctions block the possibility of spontaneous transport of macromolecular compounds from the bloodstream to the brain tissue.

Abbreviations

A – astrocyte;

AS – astrocyte stalk;

BV – blood vessel lumen;

CO – endothelial cell cytoplasmic outgrowths;

EC – endothelial cell;

P – pericyte;

RBC – red blood cell;

INTRODUCTION

The brain coordinates the functioning of all organs and systems of the body, ensuring its integrity and harmonious interaction with the environment. For its work, strict adherence to homeostasis criteria is necessary [1,2]. At the same time, the main metabolic processes that ensure the constancy of the environment occur between its tissues and the vascular system at the level of the hemomicrocirculatory bed [3,4]. The main element of the latter is the hemocapillary, whose structure determines all the physiological and biochemical processes occurring in this organ [5-7]. Taking into account the above, the goal was set: to establish the characteristics of the ultrastructural organization of endothelial cells in the capillaries of the cerebellar cortex of a domestic bull.

The study of the ultrastructural organization of endotheliocytes in the cerebellar cortex capillaries of domestic bulls (*Bos taurus*) is of significant interest both for fundamental science and for applied aspects of veterinary medicine and neurobiology. The relevance of this topic is determined by several key factors.

First, the cerebellum plays a critical role in motor coordination and balance in mammals, including bovines. [8, 9] Understanding the ultrastructural organization of capillary endothelial cells in the cerebellar cortex allows better understanding of the microcirculation and metabolism mechanisms in this important part of the brain. This, in turn, may contribute to the development of new methods for diagnostics and treatment.

Secondly, capillary endotheliocytes play a key role in maintaining the blood-brain barrier, which protects the brain from toxins and pathogens. [9-11] Studying the ultrastructural features of these cells in bovines can provide valuable data on the mechanisms of blood-brain barrier functioning in mammals in general. This is especially relevant in the context of comparative neuroscience, since data obtained from the bovine model can be extrapolated to other animal species, including humans.

A third important aspect is the potential application of the research results in veterinary practice. Diseases associated with microcirculation disorders in the cerebellum can lead to serious neurological disorders in bulls, which negatively affects their productivity and well-being. [9-11] Understanding the ultrastructural organization of endothelial cells can help in the development of new methods for the diagnosis and treatment of such diseases, which is of great practical importance for veterinary medicine.

MATRIALS AND METHODS

The object for the study was five fragments of the cerebellar cortex of a domestic bull no larger than 2.0 mm^3 in size. They were pre-fixed in a 2.0% solution of glutaraldehyde in a cacodylate buffer (pH 7.2–7.4) for 2 hours. After pre-fixation, the selected tissue fragments were washed in three portions of the same buffer. Next, they were postfixed in a 1.0% solution of osmium(VIII) oxide (diluted in cacodylate buffer, pH 7.2–7.4) for 1 hour, then the samples were dried out in alcohols, with increased concentrations, and absolute acetone. After this, they were poured into Epon-812, in accordance with the generally accepted methodology [12]. Three ultrathin sections were obtained from each fragment using an ultramicrotome (LKB-III, Sweden). The obtained sections were contrasted with 2.0% aqueous uranyl acetate solution and lead citrate solution and photographed in a Jem-1011 electron microscope (JEOL, Japan) at magnifications of 2500–3000 [13]. When writing the work, the terminology was given correspondingly to the International Histological Nomenclature [14].

RESULTS

Endothelial cells of the cerebellar cortex capillaries of the domestic bull are the primary element in the blood-brain barrier. They are flat cells elongated along the blood vessel. These cells contact each other with tight junctions and lie on a continuous basal membrane, forming a capillary bed. In the obtained electronograms, the diameter of the lumen of the latter varied from 1.0 to 5.0 μm, and the thickness of the basal membrane varied from 40 to 200 nm. The continuity of the basal membrane and the presence of tight junctions block the ability of macromolecular compounds to penetrate through the capillary wall to the brain tissue from the bloodstream. The nuclei have an ovoid shape and occupy most of the cell. In the area where they are located, its greatest height is observed, which varies from 3.5 to 4.5 μm (Figure 1).

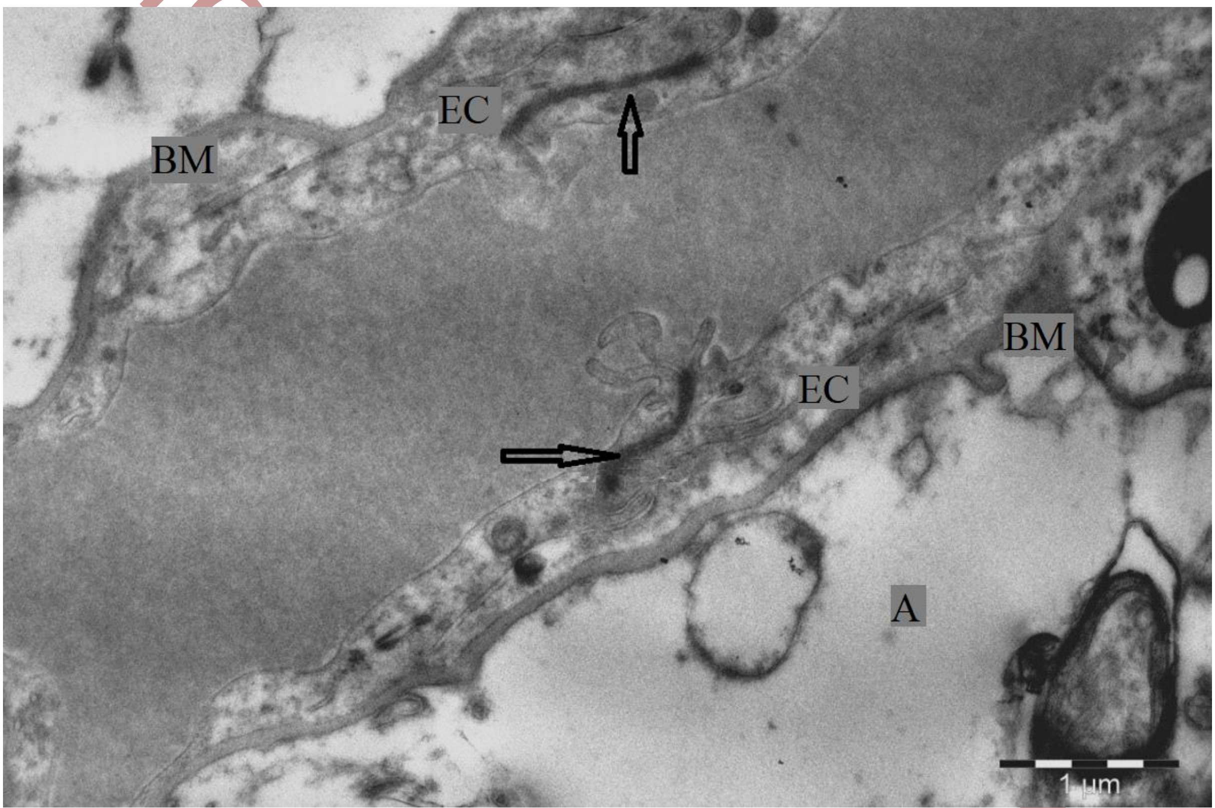

Figure 1. Ultrastructure of the basal membrane of the cortical capillary mammalian cerebellum. Electron micrograph: EC – endothelial cell; A – astrocyte; BM – basal membrane; ↑ – tight junctions.

Sometimes the surface of the nuclei has a tortuous shape due to the bends of the karyolemma. (Figure 2) The latter consists of two lipoprotein membranes, 7 to 8 nm in thickness each. The perinuclear space lies between the membranes. The membrane sometimes contains pores whose diameter varies from 70 to 100 nm. The central part of the matrix is usually electron light. Along the periphery of the nucleus, electron-dark accumulations of heterochromatin are noticeable, contacting the internal karyolemma.

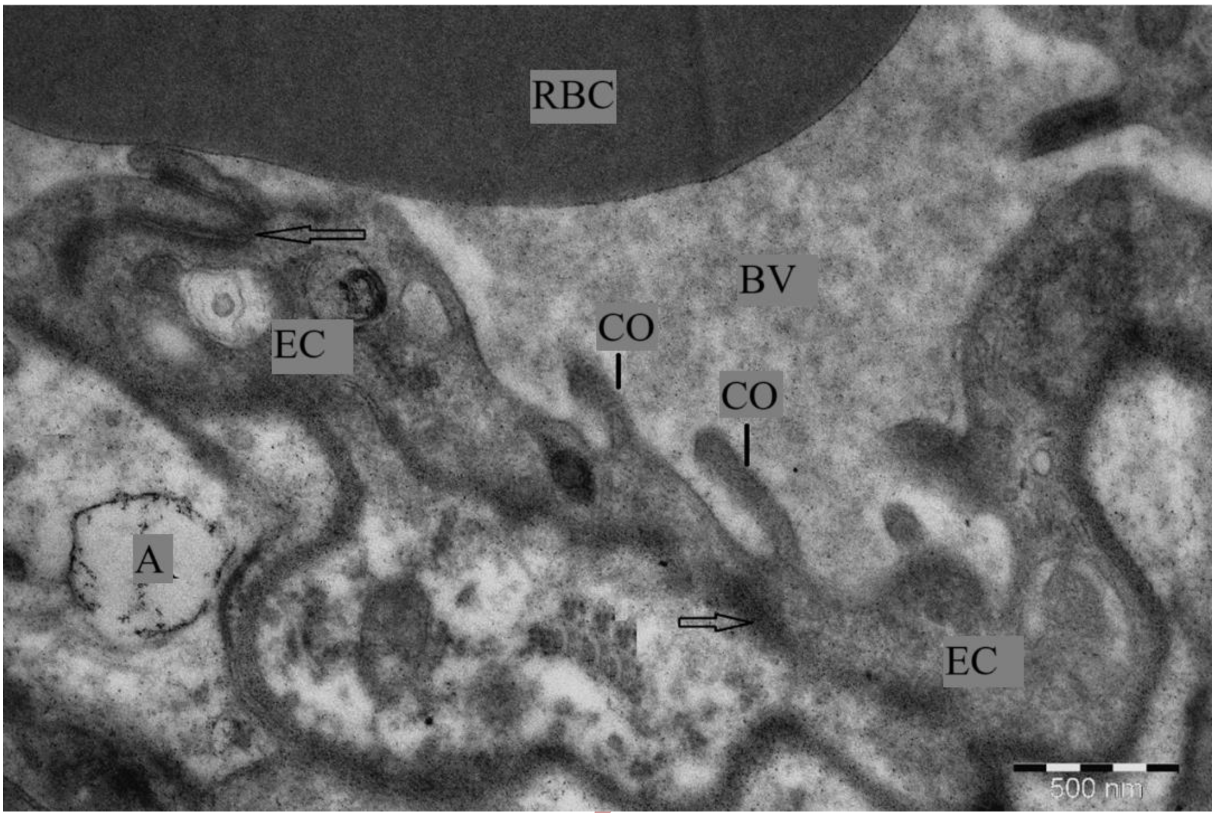

Figure 2. Ultrastructure of the blood-brain barrier of the capillary of the cerebellar cortex of a domestic bull. Electron micrograph: EC – endothelial cell; P – pericyte; A – astrocyte; BV – blood vessel lumen; E – erythrocyte; ↑—basal membrane; PL – phagolysosomes; M – mitochondria.

The cytoplasm occupies the marginal part of the cell. Its thickness is slightly lower than the central part, where the nucleus is located, and varies within 1.3–1.8 μm. Electron-light mitochondria are detected in the cytoplasm. They contain thin cristae formed by folds of the inner membrane. The outer membrane of the organelles forms their shape, which varies from round to oval. The diameter of the mitochondria is about 1.0 μm. Also in the cytoplasm, there are a significant number of ribosomes, which look like electron-dense granules with a diameter of 25 to 30 nm. In addition to individual ribosomes, polyribosomes, ribosomes clusters, can be visualized, as well as a small number of pinocytotic vesicles and single short channels of the granular endoplasmic reticulum (figure 3).

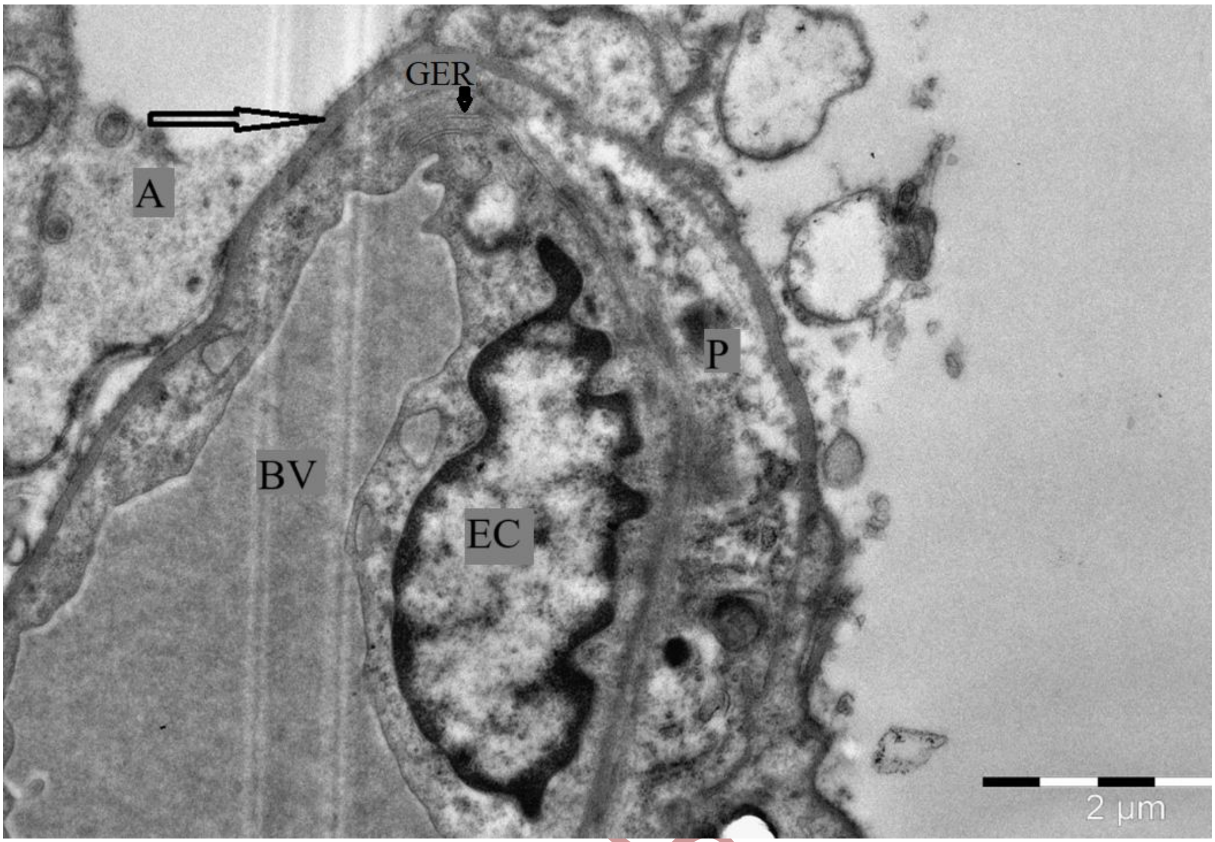

Figure 3. Ultrastructure of the endothelial cell of the capillary of the cerebellar cortex of a domestic bull. Electron micrograph:
EC - endothelial cell; P - pericyte; A - astrocyte; BV - blood vessel lumen; GER – granular endoplasmic reticulum; ↑ –basal membrane.

The cytoplasmic membrane of endothelial cells forms their outer shell. Its thickness varies within 7.5–10.0 nm in the obtained electronograms. In accordance with the location of the endotheliocytes, it forms their luminal and basal surfaces. Luminal surface facing the inside of the vessel. With an increase in the functional activity of the endothelial cell, it is able to form outgrowths and protrusions. The basal surface of the cells contacts the basal membrane (Figure 4).

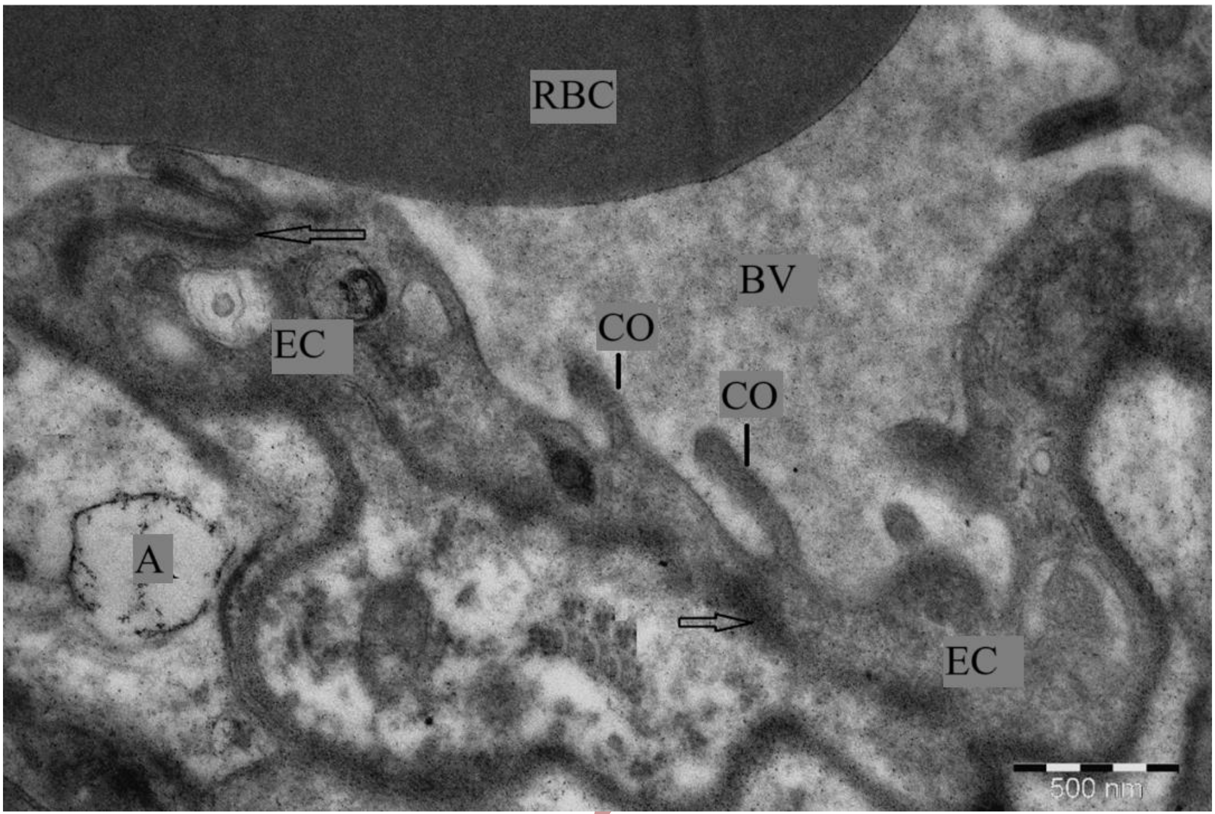

Figure 4. Ultrastructure of a mammalian cerebellar cortex capillary endothelial cell in the active phase. Electron microphotography:
EC–endothelial cell; A - astrocyte stalk; BV - blood vessel lumen; CO - endothelial cell cytoplasmic outgrowths; RBC - red blood cell; ↑ - tight junctions.

DISCUSSION

The study identified several key features that deserve detailed discussion.

First, the endothelial cells of the bovine cerebellar cortex capillaries exhibit a high mitochondrial density, indicating an intensive metabolic exchange. This may be due to the high energy requirements of endothelial cells, which provide transport of nutrients and oxygen to cerebellar neurons. [10, 15] Comparative analysis with other animal species shows that similar mitochondrial density is also typical for other mammals, which emphasizes the universality of this phenomenon. [16-20]

Second, the obtained data indicate the presence of numerous pinocytotic vesicles in the cytoplasm of endothelial cells. This indicates active transport of substances through the endothelium, which plays a key role in maintaining homeostasis of the cerebellar microenvironment. It is interesting to note that the number and size of pinocytotic vesicles can vary depending on the physiological state of the animal, which requires further research to identify regulatory mechanisms. [9,11,18]

The third important aspect is the presence of tight junctions between endotheliocytes, which form the blood-brain barrier. These structures play a critical role in preventing potentially harmful substances from entering the brain from the blood. [8,17] The results show that tight junctions in the capillaries of the bovine cerebellar cortex are highly organized, which may be due to the need for strict control over the composition of the cerebellar microenvironment.

In addition, the presence of pericytes, which are closely associated with endothelial cells, is observed. Pericytes play an important role in regulating blood flow and maintaining the structural integrity of capillaries. [17] Their presence in the capillaries of the bovine cerebellar cortex confirms their importance for microcirculation in this part of the brain.

Thus, the endothelial cells of the cerebellar cortex capillaries of the domestic bull are the primary element of the blood-brain barrier. Forming the lumen of the capillary, they contact each other with tight junctions and lie on a continuous basal membrane. The continuity of the latter and the presence of tight junctions block the possibility of spontaneous transport of macromolecular compounds from the bloodstream to the brain tissue.

CONCLUSION

In conclusion, this study highlights the complex ultrastructural organization of domestic bovine cerebellar cortical capillary endothelial cells. Revealed features, such as high mitochondrial density, active pinocytosis, tight junctions, and the presence of pericytes, indicate the high functional activity and specialization of these cells. Further studies should be aimed at studying the molecular mechanisms regulating these processes, as well as at comparative analysis with other animal species to identify common and specific features of the ultrastructural organization of endothelial cells.

References

1. Prusakov AV. Features of X-ray anatomy of the arterial vascular bed of the brain of a dachshund [Russian]. Scientific notes of the Kazan State Academy of Veterinary Medicine named after N.E. Bauman. 2014; 218(2): 215-220.
2. Prusakov AV, Zelenevsky NV, Shchipakin MV, *et al*. Blood supply to the brain of the long-tailed chinchilla (Chinchilla lanigera) [Russian]. Ippology and veterinary science. 2019; 2(32): 90-93.
3. Yashin AV, Prusakov AV. Features of the state of the microcirculatory bed and membrane digestion in newborn calves with dyspepsia [Russian]. International Bulletin of Veterinary Medicine. 2021; 2: 155-160. https://doi.org/10.17238/issn2072-2419.2021.2.155.
4. Drozdova LI. Histohematic barriers and their role in pathology [Russian]. Omsk Scientific Bulletin. 2004; S1-1 (26): 179-180.
5. Akmalova GM, Ron GI, Musina LA, Chernysheva ND. Morphological features of the structure of elements of histohematic barriers in diseases of the oral mucosa [Russian]. In the collection: Proceedings of the thirteenth Siberian Congress: "Dentistry and Maxillofacial Surgery with International Participation"; All-Russian Symposium: "New Technologies in Dentistry". 2018: 20-23.
6. Prusakov AV, Zelenevsky NV, Shchipakin MV, Bylinskaya DS, Vasiliev DV. Comparative ultrastructural organization of the blood-brain barrier of birds and mammals [Russian]. Morphology -2019 No. 6 p. 114.
7. Prusakov AV, Zelenevsky NV. Histohematic barriers of animals. St. Petersburg: Cultural and Educational Partnership, 2022: 46.
8. Karelina TV. Age-related features of synaptic activation of cerebellar Purkinje cells by the system of climbing and mossy fibers: specialty 03.03.01 "Physiology": dissertation for the degree of candidate of biological sciences. [Russian]. St. Petersburg, 2010: 125
9. Lobanov SA. Ultrastructure of the cerebellum during hypoxia. Evidence of morphological studies in medicine. [Russian] Proceedings of the International Scientific and Practical Conference. 2024: 59-63
10. Lobanov SA, Oberyukhtina EV, Cherepanov NS, Mansurova ZR. Some morphological features of the cerebellum. [Russian] Bulletin of scientific conferences. 2024; 12-2(112): 50-51
11. Mazloev AB. Microstructural features of the cerebellar cortex in newborns. [Russian] Morphology. 2019; 155(2): 182-183
12. Prusakova AV, Zelenevskiy NV, Prusakov AV, Yashin AV, Ponamarev VS. Ultra-structural organization of the gallbladder mucous membrane of Anglo-Nubian goat. Vet Res Forum. 2024; 15(3): 165-169. https://doi.org/10.30466/vrf.2024.2011081.4005.
13. Prusakova AV, Zelenevskiy NV, Prusakov AV, Yashin AV, Ponamarev VS. Organization of histo-hematic barriers of the liver in Anglo-Nubian goat. Online Journal of Animal and Feed Research. 2023;13(4): 242-245. https://doi.org/10.51227/ojafr.2023.36
14. Prusakova AV, Zelenevskiy NV, Prusakov AV, Yashin AV, Ponamarev VS. Ultrastructural organization of liver hepatocytes of the Anglo-Nubian goat. Veterinarski Glasnik. 2023; 77(2): 176-187. https://doi.org/10.2298/VETGL230615007P.

15. Hayat MA. Principles and Techniques of Electron Microscopy: Biological Applications, 4th ed. Cambridge University Press: Cambridge, UK. 2000: 543.
16. Electron Microscopy Sciences. Preparation of Post-Staining Solutions [technical protocol]. Hatfield, PA: Electron Microscopy Sciences. Accessed October 21, 2025. https://www.emsdiasum.com/docs/technical/protocols/KN_PostStainSol.pdf
17. Semchenko VV, Samuseva RP, Moiseeva MV. International histological nomenclature [Russian]. Omsk: Kolosova, 1999: 156.
18. Barkhatova VP. Neurotransmitter organization and functional significance of the cerebellum. [Russian] Annals of Clinical and Experimental Neurology. 2010; 4(3): 44-49
19. Akhmedov UB. Morphological changes in dynamics after leg amputation in the foliate region of the cerebellar vermis in dogs. Re-Health Journal. 2020; 1(5): 96-110 https://doi.org/10.24411 / 2181-0443 / 2020-10022.
20. Esakov SA. Morphofunctional organization of motor representations of facial muscles in the cortex and central nuclei of the cerebellum of a white mouse: specialty 03.03.01 "Physiology": dissertation for the degree of candidate of biological sciences. [Russian] Izhevsk, 2003: 124
21. Strizhikov VK, Ponomareva TA. Anatomical characteristics of the cerebellum in poultry. [Russian] Eurasia-2022: social and humanitarian space in the era of globalization and digitalization: Proceedings of the International Scientific Cultural and Educational Forum. 2022: 389–391
22. Karnyushko OA, Zimatkin SM. Disturbances in the morphogenesis of the cerebellar cortex of the offspring of rats with experimental cholestasis and their correction. [Russian] Bulletin of the National Academy of Sciences of Belarus. Series of Medical Sciences. 2015; 3: 95-101.
23. Luzin VI, Churilin OA, Zolotarevskaya MV. Morphological features of the rat cerebellar nuclei (literature review). [Russian] Morphological almanac named after V. G. Koveshnikov. 2020; 18(3): 97-101

Figure legends

Figure 1. Ultrastructure of the basal membrane of the cortical capillary mammalian cerebellum. Electron micrograph: EC – endothelial cell; A – astrocyte; BM – basal membrane; ↑ – tight junctions.

Figure 2. Ultrastructure of the blood-brain barrier of the capillary of the cerebellar cortex of a domestic bull. Electron micrograph: EC – endothelial cell; P – pericyte; A – astrocyte; BV – blood vessel lumen; E – erythrocyte; ↑—basal membrane; PL – phagolysosomes; M – mitochondria.

Figure 3. Ultrastructure of the endothelial cell of the capillary of the cerebellar cortex of a domestic bull. Electron micrograph:
EC – endothelial cell; P – pericyte; A – astrocyte; BV – blood vessel lumen; GER – granular endoplasmic reticulum; ↑ –basal membrane.

Figure 4. Ultrastructure of a mammalian cerebellar cortex capillary endothelial cell in the active phase. Electron microphotography:
EC–endothelial cell; A – astrocyte stalk; BV – blood vessel lumen; CO – endothelial cell cytoplasmic outgrowths; RBC – red blood cell; ↑ – tight junctions.