

**MORPHOLOGY AND MORPHOMETRY OF MOLECULAR LAYERS OF THE  
LANGUAGE OF THE POSTERIOR PART OF THE CEREBELLUM AFTER  
AMPUTATION OF THE POSTERIOR EXTREMITY IN DOGS**

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**Abstract**

In this scientific work, the morphology and morphometry of the molecular layer of the cortex of the lobule of the cerebellum lobe of the related cerebellar archicerebellum of dogs that underwent amputation of the hind limb was studied. At the initial stages of the experiment, a sharp decrease in neurocytes in this molecular layer, a thickening of the brain tissue due to edema, and a decrease in the size of basket and stellate cells were observed. In the last 60 and 90 days of the experiment, the number of neurocytes increases, the size of stellate cells increases due to compensatory hypertrophy. Morphologically in the first half of the experiment it was found that basket and stellate cells underwent dystrophy, atrophy, necrobiosis, and in the long term revealed compensatory hypertrophy and hyperchromasia.

**Keywords:** dogs, leg amputation, cerebellar cortex, cerebellar lobule, tongue lobule, molecular layer, stellate neurocytes

The relevance of the problem. The cerebellar cortex is phylogenetically divided into 3 zones: 1) archicerebellum, 2). paleocerebellum, 3) neocerebellum. The most ancient part is considered to be archicerebellum, it consists of a nodule, tongue, scrap and segments of the tonsil. The composition of paleocerebellum includes the tongue of the worm, the central lobule, the pyramid and the rupture. Neo-cerebellum consists of a pyramid of a worm, a slope, a leaf of a worm, a worm tubercle. Archicerebellum or vestibulocerebellum mainly receives its afferents from the vestibular nuclei and maintains balance and posture. In this case, the main place is occupied by the tongue (2,4,5,7). Some of the afferent fibers coming from the vestibular fibers cross the uvula (lingula), reaching the caudal part of the uvula (uvula) of the posterior part of the cerebellum (uvula).

The main functions of the cerebellum are body balance, muscle tone, coordination of slow movements, as well as coordination of reflexes responsible for the balance of the body coming from the motor center of the brain, correction of fast target movements. Each part of the cerebellum: archiocerebellum, paleocerebellum vaneocerebellum performs separate functions in the process of muscle coordination.

Archiocerebellum coordinates the activity of neurons of the vestibular nucleus of the medulla oblongata and the reticular formation. At the same time, it affects the balance of the body and the processes of orientation. Tent core neurons decrease activity upon excitation of Purkinje neurons and vice versa, inhibition of Purkinje neurons increases their activity.

The main objective of the study was the study of specific micromorphological and micromorphometric changes in the molecular layer of the cortex of the lobule of the tongue (uvula) of the posterior part of the cerebellum of the related cerebellar archicerebellum of dogs that underwent amputation of the hind limb.

Materials and methods. To achieve the goal, 35 outbred dogs weighing from 9 to 15 kg were selected, which were divided into 2 groups. 5 dogs were taken as a control group. The second group

included 30 dogs; 3 stage amputations were performed in the middle part of the right hind limb according to Pirogov. At 7, 21, 30, 60, 90 days of the experiment, the dogs were killed by emergency decapitation and the cerebellum was separated. The brain was completely immersed in a 5% solution of neutralized formalin, kept for 1 day, then cut according to the Flexig method, divided into pieces. A lobule of the tongue was cut from part of the archiocerebellum and fixed within 2 days by adding 10% neutralized formalin. The slices were passed through containers containing increasing concentrations of alcohol, dehydrated, and paraffin was poured. Continuous cutting of paraffin blocks was carried out, 5-8  $\mu\text{m}$  sections were prepared in several series, hematoxylin-eosin stained.

Morphometric studies were performed by the cytometric method according to G.G. Avtandilov (1990).

The body volume of the neuron is calculated using the formula for determining the volume of the cone according to I.N. Bogolepova (1978):  $V = 1 / 3h \times d^2 / 4$ , where V is the volume of the body of the neuron, d is the diameter of the base of the cell, h is the length of the neuron.

The results of the study. In a microscopic examination of the dogs' cerebellum, the cortical layer consists of three cytoarchitectonics: the external — molecular, the middle — ganglionic, and the internal — granular. These 3 layers consist of strictly differential neurons: molecular layer - consists of large and small round and oval shaped, located in the center of neurons with round nuclei; granular layer - consists of small, rounded neurons, with a small cytoplasm and large nuclei. Micromorphometrically, the average thickness of the molecular layer is  $161.4 \pm 5.7 \mu\text{m}$ , of the granular layer -  $187.6 \pm 6.8 \mu\text{m}$ .

Control group. Two types of neuron cells are distinguished in the molecular layer: basket-like and stellate. Basket cells are located in the lower third of the molecular layer. These are small, irregularly shaped cells measuring 10-20 microns. Their thin elongated dendritic processes are transverse to the gyrus. And long axons are located parallel to the surface of pear-shaped neurons. On the surface of pear-shaped cells, these fibers are densely arranged, forming a characteristic basket structure. Axon activity of basket neurons causes piriform inhibition.

Star-shaped neurons are located above basket-shaped neurons and have two types: small star-shaped neurons (Fig. 1), their dendrites are short, axons are poorly branched and form synapses in dendrites of pear-shaped neurons. The second type of stellate neurons is relatively larger, their dendrites are larger and widely branched, axon branches join a basket-like network around pear-shaped neurons, forming synapses with its dendrites. The basket and stellate neurons of the molecular layer form a unique unified system that transmits inhibitory nerve impulses to dendrites of Purkinje neurons.

Morphometrically, in the control group the width of the molecular layer was  $185.2 \pm 4.3 \mu\text{m}$ , the number of cells was  $84 \pm 6$ , the size of the basket cells was  $13.2 \pm 1.4 \mu\text{m}$ , and the size of stellate cells was  $22.5 \pm 2.6 \mu\text{m}$  ( table).

In the early stages of the experiment (7, 21 days), visible changes in the molecular layer lead to tissue edema, especially perivascular and pericellular edema. As a result, the basket cells are slightly reduced in size, the fibers are not visible. Stellate cells are also reduced in size due to edema, but are clearly visible from the edema around dendritic fibers (Fig. 2). Morphometrically, the molecular layer is slightly thickened, the number of cells is closer to normal, the size of basket cells is slightly increased, and the size of stellate cells is reduced (table).

On the 60th day of the experiment, it was found that the molecular layer of the posterior part of the cerebellar cortex was thickened than in previous periods, and even compared with the control group ( $194.3 \pm 6.9$ ), and the number of cells in it ( $77 \pm 6$ ) has been reduced compared to previous periods.

By this time, a thickening of the molecular layer was observed, which occurred due to edema and an increase in stellate cells in it.

days	The thickness of the molecular layer, microns	40x Number of cells	The diameter of the basket shape cells , microns	Diameter of stellate cells , microns
control group	185.2 ± 4.3	84 ± 6	13.2 ± 1.4	22.5 ± 2.6
7	187.4 ± 5.3	82 ± 5	15.2 ± 1.4	21.5 ± 2.6
21	191.7 ± 6.8	75 ± 4	17.6 ± 2.3	22.8 ± 1.9
30	204.6 ± 8.6	72 ± 3	19.5 ± 2.7	25.8 ± 2.8
60	194.3 ± 6.9	77 ± 5	18.7 ± 2.8	23.6 ± 1.7
90	192.7 ± 7.4	78 ± 4	17.6 ± 1.8	22.5 ± 1.9

At the same time, the size of basket cells increased to  $18.7 \pm 2.8 \mu\text{m}$ , and the size of stellate cells was  $23.6 \pm 1.7 \mu\text{m}$ .

At the end of the experiment, i.e. on the 90th day, the previous changes were further enhanced, the molecular layer remained wide and amounted to  $192.7 \pm 7.4$  microns. By this time, it was noticed that the arrangement of cells in the layer was much thinner and more chaotic.

It was observed that basket cells were located close to Purkinje cells in the lower layer of the formation, while stellate cells were rarely distributed on a large scale in all areas of the formation. It was noted that the sizes of these cells also stabilized somewhat less than in previous periods in accordance with the period of the experiment. The size of the basket cells was  $17.6 \pm 1.8 \mu\text{m}$ , and the size of stellate cells was  $22.5 \pm 1.9 \mu\text{m}$ .

Based on the above morphological and morphometric indicators, we can conclude that the tissue and cell structures of the molecular layer of the lobule of the tongue of the archicerebellum specifically change in dynamics. After amputation of the hind limb in the initial stages, a thickening of the layer is observed due to an increase in the size of cells and edema of the tissues of these lobules, and at the end of the experiment, the development of changes corresponding to repeated restoration is observed.

Specific changes in the cortical layer of the uvula of the posterior cerebellum were revealed microscopically, that is, the location of basket cells closer to Purkinje cells (Fig. 3), as well as the rare and chaotic arrangement of stellate cells along the layer. It was noted that the intercellular space was not the same width and that it was in harmony with glial cells.

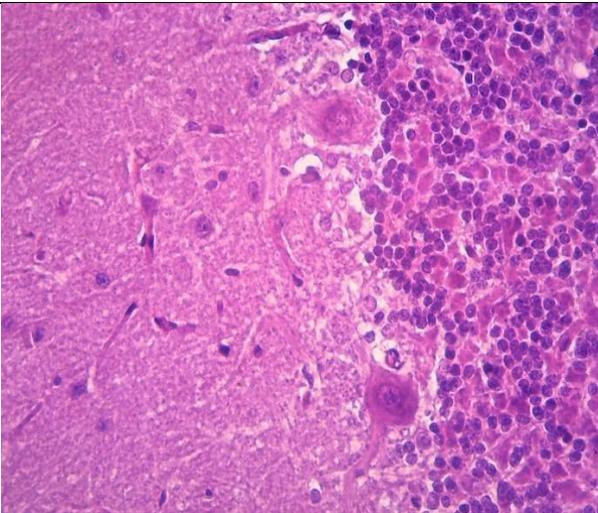
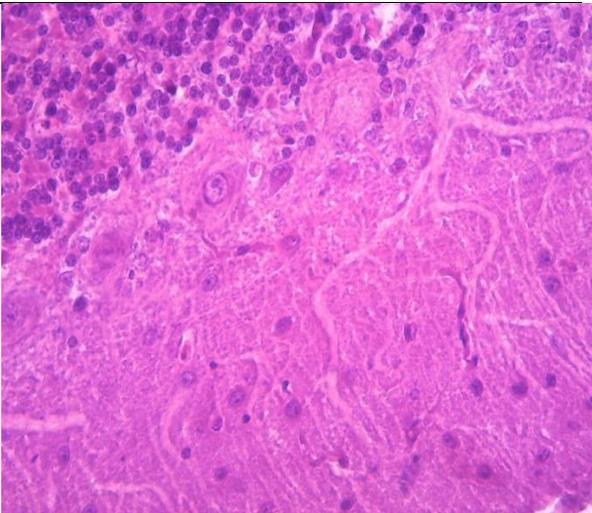
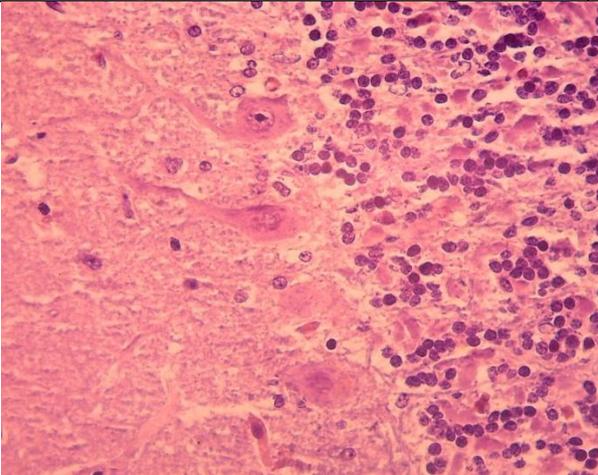
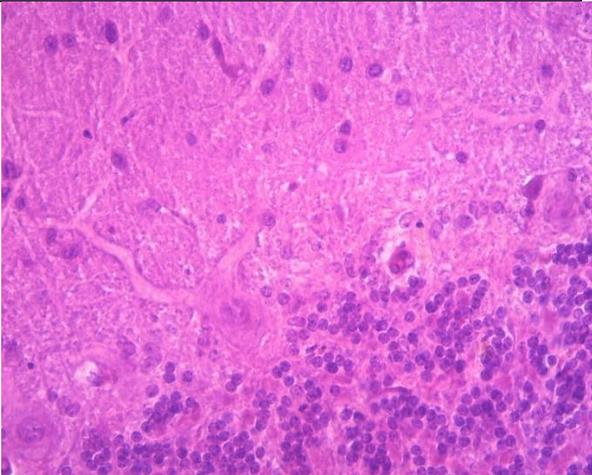
When studying the lobules of the uvula of the posterior cerebellum of the experimental animal in dynamics after amputation of the hind limb, it was found that in the first period of the experiment, i.e. after 7 days, all layers of the interstitial tissue were strongly swollen, vacuoles appeared in the edematous tissue of the molecular layer, the cells increased due to edema, and there was a large accumulation of proteins in the cytoplasm, indicating dystrophy.

Some basket cells are in a state of necrobiosis. The nuclei of these cells are wrinkled, smaller than normal and in a state of hyperchromasia.

On the 30th day of the experiment, since the molecular layer expanded due to edema, the number of cells in it decreased sharply and became erratic. The intercellular space is expanded and the penetration of granular layer neurocytes into it is observed. Some cells are located close to the Purkinje layer, while others are randomly located in the molecular layer.

By the 90th day of the experiment, the thickness of this layer also decreased due to a decrease in edema in the tissues, but in case of expansion of the blood vessels located in this layer, the edema remained around some of them. A noticeable aspect is that the nuclei of stellate cells are mainly

hypertrophied (Fig. 4), enlarged, chromatin is concentrated randomly, and the nucleus is hypertrophied.

	
<p>Fig . 1 . The location of small stellate neurocytes in the lower layer. Coloring : GE. X: 10x40.</p>	<p>Fig . 2 . H swelling of fibers and neurocytes . Coloring : GE. X: 10x40.</p>
	
<p>Fig . 3 . The location of basket neurocytes among Purkinje cells. Coloring : GE. X: 10x40.</p>	<p>Fig . 4 . Hypertrophy of the nuclei of stellate cells . Coloring : GE. X: 10x40.</p>

### Conclusion:

1. In the early stages of the experiment, after amputation of the hind limb of dogs, there is a sharp decrease in the number of neurocytes in the molecular layer of the cortex of the uvula (uvula) of the back of the cerebellum, a thickening of the brain tissue due to edema, and a decrease in the size of basket cells.

2. At the end of the experiment, on the 60th and 90th days, an increase in the number of neurocytes, an increase in the thickness of the molecular layer compared with previous periods of the experiment, even relative to the control group, and an increase in the size of stellate cells due to compensatory hypertrophy were detected.

3. Morphologically in the first half of the experiment, it was noted that both basket and stellate cells underwent dystrophy, atrophy, necrobiosis, among which neurocytes of the granular layer

were formed, the expansion of blood vessels and the development of edema in the interstitial tissue were observed.

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