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Morphology and Morphometry of Neurons of the Cerebral Cortex of the Brain Cerebellum White Rats Under the Influence of Lead Acetate

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ABSTRACT

In the experimental model we studied the morphological and morphometric features of neurons of cerebral cortex of the cerebellum of the brain sexually mature white rats under the influence of lead acetate. We applied microscopy of histological sections of the hemispheres of the cerebellum areas of the cortex with a digital microscope Axio Imager.M2 (ZEISS, Japan) with software for analysis AxioVision SE64 Rel images. 4.8.3 and ZEN 2011. Photography products we produced with the help of built-in digital camera with the increase of 40 × 10 and 100 × 10.

Keywords: cerebellar cortex, lead acetate, neurons, cytoarchitectonics.

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INTRODUCTION

The toxic effect of lead compounds is the cause of pathological conditions of the nervous system, particularly the brain. One of the structures of the brain, responsive to the action of lead-containing compounds is the cerebellum [13]. The bark of the cerebellum of human and animal brain is one's usual self and in the various pathological conditions continue to be studied intensively [3-4, 10, and 12]. However, the works devoted to the study of the structure of the cerebellar cortex contain the controversial provisions requiring their permission. Data on the effect of lead and its salts onto structure of the cerebellar cortex in postnatal ontogenesis in the available print is not enough [5-8].

The aim of the research was to study the morphometric parameters of neurons of the brain hemispheres of the cerebellum sexually mature white male rats is one's usual self conditions and under the influence of lead acetate.

TECHNIQUE

We also used in action of sexually mature male rats weighing 200-250 g. The experiment was performed on 20 animals kept on common mode vivarium. The control group consisted of 10 intact animals. The test group of 10 animals which treated for 7 days orally lead acetate Pb $(CH_3COOH)_2 \times 3H_2O$ in dose 45 mg / kg / day (in terms of lead).

The animals were killed by decapitation under anesthesia (ether and chloroform – 1:1) with compliance of principles of humanity that are set in the directives of the European Community (86/609 / EEC) and the Declaration of Helsinki, and in accordance with the rules of work with the use of experimental animals. We noted some peculiarities of the behavior of the test group animals. After intoxication of lead acetate the animals moved into the lateral position, in other time the rats had a poor appetite and they have been violated coordination, there was a general depression of animals.

The material for the study served the areas of the cerebral cortex of the brain cerebellum of white rats. For the receipt of material from the cavity of the skull with scissors cut the skin and muscle coverings, exposing the bone material. From the skull cerebellum fetched by the removal of the temporal, parietal, frontal, occipital, nasal, lacrimal, and sphenoid other bones, followed by dissection of the solid cerebral shell and soft cerebral by anatomical scissors [7].

For histological studies cerebellum we fixed in 10% neutral formalin solution, and then, it was subjected to washing with running water, dehydrated by placing the test material in alcohols of increasing concentration and embedded in paraffin by conventional methodology. Frontal sections were made 7.5 mcm thick (2 each study slice material). The sections were placed on glass slides and stained with hematoxylin-eosin. We studied 20 sections of the cerebral cortex of the brain cerebellum white rats in norm and 20 sections with lead poisoning. Each cut was performed cytoarchitectonic cerebellar cortical differentiation in accordance with its characteristic. Using a digital microscope Axio Imager.M2 (ZEISS, Japan) with the software for analysis AxioVision SE64 Rel images. 4.8.3 ZEN 2011 we measured the thickness of the layers of the cerebral cortex of the cerebellum (n = 100, 40×10 enlarging). In these layers, four visual fields were measured following morphometric parameters of cells: the cell area, minimum and maximum cell diameter, the core area, the minimum and maximum diameters of the core, with visible nucleoli (n = 240, 100×10 enlarging). It also was calculated the index of elongation cell nuclei (E) is the quotient of the maximum diameter of the core to the minimum diameter of the core. The volumes of bodies of neurons and their nuclei are calculated using the formula volume of an ellipsoid of revolution. [11] Also, the concentration of neurons was calculated using the formula:

$$K = x \times 10^{6} / 41500 \times n,$$

Where x - is the number of cells (at least 100), the n - is the number of fields of view (at least 4), 41500 is the area of each field of view, mcm ^{2}. The nuclear-cytoplasmic ratio (NCR) was calculated using the following formula

$$(NCR) = V_{nucleus} / V_{perikaryionic} - V_{nucleus},$$

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Where $V_{nucleus}$ is the volume of nucleus, $V_{perikaryionic}$ is the volume of perikaryomic.

The shooting of preparations made by means of digital microscope Axio Imager.M2 (ZEISS, Japan) with the software for analysis AxioVision SE64 Rel images. 4.8.3 and ZEN 2011 [1].

The statistical analysis of the results was used the parametric citation of the Student-t. The distributions of the studied parameters meet two mandatory conditions of use of criterion of the Student-t: Normality of distribution in both groups of comparing and the equality of two general dispersions in the comparison groups. The statistical processing of the results of the study carried out by S. Hanz with the calculation of ($x \pm s_x$), where x is the arithmetic mean, s_x is the standard deviation using the Microsoft Excel program. The assessment of the statistical hypotheses accepted level of significance $p < 0,05$ [11, 14].

MAIN PART

The study of the structural features of the cerebellar white rats confirmed the typicality its microarchitectonics in vertebrates. The bark of the cerebellum consists of three cytoarchitectonic layers arranged in the following order: 1) the outside is the molecular layer of 320,3 ± 7,17 mcm; 2) the medium layer is a layer of cells thick pear neurocytes 40,1 ± 0,60 mcm; 3) the inner is the granular layer thickness of 620,2 ± 29,66 mcm (Fig. 1).

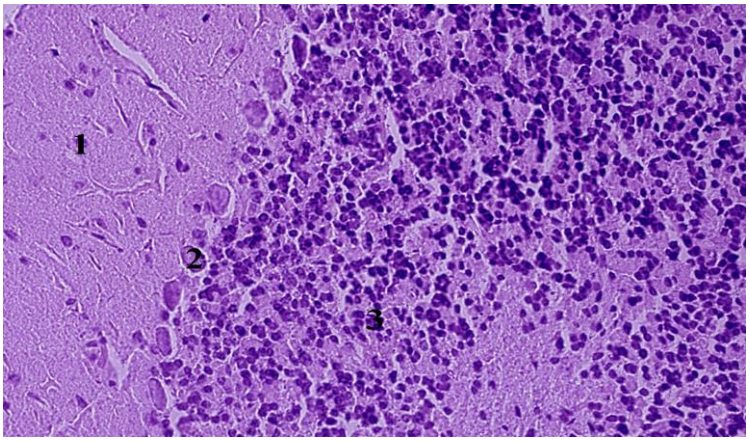

Fig. 1. The bark of the brain hemispheres of the cerebellum of white rats: 1 –the molecular layer; 2 – the layer neurocytes pear-shaped cells; 3 -the granular layer. Hematoxylin and eosin stain. Enlarging 40 × 10.

In the control the molecular layer is presented basket and stellate neurons. The perikaryonic of basket neurons round or polygonal shape, their minimum diameter was 12,1 ± 0,10 mcm and a maximum 14,5 ± 0,10 mcm. The average cell area of 199,9 ± 9,95 mcm^2, their average amount was equal to 1115,2 ± 55,75 mcm^3. The neurons contain rounded nuclei with a minimum diameter of 9,1 ± 0,04 mcm and a maximum diameter of 10,8 ± 0,09 mcm. Elongation core (E) was equal to 1,19.

The area of the nucleus was 64,3 ± 3,21 mcm^2. In the center of the core was well marked nucleolus, the volume of 469,3 ± 23,15 mcm^3. The cytoplasm of cell had a fine grain structure by the presence in its structure of the protein. The nuclear-cytoplasmic ratio (NCR) was 0,73 ± 0,04. The concentration of neurons reached 1204,8 1 mcm^2.

The stellate neurons, most of them are located at the surface of the cortex are smaller basket neurons (Fig. 2a) had an oval shape with a minimum diameter and a maximum 8,5 ± 0,08 mcm^2 and 408,3 ± 20,41 mcm^3. The neurons contained rounded nuclei poorly viewed staining with hematoxylin-eosin. The minimum core diameter was 0,07 ± 5,9 mcm, the maximum diameter was 8,3 ± 0,08 microns. The elongation core (E) is equal to 1,41. The average size and average volume of the nucleus were 50,6 ± 2,53 mcm^2, and 152,7 ± 7,64 mcm^3. The nuclear-cytoplasmic ratio (NCR) equal to 0,59 ± 0,03. Neuronal density reached 1084.3 in 1 mcm^2 (Table 1).

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Table 1: The morphometric parameters of the neurons of the cerebral cortex of the cerebellum of the brain of white rats in normal and under the lead poisoning ($x \pm s_x$)

The parameters	The molecular layer (basket cells)		The molecular layer (stellate cells)		The layer of cells pear neurocytes		The granular layer (grain-cells)	
	E	O	E	O	E	O	E	O
The diameter of the minimum core, mcm	9,1 ± 0,4	12,6 ± 0,5 *	5,9 ± 0,07	7,0 ± 0,12 *	14,6 ± 0,43	16,9 ± 0,33*	7,6 ± 0,08	8,9 ± 0,09*
The diameter of the core maximum, mcm	10,8 ± 0,09	15,3 ± 0,16 *	8,3 ± 0,08	9,7 ± 0,09*	22,4 ± 0,35	26,8 ± 0,38*	8,2 ± 0,08	9,4 ± 0,08*
The area of the kernel, mcm^2	64,3 ± 3,21	88,7 ± 0,65*	50,6 ± 2,53	68,8 ± 1,50 *	337,9 ± 7,63	402,1 ± 4,95 *	43,9 ± 0,60	46,9 ± 0,72 *
The volume of the nucleus, mcm^3	469,3 ± 23,5	1271,2 ± 63,56*	152.3 ± 7,64	248,7 ± 12,44*	2493,3 ± 23,89	4005,8 ± 40,06*	247,4 ± 12,37	389,7 ± 19,49*
The diameter of the minimum cell, mcm	12,1 ± 0,1	15,4 ± 0,20*	8,5 ± 0,08	8,7 ± 0,17	21,9 ± 0,39	27,3 ± 0,59 *	9,2 ± 0,07	11,1 ± 0,05*
The maximum diameter of the cells, mcm	14,5 ± 0,10	18,8 ± 0,20*	10,8 ± 0,11	11,3 ± 0,18	32,7 ± 0,32	39,5 ± 0,75*	10,9 ± 0,05	12,9 ± 0,07 *
The area of cell, mcm^2	199,9 ± 9,95	257,8 ± 2,33*	69,9 ± 3,49	71,2 ± 1,87	732,9 ± 12,95	879,5 ± 5,14*	73,7 ± 0,71	80,9 ± 0,99
The volume of cell, mcm^3	1115,2 ± 55,75	2333,3 ± 23,33*	408,9 ± 20,41	447,6 ± 22,38	8190,1 ± 81,89	15406,4 ± 154,06 *	483,7 ± 24,19	831,8 ± 41,59*
The gain cell nuclei index (E)	1,19	1,21	1,41	1,39	1,53	1,47	1,09	1,36
The concentration of neutrons in 1 mcm^2, (K)	1204,8	602,4	1084,3	722,9	597,4	537,2	4216,9	3493,9
The nuclear-cytoplasmic ratio (NCR)	0,73 ± 0,04	1,19 ± 0,06	0,59 ± 0,03	1,25 ± 0,06	0,44 ± 0,02	0,35 ± 0,02	1,05 ± 0,05	0,88 ± 0,04
Thickness of layer, mcm	320,3 ± 7,17		515,7 ± 7,65*		40,1 ± 0,60	47,1 ± 1,09*	620,2 ± 29,66	653,2 ± 43,15

Note: * – differences compared to controls are statistically meaningful in p <0,05. C – control group; E – experience group

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Under the intoxication of the lead acetate thickness of the molecular layer exceeded the control by 61 %. The layer differed fine pore structure. The concentration of perikaryonic decreased of molecular layer: basket neurons to 602.4 in 1 mcm^2, which is 50 % less than in the controls; stellate neurons to 722.9 1 mcm^2, which is less than the control by 33 % (Fig. 2 b). When calculating the nuclear-cytoplasmic ratio (NCR) basket neurons (1,19 ± 0,06) and stellate neurons (1,25 ± 0,06) was an increase in these parameters compared with the control at 71 % and 51 %, indicating a significant decrease cell functional activity. The minimum and maximum diameter of basket neurons increased respectively by 27,3 % and 29,7 %. The average cell area increased by%, and almost two-fold increase in the mean cell volume. The medium diameter of the nucleus increased by 38 %, and the maximum increase of 41 %. The Elongation core (E) was 1,21. The area of the core increased by 38 %, the volume of the nucleus increased almost twice.

The stellate neurons have a statistically significant increase compared with the control undergone the minimum and maximum diameter of the core, respectively, 18,6% and 16,9%, and the average area and the average nucleus increased by 35,9 % and 63,3 %. The elongation core (E) was 1,39 (Table. 1).

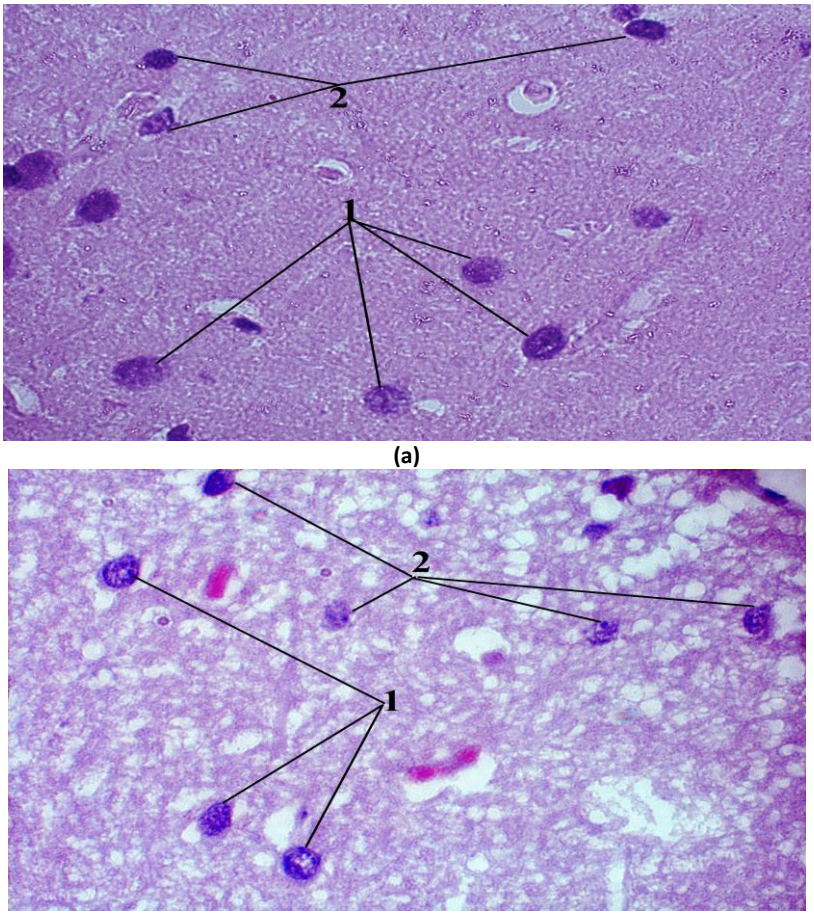
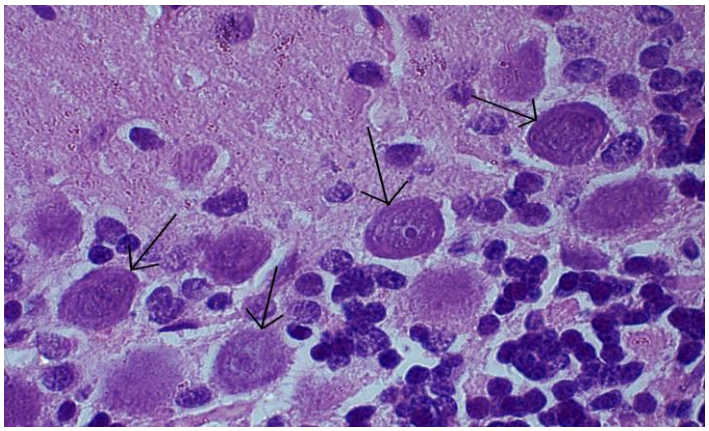


Fig. 2. The neurons in the molecular layer of the cerebral cortex of the brain cerebellum white rats a) normal; b) Under influence of the lead acetate: 1. basket cells; 2. stellate cells. Hematoxylin and eosin stain. Enlarging 100 × 10.

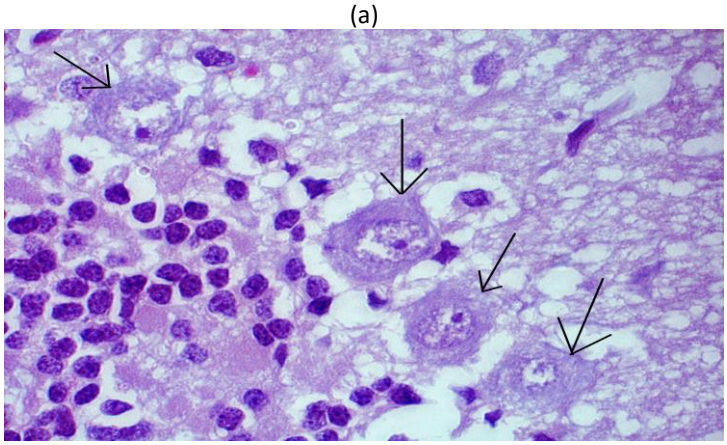
In control cells neurocytes pyriform layer formed by Purkinje cells arranged in a row above the molecular layer (Fig. 3 a). The neurons are spaced apart by the same distance, vertically oriented with respect to the surface of the cerebellar cortex. These are large cells, pear-shaped with a minimum diameter of 21, 9 ± 0, 39 mcm and maximum diameter 32,7 ± 0,32 mcm with an area of 732,9 ± 12,95 mcm2 and the volume of 8190,1 ± 81,89 mcm3. The neurons contained a kernel with the minimum and maximum diameter of 14,6 ± 0,43 mcm and 22,4 ± 0,35 mcm.

The coefficient of oblongness of kernel (E) is equal to 1,53. The Area neurons was 337,9 ± 7,63 mcm^2, and the volume of 2493,2 ± 23,89 mcm^3. The cytoplasm of cells had a coarse-grained structure. The nuclear-cytoplasmic ratio (NCR) was 0, 44 ± 0,02. The neurons concentration was 597,4 in 1 mcm^2 (Table 1).

In the study of neurocytes layer pear-shaped cells in the experimental group of animals after the influence of the lead acetate is noted the uneven distribution of Purkinje cells with ectopia into the granular layer. The thickness of the layer exceeded control about 15 %. The circuit of the perikaryonics is fuzzy, nucleus and cytoplasm have difficult borders. Around the neurons are areas of enlightenment (Fig. 3 b). The neurons' concentration was 537,2 to 1 mcm ^{2}, what is 15 % less than in the controls. The nuclear-cytoplasmic ratio (NCR) Purkinje cells (0,35 ± 0,02) compared to the control was reduced by 25 %, what indicates a significant increase in the functional activity of neurons. Minimum and maximum cell diameter compared with the control respectively increased by 24,7 % and 20,7 %, the cell area decreased by 20,1 %, at the same time they increased twice. The minimum and maximum diameter of the core increased by 15,8 % and 19,6 %. The privacy kernel coefficient (E) is equal to 1,47. The area of the core increased by 19 %, and the volume increased by half times. (Table 1).



(a)



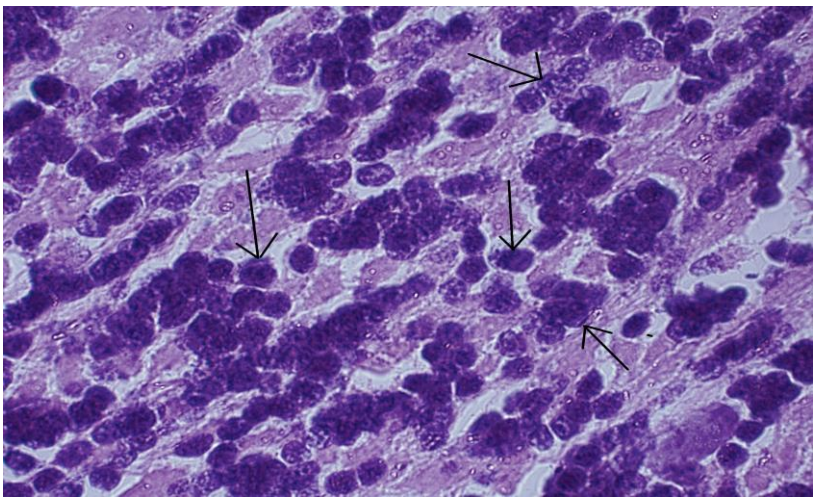
(b)

Fig. 3. The neurocytes pear-shaped cells of Purkinje cell layer of the cerebral cortex of the brain cerebellum white rats: a) Under normal; b) Under the action of lead acetate. Hematoxylin and eosin stain. Enlarging 100 × 10.

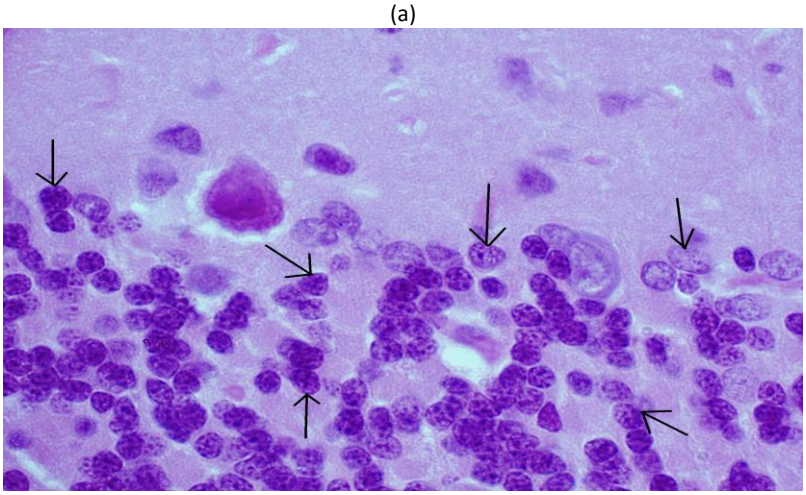
In the control group were examined in the granular layer of small neurons. This are grain-cells (Fig 4 a.). The cells grain oval minimum and maximum diameter 9,2 ± 0,07 mcm and 10,9 ± 0,05 mcm with an area of 73,7 ± 0,71 mcm^2 and the volume of 483,7 ± 24,19 mcm^3. The neurons contained large nuclei surrounded by a narrow rim of cytoplasm, their minimum diameter is 7,6 ± 0,08 mcm and a maximum diameter of 8,2 ± 0,08 mcm. Elongation core (E) is equal to 1,09. The area of the nucleus, occupied most of the cell, and accounted for 43,9 ± 0,60 mcm^2, the volume of the nucleus is equal to 247,4 ± 12,37 mcm^3. The nuclear-cytoplasmic ratio (YATSO) was 1,05 ± 0,05. The concentration of neurons reached to 4216.9 1 mm^2 (Table 1).

In the study of the granular layer in the experimental group of animals marked migration of granule cells in the molecular layer (Fig. 4 b). At the same time, statistically significant changes in layer thickness are not happening. The concentration of neurons reached 3493.9 1 mcm^2, what is 17 % less than in the controls. The nuclear-cytoplasmic ratio (NCR) neurons (0,88 ± 0,04) compared to the control was reduced by 16 %, what indicates a significant increase in the functional activity of neurons. The minimum and maximum diameters of the granular layer of neurons, respectively, increased by 20,7 % and 18,3 % cell area increased by 13,3 %, the

volume of cells was increased twice. The neurons contained large nuclei, the minimum and maximum diameter of which was increased by 17,1 % and 15 % compared with the control group of animals. The coefficient of elongation core (E) is 1,36. The volume of core compared to controls was increased by 50 % (Table 1).



(a)



(b)

Fig. 4 The cells grain granular layer of the cerebral cortex of the brain cerebellum white: a) Under normal; b) Under the action of lead acetate. Hematoxylin and eosin stain. Enlarging 100 × 10.

CONCLUSION

1. Conducted morphological and morphometric study of the molecular layer neurons of the cell layer neurons pear neurocytes and the granular layer of the cerebellum cerebral cortex of white rats under the influence of lead acetate as compared with the control revealed the following changes:

- The thickness of the molecular layer exceeded the control at 61%, with a marked decrease in the concentration of the basket perikaryonics and stellate neurons, in areas of neuronal death located the emptiness. According to the nuclear-cytoplasmic ratio (NCR) of basket (1,19 ± 0,06) and stellate neurons (1,25 ± 0,06), an increase of these parameters compared to the control at 71% and 51%, what indicates a significant decrease in cell functional activity.

- In the layer of pear neurocytes cell has changed of contour of perikaryonics, nucleus and cytoplasm have difficult borders. Purkinje cells were distributed in two rows, with ectopia into granular layer. The thickness of the control layer exceeded 15%. The decrease of the nuclear-cytoplasmic ratio (YATSO) compared with the control, indicates an increase in the functional activity of neurons, namely, increasing of the synthesis of proteins which provide vital processes in neurons and neurons themselves functioning

- In the granular layer of the most significant change is the migration of granule cells into the molecular layer. At the same time, statistically significant changes in layer thickness are not happening.

2. The findings suggest witnesses that effect of the lead acetate renders the most significant impact on the morphological and morphometric condition of neurocytes layer pear-shaped cells. Their topographic and morphometric variation leads to a restructuring of the local systems neuron-circulation that can be the basis for an explanation of changes in the nervous transmission mechanisms.

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