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Morphological characteristics of neural population of a hippocampus of Wistar rats in norm.

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ABSTRACT

The study was performed on the material of 20 Wistar rats (10 male and 10 female) weighing 220-250g. The Nissl-stained hippocampus microslides were exposed to micromorphometric researches. As a result, an absence of statistically significant interhemispheric distinctions between data of the studied parameters in the areas CA1 and CA3 of a hippocampus is established. The characteristic of the sizes of nuclei and cells of pyramidal neurons in these areas is given, and frequencies of occurrence of neurons with various sizes of nuclei and cell bodies of neurons are described.

Keywords: hippocampus, cell, neuron, morphometry.

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INTRODUCTION

The hippocampus is one of the most important structures of CNS of mammals participating in processes of acquisition and consolidation of memory traces depending on involvement of different neuromediator systems. The hippocampus participates in realization of various behavioural reactions including reactions under stress influences. Moreover, recent work on this region establishes a dynamic link between brain plasticity and cognitive experiences both across populations and within individuals(Andersen*et. al*, 2007; Chenet. al., 2011; Deuker*et. al.,* 2014).

Now the considerable volume of the researches devoted to Alzheimer's disease relates to studying of a hippocampus as this pathology is the chronic neurodegenerate disease which is characterized by development of dementia and the expressed pathomorphologic changes first of all developing in a hippocampus(Zhang *et al.,* 2016; Sanderson *et. al.,*2016).

Conceptions of pathogenetic mechanisms of stress reactions include data on activation of systems of an organism, minimizing the damaging action of a stress. Among the last the hippocampus is considered(Herman *et. al.,* 1997).

Regularities of plasticity of neurons of various structures of a brain at stimulus-response learning, processes of memory and habituation are well-known. Processes of neuronal plasticity are described also on subcortical levels (Halevas*et. al*., 2016; Guariglia*et. al.,* 2016).

The hippocampus plays an important role in processes of decrease in response of hypothalamic-pituitary-adrenal axis at a stress and at reduction of damage (Jacobson andSapolsky, 1991.). The atrophy of a hippocampus worsens its restrictive influences and conducts to prolonged response from hypothalamic-pituitary-adrenal axis on psychological stressor.

Besides, it is revealed that the dorsal region of a hippocampus is associated with dimensional orientation and possess adaptive flexibility, thereby being more stress-resistant subregion. On the contrary, the ventral hippocampus participates in the emotional reactions arising in response to stress-producing factors that leads to decrease in survivability of progenitor cells and neurogenesis, and as a result – to neurodegenerative changes. Thus, morphofunctional changes in a hippocampus play an important role at stressful influence of any character.One of manifestations of adaptation and adaptive reactions in CNS a change of sizes of neurons is considered. A number of researches is devoted to studying of change of micromorphometric parameters of neurons of a hippocampus in various conditions. At the same time, we noted that the morphometric data about neurons of intact animals, in particular, of the Wistar rats, are rather contradictory (Walsh and Cummins R.A., 1979; Tömböl*et al.,* 1979; McEwen B. S., 1998; McEwen B. S., 1999; Polman*et al.,* 2012; Hawley *et. al.,* 2012;Morch*et. al.,* 2012; Burke *et al.,* 2016; Cope, 2016; Alele, 2016;Rangasamy*et al.,* 2016; SimonyanandChavushyan, 2016; Ishihara and Fukuda T., 2016; Swanger*et al.,* 2016; Sepehriand Ganji, 2016).

Thus, it is possible to claim that the size of cells of some structure of CNS depends on specific activity of this structure.

Proceeding from this, we deemed currently important the research of morphological traits of neural population of the areas CA1 and CA3 of a hippocampus of Wistar rats at the age of 6 months.

MATHERIALS AND METHODS

The study was performed on the material of 20Wistar rats (10 male and 10 female) weighing 220-250 g. The animals were kept in cages under standard vivarium conditions with free access to food and water at 12-hour light regime and controlled temperature (22±2°C).

They were maintained in controlled environment (12:12 h light/dark cycle) and temperature (30±2ºC). All the animal experiments were performed according to the compliance with the EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

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Animals were sacrificed in carbon dioxide chamber. After sacrificing brain tissues were fixed in 96% ethanol for 24 h and then washed under tap water for 20 min. Then, the serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at 4-mm-thick using sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and subjected to Nissl-staining.

Finally, the sections were mounted and observed under a light microscope.

We determined the relative number of neurons in multiple fields of view on the total area of the pyramidal layer ofCA1 and CA3 regions (further recalculated per 10,000 µm), areas of neuron's bodies, areas of nuclei, nuclear-cytoplasmic ratio. The average quantity of neurons was calculated by randomly selecting five Nissl-stained sections at the same site from each rat. The distribution of individual neuronal sizes for each group is presented in histograms.

All measurements were taken with use of image analyzer "AxioVision"

Values are expressed as mean (± SD). Statistica 6.0 were used for statistical processing. The reliability of the results was determined by Student's t test (t) confidence level of $p<0.05$.

RESULTS

The CA1 and CA3 areas of rats at a microscopic research are clearly differentiated in both hemispheres as topographical, and on specific structure of neuronal layers.

The CA1 area is formed by the compactly located medium neurons which are characterized by a homogeneous light round nucleus, located centrally and containing basophilic nucleolus.

CA3 area consists of widely spaced large nerve cells in which the light nucleus with a single large nucleolus is noted (Fig. 1,2).

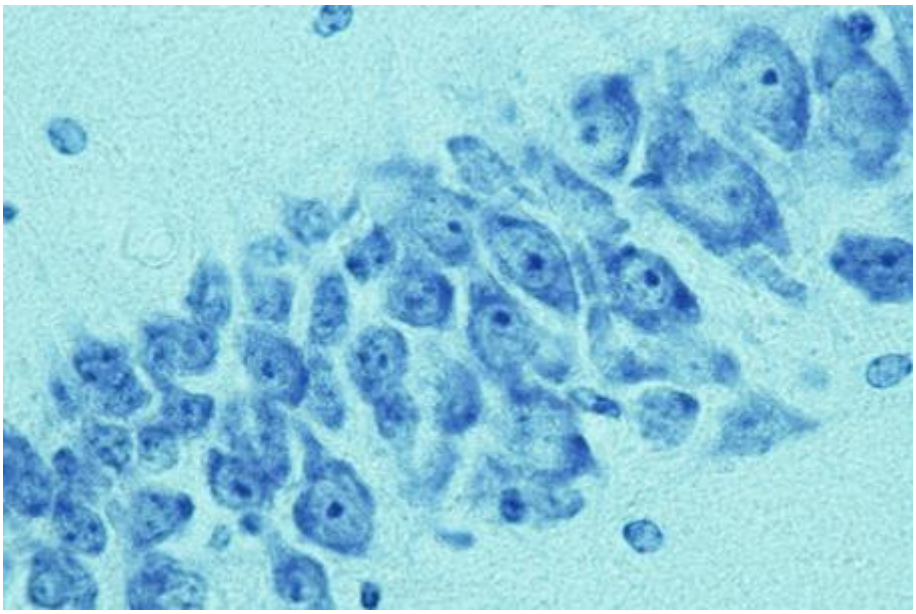

Fig 1: CA1 area of rat hippocampus. Nissl staining ×200

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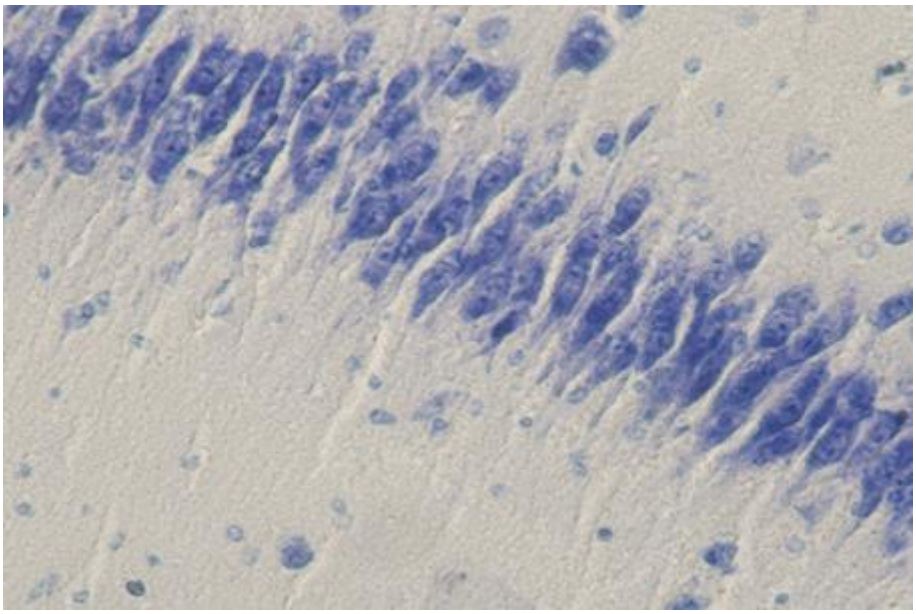

Fig 2: CA3 area of rat hippocampus. Nissl staining ×200

At the same time, averaged density of cells in the left hemisphere in the CA1 area amount 52.81±5.20 per 10000 mkm^2, and 54.58±4.82 per same area in right hemisphere.

In the CA3 area of the left hemisphere on 10000 mkm^2 the 18.21±3.12 cells and 19.40±4.52 per same area in the right hemisphere are found by us.

In the analysis of the area of cells, nuclei and nucleocytoplasmic ratio we also did not note the reliable interhemispheric distinctions (Table 1).

Table 1: Some micromorphometric characteristics of neurons of the CA1 and CA3 areas

	CA1			CA3		
	Sof nucleus, mkm^2	**Sof cell body, mkm^2**	**NCratio**	**Sof nucleus, mkm^2**	**Sof cell body, mkm^2**	**NC ratio**
Lefthemisphere	45.84±2.70	143.29±3.29	0.32±0.10	68.22±3,58	204.88±6,55	0.33±0.09
Right hemisphere	44.80±3.29	139.0±4.50	0.32±0.11	70.40±4.42	209.28±7.84	0.34±0.10

In the analysis of the frequency distribution of the area of nuclei of neurons of the area CA1 it is established that both in left, and in the right hemisphere the maximum quantity of nuclei (35%) accounts for the range of the sizes of 25-30 mkm^2. At the same, time in the CA3 area the maximum quantity of nuclei of pyramidal neurons measures in the range of 30-35 mkm^2 (22%) (Fig. 3).

At the same time in the analysis of the sizes of cells it is established that in the area CA1 the maximum quantity of neurons measures in the range of 100-110 mkm^2 (38%), and in the CA3 area we allocated 2 spikes: 110-120 mkm^2 and 150-160 mkm^2 (19% and 20% respectively) (Fig. 4).

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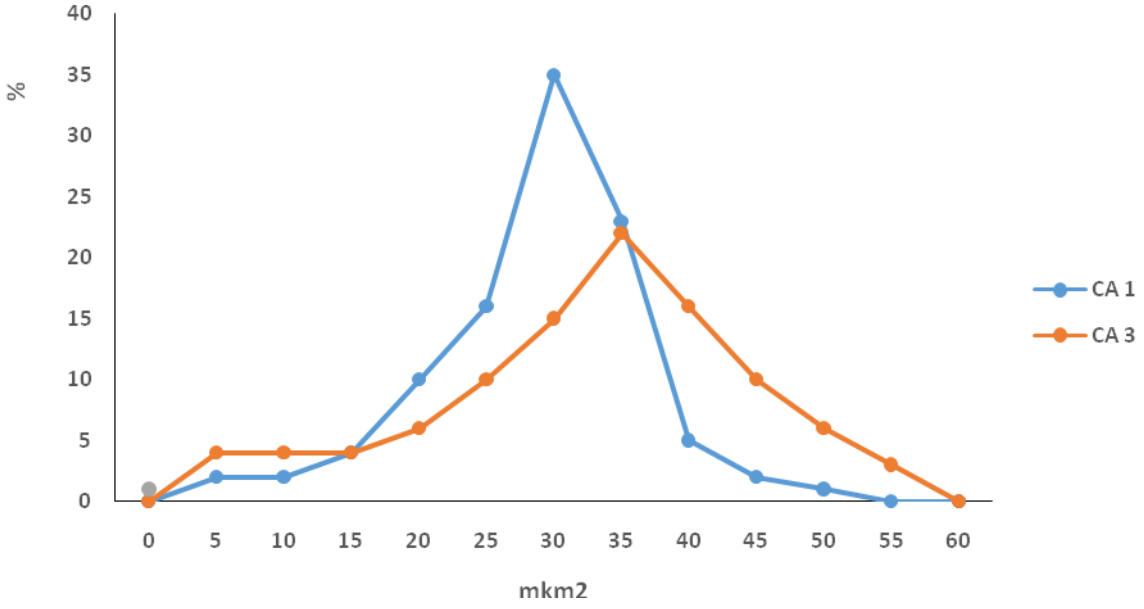

Fig 3: The frequency distribution of the sizes of nuclei in the areas CA1 and CA3 of a hippocampus of white rats.

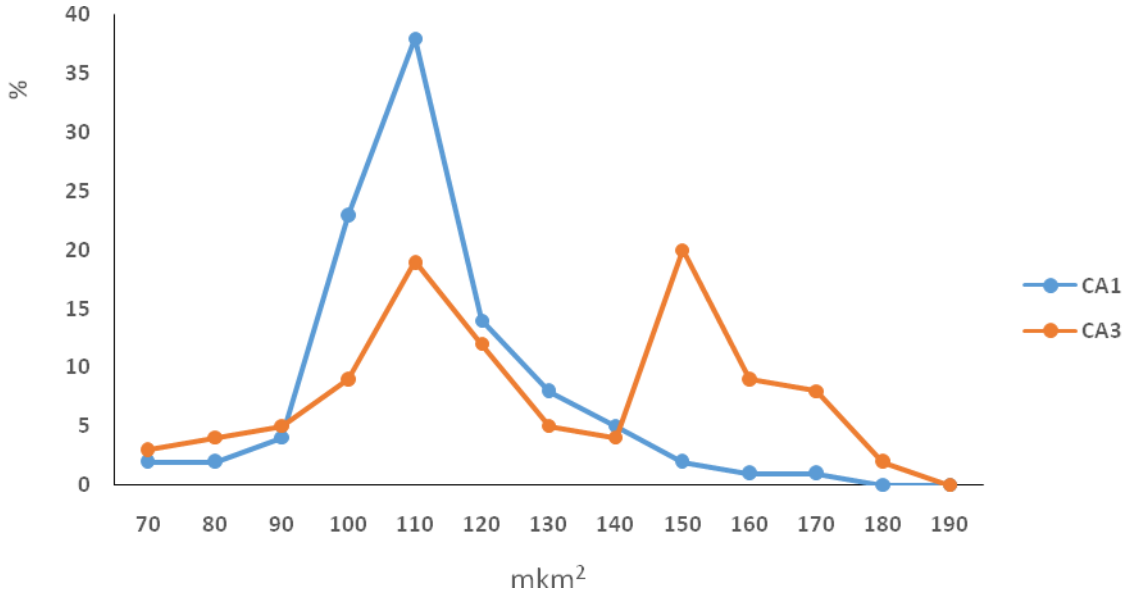

Fig 4: The frequency distribution of the sizes of cells in the areas CA1 and CA3 of a hippocampus of white rats.

DISCUSSION AND CONCLUSION

Thus, neurons of the areas CA1 and CA3 of a hippocampus form rather complex and heterogeneous structural-functional system which possesses a significant role in integrative activity of a brain of mammals. Despite numerous functional communications of a hippocampus with other structures of a CNS, heterogeneity of a structure of organ and complexity of its organization, there are not revealed by us the interhemispheric differences.

Besides, the conducted research and the analysis of literature allows to claim that some micromorphometric and morphological parameters of a hippocampus vary considerably apparently depending on an age of the studied animals.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interests regarding the publication of this paper.

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