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## MORPHOLOGY AND PATHOMORPHOLOGY

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# Morphochemical Characteristics of Hippocampal Neurons in Rats with Different Behavioral Parameters

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The hippocampus (CA3 field) was studied in rats with different behavioral characteristics. Protein contents in the neuronal cytoplasm and nuclei, as well as the size of neurons in animals predisposed to stress, poorly trained in a shuttle box, and exhibiting low locomotor activity in the open field were lower than in rats resistant to stress and characterized by high learning capacity and locomotor activity. Our results suggest that neuronal differences in the hippocampal CA3 field are associated with variations in learning capacity of animals.

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**Key Words:** rats of various strains; hippocampus; proteins; interferometry

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The hippocampus is one of the structures responsible for memory, emotions, learning, and other behavioral reactions [1,3,8,9,11]. The factors of genetically determined reactivity of animals to environmental signals can be evaluated by morphochemical characteristics of hippocampal neurons. Differences were revealed in neuroanatomical characteristics of hippocampal neurons in rats with various individual and typological parameters. For example, these neurons differ in morphological and neurohistological characteristics and ultrastructural organization of synapses [1,5,10,12].

Here we performed a comparative morphochemical study of the hippocampal CA3 field in Wistar rats, which were selected by the rate of active avoidance (AA) conditioning and differed in locomotor activity in the open field. Moreover, our study involved August and Wistar rats with different genetic resistance to stress.

## MATERIALS AND METHODS

We studied the hippocampus of Wistar rats selected by AA conditioning in a shuttle box (good and poor learners) and differed by locomotor activity in the open field (highly active and low active rats). Our study involved stress-predisposed (August rats) and stress-resistant animals (Wistar rats). The rats were decapitated under light ether anesthesia. The brain was fixed with Carnoy's fluid and processed as described elsewhere [4]. Dry weight of dense substances in the cytoplasm and nuclei of hippocampal neurons (CA3 field) was measured by means of interferometry under an Interfaco microscope. This parameter in fixed samples reflects the content and concentration of neuronal proteins [2]. Moreover, a relationship between dry weight of dense substances and size of neurons is one of the general criteria of functional activity of neurons. The area of cytoplasm and nucleus in nerve cells was measured using a MOV-1-15 ocular micrometer. We examined 150 neurons in each group of

rats. The results were analyzed by means of Protein software (Laboratory of Cytochemistry, Institute of Brain). Cresyl violet-stained sections served as a morphological control.

## RESULTS

Wistar rats with low locomotor activity in the open field are characterized by greater area of the nucleus and cytoplasm, as well as by higher content and concentration of structural proteins compared to animals with high locomotor activity (Table 1).

Comparison of morphometric parameters in good and poor learners (Table 2) showed that hippocampal neurons in poor learners were characterized by higher morphochemical indexes. Morphochemical indexes in stress-resistant Wistar rats were much higher than in stress-sensitive August rats (Table 3).

Our results indicate that morphochemical indexes are high in rats exhibiting low locomotor activity in the open field and poor AA conditioning in a shuttle box. Moreover, these indexes in stress-resistant Wistar rats are higher than in stress-sensitive August rats.

**TABLE 1.** Morphochemical Indexes of Hippocampal CA3 Neurons in Wistar Rats with High and Low Locomotor Activity in the Open Field ( $M \pm m$ )

Parameter	Locomotor activity in the open field		High/low locomotor activity, %
	high	low	
Area of the cytoplasm, $\mu^2$	55.75 $\pm$ 1.02	62.58 $\pm$ 1.11	112.3*
Area of the nucleus, $\mu^2$	52.77 $\pm$ 0.93	61.70 $\pm$ 1.21	116.9*
Protein weight in the cytoplasm, pg	58.78 $\pm$ 1.40	69.48 $\pm$ 1.37	118.2*
Protein weight in the nucleus, pg	28.58 $\pm$ 1.03	38.33 $\pm$ 1.11	134.1*
Protein concentration in the cytoplasm, pg/ $\mu^3$	1.05 $\pm$ 0.01	1.11 $\pm$ 0.01	105.7
Protein concentration in the nucleus, pg/ $\mu^3$	0.53 $\pm$ 0.01	0.63 $\pm$ 0.02	118.9*

**Note.** \* $p < 0.05$  compared to rats with high locomotor activity.

**TABLE 2.** Morphochemical Indexes of Hippocampal CA3 Neurons of Wistar Rats with Good and Poor Active Avoidance Conditioning in a Shuttle Box ( $M \pm m$ )

Parameter	Learning capacity		High/low learning capacity, %
	high	low	
Area of the cytoplasm, $\mu^2$	38.13 $\pm$ 0.7	54.71 $\pm$ 0.66	69.69*
Area of the nucleus, $\mu^2$	56.44 $\pm$ 0.6	52.18 $\pm$ 0.69	108.16
Protein weight in the cytoplasm, pg	26.36 $\pm$ 0.63	55.26 $\pm$ 0.94	47.70*
Protein weight in the nucleus, pg	19.67 $\pm$ 0.45	28.71 $\pm$ 0.86	68.51*
Protein concentration in the cytoplasm, pg/ $\mu^3$	0.69 $\pm$ 0.01	1.01 $\pm$ 0.01	68.32*
Protein concentration in the nucleus, pg/ $\mu^3$	0.35 $\pm$ 0.01	0.55 $\pm$ 0.01	63.64*

**Note.** \* $p < 0.05$  compared to rats with low learning capacity.

**TABLE 3.** Morphochemical Indexes of Hippocampal CA3 Neurons in August and Wistar Rats ( $M \pm m$ )

Parameter	Wistar	August	August/Wistar, %
Area of the cytoplasm, $\mu^2$	59.16 $\pm$ 1.06	53.16 $\pm$ 1.20	89.9*
Area of the nucleus, $\mu^2$	57.23 $\pm$ 1.07	45.15 $\pm$ 1.09	79.0*
Protein weight in the cytoplasm, pg	64.13 $\pm$ 1.38	38.81 $\pm$ 1.37	60.5*
Protein weight in the nucleus, pg	33.45 $\pm$ 1.07	16.22 $\pm$ 0.7	48.3*
Protein concentration in the cytoplasm, pg/ $\mu^3$	1.08 $\pm$ 0.01	0.73 $\pm$ 0.01	67.6*
Protein concentration in the nucleus, pg/ $\mu^3$	0.58 $\pm$ 0.01	0.48 $\pm$ 0.01	82.8*

**Note.** \* $p < 0.05$  compared to Wistar rats.

Our previous studies revealed morphochemical differences in the hippocampus of rats, which were resuscitated 10 min after cardiac arrest. These animals also differed in learning capacity. Postresuscitation damage to hippocampal neurons was more severe in good learners compared to poor learners. However, protein content in neurons was higher in animals with low learning capacity [1]. A positive correlation was found between the amount of ethanol consumption and morphochemical characteristics of hippocampal neurons. The higher was the degree of ethanol addiction, the greater was the area of neurons and protein concentration in the nucleus and cytoplasm [6].

Our results and published data on the involvement of proteins in learning, memory, and behavior [13-15] indicate that protein metabolism serves as a general criterion for the functional or pathological state of hippocampal neurons.

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