MORPHOLOGY AND PATHOMORPHOLOGY

Morphofunctional Changes in Neurons of Hippocampal Fields and Neocortical Layers in Rats Treated with Polydan

E. V. Loseva*, V. S. Evdokimova, O. V. Kurskaya, L. L. Pragina, and N. A. Tushmalova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 6, pp. 690-694, June, 2004
Original article submitted November 28, 2003

Light microscopy revealed four types of neurons differing by staining intensity and size in each of the pyramidal neuron populations in the dorsal hippocampal fields and layer V of the neocortical somatosensory area and in granular cells of the dentate fascia and stellate cells of layer II of the same cerebrocortical area in rats. Treatment with Polydan according to different protocols led to redistribution of these types of cells, which attests to stimulation of synthetic processes in these brain areas, rather than to similar sequence of the involvement of different brain areas in this process. Polydan promoted the increase in the mean number of nucleoli in the nuclei of neurons of all types, the degree of this increase being different for each type. It seems that morphological signs (staining intensity and number and size of nucleoli in the nuclei) reflect certain functional states of the neurons in the homogeneous populations. Presumably, various factors can stimulate transition of neurons from one morphofunctional status into another.

Key Words: *Polydan; hippocampal fields; neocortical layers; neuron types; nucleoli*

Positive effects of Polydan on memory in rats observed at the behavioral level [5,8] prompted more profound studies of the effects of this drug on CNS at the structural level. Polydan is a standardized mixture of DNA and RNA derivative polychlorohydrate sodium salts derived from sturgeon milt and used in oncological patients with chemotherapy-induced hemopoietic suppression [7].

We studied the effects of Polydan on structural and metabolic status of the brain during the formation of memory trace and on the morphology and function of the hippocampal and neocortical neurons in untrained animals, because these structures play the key role in the training and memory processes [1, 2,4,6].

MATERIALS AND METHODS

The study was carried out on 24 male Wistar rats (180-200 g) bred under standard vivarium conditions. The animals were divided into 4 groups (6 per group). The rats of two experimental groups were injected (once or 5 times) with 1 ml officinal Polydan solution in a dose of 0.75 mg/kg (groups E1 and E5), animals of two control groups received normal saline (1 ml of 0.9% NaCl) according to the same protocol (groups C1 and C5).

All rats were decapitated under ether narcosis 2 h after the last injection and the brain was rapidly removed.

The left cerebral hemisphere was fixed in 10% formaldehyde in phosphate buffer. Frontal sections at the

M. V. Lomonosov Moscow State University; *Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow. ***Address for correspondence:*** jazzivika@mail.ru. Evdokimova V. S.

0007-4888/04/1376-0609

level of the dorsal hippocampus (16 μ) were prepared using a cryostat (Zeiss) and stained with thionine solution with cresyl violet according to the method of Niessle.

Blocks of the dorsal hippocampus and neocortical somatosensory area were isolated from the right hemisphere and fixed in 2.5% glutaraldehyde in phosphate solution for 15 min. The blocks were cut into macrosections in the ventrodorsal direction so that they contained all hippocampal fields or all layers of the cortex. The macrosections were postfixed in 1% OsO_4 in phosphate buffer by the common method, dehydrated in ascending alcohols, and flat-parallel embedded in araldite. Semithin (1.5 μ) sections of the hippocampus and cortex were made on an ultratome (Reichert) and stained with aqueous solution of methylene blue (Merck) by the standard method.

All material was examined under an Axioplan-2 light microscope (Zeiss).

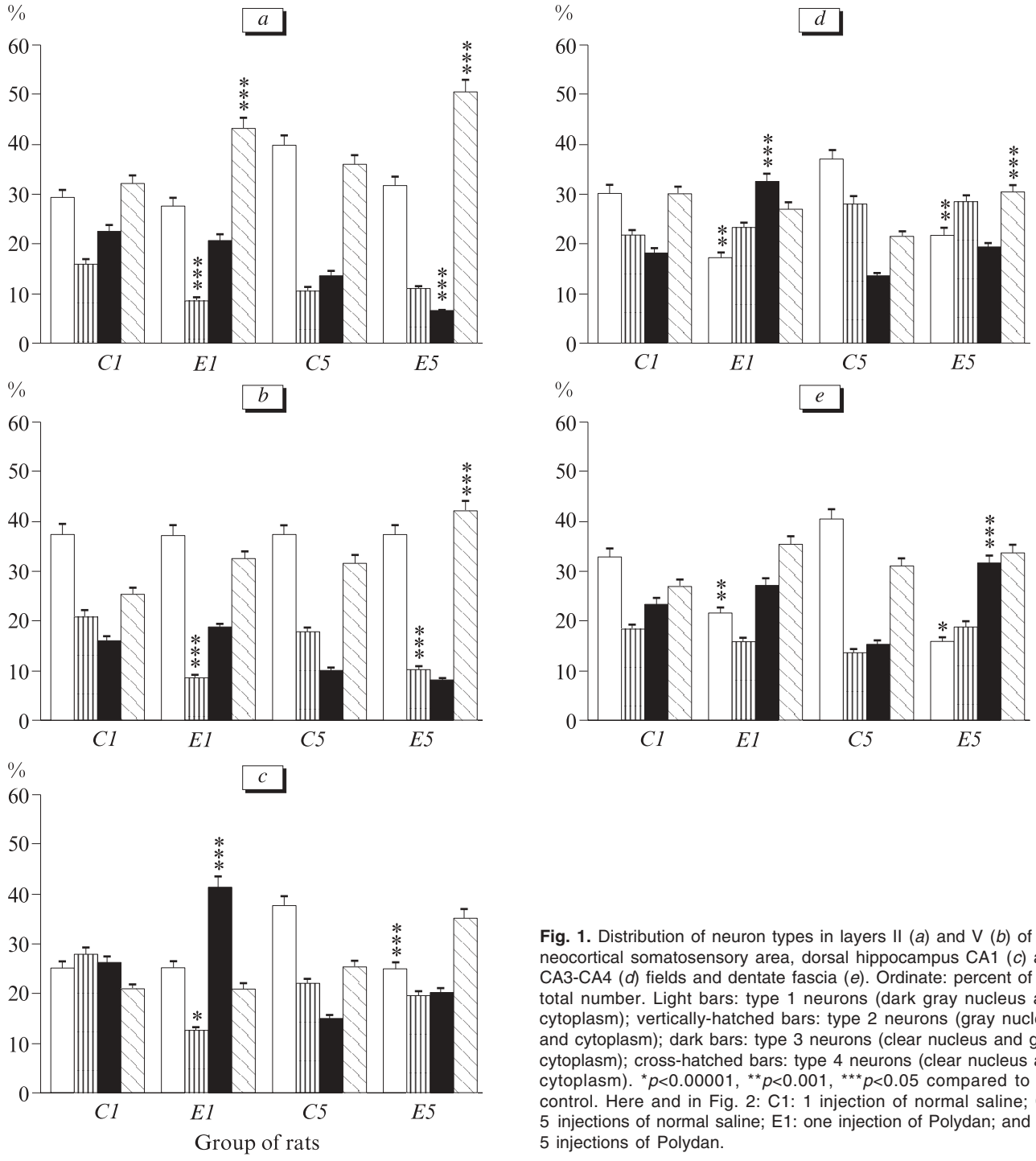

Fig. 1. Distribution of neuron types in layers II (*a*) and V (*b*) of the neocortical somatosensory area, dorsal hippocampus CA1 (*c*) and CA3-CA4 (*d*) fields and dentate fascia (*e*). Ordinate: percent of the total number. Light bars: type 1 neurons (dark gray nucleus and cytoplasm); vertically-hatched bars: type 2 neurons (gray nucleus and cytoplasm); dark bars: type 3 neurons (clear nucleus and gray cytoplasm); cross-hatched bars: type 4 neurons (clear nucleus and cytoplasm). *p<0.00001, **p<0.001, ***p<0.05 compared to the control. Here and in Fig. 2: C1: 1 injection of normal saline; C5: 5 injections of normal saline; E1: one injection of Polydan; and E5: 5 injections of Polydan.

Cell density (mean number of neurons and glia per unit of section area,1252 μ^2) was evaluated in cryostat sections of layers II and V of the neocortical somatosensory area and hippocampal fields CA1, CA3-CA4, and dentate fascia. The measurements were carried out in 50 visual fields for each rat.

Four types of cells differing by the intensity of staining were distinguished on semithin sections among the homogeneous populations of pyramidal neurons of the dorsal hippocampus and layer V of the neocortical somatosensory area, granular cells of the dentate fascia, and stellate cells of layer II of the same cerebrocortical area: type 1 cells with dark-gray nucleus and cytoplasm; type 2 with gray nucleus and cytoplasm, type 3 with clear nucleus and gray cytoplasm, and type 4 with clear nucleus and cytoplasm. The distribution of these neurons differing by the staining intensity, size, and the mean number

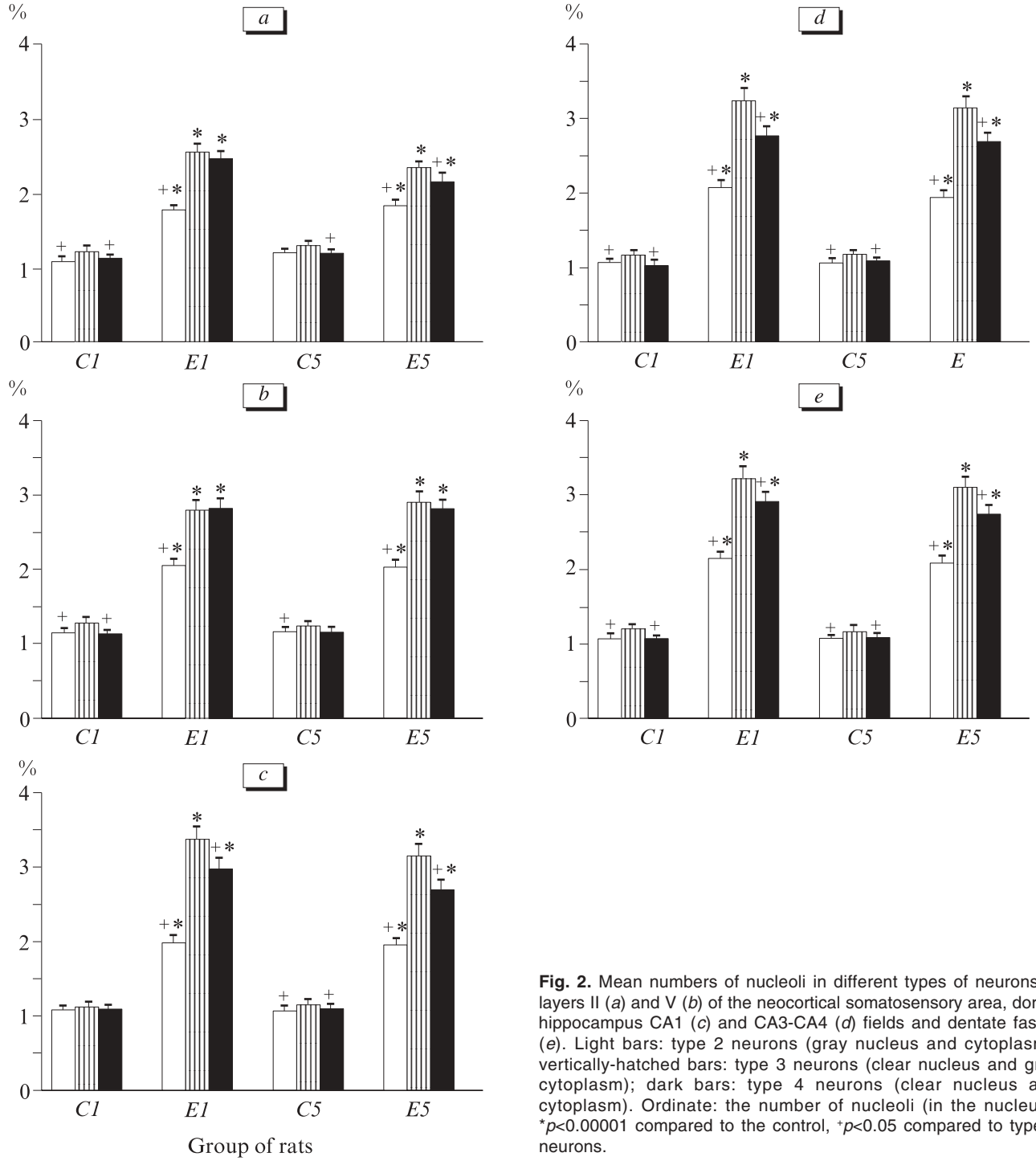

Fig. 2. Mean numbers of nucleoli in different types of neurons in layers II (*a*) and V (*b*) of the neocortical somatosensory area, dorsal hippocampus CA1 (*c*) and CA3-CA4 (*d*) fields and dentate fascia (*e*). Light bars: type 2 neurons (gray nucleus and cytoplasm); vertically-hatched bars: type 3 neurons (clear nucleus and gray cytoplasm); dark bars: type 4 neurons (clear nucleus and cytoplasm). Ordinate: the number of nucleoli (in the nucleus). $^*p<0.00001$ compared to the control, $^+p<0.05$ compared to type 3 neurons.

of nucleoli was studied (100 cells for each brain area per rat).

The results in experimental (E1 and E5) and control (C1 and C5) groups were statistically processed using Student's *t* test and Statistica software.

RESULTS

Polydan treatment led to redistribution of different types of neurons, uneven in different areas of the cortex and hippocampus and depending on the protocol of treatment. The number of dark neurons in CA1 field decreased only after 5 injections of the drug (p=0.002), while in the dentate fascia and CA3-CA4 fields it decreased both after 1 (p=0.0007, p=0.001, respectively) and 5 injections (p=0.00001, p=0.0005, respectively), and in the neocortex no changes in the distribution of neurons of this type were detected. In parallel with this, the number of gray cells in cortical layer V decreased significantly after 1 (p=0.002) and 5 (p=0.04) injections of Polydan. In layer II of gray cells this decrease was observed after 1 injection of the drug (p=0.001). In the hippocampus this effect was observed in the CA1 field after 1 injection of the drug (p=0.0001). The number of neurons with clear nucleus and gray cytoplasm increased significantly in the CA1 and CA3-CA4 fields after 1 injection (p=0.004, p=0.006, respectively), while after 5 injections it increased in the hippocampal dentate fascia (p=0.001) and neocortical layer II (p=0.04). The number of clear neurons increased significantly in neocortical layer II after treatment according to both protocols (p=0.04 after 1 injection and p=0.02 after 5 injections), while in cortical layer V it increased after 5 injections (p=0.04). In the hippocampus the number of these cells statistically significantly increased after 5 injec-

TABLE 1. Neuron Types Differing by Intensity of Staining in Layer V of the Cortical Somatosensory Area and in the CA3 Field of the Dorsal Hippocampus of Rats ($M\pm m$, n=36)

Group, neuron type			Neuron diameters, μ		Neuronal nuclei diameters, μ
			greater	minor	
Cortex	C1	type 2	26.75±0.69	22.607±0.654	16.99±0.494
		type 3	33.77±0.91*	28.704±0.848*	20.688±0.549*
		type 4	32.07±0.93**	26.921±0.703**	19.807±0.784****
	E1	type 2	27.10±0.51	23.936±0.664	16.54±0.511
		type 3	33.36±0.48*	28.429±0.59*	20.615±0.474*
		type 4	31.29±0.59**	26.683±0.793****	19.8±0.485**
	C5	type 2	27.96±0.49	24.55±0.544	17.58±0.391
		type 3	32.98±0.58*	28.84±0.615*	21.136±0.417*
		type 4	32.88±0.54*	29.331±0.415*	21.35±0.356*
	E5	type 2	28.94±0.52	25.622±0.553	18.33±0.277
		type 3	34.109±0.49*	29.745±0.541*	21.568±0.329*
		type 4	33.289±0.46*	29.193±0.451*	21.462±0.377*
Hippocampus	C1	type 2	31.178±0.762	25.939±0.54	20.2±0.544
		type 3	32.660±0.504***	30.350±0.616*	23.523±0.608***
		type 4	36.027±0.62**	31.867±0.694*	25.989±0.967*
	E1	type 2	32.66±0.671	27.7±0.987	20.75±2.117
		type 3	36.783±0.369+*	32.306±0.498**	23.506±0.575
		type 4	39.550±1.058**	34.050±0.906***	23.067±1.568
	C5	type 2	33.244±1.427	27.45±0.676	20.044±0.647
		type 3	35.394±0.306****	30.019±0.652****	23.171±0.619****
		type 4	36.521±0.675****	32.021±0.817**	23.815±0.762***
	E5	type 2	31.727±0.794	26.906±0.858	19.717±0.638
		type 3	35.042±0.711****	28.025±1.086+	21.222±0.550+
		type 4	37.764±1.273**	31.786±1.364****	25.243±1.132**

Note. n: number of members of the variation series. *p<0.00001, **p<0.0001, ***p<0.001, ****p<0.05 *vs.* type 2 neurons, +p<0.05 compared to type 4 neurons.

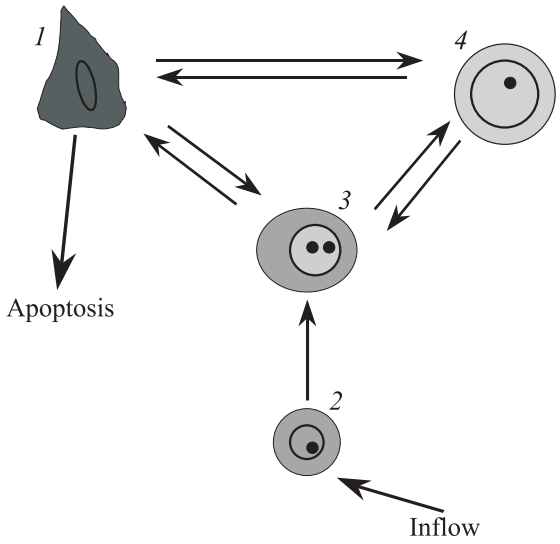

Fig. 3. Scheme of possible routes of transition of neurons of homogeneous populations from one morphofunctional status into another. *1*) neurons with dark-gray nucleus and cytoplasm; *2*) with gray nucleus and cytoplasm; *3*) with clear nucleus and gray cytoplasm; *4*) with clear nucleus and cytoplasm.

tions of Polydan only in the CA3-CA4 fields (p=0.03; Fig. 1).

The number of neurons did not change after their redistribution under the effect of Polydan, which was demonstrated by measurements of their density in cryostat sections. It seems that the neuron types distinguished in our study are not cells with different functions, but just different structural and functional states of neurons in homogeneous populations.

The number of nucleoli in the nuclei of all neuron types increased significantly (p=0.00001) in all studied areas of the cortex and hippocampus after both 1 and 5 injections of Polydan. This increase was less significant in gray and clear cells in comparison with neurons with clear nuclei and gray cytoplasm (p=0.0001). In the control groups (no Polydan treatment) the number of nucleoli in the nuclei of cells with clear nuclei and gray cytoplasm was statistically higher than in neurons of other types (p=0.05; Fig. 2). It seems that the production of ribosomal RNA (drastically increasing under the effect Polydan) is more intensive in neurons with clear nuclei and gray cytoplasm.

Cells of different types differed by size (Table 1): gray cells were the smallest, neurons with clear nucleus and gray cytoplasm were intermediate, and clear cells were the largest. The size of dark-gray cells and number of nucleoli in them were not studied, because the nuclei in these cells were shrunk and the cytoplasm and nucleoli virtually fused with condensed chromatin. According to published data, this is the appearance of cells fixed at the peak of synthetic activity [3].

The results of morphometrical analysis of neuronal redistribution, cell size and number of nucleoli after Polydan treatment suggest that the synthetic processes are least active in gray neurons, more intensive in the nucleus and nucleolar system of neurons with clear nucleus and gray cytoplasm, and in clear neurons the synthetic processes involve the cytoplasm.

It seems that the detected morphological differences in different types of neurons reflect their peculiar functional states. Based on our results, we suggest a scheme of possible neuron transition from one structural and functional state into another (Fig. 3). Transition from one morphofunctional state into another can take place under the effects of different factors.

REFERENCES

1. V. P. Bambindra and T. A. Bragina, *Structural Bases of Interneuronal Integration* [in Russian], Leningrad (1982).
2. O. S. Vinogradova, *Hippocampus and Memory* [in Russian], Moscow (1975).
3. L. B. Kalimullina, *Morfologiya*, **122**, No. 4, 75-79 (2002).
4. R. I. Kruglikov, *Neurochemical Mechanisms of Training and Memory* [in Russian], Moscow (1981).
5. L. L. Pragina, N. A. Tushmalova, E. V. Loseva, *et al.*, *Zh. Klin. Eksp. Farmakol.*, **66**, No. 6, 6-8 (2003).
6. P. V. Simonov, *Zh. Vyssh. Nervn. Deyat.*, **43**, No. 3, 514-529 (1993).
7. E. Treshchalina, M. Bychkova, and D. Bodyagin, *Vrach*, No. 5, 26-27 (1996).
8. N. A. Tushmalova, L. L. Pragina, A. N. Inozemtsev, and A. V. Asafov, *Zh. Vyssh. Nervn. Deyat.*, **49**, No. 5, 776-779 (1999).