Effect of Polidan on Ultrastructural Changes in the Cerebral Cortex and Hippocampus of Rats

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Electron microscopy and morphometry of neurons in layer V of the somatosensory area in the neocortex and CA3 field in the dorsal hippocampus showed that single and 5-fold intraperitoneal injection of Polidan was followed by ultrastructural and metabolic changes in neurons reflecting activation of protein synthesis. The number of free ribosomes decreased; the number of polysomes and count of ribosomes in polysomes and tubules of the granular endoplasmic reticulum increased. Study of mitochondria and neuropil showed that Polidan activates synthetic processes in the neocortex and hippocampus. It should be emphasized that single treatment with Polidan led to functional activation of synthetic processes, while 5-fold injection of Polidan was followed by hyperactivation of synthetic processes and depletion of ultrastructures in the neocortex and hippocampus.

Key Words: *Polidan; neocortex; hippocampus; ribosomes; mitochondria*

Polidan is used in oncology to stimulate hemopoiesis [4]. This drug often improves learning and memory [3,5]. Polidan is the extract of sturgeon milt containing a mixture of fragments of DNA and RNA sodium salts [4]. The study of the effect of Polidan on brain function in untrained animals would elucidate structural and functional bases of learning. Light microscopy showed that Polidan causes structural and functional changes in neurons of the neocortex and hippocampus in untrained rats. These changes illustrate activation of synthetic processes. Homogenous populations of neurons in the somatosensory area of the neocortex and fields of the dorsal hippocampus were studied after administration of Polidan. Both schemes of treatment with Polidan were followed by redistribution of neurons with different intensity. The observed changes were accompanied by an increase in the number of nucleoli in neurons of each type [1]. These data formed the basis for further detailed study of Polidan-induced ultrastructural changes.

Here we performed an electron microscopic morphometric study of ultrastructures in neurons of the CA3 field in the dorsal hippocampus and layer V of the somatosensory area in the neocortex of rats receiving intraperitoneal injection of Polidan.

MATERIALS AND METHODS

Experiments were performed on 24 male Wistar rats weighing 180-200 g and grown in a vivarium under standard conditions. The animals were divided into 4 groups of 6 specimens each. The rats of 2 treatment groups received single or 5-fold intraperitoneal injection of 1 ml pharmaceutical solution of Polidan (75 mg/kg). The animals of 2 control groups were injected with 1 ml 0.9% NaCl.

The animals were decapitated under ether anesthesia 2 h after the last injection. The brain was

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rapidly removed. Blocks of the dorsal hippocampus and somatosensory area of the neocortex were isolated and fixed with 2.5% glutaraldehyde in phosphate buffered saline for 15 min. They were cut in the ventrodorsal direction to obtain macrosections that included all hippocampal fields and cortical layers. Macrosections were fixed by the method of Pallade method using 2% OsO_4 in phosphate buffered saline [6], dehydrated in alcohols of increasing concentrations, and embedded in araldite (along a plane parallel). Ultrathin sections (500 Å) of layer V in the somatosensory area of the neocortex and CA3 field of the dorsal hippocampus were prepared on a Reichert ultratome. These sections were contrasted in a saturated aqueous solution of lead citrate. Ultrathin sections were examined under a JEM-100B electron microscope. The images were obtained using a CM-100 electron microscope (Philips).

The numbers of free ribosomes, polysomes, polysomal ribosomes, and ribosomes bound to tubules of the endoplasmic reticulum in pyramidal neurons were estimated in 50 fields of view for each rat. One field of view was 13.5 cm^2 (×100,000).

A visual rank study of the integrity of cristae and optical density of mitochondria (100 randomly selected mitochondria for each rat) was performed in the cytoplasm of neurons and their processes. By the integrity of cristae, mitochondria were divided into groups of structures with normal, partially destroyed, and completely destroyed cristae. We measured optical density of the matrix and estimated the number of mitochondria with an electron dense, partially lightened, and completely lightened matrix.

Study of the neuropil included calculation of profiles of processes with small, medium, and large diameter (40 fields of view for each rat). We also counted the processes with electron dense, partially

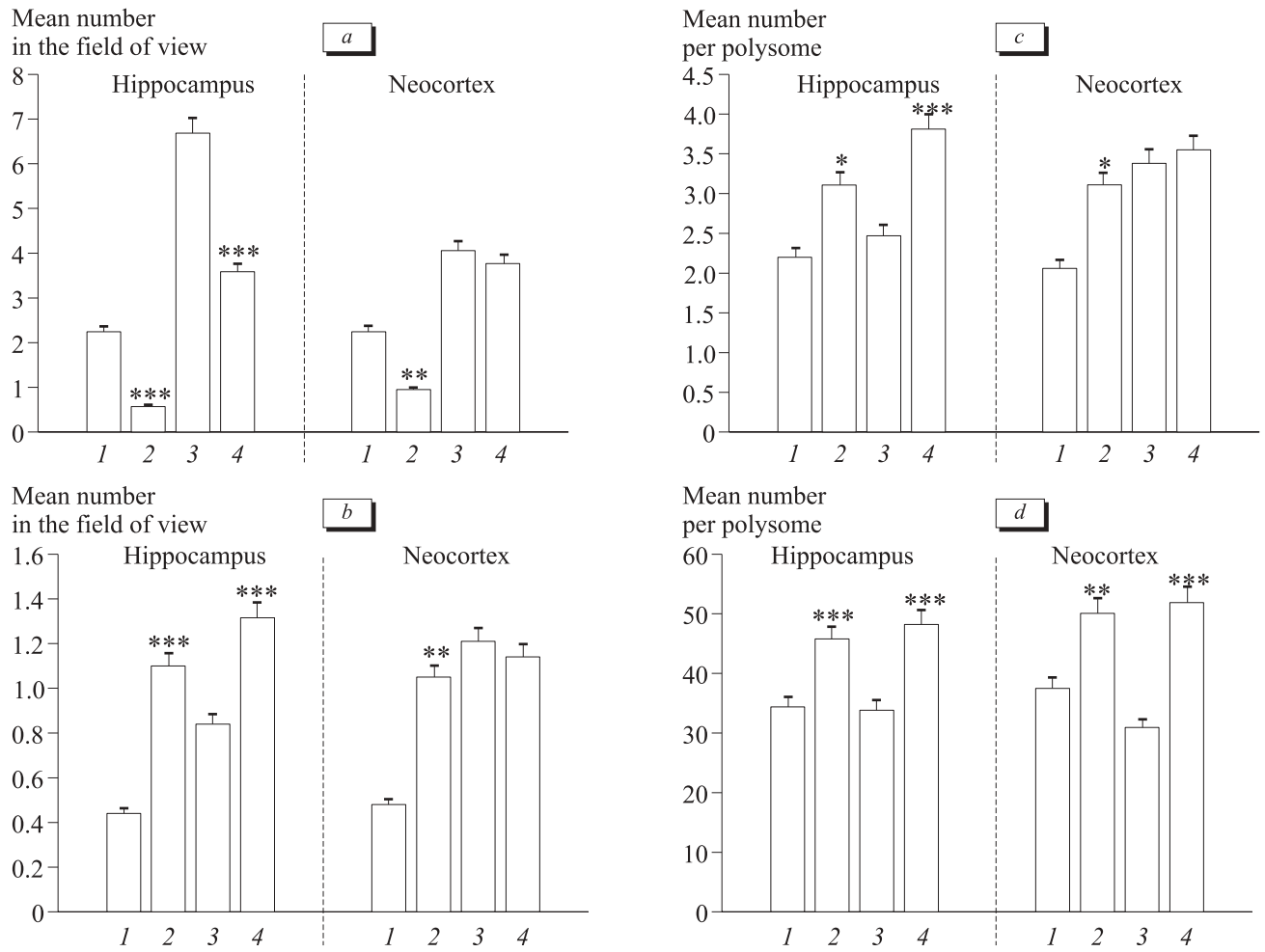

Fig. 1. Free ribosomes (*a*), polysomes (*b*), polysomal ribosomes (*c*), and endoplasmic reticulum-bound ribosomes (*d*) in neurons of layer V in the somatosensory area of the neocortex and CA3 field of the dorsal hippocampus in rats. Single administration of NaCl (*1*); single administration of Polidan (*2*); 5-fold administration of NaCl (*3*); 5-fold administration of Polidan (*4*). Here and in Fig. 2: *$p<0.05$, **$p<0.001$, and ***$p<0.000001$ compared to the control.

lightened, and completely lightened matrix. One field of view was 13.5 cm^2 (×40,000).

The results were analyzed by Student's *t* test for independent variables (Statistica software).

RESULTS

The study of the protein-synthesizing apparatus showed that single injection of Polidan causes the same changes in the hippocampus and neocortex. The number of free ribosomes decreased compared to the control. We revealed an increase in the number of polysomal ribosomes and count of polysomes and ribosomes bound to the endoplasmic reticulum. Fivefold injection of Polidan produced similar changes in the hippocampus. However, this treatment increased only the number of endoplasmic reticulum-bound ribosomes in the neocortex (Fig. 1).

Single and 5-fold injection of Polidan decreased the mean number of mitochondria with electron dense matrix in all structures of the brain. The number of mitochondria with completely lightened matrix increased under these conditions. Study of mitochondrial cristae in the neocortex revealed a decrease in the number of mitochondria with normal cristae and increase in the number of mitochondria with completely destroyed cristae. The number of mitochondria with normal cristae in the hippocampus decreased after single injection of Polidan. Fivefold injection of the drug was followed by a decrease in the number of mitochondria with normal cristae and increase in the number of mitochondria with completely destroyed cristae. These data suggest that Polidan activates energy processes in brain neurons (Fig. 2).

After administration of Polidan, ultrastructural changes were found not only in neuronal bodies,

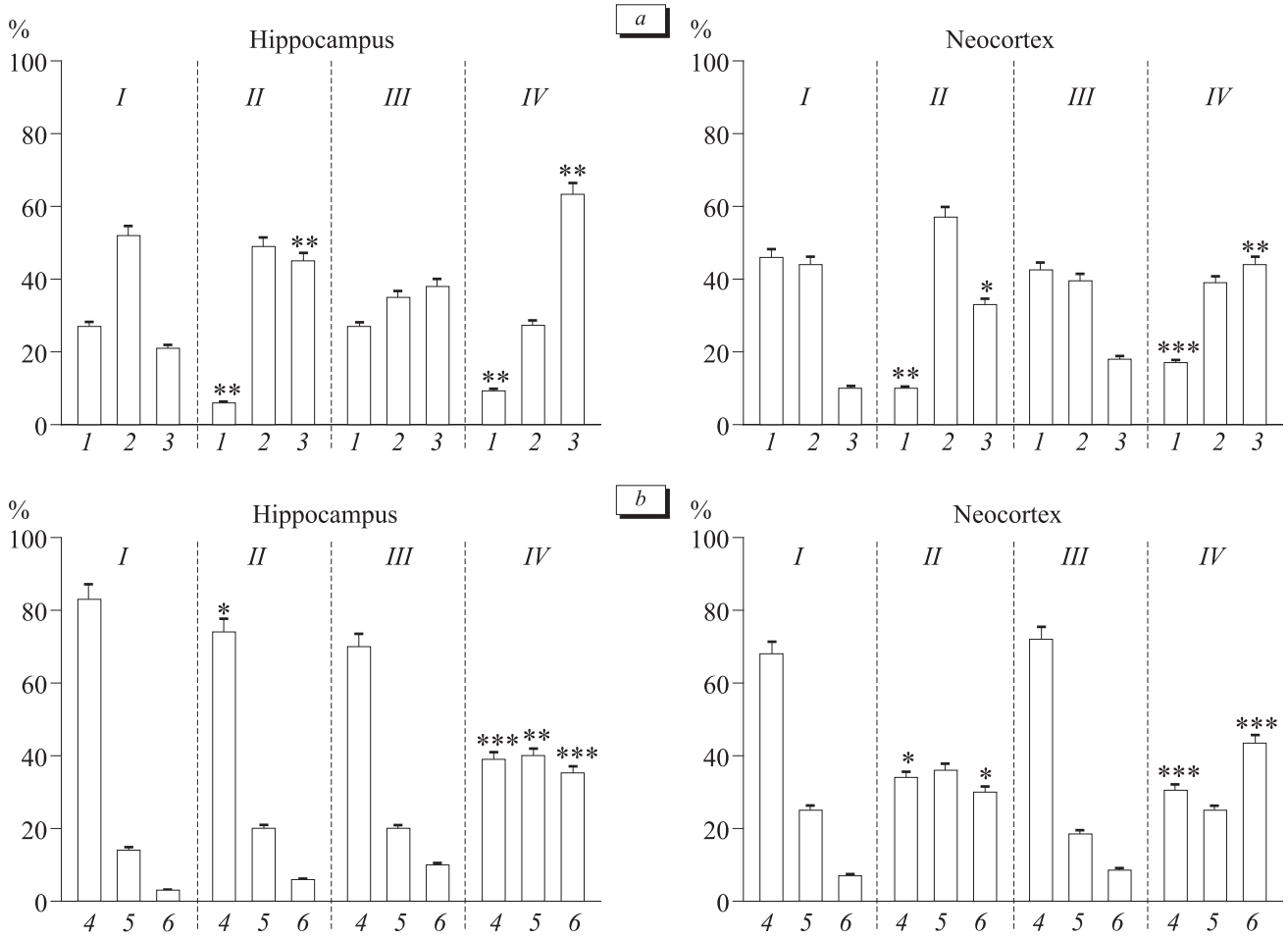

Fig. 2. Mitochondria with different lightening of the matrix (*a*) and integrity of cristae (*b*) in neurons of field CA3 in the dorsal hippocampus and layer V of the somatosensory area in the neocortex. Ordinate: number of mitochondria (% of the total number). Mitochondria with electron dense (*1*), partially lightened (*2*), and completely lightened matrix (*3*). Mitochondria with normal (*4*), partially destroyed (*5*), and completely destroyed cristae (*6*). Single administration of NaCl (*I*); single administration of Polidan (*II*); 5-fold administration of NaCl (*III*); 5-fold administration of Polidan (*IV*).

TABLE 1. Distribution of Neuron Processes in Layer V of the Somatosensory Area in the Neocortex and CA3 Field in the Dorsal Hippocampus of Rats Differing in Lightening of the Matrix (%, *M*±*m*)

Brain area, group		Profiles		
		electron dense matrix	partially lightened matrix	completely lightened matrix
Neocortex	single NaCl	12.89±1.46	55.54±2.92	31.57±2.97
	single Polidan	18.66±2.01**	50.70±2.36*	30.64±2.41*
	5-fold NaCl	15.94±1.16	48.38±1.83	35.680±2.001
	5-fold Polidan	12.80±1.19*	36.67±1.82***	50.530±2.005**
Hippocampus	single NaCl	12.42±1.40	35.51±2.18	52.07±2.29
	single Polidan	14.11±1.12*	37.22±1.58*	48.32±1.92*
	5-fold NaCl	10.96±1.02	37.20±1.55	51.84±1.61
	5-fold Polidan	7.93±0.87*	26.30±1.48*	65.77±1.61*

Note. Here and in Table 2: *p<0.05, **p<0.001, and ***p<0.000001 compared to the control (NaCl administration).

but also in the neuropil. Single treatment with the drug was followed by the appearance of new profiles of small-diameter processes in the cortex and hippocampus. The number of profiles of average-diameter processes in the neocortex decreased after 5-fold injection of Polidan. However, the number of profiles of large-diameter processes increased in the hippocampus and neocortex. It should be emphasized that changes in the neuropil observed after 5-fold injection of Polidan were not accompanied by variations in the total number of processes. However, the total number of processes increased after single treatment with Polidan (Table 1, Fig. 3). Ultrathin sections from control animals were visually similar.

Optical density of the matrix in neuronal processes in the cortex and hippocampus was modified after Polidan administration. Single injection of Polidan was followed by an increase in the number of profiles of processes with electron dense, partially lightened, and completely lightened matrix in both structures of the brain.

After 5-fold injection of Polidan the number of processes with electron dense matrix remained unchanged in the hippocampus, but decreased in the neocortex. The number of processes with partially lightened matrix decreased in the neocortex and hippocampus. These changes were accompanied by an increase in the number of processes with completely lightened matrix (Table 2).

Examination of the protein-synthesizing apparatus shows that single and 5-fold treatment with Polidan is followed by activation of synthetic processes in neurons of layer V in the somatosensory area of the neocortex and CA3 field of the dorsal hippocampus. These findings are consistent with

TABLE 2. Distribution of Neuron Processes in Layer V of the Somatosensory Area in the Neocortex and CA3 Field in the Dorsal Hippocampus of Rats Differing in the Diameter of Profiles (%, *M*±*m*)

Brain area, group	Profiles			Total number of processes in the field of view
	small diameter	mean diameter	large diameter	
Neocortex				
single NaCl	6.87±0.48	1.870±0.173	0.960±0.097	9.710±0.241
single Polidan	10.560±0.558**	1.82±0.16	1.200±0.106	13.580±0.339**
5-fold NaCl	7.420±0.316	2.590±0.139	1.270±0.067	11.280±0.168
5-fold Polidan	6.690±0.331	1.660±0.122***	1.690±0.083**	10.040±0.162
Hippocampus				
single NaCl	5.050±0.265	2.500±0.173	0.910±0.102	8.460±0.286
single Polidan	9.45±0.42***	2.925±0.192	1.010±0.098	13.380±0.405***
5-fold NaCl	6.250±0.281	2.350±0.137	1.140±0.076	9.740±0.289
5-fold Polidan	6.920±0.304	2.050±0.116	1.470±0.065*	10.440±0.309

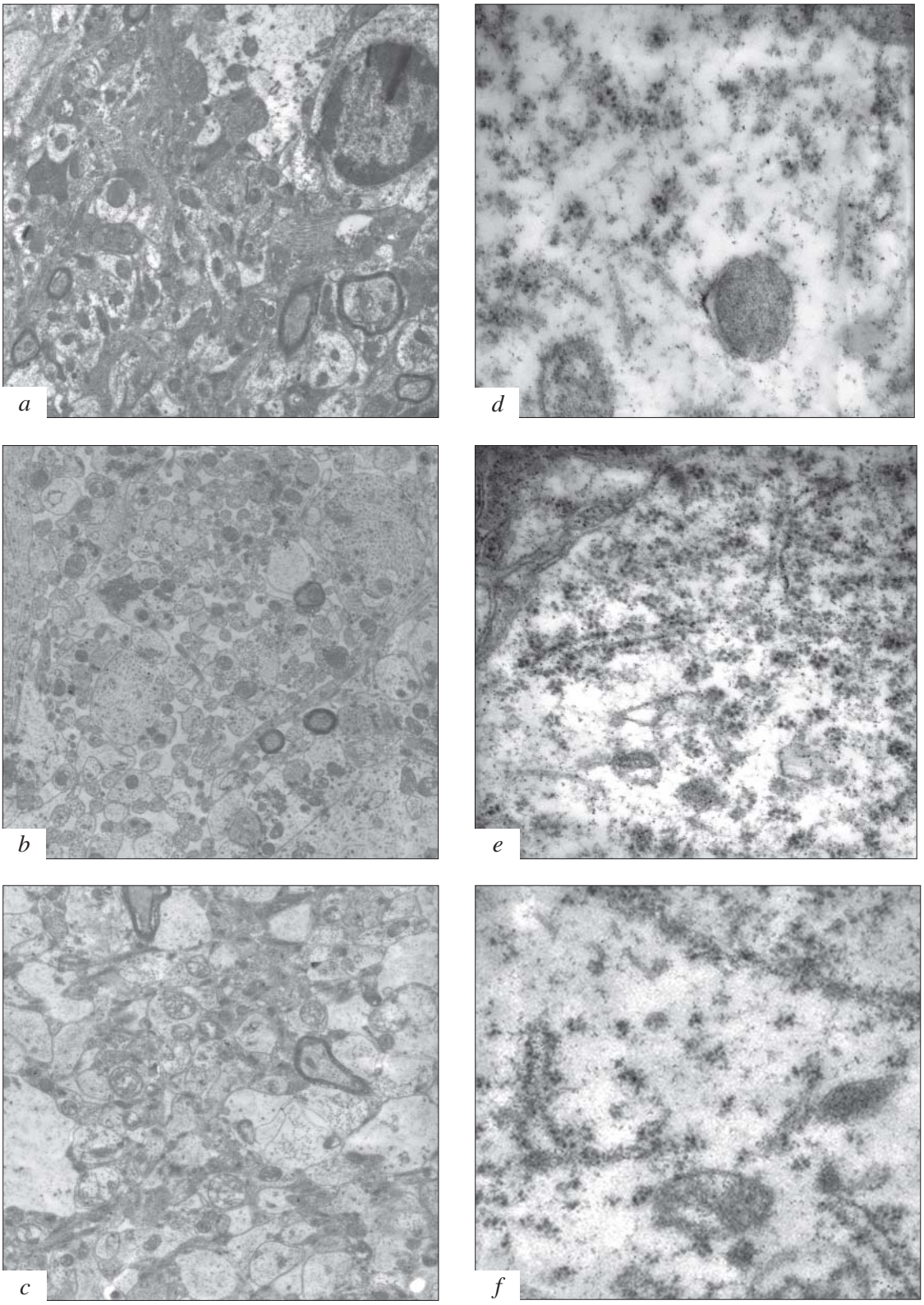

Fig. 3. Fragments of the neuropil in field CA3 of the dorsal hippocampus (*a*, *b*, *c*) and cytoplasm of pyramidal neurons in layer V of the neocortex (*d*, *e*, *f*) under control conditions (single treatment) and after single of 5-fold administration of Polidan. Control (*a*, *d*); single administration of Polidan (*b*, *e*); 5-fold administration of Polidan (*c*, *f*). ×8400 (*a*, *b*, *c*); ×52,000 (*d*, *e*, *f*).

the light microscopy data [1]. However, electron microscopy of mitochondria and neuropil showed that 5-fold injection of Polidan is followed by depletion of ultrastructures in neurons.

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