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Postnatal Organellogenesis in Pyramidal Neurons in the Cerebral Cortex in Rats

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We report here quantitative structural and histochemical evaluation of the postnatal development of organelles in neurons in the inner pyramidal layer of the frontal cortex in rat pups aged 5, 20, and 45 days form birth (four animals of each age). Growth of the perikaryon was seen during postnatal ontogeny, along with the consistent development of organelles within neurons. With age there was a progressive increase in the number of mitochondria, which became more extended in shape. Mitochondria showed progressive increases in the number and lengths of cristae. These changes were accompanied by increases in the activities of mitochondrial marker enzymes SDH and NADHDH. On day 5 after birth, the neuron cytoplasm was dominated by free ribosomes and then by rough endoplasmic reticulum. Cistern size increased progressively. Golgi complexes were not yet formed on day 5 after birth, though they were present by day 20. Lysosome numbers and size increased significantly by day 20; the activity of the lysosomal marker enzyme acid phosphatase showed a similar pattern.

Keywords: organellogenesis, pyramidal neurons, frontal cortex.

Developmental rearrangement of the cerebral cortex occurs throughout life in humans and animals, while during the growth period the body is dominated by processes of proliferation and differentiation of neural with increases in the complexity of their structure; involutionary changes occur in the elderly. The fact that the nervous system has its own structural characteristics at each age is to a certain extent due to the nature of the functional requirements imposed on it at these sages of individual body development [7]. During the postnatal period of ontogeny, there is a decrease in the density of neuron bodies, with increases in their shape and size variability. Clumps of chromatophilic substance form in the neuron cytoplasm, synthetic processes are activated in the nucleus [4], the number of nucleoli decreases [6, 7], the size of the perikaryon increases [9], and the number and length of processes increase, their branching becoming more complex [5–7, 9], and there are increases in the extent and ultrastructural complexity of organelles (endoplasmic reticulum, Golgi complex, mitochondria, and lysosomes) [7, 8, 11]. Studies at the light microscopy level revealed progressive increases in cortical thickness and pyramidal neuron size in rats at 5 and 45 days after birth, along with decreases in their density due to accelerated growth of the neuropil [1]. However, studies of the dynamics of the organelle formation in neuron perikarya in the cerebral cortex in rats during postnatal ontogeny using ultrastructural morphometry and quantitative histochemistry have been done only partially for mitochondria [11].

The aim of the present work was to carry out an ultrastructural and histochemical evaluation of postnatal organellogenesis in the bodes of neurons in the inner pyramidal layer of the frontal cortex in rats.

Materials and Methods. The study was performed on the offspring of mongrel white female rats ($n = 4$) with consideration of the "Regulations for Studies Using Experimental Animals" [3]. The study was approved by the Biomedical Ethics Committee of Grodno State Medical University (Protocol No. 1, of March 11, 2014). Animals were kept on a standard animal-house diet. Rat pups were decapitated on days 5, 20, and 45 after birth (four for each time point).

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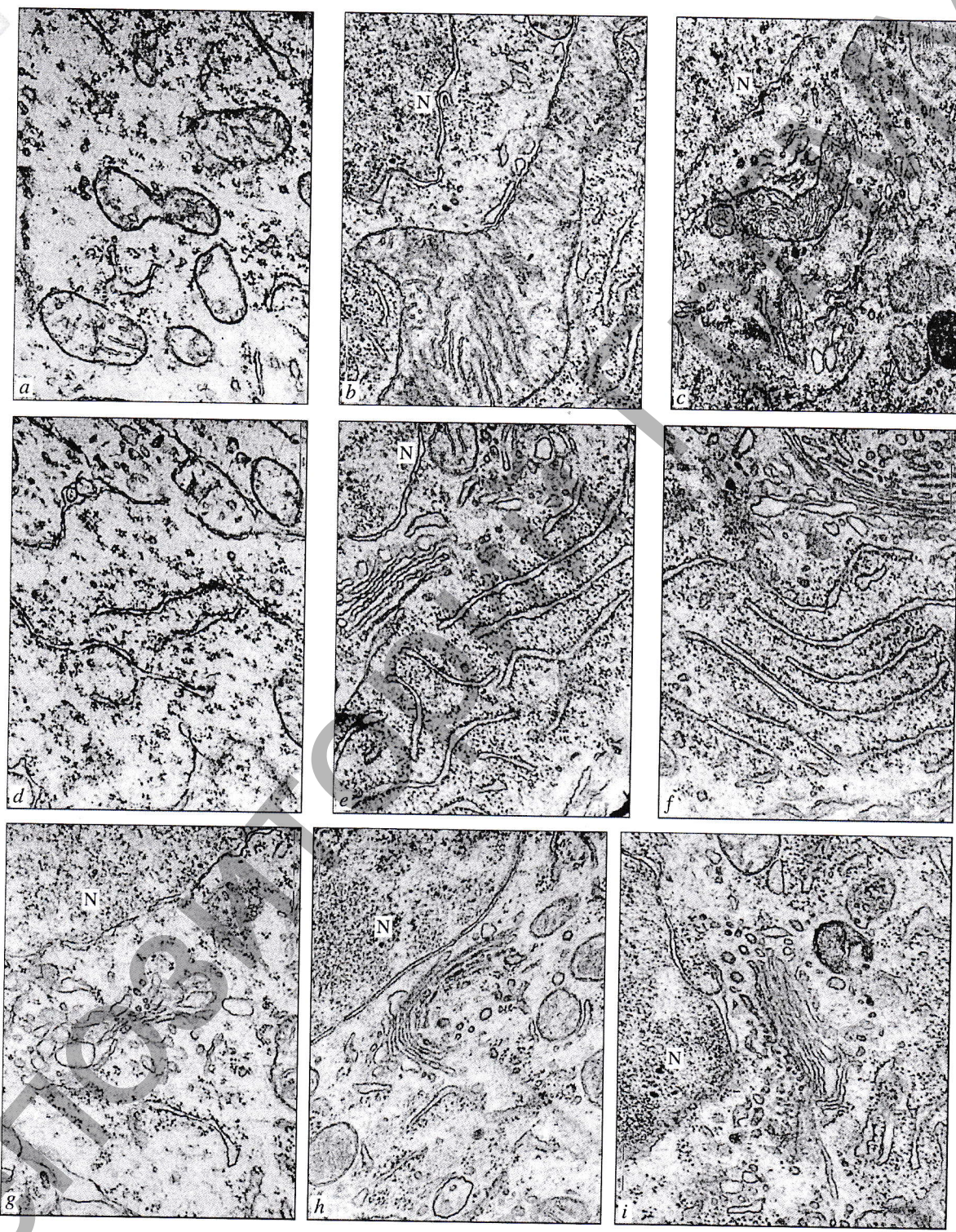

Fig. 1. Organelles in neurons in the inner pyramidal layer of the frontal cortex of the brain in rats on postnatal days 5 (*a*, *d*, *g*), 20 (*b*, *e*, *h*), and 45 (*c*, *f*, *i*). *a–c*) Mitochondria; *d–f*) rough endoplasmic reticulum; *g–i*) Golgi complex. N – nucleus. Magnification ×50000.

The frontal cortex was located on histological preparations using a stereotaxic atlas [10]. Brains were extracted rapidly after decapitation and fragments of the anterior part of the cortex were fixed in Carnaud fluid. Serial paraffin sections

TABLE 1. Ultrastructural Morphometric Measures of Neuron Organelles in Layer V of the Frontal Cortex of the Brain in Rats (Me; LQ; UQ)

Organelles	Parameter	Time after birth, days		
		5	20	45
Mitochondria	Number (per 1 μm²)	0.8 (0.6; 0.8)	1.4 (1.4; 1.6)*	1.9 (1.6; 2.2)*
	Area, μm²	0.075 (0.073; 0.08)	0.345 (0.34; 0.35)*	0.16 (0.15; 0.2)*+
	Form factor, U	0.88 (0.87; 0.89)	0.64 (0.6; 0.66)*	0.62 (0.6; 0.64)*
	Elongation factor, U	1.37 (1.26; 1.5)	4 (3; 4.5)*	2.8 (2.76; 2.82)*+
	Number of cristae per mitochondrion	4 (3; 4)	13.5 (12; 14)*	24.5 (24; 25)*+
	Total length of cristae per mitochondrion, μm	0.6 (0.45; 0.64)	3.3 (2.9; 3.6)*	4.4 (3.8; 5)*+
	Number of cristae per mitochondrion (per 1 μm²)	53 (41; 53)	39 (35; 40)	153 (125; 160)*+
	Total length of cristae in mitochondria (per 1 μm²)	8 (6; 8)	9.6 (8.8; 10)*	25 (25; 25)*+
Ribosomes	Bound (per 1 μm²)	1.6 (1.4; 1.8)*	6.8 (6; 7)*	9 (8; 9.2)*+
	Free (per 1 μm²)	13.8 (13.2; 14)	8.5 (8; 10)*	7 (6; 7.4)*+
	Total number (per 1 μm²)	15.6 (14.6; 15.8)	15.3 (14; 17)	16 (14; 16.6)
Rough endoplasmic reticulum	Extent of cisterns, μm	0.39 (0.38; 0.4)	1.1 (1; 1.1)*	2.2 (2; 2.2)*+
	Width of cisterns, μm	0.035 (0.03; 0.04)	0.1 (0.08; 0.1)*	0.071 (0.07; 0.074)*+
Golgi complex	Width of cisterns	0.15 (0.13; 0.16)	0.1 (0.08; 0.11)*	0.07 (0.06; 0.08)*
Lysosomes	Number (per 1 μm²)	0.2 (0.2; 0.2)	0.8 (0.6; 0.8)*	0.5 (0.4; 0.6)*
	Area, μm²	0.039 (0.037; 0.04)	0.1 (0.08; 0.12)*	0.1 (0.1; 0.11)*
	Form factor, U	0.88 (0.86; 0.9)	0.9 (0.87; 0.92)	0.87 (0.85; 0.9)
	Elongation factor, U	1.41 (1.4; 1.43)	1.8 (1.7; 1.9)*	1.75 (1.7; 1.8)*

Here and Table 2: *significant differences compared with postnatal day 5; +significant differences compared with postnatal day 20.

were stained with 0.1% toluidine blue by the Nissl method, the Einarsson method to detect ribonucleoproteins (RNP), or frozen in liquid nitrogen for determination of enzyme activities: succinate dehydrogenase (SDH), NADH dehydrogenase (NADHDH), and acid phosphatase (AP). Studies of histological preparations, microphotography, morphometry, and densitometry of chromogen precipitates in histological preparations were performed using an Axioscop 2 plus microscope (Zeiss, Germany), a digital video camera (LeicaDFC 320, Germany), and the image analysis program ImageWarp (Bitflow, USA).

Electron microscopy studies were performed using the required fragments of the cortex, which were placed in 1% osmium fixative in Millonig buffer pH 7.4 for 2 h at 4°C, washed in a mixture of Millonig buffer (20 ml) and sucrose (900 mg), dehydrated in increasing ethanol concentrations, a mixture of ethanol and acetone, and acetone, passed through a mixture of resin (Araldite M + Araldite H + dibutylphthalate + DMR-30) and acetone, and embedded in this embedding mixture of resins. Sections were cut on an MT-7000 ultramicrotome (RMC, USA), collected on support grids, and contrasted with uranyl acetate and lead citrate. Preparations were examined under a JEM-1011 electron microscope (JEOL, Japan) and photographed with an Olympus Mega View III digital camera (Olympus Soft Imaging Solutions, Germany). Ultrastructural morphometry was performed using the image analysis program ImageWarp (Bitflow, USA), using the cursor on the computer monitor to outline mitochondria, lysosomes, rough endoplasmic reticulum, Golgi complexes, and ribosomes, assessing their numbers, sizes, and shapes.

Mean numerical data for each animal were analyzed by nonparametric statistics in Statistica 6.0 for Windows (StatSoft Inc., USA). Descriptive statistics for each parameter included determination of the median (Me), percentile boundaries (25th, 75th), and interquartile range (IQR). Quantitative results are presented as Me – median, LQ – upper boundary of lower quartile; UQ – lower boundary of upper quartile. Differences between values at different time points in postnatal development were regarded as significant at $p < 0.05$ (Mann–Whitney U test).

Results. During postnatal ontogeny, the perikarya of neurons in the inner pyramidal layer of the cerebral cortex

TABLE 2. Activities of Enzymes in the Cytoplasm of Neurons in Layer V of the Frontal Cortex of the Rat Brain (Me; LQ; UQ), OD Units

Enzymes	Time after birth, days		
	5	20	45
Succinate dehydrogenase	–	0.14 (0.12; 0.16)	0.17 (0.16; 0.174)
NADH dehydrogenase	0.156 (0.15; 0.17)	0.2 (0.2; 0.23)*	0.24 (0.23; 0.26)*
Acid phosphatase	–	0.24 (0.22; 0.28)	0.23 (0.22; 0.236)

"–" indicates enzyme activity not determined, i.e., below detection limit.

in rats showed progressive increases in the relative content of mitochondria, by a factor of 2.5. Mitochondrial area increased five-fold from postnatal day 5 to postnatal day 20, and then decreased twofold by day 45. Mitochondria became less spherical and more extended (especially on day 20). Mitochondria showed progressive increases in the numbers and lengths of cristae (by factors of 5–7). In terms of mitochondrial area, the number of cristae was somewhat decreased on day 20 and sharply increased on day 45 (Fig. 1, Table 1). The cytoplasm of these neurons showed progressive increases in the activity of mitochondrial marker enzymes SDH and NADHDH during postnatal ontogeny (Table 2).

On postnatal day 5, the neuron cytoplasm was dominated by free ribosomes (87.5% of ribosomes). The number of free ribosomes then decreased gradually, this being accompanied by a progressive increase in the number of ribosomes bound to the rough endoplasmic reticulum. Bound ribosomes were already dominant by day 45 of postnatal development (56% of the total number) (see Fig. 1, Table 1). The extent of rough endoplasmic reticulum cisterns per unit area of cytoplasm increased progressively (by a factor of 5 from day 5 to day 45) (see Fig. 1, Table 1). The total number of ribosomes in the cytoplasm of neuron bodies showed essentially no change during postnatal ontogeny, which corresponded to the unaltered RNP content.

On postnatal day 5, Golgi complex cisterns consisted only of round vacuoles; by day 20, they had undergone a transformation to flat cisterns, which gradually became narrower (see Fig. 1, Table 1).

The relative content of lysosomes and their sizes increased significantly by day 20 of postnatal development (by a factor of 3–4), after which it showed some decrease: lysosomes became more extended (see Table 1). The cytoplasmic activity of the lysosome marker enzyme AP showed a similar change. On postnatal day 5, AP activity was very low (below the sensitivity level of the method), and reached a maximum on day 20 (Table 2).

Discussion. During postnatal ontogeny, the rat brain underwent consistent qualitative and quantitative changes in the organelles in neurons in the inner pyramidal layer of the frontal cortex. There was a progressive increase in the number of mitochondria and their areas increased sharply by day 20, after which there was some decrease. Mitochondria became more spherical in shape and more extended, and they showed progressive increases in the number and length of cristae. This was accompanied by increases in the activities of mitochondrial marker enzymes SDH (an enzyme involved in the aerobic oxidation of carbohydrates in the Krebs cycle in the neuron cytoplasm) and NADHDH (a mitochondrial enzyme involved in electron transfer, an important coupling component between the end products of carbohydrate skeleton degradation and the respiratory chain). This is evidence of a progressive increase in the functional activity of mitochondria and the supply of energy to neurons during postnatal ontogeny.

On postnatal day 5, the neuron cytoplasm was dominated by free ribosomes, which gradually bound to rough endoplasmic reticulum membranes. The extent of rough endoplasmic reticulum cisterns per unit area of cytoplasm increased progressively. The dominance of free ribosomes is evidence of an increase in protein biosynthesis for the intrinsic needs of rapidly growing neurons. Later increases in the extent of rough endoplasmic reticulum cisterns and the number of bound ribosomes are evidence of an increase in protein biosynthesis for export and transport to forming nerve endings.

On postnatal day 5, Golgi complexes were not yet formed, and consisted only of round vacuoles. They then underwent a transformation into flat cisterns, whose width gradually decreased. The relative content of lysosomes and their sizes increased significantly by postnatal day 20 and then decreased slightly with continuing postnatal ontogeny. Histochemical studies showed that the activity of the lysosome marker enzyme AP showed a similar pattern of changes [2]. These points reflect increased autophagy processes in these neurons.

Our results are entirely consistent with published data on the developmental characteristics of the ultrastructural and differentiation of organelles in the bodies of pyramidal neurons in the cerebral cortex in rats (increases in perikarya and ICD complexity of organelle structure [7, 9, 11].

Our electron microscopic and histochemical data provide the first characterization of the formation and differentiation of organelles and the development of the energy-providing and protein-synthesizing systems and the intracellular digestion and defense apparatus of neurons in the inner