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3 Effects of Antenatal Alcoholization on Brain Cortex Neurons Postnatal Development in Rats

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Abstract

The aim of the paper was to estimate histologically the consequences of alcohol consumption by rats during pregnancy on the brain cortex neurons development in their offspring. Female Wistar rats consumed a 15% solution of ethanol as a single source of drinking (4.64±2.19 g/kg/day) throughout pregnancy, control rats received aquivolume amount of water. The offspring were decapitated on the 2-, 5-, 10-, 20-, 45-, and 90th day after birth and samples of frontal brain cortex were prepared for microscopy histology, histochemistry and electron microscopy. Results: Antenatal alcohol exposure in rats increased, and then reduced the brain cortex thickness, the decrease being noted in the relative amount of brain cortex neurons and the increase in the number of their pathological forms in all time periods of the examination. Electron microscopy showed a significant reduction in the number of mitochondria per um^2 of cytoplasm and the total length of their cristae, reduction of the rough endoplasmic reticulum canal length and their clearance expansion, decrease in the bind ribosomes and increase in the free ribosomes number, expansion of the Golgi apparatus cisternae, increase in the lysosome number and size in the cytoplasm of neurons. The histochemical examination revealed the inhibition of NADH-, NADPhH, glucose-6-phosphate dehydrogenase and succinate dehydrogenases as well as activation of lactate dehydrogenase and acid phosphatase. Antenatal alcoholization led to the decrease in the expression of synaptophisin (marker of synaptogenesis) and retarded the maturation of neurons in the frontal cortex, which resulted in the increase in the expression of double cortin, and decrease in the expression of neuronal nuclear antigen. Conclusion: Alcohol consumption by rats during pregnancy induces deep and long-term histological, histochemical, immune histochemical and electron microscopy changes in the brain cortex neurons in postnatal ontogenesis in rat offspring, including early swelling and postpone shrinkage and cessation of growth of brain cortex neurons.

Key Words: Ethanol consumption, pregnancy, off spring, frontal cortex, neurons.

Introduction

Alcohol consumption during pregnancy induces the development of a number of specific disorders in offspring that are combined under the term Fetal Alcohol Syndrome (FAS), which is a part of Fetal Alcohol Spectrum Disorders (FASD) (Jones, 1973; Lemoine, 2012). According to the published data, the cerebral cortex is particularly sensitive to prenatal exposure to alcohol. Ethanol induces apoptosis, degeneration, reduction in the amount and size of brain cortex neurons, the decrease in their protein content, hypoplasia of cytoplasm, significantultrastructural abnormalities in them (Riley, Infante & Warren, 2011; Alvares. 1988; Miller, 1986; Fabriques, 1985). There is data suggesting that prenatal alcohol expose reduces the survival of neurons and disrupts their functions causing oxidative stress, DNA damage and mitochondrial dysfunction, as well as suppression of signals of insulin needed to ensure their viability, metabolism, formation of synapses and synthesis of acetylcholine (de la Monte, Wands, 2010; de la Monte, 2011; Aros, 2011). During ontogenesis alcohol induces defects in many molecular, neurochemical and cellular processes that occur during normal brain development, including disturbances of glial functions, regulation of gene expression and cell-cell interactions, increases the formation of free radicals (Alfonso-Loeches, 2011). Ethanol affects the embryonic development of the nervous system, especially the neural stem cells, destroys regulatory communications microRNA that are important for the process of maturation of neurons (Balaraman, 2012; Miranda, 2012). Prenatal alcohol exposure decreases the content of neurotrophic factors in the brain tissues of the embryo. Such changes may underlie some of CNS abnormalities related to the fetal alcohol syndrome (Heaton, 1992).

In the brain cortex of prenatall alcohol expose macaques a 60-fold increase in apoptosis was observed as compared to the controls. It can be explained by the neuropathological changes and long-term neuropsychiatric disorders in those animals (Farber, 2010).

However, systematic histological, histochemical, electron microscopic and morphometric analysis of the brain cortex neurons in dynamics of postnatal ontogenesis in animals has not been conducted.

The aim of the present study is to estimate the effect of prenatal alcohol exposure on histological, histochemical, immune histochemical and ultrastructural characteristics of frontal cortex neurons in rats at different time periods after birth.

Materials and Methods

Animals, chemicals and experimental design

25 female and 10 male Wistar rats were obtained from the breeding colony of the Grodno State Medical University. Their weight was 212±29 g. All experimental procedures complied with European Community Council Directive (86/609/EEC) for care and use of laboratory animals. Protocols were reviewed and approved by the Ethical Committee of the Grodno State Medical University (protocol No1, 11.03.2014). All efforts were made to minimize animal suffering. Rats were housed in vivarium with free access to standard laboratory food and kept under controlled environmental conditions. Rats of the experimental groups throughout pregnancy (from the day of detection of sperms in vaginal smears till delivery) received a 15% solution of ethanol as a single source of drinking, and the animals of the control group – equivolume amount of water. The average consumption of alcohol was 3.64±2.2 g/kg/day. The offspring brains of the control and alcohol groups were examined on the 2-, 5-, 10-, 20-, 45-, 90th days after birth.

All the chemicals were obtained from Sigma-Aldrich (USA).

Histology

6 controls and 6 alcoholizedrats (1 rat pap from each litter for every time period after birth) were decapitated and their brain was removed. Starting from day 45, when the sex of the animal can be distinguished, 3 males and 3 females rats from each group were elected.Samples of the brain cortex were fixed in the mixture of alcohol, chloroform and acetic acid in the ratio 6:3:1, then treated with alcohol and xilen and embedded in paraffin. 7 µm sagittal sections of the brain cortex were prepared using microtome (Leica RM2125, Germany). They were stained with 0.1% solution of thionine (the Nissl method) to assess general cytology of neurons.

Histochemistry

Pieces of brain cortex were then obtained, frozen and stored in liquid nitrogen for further analysis. 10 µm serial sagittal sections of the frozen frontal cortex were prepared using cryostat (Leica CM 1840, Germany). The activity of the oxidizing enzymes, such as succinate dehydrogenase (SDH, EC 1.3.99.1), lactate dehydrogenase (LDH, EC 1.1.1.27), glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49), NADH dehydrogenase (NADHDH, EC, 1.1.1.49) and NADPhH dehydrogenase (NADPhDH, EC, 1.6.1.1), as well as the activity of marker enzyme lysosomal acid phosphatase (AP, EC 1.4.3.4) were examined (Pearse, 1960). For a short time, for the enzyme histochemistry the cryostat sections were placed into the corresponding incubation medium, including the buffer, substrate, co-factor, if necessary, and chromogen to visualize the location of enzymatic activity, for 30 min – 5 hours, then washed and embedded in the suitable plastic medium.

Immunohistochemistry

Samples of the brain cortex were fixed in zinc-formalin at +4°C (overnight) and then embedded in paraffin. Anmicrotome (LeicaRM 2125 RTS, Germany) was used to cutsections. For immunohistochemical detection of synaptophisin (SF) we used primary polyclonal rabbit antibody Novex; for doublecortin (DCX) and neuronal nuclear antigen (NeuN) detection we used primary polyclonal rabbit antibodyAbcamab.18723 and ab.128886 (diluted 1: 400, at + 4 °C, 20 hours in a humidified chamber). Bound primary antibodies were detected using a set EXPOSE Rabbit specific HRP / DAB detection IHC kit Novex or Abcam. Neighboring sections were stained by the Nissl technique.

Light microscopy and morphometry

For the identification of frontal cortex in the brain sections the stereotaxic atlas was used (Paxinos&Watson, 2007). The examination of histological preparations, their microphotography and morphometry was carried out using microscope Axioskop 2 plus (Zeiss, Germany) equipped with digital camera (Leica DFC 320, Germany) and computer image analysis software Image Warp (Bit Flow, USA). In preparations stained by the Nissl method all visible neurons of the 5thlayer were estimated according to their type of chromatophilia (the intensity of staining of neurons cytoplasm) and divided into normochromic (normal, medium staining), hyperchromic (intense staining), hyperchromic shrinkage, hypochromic (pale staining) and cell-shadows (very pale remnants of dead neurons).Three sections of the frontal cortex brain for three fields of vision for each slice were taken. Five measurements were carried out on the thickness of the frontal cortex. Ten neurons of the 5thlayer of the frontal cortex were measured in every field of vision.

To estimate the size and shape of neuronal bodies the images of up to 30 neurons bodies on the computer monitor were outlined by mouse cursor. Maximal and minimal diameter (D), perimeter (P), square (S), as well as form-factor ($4\pi S/P2$ –parameter of sphericity and folding) and factor of elongation (maximal D/minimal D – parameter of sphericity) were calculated.

The enzyme activities or product of immune histochemical reactions were determined in cytoplasm of neurons on the optic density of chromogen obtained in the course of histochemical reactions.

Electron microscopy

For electron microscopy the small pieces of frontal brain cortex sections were taken, fixed in 1% OsO4, dehydrated and embedded in epoxy resin. An MT-7000 ultramicrotome (RMC, USA) was used for sectening. They were contrasted with uranyl acetate and lead citrate, examined using a JEM-1011 electron microscope (JEOL, Japan), and photographed with a digital camera Olympus MegaViewIII (Olympus Soft Imaging Solutions, Germany). The images of the mitochondria, lysosomes, rough endoplasmic reticulum canal, ribosomes and Golgi apparatus on the computer monitor were outlined by mouse cursor. The numbers, sizes, and shapes of these organelles were evaluated.

Statistics

The mean values obtained for every animal were processed with nonparametric statistics (because of the small number of animals in the groups) using software STATISTICA 6.0 (Stat Soft, Inc., USA). In descriptive statistics, the values of median (Me) and interquartile range (IQR) were determined. The differences were considered significant at $p<0.05$ (Mann-Whitney U-test), because it was not a normal distribution

Results

Histology

Prenatal alcohol exposure influenced the postnatal thickness of frontal brain cortex (Fig. 1). On the 2nd and 5th days after birth the cortex was thicker, as compared to controls. On the 10th postnatal day it became significantly thinner than in controls, on the 20th and 45th days the difference disappeared, but on the 90th postnatal day it became significantly thinner again (Fig. 1).

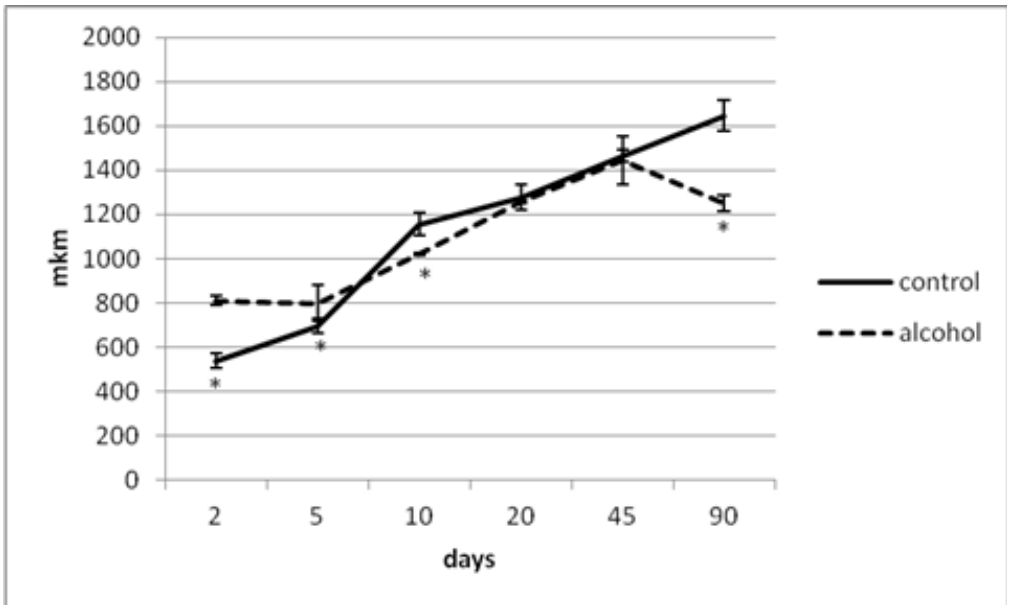

Figure 1: The thickness of the frontal cortex in rats at different time periods after birth. Data are presented as median ± interquartile range; * – $p < 0.05$, as compared to controls.

In postnatal ontogenesis (from 2 to 90 days) the density of distribution (amount per area unit) of the 5th layer neurons is regularly decreased in both groups (Fig. 2). However, during all periods of the study in the cerebral cortex of antenatally alcoholized rats a significantly lower (10-25%) amount of neurons per unit area of the section was found (Fig. 2).

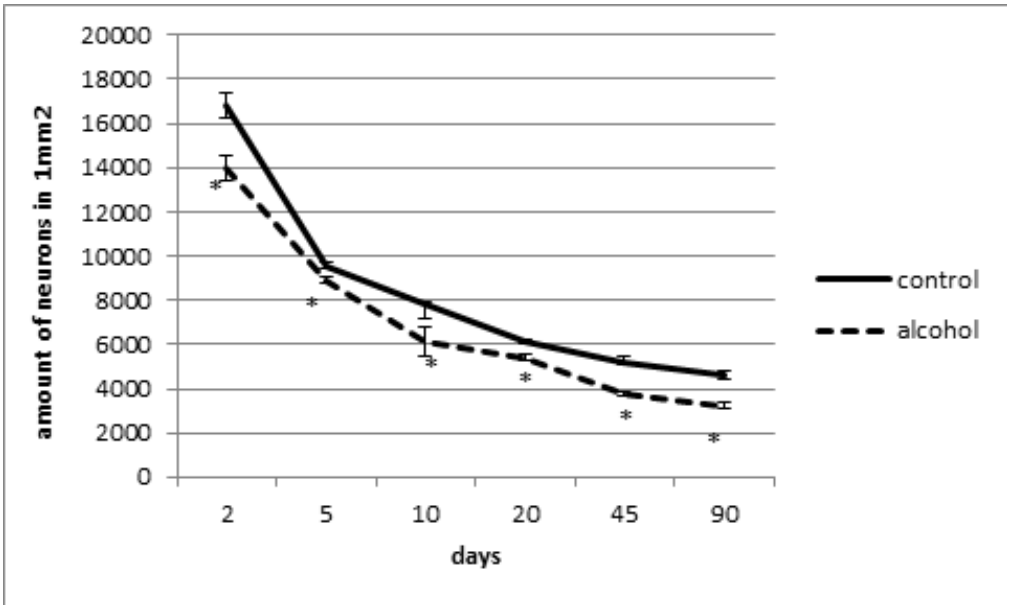

Figure 2: The density of distribution of the 5th layer neurons of the frontal cortex during postnatal ontogenesis. Data are presented as median ± interquartile range; * – $p < 0.05$, as compared to controls.

In control animals the size of the 5th layer frontal cortex neuron bodies progressively increased (4-fold) from the 2nd to the 90th postnatal day(Fig. 3) In prenatally alcoholized rats a temporary increase in the area of those neurons on the 2nd day was found. However, starting from the 20th postnatal day the area of the neuron bodies became significantly lower as compared to controls. While the size of neurons of control animals showed continuing progressive increase, in rats exposed to alcohol prenatally following the 10th postnatal day the neurons stopped their growing (Fig. 3).

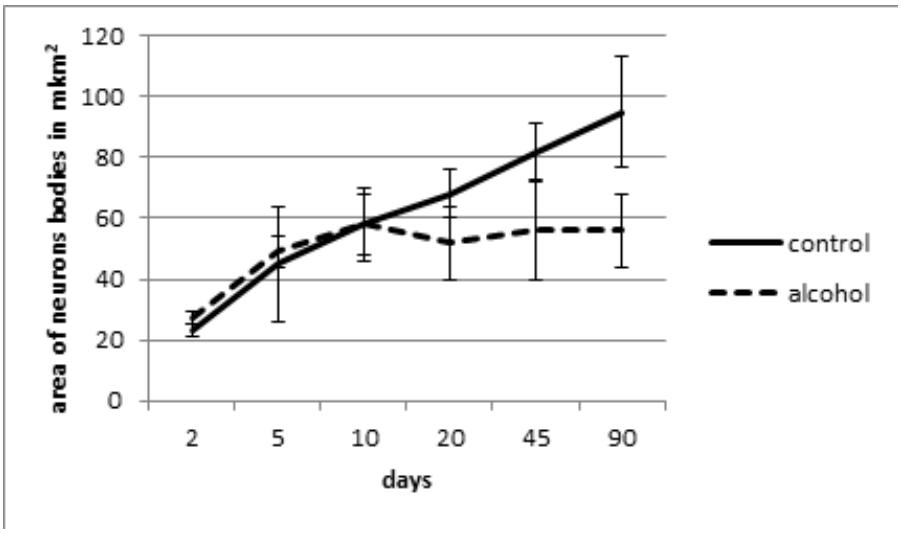

Figure 3: Dynamics of the area of perikaryon of the 5th layer neurons in the frontal cortex of rats. Data are presented as median ± interquartile range; * – $p < 0.05$, as compared to controls.

In control animals during all periods of postnatal development a normochromic neurons in preparations of frontal brain cortex prevailed (60-70%) (Fig. 4 A, 5). In prenatally alcoholized rats at all time periods of postnatal ontogenesis the number of normochromic neurons decreased significantly and the number of abnormal neurons (hyper-, hypochromic neurons and cell-shadows) increased (Fig.4 A, 5). The greatest changes were found between the 20nd and 90th postnatal days. For example, on the 90th day in the frontal cortex of prenatally alcoholized rats the amount of normochromic neurons was 2 times lower, the amount of shrinkage of hyperchromatic cells, hypochromic and cell-shadows was higher (by 66, 20 and 40 % accordingly) as compared to controls (Fig. 4, 5). The amount of shrinking hyperchromic neurons in rats exposed to alcohol increased dramatically after the 10th postnatal day and reached the maximum on the 45th and 90th postnatal days (Fig. 6). It is associated with the changes in neurons shape: increase in their elongation and decrease in form factor (sphericity).There is a negative correlation between the size of neurons and the number of shrinked neurons between the 20th and 90th days of age ($r=-0.87$—0.98; $p<0.05$).

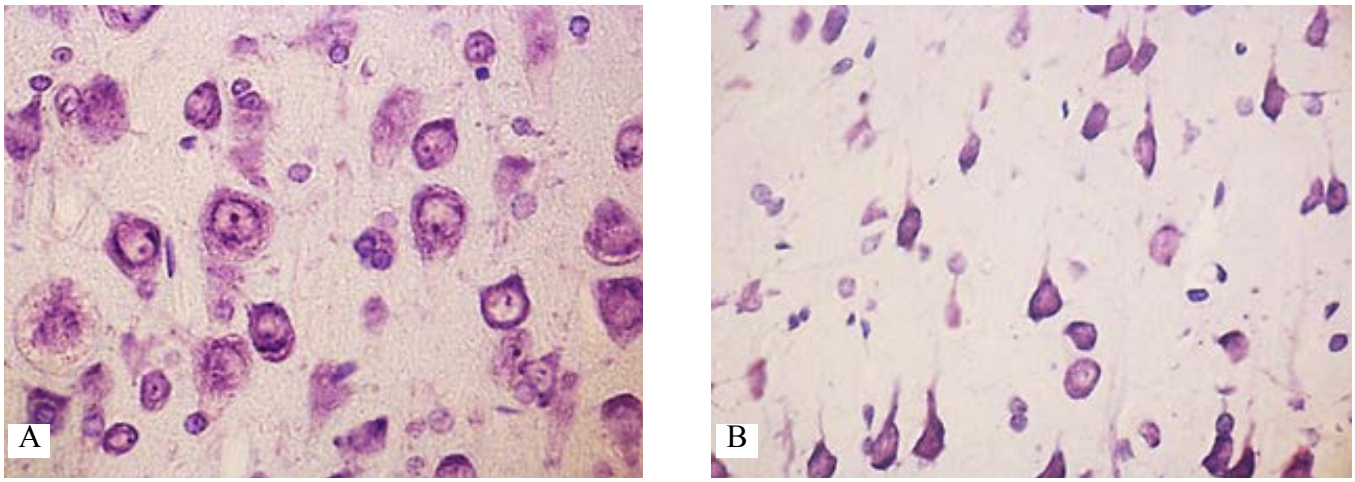

Figure 4: The 5th layer frontal cortex neurons on the 90th postnatal day in controls (A) and antenatally alcoholized rats (B).The arrow shows the shrinkage of hyperchromatic neurons. Stained by the Nissl method. Digital microphotography. Scale bars- 20 μm, magnifications- 40x.

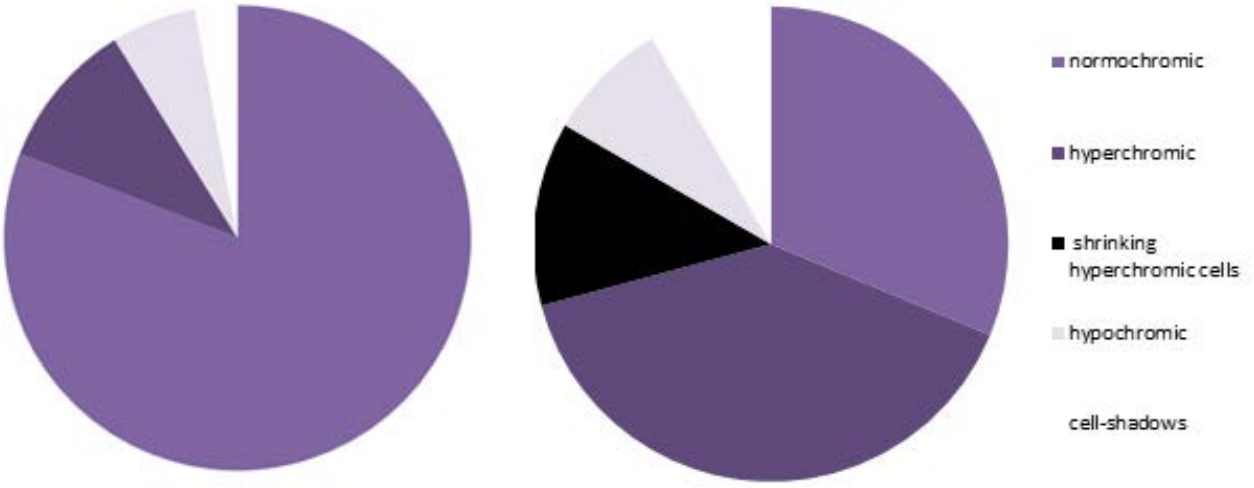

Figure 5: The percentage of neurons with different chromatophilia of cytoplasm in the frontal cortex 90-day-old rats, %.

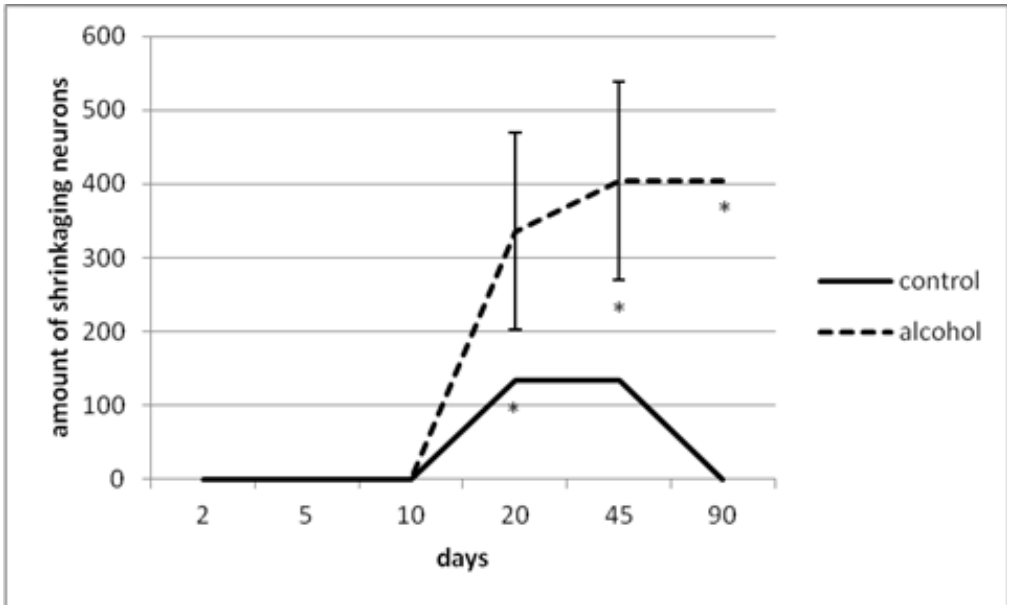

Figure 6: The amount of hyperchromic shrinkage neurons in the 5th layer frontal cortex of rats. Data is presented as median ± inter-quartile range; * – $p < 0.05$, as compared to controls.

Electron microscopy

Under lower magnification of electron microscope shrinking hyperchromic neurons looks much smaller and darker as compared to normal neurons in control rats (Fig. 7A, B). Its nucleus and plasma membrane are significantly folded. In their cytoplasm there are areas with homogeneous osmeofil content.

The mitochondria are swollen, with disrupted cristae. The number of mitochondria per unit area of cytoplasm on the 20th and 45th day issignificantly less than in controls. The mitochondria become more spherical and less elongated, and show the decrease in the number and length of cristae(Figure 7D, Table 1).

The total number of ribosomes per unit area of the cytoplasm of neurons after antenatal alcoholization was slightly higher than in the controls on the 5th day after birth (Table 1). In alcoholized rats the number of free ribosomes significantly increased, but the number of bind ribosomes decreased. The ratio of free and bind ribosomes in cytoplasm increased10 times (Fig. 7 E, F, Table 1).On the45thday after birth RER cisterns were widening and their length was significantly reduced (Figure 7 E, F, Table 1). The Golgi complex was greatly enhanced (Table 1).In some neurons the Golgi cisterns were located concentrically forming unusual cycles. Prenatal alcohol exposure increased the amount and size of lysosomes (Table 1).

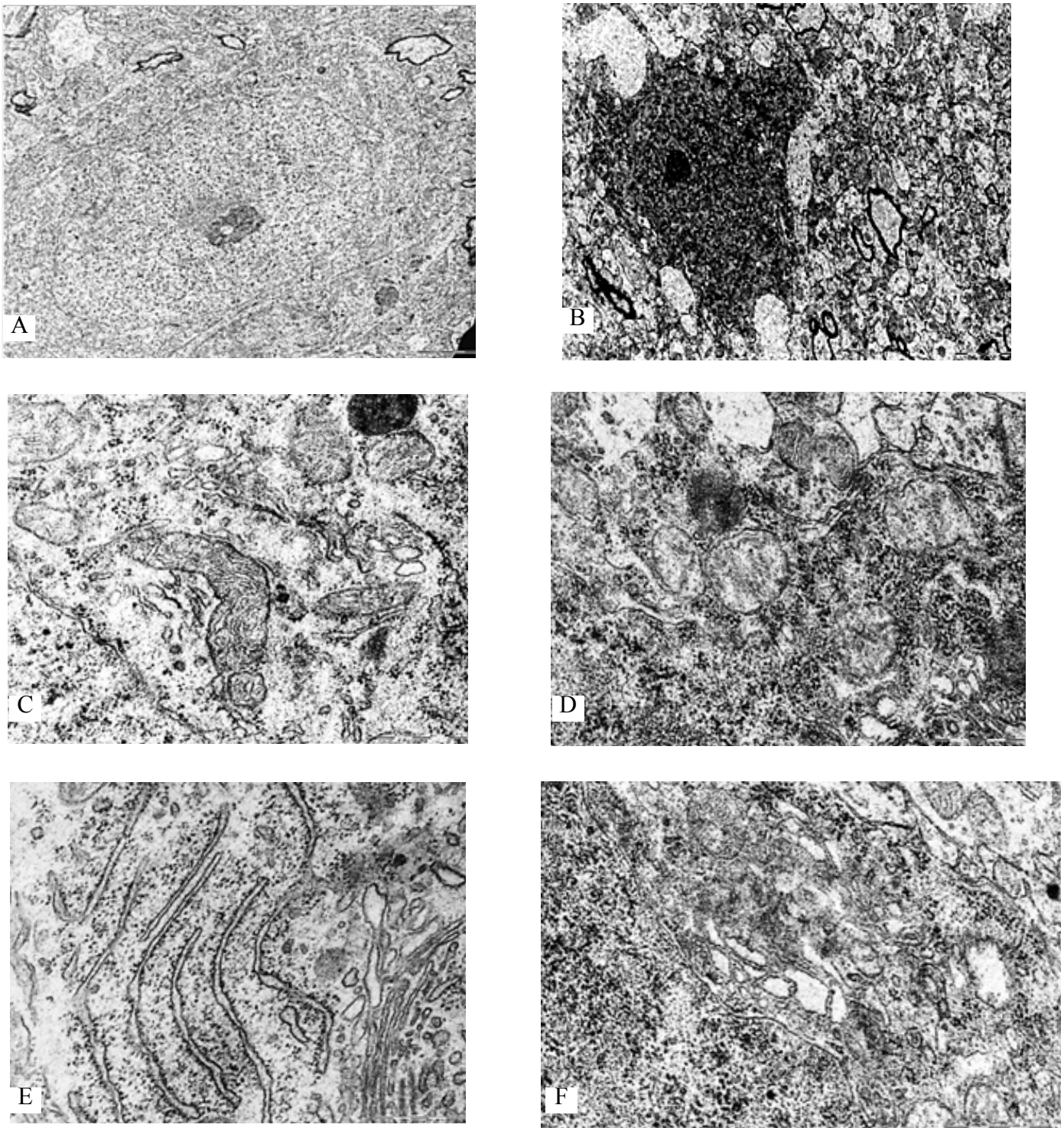

Figure7: A 5thlayer frontal cortex neurons on the 45th postnatal day in controls (A,C, E) and antenatally alcoholized rats (B,D, F).M– mitochondria, RER – rough endoplasmic reticulum, N –nucleus, L –lysosomes. Magnification: A, B – 8000; C, D, E, F – 50,000: scale segment: 0.5 μm. Electron micrographs.

Table 1: The morphometric analysis of pyramidal neurons organelles of the frontal cortex 5th layer.

Index		5th day		20th day		45th day	
		control	alcohol	control	alcohol	control	alcohol
Mitochondria	Number in 1 μm 2	0,8(0,6;0,8)	0,8(0,8;1)	1,4 (1,4;1,6)	1,1(1;1,2)*	1,9 (1,6;2,2)	1,2(1;1,2)*
	Area, μm 2	0,07(0,07;0,08)	0,076(0,07;0,08)	0,34(0,3;0,35)	0,3 (0,29;0,34)	0,16 (0,15;0,2)	0,19(0,17;0,19)
	Circularity, unit	0,88(0,87;0,89)	0,9(0,89;0,9)	0,64(0,6;0,66)	0,82 (0,8;0,9)*	0,62(0,6;0,64)	0,7(0,68;0,8)*
	Aspect, unit	1,37(1,26;1,5)	1,32(1,3;1,6)	4(3;4,5)	3(2,6;3,5)*	2,8(2,76;2,82)	2,6(2,4;2,7)*
	Number of cristaes in 1 μm 2	53(41;53)	53(27;75)	39(35;40)	27(21;28)*	153(125;160)	63(29;79)*
	Cristaes length in 1 μm 2	8(6;8)	8(4;11)	9,6(8,8;10)*	6(5;7)*	25(25;25)	13(6;16)*
	Area in cytoplasm, %	6(4;6,4)	6(5,9;8)	50(48;60)	30(29;40)*	30(24;40)	20(17;23)*
ribosomes	Number in 1 μm 2	15,6(14,6;16)	16,4(16;17,6)*	15,3(14;17)	16(14;18)	16(14;17)	16(14;17)
	Free, in 1 μm 2	13,8(13;14)	15(14,8;16)*	8,5(8;10)	14(13;15)*	7(6;7,4)	15(13;15)*
	Binding in 1 μm2	1,6(1,4;1,8)	1,4(1,2;1,4)*	6,8 (6;7)	2(1,2;2,2)*	9 (8;9,2)	1,7(1,2;2)*
RER	Length of cisterns, μm	0,39(0,38;0,4)	0,43(0,4;0,6)*	1,1(1;1,1)	1,1(1;1,1)	2,2 (2;2,2)	1,5(1;2)*
	Width of cisterns, μm	0,035(0,03;0,04)	0,04(0,03;0,05)	0,1 (0,08;0,1)	0,1 (0,09;0,11)	0,07(0,07;0,074)	0,15 (0,1;0,2)*
Golgi complex	Width of cisterns, μm	0,15(0,13;0,17)	0,15(0,14;0,17)	0,1(0,08;0,11)	0,13(0,12;0,14)*	0,07(0,06;0,08)	0,15(0,12;0,17)*
lysosomes	Number in 1 μm2	0,2(0,2;0,2)	0,4(0,4;0,4)	0,8(0,6;0,8)	1,2(1;1,4)*	0,5(0,4;0,6)	1(0,8;1,2)*
	Area, μm2	0,04(0,037;0,04)	0,045(0,04;0,05)	0,1 (0,08;0,12)	0,16(0,16;0,17)*	0,1 (0,1;0,11)	0,16(0,16;0,17)*
	Area in cytoplasm, %	0,78(0, 74;0, 8)	1,8(1,5;2)	8(5;10)	19(16;20)*	5(4;7)	16(13;20)*

Data is presented as Me (LQ; UQ); * – $p < 0.05$, as compared to controls.

Histochemistry

Histochemical investigation of frontal brain cortex of rats following prenatal alcohol exposure demonstrated the inhibition of NADH-, NADPhH, glucose-6-phosphate and succinate dehydrogenases and activation of lactate dehydrogenase and acid phosphatase in cytoplasm of 5th layer pyramidal neurons (Fig. 8, 9).

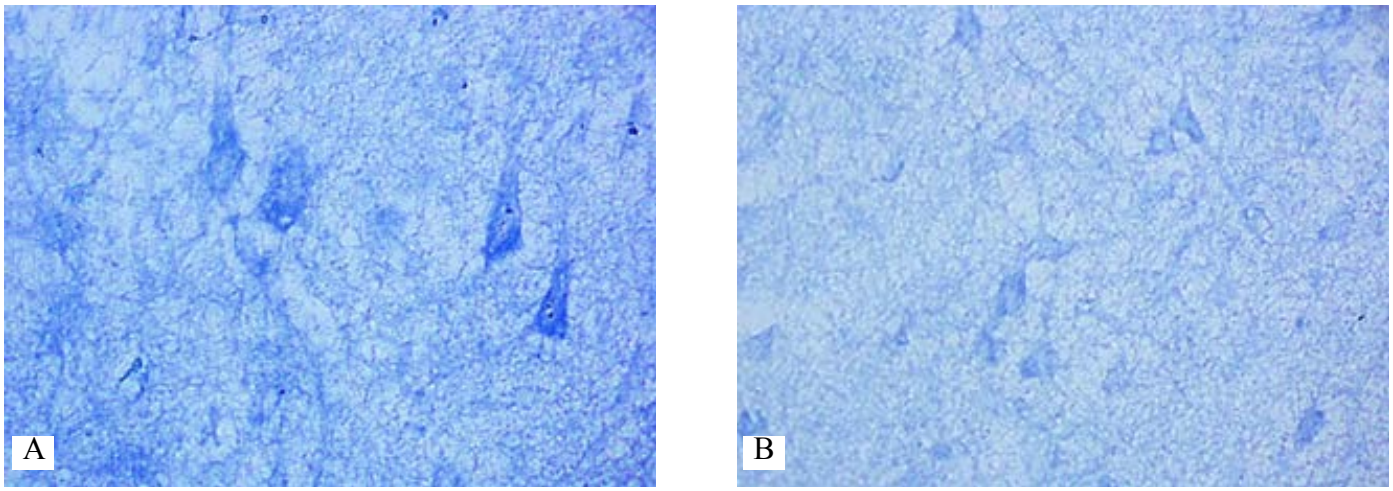

Figure8: Activity of NADH- dehydrogenase in the 5th layer frontal cortex neurons on the 45th postnatal day in controls (A) and antenatally alcoholized rats (B).Digital microphotography. Scale bars - 20 μm, magnifications – 40x.

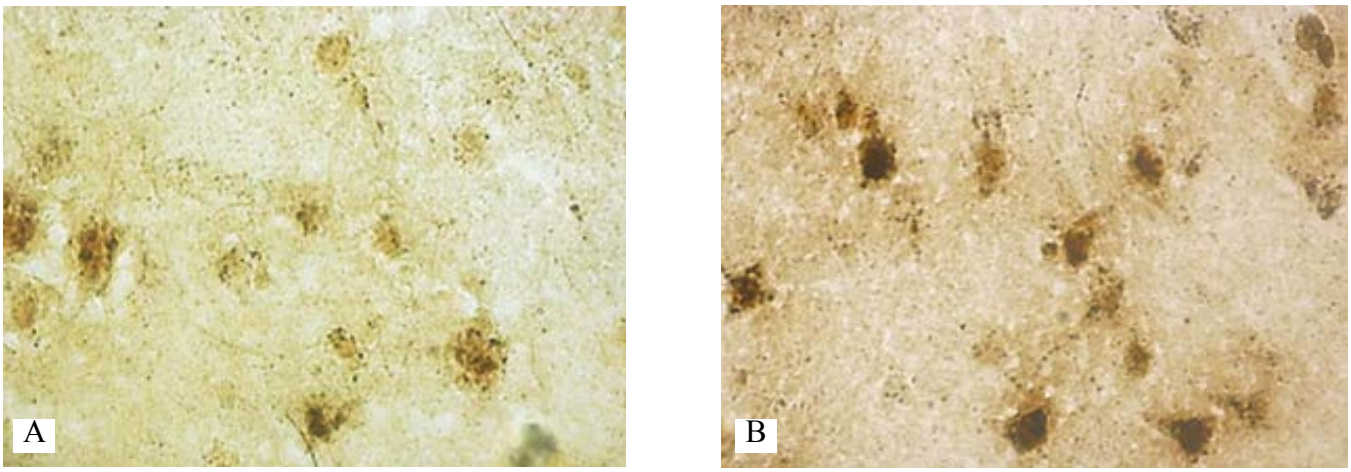

Figure 9: Activity ofacid phosphatase in the 5th layer frontal cortex neurons on the 45th postnatal day in controls (A) and antenatally alcoholized rats (B) Digital microphotography. Scale bars – 20 µm, magnifications – 40x.

Immunohistochemistry

Antenatal alcoholization dramatically decreased of synaptophys in (SF) expression in the frontal brain cortex 5th layer neuropile (Fig. 10, Table 2).

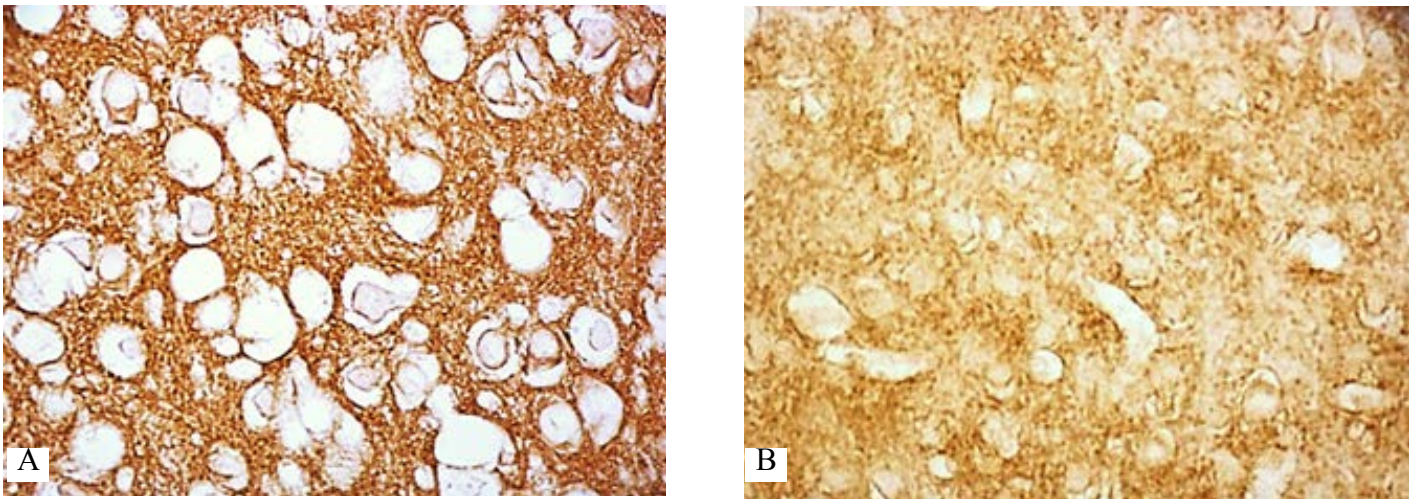

Figure 10: Expression of SF in the 5th layer frontal cortex neuropil on the 45th postnatal day in controls (A) and antenatally alcoholized rats (B).Digital microphotography. Scale bars - 20 µm, magnifications: - 40x.

Table 2: Expression of SF in the 5th layer frontal cortex neuropil.

postnatal day	control	alcohol
5	0,15±0,008	0,13±0,02*
10	0,165±0,02	0,163±0,02
20	0,28±0,02	0,22±0,01*
45	0,33±0,02	0,29±0,02*

Data are presented as Me±IUQ); * – $p < 0.05$, as compared to controls.

Antenatal alcoholization retarded the decrease in doublecortin (DCX) expression, and increased neuronal nuclear antigen (NeuN) expression in developing (5-20-th postnatal days) 5th layer frontal cortex neurons (Fig. 11, Table 3).

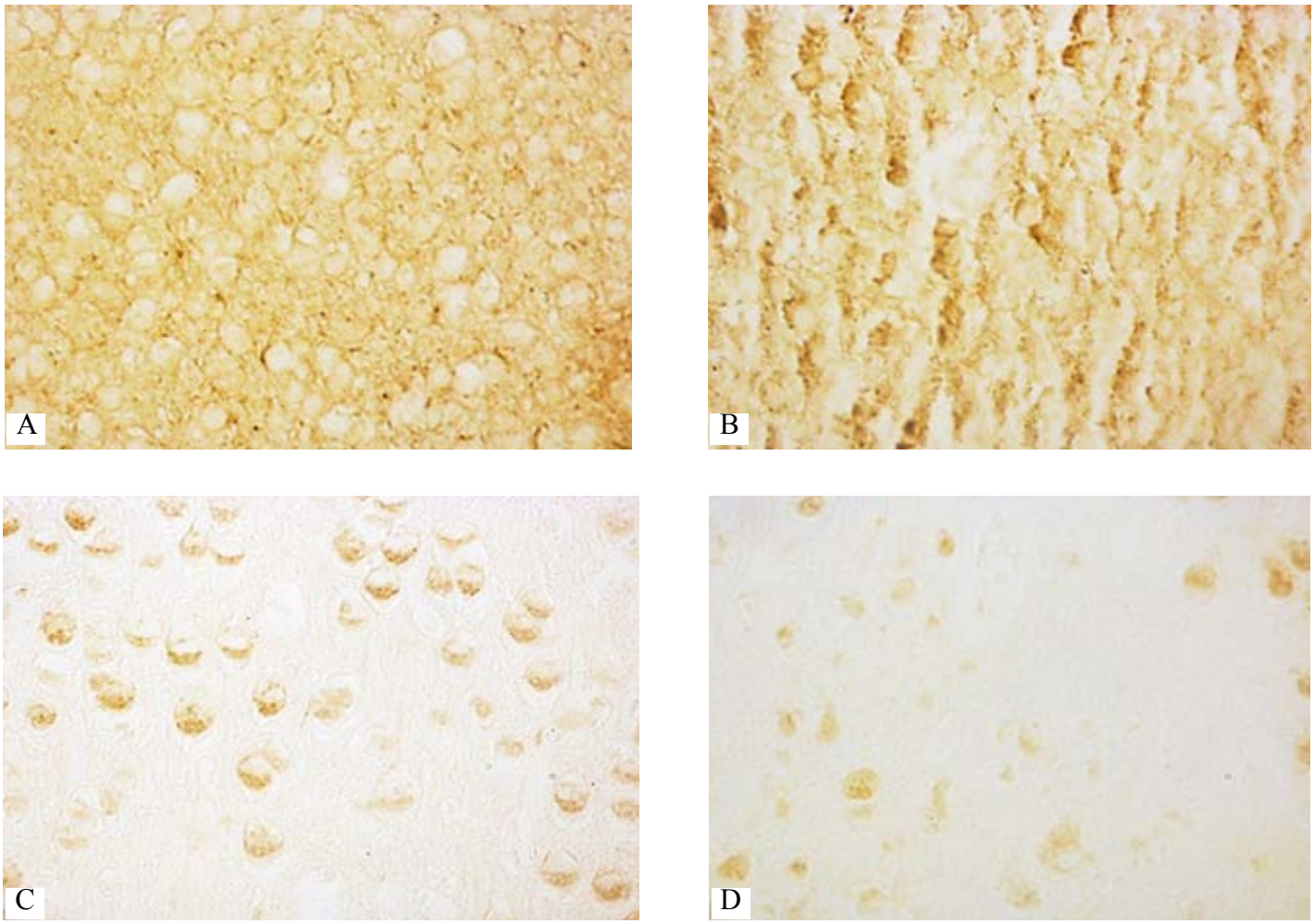

Figure 11: Expression of DCX (A,B), NeuN(C,D) in the 5th layer frontal cortex neurons on the 5th (A,B) and 20th (C, D) postnatal day in controls (A,C) and antenatally alcoholized rats (B,D). Digital microphotography. Scale bars - 20μm, magnifications: - 40x.

Table 3: Expression of DCX and NeuNin 5th layer frontal cortex neurons.

postnatal day	DCX		NeuN	
	control	alcohol	control	alcohol
5	0,12±0,006	0,21±0,006*	-	-
20	-	-	0,20±0,007	0,17±0,009*

Data are presented as Me±IUQ); * – $p < 0.05$, as compared to controls.

Discussion

The temporary thickening of the brain cortex on the 2nd and 5th postnatal days in offspring of rats who consumed alcohol during pregnancy, as compared to controls, may be associated with swelling of the cortex, which is confirmed by the pattern of perivascular edema visible in histological preparations. At that time period the highest correlation between thickness of the cortex and area of pyramidal neurons bodies was found ($r=-0.81$; $p<0.01$). A postpone reduction of the brain cortex thickness on the 90th postnatal day in antenatallyalcoholized rats can be associated with the decrease in size and shrinkage of brain cortex neurons. There was a positive correlation between the cortex thickness and size of neurons ($r=0.78$-0.96; $p<0.05$), and a negative correlation between the cortex thickness and the amount of hyperchromic shrinkage neurons ($r=-0.89$-0.94; $p<0.01$) in all periods of postnatal ontogenesis in rats prenatally exposed to ethanol.

A permanently lower amount of brain cortex neurons in all postnatal time periods studied may be due to the death of a part of neurons under the influence of alcohol during embryogenesis. Accordingly, this neurons deficiency in the brain cortex is permanent throughout postnatal development. As it is known, the weight of the brain cortex in offspring of rats exposed to alcohol during pregnancy is reduced, it becomes thinner and contains fewer neurons and glia (at G13 and G21) (Miller, 1993). These findings contradict the results of MRI showing thickening of the cerebral cortex in children and adolescents with fetal alcohol syndrome (Fernández-Jaén, 2011). Our study revealed an increased (on the 2nd, 5th postnatal days) and decrease (on the 90th postnatal day) brain cortex thickness. To explain these facts, we can assume that swelling of the gray matter occurs following antenatal alcohol exposure that is detected as a thickening of the cortex.

We observed a cessation of the growth and shrinkage of pyramidal brain cortex neurons following the 10th days of postnatal development. We cannot directly explain those phenomena. Antenatal alcoholization seems to break the normal program of postnatal development of brain cortex neurons. We found the increase in the amount of abnormal forms of the surviving neurons at all study time periods. Decrease in the number of normochromic neurons and increase in the number of pathological forms of neurons (hyper-, hypochromic neurons, shrinking hyperchromic neurons and cells shadows) in the 5th layer of the cerebral cortex was found. These different forms of abnormal neurons at the electron microscope level had disturbances in ultrastructure. Thus, hyperchromic neurons had much more free ribosomes, which ensures their hyperchromy by the Nissl method staining; the histochemical study demonstrates a higher content of RNA in those neurons cytoplasm. This testifies to the intensive biosynthesis of proteins for the own needs of neurons. Perhaps this is a way of adaptation of brain cortex neurons to compensate the depth (10-25%) as a result of antenatal alcoholization. In hyperchromicshrinking neuronsa higher electron density of hyaloplasm was found. Probably a shrinkage of the hyperchromic neurons may be seen as a failure of adaptation that leads to their subsequent death.

On the ultrastructural level, a brain cortex neurons of prenatally alcoholizedrats displayed both destructive and compensatory adaptive changes. The destructive changes in mitochondria (some of them were swelling, and showing the decrease in the density of cristae) corresponding to the decrease inactivity of their marker enzymes, succinate and NADH-dehydrogenases, in the cytoplasm of neurons at the light microscopic level. The hyperplasia of free ribosomes and hypertrophyof Golgi complex may reflect an activation of the synthetic processes in those neurons for their own needs for compensation of the damaged or lost structures to survive following the alcohol action. A reduction of bind ribosome number and RER canals indicate a decreased of protein biosynthesis for export to nerve terminals. That can disturb a functions of neurons. The significant increase in the amount and size of lysosomes in neurons accompanied by an activation of the lysosomal marker enzyme acid phosphatase (at light microscopic level) may reflect the increased autophagy for the removal of alcohol-damaged microstructures. Thus, antenatal alcoholization causes profound and diverse ultrastructural changes in the frontal cortex neurons of rat brain, which correspond to the structural and histochemical abnormalities in them at the light microscope level.

The activity of the marker aerobic oxidative enzymes (SDH, NADHDH and NADPhHDH) in brain cortex neurons cytoplasm decreased, but the marker enzyme of anaerobic glycolysis, lactate dehydrogenase increased. It indicates probably both the disturbances of energy metabolism and metabolic adaptation of neurons to alcohol, taking into consideration the alcohol-induced hypoxia.

It is known that dablcortin (Doublecortin, DCX) –a protein associated with microtubules, expresses immature neurons when they are migrating in the respective layers of the cerebral cortex and in the processes of neurons growing (Vellema, 2014).The protein NeuN (neuronalnuclearantigen) is located in the nucleus and perinuclear cytoplasm only in mature neurons(Mullen, 1992).Synaptophysin (SF) is a transmembrane glycoprotein of the presynaptic neurons membrane. SF basic functions associated with the formation of synaptic vesicles, the release of these neurotransmitters and synaptogenesis (Thiele, 2000). Immuno histochemical investigation demonstrated a disturbances of those markers, which indicates an abnormalities in the synaptogenesis and the maturation of those frontal cortex neurons after prenatal alcoholization.

Identified morphological and metabolic disturbances in the brain cortex neurons may underlie the known neurological and behavioral disorders in animals and human after antenatal alcohol exposure (Mattson, 1997).

In conclusion, alcohol consumption during pregnancy in rats induces deep long-term and irreversible structural and metabolic disturbances in the frontal cortex neurons in offspring.

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