УДК [612.825:612.821.44]:618.33]-092.9 **doi: 10.25298/2221-8785-2018-16-1-23-27**

DISTURBANCES IN BRAIN CORTEX NEURONS FOLLOWING PRENATAL ALCOHOL EXPOSURE IN RATS

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Aim. To assess the effect of antenatal alcohol exposure on histological and histochemical characteristics of brain cortex neurons in rats at different time periods after birth.

Material and methods. Experiments were performed on 25 females albino rats and their offspring by using histological and histochemical methods of study.

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. The histochemical examination revealed the inhibition of NADH-, NADPhH, G-6-PDH and SDH as well as activation of LDH and AP.

Conclusions. Alcohol consumption by rats during pregnancy induces in their offspring brain cortex neurons deep and mainly irreversible structural and metabolic disturbances.

Key words: *antenatal alcoholisation, cerebral 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) [1]. Brain 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, significant ultrastructural abnormalities in it. There are the 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 the signals of insulin needed to ensure their viability, metabolism, formation of synapses and synthesis of acetylcholine [2]. During ontogenesis alcohol induces defects in many molecular, neurochemical and cellular processes that occur during normal brain development, including disturbances of glia functions, regulation of gene expression and cell-cell interactions, increases the formation of free radicals [3]. Ethanol affects the embryonic development of the nervous system, especially the neural stem cells, destroys regulatory communications of microRNA that are important for the process of maturation of neurons [4]. However, a complex and systematic histological, histochemical and morphometric analysis of the brain cortex neurons in dynamics of postnatal development in animals following prenatal alcohol exposure has not been conducted.

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

Material and methods

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). 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 (alcohol consumption was 3.64±2.2 g/kg/day), and the animals of the control group – equivolume amount of water. The offspring brains of the control and alcohol groups were examined on the 2-, 5-, 10-, 20-, 45-, 90th days after birth (36 rats – control group and 36 rats – experimental group). For the identification of cingulate, frontal and parietal cortex in the brain sections the stereotaxic atlas was used [5]. For histological examination samples of the brain cortex were fixed in the mixture of alcohol, chloroform and acetic acid, dehydrated and embedded in paraffin. 7 μm sagittal sections of the brain cingulate, frontal and parietal cortex were stained with 0.1% solution of thionine (the Nissl method). For histochemistry pieces of brain cortex were obtained, frozen and stored in liquid nitrogen for further analysis. The activity of the oxidizing enzymes, such as succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), NADH dehydrogenase (NADHDH) and NADPhH dehydrogenase (NADPhDH), as well as the activity of the marker enzyme of lysosomes, acid phosphatase (AP) were examined in criostate section [6]. 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). The mean values obtained for every animal were processed with nonparametric statistics (because of the small number (6) of animals and absence of normal distribution in the groups) using software STATISTICA 6.0 (StatSoft, 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).

Results and discussion

Prenatal alcohol exposure influenced the postnatal thickness of a 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). The similar changes were found in cingulate, frontal and parietal cortex.

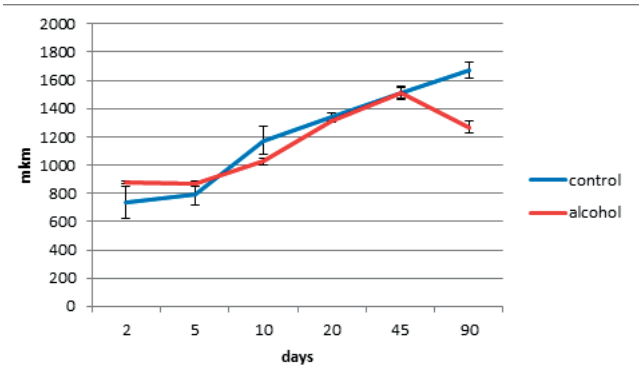

Figure 1. – The thickness of the parietal cortex in rats at different time periods after birth. Data are presented as median ± interquartile range;
**** – p<0.05, as compared to controls***

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). The similar lost of neurons was found in cingulate, frontal and parietal cortex.

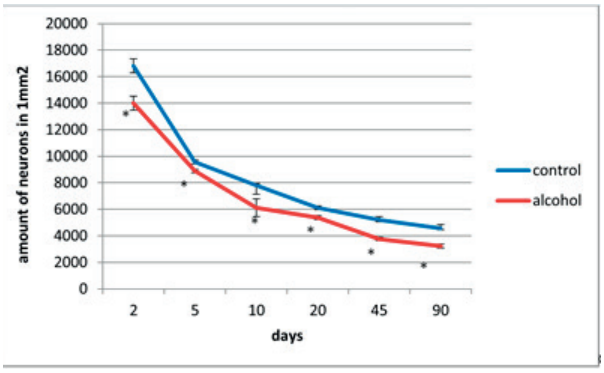

Figure 2. – The density of distribution of the 5th layer neurons of the parietal cortex during postnatal ontogenesis. Data are presented as median ± interquartile range;
**** – p<0.05, as compared to controls***

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 in all brain cortex regions studies a temporary increase in the area of those neurons bodies on the 2nd day was found. However, starting from the 20th postnatal day the area of the neuron 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 from the 20th postnatal day in all brain cortex regions studied the neurons stopped their growth (Fig. 3).

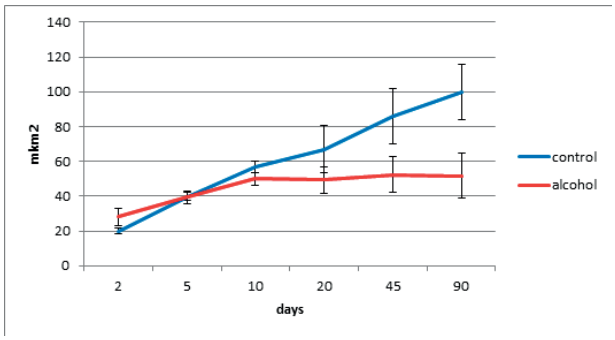

Figure 3. – Dynamics of the area of perikaryons of the 5th layer neurons in the parietal cortex of rats. Data are presented as median ± interquartile range;
**** – p<0.05, as compared to controls***

In control animals during all periods of postnatal development the normochromic neurons in preparations of brain cortex prevailed (60-70%) (Fig. 4 A, 5). In prenatally alcoholized rats at all time periods of postnatal development the number of normochromic neurons decreased significantly and the number of abnormal neurons (hyper-, hypochromic neurons and cell-shadows) increased (Fig. 4 B, 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 shrunken hyperchromatic cells, hypochromic and cell-shadows was higher (by 66, 20 and 40 % accordingly) as compared to controls (Fig. 4, 5). The amount of shrunken 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. It is associated with the changes in neurons shape: increase in their elongation and decrease in form factor. There is a negative correlation between the size of neurons and the number of shrunken neurons between the 20th and 90th days of age ($r=-0.87–0.98$; $p<0.05$). The similar changes in neurons were found in cingulate, frontal and parietal cortex.

Histochemical investigation of brain cortex of rats following prenatal alcohol exposure demonstrated the inhibition of NADH-, NADPhH, G-6-PDH and SDH and activation of LDH and AP in cytoplasm of 5th layer pyramidal neurons (Fig. 6).

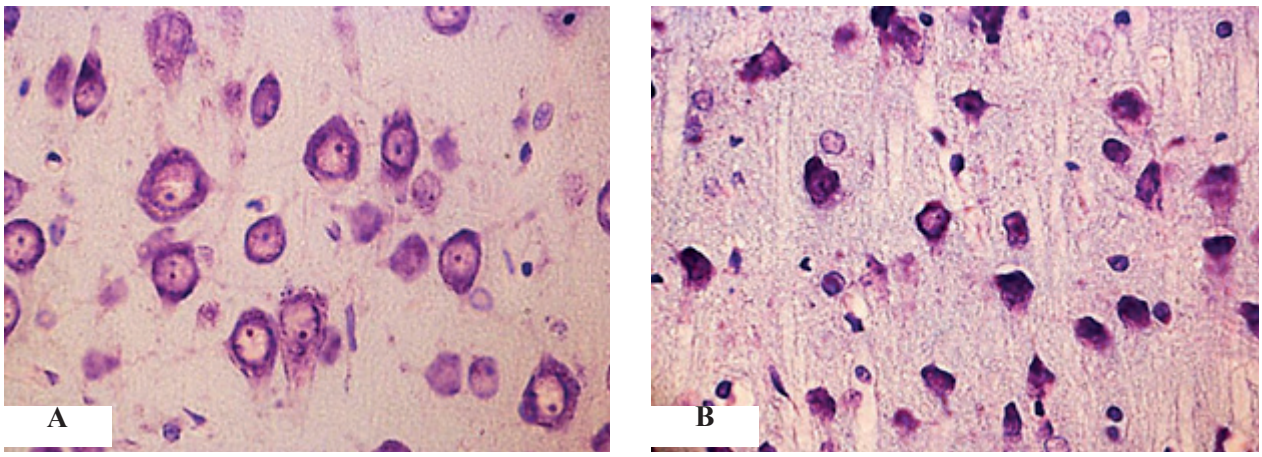

Figure 4. – The 5th layer parietal cortex neurons on the 90th postnatal day in controls (A) and antenatally alcoholized rats (B). The arrow shows the shrunken hyperchromatic neurons. Stained by the Nissl method. Digital microphotography. Scale bars - 20 μm, magnifications ×400

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

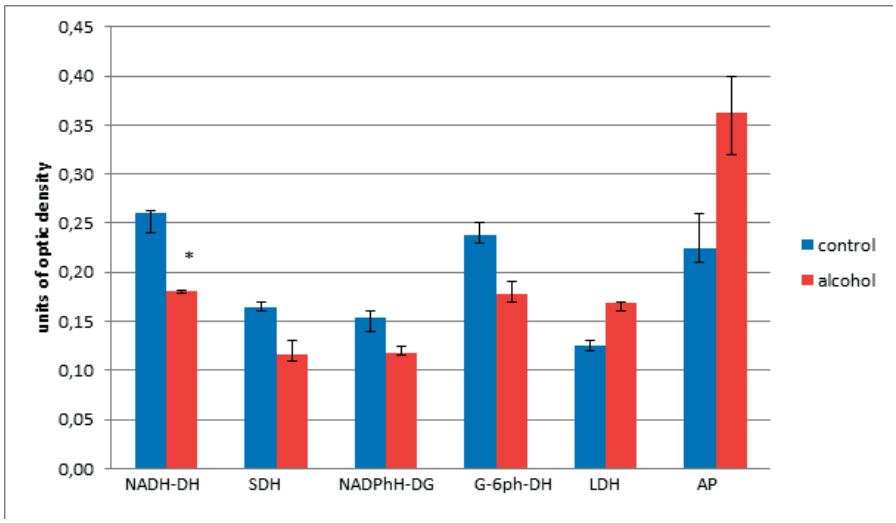

*Figure 6. – Changes of enzyme activities in cytoplasm of cingulate cortex neurons 45-day-old rats. The enzymes studied (as described in the «Material and methods») represented on the horizontal axis, the optical densities of chromogen obtained in the course of corresponding histochemical reactions are plotted on the vertical axis. Data is presented as median ± interquartile range; * – p<0.05, as compared to control*

The temporary thickening of the brain cortex on the 2nd and 5th postnatal days in the 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 size 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 antenatally alcoholized rats can be associated with the decrease in size and shrunken 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 shrunken neurons ($r=-0.89-0.94$; $p<0.01$) in all periods of postnatal ontogenesis in cingulate, frontal and parietal cortex 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. The brain cortex in offspring of rats exposed to alcohol during pregnancy becomes thinner and contains fewer neurons and glia (at G13 and G21) [7]. These findings contradict the results of Magnetic resonance imaging (MRI) showing thickening of the cerebral cortex in children and adolescents with fetal alcohol syndrome [8]. But our study revealed a temporary increased (on the 2nd, 5th postnatal days) brain cortex thickness. To explain these facts, we can assume that swelling of the gray matter occurs following antenatal alcohol exposure is detected as a thickening of the cortex under MRI.

We observed a cessation of the growth and shrinkage of pyramidal brain cortex neurons following the 10th days of postnatal development. It is seems to that antenatal alcoholization 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: the 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 brain cortex.

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, LDH increased. It indicates probably both the disturbances of energy metabolism and metabolic adaptation of neurons to alcohol, taking into consideration the alcohol-induced hypoxia [6]. 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 [9].

In conclusion, alcohol consumption during pregnancy in rats induces a dramatic deep and irreversible structural and metabolic disturbances in the brain cortex neurons in offspring.

Acknowledgements – The authors are grateful to the English translator Yanina Razvodovskaya for the correction of the manuscript.

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НАРУШЕНИЯ НЕЙРОНОВ КОРЫ МОЗГА КРЫС ПОСЛЕ ПРЕНАТАЛЬНОГО ВОЗДЕЙСТВИЯ АЛКОГОЛЯ

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Цель работы. Оценка влияния антенатальной алкоголизации на гистологические и гистохимические характеристики нейронов коры головного мозга крыс в постнатальном онтогенезе.

Материал и методы. Эксперименты выполнены на 25 самках беспородных белых крыс и родившемся от них потомстве с применением гистологических и гистохимических методов исследования.

Результаты. Антенатальная алкоголизация вызывает увеличение, а затем снижение толщины коры головного мозга крыс, уменьшение относительного количества нейронов и повышение числа патологических форм нейронов во все сроки исследования. Гистохимическое исследование выявило угнетение активности НАДН-, НАДФН-, Г-6-ФДГ и СДГ и активацию ЛДГ и КФ.

Выводы. Потребление крысами алкоголя во время беременности вызывает глубокие, часто необратимые структурные и метаболические нарушения в нейронах коры головного мозга их потомства.

Ключевые слова: *антенатальная алкоголизация, кора головного мозга, нейроны.*

Поступила: 18.05.2017 *Отрецензирована: 07.06.2017*