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# **Molecular-Cellular Targets of the Pathogenetic Action of Ethanol in the Human Brain in Ontogenesis and the Possibility of Targeted Therapy Aimed at Correcting the Effect of Pathogenic Factors**

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Additional information is available at the end of the chapter

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## **Abstract**

Prenatal exposure to ethanol has an impact on angiogenesis and synaptogenesis and formation of neurotransmitter receptors in the brain of the embryo and fetus. Compensatory mechanism of hypoxia in conditions of prenatal exposure to alcohol involves decrease in the perimeter of the vessel and the area of the vessel in the cross section and an increase in the number of vessels in the brain. A significant effect of prenatal exposure to ethanol on the development of synaptic structures in the developing brain of the fetus was expressed in the slowing down of the formation of synaptic contacts and in the reduction of their number in comparison with the norm. Shaping synaptic contact is one of the leading processes during which largely determine the future integrative brain capabilities. The properties of benzodiazepine receptors in the developing brain of the human's embryo and fetus under prenatal alcohol influence were characterized by a decrease in affinity and an increase in their density as compensatory adaptation of the fetal nervous system to the effects of alcohol. It is reflected on during synaptogenesis in the developing brain and can lay the basis of severe disorders in the unborn child. Alcohol abuse induces neuroadaptive alters of benzodiazepine receptor system in the brain in patients with alcoholism that can modulate GABA<sub>A</sub>R and mediation of GABA in the brain, which can cause alcohol addiction.

**Keywords:** alcohol, alcoholism, embryo, fetus, brain, vessel, synapse, benzodiazepine receptor, GABA

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## 1. Introduction

Prenatal alcohol exposure at moderate and higher levels increases the odds of child behavior problems with the dose, pattern and timing of exposure affecting the type of behavior problems expressed [1, 2]. Disruption in the neural activation of the prefrontal cortex (PFC) and neurobehavioral disorders were detected in children with severe prenatal exposure to alcohol (PAE) [3–6]. The developing brain is extremely sensitive to the effects of ethanol [6, 7]. The use of significant doses of ethanol during pregnancy can result in a combination of profound morphological and neurological changes called fetal alcohol syndrome (FAS) [8, 9].

The use of moderate doses of ethanol can cause abnormalities that are not associated with multiple morphological and neurological damage associated with FAS, but are associated with the development of cognitive deficits and more serious consequences in the offspring, which can be particularly pronounced in puberty [10, 11]. This formed the basis for an expanded diagnostic classification of fetal defects and a new category—neurodevelopmental disorders caused by alcohol. There is a complex relationship between the dose, nature and timing of prenatal exposure to alcohol and problems of child behavior in the future. Fetal alcohol syndrome (FAS) and fetal alcohol effects (FAE) are preventable forms of mental retardation and developmental disability caused by heavy prenatal alcohol exposure.

The human brain is arguably one of the most complicated organism living systems. This elaborate structure originates from a simple neural tube, followed by a series of differentiation processes. The possible contributions of PAE to nervous system malformations must be considered in the context of developmental timing. Neural tube defects typically occur during weeks 3–4 of human gestation [12]. Morphometric characterization of the brain at each stage not only aids in understanding this highly ordered developmental process but also provides clues to detecting abnormalities caused by genetic or environmental factors. Some observations have shown that the development of brain abnormalities: brain microencephaly, neural tube defects, hydrocephalus with various etiology and severity and cerebral vascular lesions, is not associated with complications at birth or as a result of prematurity [12].

Alcoholism of the mother can lead to the development of the FAS or FAE, which is apparent as a complex of disorders in the somatic and mental domains, reflecting impaired nervous system development [13, 14]. A number of authors have shown that the development of this syndrome is mainly due to impaired fetal brain development [15–17], starting from the earliest stages of neurogenesis and brain formation structures, which leads to a delay in migration and differentiation of neurons and some disorders of angiogenesis and synaptogenesis [15, 18–21]. The function of the blood-brain barrier (BBB) in the embryonic brain is mediated by cellular elements—endotheliocytes, developing glial cells and pericytes, and also by the noncellular structures of capillary basal membranes. Elements of BBB are under the direct influence of alcohol, with prenatal exposure to it during pregnancy in conditions of mother's alcohol abuse. In the early stages (5–6 weeks of intrauterine development), the neural tube does not have blood vessels. Neuroectodermal structures are fed from a protein-rich fluid into the neural tube. Due to their rapid growth and increase in mass, nutrients enter the newly formed blood vessels [22, 23].



At the molecular-cellular level, changes in the nervous system in the formation of alcohol dependence are associated with activation of the processes of synaptic plasticity. With the development of alcohol dependence, stimulation of neuroplasticity is considered one of the reasons for the rapid formation of a behavioral stereotype—addictive behavior. At the same time, long-term consumption of ethanol leads to a permanent disruption of synaptic plasticity, which can cause cognitive impairment, learning and memory problems, and the formation of alcoholic motivation and obsessive directed behavior in experimental animals and people with prolonged use of alcohol [24].

Neurogenesis is the basis for ensuring the plastic function of the brain and is regulated by many factors. Stimulation of neurogenesis is observed in a number of pathological conditions: brain ischemia, trauma, the development of neurodegenerative pathology, the influence of neurotoxic agents, including high doses of alcohol, prolonged use. Neurogenesis is the key adaptive function of the brain, represents one of the most important mechanisms of brain plasticity, which is expressed in an increase in the number of cells involved in the restructuring of neuronal networks. Exposure to ethanol limits early development by delaying or inhibiting the formation of postsynaptic neurons from progenitor neuronal cells (PNA) [19–21, 25].

The effects of ethanol in the early stages of development can disrupt the signaling mechanisms that regulate synaptogenesis. Negative effects of ethanol are associated also with its influences on the lipid component of neuron membranes. As lipotropic agent, ethanol is able to change the essential physico-chemical properties of cell membranes, which is reflected in the current fetal brain synaptogenesis [26, 27]. It has been shown that ethanol triggers apoptotic neurodegeneration [17] in the developing brain, when administered to infant rodents during the period of synaptogenesis, also known as the brain growth spurt period [19, 20]. Prenatal alcohol exposure inhibits neurogenesis [24, 28] and dendritic growth of newborn neurons [18].

The effects of ethanol cause neuronal death, impairment of differentiation, migration of neuronal elements and changes in neuronal plasticity, acting through various receptors and their signaling pathways [29]. Rapidly developing neural networks form synapses, mediate the communication and functioning of a multitude of synapses, through neuromediation part of them associated with a neurotransmitter gamma-aminobutyric acid (GABA), which operates via chloride-permeable GABA type A receptor channels. At an early stage of development, neurons have a high concentration of intracellular chloride, which leads to an outflow of chloride and exciting actions of GABA in immature neurons. Transmission of GABA signals is also established prior to the formation of glutamatergic transmission. Thus, GABA is the main excitatory transmitter in the early stages of development and modulates the cell cycle, the formation of cells and their migration [30–33].

The currently accepted position is that the adverse effects of ethanol are also linked with interactions with specific proteins, ion channels and receptors, leading to changes in their functions [17, 34, 35]. The ability of ethanol to interact with receptor proteins was demonstrated, which contributed to a change in neuronal excitability. GABAergic neurotransmission plays an important role in the mechanisms of action of ethanol. GABA receptors fulfill the inhibitory role in the CNS. GABA<sub>A</sub>R is an oligomeric protein complex, which contains various allosteric binding sites that modulate receptor activity, and these allosteric binding sites are the



targets for various agents, including benzodiazepines (BzD) and ethanol. Benzodiazepines, which bind to the specific sites—benzodiazepine receptors (BzDR) on the GABA receptor complex, change its conformation and affinity [35–37]. Sedative and anxiolytic effects of alcohol and benzodiazepines are based on the potentiation of inhibitory effects of GABA by the inactivation of GABA<sub>A</sub> receptors. In the experiment, it was shown that the acute effect of ethanol enhances the gain of GABAergic transmission, but chronic alcoholization increases the binding of inverse BzDR agonists and reduces GABAergic function [38, 39]. Recent data point to the existence of a relationship between the actions of ethanol and the functioning of the GABA-BzD-receptor complex.

One of the theories of alcoholism involves a shift in the general excitability of the brain as a result of reduced inhibition processes. GABA<sub>A</sub>R are modulated by the main inhibitory neurotransmitter in the central nervous system—GABA, are potential targets for alcohol and mediate the effects of ethanol [40–44]. Alcohol can activate GABA<sub>A</sub>R, possesses anxiolytic properties, and in connection with its use of this ability is a form of self-medication by patients. Decrease of GABAergic functioning was found in patients with alcoholism and persons with a high risk of alcohol addiction development [44, 45]. The sedative and anxiolytic effects of alcohol and BzD are associated with potentiating of the inhibitory effect of GABA [41, 43]. At current time has not been revealed endogenous ligands for BzDR, as for opiate receptors and others, but their role is very significant in neuropharmacology of inhibitory processes in the CNS. There are cross-reactions (tolerance and dependence) between alcohol and BzD, which confirm the interaction of ethanol with BzDR [38].

In addition to BzDR “central” type (CBR) that associated with GABA<sub>A</sub>R and having synaptic localization, known BzDR “peripheral” type (PBR), not associated with GABA<sub>A</sub>R and localized in the mitochondrial membrane, more of them are located in the glial cells of the brain.

These receptors make very important function—transfer of cholesterol into the mitochondria; this is limited step in the regulation of the neurosteroids biosynthesis. Neurosteroids are endogenous modulators of the GABA<sub>A</sub>/BzDR in the CNS [46]. BzD, anxiolytics, anesthetics and alcohol are implementing some of its effects through the PBR and regulating production of neurosteroids and their active metabolites, which are very significant for normal brain functioning [46, 47].

Understanding of the basic signaling mechanisms that regulate the excitability and inhibition of brain processes involved in the formation of alcohol addictive behavioral, the determination of the target of alcohol effects can contribute to the creation of new pharmaceutical preparations to influence these targets and to develop a potentially effective therapies to prevent the consequences of alcohol abuse and withdrawal.

In this regard, it is impossible to overestimate the importance of further studying the processes associated with angiogenesis and synaptogenesis and the formation of receptor systems in the developing human brain, in particular, the GABA-benzodiazepine receptor system under conditions of chronic effects of ethanol, their role in the development of alcohol dependence, which may contribute to further clarification of the etiopathogenesis of the disease and the search for new medications necessary for pharmacotherapeutic correction, and prevention of harmful effects of ethanol.



## **2. Neuroplastic features of vascular development, synaptic contacts and formation of benzodiazepine receptors in the developing human fetal brain under conditions of prenatal exposure to alcohol. Adaptive changes in the benzodiazepine receptor system of the human brain under the influence of chronic alcoholization**

The study of the effect of mother's alcoholism on the developing fetal brain (prenatal exposure to alcohol) was carried out in the brain tissue of embryos and human fetuses at the 7–15 week of pregnancy in accordance with the requirements of the Ethics Committee and with the consent of patients during abortion procedures under strict medical indications. About 33 embryos and fetuses were obtained from female, suffering from alcoholism and constituted the main study group. The age of women who suffered from alcoholism was 26–39 years old, and the duration of the disease was from 3 to 13 years. In all cases, according to ICD-10 criteria, alcoholism of grade II was diagnosed (ICD-10 F10.201, F10.202). The diagnosis of alcoholism was established in the Department of Addictive Conditions, the Institute of Mental Health, Tomsk National Scientific Medical Center Russian Academy of Science (RASci). The control group included samples of the brain tissue of embryos and fetuses obtained from healthy women who do not have a history of neurological or mental diseases comparable in age. Exclusion criteria were cases of adverse effects on brain development of embryos, namely exposure to radiation, chemicals, certain pharmacological agents and maternal diseases during pregnancy: influenza, rubella, toxoplasmosis and others.

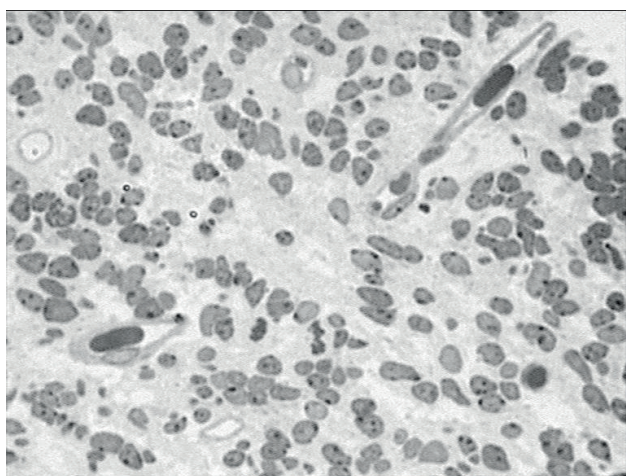
Ultrastructure of synaptic contacts and vessels of the brain tissue from embryonic and fetal brain were examined under JEM-100B and JEM-100CX electron microscopes. Electron microscopy studies addressed the intermediate layer of the wall of the forebrain, which is an accumulation of neuroblast and glioblast (including microglial cells), between which blood vessels start to grow. Morphometric analysis was performed using photographic prints from 6 to 9 cm negatives obtained from the electron microscopes. Some negatives were digitized with the scanner without intermediate paper prints. Scion Image for Windows, developed at the National Institutes of Health by Scion Corporation, was used to assess the areas of presynaptic terminals, their perimeters and the lengths of postsynaptic densities. Quantitative assessments by computerized morphometric analysis were performed by subdividing electron micrographs of embryo brain synapses into four groups, according to the period of embryo development: 7–8, 9–10, 10–11 and 11–12 weeks. This was performed in both the study group and the control group. Analyses involved five cases for each age period in the control and study groups.

### **2.1. Vesicles in the human developing brain in conditions of prenatal exposure to alcohol**

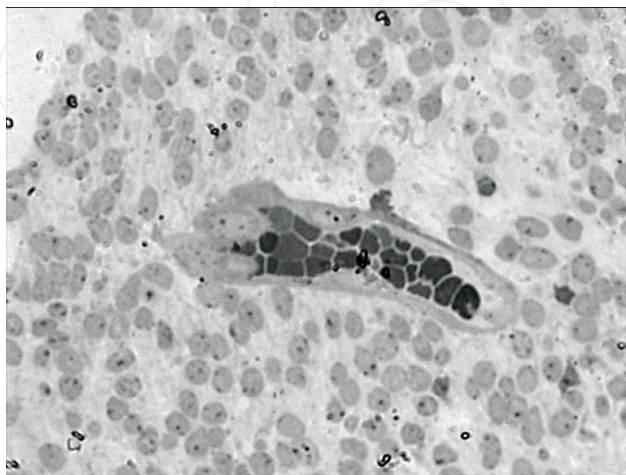
The rapidly growing neuronal structures of the developing brain of the embryo and fetus are powered by a protein-rich fluid in the lumen of the neural tube. Subsequently, this mechanism becomes inadequate when their mass increases, and the task of delivering nutrients and removing metabolic products falls on blood vessels. It is extremely important to assess the degree of alcohol exposure to vasculogenesis of the developing brain fetus under the influence of prenatal alcohol exposure associated with maternal alcoholism [48].



As our studies showed, the vessels in the developing brain of embryos and fetuses for 8–9 weeks of development under normal conditions and in the presence of prenatal exposure to alcohol consisted only of capillaries with thin walls. Endotheliocytes and pericytes are presented on microphotographs, and the lumen of the vessels was open and contained formed blood elements. On the vessels, a basal membrane, consisting of a loose fibrillar material, was visible. Morphological differences in the development of vessels between the embryos of the control and main groups during the 8–9 weeks of pregnancy were not observed. In samples of the brain tissue of the fetuses from the main experimental group, the developmental period of 10 weeks of pregnancy identified erythrocyte stasis in some forming vessels (**Figures 1 and 2**). Our data show that vessels in the human brain start to differentiate into arteries and veins from 10 weeks of gestation (**Figures 3 and 4**). Brain vessels are differentiated into arterioles, capillaries and venules. Capillary basal membranes in the main experimental and control group were already clearly visible at 12 weeks of development (**Figures 5 and 6**). In both groups, we found that the apical surfaces of endotheliocytes remained smooth, with only occasional microvillus and no

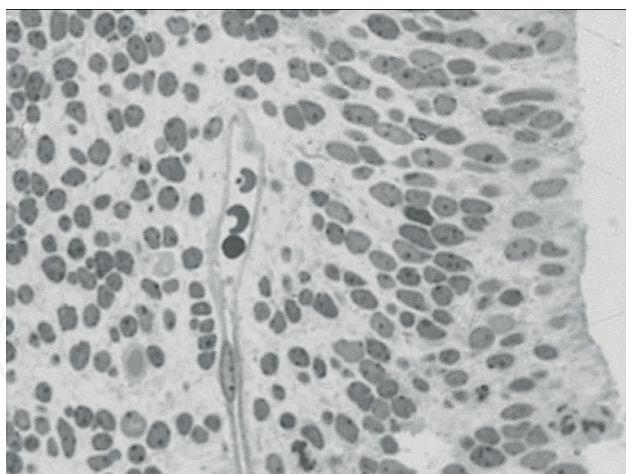


**Figure 1.** Capillaries of the intermediate layer embryonic brain. Control group, embryo 10 weeks of development. Coloring methylene blue. 740 $\times$ .

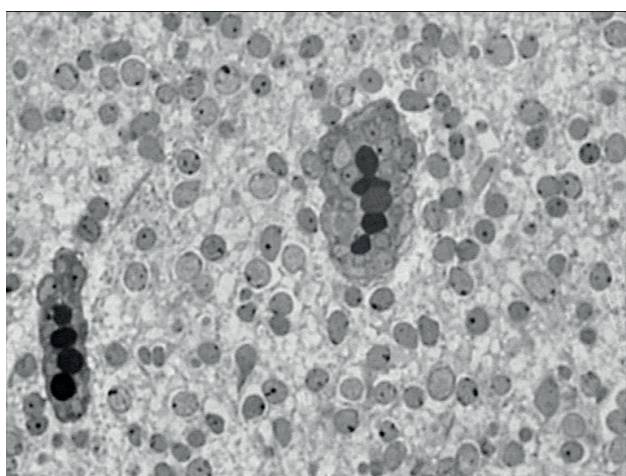


**Figure 2.** Stasis of erythrocytes in the vessel between the exact layers. Main group, embryo 10 weeks of development. Coloring methylene blue. 740 $\times$ .

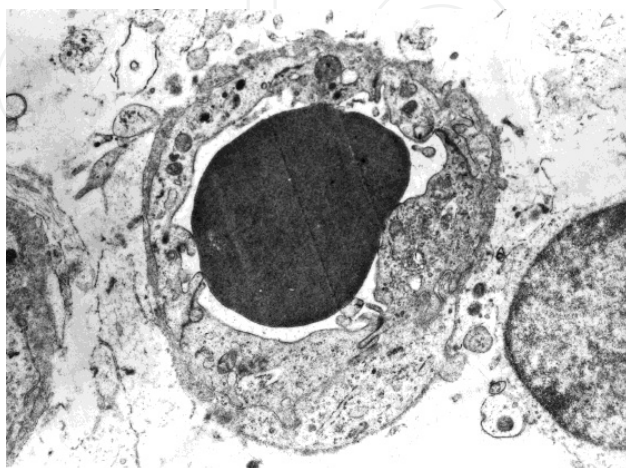




**Figure 3.** In the center of the picture, the forming venule with the shaped elements of blood in the lumen of the vessel. Control group, embryo 10 weeks of development. Coloring methylene blue, 740 $\times$ .



**Figure 4.** Two arterioles are visible in the field of vision. Control group, embryo 10 weeks of development. Coloring methylene blue, 740 $\times$ .

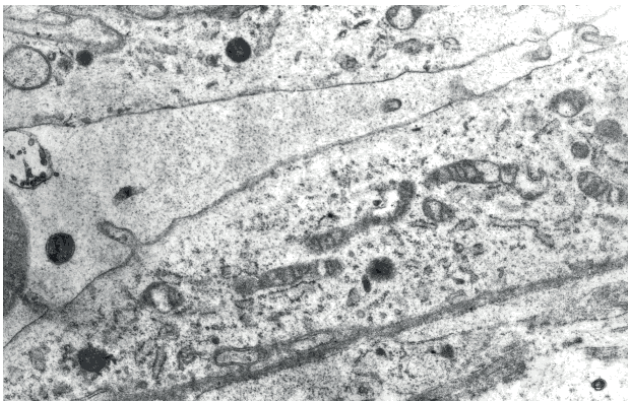


**Figure 5.** Ultrastructure of the basal membrane and capillary endothelium. The erythrocyte is visible in the lumen of the vessel. Main group, fetus 11–12 weeks of development, 10,000 $\times$ .



significant protrusions of these cells into lumens, which remained open. We studied quantitative computer morphometric and established a series of characteristics of brain tissues samples in experimental group in comparison with control group (Table 1). Mean vessel cross-sectional areas and vessel perimeters in the main experimental group were significantly reduced by 11 weeks as compared with controls. The tendency for these measures to decrease in the experimental group compared with controls persisted at 12 weeks of development. Relative vessel cross-sectional area in samples of brain tissue from the main experimental group was greater than in control group. This measure was significantly greater in this group at 11 and 12 weeks of development. The number of vessels per unit area was significantly increased in the main experimental group at weeks 11 and 12 of fetal brain gestation as compared with control group.

The first blood vessels in the human endbrain are seen at the start of week 7 of embryogenesis in the area of the ganglionic tubercle (the rudiment of the corpus striatum) and rather later in the rudiment of the neocortex (lateral wall of the lateral ventricle). The formation of blood



**Figure 6.** Basal membrane of the capillary without damage to the structure and a fragment of the cytoplasm of the endothelial cell. Main group, fetus 12 weeks of development, 45,000 $\times$ .

Measure	Control group			Experimental group		
	Week 10	Week 11	Week 12	Week 10	Week 11	Week 12
Mean cross-sectional area of vessels, $\mu\text{m}^2$	45.61 $\pm$ 0.81**	65.73 $\pm$ 2.77	59.25 $\pm$ 5.38	49.08 $\pm$ 2.61	51.82 $\pm$ 3.07*	48.26 $\pm$ 1.67
Relative cross-sectional area of vessels in brain tissue, %	0.79 $\pm$ 0.11	1.26 $\pm$ 0.11	1.38 $\pm$ 0.2	1.02 $\pm$ 0.34	5.96 $\pm$ 1003*	7.59 $\pm$ 1.44*
Number of vessels per 1 $\mu\text{m}^2$ cross-sectional area of sections	0.00017 $\pm$ 0.000023	0.000189 $\pm$ 0.000013	0.00023 $\pm$ 0.000025	0.000214 $\pm$ 0.000078	0.001137 $\pm$ 0.000189*	0.000624 $\pm$ 0.000314*
Vessel perimeter, $\mu\text{m}$	349.44 $\pm$ 18.24	492.71 $\pm$ 34.28	269.83 $\pm$ 26.0	340.58 $\pm$ 35.87	292.20 $\pm$ 16.87*	244.69 $\pm$ 16.41

\*Significant difference with control,  $p < 0.05$ .  
\*\*Significant difference compared with fetuses at 11 and 12 weeks of development,  $p < 0.01$ .

**Table 1.** Characteristics of brain vessels in normal conditions and in conditions of prenatal exposure to alcohol from week 10 to week 12 of intrauterine development ( $x \pm sx$ ).



vessels in the neocortical rudiment directly precedes the large scale migration of neuroblasts from the ventricular zone to the area of the cortical plate [22]. At 6–9 weeks of prenatal ontogenesis, developing intracerebral structures are not differentiated into arteries and veins, but have the structure of capillaries, which is consistent with our data. Endotheliocytes of intracerebral vessels are not fenestrated and contain small numbers of transport vesicles. At 8–9 weeks of gestation, vessels acquire basal membranes, which consist of a very loose fibrillar material with low electron density; there are also locations at which the endothelium makes direct contact with the intercellular space. At areas of contact between endotheliocytes and pericytes, interaction of the plasmalemmas of these cell types is seen in the form of mutual invagination [22].

We have shown that the differentiation of vessels into capillaries, venules and arterioles in the developing brain of a person begins in 10–11 weeks of pregnancy. Computer morphometric analysis showed that the main effect of alcohol on the blood vessels in the brain of the fetuses was found during the development of 11 weeks of pregnancy. An increase in the number of vessels per unit cross-sectional area of the fetal brain was observed, while the average cross-sectional area and perimeter of the vessels were reduced. Under conditions of prenatal alcohol influence, brain tissue undergoes hypoxia. Increase in the number of cerebral vessels per unit cross-sectional area is a compensatory adaptive mechanism in the development of this state.

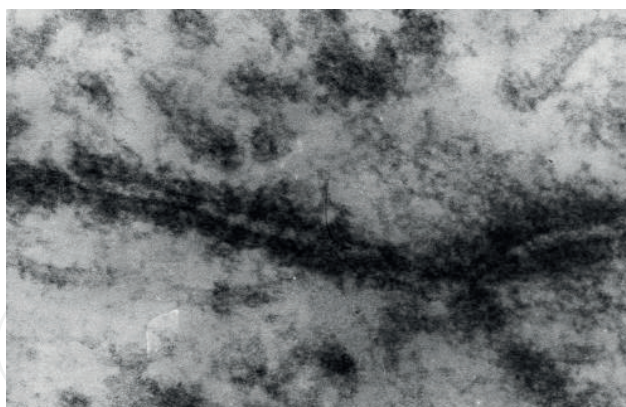
Thus, the influence of alcohol during pregnancy can significantly affect the dynamics of the cerebral circulation in the embryo and fetus, which is manifested by altering the vascularization of the developing human brain.

## **2.2. Cortical synaptogenesis in the human developing brain in conditions of prenatal exposure to alcohol**

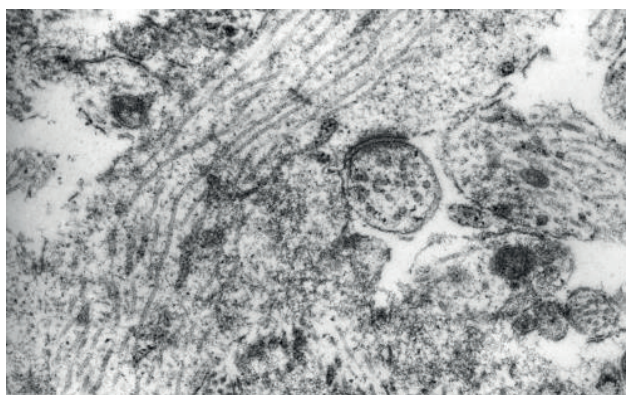
As a lipotropic agent, ethanol, is able to change the basic physicochemical properties of cell membranes, which are reflected in the current synaptogenesis of the embryonic brain in order to establish the nature of this effect, we conducted the following studies.

In human embryonic brain in the early period—7–8th week of gestation, the desmosome-like contacts were represented as we observed. Contacting membranes are in their middle part of thickening, which both sides approach to each other, forming a fissure. In these places of the thickening, the membrane can be connected. Electron-dense material is in the field of adhesion. Contacts of this type are found between dendritic processes and neuronal cells. During the development of 9–10 weeks of pregnancy, these types of contacts are less frequent. Contacts with the presence of vesicular elements have been revealed. Synaptic vesicles were rounded and had a bright center, and the diameter of these vesicles was approximately 40 nm. The width of the synaptic space of immature synapses was approximately 20 nm. The length of the area of the sealing membrane reached 0.1–0.15 microns (**Figure 7**). In the transitional stage from synapse-like contacts to their true synaptic form, single synaptic vesicles were visualized near the presynaptic membrane. Such synapses are located mainly at the lower boundary of the intermediate layer of the cerebral cortex (**Figures 8 and 9**). They can already be considered functionally competent.

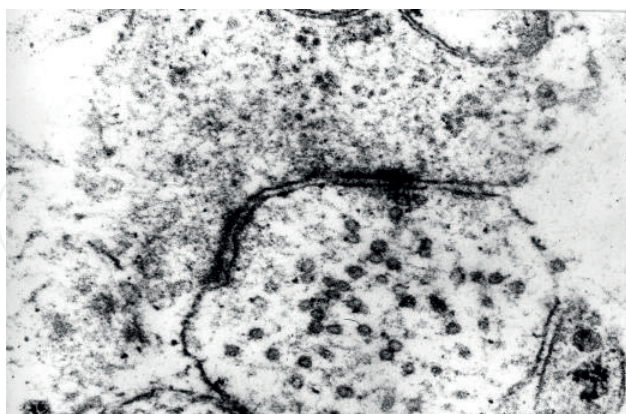




**Figure 7.** Contact with uniformly thickened membranes. Main group, the fetus of 10–11 weeks. Magnification 160,000.



**Figure 8.** The emerging synapses in the cerebral cortex the intermediate layer brain. Main group, 12-week fetus. Magnification 40,000.



**Figure 9.** Completely formed functionally competent synapse. Main group, the fetus of 11–12 weeks. Magnification 70,000.

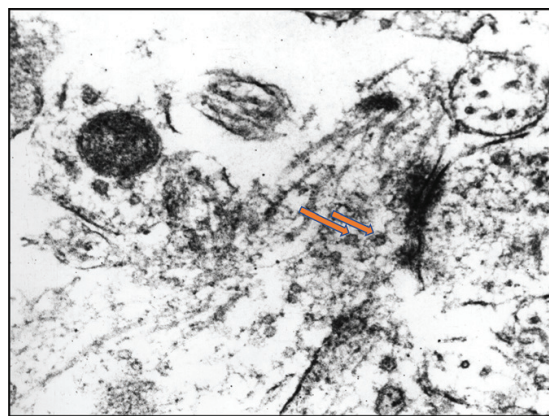
At the stage of fetal development 10–12 weeks, the number of synapses with relatively mature structures increased. They are located in the border of the ventricular and intermediate layers and in the intermediate layer of the cortical plate and nerve cells. In synaptic contacts, all the



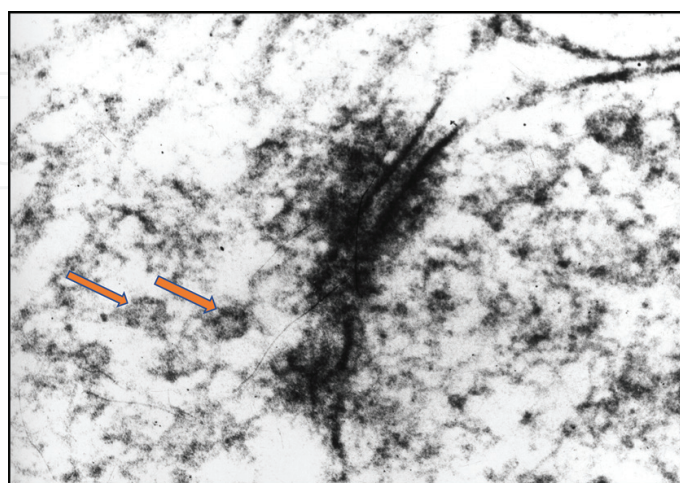
necessary components were found; from the mature synapses, their difference was the smaller number of synaptic vesicles. Synaptic contacts on neuroblasts and glioblasts have fewer synaptic vesicles compared to the synapses of the mature brain. All of the above features were inherent in both the control group and the main group of embryos and fetuses (**Figures 10 and 11**).

In the brain tissue of embryos and fetuses obtained from women suffering from alcoholism, a slowdown in the formation of synaptic structures was observed. Non-synaptic contacts in the samples of the main study group did not differ from those of control in the frequency of occurrence in the brain tissue and in its structure. The fully formed structure of the synaptic contacts is associated with the appearance of synaptic vesicles comparable with structure control; however, the area of the synapse was smaller [49].

The strong evidence we have obtained suggests that the developing brain is vulnerable to the pathogenic effects of ethanol. In the cells of the brain of embryos and fetuses from the main group of the study group, a slowing down of the process of synaptogenesis in comparison



**Figure 10.** Single synaptic vesicles in the formation of contact, the main group is a fetus of 12 weeks of development, magnified 60,000.



**Figure 11.** Single synaptic vesicles in the formation of contact, the main group is a fetus of 12 weeks of development, magnified 144,000.



with the norm was revealed, which can be critical for neurotransmitter processes in the developing human brain.

2.3. Morphometric analysis of synapses in the human developing brain in conditions of prenatal exposure to alcohol

Morphometric analysis of synaptic characteristics was performed in the study and control groups, using as a criterion the stage of development of embryos and fetuses.

In the main study group, a significant decrease in all parameters of synaptic structures was revealed in comparison with the control. More detailed analysis of synapse parameters was then performed, taking cognizance of embryo and fetus developmental period (Figures 12–14, Table 2).

We found that the length of postsynaptic density was lower in the main group compared to the control group already at the 7–8th week of gestation. At the 9th week of pregnancy, we identified synaptic contacts, especially at the upper margin of the middle layer. At this period of brain development, all synaptic parameters studied were significantly smaller in the main

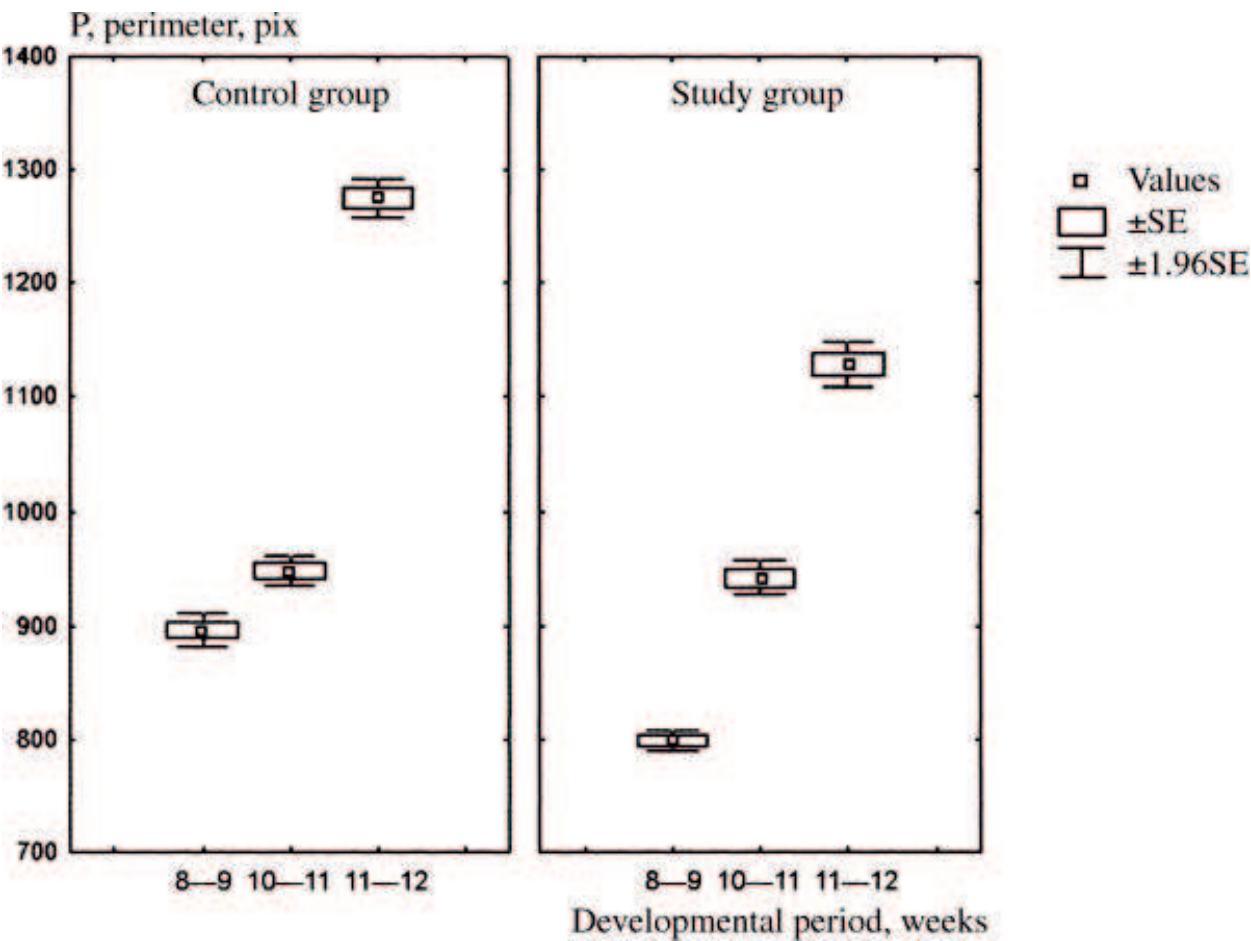
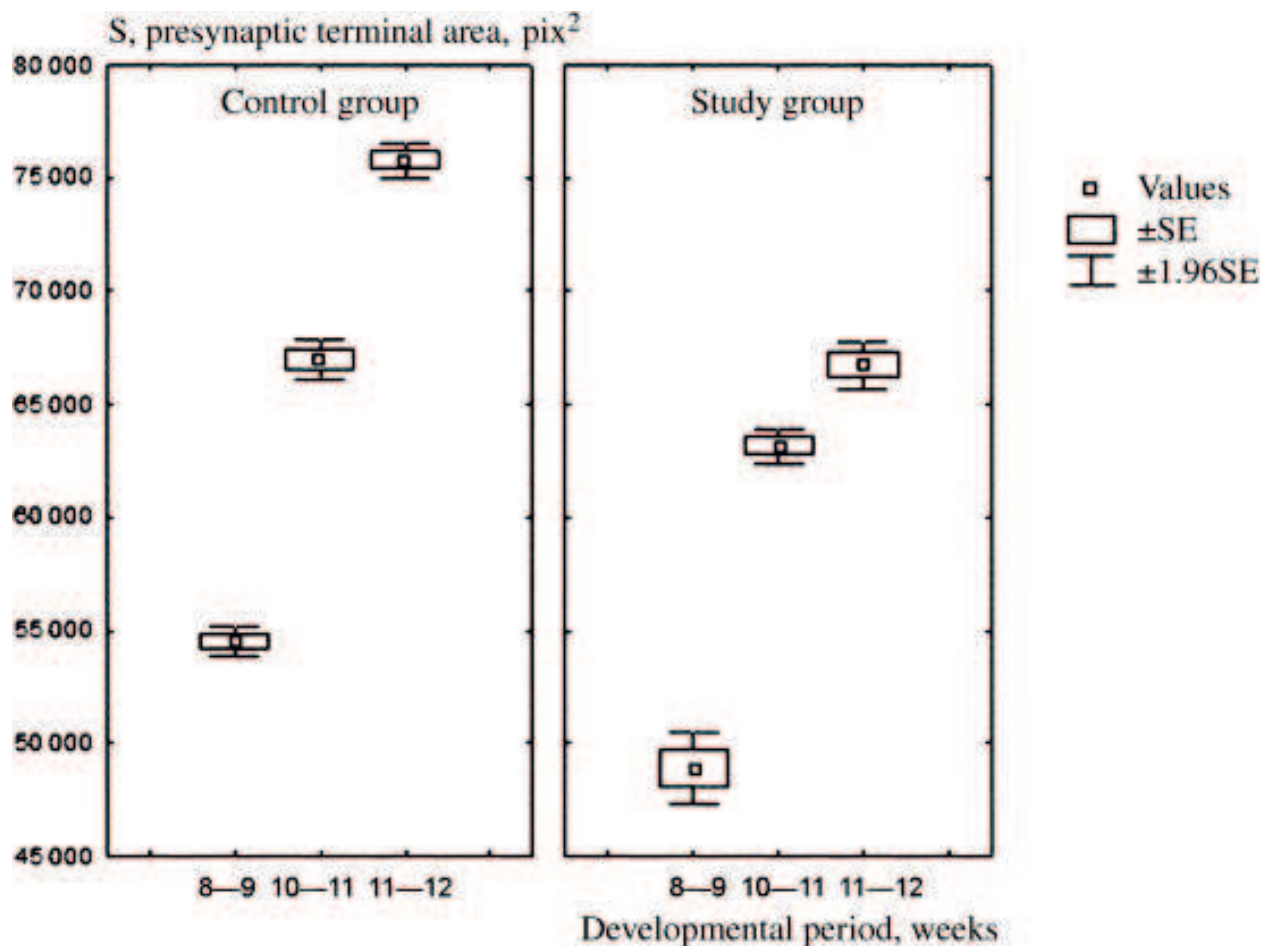


Figure 12. Morphometric values for presynaptic terminal perimeters in the control and study groups at different weeks of development.





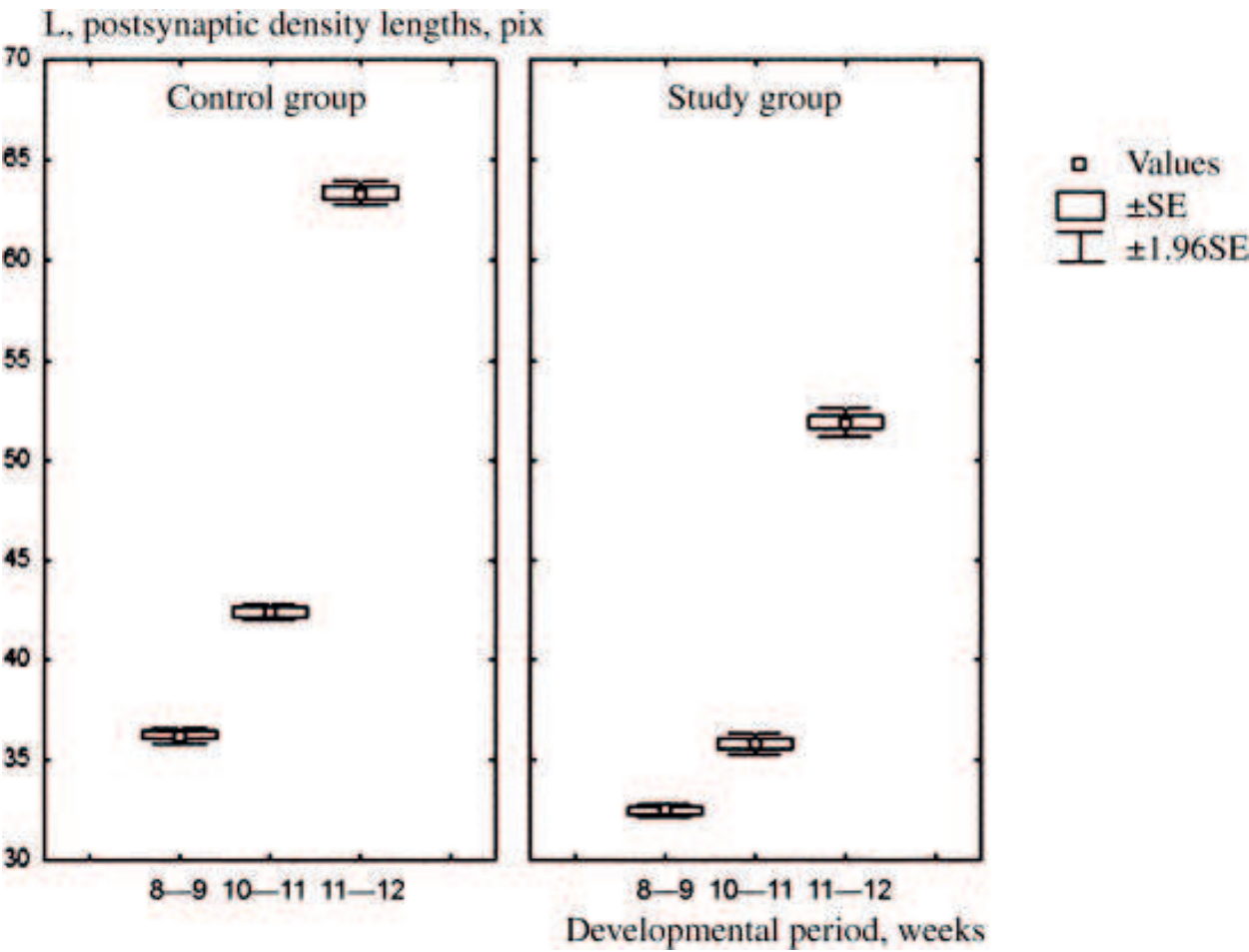
**Figure 13.** Morphometric values for presynaptic terminal areas in the control and study groups at different weeks of development.

group with respect to the control. At week 10, we also noted a decrease in all parameters of the study at the synapses; however, the presynaptic perimeters did not differ.

At 11-12 weeks of development, there was a more pronounced change in the parameters of synaptic contacts in the main group relative to the control group. Most synapses in the brain of the fetuses of 11-12 weeks of gestation are axodendritic positively bent synapses with some insignificant amount of synaptic vesicles and single mitochondria in the presynaptic terminals of the synapses.

The fully formed structure of synaptic connections with the appearance of synaptic vesicles compared to the control, but synapse core area considerably less resulting computer-morphometric analysis, we identified a delay of synapses and their structural immaturity which is probably due to a direct effect of alcohol on nerve cells, primarily due to its membranotropic action. Our morphometric studies have revealed that the prenatal influence of alcohol has a pronounced effect on the structural organization of synaptic contacts and their parametric characteristics. Our data confirm the data of other researchers obtained in studies in the culture of hippocampal tissues under the influence of a solution of ethanol [50, 51].





**Figure 14.** Morphometric values for postsynaptic density lengths in the control and study groups at different weeks of development.

Stage of development	7–8 Weeks		9 Weeks		10 Weeks		11 Weeks	
Measure	C	S	C	S	C	S	C	S
	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE
	N = 90	N = 90	N = 210	N = 210	N = 210	N = 210	N = 210	N = 210
Length of postsynaptic density	25.21 ± 3.0	23.56 ± 2.4	36.21 ± 1.56	32.45 ± 1.23*	42.37 ± 1.70	35.80 ± 2.37*	63.33 ± 2.51	51.90 ± 2.88*
Area of postsynaptic terminals	–	–	54.521 ± 2673	48.861 ± 6773*	66.964 ± 3833	63.178 ± 3168*	75.742 ± 3207	66.750 ± 4436*
Perimeter of postsynaptic terminals	–	–	896.28 ± 63.7	798.90 ± 40.09*	948.19 ± 58.2	941.56 ± 64.44	1276.02 ± 73.08	1129 ± 86.87*

Notes: C, control group; S, study group (materials from alcoholic mothers).  
\*Significant differences between study and control groups ( $p < 0.01$ ).

**Table 2.** Morphometric parameters of synapses in the human brain at different stages of embryonic development.



Thus, as a result of computer-morphometric analysis, we found a delay of synapses and their structural immaturity, which is probably linked to the direct effect of alcohol on nerve cells in the first place due to its membranotropic action.

#### 2.4. Formation of benzodiazepine receptors of the developing human brain of the fetus in conditions of prenatal exposure to alcohol

To study the formation of benzodiazepine receptors of the synaptic structures of the brain of the developing fetus in normal and prenatal influences of alcohol, BzDR were investigated by radio-receptor binding with [<sup>3</sup>H]-flunitrazepam using synaptosomal fraction obtained from the brain of fetuses and human embryos. Radioanalysis was performed in a Rack-beta scintillation  $\beta$ -counter. The dissociation constant ( $K_d$ ) and number of specific binding sites ( $B_{max}$ ) were determined by analysis of saturation curves in Scatchard coordinates. Linear Scatchard blots were analyzed in all cases which confirm the presence of only a specific population of binding sites. Distributions of parameters did not deviate from the normal, so statistical analysis of the data was performed by parametric variational statistics (Student's test) on Statistika 10.0; differences were regarded as significant at  $p < 0.05$ . Correlational relationships were assessed by Spearman analysis. Experimental work was carried out in the Department of Clinical Neuroimmunology and Neurobiology of Mental Health Research Institute, Tomsk National Research Medical Center RASci (Tomsk) and in the Laboratory of Clinical Neuromorphology and Laboratory of Clinical Biochemistry of Mental Health Research Center RASci (Moscow). All the studies were approved by the Ethics Committee of the Mental Health Research Institute.

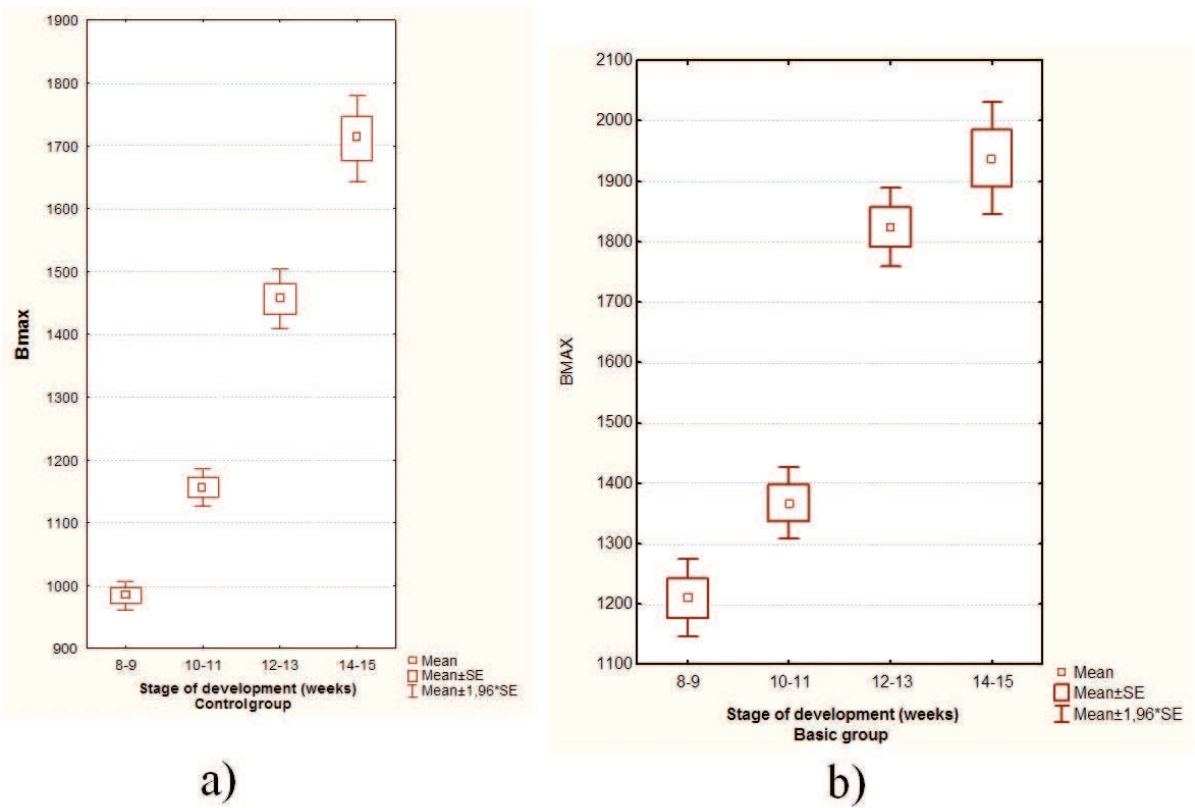
Studies of the properties of human brain BzDR at 8–9 weeks of development showed that specific [<sup>3</sup>H]-flunitrazepam binding site density ( $B_{max}$ ) was greater in the study group than the control group (**Figure 15, Table 3**). There was a decrease in receptor affinity for the [<sup>3</sup>H]-flunitrazepam, in the main study group, related to the increase in the value of  $K_d$  (**Figure 16, Table 3**). The dissociation constant— $K_d$  is inversely proportional to the receptor affinity for their ligand, that is affinity corresponds— $1/K_d$ . The observed increases in  $K_d$  indicate a decrease in the affinity of the receptors. The data obtained indicate an increase in the expression of receptors with a decrease in their affinity for the ligand in human embryo brains under the prenatal alcohol exposure.

At 10 weeks of gestation, there were not expressive changes in [<sup>3</sup>H]-flunitrazepam-binding parameters ( $K_d$  and  $B_{max}$ ) in compared groups. However, it should be noted that the dynamics of changes in receptor density is discrete, nonlinear. At this period, slight changes in the binding parameters in the control and experimental groups were noted. Density of receptors increases slightly between the 9th and 10th weeks of fetal development. There is some inhibition of growth in receptor density (**Figure 16, Table 3**), especially in the main group. This correlated with morphometric evaluation of synapses: decreases in presynaptic terminal area and postsynaptic density length in the main experimental group relative to the control group (**Table 4**).

Alcohol in the early stages of pregnancy, according to the data, negatively affects the formation of synaptic contacts and benzodiazepine receptors in the human brain, reducing the functional



activity of the brain and its development. We found that from the 12–13 weeks of pregnancy, a significant increase in receptor expression ( $B_{\max}$ ) was observed, and this trend of increasing prescription density continued during the gestation period of 14–15 weeks (**Figures 15 and 16**,



**Figure 15.** Statistical analysis of  $[^3\text{H}]$ -flunitrazepam binding parameters [ $B_{\max}$  (fmol/mg of protein) – density of binding sites] with synaptosomal membranes of human embryonic and fetuses brain in the control (a) and study (b) groups in dynamics.

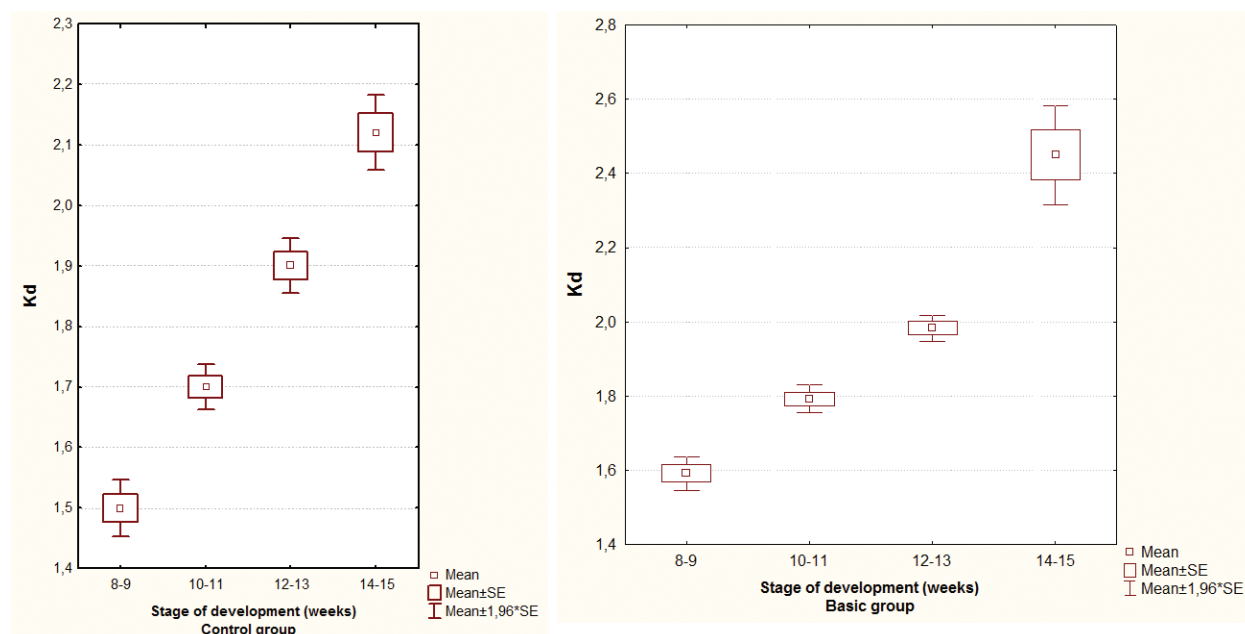
Developmental period, weeks	Control group			Study group		
	$B_{\max}$ fmol/mg protein	$K_d$ nM	n	$B_{\max}$ fmol/mg protein	$K_d$ nM	n
8–9	984.22 $\pm$ 11.64	1500 $\pm$ 0.024	9	1210.00 $\pm$ 32.79* $r = 0.47$ $p = 0.0001$	1591 $\pm$ 0.023* $r = 0.22$ $p = 0.014$	9
10–11	1156.00 $\pm$ 15.22	1700 $\pm$ 0.019	8	1367.40 $\pm$ 30.38* $r = 0.50$ $p = 0.0001$	1792 $\pm$ 0.019* $r = 0.49$ $p = 0.04$	10
12–13	1456.29 $\pm$ 24.17	1900 $\pm$ 0.023	7	1824.13 $\pm$ 33.51* $r = 0.23$ $p = 0.0001$	1982 $\pm$ 0.018* $r = 0.19$ $p = 0.014$	8
14–15	1712.00 $\pm$ 35.24	2120 $\pm$ 0.031	5	1938.17 $\pm$ 47.28* $r = 0.73$ $p = 0.005$	2450 $\pm$ 0.068* $r = 0.56$ $p = 0.0027$	6

Notes:  $B_{\max}$ ,  $[^3\text{H}]$ -flunitrazepam binding density with synaptosomal BzDR;  $K_d$ , ligand-receptor complex dissociation constant ( $[^3\text{H}]$ -flunitrazepam with synaptosomal BzDR). \*Statistically significant differences between study and control groups,  $p < 0.01$ .

**Table 3.**  $[^3\text{H}]$ -flunitrazepam binding properties with synaptosomal membranes from human embryo and fetus brains (8–15 weeks of development).



**Table 3).** However, in the experimental group, with prenatal exposure to alcohol, the affinity of the receptors decreased at all stages of the human brain development, and the increase in expression and density of receptors can be considered as compensatory adaptive brain



**Figure 16.** Statistical analysis of [ $^3\text{H}$ ]-flunitrazepam binding parameters [ $K_d$  (nM) – constant of dissociation ligand-receptor complex] with synaptosomal membranes of human embryonic and fetuses brain in the control (a) and basic groups (b) in dynamics.

Developmental period. weeks	Control group ( $M \pm SE$ )				Study group ( $M \pm SE$ )			
	$B_{\max}$	P	S	L	$B_{\max}$	P	S	L
8–9	984.22 $\pm$ 11.64	896.28 $\pm$ 63.7 $r = 0.80$ $p = 0.0006$	54.521 $\pm$ 2673 $r = 0.79$ $p = 0.0003$	36.21 $\pm$ 1.56 $r = 0.89$ $p = 0.0004$	1210.00 $\pm$ 32.79	798.90 $\pm$ 40.09 $r = 0.78$ $p = 0.0004$	48.861 $\pm$ 6773 $r = 0.64$ $p = 0.0002$	32.45 $\pm$ 1.23 $r = 0.85$ $p = 0.0007$
10–11	1156.00 $\pm$ 15.22	948.19 $\pm$ 58.2 $r = 0.77$ $p = 0.0004$	66.964 $\pm$ 3833 $r = 0.62$ $p = 0.0002$	42.37 $\pm$ 1.70 $r = 0.87$ $p = 0.0008$	1367.40 $\pm$ 30.38	941.56 $\pm$ 64.44 $r = 0.82$ $p = 0.0006$	63.178 $\pm$ 3168 $r = 0.71$ $p = 0.0001$	35.80 $\pm$ 2.37 $r = 0.88$ $p = 0.0005$
12–13	1456.29 $\pm$ 24.17	1276.02 $\pm$ 73.1 $r = 0.83$ $p = 0.0008$	75.742 $\pm$ 3207 $r = 0.76$ $p = 0.0001$	63.33 $\pm$ 2.51 $r = 0.91$ $p = 0.0003$	1824.13 $\pm$ 47.28	1129 $\pm$ 86.87 $r = 0.79$ $p = 0.0004$	66.750 $\pm$ 4436 $r = 0.70$ $p = 0.0003$	51.90 $\pm$ 2.88 $r = 0.83$ $p = 0.0008$

Notes: L, postsynaptic density length; S, presynaptic terminal area; P, presynaptic terminal perimeter; r, correlation between control and study groups between  $B_{\max}$  and P (\*),  $B_{\max}$  and S (\*\*), and  $B_{\max}$  and L (\*\*\*); p, level of significance of correlational relationships.

**Table 4.** Correlation analysis of morphometric parameters of synapses (presynaptic terminal area perimeter and area, postsynaptic density length) and [ $^3\text{H}$ ]-flunitrazepam specific binding site density (BzDR) at different developmental stages.



reaction with decreasing affinity of receptors. The change in receptor affinity is attributed to neuroplastic changes in the tissue of the developing brain due to the chronic effects of alcohol.

In ontogenesis, in the early stages of gestation, the benzodiazepine receptor system of the human brain is normally formed, starting with the 7th week of development. According to the data obtained, the density of BzDR during pregnancy 8–9 – 14–15 weeks increases by almost 200%. During prenatal influence of alcohol, associated with maternal alcoholism, we found that expression of BzDR was higher in comparison with control, at different developmental stages. The data of receptor analysis showed that the density of synaptic BzDR ( $B_{\max}$ ) correlates with the morphometric characteristics of the synapses (**Table 4**). We have shown that the affinity of receptors for the ligand during the development of the brain is somewhat reduced, which indicates the greatest sensitivity of receptors at the earliest stages of development—8–10 weeks of gestation. The prenatal influence of alcohol significantly reduced the affinity of the receptors in the experimental group, which confirms the greatest sensitivity of the BzDR to alcohol at the earliest stage of the formation of the human brain. The results of our study of the human embryonic brain in normal and under the influence of alcohol, which is associated with mother's alcoholism, indicate significant neuroplastic changes in the human brain during the early stages of its growth and development [52, 53].

Neuroplastic changes in blood vessels, synapses associated with GABAergic activity and BzDR receptors, in the developing brain under the influence of maternal alcoholism, are aimed at adapting the nervous system of the embryo and fetus to the phenomena of hypoxia, as well as functional failure of GABAergic neurotransmission. However, these adaptive changes in the human embryonic brain differ significantly from the processes of formation of angiogenesis and synaptogenesis and GABA<sub>A</sub>R neurotransmitter system of the normal human brain, which leads to various somatic disruptions and mental disorders, including the development of FAS and PAE.

## **2.5. Benzodiazepine receptor system in various structures of the human mature brain in patients with alcoholism**

Benzodiazepine receptors in different human mature brain of the alcoholics were performed using autopsy material (postmortem) obtained as a result of an urgent autopsy. Samples of autopsy material of the human brain were obtained during urgent autopsy (no later than 6 hours after the onset of death). Samples of the tissue of the prefrontal cerebral cortex, the cerebellar cortex and the head of the caudate nucleus of the brain in persons who were chronically subjected to alcoholization (based on anamnesis) and control subjects were postmortem. Samples of the brain were frozen and stored in thermoses with liquid nitrogen. A total of 126 samples from different areas of the human brain were obtained for the study of radio-receptor binding, including the basic group and the reference control group. In addition to the data of the anamnesis, the objective biological criteria for chronic alcoholization of man (fatty liver, cirrhosis, etc.) were used to form the main group. The control group included patients who did not have neurological and mental illnesses. Autopsy material was obtained only from males, and the age range was 33–54 years. Alcoholic patients were under the supervision by psychiatrists of Mental Health Research Institute and had a diagnosis according to ICD-10: F10.232; F10.302. Patients



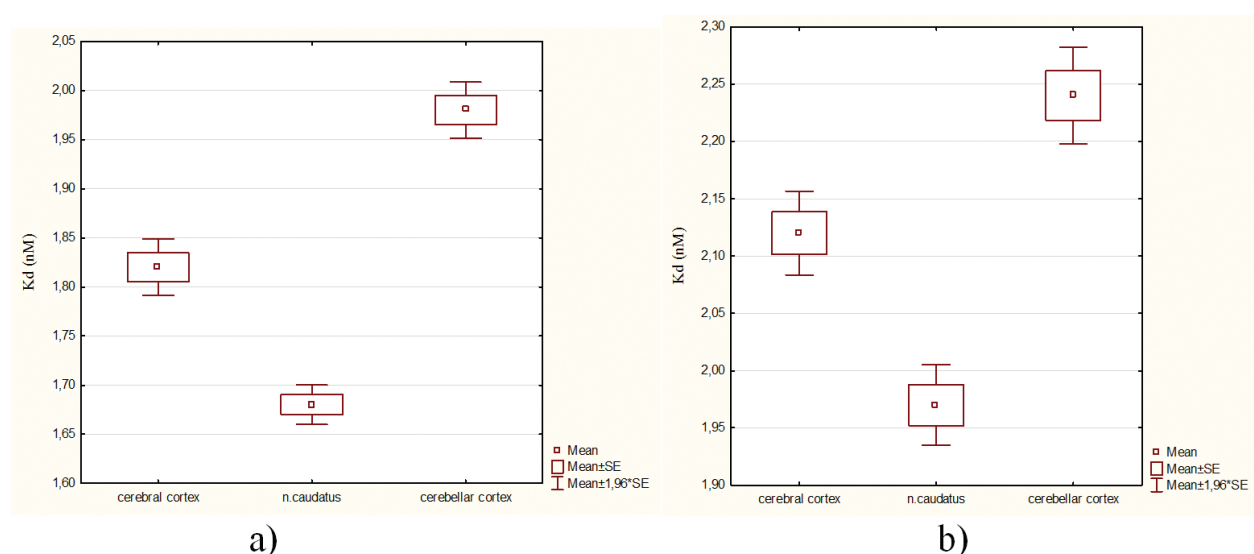
with other psychiatric disorders were not included in this study. The study included only patients whose lethal outcome occurred as a result of acute heart failure and not subjected to resuscitation measures.

The separation of tissue from human brain samples into membrane fractions (synaptosomal and mitochondrial) was carried out by preparative ultracentrifugation. The resulting membrane fractions were frozen and stored at  $t = -80^{\circ}\text{C}$ . Investigation of the properties of BzDR “central” type (CBR) and BzDRs “peripheral” type (PBR) was performed by the radioreceptor assay of binding synaptosomal and mitochondrial membranes with selective ligands. We used the parametric method (t test) using Statistika 10.0.

The experimental part of the research was carried out by us in the Laboratory of Neurobiology Mental Health Research Institute (Tomsk) and Laboratory of Clinical Biochemistry Research Center for Mental Health Sciences (Moscow). All ongoing studies were approved by the Ethics Committee.

- (I) A study of the binding characteristics of the selective ligand [ $^3\text{H}$ ]-flunitrazepam with synaptosomal fractions of membranes obtained from various regions of the human brain (postmortem) has shown that the properties of synaptosomal BzDR differ in the structures of the brain studied. The highest affinity of CBR was detected in the caudate nucleus and the lower affinity receptors have been identified in the cerebral cortex (the region of the prefrontal cortex) and in the cerebellar cortex (**Figure 17, Table 5**).

The density of the receptors in the brain structures studied was also different: the maximum receptor density ( $B_{\text{max}}$ ) was detected in the caudate nucleus, in the cerebral cortex (the region of the prefrontal cortex) and in the cerebellar cortex (**Figure 18, Table 5**). Thus, the results obtained by us testify to the heterogeneity of the CBR in various areas of the human brain in the control group. A comparative analysis of the



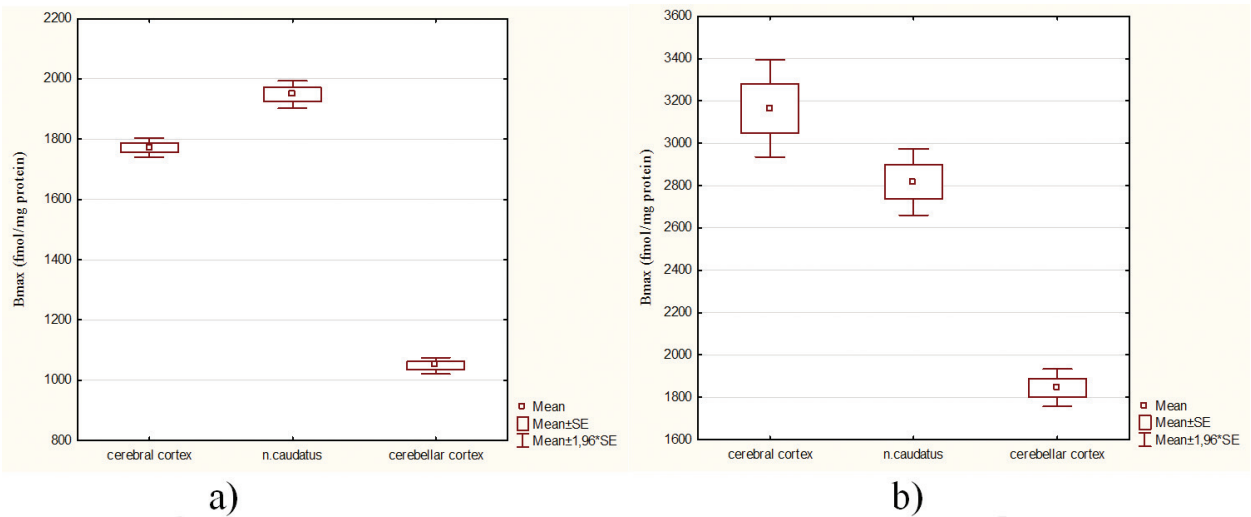
**Figure 17.** Statistical analysis of [ $^3\text{H}$ ]-flunitrazepam binding parameters [ $K_d$  (nM) – constant of dissociation ligand-receptor complex] with synaptosomal membranes in different areas of the human brain in control group (a) and study group (b) (alcoholic patients).



Area of the brain	[ <sup>3</sup> H]-flunitrazepam binding to synaptosomal membranes				[ <sup>3</sup> H]-PK-11195 binding to mitochondrial membranes			
	Control group (n = 21)		Study group (n = 21)		Control group (n = 21)		Study group (n = 21)	
	K <sub>d</sub> <sup>1</sup> (nM)	B <sub>max</sub> <sup>1</sup> (fmol/mg protein)	K <sub>d</sub> <sup>1</sup> (nM)	B <sub>max</sub> <sup>1</sup> (fmol/mg protein)	K <sub>d</sub> <sup>2</sup> (nM)	B <sub>max</sub> <sup>2</sup> (fmol/mg protein)	K <sub>d</sub> <sup>2</sup> (nM)	B <sub>max</sub> <sup>2</sup> (fmol/mg protein)
Prefrontal cortex {M ± SE}	1.82 ± 0.07	1772 ± 79	2.12 ± 0.09*	3165 ± 565*	2.45 ± 0.17	1824 ± 11	3.12 ± 0.13**	2245 ± 168**
N. caudatus {M ± SE}	1.68 ± 0.05	948 ± 112	1.97 ± 0.09*	2817 ± 386*	1.12 ± 0.09	724 ± 36	2.31 ± 0.16**	1895 ± 77**
Cerebellar cortex {M ± SE}	1.98 ± 0.1	1048 ± 67	2.24 ± 0.21*	1845 ± 217*	2.61 ± 0.21	1209 ± 98	3.32 ± 0.19**	2479 ± 123**

Notes: B<sub>max</sub><sup>1</sup>, density of binding sites [<sup>3</sup>H]-flunitrazepam with synaptosomal membranes; K<sub>d</sub><sup>1</sup>, constant of dissociation ligand-receptor complex [<sup>3</sup>H]-flunitrazepam with CBR; B<sub>max</sub><sup>2</sup>, density of binding sites [<sup>3</sup>H]PK-11195 with mitochondrial membranes; K<sub>d</sub><sup>2</sup>, constant of dissociation ligand-receptor complex [<sup>3</sup>H]PK-11195 with PBR; n, the number of cases studied. \*Statistically significant difference indicators binding [<sup>3</sup>H]-flunitrazepam and \*\*[<sup>3</sup>H]PK-11195 between study and control groups, p < 0.05.

**Table 5.** Properties of [<sup>3</sup>H]-flunitrazepam and [<sup>3</sup>H]PK-11195 binding to the synaptosomal and mitochondrial membranes from different areas of the human brain in alcoholic patients and control.



**Figure 18.** Statistical analysis of [<sup>3</sup>H]-flunitrazepam binding parameters [B<sub>max</sub> (fmol/mg of protein) – density of binding sites] with synaptosomal membranes in different areas of the human brain in control group (a) and study group (b) (alcoholic patients).

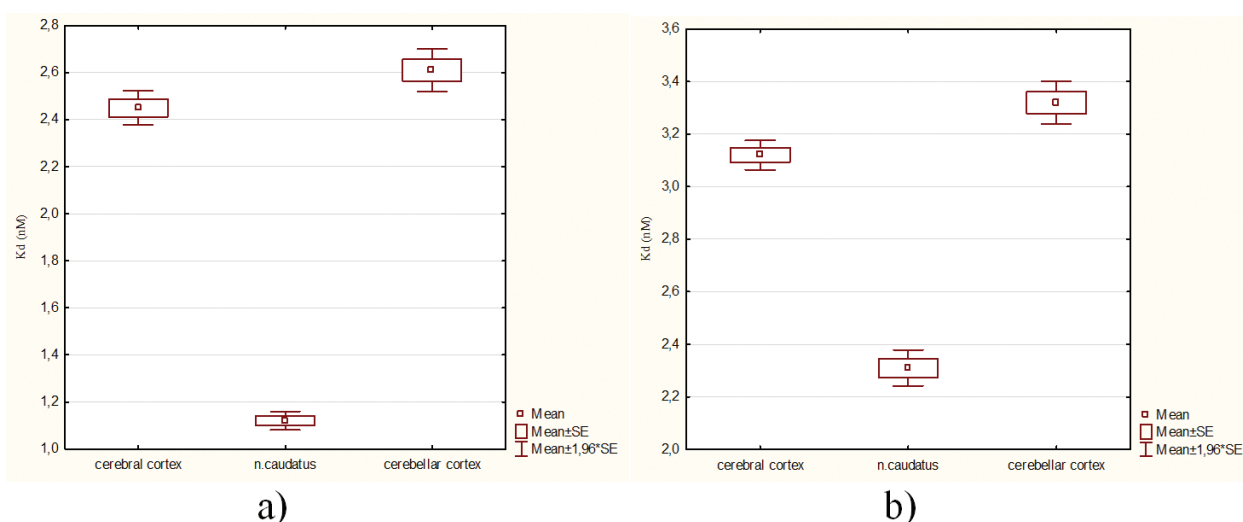
kinetic characteristics of the binding of [<sup>3</sup>H]-flunitrazepam showed a significant increase in the K<sub>d</sub> values in the studied brain structures in the patients of the main group as compared to the patients in the control group, which indicates a decrease in receptor



affinity. The largest changes in  $K_d$  were found in the cerebral cortex, the caudate nucleus and, to a lesser extent, in the cerebellar cortex (**Figures 17 and 18, Table 5**). Thus, the changes revealed by us indicate a decrease in the affinity of CBP in the brains of patients under the exposure of chronic alcoholization and an increase in their density in relation to the control group, which can be compensatory adaptive in nature [54].

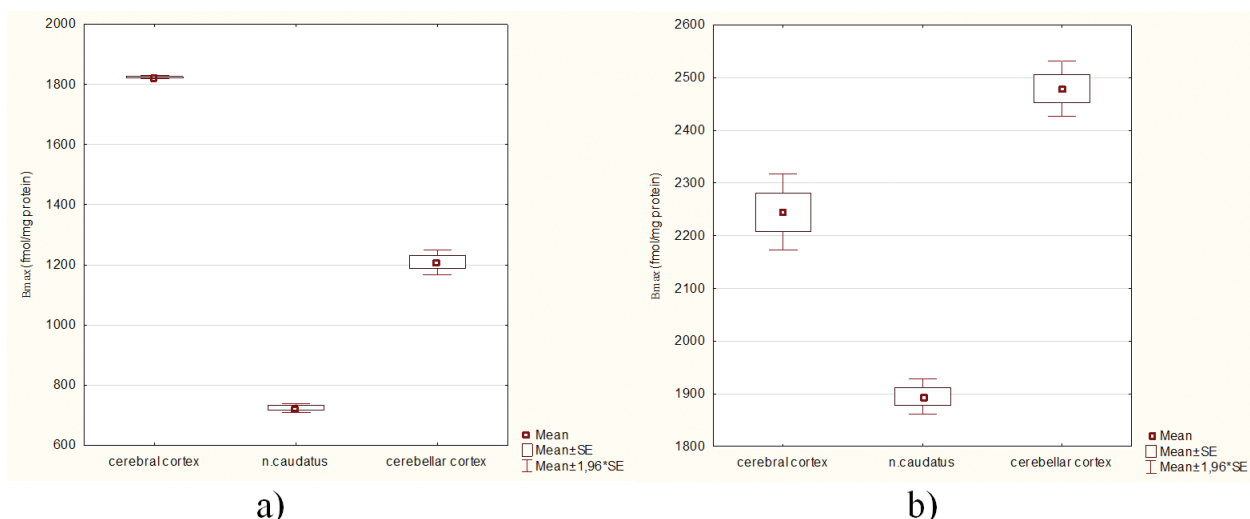
- (II) A comparative analysis of the PBR properties in the study of the binding of [ $^3\text{H}$ ]PK-11195 to the mitochondrial fraction of membranes isolated from various regions of the human brain showed that the degree of manifestation of changes in the PBR properties is not the same in the studied brain structures of patients who had alcoholism according to anamnesis. The greatest changes of PBR in comparison with the control were detected in the caudate nucleus and the cerebellar cortex (**Figures 19 and 20, Table 5**). The obtained results indicate a heterogeneous change in the properties of BzDR of selective ligands in the human brain under the influence of chronic alcoholization, which confirms the hypothesis of adaptive receptor neuroplasticity and the heterogeneity of the physiological response in various brain regions to the effect of chronic alcohol exposure [54].

The results we obtained are consistent with data from other studies showing a decrease in the function of GABA<sub>A</sub>/BzDR in the cerebral cortex in patients with alcohol dependence [36, 55]. These data confirm that the low affinity of BzDR can be a neuronal marker of the development of anxiety and conditions associated with chronic alcohol use and AAS. The study of BzDR carried out by us in various areas of the human brain (on postmortal material) showed that the properties of synaptosomal and mitochondrial receptors differ in the brain structures studied: the prefrontal cortex, the caudate nucleus and the cerebellar cortex. CBR are the sites of specific binding of ligands of benzodiazepine series, neurosteroids and alcohol to the GABA receptor, modulating its function allosteric and regulating the processes of inhibition in brain structures that affect the activity of various neurotransmitter systems, including the activity in the structures of the brain associated with the process of natural reinforcement. The higher affinity and



**Figure 19.** Statistical analysis of [ $^3\text{H}$ ]PK-11195 binding parameters [ $K_d$  (nM) – constant of dissociation ligand-receptor complex] with mitochondrial membranes in different areas of the human brain in control group (a) and study group (b) (alcoholic patients).





**Figure 20.** Statistical analysis of  $[^3\text{H}]$ PK-11195 binding parameters [ $B_{\text{max}}$  (fmol/mg of protein) – density of binding sites] with mitochondrial membranes in different areas of the human brain in control group (a) and study group (b) (alcoholic patients).

density of CBR in the caudate nucleus and the prefrontal cortex are related to their functional activity in the regulation of emotions and motivated human behavior.

The effect of ethanol causes a change in PBR not associated with  $\text{GABA}_A$ R, localized in the mitochondrial membrane, predominantly in glial cells of the brain, and providing cholesterol transfer into the mitochondria [46], thus affecting the regulation of the synthesis of neurosteroids, which are endogenous modulators of  $\text{GABA}_A/\text{BzDR}$  in the CNS [42]. Alcohol carries out some of their effects through PBR, regulating the production of neurosteroids and their metabolites, which are critical components of normal brain function [46]. Thus, PBR indirectly affects  $\text{GABAergic}$  function in the brain, mainly reacting to neurotoxic effects and various brain damage [36, 55, 56].

The data obtained by us confirm the existence of regulatory mechanisms mediating the relationship between the properties of  $\text{GABA}_A/\text{BzDR}$  caused by receptor neuroplasticity and alcohol addiction.

### 3. Conclusion

An important factor that can influence addiction liability is exposure of alcohol and other psychoactive substances during the early life period. Exposure to ethanol, early in life, can have long-lasting implications on brain function and drugs of abuse response later in life.

One of the mechanisms of action of alcohol is the ability to induce vascular spasm, which leads to hypoxia of the developing embryo and affects the retardation of development and growth of the fetus with prenatal effects of alcohol. These changes can lead to the development of fetal alcohol syndrome. Compensatory mechanism in the conditions of this pathology, leading to a decrease in the perimeter of the vessel and the area of the vessel in the cross section, is an



increase in the number of vessels in the brain [57]. Alcoholization of the mother, leading to prenatal effects of alcohol on the developing fetus, affects the dynamics of embryonic development of the circulatory system in the human brain, which manifests itself in a change in the vascularization of the growing human brain [23].

The effects of ethanol in the early stages of development can disrupt the signaling mechanisms that regulate synaptogenesis. The result was “dilution” of the structure of elementary membranes and damaged membranes are less able to establish strong contact with each other, which is probably due also to a reduced ability of cells that are in constant contact with ethanol, synthesized mediators filling synaptic vesicles. This significantly violated the formation of neuronal mechanisms underlying the susceptibility and processing of information, which in turn could adversely affect a person’s mental activity.

The data obtained by us showed a structured picture of synaptogenesis as one of the most significant periods in the formation and development of the brain, providing its functions and determining the adaptive potential in prenatal alcohol influences. The influence of prenatal ethanol on the development of synaptic structures was expressed in reduction of morphometric parameters, namely slowing the formation of synaptic contacts and reducing their formation in the brain of the embryo and fetus in the early stages of development, in contrast to the normally developing brain, which affects synaptogenesis in the developing brain of a person and can underlie fetal death or serious disorders the child in the future [23, 49, 52–54].

On the background of the decrease in the formation of synaptic structures seen here in the fetal brain during gestation under the influence of maternal alcoholism and the simultaneous decrease in the affinity of synaptosomal BzDR, the tendency to an increase in receptor density can be evaluated as neuroplastic features and compensatory reaction directed to adapting the embryo and fetus nervous system to conditions of functional insufficiency of GABAergic neurotransmission. These new data can broaden the understanding of the molecular basis of predisposition not only to alcoholism but also to various disorders associated with PAE. Children and adolescents who were under the influence of alcohol during the period of prenatal development noted functional disorders of neurocognition, self-regulation and adaptive functioning and various neurobehavioral disorders associated with PAE [58]. Plasticity of ion channels and receptors linked to ion channels regulated by neurotransmitters is significant for the realization of adaptive processes in the brain, providing synaptic plasticity for the formation and development of neural network, physiological and pathophysiological processes. Prenatal alcohol exposure (PAE) can cause irreversible physical, neurological and psychiatric impairments that are present at birth and can have lifelong implications [14, 59]. The relationship between prenatal exposure to alcohol and the frequency of behavioral disorders in children and adolescents is established. [60]. The effect remained significant compared to other variables, including environment, maternal psychopathology and some others, and can cause a different mental dysfunction associated with a violation of brain metabolism in children and adolescents in the future [61].

Similar changes in the benzodiazepine receptor binding were identified by us in the brains of patients with alcoholism also. A decrease in the ability of receptors to bind agonist ligands impairs the ligand:receptor protein ratio, leading to decreased binding of the major neurotransmitter



GABA and impairment to synaptic transmission. Our results are consistent with other studies that showed a reduction in the function of GABA<sub>A</sub>/BzDR in the prefrontal cortex in patients with alcohol dependence [36, 55]. Alcohol causes neuroplastic changes in BzDR associated with a decrease in the affinity of the receptors, a change in the conformational state of the GABA<sub>A</sub>/BzD rector complex, as a result of inhibition of the binding kinetics of BzDR by the polypeptide DBI (Diazepam Binding Inhibitor), as well as its metabolites. The endogenous peptide DBI possesses anxiogenic action and is the inverse agonist of BzDR [62]. Chronic alcohol exposure induces the expression of endogenous DBI interacting with receptors and suppresses binding affinity to [<sup>3</sup>H]-flunitrazepam.

Neuroplastic changes of GABA<sub>A</sub>R, caused by the influence of ethanol, are associated with a change in the composition of subunits of the receptor complex and change in the pharmacological sensitivity and receptor function associated with the development of tolerance to ethanol and alcohol dependence. High heterogeneity of different isoforms of subunits of the GABA<sub>A</sub> receptor ( $\alpha$ 1- $\alpha$ 6;  $\beta$ 2, $\beta$ 3) in various regions of the brain: nuclei of the basal ganglia, prefrontal cortex and limbic regions of the brain, underlies the functional differentiation of the GABA<sub>A</sub> receptor complex and provides a varying degree of modulation functions of GABA<sub>A</sub>R by ethanol in various brain structures [63]. Changes in the expression of neuronal elements induced by alcohol, leading to changes in neurotransmitter function adaptation systems in the brain associated with neuroplasticity [64].

Benzodiazepines, anxiolytics, anesthetics and alcohol are implementing some of its effects through the BzDR “central” and “peripheral” types regulating the synthesis of neurosteroids, which are critical for the provision of brain functions. Ethanol modulates GABA<sub>A</sub>/BzD receptor complex function by affecting synthesis neurosteroids *de novo* in the brain, stimulating the mitochondrial receptors of the “peripheral type” — PBR, providing the transfer of cholesterol to mitochondria and synthesizing neurosteroids, independent of the functions of the HPA axis. This mechanism can play a principal role in the central effects of alcohol. Thus, the functional activity of PBR has a modulating effect on GABAergic function in the structures of the brain, reacting to various neurotoxic effects and damage [65].

Alcohol does not have specific receptors in the brain; however, the receptor proteins are exposed to ethanol. The research of a number of authors is aimed at studying long-lasting adaptive changes (neuroplasticity), which contribute to the development of alcohol dependence. Our studies aimed at studying neuroadaptation under the influence of chronic alcohol effects on the benzodiazepine receptor system of the brain have revealed that a low affinity of BzDR can be a marker of disorders of synaptogenesis and regulatory mechanisms mediating the GABA<sub>A</sub>/BzDR bond that induces receptor neuroplasticity and alcohol addiction [41, 54, 65, 66].

BzDR “central” and “peripheral” types can be a key link to the discovery of new promising therapy for the treatment of compulsive craving for alcohol, alcohol abuse and dependence. The integration of current data and our data is necessary to define the role of GABA<sub>A</sub>R in modulating the rewarding and aversive effects of ethanol and may lead to the development of pharmacotherapy that targets GABA<sub>A</sub>/BzD receptors to treat alcoholism in human beings [65–68].



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