

Cortical Synaptogenesis in the Human Brain in Conditions of Prenatal Alcoholization

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Abstract

Objective: Shaping synaptic contact is one of the leading processes during which largely determine the future integrative brain capabilities. Prenatal exposure to ethanol may have an impact on synaptogenesis in the brain of the embryo and foetus. The purpose of this study - identify the features of synaptogenesis in the brain of embryos and fetuses in conditions of prenatal alcoholization.

Materials and Methods: 33 embryos and fetuses were obtained from female alcoholic patients. Alcoholic patients were aged 26–39 years and the duration of illness was 3–13 years. In all cases, grade II alcoholism was diagnosed (ICD - 10 F10.201, F10.202). The control group consisted of embryos and fetuses from healthy women numbering 30 people with no history of neurological or mental illnesses.

The method of electron microscopy and morphometry have been used to study peculiarities in formation of the structure of human embryo and fetus brain synapses at early stages (7 to 12 weeks) of development at mother alcoholization.

Results: Electron microscopy of material obtained from alcoholic women has shown slowing - down of formation of synaptic structure. Morphometry has revealed that under the influence of prenatal alcoholization the formation of components of synaptic brain contacts slows down at studying stages of development: the area of presynaptic terminals and their perimeter decreases, and the perimeter of postsynaptic densities decreases as well.

Conclusions: The data showed a significant effect of prenatal exposure to ethanol on the development of synaptic structures - reduction of morphometric parameters, slowing the formation of synaptic contacts on the background of reduction of their formation in the brain of the fetus in the early stages of development compared with the norm, which is reflected on during synaptogenesis in the developing brain and can lie the basis of severe disorders in the unborn child.

Graphical Abstract

Methods of electron microscopy and morphometry have been used to study peculiarities in formation of the structure of human embryo and fetus brain synapses at early stages of development at mother alcoholization.

Keywords: Alcoholism; Prenatal pathology; Brain; Embryos; Fetuses; Ultrastructure; Synapses

Abbreviations

FAS: Foetal Alcohol Syndrome; AWS: Alcohol Withdrawal Syndrome; CNS: Central Nervous System; ICD - 10: International Statistical Classification of Diseases and Related Health Problems 10th Revision

Introduction

Prenatal exposure to alcohol can lead to the development of fetal alcohol syndrome (FAS), which is apparent as a complex of disorders in the somatic and mental domains, reflecting impaired development of CNS [1-3].

The development of this syndrome often associated with alcohol withdrawal syndrome (AWS) has been shown to be associated mainly with disturbances to development of the fetal brain [4-6], starting from the very earliest stages of neurogenesis and the formation of its structures, leading to delay in neuron migration and differentiation, as well as changes in synaptogenesis [4,7-9].

However, the effects of maternal alcoholization on brain development in human embryos and the possible mechanisms of its action on nervous tissue formation and the development of synaptic structures in the brain have received insufficient study.

It has long been believed that the negative effects of ethanol are associated mainly with its influences on the lipid component of neuron membranes in the brain. In ontogenetic morphogenesis of synaptic

development contact is one of the leading processes during which largely determines the future integrative brain capabilities.

Some exogenous and endogenous factors including ethanol, entering the body of the embryo in the use of his woman during pregnancy can modify synaptogenesis [10,11] and subsequently affect the function of CNS. As lipotropic agent, ethanol is able to change the essential physico - chemical properties of cell membranes, which is reflected in the current foetal brain synaptogenesis to establish the nature of this effect, have been made now study [12,13].

The purpose of this investigation was to study the development of synaptic contacts in the brains of embryos and fetuses aged 7-12 weeks obtained from alcoholic female patients and healthy women.

Materials and Methods

The brains of human embryos and fetuses at 7-12 weeks of development were studied, obtained in compliance with the requirements of the Ethics Committee and with patients' consent during pregnancy termination procedures for medical indications. A total of 33 embryos and fetuses were obtained from female alcoholic patients and constituted the study group. Alcoholic patients were aged 26-39 years and the duration of illness was 3-13 years. In all cases, grade II alcoholism was diagnosed (ICD - 10 F10.201, F10.202). Diagnoses of alcoholism were established at the Department of Addictive States, Research Institute of Mental Health, Siberian Branch, Russian Academy of Medical Sciences. The control group consisted of embryos and fetuses from healthy women with no history of neurological or mental illnesses. Women of the control group numbering 30 people were of comparable age as alcoholic patients.

Significant information was obtained by using embryonic material only from cases in which there were no harmful influences with additional effects on embryo brain development (radiation, chemical substances, certain medical drugs, and maternal diseases during pregnancy, i.e., influenza, rubella, toxoplasmosis, etc.). Embryonic and fetal brain specimens were fixed and embedded in Araldite. Ultrathin sections were cut on an Ultracut - E (Austria) microtome, contrasted with uranyl acetate and lead citrate using the Reynolds method, and examined under JEM - 100B and JEM - 100CX electron microscopes. Possible errors were excluded by obtaining images at the same magnifications (36,000 or 48,000). This was because of the characteristics of embryo tissue, such as the small number of synapses at various stages of development and the sizes of synaptic connections themselves. Electron microscopy studies addressed the intermediate layer of the wall of the forebrain, which is an accumulation of neuro - and glioblasts (including microglial cells), between which blood vessels start to grow. Morphometric analysis was performed using photographic prints from 6-9 cm negatives obtained from the electron microscopes. Prints from negatives were made by 1:1 contact printing; images were then scanned in grayscale with a resolution of 300 dpi and stored in TIFF format without compression. 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. Totals of 15 - 20 microphotographs from different parts of the intermediate layer were used in each case. Measurement results are presented in relative units, as the number of pixels for assessment of length, and the number of image pixels squared for assessment of the areas of the structural components of synapses.

Statistics

Experimental work was carried out in the Laboratory of Neuroimmunology and Neurobiology Mental Health Research Institute (Tomsk). All the studies were approved by the Ethics Committee of the Mental Health Research Institute.

Results

In embryonic human in early period of development, starting with the 7-8th week, desmosome - like contacts are prevalent. Contacting membranes are in their middle part of this thickening, which both sides approach to each other, forming a fissure. In places of the thickening the membrane can be connected. This electron - dense material is most substantial in the field of adhesion. Contacts of this type are found between bodies of cells and different dendrites. In later terms of the development (weeks 9 - 10) indicated types of the contacts are found more seldom. Junctions with presence of vesicular elements have been distinguished. Synaptic vesicles usually had a rounded shape and bright center and their diameter was about 40 nm. The fissure width of immature synapses was about 20 nm. The length of the sealing membrane area reached 0.1 - 0.15 μm (Figure 1).

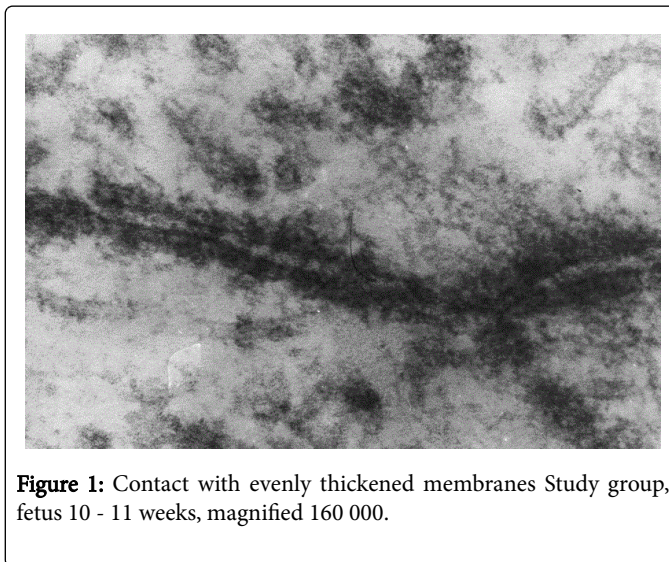


Figure 1: Contact with evenly thickened membranes Study group, fetus 10 - 11 weeks, magnified 160 000.

The emergence of single synaptic vesicles near the presynaptic membrane is believed the transitional stage from synapses - like contacts to their true synaptic form. In general, these synapses could be called functionally competent. They are located predominantly in the lower boundary of the intermediary layer of the cerebral cortex (Figure 2).

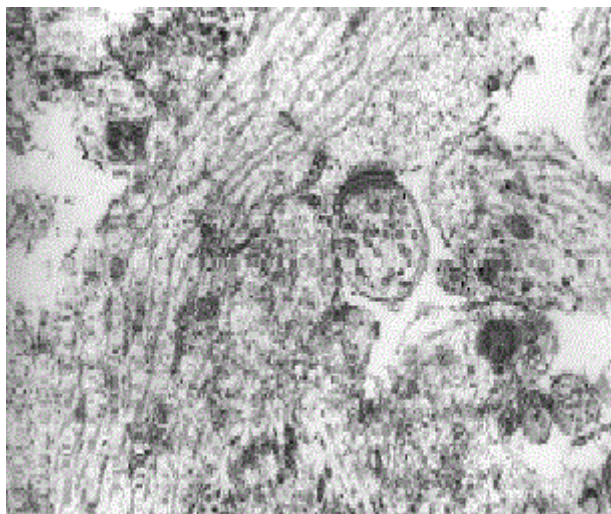


Figure 2: Functionally competent synapse Control group, fetus 10 - 11 weeks, magnified 60000.

At the stage of 10 - 12 weeks the number of synapses with relatively mature structures increases. More likely they could be found at the boundary of ventricular and intermediary layers, and in the intermediary layer of nerve cells and the cortical plate. Synaptic contacts have all the necessary components, differing from the mature brain synapses, fewer synaptic vesicles. Synaptic contacts on neuroblasts and glioblasts differed from synapses of the mature brain with smaller number of synaptic vesicles. All the above features were characterized as the control and basic groups of embryos (Figures 3 and 4).

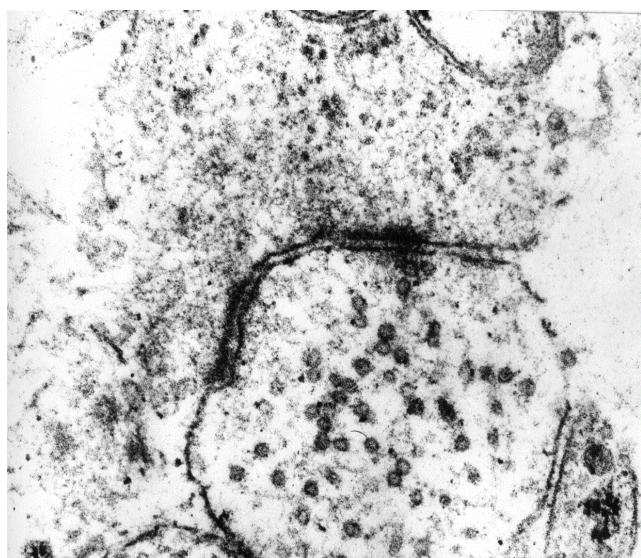


Figure 3: Completely formed functionally competent synapse Study group, fetus 11 weeks, magnified 70000.

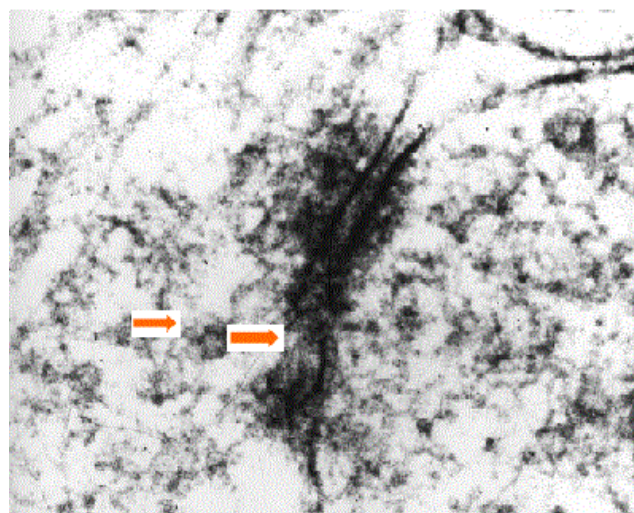
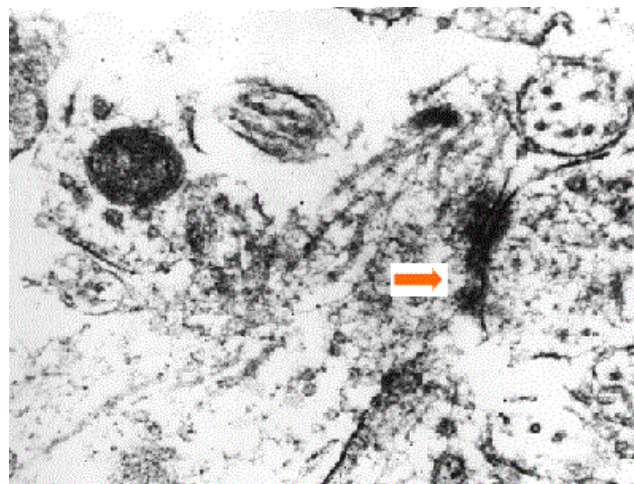


Figure 4: Single synaptic vesicles in the area of forming contact Study group, fetus 12 weeks. Above - magnified 60 000 (top electron diffraction); Under - magnified 144 000 (lower electron diffraction).

On inputs received from women - alcoholics, found slowing the formation of synaptic structures. It forms of non - synaptic compounds in frequency and structure did not differ from the controls. Fully formed structure of synaptic connections in the appearance of synaptic vesicles is compared with the control, but the area of core synapse was less.

Analysis of the morphometric parameters of synapses started with a general evaluation of the study and control groups without separating materials on the basis of development periods. Significant ($p < 0.01$) decreases in all parameters of developing synaptic structures were seen in the study group as compared with the control group.

More detailed analysis of synapse parameters was then performed, taking cognizance of embryo and fetus developmental period (Table 1 and Figures 5-7). The period 7-8 weeks of development was the most difficult for analysis, as the number of synaptic connections at this stage of development was very small. The results obtained show that at 7-8 weeks of development, postsynaptic density lengths were shorter than in controls, though the difference was not statistically significant ($p > 0.1$). Presynaptic terminal perimeters and areas could not be analysed for this period, as the presynaptic components of synaptic

contacts at this period were generally located on large dendrites. Thus, at 7-8 weeks, we found a minor decrease in postsynaptic density length in the study group, though this difference did not reach statistical significance. At nine weeks of development, there was extensive development of synaptic contacts, especially at the upper margin of the intermediate layer. The lengths of postsynaptic densities, presynaptic terminal perimeters, and presynaptic terminal areas were significantly smaller ($p < 0.01$) in the study group than in controls.

Stage of development	7 - 8 weeks		9 weeks		10 weeks		11 weeks	
	C M ± SE N = 210	S M ± SE N = 210	C M ± SE N = 210	S M ± SE N = 210	C M ± SE N = 210	S M ± SE N = 210	C M ± SE N = 210	S M ± SE N = 210
Lenths 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	-	-	54521 ± 2673	48861 ± 6773*	66964 ± 3833	63178 ± 3168*	75742 ± 3207	66750 ± 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*

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

Table 1: Morphometric parameters of synapses in the human brain at different stages of embryonic development.

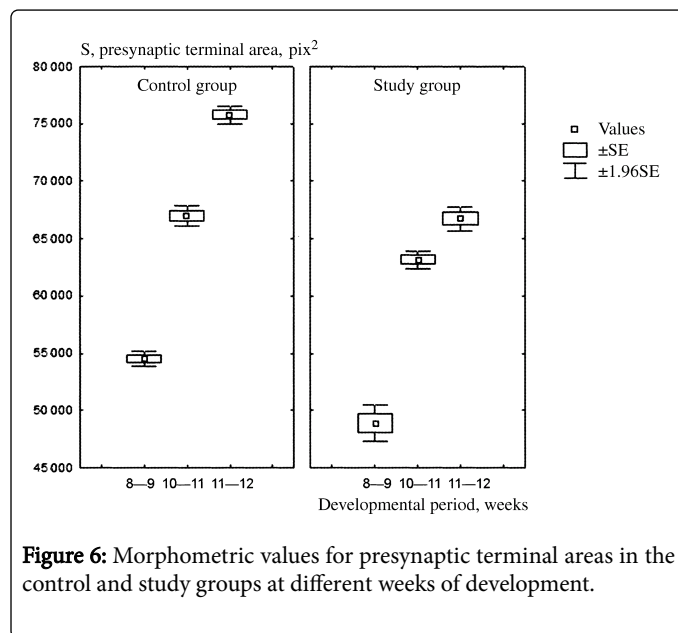
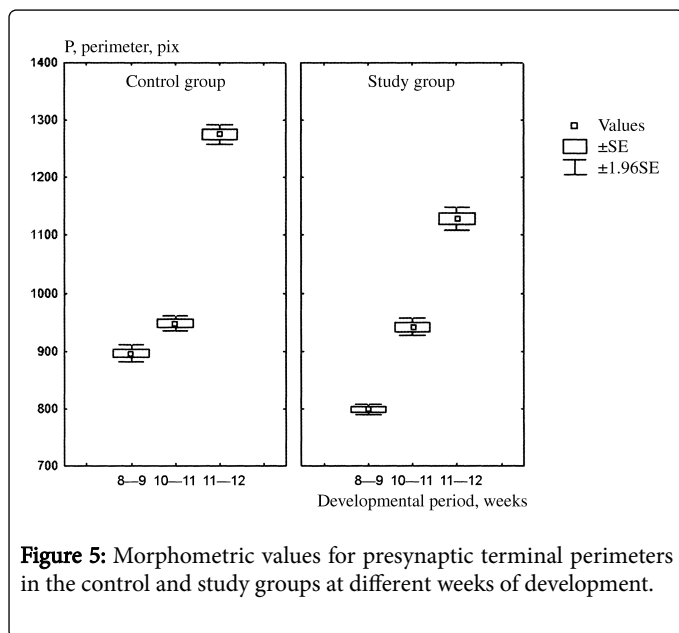


Figure 5: Morphometric values for presynaptic terminal perimeters in the control and study groups at different weeks of development.

Figure 6: Morphometric values for presynaptic terminal areas in the control and study groups at different weeks of development.

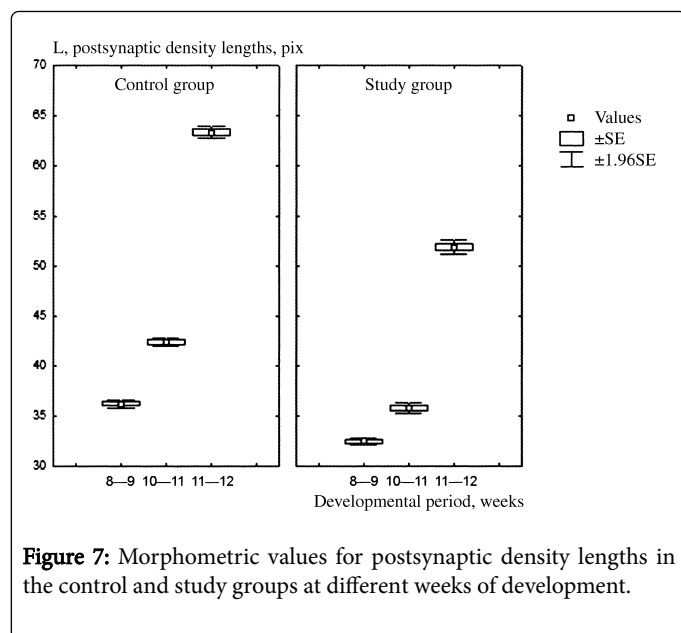


Figure 7: Morphometric values for postsynaptic density lengths in the control and study groups at different weeks of development.

The 10 - week period was also characterized by changes - reductions - in some of the morphometric parameters in the study group as compared with controls, i.e., there was shortening of postsynaptic densities and a decrease in the areas of presynaptic terminals ($p < 0.01$), though quantitatively, presynaptic terminal perimeters were not different.

At 11 - 12 weeks of development, significant differences from controls were seen in all morphometric parameters in the study group - synaptic contact postsynaptic density lengths ($p < 0.01$), presynaptic terminal areas ($p < 0.01$), and presynaptic terminal perimeters ($p < 0.01$). In addition to these quantitative parameters, it was noted that most synapses analyzed at 11 - 12 weeks were axodendritic positively flexed synapses with small numbers of synaptic vesicles and occasional mitochondria in the presynaptic parts of synapses.

Thus, morphometric analysis showed that embryo and fetus brains from alcoholic patients were characterized by delayed synapse development, reflecting structural immaturity. This may be associated with the direct effect of alcohol on nerve cells, primarily its membranotropic action.

Discussion

Compelling evidence has shown that the developing brain is vulnerable to the damaging effects of ethanol. We found that in cells of brains of embryos and fetuses from study group (obtained from women suffering from alcoholism) slowing down of formation of synaptic structures as compared with the norm may be the cause of an alteration of neuromediator transmission.

As shown by morphometric studies, alcoholism affects the development of the important from the point of view of the structural organization of the synapse parts - reduces the perimeter and the area of the presynaptic terminal. This indicates a decreased ability of synapses involved in the transmission of impulses to neighboring cells. It is a logical extension of the observed decrease in postsynaptic densities. Similar developmental disorders of synaptic contacts in the culture of hippocampal tissue making it ethanol solution was observed by other researchers [10,11].

The data showed a significant effect of prenatal exposure to ethanol on the development of synaptic structures - reduction of morphometric parameters, slowing the formation of synaptic contacts on the background of reduction of their formation in the brain of the fetus in the early stages of development compared with the norm, which is reflected on during synaptogenesis in the developing brain and can lie the basis of severe disorders in the unborn child [2-4].

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 membrane - action. The consequence of this was the "thinning" of the structure of elementary membranes and damaged membranes are less able to establish close contacts with each other, which is probably also connected with reduced ability of cells that are in constant contact with ethanol, synthesize neurotransmitters, which fill the synaptic vesicles [14]. This greatly disturbed neuronal mechanisms underlying the susceptibility and processing of information, which in turn may adversely affect the mental activity of the individual.

Prenatal exposure of ethanol to brain human embryos and fetuses in material breach formation of neuronal mechanisms underlying sensitivity and processing, which in turn can affect the mental activities of the individual, that, in turn, can affect the mental activity of the individual [15].

References

1. Frost EA, Gist RS, Adriano E (2011) Drugs, alcohol, pregnancy, and the fetal alcohol syndrome. *Int Anesthesiol Clin* 49: 119-133.
2. Jones KL, Smith DW, Hanson JW (1976) The fetal alcohol syndrome: clinical delineation. *Ann N Y Acad Sci* 273: 130-139.
3. Olson HC, Morse BA, Huffine C (1998) Development and Psychopathology: Fetal Alcohol Syndrome and Related Conditions. *Semin Clin Neuropsychiatry* 3: 262-284.
4. Bookstein FL, Streissguth AP, Connor PD, Sampson PD (2006) Damage to the human cerebellum from prenatal alcohol exposure: the anatomy of a simple biometrical explanation. *Anat Rec B New Anat* 289: 195-209.
5. Singh AK, Gupta S, Jiang Y, Younus M, Ramzam M (2009) In vitro neurogenesis from neural progenitor cells isolated from hippocampus region of the brain of adult rats exposed to ethanol during early development through their alcohol - drinking mothers *Alcohol Alcohol* 44: 185-198.
6. Olney JW, Wozniak DF, Jevtovic - Todorovic V, Farber NB, Bittigau P, et al. (2002) Glutamate and GABA receptor dysfunction in the fetal alcohol syndrome. *Neurotox Res* 4: 315-325.
7. He J, Nixon K, Shetty AK, Crews FT (2005) Chronic alcohol exposure reduces hippocampal neurogenesis and dendritic growth of newborn neurons. *Eur J Neurosci* 21: 2711-2720.
8. Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, et al. (2000) Ethanol - induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science* 287: 1056-1060.
9. Klintsova AY, Helfer JL, Calizo LH, Dong WK, Goodlett CR, et al (2007) Persistent impairment of hippocampal neurogenesis in young adult rats following early postnatal alcohol exposure *Alcohol Clin. Exp Res* 31(12): 2073-2082.
10. Bakkum BW, Benevento LA, Cohen RS (1991) Effects of light - dark and dark - rearing on synaptic morphology in the superior colliculus and visual cortex of the postnatal and adult rat *J Neurosci Res* 28: 65-80.
11. Yanni PA, Lindsley TA (2000) Ethanol inhibits development of dendrites and synapses in rat hippocampal pyramidal neuron cultures. *Brain Res Dev Brain Res* 120: 233-243.
12. Levine GH, Maglio JJ, Horwitz J (2000) Differential effects of ethanol on signal transduction. *Alcohol Clin Exp Res* 24: 93-101.

13. Narahashi T, Kuriyama K, Illes P, Wirkner K, Fischer W, et al. (2001) Neuroreceptors and ion channels as targets of alcohol. *Alcohol Clin Exp Res* 25: 182S-188S.
14. Berman RF, Hannigan JH (2000) Effects of prenatal alcohol exposure on the hippocampus: spatial behavior, electrophysiology, and neuroanatomy. *Hippocampus*. 10: 94-110.
15. Guerri C, Pascual M (2010) Mechanisms involved in the neurotoxic, cognitive, and neurobehavioral effects of alcohol consumption during adolescence. *Alcohol* 44: 15-26.