

Prenatal Alcohol Exposure and Dysfunction of Hippocampal Formation in Cognition

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Background: Fetal alcohol syndrome is associated with numerous problems in the development and function of the brain. Learning and memory deficits are among well known effects of prenatal exposure to alcohol.

Objective: This study aimed to examine the spatial working memory of 60-day old rats who were exposed to alcohol during their fetal life and to find the relation between the possible alcohol-impaired spatial memory and gestational period of exposure to alcohol.

Materials and Methods: Pregnant rats in different stages of gestation period were administered with ethanol. Using a radial arm maze, the offspring were subjected to spatial working memory training at 60 days of postnatal age.

Results: The rats exposed to ethanol during the first 10 days of fetal life indicated lower performances compared to the controls. Those receiving alcohol during the second half of pregnancy period had no problem in maze navigation. Behavior of the animals exposed to alcohol during the first and the second quarter of the gestation period demonstrated that only the latter were weak in solving maze tasks. The groups related to the third and the fourth quarter of gestation period had a similar behavior with the control group. Comparison of the animals' performances in all groups revealed that only the second quarter group was the most disadvantaged.

Conclusion: Our data indicates that the second quarter of the gestation period is more sensitive to harmful effects of alcohol on the areas of brain involved in learning and memory. Since the hippocampus is central in cognitive functions and this part of brain is highly vulnerable to alcohol effects it can be concluded that the hippocampus is mostly affected in the second quarter of prenatal life.

Key words: Fetal alcohol syndrome, Hippocampus, Learning and memory, Radial arm maze

Introduction

Fetal alcohol syndrome was first described in the early 1970s to describe a pattern of birth defects found in children of mothers who consumed alcohol during pregnancy (Jones and Smith, 1973). Fetal alcohol syndrome is defined by four criteria: maternal drinking during pregnancy; a characteristic pattern of facial abnormalities; growth retardation; and brain damage, which often is manifested by intellectual difficulties or behavioral problems (Stratton *et al.*, 1996). Alcohol or its metabolic breakdown products can interfere with brain development by altering the

production or function of natural regulatory substances that promote the orderly growth and differentiation of neurons (Michaelis and Michaelis, 1994). The regions of the brain that are most seriously affected by prenatal alcohol exposure in terms of ability to function are basal ganglia (Mattson *et al.*, 1996b), which its damage impairs spatial memory and set shifting in animals (Mattson *et al.*, 1996b; Mattson and Riley, 1999) and various cognitive processes in humans (Bannister, 1992); cerebellum (Sowell *et al.*, 1996), a structure involved in balance, gait, coordination, and cognition (Riley *et al.*, 1995); corpus callosum, a band of nerve fibers that forms the major communication link between the right and left halves of the brain; and hippocampus (Berman and Hannigan, 2000), a structure that lies deep within the temporal lobe of the brain and is involved in memory. The behavioral and cognitive

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impairments associated with fetal alcohol syndrome reflect underlying structural or functional changes in the brain (Roebuck *et al.*, 1998). Children prenatally exposed to alcohol exhibit a variety of problems with language and memory (Stratton *et al.*, 1996; Janzen *et al.*, 1995). Mattson *et al.*, (1996a) found that children with fetal alcohol syndrome ages 5 to 16 learned fewer words compared with a group of children of comparable mental age who did not have fetal alcohol syndrome. Spatial cognitive processing allows animals to form cognitive maps of their environment to facilitate learning and memory. Spatial memory has been represented as a two-part code, working (short-term) and reference (long-term) memory (Honig, 1978). Working memory is retained only long enough to complete a particular task, after which the information is discarded because it is no longer needed. In contrast, reference memory is retained for longer periods because it is needed to complete successive tasks. Spatial working memory is needed to remember information that is different in specific content over time (Olton and Papas, 1979). Acute ethanol administration has demonstrated impairments in both spatial and non-spatial working memory tests (Gibson, 1985; Givens, 1995). Just as critical periods are being described for effects of neonatal alcohol exposure (West and Goodlett, 1990; Goodlett and Johnson, 1999), there are likely to be critical periods for the effects of prenatal alcohol exposure on brain development. If so, critical periods may exist for the postnatal ameliorative effects of environmental enrichment, and these should be identified. Animal models, particularly those using rodents, are powerful tools for determining the mechanisms and outcomes of early alcohol exposure because the physiological responses to alcohol in development are similar to those in humans (Hannigan and Abel, 1996). Considering working memory tasks this study aims to examine the spatial working memory of 60-day old rats who were exposed to alcohol during their fetal life and to find the relation between the possible alcohol-impaired spatial memory and gestational period of exposure to alcohol.

Materials and Methods

Animals

Sixty eight male and female Wistar rats at 60 postnatal days of age (on arrival to the testing room) were used in this study. The experiments were principally carried out on 7 groups of rats as described later. Rats were maintained on a 12:12 light-dark cycle. The animals had free access to

drinking water and food. During the experiments, however, water was available freely but there was a restricted feeding schedule to maintain body weight of approximately 85% of free feeding levels. One hour daily feeding took place after the second trial (see below).

Breeding

Each female rat was placed with a male overnight and the cages were examined the next morning for evidence of a sperm plug. If a plug was found, the dam was assumed to be pregnant and the day was marked as day 0 of pregnancy period.

Alcohol administration

The pregnant rats were assigned to one of 7 groups based on the period of alcohol administration. Two groups of the pregnant rats were administered alcohol during the first (n=9) and second (n=11) half (10 days) of pregnancy period. Also, four groups were receiving alcohol during first (n=7), second (n=15), third (n=8) and fourth (n=6) quarter (5 days) of pregnancy period. Rats in control group (n=12) received only tap water. Ethanol (30%w/v) was solved in water and animals had free access to it orally. This procedure typically produces an alcohol concentration of about 5.7g/kg (Nio *et al.*, 1991). Pups were reared with their mothers until 30 days of postnatal age. Then, they were grouped into males and females and kept in separate cages. The animals were transferred to the test room 5 days prior to the experiments.

Apparatus

The apparatus was a radial arm maze (made by Razi Rad Company) elevated 70 cm off the floor. It consisted of 8 arms (70 × 15 cm) radiating from a central platform (30 cm in diameter). Unclear doors made of Plexiglas were located at the entrance of each arm. A small food cup was placed at the end of each arm.

Experimental design

The experiments were designed for evaluation of spatial working memory and memory persistent test. The animals were tested throughout the experiments in a test room which consisted of numerous visible visuospatial cues.

Shaping

The rats were shaped with the maze for two days prior to the experiment. During two trials of the first day, the animals were placed in the maze so

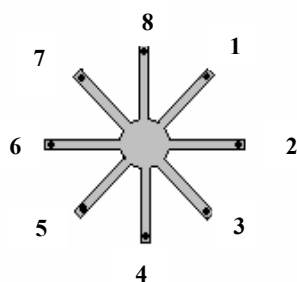


Figure 1. The radial arm maze used in this study. The maze had 8 arms separated from a central arena by movable guillotine doors. The arms were numbered clockwise from 1 to 8. Thick spots indicate the place of the food cups at the end of each arm.

that they could obtain food rewards that were scattered throughout the maze. In the first trial of the second day, they were placed in the maze and could freely search the maze and eat food rewards placed only in the food cups at the end of the arms. In the second trial of the second day, the doors controlling the entrance in the arms were closed and opened regularly to accustom rats with the movements of the doors.

Working memory procedure

During the working memory experiments the animals had to learn to visit each of eight baited arms once within a trial (Fig. 1). The maze was an open radial maze so that extramaze cues at the testing room were simply visible. Rats were given two trials per day with 4 hours inter-trial interval. The first and second trials were done at about 10 a.m. and 2 p.m., respectively. A trial started with the animal placed in the central platform with all the doors closed. After 30 seconds, all the doors were opened simultaneously and the animal was free to choose an arm and enter. Then, all the doors but that of the selected arm were closed. Once the rat had explored the selected arm and had come back into the central area, the door of the arm was closed, confining the animal for 10 seconds. This was to test spatial memory without interference from stereotypic behaviors. Then, all the doors were opened again and the same procedure was repeated. The session continued until all the baited arms were entered or 10 min had elapsed. Experiment for each animal was continued until two consecutive sessions in which the animal

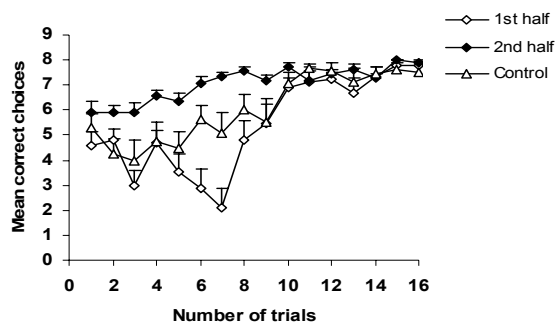


Figure 2- Graph to show the mean correct choices for each trial and group. Analysis of variance reveals a noticeable difference between the rats exposed to ethanol within the first and second half of pregnancy ($P < 0.05$). Statistical analysis applied to the first 8 trials of experiment showed that the performances were different between the first half and control group ($P < 0.01$), the second half and control group ($P < 0.05$) and between the two test groups as well ($P < 0.001$).

entered all 8 baited arms in 8 or maximum 9 selections (the criterion for the working memory tasks).

Memory persistence procedure

To examine persistency of the working memory, the animals were subjected to another set of experiments (as memory persistence test) with the same protocol 5 days after the working memory experiment finished. During the interval period the rats were kept in the condition as described for animal housing and both food and water were freely available.

Statistical analysis

The performances were evaluated in terms of number of the correct choices during each trial. One way analysis of variance followed by Student-Newman-Keuls test was applied to the data to compare the results pooled from the control group and the test group exposed to alcohol in different stages of pregnancy. All the data are presented as mean \pm S.E.M and a P value of less than 0.05 considered significant.

Results

Effect of prenatal alcohol exposure on working memory tasks

- First and second half of pregnancy period:

In the first step of this study two groups of rats receiving alcohol during first or second half of the pregnancy were subjected to working memory experiments. Comparison of the data pooled from these groups with the control group display a

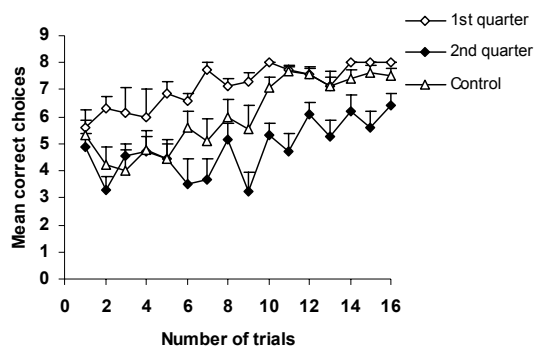


Figure 3- A comparison of performances between the control group and those exposed to alcohol during the first and second quarter of life period. Statistical analysis indicates a $P < 0.001$ for significance of the difference between performance of the second quarter group and both the control the first quarter groups.

significant difference between the rats performances over 16 trials ($F_{2,57}=4.17$, $P=0.0204$). Post hoc test indicates a higher performance of the animals exposed to ethanol during the second half of the prenatal life ($P < 0.01$). Application of analysis of variance to first 8 trials of the experiment showed a significant difference between behavior of the control group against both groups receiving alcohol ($F_{2,27}=21.596$, $P < 0.0001$). The post test shows that the control group performed better than the second half one ($P < 0.01$). Opposite was the case concerning comparison of the control and the first half groups with a higher behavior in the latter ($P < 0.05$). Figure 2 illustrates performance of the animals exposed to alcohol and the control group.

- First and second quarter of pregnancy period: The data from the first stage of the experiment showed that the ethanol administration during the first half of the fetal life results in an obvious deficit in the working memory test. Hence, the study was continued on the rats exposed to ethanol within the first half of fetal life. The trainings were conducted on two groups of rats born from the mothers receiving alcohol during the first or the second quarter of the gestation period. The findings of this stage of the experiments are represented in the figure 3. Analysis of variance revealed a general difference between the performance of the two groups and the control rats as well ($F_{2,56}=23.391$, $P < 0.0001$). Interestingly, both the control and the first quarter groups displayed further correct choices than the rats exposed to alcohol during the second quarter of prenatal life ($P < 0.01$).

- Third and fourth quarter of pregnancy period: To ensure how the animals from mothers

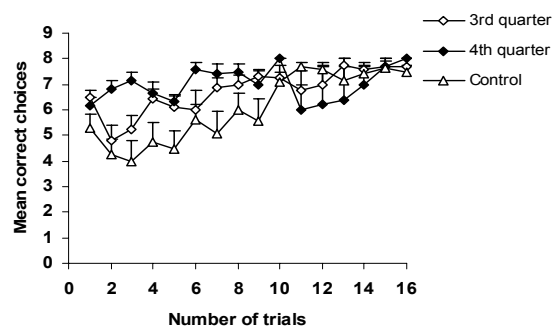


Figure 4- Comparing the performance of the control animals and those exposed to alcohol over the third and fourth quarter of the fetal life showed a similar behavior across 16 trials ($F_{2,53}=1.514$; $P=0.2294$).

receiving alcohol in the second half of the pregnancy period solve the working memory tasks we also broke the second half period to two quarters, that is, the third and the fourth quarters of pregnancy period. Thus, the working memory tests were performed on the rats exposed to alcohol during the third and the fourth period of fetal life. As illustrated in the figure 4 no statistical difference was evident between the performance of the two groups and the control rats ($F_{2,53}=1.514$, $P=0.2294$). Application of analysis of variance to the data from the control animals and all groups exposed to alcohol in different stages of prenatal life is indicating a significant difference between them ($F_{6,128}=11.38$, $P < 0.001$). Figure 5 shows that the second quarter group displays the lowest performance in searching the radial maze. This again implies that the rats receiving alcohol in the second 5 days of pregnancy period are more sensitive to ethanol impairment of spatial learning.

Effect of prenatal alcohol exposure on memory persistence tests

Five days after the last session in which the animals acquired the criterion for working memory (entry to all 8 arms of the radial maze during 8 or maximum 9 selections) the animals were introduced to another set of experiment called memory persistence test. We designed this series of experiments to understand persistency level of the learned tasks during the working memory trials. This experiment included the same protocol as in the working memory training. As is shown in figure 6 different groups began with a considerable level of remembering, however, with no improvement across the 4 trials. Nevertheless analysis of variance appeared a general variation

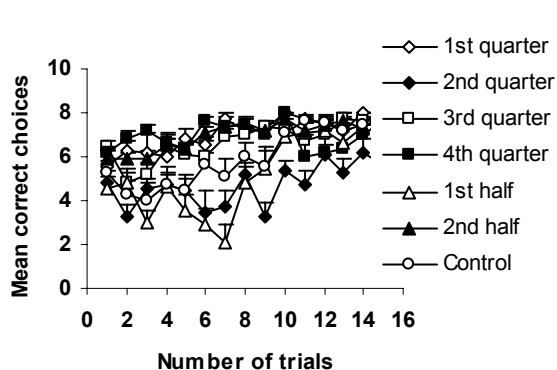


Figure 5- Performance of the control group against all groups exposed to ethanol during different stages of prenatal age. Statistical analysis indicates a general significant difference between all groups ($F_{6,128}=11.38, P<0.001$). Post hoc test shows a noticeable variation between the second quarter group and, the control ($P < 0.001$) and the first quarter ($P < 0.001$) groups.

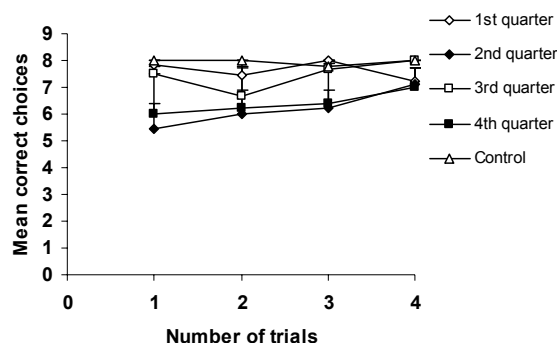


Figure 6- Memory persistence experiments in the control group and the four groups exposed to ethanol during the different periods of prenatal life. The second quarter group performed lower compared with the first ($P < 0.01$) and the third quarter ($P < 0.05$) as well as the control ($P < 0.001$) groups. Also, it was the case for the fourth quarter group where the animals exposed to alcohol in this period of pregnancy had less correct choices than the control group ($P < 0.001$).

between performance of the animals belonging to different quarters of the fetal life and controls as well ($F_{4,15}=10.546, P=0.0003$). Particularly of interest, the post hoc test indicates a noticeable difference between the second quarter group with the control ($P < 0.001$) as well as the third quarter ($P < 0.05$) groups. A pronounced variation was also evident between behavior of the fourth quarter and the control groups ($P < 0.001$).

Effects of alcohol on spatial memory acquisition in the first and second half of the prenatal life

Ethanol exposure during the first 10 days of gestation resulted in deficits in acquisition of working memory tasks. The learning problem was more obvious in the first half of the experiment; however, the animals showed to learn like controls as training progressed. The rats exposed to ethanol during the second half period of prenatal age had no problem in solving the maze tasks. To here, we concluded that the rats receiving alcohol during the first half of prenatal life are sensitive to effects of alcohol on learning deficiency.

Effects of ethanol on spatial memory acquisition in different quarters of the prenatal life

To ensure ethanol sensitivity of the first half of pregnancy further experiments were conducted on the rats born from dams receiving alcohol in this period. Two groups of two months old rats exposed to ethanol in the first and second quarter of the fetal life were tested for 16 trials. This series

of the experiment showed that the second quarter group had a lower performance in comparison to the first as well as the control group.

From these results we hypothesized that, in the first half of the gestation period, the second quarter is more liable to harmful effects of alcohol on spatial learning and memory. To make sure about this hypothesis we repeated experiments on the second quarter group ($n=9$) and the control group ($n=5$) as well. Interestingly, two different groups of the rats exposed to alcohol during second quarter of prenatal life presented an analogous behavior in working memory trainings. Also, the data from the second control group match those from the first one. Training carried out on the rats exposed to ethanol during the third and fourth period of the fetal life indicated no variation when compared to the control group. These results consistently confirm those from the animals received alcohol in the second half of prenatal life. Application of analysis of variance to the data pooled from all experiments also revealed that the second quarter group differently met our criterion in acquisition of learning and memory tasks; they displayed the lowest performance among the seven groups involved in this study.

Effects of alcohol on remembering the learned tasks

In the memory persistence experiments we tried to explain the degree of persistency of what the animals had learned in the working memory trials. In this stage, again, the second quarter group appeared to elicit the weakest performance compared to the other groups.

Discussion

Marked sensitivity of the second 5 days of prenatal life to alcohol exposure

Our findings confirm the general idea that fetal alcohol syndrome conflicts learning and memory. Behavioral deficits have been reported in animals used for testing task acquisitions in radial maze procedures. Reyes *et al.* (1989) were the first to report deficits in acquisition in two months old rats tested on eight arm radial mazes. Stone *et al.* (1996) also reported deficits in radial arm maze performance in prenatal alcohol-exposed rats. Our findings uncover that leaning problems associated with prenatal alcohol exposure are specific to a narrow period of gestation; the second quarter of fetal life. Even exposure to ethanol during a part of gestation can produce some cognitive deficit which is termed "fetal alcohol effect" (Clarren and Smith, 1978). It has been shown that during the first trimester of human pregnancy alcohol interferes with the migration and organization of brain cells (Clarren *et al.*, 1992). Alcohol drinking during the second trimester, particularly from the 10th to 20th week after conception, seems to cause more clinical features of fetal alcohol syndrome than at other times during pregnancy (Renwick and Asker, 1983). During the third trimester the hippocampus is greatly affected, which leads to problems with encoding visual and auditory information (Coles, 1991).

It also is generally accepted that early neonatal period of development in rodents shares a number of functional and maturational similarities with third trimester gestational development in humans (Dobbing and Sands, 1979), and neonatal alcohol exposure in the rat is used to model third-trimester alcohol exposure in human Fetal Alcohol Syndrome. Hall *et al.* (1994) demonstrated that gestational alcohol exposure from days 7 to 3 was sufficient to impair radial maze acquisition when rats were tested as either juveniles (26 postnatal days) or adults (80 postnatal days). This, at least partly, verifies the data presented here on the rats at 60 postnatal days exposed to alcohol during days 5 to 10 of gestation. However, Neese *et al.* (2004) reported spatial working memory deficits in female offspring born to mothers treated with ethanol during the third week of gestation in comparison from mothers treated during either the first or second weeks of gestation.

Role of hippocampus in learning and memory and its susceptibility to alcohol damages

The hippocampal system is critical for spatial learning and memory (O'Keefe and Nadel, 1978).

Lesions to the hippocampus or its afferents impair the learning of spatial tasks (O'Keefe *et al.*, 1976). Behavioral studies have supported the hypothesis that the hippocampus might be affected in children with prenatal alcohol exposure. For example, people with prenatal alcohol exposure have been reported to exhibit deficits in spatial memory as well as other memory functions associated with the hippocampus (Uecker and Nadel, 1996). In fact, even prior to the definition of fetal alcohol syndrome, it was known that placentally transferred alcohol accumulates in the fetal hippocampus of rodents and primates (Ho *et al.*, 1972). Because prenatal exposure to ethanol alters hippocampal anatomy and because the hippocampus is critically involved in spatial cognitive processing, it can be predicted that prenatal exposure to ethanol would impair spatial cognitive processing (Blanchard and Riley, 1987; Hall *et al.*, 1993; Reyes *et al.*, 1989). Numerous studies indicate that it is, in fact, the case. The exact mechanism for ethanol's effects on the hippocampal system has not yet been determined. One possibility is that ethanol decreases the spatial specificity of hippocampal pyramidal neurons or place cells (White and Best, 2000). Also, ethanol's blockade of N-methyl-D-aspartate receptors in the hippocampus could produce similar cognitive impairments (Lovinger *et al.*, 1989).

Taken together, the present results demonstrate that drinking alcohol during pregnancy undermines cognitive ability in offspring. Particularly, we found that the second 5 days of the gestation period in rats is more sensitive to deleterious effects of alcohol on spatial learning and memory. Since the hippocampus plays a central role in spatial learning and memory and confirm the vulnerability of this area of brain to alcohol during fetal life, it can be concluded that weak performance of the second quarter group in this study is due to serious damage to hippocampus.

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