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A Critical Period for Deleterious Effect of Prenatal Alcohol Exposure on Working Memory

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Abstract: The present study aimed to locate a critical period for ethanol-induced spatial task impairments in the animals exposed to alcohol during the fetal life. Two month old rats born from mothers treated with alcohol (5.7 g kg⁻¹) during the 1st half (FH) and 2nd half (SH) and, the 1st (FQ), 2nd (SQ), 3d (TQ) and 4th quarter (FoQ) of gestational period were subjected to the working memory task. The experiments were carried out on an eight arm radial maze across 16 trials. The data presented here indicate that the animals in the FH group displayed a poor performance while those in the SH group had no problem in performing the maze task. Thus, it was concluded that the FH of pregnancy period is more sensitive to toxic effects of ethanol on the spatial task. Also, the FQ and SQ group's performance indicated that the latter was significantly weak in solving the maze task. The animals related to the TQ and FoQ groups displayed a similar behavior with the control rats. Findings of this study demonstrated that drinking alcohol during pregnancy underlies cognitive phenomena in offspring. Noticeably, the second 5 days of the fetal life in the rats is more susceptible to deleterious effects of alcohol on the spatial tasks.

Key words: Critical period, fetal alcohol syndrome, radial maze, working memory

INTRODUCTION

Fetal alcohol syndrome, related to alcohol exposure during pregnancy, was first defined to describe a spectrum of birth defects that can negatively affect a child's growth, cognition, physical appearance and behavior over the lifespan (Jones and Smith, 1973; Sokol *et al.*, 2003). Although all organ systems seem to be sensitive to alcohol's effects, the brain is particularly vulnerable (Riley *et al.*, 2004). During fetal life, embryonic cells destined to become brain neurons grow in number, move to their ultimate locations and mature into a wide variety of functionally distinct neuronal cell types, eventually forming connections with other brain cells in a predetermined pattern. The mechanisms that underlie alcohol-induced fetal brain damage have been studied in experimental animals and in cultured nerve cells (Michaelis and Michaelis, 1994). Alcohol or its metabolic breakdown products can interfere with brain development by altering the production or function of natural regulatory substances that help promote the orderly growth and differentiation of neurons (Michaelis and

Michaelis, 1994). Particularly, fetal alcohol syndrome often is manifested by intellectual difficulties (Stratton *et al.*, 1996) neurobehavioral problems (Mengel *et al.*, 2006). Mattson *et al.* (2007) 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. Also, children of mothers who drank heavily during pregnancy perform poorly on tasks that involve learning spatial relationships among objects (Uecker and Nadel, 1996). Experimentally, ethanol has profound effects on the spatial ability of rats and these effects seem to mimic the spatial deficits produced by hippocampal lesions (Matthews and Morrow, 2000). The behavioral and cognitive impairments associated with fetal alcohol syndrome reflect underlying structural or functional changes in the brain (Roebuck *et al.*, 1998).

Critical periods have been described for effects of neonatal alcohol exposure (West and Goodlett, 1990; Goodlett and Johnson, 1999). Alcohol metabolism is associated with increased susceptibility to cell damage caused by harmful substances called free radicals that kill

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sensitive populations of brain cells at critical times of development in the first trimester of pregnancy (Cartwright and Smith, 1995; Chen and Sulik, 1996). Hence, there are likely to be critical periods for the effects of prenatal alcohol exposure on brain development.

Both learning disability (Howell *et al.*, 2006) and memory loss (Brust, 2008) are samples of alcohol cognitive disorders. Acute ethanol administration has demonstrated deficits in both spatial and nonspatial working memory tests (Gibson, 1985; Givens, 2006). Spatial working memory is needed to remember (task-specific) information that is different in specific content over time (Olton and Papas, 1979). Prenatal exposure to ethanol impairs spatial learning and spatial memory in juvenile (Blanchard *et al.*, 1987) and adult rats (Reyes *et al.*, 1989; Gianoulakis, 1990). Since the physiological responses to alcohol in development are similar in animals and humans, animal models are useful tools for determining the mechanisms and outcomes of early alcohol exposure (Hannigan and Abel, 1996).

Critical period for deleterious effects of prenatal alcohol exposure on cognitive function is not handled. Considering working memory the present study explores a critical period for alcohol impaired spatial memory in two month old rats prenatally exposed to ethanol.

MATERIALS AND METHODS

Animal breeding and alcohol administration: One or two female rats were placed with a male overnight. If pregnancy was confirmed the day was marked as Gestational Day (GD) 0. The pregnant rats were housed singly in cages and assigned to one of seven gestational groups. Six groups were administered with alcohol. Ethanol (30% w/v) was administered to produce a mean blood concentration of 5.7 g kg^{-1} (Nio *et al.*, 1991). Two of six groups the pregnant rats were received alcohol from GD1 to GD10 (first half; FH), GD11 to GD20 (second half; SH). Another four groups were those receiving alcohol during GD1 to GD5 (first quarter; FQ), GD6 to GD10 (second quarter; SQ), GD11 to GD15 (third quarter; TQ) and GD16 to GD20 (fourth quarter; FQ). The other group was a no treatment control, thus totaling seven groups. Number of the animals in each group were as follows: Control, 12; FH, 9; SH, 11; FQ, 7; SQ,15; TQ, 8 and FoQ, 6. The offspring were reared with their mothers until 30 postnatal day of age. The animals transferred to the test room 5 days before beginning experiments. After acclimation period, rats were divided randomly into seven groups and were kept in separate cages by sex.

Experimental subjects: Sixty eight male and female Wistar rats were included in this study. All animals were 2 month

of age when the experiments began. Animals were housed in Plexiglas cages (four animals per box) in same sex groups and maintained on a 12:12 h light:dark cycle. Food and water were provided *ad libitum* except for the periods of behavioral testing. At that time, the daily diet was reduced but care was taken to ensure that an animal's weight never dropped below 80% of their free feeding body weight.

Radial maze: All testing took place on an eight arm radial arm maze. The central platform was an octagon-shaped chamber that was 30 cm in diameter and 25 cm high. Eight 70 cm long, 15 cm wide arms were radiated from the platform at intervals of 45°. Guillotine doors were aligned in front of each arm. The doors could be raised or lowered in order to control access to maze arms. The radial maze was mounted on 70 cm legs and located in an experimental room rich with extra-maze cues, such as rack, window, stool, computer and the experimenter.

Experimental procedure: Radial maze testing consisted of two phases: shaping and working memory performance.

Shaping: The animals were firstly adapted to navigating the maze for two days prior to the testing. The rats were placed individually in the center of the maze and allowed to explore for 10 min/session twice each day. During two trails of the first day no doors separated the central platform and the arms and, the animals were allowed to explore the maze and obtain food rewards scattered throughout. In the first trial of the second day, rats were placed in the maze with all guillotine doors open 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 performance: During the working memory experiments, the animals learned to visit each of the eight arms once within a trial. The end of each arm was baited with a food reward. Rats were given two trials/day with 3-4 h intertrial intervals. A trial started with the animal placed in the central platform with all doors closed. After 30 sec, all doors were opened simultaneously and the animal was free to choose an arm to enter. Then, all doors but that of the selected arm were closed. Once the rat explored the selected arm and returned to the central area, the door of the arm was closed, confining the animal for 10 sec in order to test spatial memory without interference with stereotypic behaviors. Then, all 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. Experiments for each animal continued at least until two consecutive sessions in which the animal entered all 8 baited arms in 8 or a maximum of 9 selections (the criterion for the working memory tasks).

Statistics: The performances were evaluated in terms of the number of correct choices during each trial and, the number of required trials to reach the criterion (as described before). One-way analysis of variance (ANOVA) followed by the Tukey's test was applied to the data. All data are presented as Mean±SEM and p<0.05 was considered significant.

RESULTS

It is well known that alcohol administration during gestation period results in offspring with deficits in learning and memory abilities. To find the time within which alcohol display the highest efficacy on the radial maze performances, groups of pregnant rats were exposed to a same dose of ethanol during different periods of gestation.

Performance of the rats exposed to alcohol during the first and second half of the fetal life: Two groups of animals born from mothers exposed to alcohol during the first and second half of pregnancy period were introduced to the working memory task. In the correct choice task, while the control and the SH groups similarly improved their learning over the experiments the rats in the FH group showed an impairment in learning the task. As shown in Fig. 1, the difference is more apparent in the first half of the experiment. According to analysis of variance the animals in the control, FH and SH groups performed considerably different ($F_{2, 47} = _{5.64}$, p<0.006). The post hoc Tukey test indicated that the animals in the FH group were significantly inferior to their SH counterpart (p<0.05). As shown in Fig. 2 the three groups of the animals displayed no different in hitting the criterion ($F_{2, 29} = 1.7$, p<0.19).

Performance of the rats exposed to alcohol during different quarters of the fetal life: The results of the first step of experiments led us to conclude that the first half of fetal life is susceptible to alcohol induced impairments in the working memory testing. Thus, this study was continued on the rats exposed to alcohol during the first and second quarters of the pregnancy period. Additionally, offspring from mothers exposed to ethanol during the third and forth quarters of the prenatal age were included in the experiments. Analysis of the correct entries made by the ethanol administered and control animals indicated variations among behavior of all groups
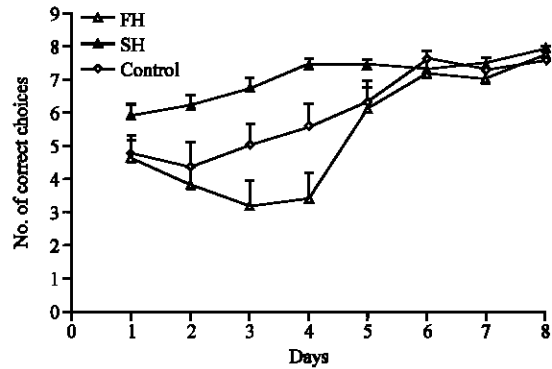

Fig. 1: Data represent acquisition of mean correct choices across 16 trials in the control and alcohol treated animals during the first and second half of the fetal life. The animals in the first half (FH) group had a significant poor performance compared to the second half (SH) groups. Each points represents average performance of two trials/day
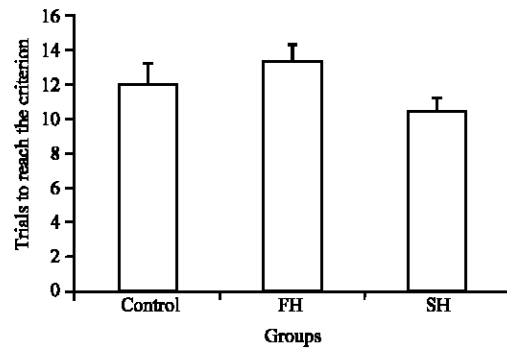

Fig. 2: Average number of trials required by the vehicle and alcohol treated rats at the first half (FH) and the second half (SH) of the fetal life during 16 trials of training on the spatial maze. No difference was evident between the animals in different groups

($F_{4, 49} = 23.29$, p<0.0001). However, the radial maze navigation of the control rats and FQ, TQ and FoQ groups demonstrated that these groups selected the correct arms in an analogous pattern. The rats in SQ group, on the other hand, showed an obvious impairment in learning the working memory task so that they could not noticeably improve their performance over experiment. The post hoc test indicates a considerable difference between the control and SQ groups (p<0.004) and, between the SQ group and the other three groups (p<0.0001) in correct choices (Fig. 3).

Concerning the criterion, statistics reveals a general difference between behavior of the control group and those exposed to ethanol during different quarters of the
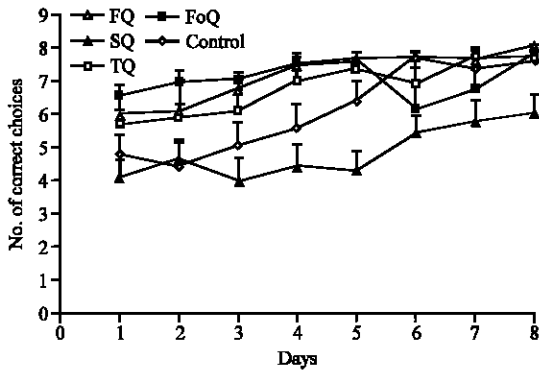
Fig. 3: Data represent acquisition of mean correct choices across 16 trials in the control and the alcohol administered rats during different quarters of the pregnancy period. The SQ group showed the weakest performance among all groups whit significant lower behavior against the control and FQ groups. Each points represents average performance of two trials/day
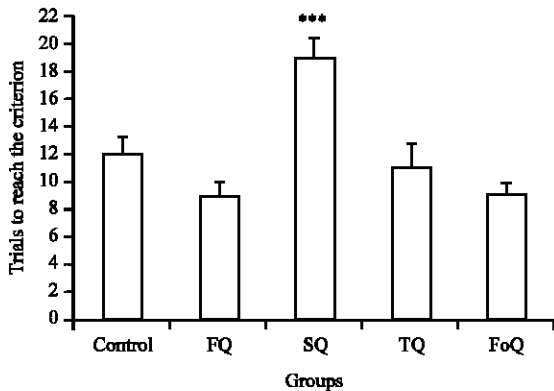

Fig. 4: Average number of trials required by the vehicle and alcohol treated rats at different quarters of the fetal life during 16 trials of training on the spatial maze. All group preformed almost equally except the second quarter (SQ) group which required more trials to hit the criterion. ***p<0.001, compared to the other groups, FQ: first quarter, TQ: third quarter, FoQ: forth quarter

prenatal age ($P_{4, 42}$ = 10.04; p<0.0001). Tukey's post test indicated a significant difference between the second quarter (SQ) and control, third quarter (TQ) and forth quarter (FoQ) groups (p<0.001). These results resemble those in the correct choice task.

It is noteworthy to point out that to ensure inferiority of the SQ group we added a further group of rats (n = 6) to the preceding one (n = 9) increasing the number of the animals to 15. The same procedure was applied to the animals yielding almost equivalent results. The data presented here is pooled from all 15 rats (Fig. 4).

DISCUSSION

Sufficient evidence has demonstrated alcohol induced deficits in both structure and function of the brain. In regard to the function, alcohol particularly influences leaning and memory phenomena. Using radial arm maze (Reyes *et al.*, 1989), Morris water maze (Kim *et al.*, 1997) and T-maze (Lochry and Riley, 1980) prenatally effects of alcohol on different aspects of spatial memory have been tested. Overall, reports express that alcoholism results in impairment of learning and memory. Here, results of the present study try to elucidate a critical period for deleterious effects of prenatally alcohol exposure on the spatial memory.

Different performance of the FH and SH groups of rats in acquisition of the working memory task: The results of experiments on the FH and SH groups of rats revealed that the animals in the former group are more vulnerable to prenatal toxic effect of ethanol than do the latter where the FH group showed an apparent deficit in acquisition of working memory tasks. The problem learning was more obvious in the first half of the experiment and 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. Consequently, it was concluded that receiving alcohol during the first half of prenatal life interferes with spatial learning and memory at postnatal age.

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 mothers receiving alcohol in this period. Then, two groups of two months old rats exposed to ethanol in the FQ and SQ of the fetal life were tested for 16 trials. These experiments documented that the SQ group had a lower performance in comparison to the FQ as well as control groups. From these results it is 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 further test this idea a second group make of the SQ rats were subjected to the working memory task. Interestingly, two different groups of the rats exposed to alcohol at SQ of prenatal age presented an analogous behavior in working memory trainings as did

the first SQ group. On the other hand, performances of the rats exposed to ethanol at the TQ and FoQ of prenatal age indicated no variation when compared to the control group consistently confirming the performance of the alcohol administered animals at SH of the fetal life. Also, the data on the criterion matches those of the correct arm entries where the SQ group differently met the criterion in acquisition of learning and memory tasks with the lowest performance among the seven groups involved in this study.

Higher susceptibility of the second quarter group to harmful effects of alcohol on the spatial task: Findings of this study confirm the general idea that fetal alcohol syndrome conflicts learning and memory. Behavioral deficits have also 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 maze. Stone *et al.* (1996) also reported deficits in radial arm maze performance in prenatal alcohol-exposed rats. The present results uncover that learning problems associated with prenatal alcohol exposure are specific to a narrow period of gestation; the second quarter of fetal life. Clarren *et al.* (1978) have reported that during the first trimester of human pregnancy alcohol interferes with the migration and organization of brain cells. Also, it is demonstrated that heavy drinking during the second trimester, particularly from the 10th to 20th week after conception, cause more clinical features of fetal alcohol syndrome than at other times during pregnancy (Renwick and Asker, 1983). 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). These findings verify the data presented here that the second 5 days of gestation could be a critical period for the effects of alcohol, at least, on the spatial task. However, Neese *et al.* (2004) reported spatial working memory deficits in female offspring born of mothers treated with ethanol during the third week of gestation in comparison to mothers treated during either the first or second weeks of gestation. On the other hand, Byrnes *et al.* (2004) found no critical period of vulnerability for ethanol-induced deficits in spatial learning or hippocampal synaptic plasticity in young adult guinea pig.

The hippocampus is critical for spatial learning and memory and lesions to the this area or its afferents impair the learning of spatial tasks (O'Keefe *et al.*, 1975). Animal studies have long suggested that hippocampus is affected by prenatal alcohol exposure (Berman and Hannigan, 2000). 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). 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 *et al.*, 1987; Reyes *et al.*, 1989). Taken together, the findings presented here demonstrate that drinking alcohol during pregnancy underlies cognitive phenomena in offspring. Particularly, the second 5 days of the gestation period in rats is prone to deleterious effects of alcohol on spatial learning and memory.

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