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Spatial Learning in Rats Exposed to Acute Ethanol Intoxication on Gestational Day 8

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MINETTI, A., M. P. AROLFO, M. B. VIRGOLINI, J. D. BRIONI AND S. FULGINITI. *Spatial learning in rats exposed to acute ethanol intoxication on gestational day 8.* PHARMACOL BIOCHEM BEHAV 53(2) 361-367, 1996.—Pregnant Wistar rats were treated on gestational day 8 (GD 8) with two IP injections of either ethanol (2.9 g/kg in 24% v/v saline solution) or saline. Offspring were tested in the water-maze task at 45 or 90 days of age. The escape latencies of rats trained with a submerged escape platform at a fixed location were similar between control and experimental rats. Analyses of responses on a probe trial carried out 10 days after the training period, revealed that 90-day-old females prenatally exposed to alcohol were less likely to swim in the target region. No differences were observed in this free-swim trial in 45- and 90-day-old male, and 45-day-old female animals. Binding studies of low-affinity $GABA_A$ sites in the hippocampus showed an increase in affinity of [^{3}H]$GABA_A$ for their binding sites in 90-day-old female offspring prenatally intoxicated with ethanol. Our results demonstrate that acute intoxication with ethanol on GD 8 did not modify acquisition but impaired the retention of spatial learning only in adult female rats. It is possible that the impaired retention will be consequence of higher $GABA_A$ receptor affinity.

Prenatal ethanol intoxication Spatial learning Memory $GABA_A$ receptors Hippocampus

PRENATAL alcohol exposure in humans can produce a variety of physical, physiological, and behavioral abnormalities (31), which have been termed Fetal Alcohol Syndrome (FAS) (17). Chronic ethanol exposure during pregnancy in rats and mice produces alterations similar to those observed in humans (2,10,19,31,33). Among the behavioral dysfunctions, these animals exhibit hyperactivity and learning disabilities. Alterations in the development of several neurotransmitter systems involved in the regulation of learning and memory in different animal models of FAS (9,11,30,34) have also been demonstrated, as well as neural changes that could be responsible for the behavioral deficits observed following prenatal ethanol exposure. Thus, reductions in hippocampal pyramidal cell number (5,38), alterations in hippocampal mossy fiber (37), and a decrease in the density and arrangement of dendritic spines (1) have been described.

Acute exposure to alcohol during a critical period of fetal development induces teratogenic effects in mice (32,36). In the rat, acute intoxication with alcohol during gestational day 8 (GD 8)—period of gastrulation—induces dose-dependent morphological and behavioral alterations in the offspring (23,35), as well as altered reactivity to several pharmacological agents (13,14,24), which induced modification in some monoaminergic functions.

It is well known that the hippocampus plays an important role in spatial learning and memory (25,28,29). Several studies suggest that cholinergic septohippocampal pathway [see (8)] and glutamatergic system [see (27)] are involved in regulating spatial learning. Other neurotransmitter systems, like the GABAergic [see (8)] and serotonergic [see (22)], also modulate this kind of learning. Therefore, there is evidence indicating that GABA may affect memory processes through its effects on cholinergic function. It was shown that GABAergic agonists reduce hippocampal cholinergic activity and impair retention of spatial information (7). The effects of GABAergic drugs on memory storage are mediated, at least in part, by the septum, amygdala, and hippocampus (3). As regards the serotonergic system, several evidence suggests that serotonin (5-HT) exerts an inhibitory influence on spatial learning and memory [see (22)].

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Spatial learning has been ultimately evaluated in a navigation task (26), a task sensitive to hippocampal dysfunction (25). Here, rats quickly learn to locate and climb onto a submerged platform using only distal cues to the platform position, available within the testing room. Previous studies have shown impaired performance on the Morris water task in young and adult rats chronically exposed to alcohol during pregnancy (6,15). In a previous work, we have found different behavioral responses induced by 5-HT agonists in 45- and 90-day-old offspring prenatally exposed to ethanol on GD 8 (14).

Considering the above-mentioned evidence, we studied the effects of acute ethanol intoxication on GD 8 on acquisition and retention of spatial information, using the Morris water-maze task (26), in 45-day-old and 90-day-old male and female offspring. Furthermore, to determine whether possible alterations in spatial learning correlates with changes in GABA function, the binding of low-affinity $GABA_A$ sites in hippocampus was also determined.

METHOD

Subjects

The procedure used to expose rats to ethanol during GD 8 has been previously described (13). Briefly, parent animals were male and nulliparous female Wistar rats (90–120 days old). They were housed in a 12 L : 12 D cycle (light onset: 0700 h), provided with food and water ad lib, and under constant temperature conditions (22 ± 1°C). In the evening of the proestrus day, they were housed overnight with male rats. The presence of spermatozoa in the vaginal smears was taken as an index of pregnancy, and was referred to as gestational day 1 (GD 1). On GD 8, dams were randomly divided into two groups: control group (CG) containing nine rats, and experimental group (EG) containing eight rats. While the EG received two IP injections of ethanol (2.9 g/kg in 24% v/v saline solution), spaced by an interval of 4 h (1000 and 1400 h), the CG received an identical volume of saline. Injections were administered with considerable care to avoid trauma to the uterus. This last group was maintained without food and water while the time alcohol-treated rats exhibited an inhibition of feeding habits (aproximately 12 h). Throughout gestation, pregnant females were fed with a lab chow maternity diet (Nutrimentos S.A., pregnancy lab chow). Maternal blood ethanol levels have been previously reported (13). Briefly, they were determined by gas chromatography at different times after alcohol injections; the highest alcohol levels were observed 60 min after the second dose (457 ± 12 mg/dl, mean ± SEM).

Within 24 h after delivery (postnatal day 1, PD 1), all pups were weighed. Litters were then randomly culled to five males and five females whenever possible. Offspring were weaned at 25 days of age and housed in groups of 8–12 rats according to sex and treatment. At 45 or 90 days of age, control and experimental male and female offspring (one or two animals from each litter) were trained in the Morris water maze.

Behavioral Studies

Apparatus. The water maze was a circular, galvanized-steel tank measuring 1.80 m in diameter and 0.50 m in height, filled to a depth of 20 cm with 25°C water (4). Attached to the rim of the tank along 1.70 m of its circumference was a white strip extending 20 cm from the rim which served as a salient cue. Other distal cues were also available in the environment surrounding the tank. Four points equally spaced around the perimeter of the tank were arbitrarily selected as starting locations. On this basis, the tank was divided into four equal quadrants in a clockwise order (target, adjacent, opposite, and adjacent). Located in the center of one of these quadrants was a 14 × 14 × 19 cm (w/l/h) Plexiglas platform (i.e., its surface was 1 cm below the water level). The platform remained in a fixed location throughout the training period of the place-trained animals.

Place-training procedure. Place-trained animals were required to locate a submerged platform that was not visible to the rat, but was always located in the same position with respect to the spatial environment, thereby allowing the rat to determine the platform location by using distal cues. A trial began when the rat was placed in the pool facing the wall of the tank at one of the starting positions, which varied from trial to trial in a quasirandom order. The rat was allowed to swim until it located and climbed onto the escape platform. Rats were gently guided to the platform if they failed to locate it within 90 s. The rat remained on the platform for 20 s before being removed. Two additional trials were given with 5–10-s intertrial intervals. Following the third trial, the rat was returned to its home cage. The rats received a total of 12 trials during 4 consecutive days of training. Escape latencies were measured registering the time after being released into the pool until the rat escaped onto the platform. All trials were videotaped through wide angle lens attached to a camera mounted above the tank.

Free-swim trials. This 30-s trial was carried out 10 days after the completion of the four training sessions. The four possible positions of the platform and the limits of the four quadrants were marked on the video screen to indicate their exact surface area. The escape platform was removed from the tank and the rat was allowed to swim for a 30-s period. From video tapes made during this free-swim trial it was possible to calculate three measures of spatial behavior: 1) latency to cross the target platform location: the time in seconds to cross the target platform marked on the screen; 2) quadrant time: the number of seconds spent by the rat in each of the four quadrants in which the tank was divided; and 3) platform crossings: the number of times the rat traversed the former position of the escape platform as well as the crossings made on the three remaining platform locations (one for each of the remaining quadrants). These three measures were used to assess the degree of spatial bias of the rats toward the target quadrant during the free-swim trial.

Neurochemical Study

Binding assays. Low-affinity [^{3}H]GABA binding was determined in 45- and 90-day-old female offspring according to the method of Enna and Snyder (12), with minor modifications. Rats were previously habituated to the manipulations that precede killing four times daily for 7 days. After this period, animals were killed, the brain rapidly removed, and the hippocampus of two or three rats (for females 45 and 90 days old, respectively) dissected on ice, were pooled and used for each experiment. The fresh tissue was homogenized in 10 vol of 0.32 mol/l sucrose and centrifuged at 0°C, 900 × *g* for 10 min; the supernatant was centrifuged at 11,000 × *g* for 20 min. The resultant pellet was submitted to hypotonic shock in 12 vol of water and centrifuged at 20,000 × *g* for 30 min. The final crude membrane fraction was stored at −20°C for at least 24 h before assay. Frozen membranes were resuspended in 50 mmol/l Tris-HCl buffer (pH 7.1) containing 0.5 g/l Triton X-100, incubated at 37°C for 30 min and centrifuged at

0°C, 100,000 × *g* for 30 min. The resulting pellet was washed twice by resuspension in Tris-HCl buffer. Specific [^{3}H]GABA (100.0 Ci/mmol, DuPont, New England Nuclear, Boston, MA) binding was determined in aliquots of crude membrane fraction (0.08–0.15 mg protein) incubated for 5 min at 0°C with 0.6 ml buffer containing [^{3}H]GABA. The concentration of [^{3}H]GABA varied between 10–600 nmol/l (low-affinity receptor); [^{3}H]GABA was maintained constant (10 nmol/l), whereas the concentration of nonradioactive GABA varied. The reaction was stopped by centrifugation of the vials at 17,000 × *g* at 4°C. The supernatant was discarded and pellets rapidly rinsed with 1 ml ice-cold buffer. The pellets were transferred to vials to count the radioactivity in 3 ml of a solution containing tolueno-Triton X-100–2,5 diphenyloxazole (PPO, Sigma Chemical Co., St. Louis, MO). The nonspecific binding, assayed in the presence of 1 mmol/l GABA, was subtracted from the total radioactivity, yielding values of specific binding. Protein was determined by the method of Lowry et al. (20). The data were analyzed using Scatchard analysis of saturation.

Statistics

Spatial learning acquisition data were analyzed by an analysis of variance (ANOVA) with repeated measures followed by the Fisher PLSD test for individual mean comparisons. The statistical analysis used for the free-swim data was a multivariated ANOVA (MANOVA) for all the quadrants. Binding results were analyzed by a two-way ANOVA and subsequent post hoc comparisons were performed using the Fisher test.

RESULTS

Maternal Data

As previously reported (13,14), acute ethanol treatment of pregnant rats did not significantly affect body weight gain during pregnancy, gestational lenght, litter size, or body weight of pups at birth.

Place-Training Performance

Escape latencies during the 12 trials for all groups (Fig. 1) were analyzed using four-way ANOVA (group × sex × age × trial) with trials as a repeated measure. The analysis revealed a significant effect of age, $F(1, 62) = 4.6$, $p < 0.03$, and a repeated measure trial effect, $F(11, 682) = 71.8$, $p < 0.0001$. However, there was no group, sex, and interaction between groups effects. When we compared performance in individual groups, we observed no differences in acquisition. Escape latencies during the 12 training trials, in 45-day-old male offspring, are shown in Fig. 1A. Both groups of rats reduced their escape latencies over trials, but no differences between control and experimental rats were observed in the acquisition phase. When a two-way ANOVA with repeated measures was made, there was a repeated measure trial effect, $F(11, 176) = 14.0$, $p < 0.0001$, but there was neither group nor group × trial interaction effects. Similar results were obtained in 90-day-old male offspring. No differences in escape latencies during the 12 training trials were observed between groups (Fig. 1B). Both control and prenatally ethanol exposed 90-day-old male groups showed decreased escape latencies over trials. There was a repeated measure trial effect, $F(11, 154) = 19.4$, $p < 0.0001$, but there was neither group nor group × trial interaction effects.

In 45-day-old females, escape latencies diminished over trials in control as well as in experimental rats (Fig. 1C). There was a repeated measure trial effect, $F(11, 165) = 15.5$, $p < 0.0001$, but there was no group or group × trial interaction effects. Control and experimental 90-day-old female rats did not differ during place training (Fig. 1D); both groups exhibited reduced escape latencies over trials. There was a repeated measure trial effect, $F(11, 187) = 27.9$, $p < 0.0001$, but there was neither group nor group × trial interaction effects.

Free-Swim Trials Performance

Figure 2 shows the time all groups spent in the four quadrants. A MANOVA [sex, age, sex × age and group (sex × age)] in the target quadrant (PP1) showed a significant sex × age interaction, $F(1, 62) = 4.4$, $p < 0.05$. We did not find differences among sex, age, and group (sex × age). In the other quadrants, the MANOVA did not show significant differences. Figure 2 shows the time spent by 45- and 90-day-old male and female offspring in the four quadrants during the free-swim trial carried out 10 days after the last day of training. As a matter of interest, we studied if there was a difference in the time spent between quadrants and the analysis showed a significant quadrant effect, $F(3, 186) = 52.5$, adjusted $p < 0.001$ (Huyhn and Feldt). As shown in Fig. 2A, B, and C, control and experimental rats from 45- and 90-day-old male and 45-day-old female offspring spent more time searching in the target quadrant as compared to the other quadrants.

The results were somehow different when 90-day-old female rats were analyzed. Although there was no difference among groups in the MANOVA, we analyzed this group of rats in particular due to the fact that in previous studies, we have found that in another group of 90-day-old female offspring, the prenatally exposed to ethanol rats were impaired in the retention of spatial information 10 days after the acquisition period. This was reflected by the greater escape latencies to reach the platform [CG = 8.5 ± 0.7 (n = 8), EG = 16.1 ± 2.9 (n = 8)]. The MANOVA in the 90-day-old female offspring showed a significant quadrant × group interaction, $F(3, 51) = 3.2$, adjusted $p < 0.05$ (Huyhn and Feldt). When we analyzed each quadrant, a significant difference between control and experimental groups in the target quadrant, $F(1, 17) = 6.9$, $p < 0.02$, was observed. These results indicate that only 90-day-old control females exhibited spatial bias towards the target quadrant 10 days after acquisition (Fig. 2D). The time spent by control rats in the target quadrant was significantly more than the time spent by the prenatally alcohol intoxicated females in the same quadrant. Furthermore, the experimental group swam approximately the same time in all quadrants, despite the fact that their escape latencies did not differ from those of control group during the place training procedure. Table 1 shows no difference in the latency to reach the former target platform position, although the number of target crossings made by the prenatal ethanol treated group was smaller than the control group one. The target crossings are correlated with the less time spent in the same quadrant and it also reflects a memory impairment. No differences among groups were found in latency to cross the former platform position and the number of target crossings in 45- and 90-day-old male and 45-day-old female offspring (data not shown).

Binding Data

This results were analyzed using two-way ANOVA (group × age), which revealed a significant group × age interaction effect, $F(1, 19) = 4.9$, $p < 0.05$, without group or age ef-

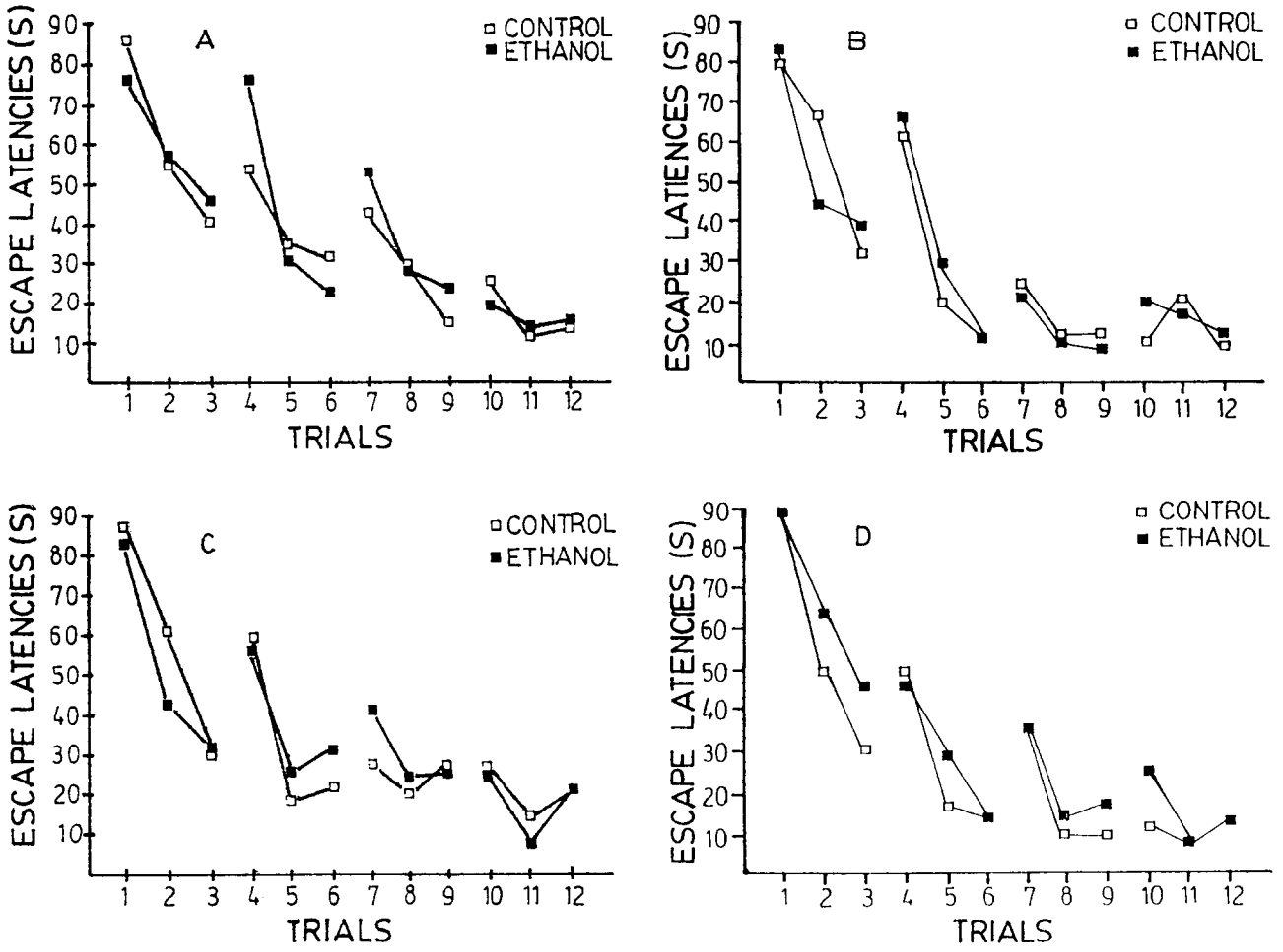

FIG. 1. Escape latencies in seconds for male of 45 (A) and 90 (B) days, and female offspring of 45 (C) and 90 (D) days of age. Control and prenatally ethanol-treated rats were place-trained with three trials per day during 4 consecutive days. Data represent the mean time of 8–10 animals per group.

fects. As observed in Table 2, low-affinity $GABA_A$ binding measured in hippocampus was similar in control and experimental 45-day-old females. There were neither differences between groups in the total number of low-affinity [^{3}H]GABA binding sites (B_{max}) nor in the affinity of [^{3}H]GABA for their binding sites (K_d). However, prenatally treated females with ethanol showed, at 90 days old, a higher K_d without change in B_{max} when compared with control group.

DISCUSSION

Our results show that acute prenatal ethanol intoxication did not affect acquisition of spatial information, because the alcohol-exposed animals learned to locate a submerged platform in the water-maze task as well as control rats. However, retention of spatial learning was impaired in 90-day-old female offspring prenatally treated with ethanol on GD 8. They did not exhibit spatial bias towards the target quadrant 10 days after acquisition. In 45-day-old male and female rats, and 90-day-old male offspring, acute prenatal exposure to alcohol did not alter spatial memory in this task.

Previous studies have demonstrated that offspring to mothers chronically exposed to ethanol exhibited impaired performance in the Morris task. These works showed that 22-day-old male and female offspring, whose mothers received ethanol from day 6 to 20 during pregnancy, found it difficult to locate a submerged platform in a water tank (6). Likewise, animals exposed to ethanol through all gestation, and tested at 40, 60, and 90 days old in the Morris task, have also showed deficit in the spatial performance (15). When ethanol was administered during postnatal days 4–10 it induced disability to perform the maze task in young male and female offspring (19–30 days of age) (16), and only in adult 90-day-old female rats (18). The difference between those investigations that have found a deficit in the acquisition of spatial learning at different ages, and our present data might be attributed to the distinct patterns of alcohol exposure—mainly time of administration—as well as environmental factors during the experiments.

Our experiments indicate an impaired retention of a spatial learning task in 90-day-old female offspring prenatally treated with alcohol. Other studies also demonstrated that 40-, 60-, and 90-day-old offspring chronically exposed to ethanol during pregnancy performed poorly on the first trial of each day

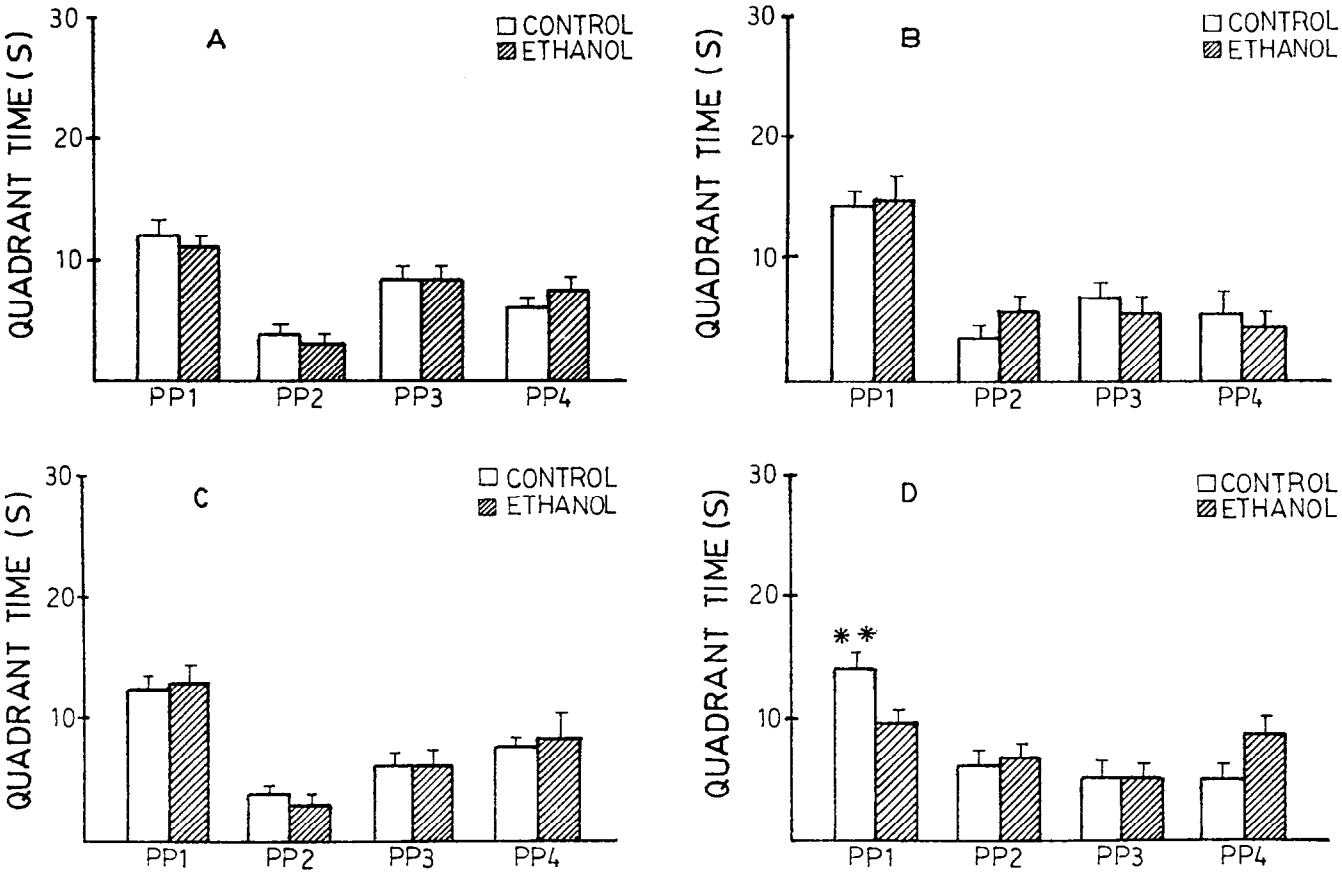

FIG. 2. Time spent in the four quadrants of the pool during the free-swim trial, carried out 10 days after training period, by males of 45 (A) and 90 (B) days, and female offspring of 45 (C) and 90 (D) days old. Quadrants were designated in a clockwise order: PP1 target, PP2 adjacent, PP3 opposite and PP4 adjacent. Data represent the mean ± SEM time in seconds (n = 8–10 animals per group). **$p < 0.02$, as compared to the time spent in the target quadrant by the experimental rats.

of testing in the Morris task, and when the platform was removed, they searched for it in all four quadrants of the pool, which suggests impaired retention of spatial information (15). Our data is consistent with the notions that alcohol differentially impair cognition in male or female rats. Thus, spatial information processing was more affected by chronic prenatal alcohol exposure in female than in male offspring (6). The performance in the water maze task has also been impaired in adult female but not in adult male rats exposed to alcohol during 4–10 postnatal days (18). Moreover, deficits in the performance on a two-way shock-avoidance task have

TABLE 1

EFFECT OF PRENATAL ETHANOL EXPOSITION IN 90-DAY-OLD FEMALE OFFSPRING IN THE LATENCY TO CROSS AND THE NUMBER OF CROSSINGS OF THE TARGET PLATFORM LOCATION DURING THE 30-s FREE-SWIM TRIAL

Group	n	Latency	Crossings
Control	9	8.1 ± 1.2	2.1 ± 0.3
Ethanol	10	13.6 ± 2.8	1.2 ± 0.3*

Results are expressed as the mean ± SEM time in seconds for latency and number of platform crossings.

n = number of animals per group.

*$p < 0.05$ significantly different from the control group.

TABLE 2

EFFECTS OF PRENATAL ETHANOL EXPOSITION IN FEMALE RATS OFFSPRING ON LOW-AFFINITY [^{3}H]GABA BINDING IN HIPPOCAMPUS

Group	Age	n	B_{max} (pM/mg protein)	K_d (nM)
Control	45	6	1.77 ± 0.30	28.2 ± 7.8
	90	5	1.56 ± 0.29	22.0 ± 2.9
Ethanol	45	5	1.82 ± 0.45	24.0 ± 2.2
	90	7	2.00 ± 0.48	42.5 ± 7.0*†

Values represent the means ± SEM.

n = number of animals per group.

*$p < 0.05$ compared to control group of the same age.

†$p < 0.05$ compared to ethanol group of 45 days of age.

been demonstrated only in female rats chronically exposed to ethanol in utero (2). This greater vulnerability to alcohol for the female is difficult to interpret. However, considering that the hippocampus development is sexually dimorphic (21), perinatal alcohol could differentially affect the hippocampus, depending on the sex. Our experiments concern an acute intoxication with alcohol that produces high blood alcohol levels during gastrulation period. In previous works, we found that male and female offspring exhibited a similar sensitivity to the hypnotic effect of ethanol and no sexual differences on behavioral response to 5-HT_1 agonist (13,14). However, in recent experiments we observed that the reactivity to the ansiolytic effect of ethanol measured in the plus-maze test is higher in adult females compared with male offspring submited to the same treatment. Also, cortical endogenous levels of 5-HT are lower in experimental animals than in controls only in adult males and not in females (unpublished results). This evidence will indicate that different effects on males and females are possible according to the behavioral and neural system studied.

In this study an impaired retention in adult females but not in younger animals prenatally treated with ethanol was observed. In previous works we have found that at 45 days old the experimental offspring exhibited an increased behavioral response to 5-HT_1 and 5-HT_2 agonists, and that at 90 days old they showed a diminished behavioral reactivity to 5-HT agonists as compared to controls. Also, the hypnotic effect of ethanol was different and opposite in 45- and 90-day-old experimental offspring (14,13). Deficits presented in spatial memory processes by chronic exposure to ethanol in utero, are also more severe in adult life (15).

On the other hand, our biochemical data indicate that 90-day-old, but not 45-day-old, females prenatally exposed to alcohol on GD 8 showed a significant increase in affinity of hippocampal low-affinity $GABA_A$ binding sites with respect to the control rats. No differences were found in the total number of hippocampal low-affinity $GABA_A$ binding sites between control and experimental female 45-day-old rats. Brioni et al. (7) have demonstrated that pretraining muscimol ($GABA_A$ agonist) injections in the septum impaired retention of spatial information but not acquisition. These evidences allow us to suggest that the greater affinity of hippocampal low-affinity $GABA_A$ binding sites observed in 90-day-old female intoxicated with ethanol on GD 8 might be responsible, in part, for the impaired retention exhibited in this animals. Furthermore, we did not find differences in low-affinity $GABA_A$ binding between control and experimental 45-day-old female rats; coincidentally, at this age we did not observe alterations in acquisition and retention of spatial learning either.

In conclusion, our results demonstrate that acute intoxication with ethanol during GD 8 impaired the retention of spatial information in adult female offspring, without altering the acquisition of the learning in all groups. Also, the same group of rats presented alterations in low-affinity $GABA_A$ binding, which could probably explain the impairment in the retention of spatial information. However, the study of other neurotransmitter systems on several neural structures that also regulate the spatial learning and memory, are needed to clarify the possible mechanism involved in the altered memory observed.

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REFERENCES

1. Abel, E. L.; Jacobson, S.; Sherwin, B. T. In utero alcohol exposure: Functional and structural brain damage. Neurobehav. Toxicol. Teratol. 5:362–366; 1983.
2. Abel, E. L. Prenatal effects of alcohol on adult learning in rats. Pharmacol. Biochem. Behav. 10:239–243; 1979.
3. Ammassari-Teule, M.; Pavone, F.; Castellano, C.; McGaugh, J. L. Amygdala and dorsal hippocampus lesions block the effects of GABAergic drugs on memory storage. Brain Res. 551:104–109; 1991.
4. Arolfo, M. P.; Brioni, J. D. Diazepam impairs place learning in the Morris water maze. Behav. Neural Biol. 55:131–136; 1991.
5. Barnes, D. E.; Walker, D. W. Prenatal ethanol exposure permanently reduces the number of pyramidal neurons in rat hippocampus. Dev. Brain Res. 227:333–340; 1981.
6. Blanchard, B. A.; Riley, E. P.; Hannigan, J. H. Deficits on a spatial navigation task following prenatal exposure to ethanol. Neurotoxicol. Teratol. 9:253–258; 1987.
7. Brioni, J. D.; Decker, M. W.; Gamboa, L. P.; Izquierdo, I.; McGaugh, J. L. Muscimol injections in the medial septum impair spatial learning. Brain Res. 522:227–234; 1990.
8. Decker, M. W.; McGaugh, J. L. The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. Synapse 7:151–168; 1991.
9. Detering, N.; Edwards, E.; Ozand, P.; Karahasan, A. Comparative effects of ethanol and malnutrition on the development of catecholamines neurons: Changes in specific activities of enzymes. J. Neurochem. 34:297–307; 1980.
10. Díaz, J.; Samson, H. H. Impaired brain growth in neonatal rats exposed to ethanol. Science 208:751–753; 1980.
11. Druse, M. J. Effects of maternal ethanol comsumption on neurotransmitter and lipids in offspring. Neurobehav. Toxicol. Teratol. 3:81–87; 1981.
12. Enna, S. J.; Snyder, S. H. Influence of ions, enzymes and detergents on gamma-aminobutyric acid receptor binding in synaptic membranes of rat brain. Mol. Pharmacol. 13:442–453; 1977.
13. Fulginiti, S.; Artinian, J.; Cabrera, R.; Contreras, P. Response to an ethanol challenge dose on sleep time and blood alcohol level in Wistar rats prenatally exposed to ethanol during gestational day 8. Alcohol 6:253–256; 1989.
14. Fulginiti, S.; Vigliecca, N. S.; Minetti, S. A. Acute ethanol intoxication during pregnancy: Postnatal effects on the behavioral response to serotonin agents. Alcohol 9:523–527; 1992.
15. Gianoulakis, C. Rats exposed prenatally to alcohol exhibit impairment in spatial navigation test. Behav. Brain Res. 36:217–228; 1990.
16. Goodlett, C. R.; Kelly, S. J.; West, J. R. Early postnatal alcohol exposure that produces high blood alcohol levels impairs development of spatial navigation learning. Psychobiology 15:64–74; 1987.
17. Jones, K. L.; Smith, D. W.; Ulleland, C. N.; Streissguth, A. P. Pattern of malformation in offspring of chronic alcoholic mothers. Lancet 1:1267–1271; 1973.
18. Kelly, S. J.; Goodlett, C. R.; Hulsether, S. A.; West, J. R. Impaired spatial navigation in adult female but not adult male rats exposed to alcohol during the brain growth spurt. Behav. Brain Res. 27:247–257; 1988.
19. Lochry, E. A.; Riley, E. P. Retention of passive avoidance and T-maze escape in rats exposed to alcohol prenatally. Neurobehav. Toxicol. 2:107–115; 1980.
20. Lowry, O.; Rosebrough, N. I.; Farr, A. L.; Randall, R. J. Pro-

tein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
21. Loy, R.; Milner, T. A. Sexual dimorphism in extent of axonal sprouting in rat hippocampus. Science 208:1282–1284; 1980.
22. McEntee, W. J.; Crook, T. H. Serotonin, memory, and the aging brain. Psychopharmacology (Berlin) 103:143–149; 1991.
23. Molina, J. C.; Ferreyra Moyano, H.; Spear, L. P.; Spear, N. E. Acute alcohol exposure during gestational day 8 in the rat: Effects upon physical and behavioral parameters. Alcohol 1:459–464; 1984.
24. Molina, J. C.; Hoffman, H.; Spear, L. P.; Spear, N. E. Sensorimotor maturation and alcohol responsiveness in rats prenatally exposed to alcohol during gestational day 8. Neurotoxicol. Teratol. 9:121–128; 1987.
25. Morris, R. G. M.; Garrud, P.; Rawlins, J. L. P.; O'Keefe, J. Place navigation impaired in rats with hippocampal lesions. Nature 297:681–683; 1982.
26. Morris, R. G. M. Spatial localization does not require the presence of local cues. Learn. Motiv. 12:239–260; 1981.
27. Morris, R. G. M. Synaptic plasticity and learning: Selective impairment of learning in rats and blockade of long-term potentiation in vivo by the *N*-methyl-D-aspartate receptor antagonist AP5. J. Neurosci. 9:3040–3057; 1989.
28. O'Keefe, J.; Nades, L. The hippocampus as a cognitive map. Behav. Brain Sci. 2:487–533; 1979.
29. Olton, D. S.; Becker, J. T.; Handelmann, G. E. Hippocampus, space, and memory. Behav. Brain Sci. 2:313–365; 1979.
30. Rawat, A. K. Developmental changes in the brain levels of neurotransmitters as influenced by maternal ethanol consumption in the rat. J. Neurochem. 28:1175–1182; 1977.
31. Streissguth, A. P.; Landesman-Dwyer, S.; Martin, J.; Smith, D. W. Teratogenic effects of alcohol in humans and laboratory animals. Science 209:353–361; 1980.
32. Sulik, K. K.; Johnston, M. C. Sequence of developmental alterations following acute ethanol exposure in mice craniofacial features of the fetal alcohol syndrome. Am. J. Anat. 166:257–269; 1983.
33. Sulik, K. K.; Johnston, M. C.; Webb, M. A. Fetal alcohol syndrome: Embryogenesis in a mouse model. Science 214:936–938; 1981.
34. Tajuddin, N.; Druse, M. J. Effects of in utero ethanol exposure on cortical 5-HT_2 binding sites. Alcohol 5:461–464; 1989.
35. Viglecca, N. S.; Fulginiti, S.; Minetti, S. A. Acute ethanol exposure during pregnancy in rats: Effects upon a multiple learning task. Alcohol 6:363–368; 1989.
36. Webster, W. S.; Walsh, D. A.; Lipson, A. H.; McEwen, S. E. Teratogenesis after acute alcohol exposure in inbred and outbred mice. Neurobehav. Toxicol. 2:227–234; 1980.
37. West, J. R.; Hodges, C. A.; Black, A. C. Prenatal exposure to ethanol alters the organization of hippocampal mossy fibers in rats. Science 211:957–959; 1981.
38. West, J. R. Long-term effects of development exposure to alcohol. Neurotoxicology 7:245–256; 1986.