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Research paper

Impairments of spatial learning and memory in rat offspring with fetal growth restriction ☆

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Abstract

Objective: Throughout the world, fetal growth restriction(FGR) is one of the most severe complications occurring during pregnancy. It is subsequently associated with neurologic abnormalities in chldren. Our aim was to investigate the spatial learning and memory ability of rat offspring born with FGR. **Methods:** A rat model of FGR was constructed using the method of passive smoking. Spatial learning and memory were studied in rat offspring born with FGR by assessing the animals' performance using the Morris water maze task. **Results:** At 1- and 2- months of age, both female and male offspring rats showed impairment of performance, while at 4 months of age, only female rats showed impaired performance. The FGR offspring spent a longer time swimming and used inefficient strategies($P < 0.05$, respectively). However, there were no significant maze performance FGR effects in the 4 month old male rats. In all groups of FGR offspring, irrespective of age or sex, the time spent in the platform quadrant by the rat was significantly less than that in the control group($P < 0.05$). **Conclusion:** The Morris water maze performance decreased in rat offspring born with FGR. It is suggested that FGR can cause impairments of spatial learning and memory in young animals.

Key words: Fetal growth restriction; Learning and memory; Pregnancy

INTRODUCTION

Worldwide, fetal growth restriction(FGR), also called intrauterine growth restriction(IUGR), is one of the most common complications occurring during pregnancy, with complication rates of up to 6% of all pregnancies[1]. FGR is associated not only with a marked increased risk in perinatal mortality and morbidity, but also with long-term outcome risks[2]. In the survivors there is increased susceptibility to diabetes and cardiovascular disease in adulthood[3]. Therefore, in recent years obstetricians and pediatricians have paid more attention to the results of FGR research. During the fetal period the central nerve system is developing rapidly and is easily affected by a variety of factors, which may lead to abnormal higher nervous system activity in the offspring. In our research, a FGR model was constructed. At different postnatal periods the FGR offspring performed the Morris water maze task and we investigated the impact of FGR on spatial learning and on memory capacity.

MATERIALS AND METHODS

The protocol for this study was approved by the Ethical Committee of the Medical College of Xi' an Jiaotong University.

Rat model of FGR

In this study, adult healthy Sprague-Dawley rats were provided by the Animal Experiment Center of Medical College of Xi' an Jiaotong University. The body weight of the rats ranged from 230 g to 260 g. Animals

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were placed in cages overnight at the ratio 1:2, male to females. Normal saline vaginal smears were performed and the presence of sperm was used to designate day 0 of pregnancy. All pregnant rats were individually housed in our Institute for Laboratory Animal Research, in a temperature-controlled environment with a controlled light and dark cycle. Temperature was controlled at the range of 25-30℃, and the humidity was 85%. The light-dark cycle was 12:12 h(light on at 7:00 a.m.). All pregnant females had ad libitum access to a standard diet and water throughout all experiments. Twenty pregnant rats were chosen randomly to undergo passive smoking for 1h in a cage at 8:00 a.m. and 4:00 p.m. from the 1st to the 18th days of pregnancy. Meanwhile, another 20 pregnant rats were put into a cage to inhale air under the same conditions as a control group. Deliveries were spontaneous. A neonate from the passive smoke group was considered to be part of the FGR group if its body weight was lower than the 10th percentile of the average value of the air-inhaled group of the same age(control group). At 1-, 2- and 4-month after delivery, 10 age and sex matched offspring from each group performed the Morris water maze task.

The detection of Morris water maze

Behavioral tests took place in a water maze using procedures described by Morris[4]. A round black tank (120 cm in diameter and 55 cm in depth) was filled with water. The pool was divided into four quadrants. A round plexiglass escape platform (8 cm in diameter) was placed 1 cm beneath the surface of the water at the center of a designated quadrant. The distance from the platform center to the pool edge was 35 cm. The location of the platform was the same throughout the training days. Water temperature was maintained at 23 ± 1℃ and water was changed and the tank cleaned daily. The position and orientation of the pool in the testing room remained unchanged throughout the study, and thus, both geometric and landmark cues were maintained constant[5].

The Morris water maze task consisted of two tests. The place navigation test lasted for four and a half days, with 2 sessions per day, and each session was comprised of four trials with an inter-trial interval of 60 s. The inter-session interval on a single day was 2 h. Tests were conducted between 8:00 and 12:00 a.m., and between 2:00 and 6:00 p.m. One day before the spatial training commenced, all rats underwent pre-training to familiarize them with the requirements of the test. During the trial, each rat was left in the water facing the wall of the pool and had 120 s in which to find the platform. If the rat did not find the hidden platform within a period of 120 s it was gently placed there by the observer for 10 s and the performance score(escape latency) was marked as "120 s". The initial position in which the animal was left in the tank was one of the four cardinal compass points of the pool quadrants and varied among trials in a pseudorandom manner. The spatial probe test on the afternoon of the fifth training day was comprised of only one single trial lasting 120 s. The platform was removed from the pool, and each rat entered the pool from the quadrant opposite to the one containing the platform(the "platform" quadrant) in previous testing sessions.

The swim path of a rat during each trial was recorded by a video camera mounted above the center of the pool and connected to a commercial video/computer system(Beijing Logon Science and Technology). The escape latency for finding the platform and the following four swimming strategies were recorded:①marginal: swimming along the pool edge, ②random: swimming randomly, ③ tendency: swimming around but generally towards the platform area, and ④ straight: swimming straight towards the platform. In the second test, the time that the rat spent within 120 s on the "platform" quadrant was additionally recorded.

Statistical analysis

The results are expressed as means ±SEM. Repeated-measure analysis of variance was used to compare the latency of different groups. Treatment, age, and sex were the three between-subject factors. One-Way ANOVA tests were used to evaluate statistical differences for the time spent on the "platform" quadrant among different groups. Swimming strategies were analyzed by Mann-Whitney non-parametric tests. A 2-sided P value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 13.0 software.

RESULTS

Latencies across sessions for all the animal groups are shown in ***Fig. 1***. All rats improved in performance during the tests, as indicated by the progressive reduction in escaping latencies over the sessions(F= 90.968, P < 0.05). For the three between-subject factors (treatment, age, and sex), the interaction of sex × age was significant(F = 5.223; P< 0.05). However, interactions of treatment × age(F = 0.391, P> 0.05), treatment × sex(F = 0.341, P > 0.05), and treatment × age × sex(F = 0.285, P> 0.05) were not significant.

To analyze the treatment main effects, we examined the differences between FGR and control groups for each age and sex combination. Interactions between testing sessions and treatment were not significant. The treatment main effect was significant for the 1-month male group(F = 7.859, P< 0.05), 1-month female group (F = 5.015, P< 0.05), 2-month male group(F = 5.238,

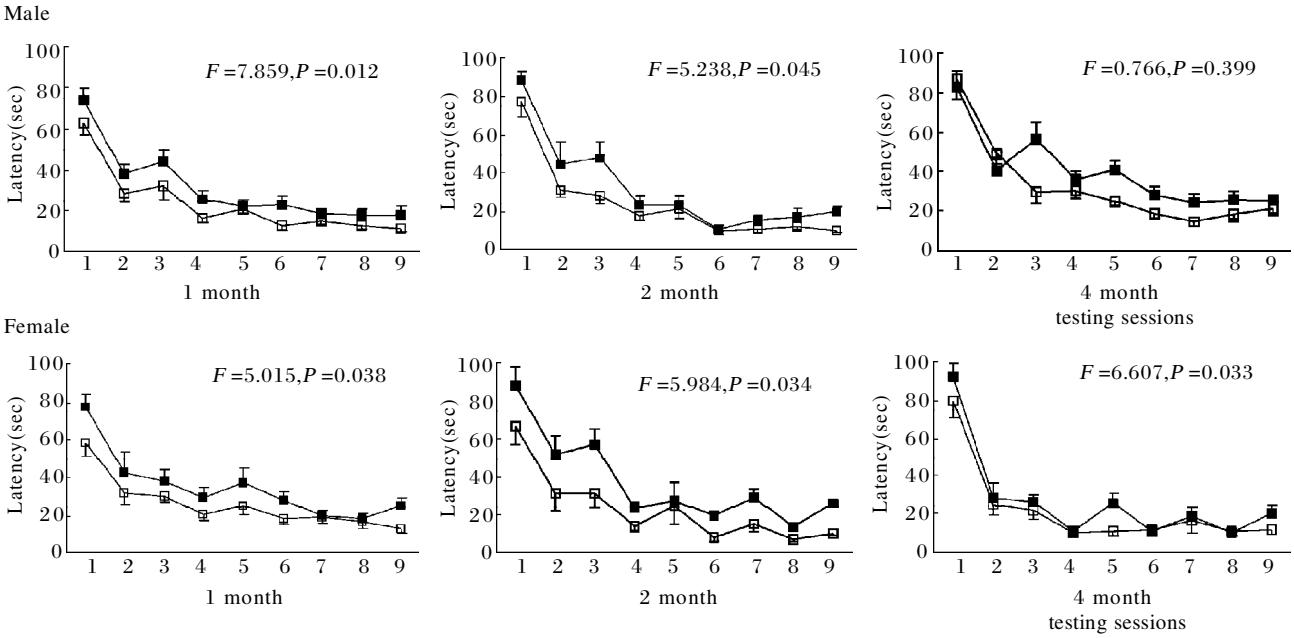
$P < 0.05$), 2-month female group($F = 5.984$, $P < 0.05$), 4-month female group($F = 6.607$, $P < 0.05$), but not for the 4-month male group($F = 0.766$, $P > 0.05$).

Examination of the strategy profiles used by each animal group revealed that the FGR group rats used non-effective strategies(random strategy or marginal strategy) more frequently but less effective strategies (tendency strategy or straight strategy) less frequently than control group rats except in the 4-month-old male group(***Fig. 2***).

In the last test, we observed that the times spent in the "platform" quadrant by the rats in FGR groups were significantly less than those in the control groups (***Table 1***).

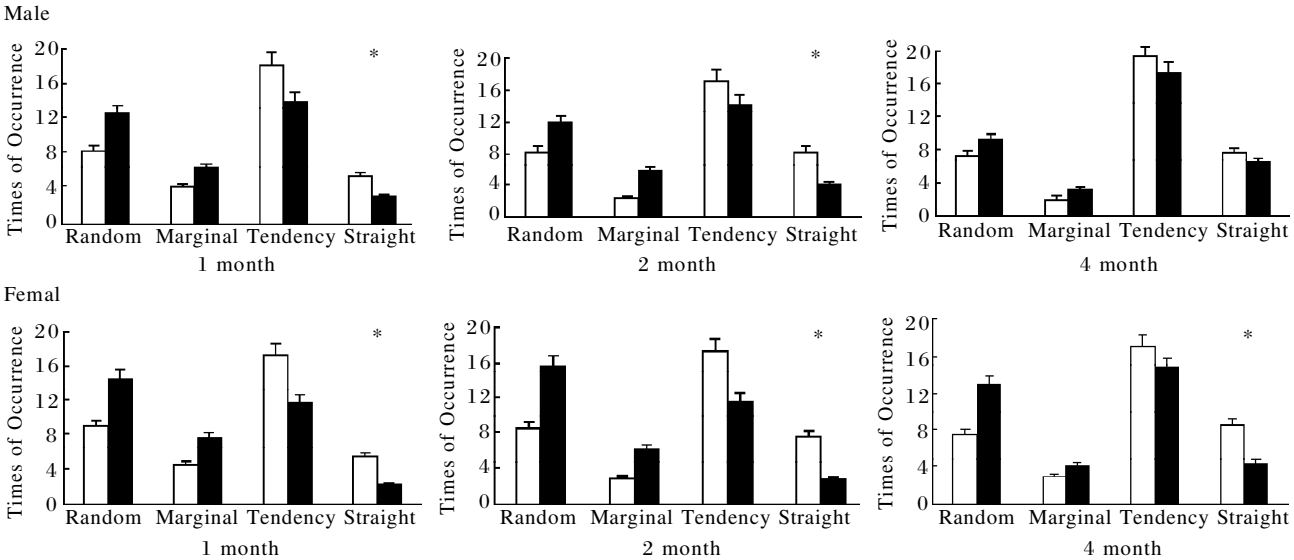
DISCUSSION

FGR is an important cause of perinatal mortality and morbidity[6] and is subsequently associated with neurologic abnormalities[7,8]. Obstetricians and pediatricians have a great interest in the impact of FGR on the offspring. Since ethical and methodological constraints limit experimental studies of human pregnancy, animal models of FGR have been used in many research studies. The passive smoking model of FGR in rats was used in this study, and an impact of FGR on the neural development of the offspring was detected.



The error bars indicate the standard errors of the mean.

Fig. 1 Average escape latency during the 9 testing sessions for FGR(filled squares) and normal(open squares) offspring



The error bars indicate the standard errors of the mean. *Significant differences between FGR and control groups($P < 0.05$).

Fig. 2 Average incidence of each of the four strategies used by the FGR group(solid bars) and control group(open bars)

Table 1 **Average time spent in the platform quadrant in the Morris water maze probe tests(means ± SEM)**

Group	N	1-month		2-month		4-month	
		Male	Female	Male	Female	Male	Female
FGR	10	31.85 ± 2.38*	31.22 ± 2.05*	31.52 ± 1.36#	34.45 ± 2.15#	33.65 ± 1.22#	35.18 ± 2.08#
Control	10	41.15 ± 2.70	40.69 ± 2.68	46.97 ± 3.66	47.72 ± 3.21	41.85 ± 2.11	43.88 ± 1.27

*$P < 0.05$, #$P < 0.01$, vs control group.

In this study, we first investigated the effects of FGR on the spatial learning and memory ability of 1-, 2- and 4-month-old offspring rats. We found that with the 1- and 2-month-old FGR offspring groups, of either sex, the latency to find the platform in the water maze task was longer than for the corresponding control group. In addition, FGR offspring were inclined to using ineffective strategies(random or marginal strategy), and less inclined to use effective strategies(tendency or straight strategy). However, in 4-month-old offspring, such an effect was only observed in females. These results indicate that FGR impairs the learning ability of the offspring. Our conclusion is similar to that of Leitner[9] and his coworkers, whose research showed that spatial orientation in IUGR children(6-years-old) was inferior to their age-matched controls, and they thought this possibly contributed to their potential learning difficulties. Learning difficulties are frequently diagnosed in children born with FGR. A study by Geva *et al*[10] showed learning difficulties accompanied by lower academic achievement were more prevalent in children with IUGR. Recently, other reports showed that children born with FGR had long-term cognitive impairments and learning difficulties in school[11-13].

The present data also show that FGR can impair the memory capacity of offspring. The staying times spent in the "platform" quadrant by rats in the FGR groups were shorter than those in control groups. A few recent studies have provided similar results on memory abilities in FGR offspring. Black *et al*[14] studied recognition memory in FGR neonates. Frisk and colleagues[15] examined working memory in children, and Isaacs *et al*[16] evaluated daily memory in adolescents born with FGR. Those studies are agreement with our results. Recently, research by Geva and colleagues[17] found that children with IUGR had short-term memory difficulties that hindered both the serial verbal processing system and simultaneous processing of high-load visuo-spatial stimuli. However, a systematic evaluation of the various memory systems in children with FGR has not yet been conducted.

It is of interest that we observed a difference in the learning ability of male and female 4-month-old FGR offspring. FGR had a marked effect on the cognitive performance of 4-month female rat offspring. These female animals used inefficient strategies and spent a longer time to find the platform compared to their control counterparts. No significant effects were observed in the 4-month-old male groups. Similarly, previous studies have reported a sex-dependent difference in the response to harmful prenatal environments. Exposure to harmful factors such as alcohol, toluene, or MR during pregnancy caused poor performance in the water maze, but only in the female offspring of the exposed rats[18-20]. It has been proposed that males generally use a single type of cue(geometric) in spatial learning, while females depend on multiple cue types[5]. Thus, strategies used by males are considered less complicated than those used by females and therefore the performance of males in the water maze may be better than that of females. This difference between the sexes is not apparent in younger rats. It has been reported that sexual development of female rats occurs later than that of males[21] and estrogen has been closely associated with the neural plasticity that occurs during learning[22]. The more prolonged sexual maturation in females may lead to a higher sensitivity to FGR, and the consequences are more obvious when females have just become sexually mature.

Learning and memory are essential brain functions, and the hippocampus is known to play an important role in many types of both functions. Recent reports point to an increased susceptibility to learning and memory deficits among children born prematurely who had been diagnosed with IUGR. As is known, FGR has a negative effect on brain development[23, 24], but the pathogenic mechanisms are not clear. The deficits in learning and memory abilities in FGR offspring are associated with abnormalities of the brain, especially the hippocampus. Multiple studies have documented the adverse effects of FGR on the hippocampus and surrounding structures. FGR can affect brain development in a way which could lead to neurological and behavioral deficits in the postnatal animal. Models of various animal species with FGR were studied and demonstrated specific susceptibility and alterations of the hippocampal formation and its related neural structures. Mallard *et al*[25] found that the number of neurons was reduced in the hippocampus and the cerebellum in the postnatal guinea-pig following IUGR. Tatli[26] and colleagues proved that FGR decreased brain weights of offspring rats and increased oxidative damage to lipids and proteins from some CNS areas. Additionally, experimental studies in different animal

models of FGR have shown neuronal degeneration in the hippocampal pyramidal neurons and loss of dendritic branches and density of granular neurons in the dentate gyrus, with an overall reduction of cellularity by 30%[27], which resulted in a reduced overall hippocampal volume when measured by MRI[28].

In summary, our research shows that FGR impairs spatial learning and memory ability of the offspring, and that the observed impairments are gender-and age-specific. The mechanism of these impairments probably relates to abnormalities in the hippocampus. The results of the present study point to the need for further research to elucidate the neuromolecular and neurocellular bases of these effects, and perhaps provide clues for possible preventive measures against these impairments.

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