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Age- and gender-dependent impairments of neurobehaviors in mice whose mothers were exposed to lipopolysaccharide during pregnancy

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ABSTRACT

Lipopolysaccharide (LPS)-induced intrauterine infection has been associated with neurodevelopmental injury in rodents. The purpose of the present study was to analyze the dynamic changes of neurobehaviors in mice whose mothers were exposed to LPS during pregnancy. The pregnant mice were intraperitoneally (i.p.) injected with LPS (8 μg/kg) daily from gestational day (gd) 8 to gd 15. A battery of neurobehavioral tasks was performed in mice at postnatal day (PND) 70, 200, 400 and 600. Results showed that the spatial learning and memory ability, determined by radial six-arm water maze (RAWM), were obviously impaired in two hundred-day-old female mice and four hundred-day-old male mice whose mothers were exposed to LPS during pregnancy. Open field test showed that the number of squares crossed and peripheral time, a marker of anxiety and exploration activity, were markedly increased in two hundred-day-old female mice following prenatal LPS exposure. In addition, prenatal LPS exposure significantly shortened the latency to the first grid crossing in six hundred-day-old female offspring. Moreover, sensorimotor impairment in the beam walking was observed in two hundred-day-old female mice whose mothers were exposed to LPS during pregnancy. Species-typical behavior examination showed that prenatal LPS exposure markedly increased weight burrowed in seventy-day-old male offspring and six hundred-day-old female offspring. Correspondingly, prenatal LPS exposure significantly reduced weight hoarded in two hundred-day-old female offspring. Taken together, these results suggest that prenatal LPS exposure induces neurobehavioral impairments at adulthood in an age- and gender-dependent manner.

1. Introduction

Lipopolysaccharide (LPS) is a toxic component of cell walls in gram-negative bacteria and is widely present in the digestive tracts of humans and animals (Jacob et al., 1977). Humans are constantly exposed to low levels of LPS through bacterial infection. Gastrointestinal distress and excess alcohol intake are known to increase uptake of LPS from gastrointestinal tract into blood (Fukui et al., 1991; Zhou et al., 2003). High levels of LPS have also been detected in women with bacterial vaginosis (Platz-Christensen et al., 1993). In human, Gram-negative bacterial infections are a recognized cause of embryo loss and preterm labor (Romero et al., 1988). Mimicking maternal infection by exposing the pregnant rodents to LPS at early gestational stages resulted in embryonic resorption and fetal death (Gendron et al., 1990; Ogando et al., 2003). Maternal LPS exposure at middle gestational stages caused teratogenesis, fetal death and preterm delivery (Leazer et al., 2002; Zhao et al., 2008). In addition, several studies showed that maternal LPS exposure at late gestational stages led to fetal death, growth restriction, skeletal development retardation, and preterm labor (Rivera et al., 1998; Buhimschi et al., 2003; Xu et al., 2005, 2006, 2007b).

Maternal inflammation during pregnancy resulted in a higher incidence of psychiatric disorders with a presumed neurodevelopmental origin in the offspring, including schizophrenia and autism. Substantial clinical studies have showed that the mothers of newborns with schizophrenia or brain white matter lesions had higher levels of pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukin-1beta (IL-1β) and interleukin-6 (IL-6) in their amniotic fluid and maternal serum than did mothers delivered of newborns without schizophrenia or brain white matter lesions (O'Callaghan et al., 1994; Naudin et al., 1997; Yoon et al., 1997a). Another study found that the expression of TNF-α, IL-1β and IL-6 is also much higher in brains with periventricular leukomalacia (PVL) than in those without PVL (Yoon et al., 1997b). Mimicking intrauterine infection and inflammation by maternal

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LPS exposure during pregnancy significantly increased the levels of TNF-α, IL-1β and IL-6 in maternal serum, fetal liver and amniotic fluid, TNF-α and IL-10 in fetal brain in rodents (Ashdown et al., 2006; Xu et al., 2007a). Several studies showed that maternal LPS (120 μg/kg, i.p.) exposure at late gestational stages markedly impaired the learning abilities and social behavioral performance in adulthood (Golan et al., 2005; Golan et al., 2006; Hava et al., 2006). Another study found that maternal IL-6 exposure at middle and late gestational stages resulted in inflammatory neurodegeneration in hippocampus with NMDA/$GABA_A$ dysregulation and impaired spatial learning (Samuelsson et al., 2006).

In the present study, we aimed to analyze the dynamic changes of neurobehavioral impairments in adult offspring whose mothers were exposed to LPS (8 μg/kg, i.p.) at middle gestational stages. We found that prenatal LPS exposure induces neurobehavioral impairments at adulthood in an age- and gender-dependent manner.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8) was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were from Sigma or as indicated in the specified methods.

2.2. Animals and treatments

CD-1 mice (male mice: 28–30 g; female mice: 24–26 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. The presence of a vaginal plug was designated as gestational day (gd) 0. Twenty pregnant mice were randomly divided into two groups. In LPS group, the pregnant mice received an intraperitoneal (i.p.) injection of LPS (8 μg/kg) daily from gd 8 to gd 15. The normal saline-treated pregnant mice served as controls. Within 24 h after birth, excess pups were removed, so that four males and four females were kept per dam. At postnatal days 21 (PND 21), pups were separated from their siblings and housed five to a cage. A male mouse and a female mouse per litter were given the behavioral tests. The same mice in each group were tested at PND 70, 200, 400, 600. Some mice with overt blindness and large abdomen were removed before behavioral tests. The battery of behavioral tasks lasted for twelve days. Except for nesting and hoarding, each task was carried out during the light phase and in the following order: Species-typical behaviors (days 1 and 2); Open-field test (day 3); Beam walking (day 4); Tightrope (day 5); RAWM (days 6–12). All tasks were performed in the feeding room in order to preserve the adaptation to the environment. The mice were allowed free access to food and water at all times and were maintained in a 12-h light/dark schedule and in a controlled temperature (20–25 °C) and humidity (50% ± 5%) environment for a period of one week before use. All animal experiments were carried out in accordance with the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Radial six-arm water maze (RAWM)

The protocol for RAWM was designed according to the literatures (Morgan et al., 2000; Chen et al., 2004). The maze consisted of a circular black pool (1 m in diameter, 21 cm in height) with six swim arms (30.5 cm × 19 cm × 21 cm) that radiated out from a central area (40 cm in diameter) and was full of water (19–22 °C). A submerged platform (10 cm in diameter and 15 cm in height) was located at the end of one of these alleys and at a distance of 4.5 cm from three walls. In addition to the experimenter, three spatial cues of different size and shape were surrounded the maze at a height of 150 cm and a distance of 120 cm from the outer wall of the maze. Each day, the mice learned the location of the submerged platform, which was placed in the same arm on each trial within a day and in a different arm across days, during four consecutive acquisition trials (trials 1–4) followed 30 min later by a retention trial (trial 5). In each trial, the mouse was started in one of four random entry arms not including the arm containing the platform and its opposite alley and allowed to swim for 60 s to find the escape platform. Upon entering an incorrect arm (four paws within the swim alley) or failing to select an arm, the mouse was gently dragged back to the start arm for that trial and counted an error. All mice spent 30 s on the platform following the trial before beginning the next trial. After the fourth trial had been completed, the mouse was dried with a towel, placed in its home cage under a 150-W floodlight for 30 min, then returned to the maze and performed the retention trial with the identical start alley as trial 4. The number of errors and the latency (the time from entering the water to finding the platform) were recorded. Due to the altered location for each mouse entering the water through trials 1–4 daily, and the different swimming orientation of the mice, the performance in trial 1 was not always worse than that in trials 2–4, so the number of errors and the latency in the learning period (trials 1–4) daily was averaged and was considered as the learning performance each day.

2.4. Open field

To determine spontaneous motor activity and anxiety, an open-field procedure was designed (Tong et al., 2007). An open, black wooden box (81 cm × 81 cm interior area) with 28-cm high walls was used. The box floor was painted with white lines (3 mm wide) to form 16 equal squares (20 cm × 20 cm) with a colored box (8 cm × 5 cm × 3 cm) in the center of the area. Illumination was provided by a 40-W white light placed 2.80 m above the center of the field. The mouse was introduced to one of the four corners in the field, facing the wall, and was permitted to explore the environment for 5 min ad libitum. During this time, squares crossed, latency to the first grid crossing and peripheral time (the time spent in the 12 peripheral squares) were recorded. Moreover, the area was cleaned with stupe before the next mouse was tested.

2.5. Beam walking

The apparatus consisted of a 51-cm-long wooden beam (1 cm wide) and two vertical supports (50 cm in height). Each mouse was given three successive trials and perpendicularly placed on the center of the beam. There was 60 s in a maximum duration of each trial. The balance time, during which the mice did not fall from the beam, was recorded as each of the three trials. If the mice remained on the beam for the duration of the trial or escaped to either of the platforms, it was recorded as 60 s. The mean time of the three trials was recorded.

2.6. Tightrope

In the task, each mouse was forced to grasp and suspend from a tightrope. A taut and tiny cotton rope (2 mm in diameter with an ink mark at 5-cm intervals along its length) was stretched across a tank (100 cm in diameter, 30 cm in height) half-filled with water (at 19–22 °C). Before the start of the test, each mouse was placed into the water for 5 s. During a 60-s trial, the mouse was raised to grasp the center of the rope with its forepaws, and then slowly released in order to support its own weight by means of its grip. The suspension time and the number of markers crossed were separately recorded. Once a mouse had fallen off the rope, or stayed on the rope for up to 60 s, it was immediately removed to a home cage and allowed to rest for 30 s before the next trial. The suspension time and the number of markers crossed in three trials were averaged for performance each mouse. Among the mice with the same average suspension time, some were immovably suspended on the tightrope, whereas the others could not only suspend on the tightrope, but also move laterally and horizontally. In order to distinguish these mice, we used a transformed score [=(average suspension time) + 10(average number of the marker crossed)].

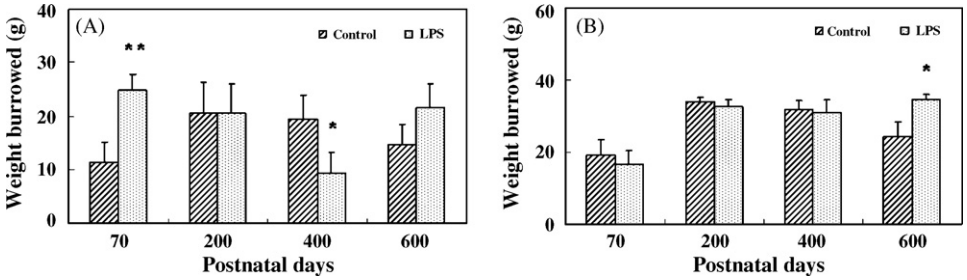

Fig. 1. Effects of prenatal LPS exposure on burrowing behaviors in mice. Pregnant mice were administered with LPS (8 μg/kg, i.p.) daily from gd 8 to gd 15. The weight burrowed of male (A) and female (B) offspring was tested at PND 70, 200, 400, 600. Data were expressed as means ± SEM of ten mice. $^*P < 0.05$, $^{**}P < 0.01$ as compared with controls.

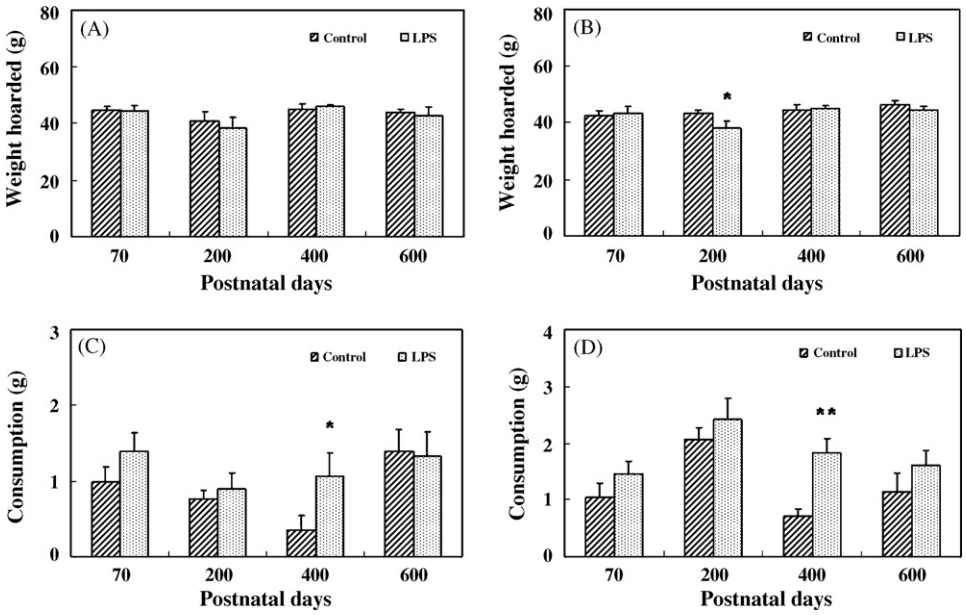

Fig. 2. Effects of prenatal LPS exposure on hoarding behaviors in mice. Pregnant mice were administered with LPS (8 μg/kg, i.p.) daily from gd 8 to gd 15. The weight hoarded and consumption of male (A, C) and female (B, D) offspring were examined at PND 70, 200, 400, 600. Data were expressed as means ± SEM. $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with controls.

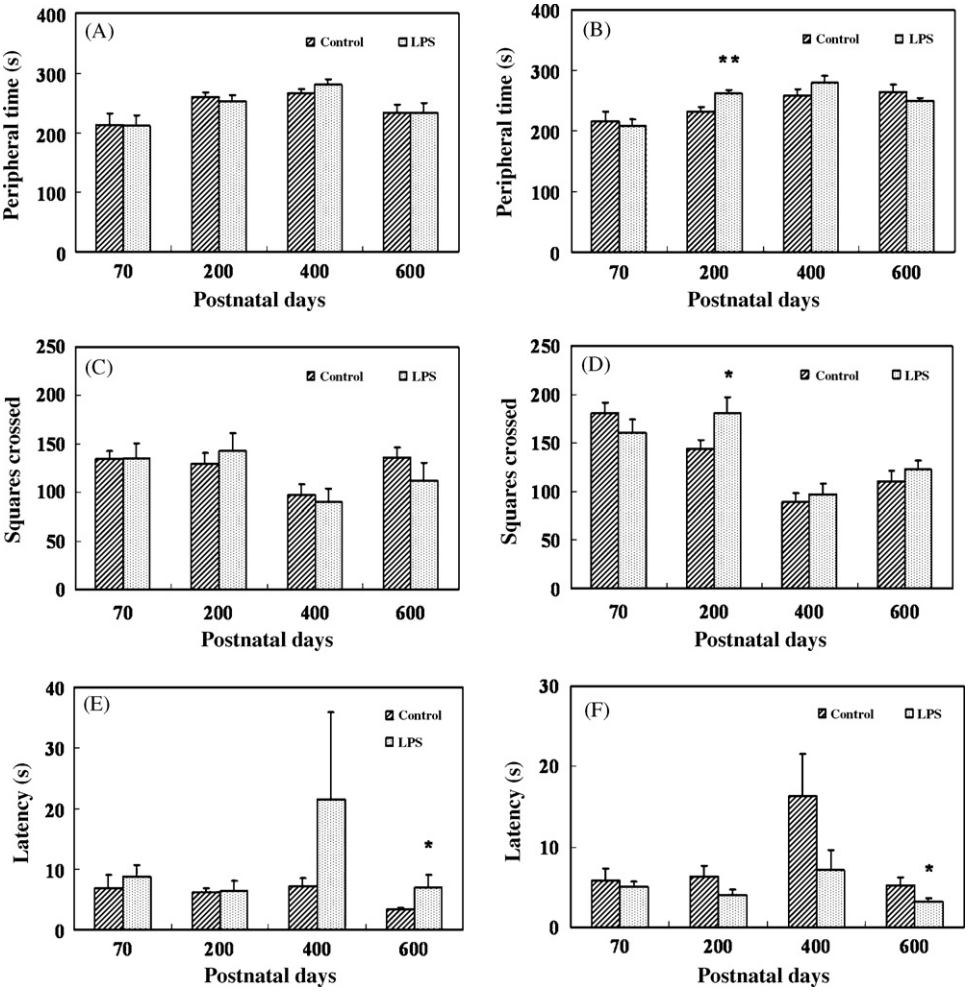

Fig. 3. Effects of prenatal LPS exposure on exploration and anxiety behaviors in mice. Pregnant mice were administered with LPS (8 μg/kg, i.p.) daily from gd 8 to gd 15. Peripheral time, the number of squares crossed and latency to the first grid crossing of male (A, C, E) and female (B, D, F) offspring were examined at PND 70, 200, 400, 600. Data were expressed as means ± SEM. $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared with controls.

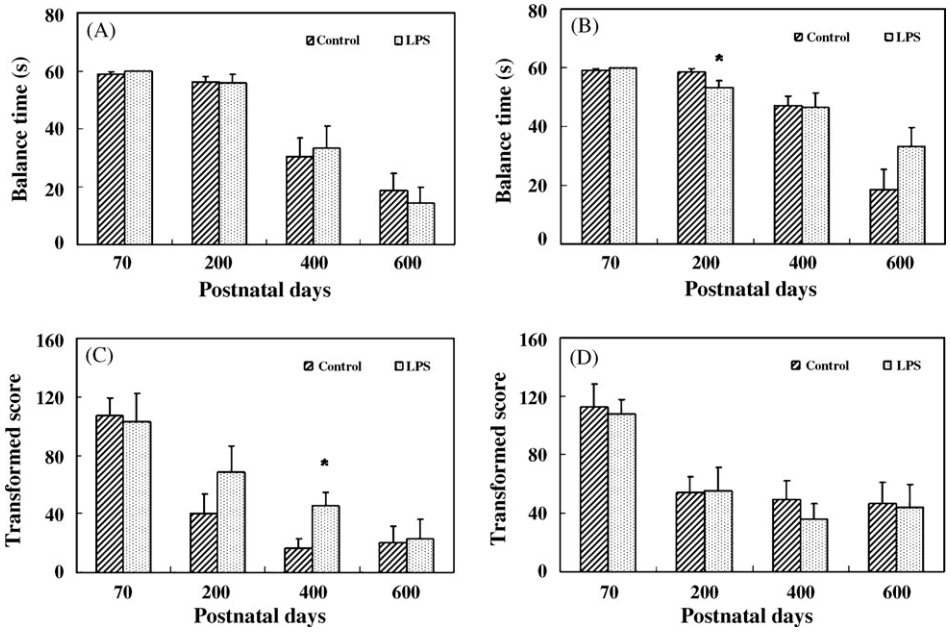

Fig. 4. Effects of prenatal LPS exposure on sensory and motor function in mice. Pregnant mice were administered with LPS (8 μg/kg, i.p.) daily from gd 8 to gd 15. Balance time and transformed scores of male (A, C) and female (B, D) offspring were examined at PND 70, 200, 400, 600. Data were expressed as means ± SEM. $n = 10$. *$P < 0.05$, as compared with controls.

2.7. Species-typical behaviors

The apparatus and procedure were designed according to previous studies with some modifications (Deacon et al., 2002, 2003).

2.7.1. Burrowing

The experimental apparatus was composed of a plastic cage, which was the same as the home cage, contained a plastic tube (4 cm in diameter, 10 cm long) and a bright iron tube with semi-cylinder shape (5 cm in diameter, 12 cm long). In order to aggregate food within the iron tube, two bars of 1-cm height were transversely pasted at the floor of the iron tube at 1-cm distance from each end, and the space between two bars was full of 40-g soybean. Each mouse (not deprived of food) was placed into single cage during the light period. And then the soybean displaced from the tube was weighed 2 h later.

2.7.2. Hoarding

Mice were housed in wooden boxes with wire mesh lids and wood shaving bedding (30 cm × 20 cm × 15 cm), each attached to a 60-cm-long wire mesh tube, at the far end of which was placed 50 g of normal diet food pellets. Mice were individually housed in the boxes just before the beginning of the dark cycle and had free access to water. The next morning, the pellets found in the box were weighed and were considered as the weight hoarded. Moreover, the consumption, including the weight eaten and crunched, was also calculated (the consumption = initial weight − the weight hoarded).

2.7.3. Nesting

All mice were individually housed with food, water and new sawdust bedding. Six pieces of white papery cloth (4 cm × 4 cm), which was used to build a nest, were evenly placed in each cage. After the overnight, the nests were scored according to the following standards: 0, no visible crater of sawdust and no papery cloth; 1, sawdust crater alone and no shredded cloth; 2, sawdust crater with shredded or whole papery cloth gathered around and in the crater; 3, sawdust crater with shredded or whole papery cloth gathered around and in the crater forming a cup-shaped nest; 4, the shredded papery cloth forms a ball-shaped nest covering the mouse.

2.8. Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM). To evaluate the difference between control group and LPS group, data analysis was performed by two-way ANOVA for repeated measures. When $P < 0.05$ or a lower value, it was considered to be a significant difference.

3. Results

3.1. Effects of prenatal LPS exposure on species-typical behaviors at adulthood

To investigate the effects of prenatal LPS exposure on species-typical behaviors at adulthood, the pregnant mice were administered with LPS daily from gd 8 to gd 15. No signs of maternal toxicity were observed in dams treated with LPS. As shown in Fig. 1A and B, weight burrowed was significantly increased in seventy-day-old male mice and six hundred-day-old female mice whose mothers were administered with LPS during pregnancy, whereas prenatal LPS exposure decreased weight burrowed in male mice at PND400. The effects of prenatal LPS exposure on hoarding behavior were presented in Fig. 2. Results showed that weight hoarded was markedly decreased in two hundred-day-old female mice whose mothers were exposed to LPS during pregnancy. Interestingly, prenatal LPS exposure obviously increased consumption in male and

Table 1
Effects of prenatal LPS exposure on learning and memory performances in males over seven consecutive days.

Index	PND 70		PND 200		PND 400		PND 600	
	Control	LPS	Control	LPS	Control	LPS	Control	LPS
Latency in trials 1–4	32.4 ± 2.1	27.8 ± 2.1	34.6 ± 1.9	33.7 ± 1.9	34.5 ± 1.6	40.0 ± 1.7*	30.2 ± 1.7	33.9 ± 1.8
Errors in trials 1–4	4.3 ± 0.3	3.8 ± 0.3	4.4 ± 0.2	4.5 ± 0.2	3.8 ± 0.2	4.7 ± 0.2**	4.0 ± 0.2	4.1 ± 0.2
Latency in trials 5	33.5 ± 2.7	24.7 ± 2.5*	32.4 ± 2.8	31.5 ± 2.7	32.2 ± 2.7	38.5 ± 2.6*	23.9 ± 2.5	24.1 ± 2.4
Errors in trials 5	5.2 ± 0.5	3.3 ± 0.4**	4.7 ± 0.5	4.5 ± 0.5	3.9 ± 0.4	4.9 ± 0.4*	3.5 ± 0.4	3.3 ± 0.4

*$P < 0.05$, **$P < 0.01$ as compared with controls.

Table 2
Effects of prenatal LPS exposure on learning and memory performances in females over seven consecutive days.

Index	PND 70		PND 200		PND 400		PND 600	
	Control	LPS	Control	LPS	Control	LPS	Control	LPS
Latency in trials 1–4	29.4 ± 2.1	26.1 ± 2.0	23.5 ± 1.8	33.2 ± 1.5**	34.8 ± 1.8	32.0 ± 1.6	38.4 ± 1.6	31.6 ± 1.5**
Errors in trials 1–4	4.2 ± 0.3	3.8 ± 0.3	3.2 ± 0.3	4.4 ± 0.2**	4.0 ± 0.2	4.4 ± 0.2	4.3 ± 0.2	3.9 ± 0.2
Latency in trials 5	29.8 ± 2.7	23.3 ± 2.4*	21.9 ± 2.2	30.3 ± 2.6*	27.7 ± 2.5	28.9 ± 2.6	32.8 ± 2.7	31.4 ± 2.7
Errors in trials 5	4.9 ± 0.5	3.8 ± 0.5*	3.5 ± 0.4	4.8 ± 0.5*	3.7 ± 0.4	4.5 ± 0.5	4.4 ± 0.4	4.6 ± 0.5

*$P < 0.05$, **$P < 0.01$ as compared with controls.

female mice at PND400. There was no significant difference on the nesting scores between LPS-treated mice and controls at all time points (data not shown).

3.2. Effects of prenatal LPS exposure on exploration and anxiety activities at adulthood

The effects of prenatal LPS exposure on exploration and anxiety activities at adulthood were detected by open field test. As shown in Fig. 3, the average peripheral time and the number of squares crossed were significantly increased only in middle-aged females (PND200) whose mothers were exposed with LPS during pregnancy, whereas prenatal LPS exposure had little effect on the average peripheral time and the number of squares crossed in males and young (PND70) and old females (from PND400 to PND600). Interestingly, latency to the first grid crossing was markedly decreased in six hundred-day-old female mice whose mothers were exposed to LPS during pregnancy (Fig. 3F). By contrast, prenatal LPS exposure markedly prolonged the latency to the first grid crossing in male mice at the age of six hundred days (Fig. 3E).

3.3. Effects of prenatal LPS exposure on sensorimotor function at adulthood

As shown in Fig. 4A and B, balance time was shorter in two hundred-day-old female offspring whose mothers were exposed to LPS during pregnancy, whereas no significant difference on balance time was observed in males and young (PND70) and old females (from PND400 to PND600) whose mothers were exposed to LPS during pregnancy. In addition, prenatal LPS exposure significantly increased transformed score of tightrope test in male offspring at the age of four hundred days as compared with the controls (Fig. 4C and D).

3.4. Effects of prenatal LPS exposure on learning and memory performance at adulthood

The effects of prenatal LPS exposure on learning and memory performances were examined by RAWM. ANOVA with repeated measures showed that average errors during the retention phase were markedly reduced in seventy-day-old male mice following prenatal LPS exposure (Table 1). In contrast, latency during the learning phase [$F_{(1,14)} = 13.602$, $P < 0.01$], the number of errors during the learning phase [$F_{(1,14)} = 9.557$, $P < 0.01$] and latency during the memory phase [$F_{(1,14)} = 6.243$, $P < 0.05$] were significantly increased in two hundred-day-old female mice whose mothers were exposed to LPS during pregnancy (Table 2). Moreover, prenatal LPS exposure had mild effect on the number of errors during the learning phase in four hundred-day-old male mice [$F_{(1,18)} = 3.857$, $P = 0.065$; Table 1]. However, no significant differences in learning and memory performances between the controls and LPS treatments were observed in six hundred-day-old male or female mice (Tables 1 and 2). We also analyzed average errors and latency over seven consecutive days by *t*-test. Results showed that prenatal LPS exposure significantly increase average errors and latency during the learning and retention phase in four hundred-day-old male mice and two hundred-day-old female mice (Tables 1 and 2). Interestingly, prenatal LPS exposure obviously decreased average errors and latency during the retention phase in seventy-day-old male offspring and in seventy-day-old females (Tables 1 and 2). In addition, latency during the learning phase was markedly decreased in six hundred-day-old female mice whose mothers were exposed to LPS during pregnancy (Table 2).

4. Discussion

In the present study, we analyzed the dynamic changes of neurobehavioral impairments in adult offspring whose mothers were exposed to LPS daily from gd 8 to gd 15. A battery of neurobehavioral tasks was performed in mice at PND 70, 200, 400 and 600. Our results showed that prenatal LPS exposure resulted in the impairments of neurobehaviors, such as species-typical behaviors, exploration and anxiety, sensorimotor, and learning and memory at adulthood in an age- and gender-dependent manner.

The species-typical behaviors, including burrowing, hoarding, and nesting, have been widely studied in rodents. It has been demonstrated that a successful achievement of the species-typical behaviors depends on the intact of the hippocampus and prefrontal cortex in mice (Deacon et al., 2002, 2003). Recent studies showed that administration of sub-pyrogenic doses LPS to adult mice induced impairments in species-typical behaviors that were similarly affected by hippocampal lesions (Deacon et al., 2002; Teeling et al., 2007). In the present study, we investigated the effects of prenatal LPS exposure on the species-typical behaviors in mice at adulthood. Results showed that impacts of burrowing behavior were observed in seventy-day-old male mice, four hundred-day-old male mice and six hundred-day-old female mice whose mothers were administered with LPS at middle gestational stages. In addition, prenatal LPS exposure induced hoarding impairments in two hundred-day-old females.

Exploration and anxiety activities were determined by open field task or elevated plus maze. Several studies indicated that six hundred-day-old male mice whose mothers were exposed to LPS (120 μg/kg, i.p.) at late gestational stages covered longer distances and entered frequently to the center of the field (Golan et al., 2006; Hava et al., 2006), whereas no significant difference in exploratory behavior was observed between the control male mice and eight-month-old male mice whose mothers were exposed to LPS (120 μg/kg, i.p.) at late gestational stages (Golan et al., 2005). These results are in agreement with ours, in which there was no significant difference in exploratory behavior between two hundred-day-old male mice and the controls whose mothers were exposed to LPS (8 μg/kg, i.p.) at middle gestational stages. However, maternal LPS exposure at middle gestational stages induced an increase in peripheral time and squares crossed in two hundred-day-old female mice. In addi-

tion, latency to the first grid crossing was markedly increased in six hundred-day-old females whose mothers were exposed to LPS daily from gd 8 to gd 15. To get more insights into the emotional phenotype displayed by LPS-exposed offspring, the elevated plus maze test is required to be performed in further studies.

Effects of prenatal LPS exposure on sensorimotor function at adulthood remain controversial. Several studies indicated that maternal exposure to a single dose of LPS at gd 17 had no effect on sensory and motor function in eight-month-old males and twenty-month-old male mice (Golan et al., 2005, 2006). In the present study, we found that sensorimotor impairment in the beam walking was observed in two hundred-day-old female mice whose mothers were exposed to LPS daily from gd 8 to gd 15. Our results are in agreement with those by others (Borrell et al., 2002; Fortier et al., 2007; Romero et al., in press), in which maternal LPS challenge throughout gestation or at middle and late gestational stages markedly disrupted sensorimotor gating at adulthood in rats.

Numerous studies have demonstrated that the hippocampus mediates allocentric spatial navigation. The prefrontal cortex is critical to acquiring the rules that govern performance in particular tasks, while the dorsomedial striatum mediates egocentric spatial orientation (Devan et al., 1996; Oliveira et al., 1997). Currently, the morris water maze (MWM) and the radial six-arm maze (RAWM) were widely used to evaluate spatial learning and memory abilities in rodents (Morgan et al., 2000; Vorhees et al., 2000; Vann and Aggleton, 2003; Colón-Cesario et al., 2006; Huang et al., 2006; Su et al., 2007). RAWM consists of the variable spatial complexity of radial arm maze and the efficient learning of MWM. One distinct advantage of RAWM is the ability to assess both reference and working memory errors simultaneously (Hyde et al., 1998). Several studies showed that maternal LPS (120 μg/kg, i.p.) exposure at gd 17 induced the impairments of the learning and memory performance detected by MWM, which was associated with specific histopathological injury in the hippocampus region in adult offspring (Golan et al., 2005, 2006). Another study showed that maternal IL-6 exposure at middle and late gestational stages caused impairments in spatial learning (Samuelsson et al., 2006). In line with the previous studies, the present study also found that the spatial learning and memory ability, determined by radial six-arm water maze (RAWM), were obviously impaired in two hundred-day-old female mice and four hundred-day-old male mice whose mothers were exposed to LPS (8 μg/kg, i.p.) daily from gd 8 to gd 15. These results suggested that prenatal LPS exposure induced impairments in the spatial learning and memory ability occurred earlier in females than in males. However, there was no significant difference in four hundred-day-old female mice or six hundred-day-old male mice between LPS treatment group and the control group. The same mice had been given RAWM test at different ages, so some tasks were learned in aged mice. Additional experiment is required to determine whether prenatal LPS exposure induces learning and memory impairments in test-naïve mice at different ages.

In summary, our results indicated that prenatal LPS exposure induced neurobehavioral impairments at adulthood in an age- and gender-dependent manner. The decreases of the spatial learning and memory performance were observed in four hundred-day-old males. The impairments of the anxiety activity occurred in six hundred-day-old females. Burrowing behavior was impaired in seventy-day-old males and six hundred-day-old females. In two hundred-day-old females, all the neurobehaviors, including hoarding behavior, sensorimotor function, anxiety and exploration activities, and the spatial learning and memory performance, were impaired.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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References

Ashdown, H., Dumont, Y., Ng, M., Poole, S., Boksa, P., Luheshi, G.N., 2006. The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. Mol. Psychiatry 11, 47–55.

Borrell, J., Vela, J.M., Arevalo-Martin, A., Molina-Holgado, E., Guaza, C., 2002. Prenatal immune challenge disrupts sensorimotor gating in adult rats. Implications for the etiopathogenesis of schizophrenia. Neuropsychopharmacology 26, 204–215.

Buhimschi, I.A., Buhimschi, C.S., Weiner, C.P., 2003. Protective effect of N-acetylcysteine against fetal death and preterm labor induced by maternal inflammation. Am. J. Obstet. Gynecol. 188, 203–208.

Chen, G.H., Wang, Y.J., Wang, X.M., Zhou, J.N., 2004. Accelerated senescence prone mouse-8 shows early onset of deficits in spatial learning and memory in the radial six-arm water maze. Physiol. Behav. 82, 883–890.

Colón-Cesario, W.I., Martínez-Montemayor, M.M., Morales, S., Félix, J., Cruz, J., Adorno, M., Pereira, L., Colón, N., Maldonado-Vlaar, C.S., Peña de Ortiz, S., 2006. Knockdown of Nurr1 in the rat hippocampus: implications to spatial discrimination learning and memory. Learn. Mem. 13, 734–744.

Deacon, R.M., Croucher, A., Rawlins, J.N., 2002. Hippocampal cytotoxic lesion effects on species-typical behaviours in mice. Behav. Brain Res. 132, 203–213.

Deacon, R.M., Penny, C., Rawlins, J.N., 2003. Effects of medial prefrontal cortex cytotoxic lesions in mice. Behav. Brain Res. 17, 139–155.

Devan, B.D., Goad, E.H., Petri, H.L., 1996. Dissociation of hippocampal and striatal contributions to spatial navigation in the water maze. Neurobiol. Learn. Mem. 66, 305–323.

Fortier, M.E., Luheshi, G.N., Boksa, P., 2007. Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy. Behav. Brain Res. 181, 270–277.

Fukui, H., Brauner, B., Bode, J.C., Bode, C., 1991. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. J. Hepatol. 12, 162–169.

Gendron, R.L., Nestel, F.P., Lapp, W.S., Baines, M.G., 1990. Lipopolysaccharide-induced fetal resorption in mice is associated with the intrauterine production of tumour necrosis factor-alpha. J. Reprod. Fertil. 90, 395–402.

Golan, H.M., Lev, V., Hallak, M., Sorokin, Y., Huleihel, M., 2005. Specific neurodevelopmental damage in mice offspring following maternal inflammation during pregnancy. Neuropharmacology 48, 903–917.

Golan, H., Stilman, M., Lev, V., Huleihel, M., 2006. Normal aging of offspring mice of mothers with induced inflammation during pregnancy. Neuroscience 141, 1909–1918.

Hava, G., Vered, L., Yael, M., Mordechai, H., Mahoud, H., 2006. Alterations in behavior in adult offspring mice following maternal inflammation during pregnancy. Dev. Psychobiol. 48, 162–168.

Huang, F.L., Huang, K.P., Wu, J., Boucheron, C., 2006. Environmental enrichment enhances neurogranin expression and hippocampal learning and memory but fails to rescue the impairments of neurogranin null mutant mice. J. Neurosci. 26, 6230–6237.

Hyde, L.A., Hoplight, B.J., Denenberg, V.H., 1998. Water version of the radial-arm maze: learning in three inbred strains of mice. Brain Res. 785, 236–244.

Jacob, A.L., Goldberg, P.K., Bloom, N., Degenshein, G.A., Kozinn, P.J., 1977. Endotoxin and bacteria in portal blood. Gastroenterology 72, 1268–1270.

Leazer, T.M., Barbee, B., Ebron-McCoy, M., Henry-Sam, G.A., Rogers, J.M., 2002. Role of the maternal acute phase response and tumor necrosis factor alpha in the developmental toxicity of lipopolysaccharide in the CD-1 mouse. Reprod. Toxicol. 16, 173–179.

Morgan, D., Diamond, D.M., Gottschall, P.E., Ugen, K.E., Dickey, C., Hardy, J., Duff, K., Jantzen, P., DiCarlo, G., Wilcock, D., Connor, K., Hatcher, J., Hope, C., Gordon, M., Arendash, G.W., 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 408, 982–985.

Naudin, J., Capo, C., Giusano, B., Mège, J.L., Azorin, J.M., 1997. A differential role for interleukin-6 and tumor necrosis factor-alpha in schizophrenia? Schizophr. Res. 26, 227–233.

O'Callaghan, E., Sham, P.C., Takei, N., Murray, G., Glover, G., Hare, E.H., Murray, R.M., 1994. The relationship of schizophrenic births to 16 infectious diseases. Br. J. Psychiatry 165, 353–356.

Ogando, D.G., Paz, D., Cella, M., Franchi, A.M., 2003. The functional role of increased production of nitric oxide in lipopolysaccharide-induced embryonic resorption in mice. Reproduction 125, 95–110.

Oliveira, M.G., Bueno, O.F., Pomarico, A.C., Gugliano, E.B., 1997. Strategies used by hippocampal- and caudate-putamen-lesioned rats in a learning task. Neurobiol. Learn. Mem. 68, 32–41.

Platz-Christensen, J.J., Mattsby-Baltzer, I., Thomsen, P., Wiqvist, N., 1993. Endotoxin and interleukin-1 alpha in the cervical mucus and vaginal fluid of

pregnant women with bacterial vaginosis. Am. J. Obstet. Gynecol. 169, 1161–1166.
Rivera, D.L., Olister, S.M., Liu, X., Thompson, J.H., Zhang, X.J., Pennline, K., Azuero, R., Clark, D.A., Miller, M.J., 1998. Interleukin-10 attenuates experimental fetal growth restriction and demise. FASEB J. 12, 189–197.
Romero, E., Guaza, C., Castellano, B., Borrell, J., in press. Ontogeny of sensorimotor gating and immune impairment induced by prenatal immune challenge in rats: implications for the etiopathology of schizophrenia. Mol. Psychiatry doi:10.1038/mp.2008.44.
Romero, R., Roslansky, P., Oyarzun, E., Wan, M., Emamian, M., Novitsky, T.J., Gould, M.J., Hobbins, J.C., 1988. Labor and infection. II. Bacterial endotoxin in amniotic fluid and its relationship to the onset of preterm labor. Am. J. Obstet. Gynecol. 158, 1044–1049.
Samuelsson, A.M., Jennische, E., Hansson, H.A., Holmäng, A., 2006. Prenatal exposure to interleukin-6 results in inflammatory neurodegeneration in hippocampus with NMDA/GABA(A) dysregulation and impaired spatial learning. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290, R1345–1356.
Su, K.Y., Chien, W.L., Fu, W.M., Yu, I.S., Huang, H.P., Huang, P.H., Lin, S.R., Shih, J.Y., Lin, Y.L., Hsueh, Y.P., Yang, P.C., Lin, S.W., 2007. Mice deficient in collapsin response mediator protein-1 exhibit impaired long-term potentiation and impaired spatial learning and memory. J. Neurosci. 27, 2513–2524.
Teeling, J.L., Felton, L.M., Deacon, R.M., Cunningham, C., Rawlins, J.N., Perry, V.H., 2007. Sub-pyrogenic systemic inflammation impacts on brain and behavior, independent of cytokines. Brain Behav. Immun. 21, 836–850.
Tong, H., Chen, G.H., Liu, R.Y., Zhou, J.N., 2007. Age-related learning and memory impairments in adult-onset hypothyroidism in Kunming mice. Physiol. Behav. 91, 290–298.
Vann, S.D., Aggleton, J.P., 2003. Evidence of a spatial encoding deficit in rats with lesions of the mammillary bodies or mammillothalamic tract. J. Neurosci. 23, 3506–3514.
Vorhees, C.V., Inman-Wood, S.L., Morford, L.L., Broening, H.W., Fukumura, M., Moran, M.S., 2000. Adult learning deficits after neonatal exposure to D-methamphetamine: selective effects on spatial navigation and memory. J. Neurosci. 20, 4732–4739.
Xu, D.X., Chen, Y.H., Wang, H., Zhao, L., Wang, J.P., Wei, W., 2005. Effect of N-acetylcysteine on lipopolysaccharide-induced intra-uterine fetal death and intra-uterine growth retardation in mice. Toxicol. Sci. 88, 525–533.
Xu, D.X., Chen, Y.H., Zhao, L., Wang, H., Wei, W., 2006. Reactive oxygen species are involved in lipopolysaccharide-induced intra-uterine growth restriction and skeletal development retardation in mice. Am. J. Obstet. Gynecol. 195, 1707–1714.
Xu, D.X., Wang, H., Ning, H., Zhao, L., Chen, Y.H., 2007a. Maternally administered melatonin differentially regulates lipopolysaccharide-induced proinflammatory and anti-inflammatory cytokines in maternal serum, amniotic fluid, fetal liver, and fetal brain. J. Pineal Res. 43, 74–79.
Xu, D.X., Wang, H., Zhao, L., Ning, H., Zhang, C., Chen, Y.H., 2007b. Effects of low-dose lipopolysaccharide (LPS) pretreatment on LPS-induced intra-uterine fetal death and preterm labor. Toxicology 234, 167–175.
Yoon, B.H., Jun, J.K., Romero, R., Park, K.H., Gomes, R., Choi, J.H., Kim, I.O., 1997a. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1[beta], and tumor necrosis factor-[alpha]), neonatal brain white matter lesions, and cerebral palsy. Am. J. Obstet. Gynecol. 177, 19–26.
Yoon, B.H., Romero, R., Kim, C.J., Koo, J.N., Choe, G., Syn, H.C., Chi, J.G., 1997b. High expression of tumor necrosis factor-[alpha] and interleukin-6 in periventricular leukomalacia. Am. J. Obstet. Gynocol. 177, 406–411.
Zhao, L., Chen, Y.H., Wang, H., Ji, Y.L., Ning, H., Wang, S.F., Zhang, C., Lu, J.W., Duan, Z.H., Xu, D.X., 2008. Reactive oxygen species contribute to lipopolysaccharide-induced teratogenesis in mice. Toxicol. Sci. 103, 149–157.
Zhou, Z., Wang, L., Song, Z., Lambert, J.C., McClain, C.J., Kang, Y.J., 2003. A critical involvement of oxidative stress in acute alcohol-induced hepatic TNF-alpha production. Am. J. Pathol. 163, 1137–1146.