Behavioural Brain Research 218 (2011) 267–279

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Acceleration of age-related learning and memory decline in middle-aged CD-1 mice due to maternal exposure to lipopolysaccharide during late pregnancy

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ARTICLE INFO

Article history:
Received 4 April 2010
Received in revised form 24 August 2010
Accepted 1 November 2010
Available online 9 November 2010

Keywords:
Aging
Alzheimer's disease
Lipopolysaccharide
Model
Mouse
Pregnancy

ABSTRACT

Previous studies have shown that inflammation process involves pathogenesis of Alzheimer's disease (AD). But, the natural AD model of inflammation has not been obtained yet. In the present study, CD-1 mothers intraperitoneally received a 50 μg/kg lipopolysaccharide (LPS) or normal saline daily during gestational days 15–17. Body weight of the offspring was recorded at ages of 4–33 weeks. A different battery of behavioral tasks was, respectively, completed at ages of 35, 290 and 400 days. The results showed that there was no significant difference in body weight between LPS-treated and control mice during ages of 4–33 weeks. LPS-treated offspring had similar anxiety and locomotor behaviors, and spatial ability of learning and memory at the age of 35 days compared to the controls. At an age of 290 days, the LPS-treated offspring had similar sensorimotor ability, locomotor activity and anxiety, species-typical behaviors, and spatial ability of learning and memory. At an age of 400 days, there were similar sensorimotor ability, locomotor activity and anxiety between the LPS-treated offspring and controls. However, there were impaired species-typical behaviors, and spatial and non-spatial abilities of learning and memory in the LPS-treated offspring. Our results suggested that maternal exposure to LPS in adequate dose in late gestation can deliver term offspring which experience a normal duration of development and maturation, and an accelerated aged-related impairment in memory (spatial and non-spatial) and species-typical behaviors in middle-aged. These meet with the criteria of AD model in behaviors.

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1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly. It is a complex, multifactorial and heterogeneous mental illness characterized by age-related and gradual onset with progressive and irreversible multiple cognitive functions. Memory impairment appears in the earliest stage of this disease. Histopathologically, the hallmarks are a number of amyloid plaques and neurofibrillary tangles, composed by the extracellular and intracellular accumulation of the amyloid peptide (Aβ) and the hyperphosphorylated protein Tau, respectively, in the brain. However, AD etiopathogenesis remains mysterious. Up to date, the Aβ hypothesis derived from studies of familial AD has provided a window to understand the pathogenesis, and resulted in establishment of the most useful model of AD etiology. But the etiology of 95% sporadic AD patients in the upstream of increased Aβ burden remains unknown.

Over the past several decades, the pathogen hypothesis has repeatedly been proposed, with speculation that pathogens acts as a trigger or co-factor for AD [38,55]. However, data supporting the pathogen hypothesis are scarce and often contradictory, and explanation of the considerable delay between initial infection and emergence of AD symptoms remains inconvincible [55]. Recently, inflammation hypothesis of AD etiology has been established by both experimental and clinic data. It suggests that neuroinflammatory response triggers and follows the Aβ increment in the AD brain [34,50], and impairs memory processes [32]. Lipopolysaccharide (LPS), the cell wall component of a Gram-negative bacteria which is recognized by the immune system and causes the production of cytokines [36], can cause immediate neuroinflammatory response and memory impairments after acutely or chronically peripheral administration or specific injection into brain regions, such as hippocampus [32,34]. However, these results are insufficient to explain why late-onset cognitive impairment follows a normal cognition during the first 5 or 6 decades in AD patients, considering the course of LPS-inducing inflammation. A new hypothesis that emphasizes the factors in fetal origins of adult disease, including AD, has recently been proposed [4,76]. According to this new hypothesis, the disadvantageous early fetal environment, such as bacterial or viral infections, associates etiologically with later

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doi:10.1016/j.bbr.2010.11.001

adult diseases. If the AD etiology involves congenitally infective hypothesis, the lipopolysaccharide or proteins coming from the surfaces of the bacteria or viruses, LPS, may persistently and mildly induce the generation of a number of inflammatory mediators including interleukin (IL)-1β, IL-6 and tumor necrosis factor α (TNF-α) [64], which may be responsible for synaptic dysfunction and increased Aβ load in the AD brain [33,50,63,73]. Behaviorally, the congenitally infected individuals will demonstrate earlier age-related impairment of learning and memory [12]. In fact, this modified hypothesis integrates all hypotheses of AD etiology mentioned above. In order to confirm this fetal origin hypothesis of AD etiology, it is of great interest to establish a mouse model of maternal exposure to LPS in lower dose, which may result in a chronic neuroinflammatory response, to mimic the pathogenesis process of AD in individuals insulted by maternal or prenatal infections.

It has been well documented that adulthood stimulation of the immune system by inflammation, even at low levels, can induce deficits in learning and memory [13,40]. In humans, male participants being given a low dose of LPS show deficits in both immediate and delayed recall of verbal and nonverbal information, and impairment in word list learning [54]. Using pyrogenic doses, research has shown that LPS administration impairs learning and memory in hippocampus-dependent tasks, such as MWM, contextual fear conditioning and two-way active avoidance conditioning in animals [2,52,59,66,67]. The impaired memory in hippocampus-dependent tasks due to LPS treatment may be mediated by proinflammatory cytokines, such as IL-1β, IL-6, TNF-α and prostaglandins [32,63,65]. Current evidence has suggested LPS injection, either peripherally or intracerebrally, in vivo stimulates a brain inflammatory response [41,43]. Furthermore, LPS-induced cognitive impairment may involve the enhancement of β-amyloidogenesis, via increased β- and γ-secretase activities, and decrease α-secretase activity [31,42,60,62].

It is becoming ever more evident that neonatal exposure to infectious agents also impairs development and function of physiological systems throughout an individual's lifespan [37,48,53]. Neonatal exposure to LPS in rats influences reactivity to stress, immune regulation, and susceptibility to the cognitive deficits associated with immune activation in adulthood [5–7,35,58]. It was also reported that neonatal LPS-treated adult rats show reduced exploration activity and motor behaviors, impaired memory, and altered responses to cerebral ischemia [5,39,68,69].

If the infection/inflammation takes place in antenatal period, there is obviously increased risk of preterm delivery, preterm or term brain injury, neurological disorders, and mental retardation in the offspring [17,47,51,61,75]. Even systemic subclinical maternal infection also contributes to undesirable consequences [56], resulting in motor defects and developmental or behavioral disabilities for some surviving infants [23,72,77]. Maternal infection appears to specifically affect advanced functions (e.g., learning and memory, social interaction) in adult offspring, though sensory and motor development remain normal for the same adult offspring [24,26]. However, whether maternal infection leads to consequences of neurodevelopmental origin may depend on the critical windows of pregnancy and intensity of inflammation [21]. So far, different rodent models of maternal infection using LPS have been based on different time points, doses and durations, hence, it is difficult to effectively compare respective results. In Wistar rats, when mothers received subcutaneously LPS on gd 15–19 with increasing doses (20, 20, 40, 40 and 80 μg/kg), their offspring showed reduction in body weight and sensorimotor ability, and increase in anxiety at 3-month old [3]. Single LPS injection to C57BL/6 mother (gd 17, 200 μg/kg, i.p.) led to increased anxiety, reduced social interaction and less aggression in 8-month-old offspring [24]. An even lower dose injection of LPS (120 μg/kg, i.p.) to pregnant C57BL/6 mice on gd 17 also changed distinct forms of learning and memory (impaired spatial memory and increased object recognition memory) with morphological impairment of hippocampal pyramidal cells in their adult offspring [26]. In Sprague–Dawley rats, administration of 100 μg/kg LPS (i.p.) to mothers on gd 15 and 16 disrupted hippocampal synaptic transmission in their offspring on postnatal days 20–25 [45]. However, maternal LPS exposure (50 μg/kg, i.p.) during gd 18 and 19 in Sprague–Dawley rats resulted in behaviors relevant to schizophrenia in adult (about 2 months) offspring [20]. Younger fetal age appears be more tolerant to maternal inflammation induced by LPS. For example, it was reported that the C57BL/6 offspring at age of about 3 months showed impairment only in object recognition memory (with normal spatial learning and memory) when their mother had been treated subcutaneously with 300 μg/kg LPS on gd 8 [13].

Currently, there are limited data to show aging effects of maternal infection on neurological functions following brain maturation in the offspring. In one study [44], Sprague–Dawley rats were injected i.p. with LPS at 10,000 endotoxin units/kg on gd 10.5–11, the maternal LPS-exposed offspring showed normal development and aging with regard to number of dopaminergic neurons, and fluctuant locomotor activity at ages of 3 and 16 months. In another study [25], C57BL/6 mice received 120 μg/kg LPS injection (i.p.) on gd 17, their aged offspring (20 months) had no increased risk of cell death, and demonstrated normal performances in MWM, elevated plus maze, passive avoidance, motor function, and exploration tasks. Even with a higher-dose LPS exposure during pregnancy, the offspring may still experience a normal cognition development. For instance, after maternal exposure to 790 μg/kg LPS at mid-gestation of days 8, 10 and 12, the offspring Sprague–Dawley rats at ages of 10 months and older showed impairment of spatial learning and memory, but did not at an age of 3 months [30]. Unfortunately, the offspring's body weight data throughout their lifetime was not captured in that report. Therefore, it is not clear whether the offspring experienced a normal growth during infanthood to adulthood.

Therefore, it is possible to establish a mouse model of AD using offspring whose mother i.p. receive an injection of low-dose LPS in late stage of late pregnancy. In this study, a battery of behavior tasks was employed to evaluate whether there is an accelerated decline of learning and memory in the middle-aged mice with maternal exposure to low-dose LPS in late stage of embryo after normal development of sensorimotor and cognitive functions.

2. Methods

2.1. Animals and treatments

CD-1 mice (9 male mice: 30–34 g; 9 female mice: 28–30 g) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing) whose foundation colonies were all introduced from Charles River Laboratories, Inc. (USA). The colony was maintained at 23–25 °C on a 12-h light–dark cycle (lights on at 7:00 a.m.). Following a 1-week acclimation to the colony room, the males and females were paired into breeders. The presence of a vaginal plug was designated as gestational day (gd) 0. All pregnant mice were randomly divided into two groups. In LPS group, the pregnant mice received an intraperitoneal (i.p.) injection of LPS (50 μg/kg) daily from gd 15 to gd 17. The normal saline-treated pregnant mice served as controls. On the postnatal day 21, pups were separated from their mothers and siblings, and were housed in plastic cages (25.5 cm × 15 cm × 14 cm, with wood-shaving bedding). Considering social isolation adversely affects neural morphology and memory performance, each cage housed 4–5 mice of the same sex, and was maintained at constant temperature of 23–25 °C and humidity of 55 ± 5%. All offspring mice received standard rodent diet and tap water ad libitum on a 12-h light–dark cycle (lights on at 7:00 a.m.) throughout all tasks and life time.

2.2. General procedures

One male and one female offspring mice per litter were measured for body weight and assessed for complete behaviors at 35-day age, with 9 males and 9 females in each group. The offspring mice with one male and one female per litter from both groups were completed with the second and third batteries of behav-

ioral tests at ages of 290-day (adult) and 400-day (middle-aged) by turns. The body weight of each mouse was recorded at ages of 4, 7, 10, 14, 18, 22, 26, 30 and 33 weeks. Before the start of the third battery of behavioral tests, two mice from LPS-treated group and one mouse from control group had died, and two mice from each group were removed due to turning in situ or only swimming along the tank wall in the Morris water maze (MWM). The final number of mice was 14 in the LPS group (8 males, 6 females) and 15 in the control group (8 males, 7 females) going into the third batteries of tests at an age of 400 days. Except for nesting and hoarding, each task was carried out during the light phase. Considering the limitation of cognitive tasks in longitudinal study, e.g., retest effects [57], different batteries of behavioral tasks were used at different stages of detection in this study, where more sensitive radial six-arm water maze (RAWM) was used for adult mice and less sensitive MWM was used for middle-aged mice [10]. The battery of behavioral tasks consisted of open-field and RAWM at an age of 35 days. The battery of tasks at the age of 290 days included sensorimotor-based tasks (beam walk, tightrope and open field), species-typical behaviors (hording, burrowing and nesting) and cognitive task (RAWM), and was carried out in the following order: hording (day 1), burrowing (day 2), nesting (day 3), open field (day 4), beam walking (day 5), tightrope (day 7) and RAWM (days 8–14). At the age of 400 days, the battery of tasks contained sensorimotor tasks (beam walking and tightrope), anxiety-based tasks (open field, elevated plus maze and black–white alley), species-typical-behavior tasks (hording, burrowing and nesting) and cognitive tasks (novel-object recognition and MWM) in the following order: hording (day 1), burrowing (day 2), nesting (day 3), open field (day 4), beam walking (day 5), tightrope (day 7), elevated plus maze (day 8), black–white alley (day 9), novel-object recognition (days 10–13) and MWM (days 14–23). All tasks were performed in the feeding room in order to preserve adaptation to the environment. All animal experiments were carried out in compliance 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. Behavioral tests

2.3.1. Sensorimotor behaviors

2.3.1.1. Beam walking. A 110-cm-long steel rods (diameter 10 mm), with each end attached to a platform (diameter 10 cm), was supported by two vertical poles and elevated 50 cm above the water surface in a black circular tank of 150 cm in diameter. Each animal was given three successive trials and perpendicularly placed on the center of the beam. Each trial was maintained for a maximum of 60 s. The balance time, during which the animal did not fall from the beam, was recorded for each of the three trials. If the animal remained on the beam for the duration of the trial or escaped to either of the two platforms, it was recorded as 60 s. The mean time recorded for the three trials was used in the statistical analysis.

2.3.1.2. Tightrope. In this task, a taut, tiny cotton rope (2 mm in diameter) was stretched across a tank (100 cm in diameter, 30 cm in height) half-filled with water (at 21 °C). First, each mouse was placed in the water for 5 s. During a 60-s trial, the mouse was raised to grasp the center of the rope with forepaws, and then released slowly so that the mouse could support its own weight by means of its grip. The suspension time was recorded. Once a mouse had fallen into the water, or stayed on the rope for up to 60 s, it was immediately removed to a holding cage and allowed to rest for 30 s before the next trial (three subsequent trials on a single day).

2.3.2. Anxiety-based tasks

2.3.2.1. Open field. An open, black wooden box (81 cm × 81 cm floor, and 28-cm wall height) was used. The box floor was painted with white lines (3 mm wide) to form 16 equal squares (20 cm × 20 cm each). Illumination was provided by a 40-W white light placed 2.80 m above the center of the field. For each trial, an animal was placed into one of the four corners, facing the wall, and was permitted to explore the environment for 5 min ad libitum. Then, time taken by the animal to cross the first square, number of squares crossed, and peripheral time (the time spent in the 12 peripheral squares) were recorded. The area was cleaned with water before the next mouse was tested.

2.3.2.2. Elevated plus maze. The black maze was made of Plexiglas and consisted of two opposite closed arms (30 cm × 5 cm) enclosed with walls (15 cm in height) and two opposite open arms (also 30 cm × 5 cm, without edges) and formed the shape of a cross. The whole apparatus had a central arena (5 cm × 5 cm) and was elevated to 80 cm above the floor by a tripod. Each mouse was placed in the central arena of the maze facing an open arm, and observed for 5 min. The number of entries into the open arms and the time spent on the open arms were measured during a single trial. The maze was cleaned with water after each mouse.

2.3.2.3. Black–white alley. A narrow galvanized iron box (120 cm × 9 cm × 30 cm, with one half painted black and the other half was painted white) was used to form a long black–white alley without top [8]. Each mouse was placed into the black half facing the end wall. The latency to enter the white alley was recorded during a single 90 s trial. If the mouse never entered the white alley the latency was recorded as 90 s.

2.3.3. Species-typical-behavior tasks

2.3.3.1. Hoarding. Mice were housed in wooden boxes (30 cm × 20 cm × 15 cm) with wire mesh lids and wood shaving bedding [9]. Each box was attached to a wire mesh tube of 60 cm long, with 50 g of normal diet food pellets (each around 2 g) placed at the far end of tube. Mice were individually housed in the boxes just before the start of the dark cycle and had unlimited access to water. The pellets found in the box the next morning were weighed and taken as the weight hoarded.

2.3.3.2. Burrowing. The plastic cage, similar to the home cage, contained a plastic tube (4 cm in diameter, 10 cm long) serving to enrich the environment by providing an alternative refuge for the mice, and a bright iron tube with semi-cylinder shape (5 cm in radius, 12 cm long) [9]. In order to gather 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 the bars was filled with maize of 40 g. At least 2 h before the start of the dark period, each mouse (not deprived of food) was placed into individual experimental cage, and the weight displaced from the tube was measured 2 h later.

2.3.3.3. Nesting. Mice were individually housed overnight, with food, water and new sawdust bedding. Six pieces of white papery cloth (5 cm × 5 cm) were evenly placed in each cage for mice to make nests [9]. After the overnight test, the nests usually consisted of a shallow crater of sawdust, mixed, or sometimes covered with shredded or whole papery cloth. They were scored according to the following scale: (0) no visible crater of sawdust, no papery cloth; (1) sawdust crater alone, no shredded cloth; (2) sawdust crater with shredded or whole papery cloth gathered around and mixed in the crater; (3) sawdust crater with shredded or whole papery cloth gathered around and mixed in the crater forming a cup-shaped nest; and (4) the shredded papery cloth forms a ball-shaped nest covering the mouse.

2.3.4. Cognitive tasks

2.3.4.1. RAWM. A black circular tank (100 cm in diameter, 21 cm in height) filled with 20–21 °C water was placed on a steel rack, and rounded by a white cloth curtain with three black cardboards of different shapes (circle, triangle and square, respectively) hung equidistantly [10]. The tank had six swimming alleys (30.5 cm × 19 cm × 21 cm) that radiated out from a 40-cm-diameter center area. A black escape platform (diameter 10 cm) was submerged 1.0 cm below the surface of the water. The mice underwent five trials a day for 7 days. The sequences of starting points differed every day, but the location of the platform and experimenter were kept the same. The time allowed to find the platform was measured up to a maximum of 60 s. Upon locating the platform, the mouse was left there for 30 s prior to the next trial. If the mouse failed to locate the platform within 60 s, it was guided to the platform and allowed to stay there for 30 s. The number of errors and the escape latencies (time that each mouse took to locate the platform) were recorded, averaged daily and used later for statistical analysis.

2.3.4.2. MWM. A circular black tank (150 cm in diameter, 30 cm in height) was filled daily with fresh tap water (21–22 °C) to a depth of 15 cm [10]. A black escape platform (10 cm diameter, height 24 cm) was placed in one of the four quadrants of the pool. The tank was placed in the same environment as being described for the RAWM task. A camera was hung on top of the center of the pool to monitor swim patterns, and port the images into a computer. An image analysis software was developed and used to automate calculations of escape latency (in unit of s), distance (in unit of cm), velocity (in unit of cm/s), and percentage of time spent in each zone of the arena.

Place learning. The platform was kept in a fixed position (quadrant 2) and submerged 1.0 cm below the surface of the water. On each trial, an animal was put into the water, facing the wall, and at randomly assigned one of four different starting positions spread around the pool's perimeter (one position per quadrant). The test lasted 10 days with each mouse receiving four successive trials a day. The time allowed to find the submerged platform was a maximum of 60 s. Upon locating the platform, each mouse was kept there for 30 s. If the platform was not found within the 60 s, a mouse was guided to the platform and kept there for 30 s. Thereafter, each animal was transferred to its cage until the next trial was started. After all of the mice completed this trial, the next trial began by following the same order of the mice.

Probe trial. On the last day, an additional trial was given as a probe trial when the platform was removed. The animals were placed in the quadrant where the platform was previously located in the fourth starting position and were put through a single test-free swimming for 60 s. The percentage of distance and time spent in the quadrant where the platform was previously located (target quadrant) was used as a measure of spatial memory.

2.3.4.3. Novel-object recognition. The protocol was designed according to previous studies of rat with partial modification [22]. The Y-shaped apparatus placed in a sound-proof room has three equidistant arms, one is a start arm and the other two are object arms. The start arm (30 cm in length and 10 cm in width) contained a guillotine door 10 cm away from the end and provided a start box area, within which the mouse could be confined at the start of the sample and choice phases of a given trial. The door is closed when mouse is exploring. Each object arm was 23 cm long, 10 cm

Table 1
The body weights (g) of CD-1 mice with maternal LPS exposure in different ages.

Age (weeks)	LPS		Control	
	Male (n = 9)	Female (n = 9)	Male (n = 9)	Female (n = 9)
4	20.2 ± 0.97	18.4 ± 0.63	18.8 ± 0.97	18.1 ± 0.60
7	31.6 ± 0.77	25.4 ± 0.67	31.9 ± 0.77	25.6 ± 0.64
10	35.5 ± 0.99	28.1 ± 0.77	36.3 ± 0.99	28.6 ± 0.73
14	38.5 ± 1.25	30.4 ± 1.28	39.7 ± 1.25	30.8 ± 1.21
18	40.4 ± 1.21	32.2 ± 1.47	42.9 ± 1.21	32.4 ± 1.40
22	40.7 ± 1.55	32.9 ± 1.48	43.9 ± 1.55	32.4 ± 1.41
26	41.2 ± 1.61	34.5 ± 1.91	44.2 ± 1.61	34.2 ± 1.82
30	42.0 ± 1.72	34.6 ± 1.93	44.4 ± 1.71	34.5 ± 1.84
33	42.7 ± 1.72	34.5 ± 1.79	44.6 ± 1.71	34.0 ± 1.71

wide and 40 cm high. The floor and the inside walls are black. The apparatus was rounded by a black cloth curtain. A video camera (SONY SSC-DC488P) was mounted above the apparatus so that the activities of mice can be captured and recorded. A light provided a constant illumination of about 150 lx at the center of the apparatus.

In the acclimation phase, the mice were allowed to freely explore the Y-shaped apparatus with no object for 5 min per day for 3 days. In the sample phase, the mice were returned to the home cage staying there for 2 min following each exploration of the apparatus with no object for 1 min. Two identical familiar (or sample) objects (A1 and A2) were placed in the left and right corners of the apparatus, respectively. Each animal was placed at the start arm with the guillotine door open. As soon as the animal completely entered the object arms, the guillotine door was closed and timing began. After the planned sample-object exposure time (5 min), the animal was removed from the apparatus and returned to the colony. After a 10-min interval, the animal was reintroduced into the apparatus for another 5-min period of exploration (choice phase 1), replacing one of the sample objects with a novel object (B) and the other sample object with A3. After a 24-h delay (choice phase 2), the object B was replaced by the sample object A4 and A3 was replaced by another novel object (C) (see Fig. 1), and the animal was reintroduced into the apparatus for a 3-min exploration. The apparatus and objects were cleaned with water following exploration of each animal.

The video-image analysis was completed by an operator who was blind to the experimental design using software (XNote Stopwatch 1.39). It was considered exploration if an animal directed its nose to the objects at a distance of no longer than 1 cm and/or touched it with its nose. The exploring-time for the familiar (T_F) or the novel object (T_N) within the first 1 min during choice phase was recorded separately. The preferential index for novel object (PI_N) in the choice phase was defined as $T_N/(T_F + T_N) \times 100\%$.

2.4. Statistical analysis

Before the statistical tests were performed, the data were explored to reveal their feature of distribution. The results were expressed as mean ± means of standard error (S.E.M.) for the parametric data or median (25th/75th quartile) for the nonparametric data. When the distribution of data was normal, the analysis was performed using the one-way analysis of variance (ANOVAs) with treatment as independent variables. When the distribution of data was non-normal, the Kruskal–Wallis H test was employed. For test data from the RAWM and MWM tasks and body weight, the Repeated Measures Analysis of Variance (rm-ANOVAs) was employed with Fisher's least-significant difference test for post hoc analysis. To avoid potential obstruction resulting from the differential patterns of age-related changes in the different sexes, a "single-sex" ANOVAs/rm-ANOVAs/H test (analysis for each sex with age as independent variables) was used to reveal any significant age effects [10]. All analyses were conducted by statistical software, SPSS 13.0 for Windows. Significance was assumed when $P < 0.05$.

3. Results

3.1. Body weight

The "single-sex" rm-ANOVAs results, as summarized in Table 1, indicated that body weight was similar between LPS-treated and control mice for both males [$F_{(1,14)} = 1.098$, $P = 0.310$] and females [$F_{(1,14)} < 0.1$].

3.2. Behaviors in mice at age of 35 days

3.2.1. Open field

The LPS treatment did not affect the latency in sexes combined [$X^2_{(1,34)} = 0.823$, $P = 0.364$], males [$X^2_{(1,16)} < 0.1$] and females [$X^2_{(1,16)} = 0.633$, $P = 0.426$], nor did it significantly affect the peripheral time and squares crossed in the sexes combined [$F_{(1,34)} = 3.728$, 1.117; $P = 0.061$, 0.298], with males [$F_{(1,16)} = 3.055$, 0.154; $P = 0.100$, 0.700] and females [$F_{(1,16)} = 0.940$, 1.333; $P = 0.345$, 0.263] (see Table 2).

3.2.2. RAWM

3.2.2.1. Learning phase.

Latency. The progressively shortened latency over time [$F_{(6,204)} = 22.675$, $P < 0.001$; see Fig. 2A] suggests that the mice were able to learn the task. For all mice combined, the post hoc analysis showed that the latencies in days 2–4 were significantly shorter than that in day 1 (Ps < 0.05), reaching the shortest levels by days 5–7 (relative to day 4, Ps < 0.01). The rm-ANOVA results showed that LPS-treatment insignificantly affected on the latency for both sexes combined [$F_{(1,34)} = 1.612$, $P = 0.213$], males [$F_{(1,16)} < 0.1$] and females [$F_{(1,16)} = 2.763$, $P = 0.116$] (see Fig. 2B).

Errors. The number of errors progressively declined daily [$F_{(6,204)} = 8.100$, $P < 0.001$; see Fig. 2C]. For all mice combined, the errors in days 5–7 were significantly fewer than those in days 1–4 (Ps < 0.001). LPS treatment insignificantly affected the errors counts for sexes combined [$F_{(1,34)} = 1.200$, $P = 0.281$] and males [$F_{(1,16)} = 0.188$, $P = 0.671$], but significantly affected the females [$F_{(1,16)} = 4.879$, $P = 0.042$] (see Fig. 2D).

3.2.2.2. Memory phase.

Latency. The latency progressively shortened daily [$F_{(6,204)} = 6.940$, $P < 0.001$; see Fig. 2E]. For all mice combined, the latencies in days 3–4 were significantly shorter than that in day 1 (Ps < 0.05), reaching the shortest levels by days 5–7 (relative to day 1, Ps < 0.01). The rm-ANOVA results showed that LPS treatment insignificantly impacted the latency for both sexes combined [$F_{(1,34)} < 0.1$], males [$F_{(1,16)} = 0.451$, $P = 0.511$] and females [$F_{(1,16)} = 0.120$, $P = 0.733$] (see Fig. 2F).

Errors. The number of errors progressively declined with days [$F_{(6,204)} = 4.595$, $P < 0.001$; see Fig. 2G]. For all mice combined, the errors in days 3–4 were significantly fewer than that in day 2 (Ps < 0.05), reaching the lowest levels by days 5–7 (relative to day 2, Ps < 0.001). The rm-ANOVA showed that LPS treatment insignificantly impacted the error counts for the combined sexes [$F_{(1,34)} < 0.1$], males [$F_{(1,16)} = 0.280$, $P = 0.604$] and females [$F_{(1,16)} = 0.469$, $P = 0.503$] (see Fig. 2H).

3.3. Behaviors in mice at age of 290 days

3.3.1. Sensorimotor tasks

The performance is shown in Table 2. No significant treatment effect to the balance time was observed in the beam walking task for the combined sexes [$F_{(1,34)} = 0.258$, $P = 0.615$], males [$F_{(1,16)} = 0.393$, $P = 0.539$] and females [$F_{(1,16)} = 1.668$, $P = 0.215$]. No significant treatment effect to the suspension time was seen either in the tightrope task for the combined sexes [$F_{(1,34)} = 0.258$, $P = 0.615$], males [$F_{(1,16)} = 0.269$, $P = 0.611$] and females [$F_{(1,16)} = 2.096$, $P = 0.167$].

3.3.2. Open field

The performance is presented in Table 2. No significant effects of treatment were found in the peripheral time and latency test results for the combined sexes [$F_{(1,34)} < 0.1$; $X^2_{(1,34)} = 2.201$, $P = 0.138$], males [$F_{(1,16)} = 2.222$, $P = 0.156$; $X^2_{(1,16)} = 3.359$, $P = 0.116$] and females [$F_{(1,16)} = 1.572$, $P = 0.226$; $X^2_{(1,16)} < 0.1$]. No significant effect of treatment was found either in the number of squares crossed test for the combined sexes [$X^2_{(1,34)} = 2.497$, $P = 0.114$] and females [$X^2_{(1,16)} < 0.1$]. But, the number of squares crossed by the

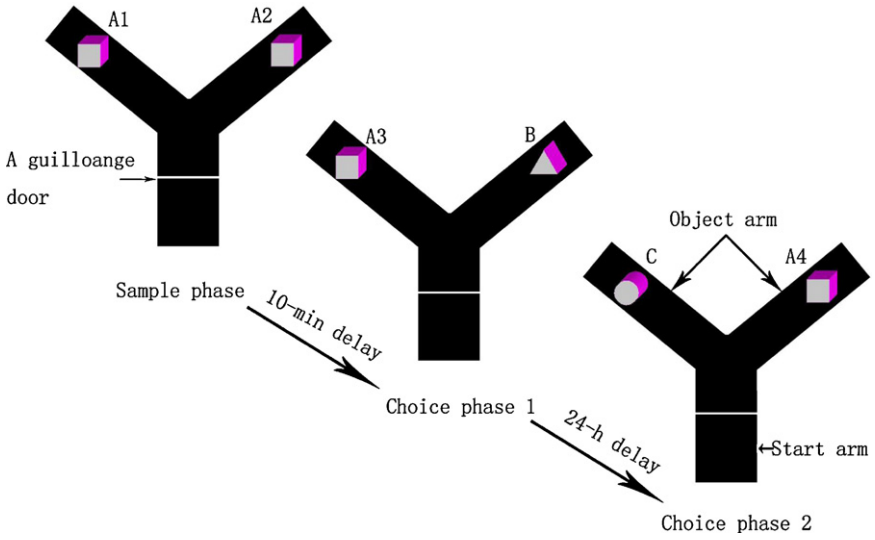

Fig. 1. A schematic summary of the protocol in the novel object recognition task. In the sample phase, identical objects (A1 and A2) were placed in the left and right object arms, respectively. In the choice phase 1, a sample object was replaced with a novel object (B) and the other with A3 after a 10-min interval. In the choice phase 2, the object B was replaced with sample object A4 and the familiar object A3 was replaced by novel object C after a 24-h delay.

LPS males was less than that by the control males [$X^2_{(1,16)} = 3.951$, $P = 0.047$].

3.3.3. Species-typical-behavior tasks

Table 2 shows the performance in the hording, burrowing and nesting tasks. There were no major differences in the weight hoarded [$X^2_{(1,34)} = 0.547$, $P = 0.459$], weight burrowed [$X^2_{(1,34)} = 0.021$, $P = 0.884$] and nesting ability [$X^2_{(1,34)} = 2.754$, $P = 0.097$] between the LPS mice and the control ones. The "single-sex" ANOVAs also indicated that LPS-exposure did not significantly affect the weight hoarded [$X^2_{(1,16)} = 0.566, 0.154$; $P = 0.452, 0.695$] and burrowed [$X^2_{(1,16)} = 2.388, 1.387$; $P = 0.122, 0.239$], and the nesting ability [$X^2_{(1,16)} < 0.1$; $X^2_{(1,16)} = 2.707$, $P = 0.117$] of both males and females.

3.3.4. RAWM

Learning phase. Latency and number of errors progressively decreased daily for all mice combined [$F_{(6,204)} = 68.164$ and 68.077, $Ps < 0.001$]. There were no major differences in latency and number of errors between the LPS-exposure group and the control group for the combined sexes [$F_{(1,34)} = 1.834, 2.255$; $P = 0.184$, $P = 0.142$; see Fig. 3A and C], males [$F_{(1,16)} = 0.176$, $P = 0.680$; $F_{(1,16)} = 0.183$, $P = 0.674$] and females [$F_{(1,16)} = 3.832$, $P = 0.066$; $F_{(1,16)} = 3.329$, $P = 0.085$] (see Fig. 3B and D).

Memory phase. Latency and error counts progressively decreased daily [$F_{(6,204)} = 20.270$ and 14.133, $Ps < 0.001$]. There were no significant differences in latency and number of errors between the LPS group and the control group for the combined sexes [$F_{(1,34)} = 2.474$, 1.264; $P = 0.125, 0.269$; see Fig. 3E and G], males [$F_{(1,16)} = 1.563$, 0.802; $P = 0.229, 0.341$] and females [$F_{(1,16)} = 0.581, 0.415$; $P = 0.456$, 0.527] (see Fig. 3F and H).

3.4. Behaviors in mice at age of 400 days

3.4.1. Sensorimotor tasks

The performance is presented in Table 2. There were no major differences in balance time and suspension time results in the beam walking task between the LPS group and the control group for the combined sexes [$X^2_{(1,27)} = 0.120$, $P = 0.729$; $F_{(1,27)} = 2.615$, $P = 0.118$], males [$X^2_{(1,14)} = 0.121$, $P = 0.728$; $F_{(1,14)} = 2.363$, $P = 0.155$] and females [$X^2_{(1,11)} < 0.1$; $F_{(1,11)} < 0.1$] (see Table 2).

3.4.2. Anxiety-based tasks

3.4.2.1. Open field. There were significant differences in latency, peripheral time and squares crossed between the LPS group and the control group for the combined sexes [$F_{(1,27)} = 1.564$, 0.817 and 0.320; $P = 0.222$, 0.374 and 0.576], males [all $F_{(1,14)} < 1.0$, $Ps > 0.661$] and females [$F_{(1,11)} = 1.414$, 3.413 and 0.015, $P = 0.259$, 0.960 and 0.905] (see Table 2).

3.4.2.2. Elevated plus maze. The time spent on the open arm(s) and number of entries to the open arm(s) were not significantly affected by LPS exposure for the combined sexes [$F_{(1,27)} = 0.189$, $P = 0.667$; $X^2_{(1,27)} = 0.261$, $P = 0.610$], males [$F_{(1,14)} = 2.718$, $P = 0.121$; $X^2_{(1,14)} = 2.389$, $P = 0.122$] and females [$F_{(1,11)} = 0.948$, $P = 0.351$; $X^2_{(1,11)} = 0.762$ $P = 0.383$] (see Table 2).

3.4.2.3. Black–white alley. No significant effect was found from LPS treatment to the latency to enter the white alley and the time spent in the white alley for the combined sexes [$F_{(1,27)} = 1.336$, 1.050; $P = 0.259, 0.315$] and females [$F_{(1,11)} = 2.220, 0.003$; $P = 0.164$, 0.956]. But the time spent in the white alley by the LPS-treatment males was significantly longer than that by the control males [$F_{(1,14)} = 5.771$, $P = 0.031$] (see Table 2).

3.4.3. Species-typical-behavior tasks

The performances in the hording, burrowing and nesting tasks are presented in Table 2. The weight hoarded by the LPS-treated group was significantly higher than that by the control group for the combined sexes [$X^2_{(1,27)} = 5.662$, $P = 0.017$], but only marginally higher when the sexes were separated [males: $X^2_{(1,14)} = 3.574$, $P = 0.059$; females: $X^2_{(1,11)} = 3.458$, $P = 0.063$]. Overall, the LPS-treated mice burrowed more weight than the control mice [$X^2_{(1,27)} = 7.444$, $P = 0.006$], though this treatment effect was only found among the males [$X^2_{(1,14)} = 4.418$, $P = 0.036$]. The score of nesting by the LPS mice was lower than that by the control mice [$X^2_{(1,27)} = 14.089$, $P < 0.001$]. This treatment effect to nesting was observed among both the males [$X^2_{(1,14)} = 5.596$, $P = 0.018$] and the females [$X^2_{(1,11)} = 7.934$, $P = 0.005$].

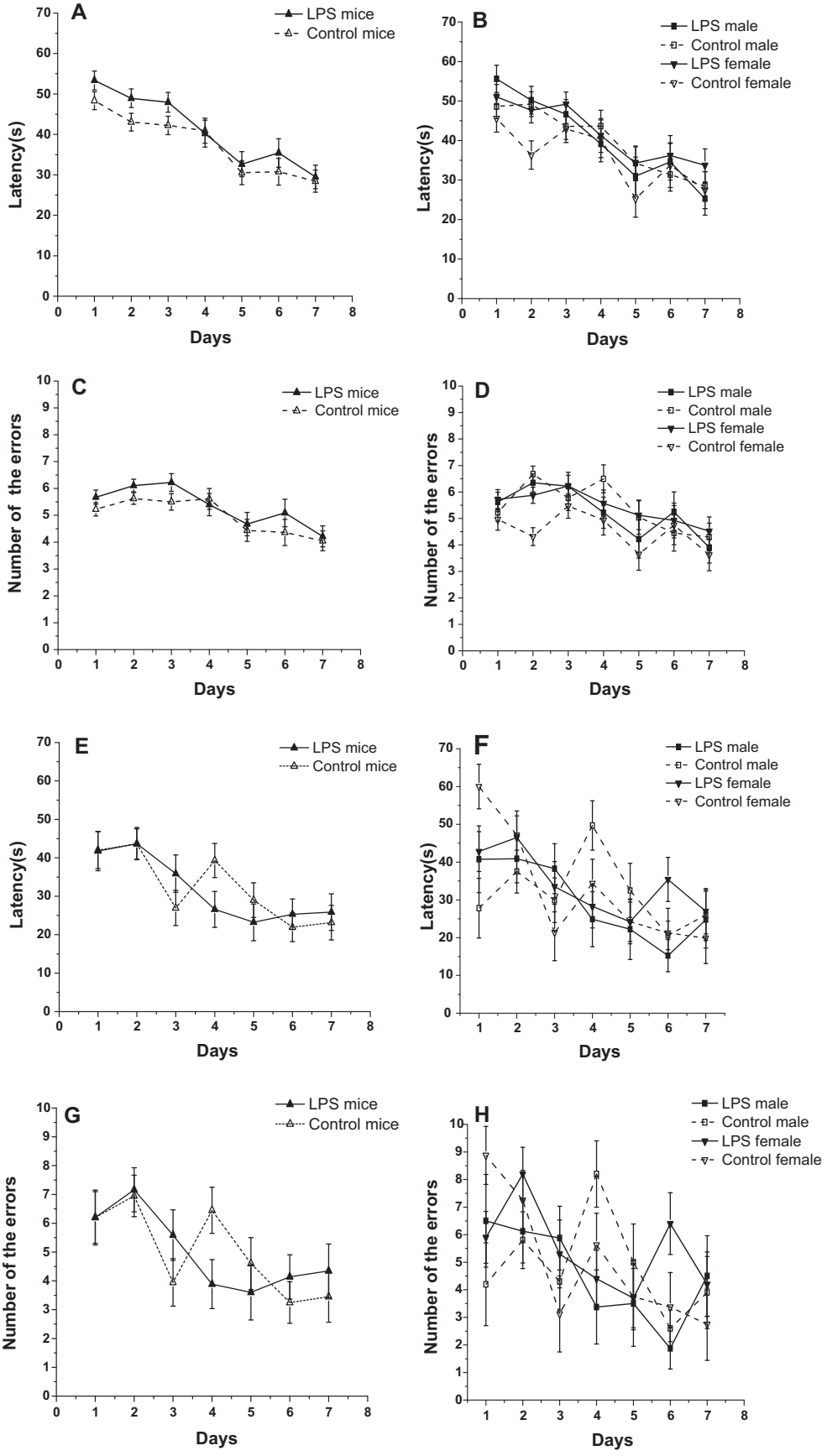

Fig. 2. The performance of 35-day CD-1 offspring with maternal LPS exposure in the RAWM. The latency (A and B) and errors (C and D) for different-treatment offspring during the learning phase (trials 1–4), and latency (E and F) and errors (G and H) during the memory phase (trial 5). There were no treatment effects on all measures in all mice (A, C, E and G) and males or females (B, D, F and H). The number was 18 for both LPS and control groups with 9 in each sex per group. The bars standing represent for S.E.M.

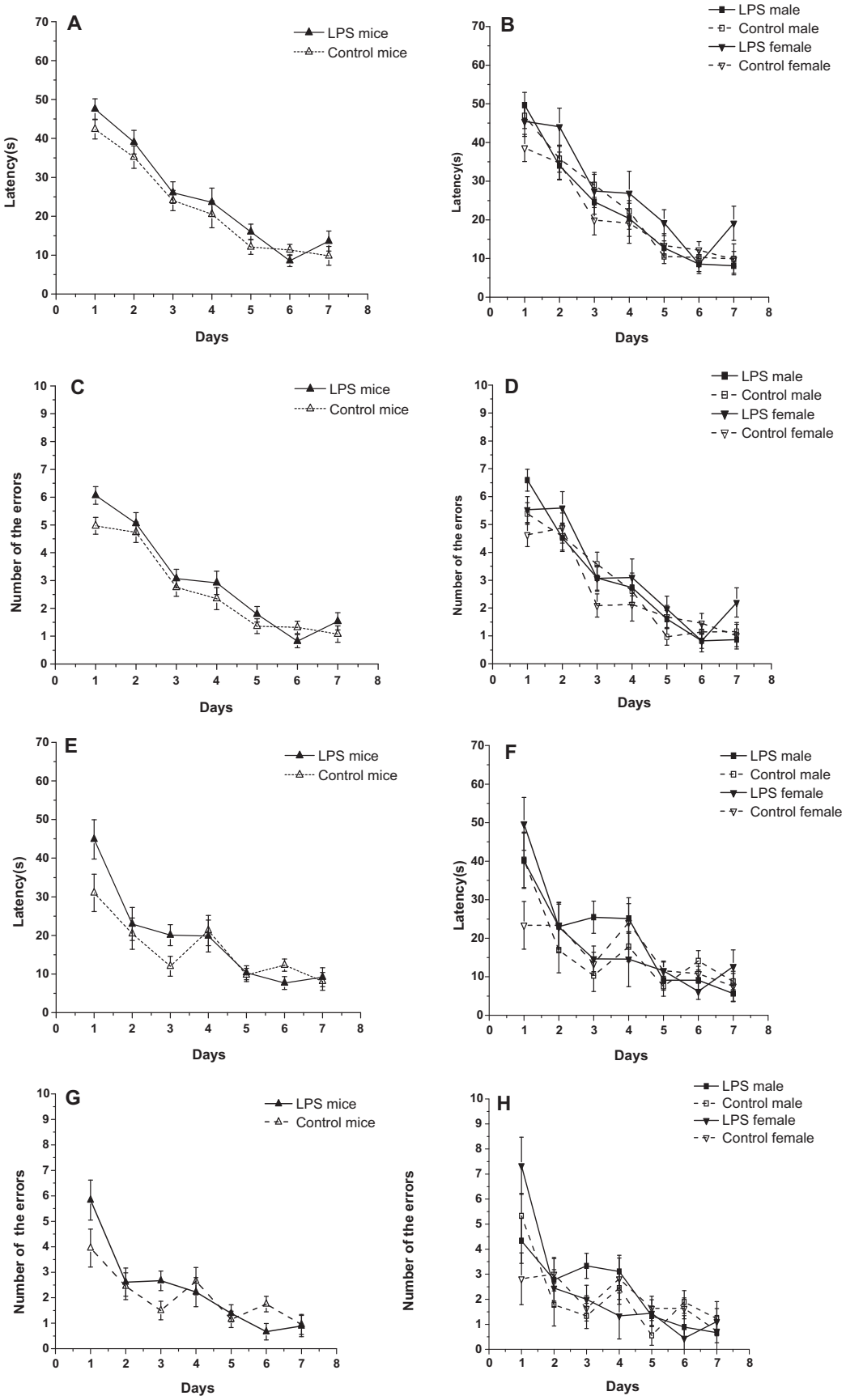

Fig. 3. The performance of 290-day CD-1 mice with maternal LPS exposure in the RAWM. The latency (A and B) and errors (C and D) for different-treatment offspring during the learning phase (trials 1–4), and latency (E and F) and errors (G and H) during the memory phase (trial 5). There were no treatment effects on all measures in the combined sexes (A, C, E and G) and males or females (B, D, F and H). The number was 18 for both LPS and control groups with 9 in each sex per group. The bars standing represent for S.E.M.

Table 2
The performance of CD-1 mice with maternal LPS exposure in non-cognitive tasks.

Tasks	Measure	LPS			Control		
		Total	Males	Females	Total	Males	Females
35-day CD-1 mice (n = 18 in the LPS-treatment and control groups with n = 9 in each sex)							
Open-field	Latency (s)	19.0 (9.0/33.8)	8.0 (2.1/31.0)	21.0 (16.6/36.3)	12.5 (6.8/20.8)	10.0 (3.0/16.0)	16.5 (10.8/35.8)
	Peripheral time(s)	282.8 ± 4.7	288.8 (268.0/298.8)	288.5 (276.3/296.0)	270.9 ± 4.0	267.5 (251.8/290.0)	271.5 (258.8/286.0)
	Squares crossed	104.4 ± 9.8	110.1 ± 15.9	99.9 ± 12.0	118.1 ± 7.2	117.5 ± 11.1	118.7 ± 12.0
290-day CD-1 mice (n = 18 in the LPS-treatment and control groups with n = 9 in each sex)							
Open-field	Latency (s)	9.0 (7.0/12.5)	9.0 (7.0/15.5)	8.0 (6.0/13.0)	7.5 (5.0/10.5)	6.0 (4.5/9.5)	9.0 (5.0/11.0)
	Peripheral time (s)	246.3 ± 5.7	251.6 ± 8.9	241.1 ± 8.9	245.0 ± 6.83	232.0 ± 8.9	255.6 ± 8.1
	Squares crossed	95.5 (65.6/120.0)	92.0 (71.5/110.0)*	99.0 (57.0/128.5)	111.5 (92.0/119.3)	112.0 (98.0/142.5)	111.0 (89.0/117.0)
Beam walking	Balance time (s)	43.0 ± 2.9	42.1 ± 4.6	43.9 ± 3.8	45.1 ± 3.0	38.8 ± 2.5	51.3 ± 4.3
Tightrope	Suspension time (s)	46.0 ± 2.6	45.6 ± 4.8	46.3 ± 2.2	46.8 ± 2.4	42.5 ± 3.6	51.2 ± 2.5
Hoarding	Weight hoarded (g)	3.2 (0.0/9.4)	4.9 (2.5/9.9)	0.0 (0.0/10.1)	1.2 (0.1/5.4)	3.1 (0.3/2.9)	0.7 (0.0/1.6)
Burrowing	Weight displaced (g)	9.3 (4.7/31.0)	25.1 (6.3/32.9)	7.1 (2.8/22.0)	13.3 (2.5/34.3)	3.2 (1.5/25.1)	26.9 (4.5/36.8)
Nesting	Nesting scores	1.5 (1.0/2.0)	1.5 (1.0/2.0)	1.5 (0.8/2.0)	1.5 (1.0/1.9)	1.0 (0.5/2.8)	1.5 (1.0/2.0)
400-day CD-1 mice (n = 14 in the LPS group with 8 males and 6 females; n = 15 in control group with 8 males and 7 females)							
Open-field	Latency (s)	3.8 ± 0.6	3.8 ± 0.8	3.7 ± 1.1	5.0 ± 0.7	4.2 ± 0.5	5.9 ± 1.5
	Peripheral time (s)	250.9 ± 4.5	253.6 ± 6.9	247.2 ± 5.5	255.8 ± 2.9	251.0 ± 3.4	261.3 ± 4.0
	Squares crossed	149.0 ± 5.9	137.3 ± 4.7	164.7 ± 9.3	153.6 ± 5.1	145.0 ± 7.5	163.4 ± 5.0
Beam walking	Balance time (s)	60.0 (48.7/60.0)	58.1 (31.1/60.0)	60.0 (57.3/60.0)	60.0 (46.3/60.0)	60.0 (42.7/60.0)	60.0 (60.0/60.0)
Tightrope	Suspension time (s)	38.5 ± 4.6	34.5 ± 5.8	46.4 ± 6.4	46.9 ± 3.6	46.7 ± 3.6	47.6 ± 4.7
Elevated plus maze	Time on the open arm(s)	29.5 ± 7.6	19.7 ± 7.6	42.4 ± 11.7	34.1 ± 7.3	40.7 ± 10.2	26.6 ± 11.1
	Number of entries to the open arms	2.0 (0.8/3.3)	1.0 (0.0/3.0)	2.5 (2.0/5.0)	2.0 (0.0/5.0)	2.5 (1.8/5.0)	2.0 (0.0/3.0)
Black–white alley	Latency to enter the white alley	5.0 ± 0.6	5.2 ± 0.5	4.8 ± 0.7	5.9 ± 0.7	4.8 ± 0.5	7.1 ± 1.3
	Time spent in the white alley	49.5 ± 2.6	54.3 ± 2.3*	44.7 ± 4.5	46.1 ± 2.3	47.2 ± 1.9	45.0 ± 4.4
Hoarding	Weight hoarded (g)	15.1 (5.7/20.9)*	15.1 (9.9/16.1)	11.8 (3.8/32.4)	4.9 (1.0/10.4)	5.1 (4.0/9.2)	1.6 (0.0/15.9)
Burrowing	Weight displaced (g)	12.5 (5.6/21.3)*	14.0 (6.8/21.5)*	9.9 (5.1/20.5)	4.3 (1.4/6.1)	3.2 (1.4/5.6)	5.5 (3.9/10.6)
Nesting	Nesting scores	2.0 (1.4/2.0)*	2.0 (1.6/2.0)*	2.0 (1.0/2.0)*	3.0 (2.0/3.0)	2.8 (2.0/3.0)	3.0 (2.5/3.0)

Compared to corresponding control mice *P < 0.05, **P < 0.01.

3.4.4. Cognitive tasks

3.4.4.1. MWM. Fig. 4 shows the performance of mice in finding the submerged platform in the MWM.

Place learning. The latency shortened progressively daily for all mice combined [$F_{(9,243)} = 5.166$, $P < 0.001$; see Fig. 4A]. More specifically, the latency on day 3 was significantly shorter than that on day 1 ($P = 0.036$), and latencies on day 4 and onwards are even shorter than that on day 3 (Ps < 0.05). The rm-ANOVA indicated that the LPS-treated mice had longer latencies than the control mice [$F_{(1,27)} = 9.551$, $P = 0.005$, see Fig. 4A], for both males [$F_{(1,14)} = 4.809$, $P = 0.046$] and females [$F_{(1,11)} = 5.699$, $P = 0.036$] (see Fig. 4B). The cumulative distance also declined daily [$F_{(9,243)} = 5.452$, $P < 0.001$; see Fig. 4C]. The post hoc analysis showed that the distances on days 2–8 were significantly shorter than those on day 1 (Ps < 0.05), so was the distance on day 10 relative to that on day 8 ($P = 0.022$). The LPS-treated mice had significantly longer distances than the control mice [$F_{(1,27)} = 7.958$, $P = 0.009$]. This treatment effect mainly occurred among the females [$F_{(1,11)} = 5.396$, $P = 0.040$], not obvious among the males [$F_{(1,14)} = 2,835$, $P = 0.114$] (see Fig. 4D). There was similar swimming velocity between the LPS-treated and control groups for the combined sexes [$F_{(1,27)} = 0.208$, $P = 0.652$, see Fig. 4E], males [$F_{(1,14)} = 0.405$, $P = 0.535$] and females [$F_{(1,11)} = 2.565$, $P = 0.138$] (see Fig. 4F).

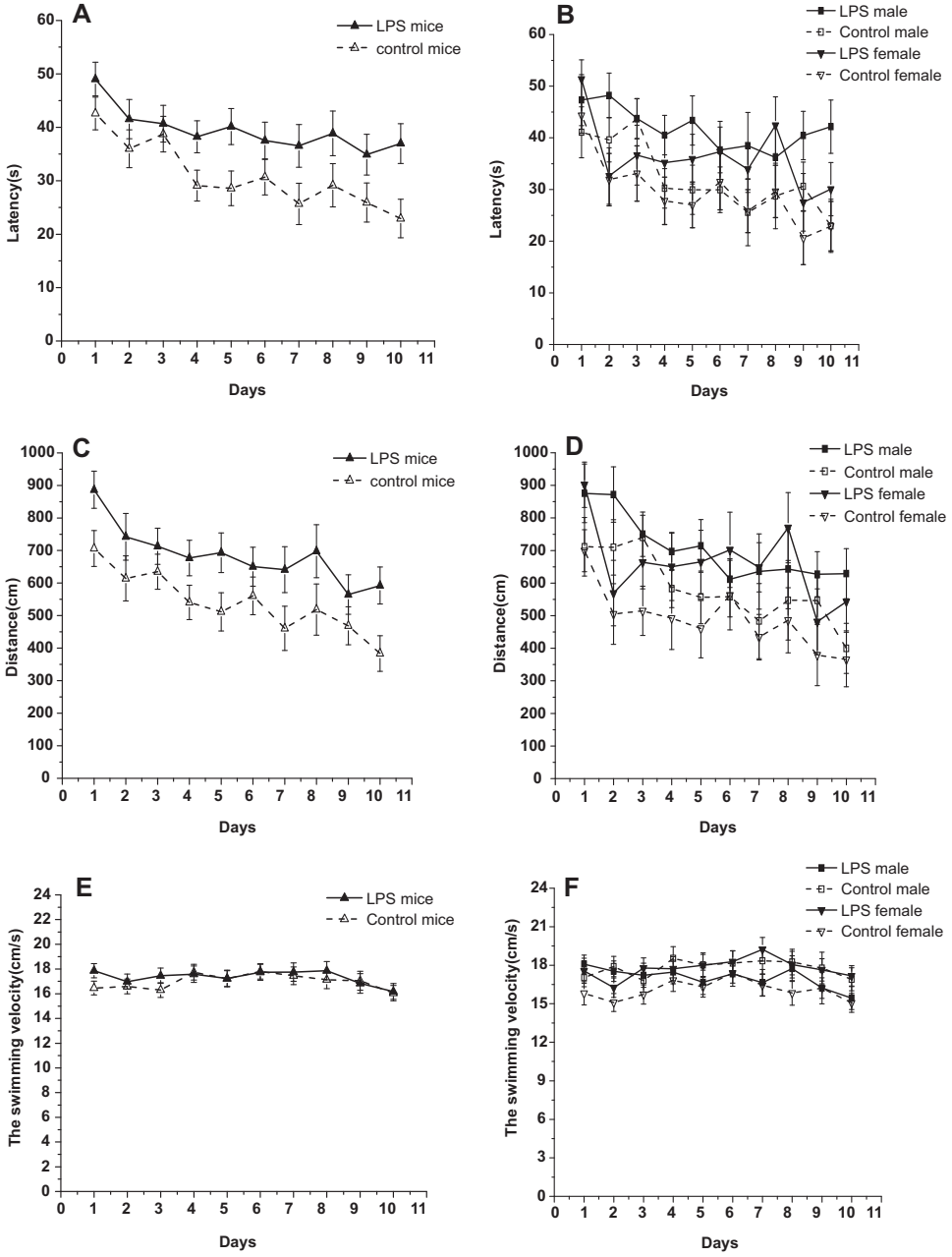

Fig. 4. The performance of 400-day CD-1 mice with maternal LPS exposure in the MWM. The latency (A and B), swimming distance (C and D) and swimming velocity (E and F) during place learning are shown by the combined sexes (A, C and E) and separated sex (B, D and E), respectively. The percentages of distance (G and H) and time (I and J) in the target quadrant are shown by the combined sexes (G and I) and separated sex (H and J). LPS-treated mice had significantly longer latency (A) and swimming distance (C) in the place learning test than the control ones, for males and females (latency, B) or females (swimming distance, D). The swimming velocity was similar between LPS group and control group for the combined (E) or separated (F) sexes. The LPS group had lower percentage of distance and time than the controls in the target quadrant during probe trial for the combined sexes (G and I), but the treatment effect was not significant for the separated sex (H and J). The number was 14 in the LPS group (8 males, 6 females) and 15 in the control group (8 males, 7 females). The bars standing represent for S.E.M. # Compared to the control group, $P < 0.05$.

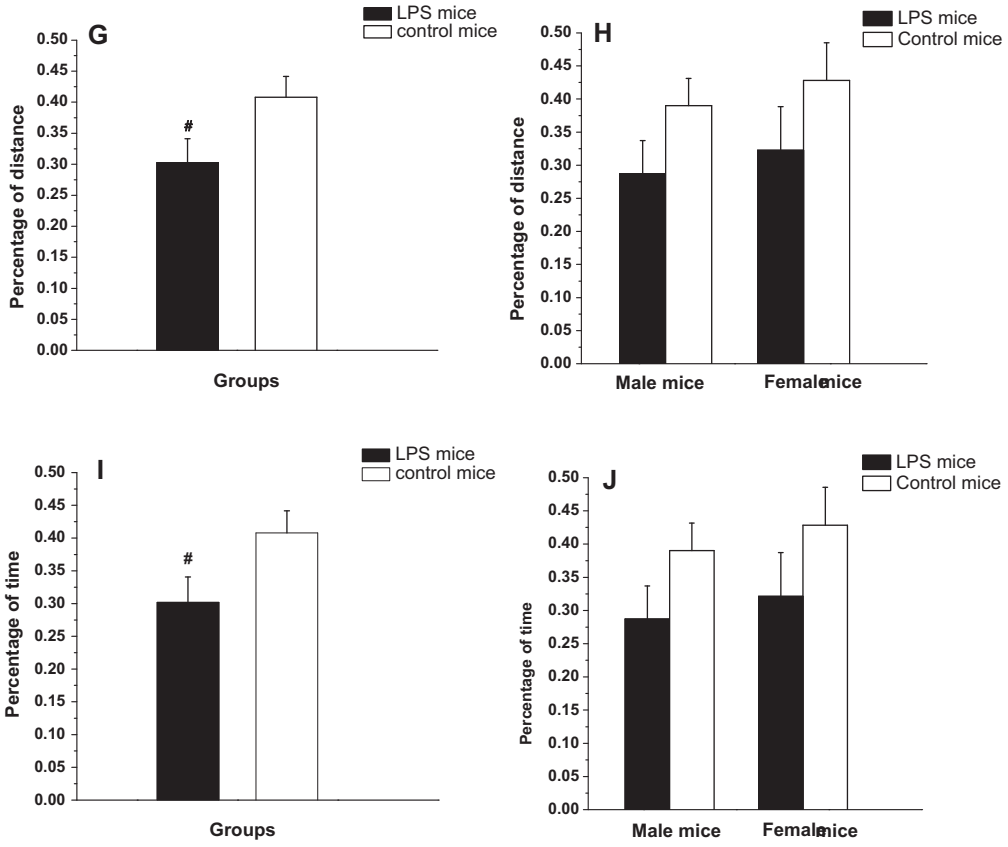

Fig. 4. (*Continued*).

Probe trial. The percentage of both distance and time in the target quadrant showed significant effects of treatment for the combined sexes [$F_{(1,27)} = 4.256, 4.320$; $P = 0.049, 0.047$; see Fig. 4G and I], being less for the LPS-treated mice. After separating the sexes, no treatment effect was found on the percentages of distance and time in the target quadrant for males [$F_{(1,14)} = 2.521, 2.530$; $P = 0.135, 0.134$] or females [$F_{(1,11)} = 1.488, 1.530$; $P = 0.248, 0.242$] (see Fig. 4H and J).

3.4.4.2. Novel object recognition. In the testing period, only 51–53% of exploratory time was spent in the novel object by the LPS-treated mice with 10-min and 24-h delays, respectively. In comparison, the control mice spent 65–70% in the novel object for the same tests. The difference was significant among the combined sexes [$F_{(1,27)} = 4.827, 8.750$; $P = 0.037, 0.006$; see Fig. 5A and C]. In the 10-min delay task, the treatment effect was mainly observed from the males [$F_{(1,14)} = 6.063$, $P = 0.027$; Fig. 5B]. In the 24-h delay task, however, the treatment effect was mainly attributable to the females [$F_{(1,11)} = 6.181$, $P = 0.030$; Fig. 5D].

4. Discussion

This study, to our best knowledge, focused on some unique aspects of accelerated age-related impairment of learning and memory using mouse model of repeatedly maternal lower-dose LPS exposure during late pregnancy (gd 15–17). Our results indicated that the body weight was similar between maternal LPS-exposure and control CD-1 mice at different time points across a period of 33 weeks. At an age of 5 weeks, the LPS-exposure CD-1 mice showed similar locomotor activity and anxiety in the open field, and spatial ability of memory in the RAWM task, as the normal saline-treatment CD-1 mice, with an exception of poorer learning ability in the female LPS mice. Comparing to the control mice, the LPS-exposure mice showed similar sensorimotor abilities in the beam walking and the tightrope tasks, locomotor activity (perhaps more decline among male LPS mice) and anxiety in the open field task, species-typical behaviors in the hording, burrowing and nesting tasks, and spatial ability of learning and memory in the RAWM task at an age of 290 days. Compared to the control mice at the same age of 400 days, LPS-exposure mice had similar sensorimotor abilities in the beam walking and tightrope tasks, similar locomotor activity in the open field task, similar anxieties in the open field and elevated plus maze, slight declined anxiety in the black-white alley task for males, increased hording and burrowing activities and decreased nesting ability in the species-typically behavioral tasks, and decreased abilities of learning and memory in the novel object recognition and MWM tasks. These results suggested that the CD-1 mice, whose mother repeatedly exposed to LPS in lower-dose during late embryo stage, had relatively normal development and maturity in physical state and nervous system functions. For example, they showed relatively normal sensorimotor ability, emotional state, species-typical behaviors, and spatial

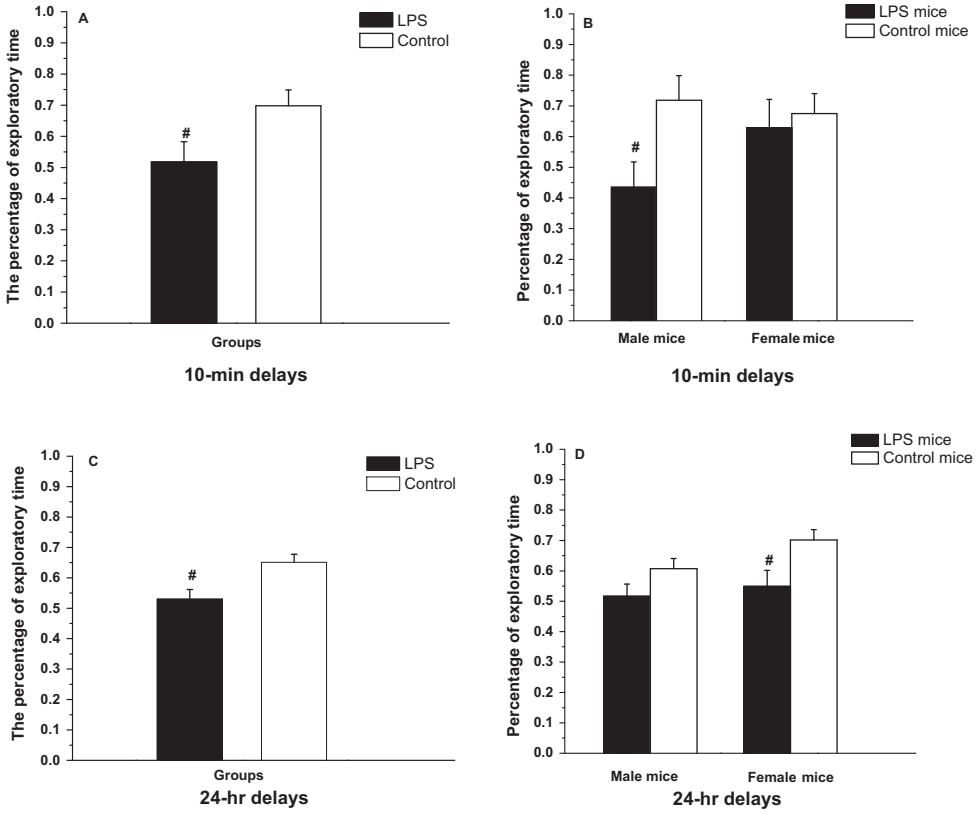

Fig. 5. The preferential index for novel object (PI_N) in the novel object recognition task. The maternally LPS-treated mice had lower PI_N after both the 10-min and 24-h delays in sexes combined (A and C). The treatment effect was mainly attributable to the males in the 10-min delay (B), and the females in the 24-h delay (D). The bars standing represent for S.E.M. The number was 14 in the LPS group (8 males, 6 females) and 15 in the control group (8 males, 7 females). # Compared to the control group, $P < 0.05$.

cognitive function. These normal physiological functions preserved at least to an age of 290 days (9.5 months approximately). However, they showed an accelerated age-related deterioration of spatial learning and memory, long-term object recognition memory and species-typical behaviors, one group of hippocampus-dependent behaviors.

Memory, (especially episodic memory impairments), occur as a result of normal aging across many species, including humans and rodents [11,27]. However, the onset age of memory impairments remains to be identified [49,57]. Generally, the age-related decline of learning and memory begins relatively early in adulthood [57]. Though, not all aspects of memory function exhibit early age-related decline. In humans, the spatial visualization and matrix reasoning decline before age 25 along with neurobiological changes, such as declines in dopaminergic functions, total synapses in cerebral cortex and cortical blood perfusion, and increase in astrocyte cell volume [1,19]. In accelerated senescence prone mouse-8, the decline of spatial learning and memory in the RAWM was reported to start at 5-month old [10]. For C57Bl/6J mice, a longer lifetime inbred mouse, middle-aged (14 months) females show decreased learning ability in the IntelliCage task [46]. Our unpublished data indicated that 12-month CD-1 mice and 12.5-month Kunming mice had shown poorer performance than the same strain mice aged 7 months in the RAWM. Other study also reported that both male and female 12-month CD-1 mice had lower abilities of learning and memory in a 3D maze [18]. Therefore, the normally control 400-day (about 13.5 months) CD-1 mice used in the present study should had already reached the onset age of spatial memory impairment.

It has gained growing acceptance for the fetal origins of adult disease hypothesis [76], which causally associates a disadvantageous early fetal environment with later adult diseases, including ischemic heart disease, obesity, hypertension, hyperlipidaemia, noninsulin-dependent diabetes mellitus, and degeneration diseases of nervous system (e.g., AD and Parkinson's disease). It is believed that prenatal insult of inflammation, due to intrauterine infection during development of the embryo or maternal infection during pregnancy, can lead to life-long changes in a number of physiological processes [39], such as physiological responses to environmental challenges and alter predisposition to pathology later in life [58]. When a lower dose of LPS (8 μg/kg) was given (i.p.) daily to pregnant CD-1 mice for 8 days during mid-gestation (gd 8–15), as reported in our previous study [74], the offspring had impaired spatial learning and memory in RAWM at ages of about 7 months or older after a normal maturation.

In this study, the pregnant CD-1 mothers were i.p. given 50 μg/kg of LPS daily during late gestation (gd 15–17) to mimic the prenatal inflammation. A normal physical development and maturation was observed in the offspring at an age of 33 weeks by a dynamic measure of body weight. Compared to the control, there were no changes in any of sensorimotor ability, locomotor activity, and anxiety in the LPS-treated offspring up to age of 400 days (about 13.5 months). And, there were no changes in spatial ability of learning and memory, and species-typical behaviors up to an age of 290 days (about 9.5 months). However, there were significant impairment of spatial and non-spatial learning and memory, and species-typical behaviors in the 400-day offspring of maternal LPS exposure when compared to control mice of the same age. Due to our inability to repetitively measure spatial learning and memory for the RAWM and MWM tasks, both of which are hippocampus-dependent, we used RAWM to detect the spatial ability of learning and memory in the offspring CD-1 mice at the age of 290 days, and MWM at the age of 400 days. Note that we had shown RAWM

task being more sensitive for displaying impaired spatial ability of learning and memory than MWM task in our previous studies [10]. In order to obtain a clear conclusion, we employed the RAWM task at a time point when the mice have better memory ability (at age of 290 days), and the MWM task at a time point when the mice have poorer memory ability (at age of 400 days). As the LPS-treatment effect was not shown in the more sensitive RAWM task for the offspring at 290-day age, it suggests that the spatial ability of learning and memory in the offspring at that age was not affected by repeated maternal LPS-exposure during late gestation. Similarly, as reduced performance was observed in less sensitive MWM task for the LPS-treated offspring at the 400-day age when compared to control mice of the same age, it suggests that an accelerated age-related decline in spatial ability of learning and memory was affected by the maternal LPS-exposure during late gestation. For the novel object recognition task, if the interval after training is more than 3 h, it would be the hippocampus-dependent [29]. So far, very little has been understood about the age-related change of object recognition memory in mouse. In this study, the LPS-treated offspring at 400-day age showed reduced object recognition memory in tests with both 10-min and 24-h delays when compared to control mice of the same-age. It suggests that maternal inflammation accelerates age-related decline in hippocampus-dependent non-spatial memory in the offspring.

It has been reported that a successful completion of the species-typical behaviors (such as hoarding, burrowing and nesting) by mice depends on the intact of the hippocampus and medical prefrontal cortex [14,15]. In humans, aging appears to enhance the prevalence of hoarding behavior [70]. Our previous study of senescence-accelerated prone mouse 8 revealed that age-related improvement in hoarding and burrowing ability took place at an age of 7 months or older, and degradation in nesting ability at different ages than the age-matched senescence-resistant mouse 1 [9]. For the effect of inflammation on the species-typical behaviors, it is reported that impaired burrowing behavior could be induced by scrapie [16,28] and LPS at doses insufficient to induce a fever response [71]. At this study, middle-aged (400 days) CD-1 mice of repeatedly maternal LPS exposure, with lower-dose exposure during latter embryo stage, exhibited increased hording and burrowing activities, and decreased nesting ability in the species-typically behavioral tasks. These findings were coincident with the result from aging mouse model [9], but contrary to the result from mice acquired subpyrogenic-dose LPS-exposure [71].

In summary, we reported that the CD-1 offspring, whose mothers i.p. received an injection of 50 μg/kg LPS daily during gd 15–17, showed a relative normal duration of development and maturation in physical and neural functions, and an accelerated aged-related impairment in hippocampus-dependent memory tasks (spatial and non-spatial) and species-typical behavior tasks. This mouse model of prenatal inflammation seems to meet with the criteria of AD model, at least in behaviors. Further research is needed to explore, whether the pathological characteristic in the brain of this model is in accordance with that in AD.

Conflict of interest

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

Acknowledgments

This work was financially supported by the National Foundation of Nature Science of China (30872730), Specialized Research Fund for the Doctoral Program of Higher Education (20070366004) and the Major Projects for Fund of Nature Science Study in Anhui Universities (ZD2008007-1).

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