Inflammatory insult during pregnancy accelerates age-related behavioral and neurobiochemical changes in CD-1 mice

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Abstract Data shows that inflammation during pregnancy significantly exerts a long-term influence on offspring, such as increasing the risk of adult cognition decline in animals. However, it is unclear whether gestational inflammation affects the neurobehavioral and neurobiochemical outcomes in the mother-self during aging. In this study, pregnant CD-1 mice

intraperitoneally received lipopolysaccharide (LPS) in two doses (25 and 50 g/kg, respectively) or normal saline daily during gestational days 15–17. At the age of 15 months, a battery of behavioral tasks was employed to evaluate their species-typical behaviors, sensorimotor ability, anxiety levels, and spatial learning and memory abilities. An immunohistochemical method was utilized preliminarily to detect neurobiochemical indicators consisting of amyloid- β , phosphorylated tau, presynaptic proteins synaptotagmin-1 and syntaxin-1, glial fibrillary acidic protein (GFAP), and histone-4 acetylation on the K8 site (H4K8ac). The behavioral results showed that LPS exposure during pregnancy exacerbated a decline in 15-month-old CD-1 mice's abilities to nest, their sensorimotor and spatial learning and memory capabilities, and increased their anxiety levels. The neurobiochemical results indicated that gestational LPS exposure also intensified age-related hippocampal changes, including increased amyloid- β_{42} , phosphorylated tau, synaptotagmin-1 and GFAP, and decreased syntaxin-1 and H4K8ac. Our results suggested that the inflammatory insult during pregnancy could be an important risk factor for the development of Alzheimer's disease, and the H4K8 acetylation might play an important role in the underlying mechanism. This study offers a perspective for improving strategies that support healthy development and successful aging.

Xue-Yan Li and Fang Wang are the co-first author in this paper.

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Introduction

From a medical viewpoint, one of the main medical challenges of the future is the world's progressively increasing elderly population. Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disease that is the leading cause of dementia among the elderly (Katzman 2008). Though the cause of AD remains unclear, age and female sex are regarded as major risk factors for AD.

In humans and rodents, age-associated memory impairment (AAMI) is a common phenomenon, and it may transform to AD via unclear mechanisms. To date, the generally accepted notion is that exposure to stressors (such as inflammation) during early development can accelerate AAMI progression (Hao et al. 2010; Sterlemann et al. 2010). In addition to early development, however, the greatest change that a woman will undergo is her reproductive experience, including pregnancy and maternal behaviors (Kinsley et al. 2008). This exposure to sharply altered hormone levels during pregnancy and the postpartum period can result in obviously reactive changes in the brain, including changes in the increment of cell body size, the extension of dendritic branches, and alteration of neurogenesis in several brain regions, such as the forebrain and hippocampus (Prange-Kiel and Rune 2006; Tomizawa et al. 2003). Although the hormone surges are transient during pregnancy and the postpartum period, the effects of the changes induced by the reproductive experience on the brain's functions and behaviors are life-long (Kinsley et al. 1999). For example, the emerging data indicate that the normal reproductive experience or motherhood can mitigate AAMI (Gatewood et al. 2005) and alter the cellular response to estrogens in older hippocampi in rats (Barha and Galea 2011). These results suggest that the normal reproduction experience has a long-lasting and extensive influence on the AAMI in female rodents (Li et al. 2013).

Despite the above-outlined benefits of pregnancy, the state is still one wherein females are vulnerable to infections from bacteria and viruses. Some of the most common conditions during pregnancy are respiratory tract, enteric, urinary, and periodontal infections. These infections can induce inflammatory responses, which are then accompanied with increased levels of circulating proinflammatory cytokines, such as interleukin-1 β , interleukin-6, and tumor necrosis factor- α . Lipopolysaccharides (LPS), the cell wall components of

Gram-negative bacteria, can be recognized by activating the immune system and inducing the expression of proinflammatory cytokines (Dantzer 2004). For this reason, LPS are commonly employed to mimic bacterial infections in experimental animals. The systemic inflammation mediated by the LPS can trigger a set of cellular and molecular events that are able to initiate neuroinflammation to affect cognitive function (Arai et al. 2001; Cunningham et al. 2009; Sparkman et al. 2006) and reduce adult neurogenesis (Fujioka and Akema 2010; Monje et al. 2003). It has been well documented that pregnant animals have an increased sensitivity to LPS compared to non-pregnant ones (Faas et al. 2003; Kunnen et al. 2014). Furthermore, studies have also demonstrated that pregnant rats exhibit much more intense and persistent inflammation than non-pregnant rats when exposed to even a very low dose of LPS (Faas et al. 1995; Faas et al. 1994).

There is a physiologic regulation of the innate immune responses to adapt the fetal allografts during pregnancy. The primary adaptation is a change in the production of cytokines. The cytokines can be divided into two functional groups, including Th1 reactions (cell-mediated immunity) and Th2 reactions (humoral immunity). During a normal pregnancy, the balance of Th1 and Th2 cytokines is strongly shifted toward Th2 activity, and this plays a potentially protective role. However, Th1/Th2 activity balance is followed by a progressive shift toward Th1 predominance late in gestation, which is when abnormal levels may initiate and intensify the cascade of inflammatory cytokine production involved in adverse pregnancy outcomes (Challis et al. 2009).

However, information about whether gestational inflammations affect the mother's own brain in terms of aging is still elusive. Studies have suggested that hypertensive pregnancy disorders have an increased, sex-specific risk of cognitive decline and dementia among women 5 to 10 years after the hypertensive pregnancy (Aukes et al. 2007; Postma et al. 2014a; b). Moreover, a recent study has shown that hypertensive pregnancy disorders are associated with worse performance in tests of processing speed and smaller brain volumes decades later, when compared with women with histories of normotensive pregnancies (Mielke et al. 2016). Other studies have demonstrated that stress during pregnancy durably hampers maternal body-weight growth, increases anxiety-like behaviors, and affects reactivity to stress in mothers more than 1 month after the stress

exposure (Darnaudéry et al. 2004). These data imply a long-term, significant impact of adverse pregnancy on behaviors in mothers. Given normal reproduction experience and the adverse gestational conditions mentioned above can chronically affect cognitive functioning or other behaviors (Li et al. 2013; Mielke et al. 2016; Darnaudéry et al. 2004), so it is plausible to hypothesize that gestational inflammation can impact the AAMI and corresponding neural substrates in the mother-self during aging.

As noted, aging is key risk factor for AD, and studies have suggested that AD has a long preclinical stage from aging to the emergence of clinical symptoms (Sperling et al. 2014). In general, mild cognitive impairment (MCI), especially amnesiac MCI, is accepted as an intermediate state between normal aging and dementia. The AD neuropathology is an age-dependent progressive process that perhaps lasts decades before the neuropathological diagnostic criteria can be met; it ultimately leads to synaptic loss and neuronal damage in cortical areas of the brain essential for cognitive functions (Sperling et al. 2014; Swerdlow 2007). It has been shown that the accumulation of amyloid- β ($A\beta$), hyperphosphorylation of tau protein, and circuit-specific changes of synaptic connections, such as changed synaptic proteins, appear contribute to AAMI (Chen et al. 2007b; Clinton et al. 2010). For $A\beta_{42}$ and $A\beta_{40}$, two major $A\beta$ isoforms, $A\beta_{42}$ is more insoluble and prone to aggregation (Miners et al. 2014). The insoluble $A\beta_{42}$ progressively increases with age and further aggravates in the AD brain, which is accompanied with cognitive decline (Darusman et al. 2014; Fukumoto et al. 2004; Yue et al. 2014). Increasing evidence has shown that $A\beta$ oligomers trigger neurotoxicity, probably via phosphorylated tau (p-tau) (Selenica et al. 2013).

Synaptotagmin-1 (Syt-1) and syntaxin-1 (Stx-1) are important presynaptic active-zone proteins of the brain and mediate fast neurotransmitter release (Jurado 2014; Paddock et al. 2011). Our previous and other studies have shown that the hippocampal level of Syt-1 increases with aging and is associated with AAMI in senescence-accelerated prone mice P8 (SAMP8) (Chen et al. 2007b; Tong et al. 2015), whereas Stx-1 levels decrease in normal-aged rodents (Tong et al. 2015; VanGuilder et al. 2011) and transgenic AD mice (Wirths and Bayer 2010). So, $A\beta_{42}$, p-tau, Syt-1, and Stx-1 can be regarded as age-related neurobiochemical indicators linked to AAMI.

Except for these biomarkers, it has been widely accepted that aging is accompanied by low-grade, chronic neuroinflammation. Astrocytes and microglia are fundamental in defending the brain against infection and inflammation. An increasing number of studies suggest that astrogliosis is an early phenomenon and an important pathologic change in AD development (Carter et al. 2012). Glial fibrillary acidic protein (GFAP) is the main intermediate filament protein but also a specific marker of mature astrocytes (Hayakawa et al. 2007; Walz and Lang 1998). Data have shown that the expression of GFAP mRNA and protein during aging progressively increases in humans and rodents (Cotrina and Nedergaard 2002; Goss et al. 1991; Hayakawa et al. 2007). The enlargement of astrocytic bodies and increased GFAP expression are indications of reactive gliosis, a process highly related to brain damage and aging.

Studies have indicated that AAMI is correlated with the remodeling of chromatin via histone acetylation (Peleg et al. 2010; Zhao et al. 2013), which causes the electrostatic affinity between neighboring histones and the deoxyribose nucleic acid to dwindle and promote a more open chromatin structure that leads to permissive memory-related gene transcription (Brownell and Allis 1996; Kouzarides 2007). Several acetylated sites of histone (H) residues in the promoter region have been shown to be important for learning, memory, and synaptic plasticity (Graff et al. 2011; Mendelsohn and Larrick 2012). Specifically, H4 acetylation on the K8 site (H4K8ac) is involved in the formation of long-term memories (Intlekofer et al. 2013; McQuown et al. 2011). Recently, our studies have also indicated that the oral long-term administration of acarbose or 1-Deoxynojirimycin can alleviate age-related impairments related to spatial learning and memory in SAMP8 mice (Chen et al. 2015; Tong et al. 2015), and the increased H4K8ac may be associated with this alleviation (Chen et al. 2015; Yan et al. 2015).

It is therefore of great interest to investigate the effects of gestational inflammation on AAMI and to explore preliminarily the relevant changes of neurobiochemistry in female CD-1 mice. In this study, we address several issues. First, we used a battery of tasks to explore whether exposure to LPS during pregnancy can affect age-related behavioral changes, focusing on AAMI, in female CD-1 mice. Second, we examined whether LPS exposure during pregnancy can affect AAMI-related biochemical indicators, including $A\beta_{42}$,

p-tau, presynaptic proteins Syt-1 and Stx-1, and GFAP in the layers of different hippocampal subregions. Third, we investigated whether LPS treatment during pregnancy exacerbates the reduction of H4K8ac levels in older hippocampi.

Materials and methods

Materials

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

Animals and treatment

The CD-1 mice (7–8-weeks old, 30 males and 60 females) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd., whose foundation colonies were all introduced from Charles River Laboratories, Inc. The colony was maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.) environment with a controlled temperature (24 ± 2 °C) and humidity (55 ± 5 %). Water and food was available ad libitum. After 2 weeks' acclimation to the colony room, the males and females were paired (1:2) into breeders. The presence of a vaginal plug on the next morning was designated as gestational day (gd) 0. Forty-five pregnant mice were obtained on the next day and randomly divided into three groups: higher-dose LPS (HD-LPS, $n = 12$), lower-dose LPS (LD-LPS, $n = 12$), and a control (CON, $n = 21$) group. During gd 15–17, the HD-LPS or LD-LPS groups, respectively, received intraperitoneal injections of LPS (50 or 25 µg/kg) daily, and the CON group received an injection of normal saline. To avoid stress, these mothers were not separated from their offspring until postnatal day 21, and then they were housed in polypropylene cages with 4–5 mice per cage. At age 6 months, 10 mothers randomly selected from the CON group were identified to serve as the younger-control group (Y-con) to complete the behaviors, and the 11 residual mice were assigned into the older-control group (O-con). Before beginning the behavioral tasks at 15 months old, mice with movement disorders, hair removal, or any visible tumors were excluded. Finally, 10 mice in each group entered the study. The animals were treated 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.

Behavioral tests

Species-typical behavior tasks

Burrowing The plastic home cage contained a plastic tube (diameter 4-cm, length 10-cm), so as to enrich the environment by providing an alternative refuge and an iron tube with semi-cylinder shape (radius 5-cm, length 12-cm) [50]. Two bars of 1-cm height were transversely attached to the floor of the iron tube 1-cm from each end, and the inter-bar space was filled with 40-g of corn. Each mouse was placed into an individual experimental cage, and the weight displaced from the tube was measured 2-h later.

Nesting Each mouse was housed overnight in the home cage with new sawdust bedding and six pieces of white, papery cloth (5×5 cm²), which the mice could use to make nests (Chen et al. 2005). After an overnight, the quality of nests was scored as: 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 in the crater; 3, sawdust crater with shredded or whole papery cloth gathered around and in the crater, forming a cup-shaped nest; and 4, the shredded papery cloth formed a ball-shaped nest covering the mouse.

Sensorimotor tasks

Beam walking A 110-cm-long steel rod (diameter 1 cm), with each end attached to a platform (diameter 20-cm), was supported by two vertical poles and elevated 50 cm above the water surface of a black circular tank 150-cm in diameter. Each mouse was placed perpendicularly on the center of the beam and stayed there for a maximum of 60 s in each trial for three successive trials. Balance time, during which the animal did not fall from the beam, was recorded in each trial. If the animal remained on the beam up to 60 s, or escaped to a platform, it was recorded as 60 s. The mean of the balance times in the three trials was entered for statistical analysis.

Anxiety-based tasks

Open field The apparatus was a black wooden box (floor $81 \times 81 \text{ cm}^2$, wall height 28-cm) with 16 equal squares (each $20 \times 20 \text{ cm}^2$) formed with white-painted lines (width 3-mm) in the floor. A 40-W white light was placed 2.80 m above the center of the field to provide illumination. The animal was placed in a corner square facing the wall and was permitted to freely explore the environment for 5 min. Then, the latency (the time taken by the animal to leave the corner square), number of squares crossed, and peripheral time (the time spent in the 12 peripheral squares) were recorded. The area was cleaned with 70 % ethanol before the next mouse was tested.

Elevated plus maze The elevated plus maze was made of Plexiglas and elevated by a tripod to 80 cm above the floor. The apparatus consisted of two opposite closed arms ($30 \times 5 \text{ cm}^2$) enclosed with walls (height 15-cm) and two opposite open arms ($30 \times 5 \text{ cm}^2$, without walls), forming the shape of a cross with a central arena ($5 \times 5 \text{ cm}^2$). Each mouse was placed in the central arena of the maze facing an open arm and observed for 5 min. The latency (the time before first leaving the central arena), number of entries into the open arms, and time spent on the open arms were recorded during a single trial. The maze was cleaned with 70 % ethanol after each mouse.

Spatial learning and memory tasks

Six-radial arm water maze (RAWM) The apparatus and procedure has been described [56]. The maze consisted of a circular black tank (diameter 100-cm, depth 21-cm) and six swim arms ($30.5 \times 19 \times 21 \text{ cm}^3$) that radiated out from a 40-cm-diameter center area. The tank was placed on a steel rack (30-cm high) and filled with water (20–22 °C) to 16-cm depth. A black escape platform (diameter 10-cm) was submerged 1.0 cm below the water surface. In addition, the tank was surrounded by a white cloth curtain 100 cm away from the exterior-wall, with three black cardboards of different shapes (circle, triangle, and square) hung equidistantly on the interior of the curtain, to act as cues guiding spatial navigation. The mice underwent four consecutive acquisition trials (Trials 1–4) and one memory retention trial (Trial 5) each day. The sequences of starting points were different from day to day, but the location of the

platform and experimenter remained unchanged. In Trials 1–4, the mouse was respectively released into water from one of four random entry alleys, not including the alley containing the platform and its opposite alley. In each trial, the mouse had 60 s to find the platform. Upon finding the platform, the mouse stayed there for 30 s prior to the next trial. If the mouse failed to locate the platform within 60 s, it would be guided to the platform and remained there for 30 s. Upon entering an incorrect alley with its whole body, or when failing to select any alleys within 10 s, the mouse was gently dragged back to the start alley and the attempt was recorded as an error. After Trials 1–4 were completed, the mouse was dried by electric heater and placed in the home cage under a 150 W floodlight for 30 min, and then returned to the maze to complete Trial 5, using the same starting-alley as used in Trial 4. The number of errors and latency (the time from entering the water to finding the platform) was recorded and averaged daily for data analysis.

Preparation of tissue samples

Fifteen days after the behavioral testing (avoiding of the effects of the behavioral tests on biochemical indicators), the mice were anesthetized with halothane. After cervical dislocation, brains were immediately removed and bisected in the mid-sagittal plane on ice and then immersed in 4 % paraformaldehyde at 4 °C for 3 days and paraffin-embedded for pathological staining and immunohistochemistry. Fixated brains were sectioned in the coronal plane at 6- μm thickness from tissue paraffin blocks using a microtome.

Pathological staining

Improved Bielschowsky staining This method has shown a good sensitivity for neurofibrillary tangles, and it is used as a single stain for the diagnosis of AD. The improved Bielschowsky staining kit was purchased from SenBeiJia Biological Technology Co., Ltd. (Nanjing, China). The tissue sections were placed into fresh Bielschowsky silver nitrate solution for 30 min at 37 °C after dewaxing and hydration. After being washed thoroughly with distilled water, slides were treated with a reducing agent for seconds, until the section showed up as yellow. Then, the slices were titrated with ammoniacal silver nitrate solution for 30 s and reduced again for 2 min, during which the reducing solution was changed twice, rendering the slice's appearance a brownish

yellow. Slides were then rinsed in distilled water, modulated to color with gold chloride solution for 3 min, fixed in sodium thiosulfate, and dehydrated through alcohols and xylene in turn.

Immunohistochemistry

The streptavidin-biotin-peroxidase complex (SABC) method was applied, as described [39]. The sections of hippocampus were dewaxed and hydrated and then washed with running tap water for 3 min. After washing, the sections were quickly treated with periodate inactivated enzyme for 1 min to deactivate the endogenous peroxidase. Sections were treated with a sodium citrate buffer (0.01 mol/L, pH 6.0) for 10 min in a microwave to facilitate antigen retrieval. To minimize non-specific binding reactions, sections were treated with 0.4 % Triton X-100, H₂O₂ and 5 % fetal bovine before incubation with specific antibodies. Specific antibodies for A β ₄₂ (1:200), p-tau (1:500), Syt-1 (1:400), Stx-1 (1:400), GFAP (1:500), and H4K8ac (1:1000), all purchased from Abcam (Cambridge, MA, USA), were added for overnight at 4 °C. The negative control was set for each specific protein and went through the entire staining procedure but without a primary antibody. Then, the sections were incubated with a biotinylated secondary antibody (biotinlabeled goat anti-rabbit IgG) diluted 1:200 in PBS for 1 h at 37 °C and subsequently treated with SABC (Wuhan Boster Bioengineering Limited co., China) at 37 °C for 30 min, before visualized by a diaminobenzidine detection kit. In order to avoid nonspecific staining, our sections were stained in one formal test under the same experimental conditions for the test of each protein for all groups during immunohistochemical staining.

Image capture

The images of whole the dorsal hippocampus (4 × 10) and the subregions (20 × 10) were captured by the digital camera (Nikon, Japan) equipped on the optical microscope (Olympus, Japan) and saved using MetaMorph image acquisition and processing software (Universal Imaging, USA). The proteins were analyzed in each layer in three subregions using the high-magnification pictures in the following order: Hilus (HL), granule cell layer (GL), and molecular layer (ML) in the dentate gyrus (DG); ML, radiation layer (RL), pyramidal cell layer (PL), and original layer (OL)

in the CA1; and ML, stratum lucidum (SL), PL, and OL in the CA3. The target area of each image was placed in the center field of the microscope to ensure greater clarity and to avoid the possible influence of light in the field. Identical camera and microscope settings were used during the process of capturing of images and analyzing the slides.

Image analysis

The intensity of immunoreactivity in each protein was measured using semi-quantitative densitometric analysis with Image-Pro Plus 6.0 image software. The background correction was performed according to a previously described formula prior to analysis [34]. The images used for protein density estimation were used in the analysis of regional optical density (OD). The randomized areas selected in each layer in hippocampal subregions were named as areas of interest (AOIs), where the immunoreactivity was expressed with OD values. The average optical density (AOD) was calculated according to OD/AOI in each layer, which was regarded as the indicator of relative levels of specific proteins. Image analyses were performed under a blind principle without knowledge of grouping.

Statistical analysis

The results were expressed as mean $\pm$ standard error of mean (SEM) for the parametric data or as the 50th (25th/75th) quartiles for nonparametric data. The data from the RAWM were analyzed using repeated measures analysis of variance (rm-ANOVAs), with day, age, or treatment as independent variables. To determine main effects of LPS exposure, one-way analysis of variance (ANOVA) was performed for normal distribution data. Post hoc analyses were employed using Fisher's least significant difference test for a priori designed pair-wise comparisons (CON vs. two LPS groups). When the distribution of data was non-normal, the Kruskal–Wallis H test was employed with an extended *t* test for pair-wise analysis. The student's *t* test was employed for the analysis of age effect. Pearson's correlation test was used to analyze the correlation among the average errors and latency in all trials of the learning or memory phase in the RAWM and the relative level of each specific protein in whole dorsal hippocampus. *P* < 0.05 was accepted as statistically significant. SPSS 16.0 for Windows was used for the statistical analyses.

Results

Species-typical-behavior tasks

The weights of burrows were similar between the O-con and the Y-con groups [$t = 2.51$, $P = 0.022$], and LPS treatment has an insignificant effect on the weights burrowed [$F_{(2, 27)} = 0.578$, $P = 0.568$] (see Table 1). In the nesting task, the O-con group exhibited lower scores than the Y-con group [$t = 2.640$, $P = 0.015$]. LPS treatment significantly affected nesting scores [$F_{(2, 27)} = 4.858$, $P = 0.016$]. The post hoc analysis indicated that the HD-LPS ($P = 0.004$), not the LD-LPS ($P = 0.178$), had lower nesting scores than the O-con group (Table 1).

Beam walking

The O-con group had significantly shorter balance time on the beam than the Y-con group [$t = 2.18$, $P = 0.043$, Table 1]. The LPS treatment's effect on the balance time was significant [$F_{(2, 27)} = 3.507$, $P = 0.044$], that is, the HD-LPS had shorter balance times than the O-con group ($P = 0.021$).

Anxiety-based tasks

Open field

The O-con group exhibited significantly longer latency [$Z = -2.937$, $P < 0.001$] and peripheral time [$t = 6.421$, $P < 0.001$] and fewer squares

crossed [$t = 4.049$, $P = 0.006$] than the Y-con group (Table 1). The LPS treatment effects on the peripheral time [$F_{(2, 27)} = 4.300$, $P = 0.024$] and squares crossed [$F_{(2, 27)} = 3.912$, $P = 0.032$] were significant. The HD-LPS had longer peripheral time ($P = 0.007$) and fewer squares crossed ($P = 0.013$), and the LD-LPS had fewer squares crossed ($P = 0.048$) than the O-con group (see Table 1).

Elevated plus maze The O-con group showed significantly longer latency [$Z = -3.129$, $P = 0.002$] and fewer numbers of entries into the open arm(s) [$Z = -2.321$, $P = 0.020$] than the Y-con group (Table 1). The LPS treatment had an insignificant effect on the latency, number of entries into the open arm(s), and time spent on the open arm(s).

RAWM

Learning phase

Age effects The latency [$F_{(9, 162)} = 17.940$, $P < 0.001$] and number of errors [$F_{(9, 162)} = 30.023$, $P < 0.001$] progressively declined over days for control mice as a combined group (Fig. 1a, c), indicating these mice were able to learn the task. The rm-ANOVA results showed that the O-con group had significantly longer latency [$F_{(1, 18)} = 4.727$, $P = 0.043$] and more errors [$F_{(1, 18)} = 5.497$, $P = 0.031$] than the Y-con group.

Table 1 Performance of the CD-1 mice in the non-cognitive behavioral tests

Task	Parameters	HD-LPS	LD-LPS	O-con	Y-con
Hoarding	Weight hoarded (g)	21.1 ± 0.7	21.0 ± 0.9	22.6 ± 0.7	25.2 ± 0.9
Nesting	Nesting scores	2.1 ± 0.2 ^{##}	2.6 ± 0.2	3.0 ± 0.2*	3.7 ± 0.2
Beam walking	Balance time (s)	22.8 ± 3.1 [#]	34.9 ± 4.7	37.0 ± 4.4*	49.1 ± 3.5
Open field	Latency (s)	11.0(6.0/17.0)	12.5(8.5/18.7)	6.5(4.0/11.5)**	3.5(2.0/4.0)
	Peripheral time (s)	289.9 ± 11.5 ^{##}	282.4 ± 11.1	272.8 ± 16.0**	228.6 ± 27.8
	Number of squares crossed	68.6 ± 30.4 [#]	76.9 ± 28.0 [#]	106.1 ± 35.6**	146.5 ± 19.8
Elevated plus maze	Latency (s)	10.5 (6.5/12.2)	8.0(3.7/12.2)	10.0(3.7/13.2)**	2.0(1.7/4.2)
	Time on the open arm(s)	1.0(0.0/2.0)	1.0(0.8/2.0)	1.0(0.0/1.5)*	2.5(1.0/3.0)
	Number of entries to the open arms	10.0(0.0/28.5)	12.50(5.3/29.3)	12.5(0.0/28.7)	29(0.0/28.5)

Data were expressed by mean ± S.E.M.

O-con old controls, Y-con young controls

Denotes significant difference of the O-con group compared to the Y-con ($P < 0.05$, ** $P < 0.001$)

[#] Denotes significant difference of the comparison between the LPS group and the O-con group ([#] $P < 0.05$; ^{##} $P < 0.001$)

LPS treatment effects The latency [$F_{(9, 243)} = 11.087$, $P < 0.001$] and errors [$F_{(9, 243)} = 22.109$, $P < 0.001$] also progressively declined daily for all 15-month-old mice combined (Fig. 1b, d). The LPS treatment significantly affected the mice's latency [$F_{(2, 27)} = 3.518$, $P = 0.044$] and errors [$F_{(2, 27)} = 3.693$, $P = 0.038$]. The post hoc analyses showed that only the HD-LPS group had significantly longer latency ($P = 0.013$) and more errors ($P = 0.011$) than the O-con group.

Memory phase

Age effects The latency [$F_{(9, 162)} = 21.685$, $P < 0.001$] and number of errors [$F_{(9, 162)} = 18.569$, $P < 0.001$] declined progressively over days for all control mice combined (Fig. 1e, g). The O-con mice had significantly longer latency [$F_{(1, 18)} = 6.601$, $P = 0.019$] and more errors [$F_{(1, 18)} = 4.801$, $P = 0.042$] than the Y-con mice.

LPS treatment effects The latency [$F_{(9, 243)} = 7.940$, $P < 0.001$] and number of errors [$F_{(9, 243)} = 14.445$, $P < 0.001$] also progressively declined daily for all older mice combined (Fig. 1f, h). There were significant differences in the latency [$F_{(2, 27)} = 3.507$, $P = 0.044$] and number of errors [$F_{(2, 27)} = 3.537$, $P = 0.043$] among three older groups. The post hoc analyses showed that significantly longer latency ($P = 0.014$) and more errors ($P = 0.015$) only occurred in the HD-LPS group, compared to the O-con group.

The results of histopathological staining

In the Bielschowsky staining, cell nuclei and nerve fibers presented as deep black and the background showed light black. No neurofibrillary tangles were observed in the older or LPS treatment hippocampi.

Immunohistochemistry

$A\beta_{42}$ and p-tau in different hippocampal strata

The $A\beta_{42}$ AOD values are shown in Table 2, and p-tau data are displayed in Fig. 2. The $A\beta_{42}$ expression was observed in every layer (Supplementary Fig. S1), whereas p-tau was primarily expressed in the cellular layer of the hippocampus (Supplementary Fig. S2). The O-con group had significantly higher contents of $A\beta_{42}$ in the DG-HL [$t = 2.806$, $P = 0.010$], DG-ML [$t = 2.677$,

Fig. 1 Performances of differently-treated CD-1 mice in the RAWM. The latency (a, b) and number of errors (c, d) during the learning phase (Trials 1–4), the latency (e, f), and number of errors (g, h) during the memory phase (Trial 5). There were significant age and treatment effects on two measures during both the learning and memory phases. The sample number was 40 for the four groups, with 10 mice in each group. The bars standing represented for SEM compared to the Y-con mice $^{\#}P < 0.05$ and compared to the O-con mice $^{*}P < 0.05$

$P = 0.015$], CA1-ML [$t = 2.327$, $P = 0.032$], CA1-RL [$t = 2.765$, $P = 0.013$], CA1-PL [$t = 3.230$, $P = 0.005$], CA3-SL [$t = 2.510$, $P = 0.022$], and CA3-PL [$t = 3.035$, $P = 0.007$] than the Y-con mice, as well as higher p-tau levels in the DG-GL [$t = 3.204$, $P = 0.005$] and CA1-PL [$t = 3.241$, $P = 0.005$]. The LPS treatment effects were significant on the $A\beta_{42}$ contents in the DG-HL [$F_{(2, 27)} = 4.370$, $P = 0.023$], CA1-RL [$F_{(2, 27)} = 3.571$, $P = 0.043$], CA1-PL [$F_{(2, 27)} = 3.628$, $P = 0.041$], CA3-ML [$F_{(2, 27)} = 3.797$, $P = 0.036$], and CA3-SL [$F_{(2, 27)} = 4.557$, $P = 0.020$], and the p-tau levels in the CA1-PL [$F_{(2, 27)} = 4.960$, $P = 0.015$] and CA3-PL [$F_{(2, 27)} = 3.667$, $P = 0.039$]. Compared to the O-con mice, the HD-LPS held higher levels of $A\beta_{42}$ or p-tau in all aforementioned subregion layers ($P_s < 0.05$), and the LD-LPS only had significantly higher $A\beta_{42}$ in the CA3-ML ($P = 0.030$) and higher p-tau in the DG-GL ($P < 0.05$).

Syt-1 and Stx-1 in different hippocampal layers

The Syt-1 and Stx-1 were expressed in all layers of hippocampus (Supplementary Fig. S3 and S4). Compared to the Y-con mice (Table 2), the O-con group had higher levels of Syt-1 in all layers except for the DG-GL and CA3-PL ($P_s < 0.05$) and lower levels of Stx-1 in all strata besides the CA1-RL, CA1-OL, and CA3-ML ($P_s < 0.05$). The LPS treatment made Syt-1 and Stx-1 levels have significant differences in most hippocampal layers, i.e., Syt-1 in the DG-ML [$F_{(2, 27)} = 3.884$, $P = 0.027$], CA1-ML [$F_{(2, 27)} = 4.175$, $P = 0.001$], CA1-RL [$F_{(2, 27)} = 8.624$, $P = 0.015$], CA1-OL [$F_{(2, 27)} = 4.993$, $P < 0.001$], and CA3-MS [$F_{(2, 27)} = 1.270$, $P < 0.001$], and Stx-1 in the DG-HL [$F_{(2, 27)} = 5.054$, $P = 0.014$], DG-GL [$F_{(2, 27)} = 16.609$, $P < 0.001$], CA1-PL [$F_{(2, 27)} = 9.770$, $P < 0.001$], CA3-PL [$F_{(2, 27)} = 9.268$, $P < 0.001$], and CA3-OL [$F_{(2, 27)} = 6.116$, $P = 0.006$]. The HD-LPS had significantly increased Syt-1 in the DG-ML, CA1-ML, RL and OL), and CA3-ML ($P_s < 0.05$), and decreased Stx-1

Table 2 The relative levels (indicated by AOD of immunohistochemical staining) of A β ₄₂, Syt-1, and Stx-1 in different layers of hippocampus ($\times 10$)

Proteins	Groups	Dentate gyrus				CA1				CA3			
		Hilus	GL	ML	ML	ML	RL	PL	OL	ML	SL	PL	OL
A β ₄₂	Y-con	1.0 $\pm$ 0.05	1.3 $\pm$ 0.08	1.0 $\pm$ 0.06	0.9 $\pm$ 0.5	0.9 $\pm$ 0.04	1.1 $\pm$ 0.06	0.9 $\pm$ 0.06	0.9 $\pm$ 0.07	0.8 $\pm$ 0.06	0.9 $\pm$ 0.07	1.2 $\pm$ 0.06	0.9 $\pm$ 0.06
	HD-LPS	1.5 $\pm$ 1.05 ^{##}	1.4 $\pm$ 0.08	1.1 $\pm$ 0.03	1.0 $\pm$ 0.10	1.3 $\pm$ 0.08 [#]	1.8 $\pm$ 0.10 ^{##}	1.0 $\pm$ 0.01	1.4 $\pm$ 0.08 ^{##}	1.1 $\pm$ 0.04 [#]	1.4 $\pm$ 0.08 ^{##}	1.7 $\pm$ 0.09	1.2 $\pm$ 0.07
	LD-LPS	1.3 $\pm$ 0.07	1.3 $\pm$ 0.10	1.0 $\pm$ 0.04	1.0 $\pm$ 0.03	1.0 $\pm$ 0.06	1.6 $\pm$ 0.09	1.0 $\pm$ 0.04	1.2 $\pm$ 0.07	1.1 $\pm$ 0.05 [#]	1.2 $\pm$ 0.07	1.5 $\pm$ 0.05	1.1 $\pm$ 0.06
	O-con	1.2 $\pm$ 0.06 [*]	1.3 $\pm$ 0.12	1.2 $\pm$ 0.06 [*]	1.0 $\pm$ 0.04 [*]	1.1 $\pm$ 0.05 [*]	1.4 $\pm$ 0.07 ^{**}	1.0 $\pm$ 0.06	1.1 $\pm$ 0.04 [*]	0.9 $\pm$ 0.09	1.1 $\pm$ 0.04 [*]	1.5 $\pm$ 0.09 ^{**}	1.0 $\pm$ 0.06
Syt-1	Y-con	0.9 $\pm$ 0.08	1.0 $\pm$ 0.08	1.0 $\pm$ 0.08	0.9 $\pm$ 0.05	1.1 $\pm$ 0.07	1.1 $\pm$ 0.08	1.1 $\pm$ 0.06	1.1 $\pm$ 0.11	1.0 $\pm$ 0.05	1.1 $\pm$ 0.11	1.3 $\pm$ 0.10	1.2 $\pm$ 0.16
	HD-LPS	1.6 $\pm$ 0.07	1.3 $\pm$ 0.06	1.6 $\pm$ 0.06 [#]	1.5 $\pm$ 0.06 [#]	1.7 $\pm$ 0.05 ^{##}	1.4 $\pm$ 0.07	1.6 $\pm$ 0.08 [#]	1.5 $\pm$ 0.14	1.6 $\pm$ 0.07 [#]	1.5 $\pm$ 0.14	1.4 $\pm$ 0.06	1.5 $\pm$ 0.14
	LD-LPS	1.4 $\pm$ 0.13	1.2 $\pm$ 0.09	1.6 $\pm$ 0.07 [#]	1.5 $\pm$ 0.07 [#]	1.4 $\pm$ 0.08	1.2 $\pm$ 1.02	1.2 $\pm$ 0.11	1.3 $\pm$ 0.10	1.3 $\pm$ 0.12	1.3 $\pm$ 0.10	1.2 $\pm$ 0.12	1.3 $\pm$ 0.15
	O-con	1.3 $\pm$ 0.11 ^{**}	1.2 $\pm$ 0.10	1.4 $\pm$ 0.09 [*]	1.2 $\pm$ 0.09 ^{**}	1.4 $\pm$ 0.07 [*]	1.3 $\pm$ 0.07 [*]	1.2 $\pm$ 0.14 [*]	1.5 $\pm$ 0.11 [*]	1.3 $\pm$ 0.10 [*]	1.5 $\pm$ 0.11 [*]	1.3 $\pm$ 0.07	1.3 $\pm$ 0.11 [*]
Stx-1	Y-con	2.1 $\pm$ 0.04	2.1 $\pm$ 0.02	1.9 $\pm$ 0.02	1.7 $\pm$ 0.03	1.8 $\pm$ 0.04	1.9 $\pm$ 0.03	1.9 $\pm$ 0.04	2.3 $\pm$ 0.04	1.9 $\pm$ 0.04	2.3 $\pm$ 0.04	1.9 $\pm$ 0.02	2.2 $\pm$ 0.03
	HD-LPS	1.7 $\pm$ 0.04 [#]	1.8 $\pm$ 0.03 ^{##}	1.7 $\pm$ 0.04	1.5 $\pm$ 0.02	1.7 $\pm$ 0.03	1.6 $\pm$ 0.04 ^{##}	1.8 $\pm$ 0.03	1.7 $\pm$ 0.06	1.7 $\pm$ 0.06	2.0 $\pm$ 0.03	1.6 $\pm$ 0.04 ^{##}	1.9 $\pm$ 0.02 [#]
	LD-LPS	1.8 $\pm$ 0.03	1.9 $\pm$ 0.02 [#]	1.7 $\pm$ 0.03	1.6 $\pm$ 0.04	1.7 $\pm$ 0.05	1.7 $\pm$ 0.04	1.8 $\pm$ 0.02	1.6 $\pm$ 0.02	1.6 $\pm$ 0.02	2.0 $\pm$ 0.03	1.7 $\pm$ 0.02 [#]	2.0 $\pm$ 0.02
	O-con	1.9 $\pm$ 0.03 ^{**}	2.0 $\pm$ 0.02 [*]	1.7 $\pm$ 0.03 ^{**}	1.6 $\pm$ 0.04 [*]	1.7 $\pm$ 0.03	1.8 $\pm$ 0.03 [*]	1.8 $\pm$ 0.03	1.8 $\pm$ 0.03	1.8 $\pm$ 0.03	2.1 $\pm$ 0.04 [*]	1.8 $\pm$ 0.04 [*]	2.0 $\pm$ 0.03 [*]

Data were expressed by mean $\pm$ S.E.M.

GL granule cell layer, ML molecular layer, RL radiation layer, PL pyramidal cell layer, OL original layer, SL stratum lucidum

Denotes significant difference of the O-con group compared to the Y-con ($P < 0.05$; ** $P < 0.001$)# Denotes significant difference of the comparison between the LPS group and the O-con group ([#] $P < 0.05$; ^{##} $P < 0.001$)

Fig. 2 The p-tau levels in the hippocampal subregions in the differently-treated CD-1 mice. The subregions included dentate gyrus (DG), CA1, and CA3. Data were expressed by mean $\pm$ SEM. Asterisk denotes the significant difference of the O-con group compared to the Y-con group (* $P < 0.05$, ** $P < 0.001$). Number sign denotes the significant difference of the comparison between the LPS groups and the O-con group (# $P < 0.05$; ## $P < 0.001$). AOD average optical density

in the DG-HL, DG-GL, CA1-PL, CA3-PL, and CA3-OL than the O-con (P s < 0.05). So did the LD-LPS for Syt-1 in the DG-ML and CA1-ML (P s < 0.05), and Stx-1 in the DG-GL and CA3-PL (P s < 0.05).

GFAP in different hippocampal subregions

The GFAP positive astrocytes were morphologically enlarged in the older hippocampus (Supplementary Fig. S5). Compared to the Y-con group (Fig. 3), the O-con mice had higher immunoreactivity in the DG [$t = 2.298$, $P = 0.034$], CA1 [$t = 2.930$, $P = 0.009$] and CA3 [$t = 2.499$, $P = 0.024$]. The LPS treatment significantly affected GFAP levels in the CA1 [$F_{(2, 27)} = 4.589$, $P = 0.019$] and CA3 [$F_{(2, 27)} = 3.472$, $P = 0.045$]. Compared to the O-con, the HD-LPS showed higher levels of GFAP in the CA1 and CA3 (P s < 0.05) and the LD-LPS in the CA1 ($P < 0.05$).

H4K8ac in different hippocampal layers

The H4K8ac was primarily expressed in the cellular layer of the dorsal hippocampus, which was indicated as immunohistochemical staining (Supplementary Fig. S6). Compared to the Y-con mice (Fig. 4), the O-con group had significantly lower levels of H4K8ac in the DG [$t = 2.542$, $P = 0.020$], CA1 [$t = 3.674$, $P = 0.002$], and CA3 [$t = 2.156$, $P = 0.045$]. The LPS treatment significantly affected the H4K8ac levels in the

Fig. 3 The GFAP levels in the hippocampal subregions in the differently-treated CD-1 mice. The other information is identical to Fig. 2

DG [$F_{(2, 27)} = 7.996$, $P = 0.002$], CA1 [$F_{(2, 27)} = 6.394$, $P = 0.005$], and CA3 [$F_{(2, 27)} = 7.943$, $P = 0.002$]. Further, the HD-LPS had lower levels of H4K8ac in the DG-PL, CA1-PL, and CA3-PL than the O-con group (P s < 0.05). This was also the case for the LD-LPS in the CA1-PL and CA3-PL ($P < 0.05$).

Relationship between spatial cognitive performance and protein levels in the hippocampus

Table 3 displays the Pearson's correlation coefficients between the hippocampal protein levels, indicated as mean in all subregion-layers, and the RAWM performances for all mice combined. Positive correlations

Fig. 4 The H4K8ac levels in the hippocampal subregions in the differently-treated CD-1 mice. The other information is identical to Fig. 2

Table 3 Pearson's correlation coefficients between performance in the six-radial arm water maze and protein levels in the hippocampus

Protein contents	Learning phase		Memory phase	
	Latency	Number of errors	Latency	Number of errors
A β ₄₂	0.281(0.083)	0.314(0.052)	0.476(0.002)**	0.500(0.001)**
P-tau	0.396(0.012)*	0.434(0.005)**	0.492(0.001)**	0.540(0.000)**
Syt-1	0.131(0.425)	0.156(0.343)	0.319 (0.048)*	0.347(0.031)*
Stx-1	-0.426(0.006)**	-0.455(0.003)**	-0.521(0.001)**	-0.549(0.000)**
GFAP	0.567(0.001)**	0.568(0.001)**	0.578(0.000)**	0.643(0.000)**
H4K8	-0.346(0.029)*	-0.367(-0.020)*	-0.481(0.002)**	-0.504(0.001)**

Denotes significant correlation coefficients ($P < 0.05$; ** $P < 0.01$)

between A β ₄₂ levels and the errors and latency were significant in the memory phase (P s = 0.001 and 0.002) and marginal in the learning phase (P s = 0.052 and 0.083). The levels of both the p-tau and the Syt-1 positively correlated with the errors and latency in the learning and memory phases (P s < 0.05). The Stx-1 level negatively correlated with the errors and latency in the learning and memory phases (P s < 0.05). So did the H4K8ac level (P s < 0.05).

Discussion

LPS exposure during pregnancy intensified the age-related changes of behaviors in CD-1 mice

The current experiment was designed to explore whether an inflammatory insult during pregnancy can exacerbate age-related changes of behaviors in CD-1 mice using the model of repeated LPS exposure during late pregnancy (Chen et al. 2011). Regarding the species-typical behaviors, normal burrowing and nesting abilities appear to depend on an intact hippocampus (Chen et al. 2005). In the current study, no significant age or LPS treatment effects were observed in the mice's burrowing activity. However, the O-con mice had poorer nesting abilities than the Y-con ones, and the HD-LPS had poorer nesting ability than the O-con group. These data demonstrated that repeated LPS exposure during late pregnancy exaggerated the age-related decrement of nesting ability in female CD-1 mice. On the beam-walking test, the O-con mice had significantly shorter balance times than the Y-con mice, indicating that the sensorimotor ability was impaired in 15-month-old CD-1 female mice. Interestingly, LPS treatment dose-dependently (i.e., in higher doses)

intensified this age-related impairment of sensorimotor ability (Table 1). Generally, anxiety behaviors will change with age in rodents and humans (Boguszewski and Zagrodzka 2002; Chen et al. 2007a; Lamberty and Gower 1993). In the current study, a significant age effect was observed in the open field and elevated plus maze (Table 1) tests. Compared to the Y-con group, the O-con mice showed longer latency and peripheral times in the open field, and longer latency and fewer numbers of entering into the open arms in the elevated-plus maze, indicating an increased level of anxiety in the older CD-1 mice. Few studies have examined that the effect of gestational stress exposure on anxiety in the middle age and onwards. In this study, repeated LPS exposure during late gestation augmented the increased anxious behaviors in the open field, but not in the elevated-plus maze in the older female mice, especially under higher doses, suggesting that LPS exposure during late gestation could affect task-specific anxiety late in life.

In general, cognitive functions gradually decline during normal aging in humans and rodents, especially in certain types of memory, such as spatial, working, and long-term memories (Yassa et al. 2011). The RAWM is a common task employed to test the hippocampus-dependent spatial memory (Chen et al. 2004). In the current study, the O-con group showed decreased abilities of spatial learning and memory in the RAWM compared to the Y-con mice, which was in line with the previous results (Cao et al. 2013; Ennaceur et al. 2008). For example, our previous data showed that the decline of spatial learning ability in the RAWM in CD-1 mice emerged at age 12 months (Cao et al. 2013). Another study also confirms that middle-aged (12-month-old) CD-1 mice have poorer performance of learning and memory in a 3D maze task (Ennaceur et al. 2008). Interestingly, LPS exposed mice performed worse

in the RAWM than the O-con mice, especially with higher-dose LPS exposures. These findings implied that gestational inflammation could accelerate the age-related impairment of spatial learning and memory in CD-1 mice, which is able to increase the risk of AD late in life.

LPS exposure during pregnancy intensified AD-like pathology and AAMI-related changes of Syt-1, Stx-1, and GFAP

AD has a long preclinical stage from aging to the emergence of clinical symptoms, and the MCI has been considered the intermediate stage between normal brain aging and dementia (Markesbery 2010; Yi et al. 2015). Based on the recent evidence, cognitive decline in AD occurs prior to the presence of amyloid plaques and neurofibrillary tangles, while just the soluble A β oligomers and hyperphosphorylated tau impair the cognitive functions (Lesne et al. 2006; Pater 2011). In our study, although the silver staining did not show neurofibrillary tangles, the biochemical changes observed in the two LPS groups were in accordance with AD-like pathology. The LPS-exposed mice revealed an enhancement in the intensity of immunoreactivity for A β ₄₂ and p-tau in the hippocampus, indicating a possibility; that is, adverse pregnancies may result in a trend prone to forming AD-like pathology in aged mice. Given that soluble A β oligomers and hyperphosphorylated tau were detrimental to cognitive function, the adverse experience of pregnancy (gestational inflammatory reaction) could predispose a deteriorated AAMI.

Syt-1 is considered a calcium sensor for fast chemical synaptic transmission (Koh and Bellen 2003), and Stx-1 is one key component of the SNARE complex (soluble NSF-attachment protein receptor) (Jurado 2014). The two key neurotransmission-related presynaptic proteins are involved in synaptic plasticity-related events and are essential for memory formation (Jurado 2014). The current results indicated that age significantly impacts the levels of Syt-1 and Stx-1 in the hippocampus of older CD-1 mice (i.e., aging increased Syt-1 and reduced Stx-1 in the hippocampus). Moreover, our data also showed that the increased Syt-1 and decreased Stx-1 were associated with decreased ability in terms of spatial learning and memory in the RAWM. These results were in line with previous reports (Chen et al. 2007b; Tong et al. 2015; VanGuilder et al. 2011). As hypothesized, the LPS treatment dose-dependently accelerated the changes of Syt-1 and Stx-1 in the

hippocampus of the older CD-1 mice, suggesting that exposure to LPS during pregnancy could accelerate the age-related changes of Syt-1 and Stx-1.

Chronic neuroinflammation may be an important mechanism underlying AAMI in the rodents and humans (Alley et al. 2008; Gorelick 2010). Neuroinflammation is also involved in the development of amyloid plaques in AD. During aging, microglia and astrocytes, the two major cell effectors contribute to chronic activation of neuroinflammation, overexpression of proinflammatory cytokines, and reactive oxygen species. Animal studies show that inhibiting astrocytic activation using anti-inflammatory minocycline can alleviate the hepatectomy-related long-term impairment of spatial memory in 19-month-old C57BL/6 mice (Jin et al. 2014) and improves cognitive impairment in AD models (Choi et al. 2007). Astrocytes become highly reactive in response to neuroinflammation, and the activation of astrocytes is characterized by hypertrophy and cell proliferation, which is associated with GFAP hyper-expression (Eng et al. 2000). In our experiment, only weak GFAP positive astrocytes could be seen in the Y-con mice's hippocampi. However, the GFAP immunoreactivity apparently increased in the O-con group relative to the Y-con group, with the hypertrophy of cell bodies and processes, indicating that astrocytes were activated in the hippocampus with age. As expected, the gestational LPS exposure also dose-dependently worsened hyper-activity of astrocytes in the older hippocampus, as indicated by increased immunoreactivity of GFAP in the HD-LPS group relative to the O-con group (Supplementary Fig. S5).

LPS exposure during pregnancy intensified H4K8ac reduction in aging hippocampi

Existing evidence indicates that histone hypoacetylation mediates the silencing of neuroplasticity genes in the aging rodent's hippocampus, which is correlated with AAMI (Blalock et al. 2003; Loerch et al. 2008). Histone acetylation is controlled by the relative activities of histone acetyltransferases (HAT) and deacetylases (HDAC). In the aging or neurodegenerating brain, increased expression of HDAC2 coincides with reduced histone acetylation and reduced gene expression, which are related to declines in synaptic plasticity and poor memory performance (Graff and Tsai 2013). Sodium butyrate (NaB), an HDAC inhibitor, can enhance the hippocampal H4K8ac level, which improves the gene expression necessary for synaptic plasticity and memory formation (Foley et al.

2014; Itzhak et al. 2013). Another study also shows that NaB treatment can induce increments of H4K8ac, which is parallel with better performance in location memory tasks (Intlekofer et al. 2013). In this study, we found a reduction of H4K8ac in cell layers of three hippocampal subregions in the O-con mice, compared to the Y-con mice. This age-related reduction of H4K8ac was in accordance with our recently published results from SAMP8 mice (Chen et al. 2015; Yan et al. 2015). Excitingly, the gestational LPS exposure augmented this age-related reduction of H4K8ac levels in the older hippocampi, especially in the higher doses, suggesting that declining H4K8 acetylation might play an important role in the underlying mechanism of how LPS exposure during pregnancy accelerates AAMI.

Summary

Collectively, our findings showed that CD-1 mice who received LPS (especially at the higher dose) during pregnancy revealed a significant augmentation of age-related behavioral changes, including decreased abilities of nesting and sensorimotor, increased anxiety, and reduced abilities for spatial learning and memory. The latter was associated with an elevation of A β 42 load p-tau level, hyper-activity of astrocytes, changed levels of presynaptic proteins (increased Syt-1 and decreased Stx-1), and a reduction of H4K8 acetylation in the dorsal hippocampus. To our best knowledge, for the first time, we put forward a notion that the inflammation exposure during pregnancy can deteriorate AAMI and age-related biochemical changes. Although this study is limited by the imprecise, semi-quantitative method of immunohistochemistry, it did reveal the possibility that adverse pregnancies might contribute to females' unsuccessful aging and age-related neurodegenerative diseases (e.g., AD), but the precise mechanism of this hypothesis needs further research. On the other hand, would LPS exposure have the same effect on virgin females? This is a very important and interesting topic that will be explored in our future experiment. This work was supported financially by the National Natural Science Foundation of China (81370444), the Natural Science Foundation for the Youth of China (81301094), the College Natural Science Foundation of Anhui Province (KJ2015A126), and the College Science Foundation of Anhui Medical University (2015xkj024).

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Compliance with ethical standards

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

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