

Research Article

Effects of Gestational Inflammation with Postpartum Enriched Environment on Age-Related Changes in Cognition and Hippocampal Synaptic Plasticity-Related Proteins

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Received 19 March 2020; Revised 26 October 2020; Accepted 4 November 2020; Published 19 November 2020

Academic Editor: Michele Fornaro

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Increasing evidence indicates that exposure to inflammation during pregnancy intensifies the offspring's cognitive impairment during aging, which might be correlated with changes in some synaptic plasticity-related proteins. In addition, an enriched environment (EE) can significantly exert a beneficial impact on cognition and synaptic plasticity. However, it is unclear whether gestational inflammation combined with postnatal EE affects the changes in cognition and synaptic plasticity-related proteins during aging. In this study, pregnant mice were intraperitoneally injected with lipopolysaccharides (LPS, 50 $\mu\text{g}/\text{kg}$) or normal saline at days 15–17 of pregnancy. At 21 days after delivery, some LPS-treated mice were randomly selected for EE treatment. At the age of 6 and 18 months, Morris water maze (MWM) and western blotting were, respectively, used to evaluate or measure the ability of spatial learning and memory and the levels of postsynaptic plasticity-related proteins in the hippocampus, including postsynaptic density protein 95 (PSD-95), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) GluA1 subunit, and Homer-1b/c. The results showed that 18-month-old control mice had worse spatial learning and memory and lower levels of these synaptic plasticity-related proteins (PSD-95, GluA1, and Homer-1b/c) than the 6-month-old controls. Gestational LPS exposure exacerbated these age-related changes of cognition and synaptic proteins, but EE could alleviate the treatment effect of LPS. In addition, the performance during learning and memory periods in the MWM correlated with the hippocampal levels of PSD-95, GluA1, and Homer-1b/c. Our results suggested that gestational inflammation accelerated age-related cognitive impairment and the decline of PSD-95, GluA1, and Homer-1b/c protein expression, and postpartum EE could alleviate these changes.

1. Introduction

Aging results in the increased prevalence of age-related diseases such as Alzheimer's disease (AD), Parkinson's disease, and vascular dementia [1, 2]. Age-related cognitive decline (ARCD) is the most prominent feature of aging and can be affected by many factors such as genetics, sex, age, living environment and styles, drug and substance use, exposures to inflammation, and psychosocial stresses [3].

Pregnancy is a complex and challenging period. Compared with nulliparity, normal pregnancy and fertility experience may help learning and memory during the lifetime as well as reduce neuronal aging [4]. At the same time, pregnancy is the most sensitive cognitive period and can increase the vulnerability to external harmful factors such as inflammation caused by bacteria or viruses. Lipopolysaccharide (LPS) is widely used in experimental models of bacterial infection. It can induce neuroinflammatory responses and

produce proinflammatory cytokines such as interleukin-1 β and tumor necrosis factor- α , which can affect the normal function of the brain and accelerate neuronal aging through specific signaling pathways [5–7]. Our previous evidence indicates that maternal exposure to LPS during pregnancy accelerates age-related spatial learning and memory impairment in the CD-1 offspring [8, 9], and this inflammation also affects cognition in middle-aged mothers [10]. Nevertheless, evidence of whether the mothers have the same adverse effects in young and old age remains scarce.

An enriched environment (EE) is defined as the addition of social, physical, and somatosensory stimulation into an animal's environment via larger group housing, extra objects, and running wheels. EE has been found to provide clear benefits for cognitive aging, particularly evident in aged animals. For instance, lifelong EE can prevent ARCD in recognition and spatial and working memory in male Wistar rats [11]. Exposure of aged C57BL/6 mice to EE counteracts the decline in long-term memory for the social transmission of food preference during the normal aging process [12]. These beneficial effects of EE have been related to reduced age-related changes in hippocampal dendritic branching, neurogenesis, gliogenesis, spine density, and neural plasticity, including its epigenetic underpinnings [13–22]. For example, early running exercise in adult rats can increase dendritic spine density, improve synaptic plasticity, promote neuronal activity, and enhance associative learning and memory in rats [23]. Exposure to stimulating environmental conditions preserves remote recall of declarative memory abilities in aged C57BL/6 mice by promoting system consolidation through the activation of epigenetic regulatory processes [12]. However, only few studies have explored the effects of EE on LPS-induced cognitive decline. EE alleviates learning and memory impairment as well as hippocampal proinflammatory cytokine changes induced by LPS in young Wistar rats [24]. EE rescues behavioral and neurophysiological effects induced by a prenatal maternal LPS infection in young adult Sprague–Dawley rats, including disruptions in both social engagement and spatial discrimination and downregulating genes critical to synaptic transmission and plasticity [25]. Nevertheless, it remains unclear whether postpartum EE could ameliorate cognitive change induced by gestational exposure to LPS during aging.

Cognitive decline with aging could be associated with altered levels of synaptic plasticity-related proteins. Increasing studies have implicated that alterations in hippocampal synaptic plasticity are involved in ARCD, although the underlying mechanisms are not completely clear. Changes in synaptic protein levels can affect synaptic plasticity and thus may have an adverse effect on cognitive ability. Our previous studies have indicated that the alteration of some synaptic plasticity-related proteins in the hippocampus, such as Syt1, Munc18-1, and SNAP-25, are associated with ARCD [9, 26]. To date, many synaptic plasticity-related proteins have been identified, but their life span expression profiles and their effects on ARCD remain elusive.

The postsynaptic density protein 95 (PSD-95), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) GluA1 subunit, and Homer-1b/c are expressed

on the synaptic membrane. They play an indispensable role in synaptic plasticity and the formation and maintenance of learning and memory [27–29]. Many factors such as age, inflammation, and social psychological environment could affect the expressions of PSD-95, GluA1, and Homer-1b/c in the brain and thus may have an impact on cognitive function. For instance, long-term postweaning social isolation produces marked deficits in spatial learning and memory and significantly decreases the protein expression levels of PSD-95 and GluA1 in the hippocampus [30]. Aerobic exercise can improve spatial memory and increase glutamatergic proteins (NMDA receptor and PSD-95) within the hippocampus of aging rats [31]. Intracerebroventricular LPS injection could induce deficits in spatial learning and memory and memory acquisition of the passive avoidance response and also loss of PSD-95 expression in the hippocampus [32]. Compared to the adult (age: 6 months) Long Evans rats, aged rats (age: 24 months) present deficits in learning, reverse memory, and retention and show increased Homer 1b/c expression in the CA1 hippocampus subfield [28]. To our best knowledge, no study yet has explored the effects of gestational inflammation with postpartum EE on the hippocampal expression of PSD-95, GluA1, and Homer-1b/c.

Therefore, we aimed to explore the following: (1) age-related cognitive changes and the expression levels of synaptic plasticity-related proteins including PSD-95, GluA1, and Homer-1b/c in the brain; (2) the effects of gestational inflammatory stimuli (LPS) with postpartum EE on age-related changes of cognition and these synaptic proteins; and (3) the correlations between the synaptic protein levels in the hippocampus and learning and memory impairment.

2. Materials and Methods

2.1. Animals and Treatments. The CD-1 mice (8 weeks) were purchased from the Model Animal Research Center of Nanjing University. All animals were fed in the laboratory for 2 weeks to adapt to the environment. Control feeding environmental conditions consisted of constant temperature ($22 \pm 1^\circ\text{C}$) and humidity ($50 \pm 5\%$) with a 12 h light/dark cycle (lights on at 0700). The male and female mice were paired (1:2) into breeders, and the females were checked for vaginal plugs every morning. The presence of a vaginal plug was designated as gestational day 0. Pregnant CD-1 mice were reared in a single cage and intraperitoneally injected with LPS ($50 \mu\text{g}/\text{kg}$, Sigma) or the same volume of saline (control group, CON) every day at gestational days 15–17. The female mice gave birth normally and were separated from their offspring 21 days after birth. Then, these mothers receiving LPS were randomly administered either no treatment (LPS-alone group, LPS) or EE treatment (LPS+EE group, LPS-E). Six mice were randomly selected from each group (CON, LPS, and LPS-E) when the animals were, respectively, 6 and 18 months old, and related tests were then completed. All experimental procedures were carried out in accordance with the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.2. Enriched Environment. For the LPS-E, just after stopping breastfeeding, the mice were housed in a larger cage ($52 \times 40 \times 20 \text{ cm}^3$) for enriching social life in the group. Different toys or novelty objects were placed in the cage every week, such as rings, pipes, and suspenders, to encourage the females to escape and/or seek refuge, strengthen exercise, and adapt to novel and enriched environments until the end of the behavioral experiment [33].

2.3. Morris Water Maze. The MWM task was used to detect the spatial ability of learning and memory [34, 35]. The device, a circular black tank with a diameter of 150 cm and a height of 30 cm, was filled with water ($22 \pm 2^\circ\text{C}$) and contained a black cylindrical platform (10 cm in diameter, 24 cm in height). The tank was surrounded by a white cloth curtain with three black clues (circular, triangular, and square). The camera system was installed above to record the movement track of mice, and the image was subsequently analyzed by ANY-maze software (Stoelting, United States). The experimental process was divided into two stages—a place navigation trial (learning phase) and a probe trial (memory phase). In the place navigation trial, the platform 1 cm below the surface of the water was fixed to the target quadrant (defined as the target quadrant). The experiment lasted for 7 days with 4 trials each day. Before the first trial, the mouse was put on the platform for 30 s, then released into the water from different starting positions, facing the wall (except for the target quadrant), and was allowed a maximum of 60 s swimming to find the platform. Then, the mouse was placed on the platform for 30 s. If the platform was not found within the 60 s, the mouse was guided to the platform and kept there for 30 s. One hour after the last place navigation trial on the last day, a 60 s probe trial was completed, and the mouse was put into water from the quadrant opposite to the target quadrant without the platform. Owing to commonly declined swimming speed which is accompanied with increased swimming latency in the aged animals, the average swimming distance during the place navigation was considered the index for learning ability, and the percentage of the target quadrant swimming distance to the total distance in the probe trial was considered the index for memory ability.

2.4. Tissue Preparation. Fifteen days after the behavior experiment was completed, the mice were anesthetized by 3% halothane inhalation. After cervical dislocation and decapitation, the hippocampus was quickly isolated on ice and frozen in a -86°C cryopreservation refrigerator for subsequent western blotting.

2.5. Western Blotting. The western blot experiment was carried out according to a previously described method. Proteins were extracted by protein neutral lysis buffer (RIPA), and the SDS-PAGE protein loading buffer was heated in a boiling water bath for 10 min to completely denature the protein. After the sample was cooled at room temperature, the proteins were separated using SDS-PAGE (80 v/30 min and 120 v/1 h). Then, the protein components were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, USA) and blocked with 5% skim milk in TBS for 2 h at room

temperature. Probing was performed using the following primary antibodies: AMPAR GluA1 (1:1000, Abcam, ab31232), PSD-95 (1:1000, Abcam, ab18258), Homer1b/c (1:2000, Abcam, ab97593), and β -actin (1:1000, Zs-BIO, TA-09) overnight at 4°C . Blots were subsequently incubated with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit IgG: 1:5000, Zs-BIO, ZB-2301; goat anti-mouse IgG: 1:5000, Zs-BIO, ZB-2305) for 2 h at room temperature. The immunolabeled protein bands were detected using the ECL Plus Detection Kit (Thermo, USA). Graphs of blots were obtained in the linear range of detection and were quantified for the level of specific induction by scanning laser densitometry. The densitometric analysis of immunoreactivity was conducted using the ImageJ software (Media Cybernetics, USA) to calculate the relative expression.

2.6. Statistical Analysis. The results were presented as mean \pm standard deviation for normally distributed data. The data were analyzed using repeated-measure analysis of variance (rm-ANOVA) in the learning task of MWM. One-way analysis of variance (ANOVA) was performed for normally distributed data. Post hoc analyses were performed using Fisher's least significant difference test. The independent-samples t -test was employed for the analysis of the age effect. When the distribution of data was nonnormal, Kruskal-Wallis H test was employed with an extended t -test for pairwise analysis. Then, Pearson's correlation test was used to analyze the correlations between the relative levels of hippocampal synaptic proteins and MWM performance. Statistical analyses were performed with SPSS 19.0 for Windows. Significance was set to $P < 0.05$.

3. Results

3.1. Morris Water Maze

3.1.1. Learning Phase

(1) *Age Effects.* In the learning phase, the swimming distance ($F_{(6,60)} = 78.967$, $P_s < 0.001$) progressively declined over days for control mice, indicating that these mice were able to learn the task. The rm-ANOVA results showed that the 18-month control group had a significantly longer distance ($F_{(1,10)} = 20.781$, $P < 0.001$) than the 6-month control group (Figure 1(a)).

(2) *Treatment Effects.* For the 6-month mice, the swimming distance ($F_{(6,60)} = 10.867$, $P_s < 0.001$) progressively declined over days in the learning phase. However, there were no significant differences in swimming distances ($F_{(2,15)} = 0.313$, $P = 0.736$) among different-treatment groups (Figure 1(c)). For the 18-month-old mice, the swimming distance ($F_{(6,60)} = 209.946$, $P_s < 0.001$) progressively declined over days in the learning phase. There were significant differences in swimming distances ($F_{(2,15)} = 40.745$, $P < 0.001$) among different-treatment groups. Post hoc analysis revealed that the 18-month-old LPS group had a significantly longer swimming distance than the 18-month-old CON and LPS-E groups (both $P_s < 0.001$). Moreover, the 18-month-old

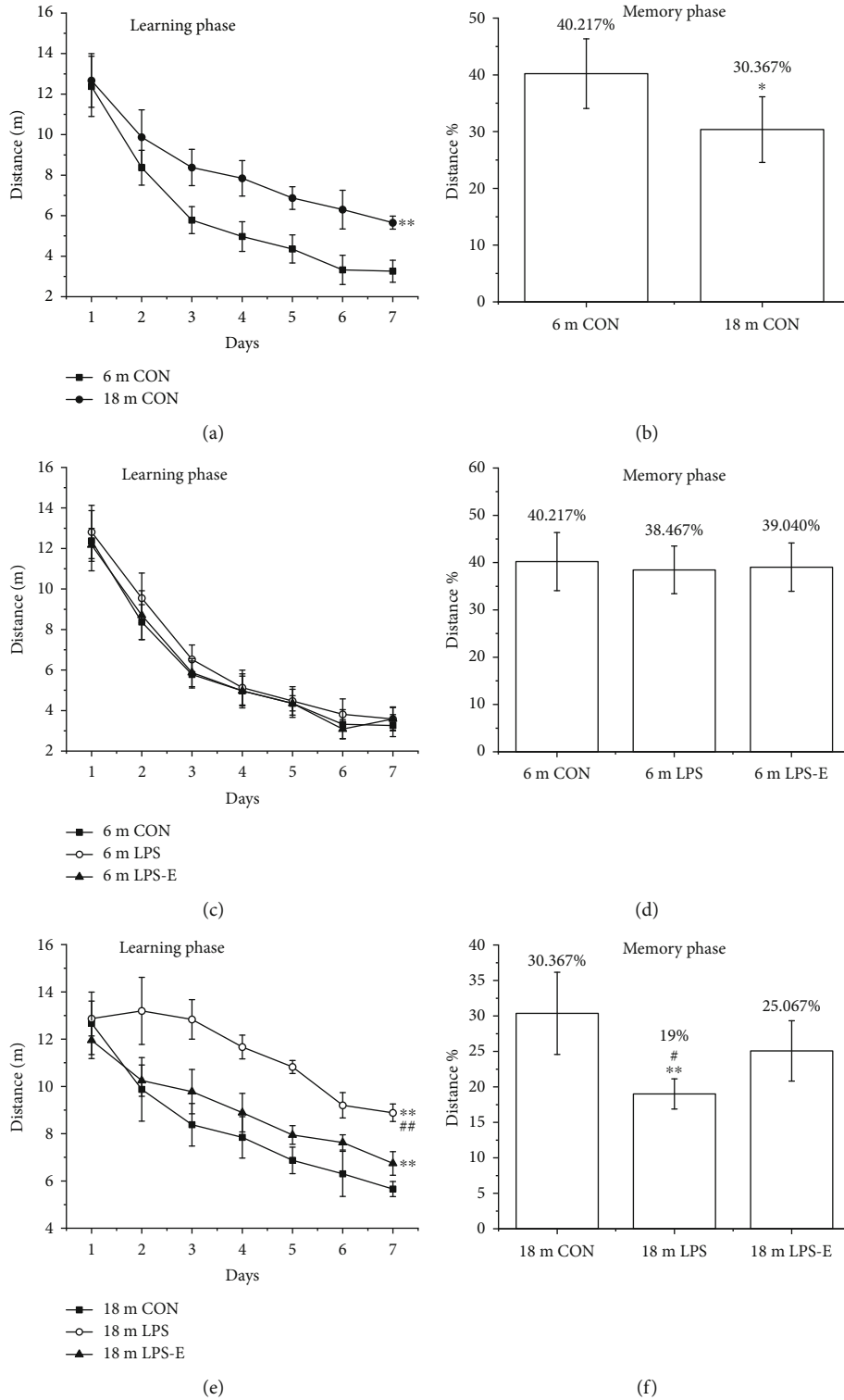


FIGURE 1: The performance of female CD-1 mice in the MWM task. (a, b) Show the effect of age, (c, d) show the effect of treatments at age 6 months, and (e, f) show the effect of treatments at age 18 months. The naturally older females had a significantly longer swimming distance in the learning phase (a) and smaller percent of the swimming distance in the target quadrant in the memory phase (b). In the 6-month-old mice, those with diverse treatments had similar performance of learning (c) and memory (d). In the 18-month-old mice, those exposed to gestational inflammation had a significantly longer learning swimming distance (e) and smaller memory distance percent (d), and the mice additionally exposed to enriched environment postpartum onward showed significant improvement in both cognitive measures (e, f) but still had a significantly longer learning swimming distance than the normal controls. The sample size was six in each group. The data are presented as mean \pm standard deviation. * denotes comparison to the young or the same-age controls, * $P < 0.05$, ** $P < 0.01$; # denotes comparison to the LPS-E group, # $P < 0.05$, ## $P < 0.01$.

LPS-E group had a significantly longer swimming distance than the same-age CON group ($P_s = 0.001$, Figure 1(e)).

3.1.2. Memory Phase

(1) *Age Effects.* The percentage of the swimming distance within the target quadrant was >25% in the 6- and 18-month-old CON animals, indicating that these mice had memory effect on the task. Compared with the 6-month-old CON mice, the percentage of the swimming distance within the target quadrant decreased significantly in the 18-month-old CON group ($t = 2.860$, $P = 0.01$, Figure 1(b)).

(2) *Treatment Effects.* As seen from the overall analysis (Figure 1(d)), there were no significant differences in the percentage of the swimming distance within the target quadrant among different-treatment groups in the 6-month-old mice ($F_{(2,15)} = 0.404$, $P > 0.05$). The overall analysis of the 18-month-old mice is presented in Figure 1(f). There were significant differences in the percentage of the swimming distance ($F_{(2,15)} = 10.372$, $P < 0.01$) within the target quadrant among different-treatment groups. Post hoc analysis revealed that the percentage of the swimming distance within the target quadrant in the 18-month-old LPS group was significantly lower than that in the same-age CON and LPS-E groups ($P < 0.01$ and $P = 0.028$, respectively). But, there was insignificant difference between the CON and LPS-E groups ($P = 0.051$).

3.2. Levels of PSD95, GluA1, and Homer-1b/c in the Hippocampus

3.2.1. *Age Effects.* In this study, western blotting was used to detect the levels of PSD-95, GluA1, and Homer-1b/c in the hippocampus (Figure 2(a)). The levels of PSD-95 ($t = 24.159$, $P < 0.001$), GluA1 ($t = 13.549$, $P < 0.001$), and Homer-1b/c ($t = 18.404$, $P < 0.001$) were significantly lower in the 18-month-old CON group relative to the 6-month-old CON group (Figure 2(b)).

3.2.2. *Treatment Effects.* For the 6-month-old mice, the overall analysis showed that there were no significant differences (Figure 2(c)) in the hippocampal levels of PSD-95 ($F_{(2,15)} = 0.935$, $P = 0.414$), GluA1 ($F_{(2,15)} = 1.167$, $P = 0.338$), and Homer-1b/c ($F_{(2,15)} = 0.068$, $P = 0.935$) among different-treatment groups. However, for the 18-month-old mice, different treatments significantly affected the levels of hippocampal PSD-95 ($F_{(2,15)} = 322.940$, $P < 0.001$); GluA1 ($F_{(2,15)} = 321.431$, $P < 0.001$); and Homer-1b/c ($F_{(2,15)} = 392.495$, $P < 0.001$). Post hoc analysis showed that the levels of PSD-95, GluA1, and Homer-1b/c in the 18-month-old LPS group were significantly lower than those in the same-age control group and LPS-E group ($P_s < 0.01$). The levels of PSD-95, GluA1, and Homer-1b/c significantly reduced in the LPS-E group compared to the CON group ($P_s < 0.01$, Figure 2(d)).

3.3. *Correlations between Performances of MWM and Synaptic Proteins.* Regarding the 6- and 18-month-old mice,

the hippocampal levels of PSD-95, GluA1, and Homer-1b/c were significantly associated with learning and memory in the MWM task (Table 1).

Upon combining the 6-month-old mice of all groups, the hippocampal levels of PSD-95 ($r = -0.513$, $P = 0.030$; $r = 0.126$, $P = 0.618$), GluA1 ($r = -0.609$, $P < 0.001$; $r = 0.540$, $P = 0.021$), and Homer-1b/c ($r = -0.483$, $P = 0.042$; $r = 0.560$, $P = 0.016$) correlated negatively with the swimming distance in the learning phase and positively with the percentage of the swimming distance within the target quadrant.

For all the 18-month-old mice, the hippocampal PSD-95, GluA1, and Homer-1b/c in the control group correlated negatively with the learning swimming distance ($r = -0.910$, -0.671 , and -0.909 ; $P = 0.012$, 0.144 , and 0.012 , respectively) and positively with the memory percentage of the swimming distance within the target quadrant ($r = 0.856$, 0.639 , and 0.887 ; $P = 0.029$, 0.172 , and 0.019 , respectively). Similarly, for the LPS group, the hippocampal PSD-95, GluA1, and Homer-1b/c correlated negatively with the learning swimming distance ($r = -0.777$, -0.831 , and -0.735 ; $P = 0.069$, 0.040 , and 0.096 , respectively) and positively with the memory percentage of the swimming distance ($r = 0.918$, 0.931 , and 0.970 ; $P = 0.010$, 0.007 , and 0.001 , respectively). Furthermore, the hippocampal PSD-95, GluA1, and Homer-1b/c in the LPS-E group correlated negatively with the learning swimming distance ($r = -0.841$, -0.913 , and -0.671 ; $P = 0.036$, 0.011 , and 0.145 , respectively) and positively with the memory percentage of the swimming distance ($r = 0.833$, 0.696 , and 0.825 ; $P = 0.040$, 0.125 , and 0.043 , respectively).

4. Discussion

4.1. *Effects of Gestational Inflammation and Postpartum Enriched Environment on ARCD.* The hippocampus plays an important role in normal learning and memory consolidation, but it is very fragile during aging [36]. MWM has been widely applied in the laboratory to assess spatial learning and memory in rodents [37]. The completion of MWM depends on the integrity of the hippocampal structure and function [38]. In this study, MWM was used to access hippocampus-dependent spatial learning and memory in CD-1 mice. We found that 18-month-old control CD-1 mice had impaired spatial learning and memory relative to 6-month-old control mice, which was consistent with previous findings [10, 39]. In fact, our previous study showed that the onset of cognitive impairment during aging in CD-1 mice began early at age 12 months and gradually worsened with aging [39].

LPS is a potent endotoxin that can provide a persistent inflammatory stimulus. LPS-induced neuroinflammation is commonly used in animal models of AD research [40]. Extensive research suggests that systemic LPS triggers neuroinflammation with consequent development of behavioral and cognitive deficits at different ages in rodents [41, 42]. Our previous studies also show that exposure to LPS in late pregnancy accelerated the impairment of spatial learning and memory in the offspring of CD-1 mice from middle age (12-month-old) onward [8, 9, 39] and the mothers at midlife (15-month-old) [10]. In the present study, we assessed spatial learning and memory in 6-month-old and

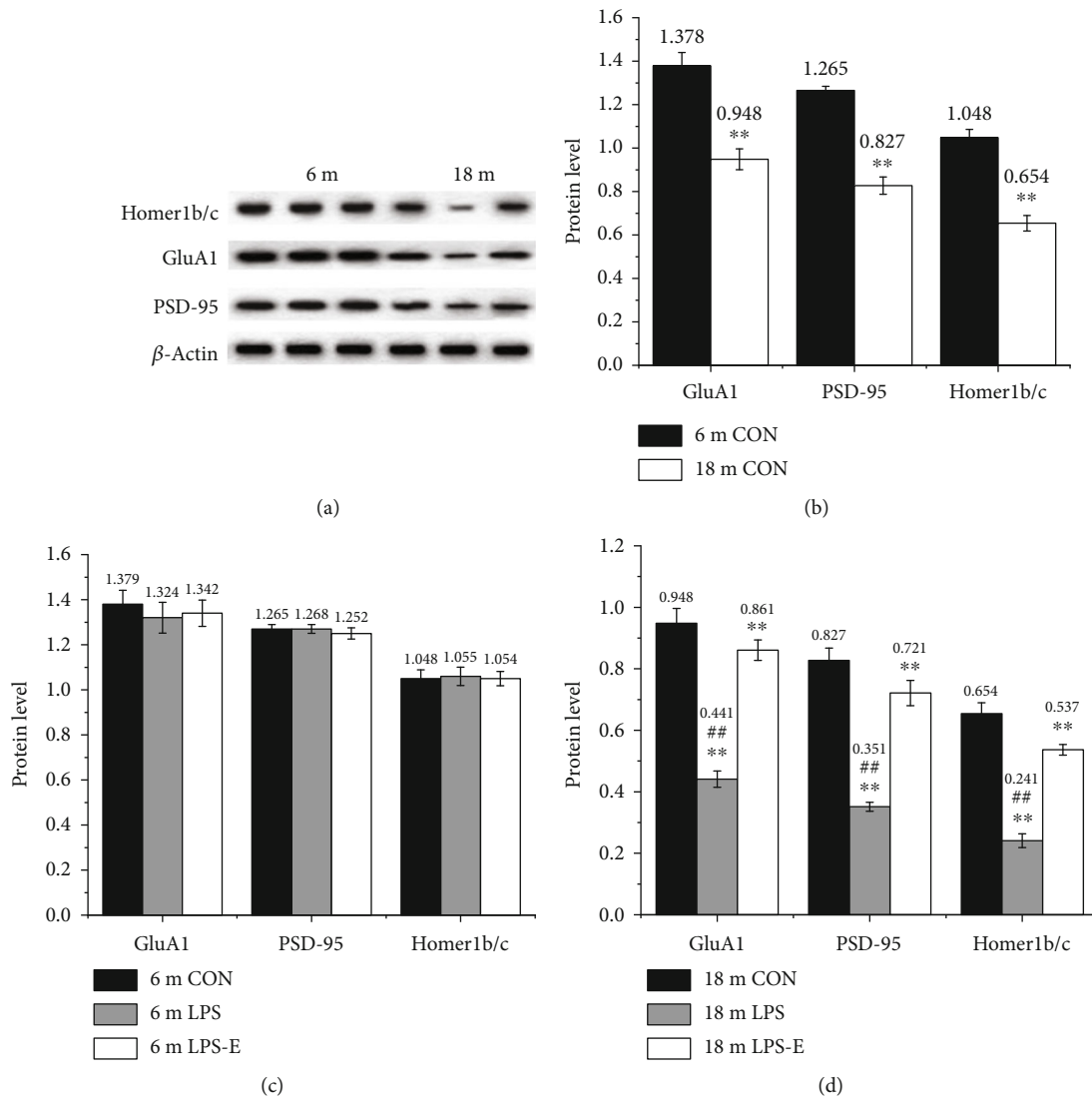


FIGURE 2: The results of hippocampal synaptic proteins. (a) Shows the protein bands after western blotting; (b) shows the effect of age, and (c, d) show the effect of treatments, respectively, at age 6 and 18 months, on the three synaptic proteins. The older female mice had lower hippocampal levels in PSD-95, GluA1, and Homer-1b/c than the younger females (b). In the 6-month-old mice, the diverse treatment groups had similar levels of these proteins (c); however, in the 18-month-old mice, female mice with inflammatory insult during pregnancy had obviously low levels (less than one-half) of hippocampal PSD-95, GluA1, and Homer-1b/c than the controls, and a marked improvement occurred in the females with enriched environment postpartum onward but did not reach that level in the controls (d). The sample size was six in each group. The data are presented as mean \pm standard deviation. * denotes comparison with the young or the same-age controls, * $P < 0.05$, ** $P < 0.01$; # denotes comparison with the LPS-E group, # $P < 0.05$, ## $P < 0.01$.

18-month-old mice that were exposed to LPS in late pregnancy. The results showed that the ability of spatial learning and memory in these mice with gestational exposure to LPS was similar to that of the controls at the age of 6 months. However, at the age of 18 months, these mice with gestational exposure to LPS had a worse cognitive ability than the identical-age controls. Therefore, our evidence indicates that neuroinflammation during pregnancy could accelerate the impairment of spatial learning and memory from midlife to late life in CD-1 mice.

EE is considered to have favorable effects on normal cognitive development and thus may potentially reverse the effects of cognitive decline induced by some factors such as

age, life stress, and neuroinflammation. To our best knowledge, the present study is the first to explore whether EE could affect changes in spatial learning and memory induced by gestational exposure to LPS during aging in CD-1 mice. The results showed that the young (6-month-old) LPS-E mice had similar spatial learning and memory as the control or LPS mice. At the age of 18 months, LPS mice showed worse performance of spatial learning and memory relative to CON mice, and LPS-E mice had significantly better spatial learning and memory than LPS mice. Moreover, LPS-E mice had worse spatial learning and similar spatial memory compared with CON mice, suggesting that EE could rescue spatial memory impairment but not spatial learning. This

TABLE 1: The correlations between levels of hippocampal synaptic proteins and performances in the Morris water maze test.

Proteins	Learning phase Distance $r(P)$	Memory phase Distance% $r(P)$
<i>PSD</i>		
6 m (all mice)	-0.513 (0.030)*	0.126 (0.618)
18 m CON	-0.910 (0.012)*	0.856 (0.029)*
18 m LPS	-0.777 (0.069)*	0.918 (0.010)*
18 m LPS-E	-0.841 (0.036)*	0.833 (0.040)*
<i>GluA1</i>		
6 m (all mice)	-0.609 (0.000)**	0.540 (0.021)*
18 m CON	-0.671 (0.144)	0.639 (0.172)
18 m LPS	-0.831 (0.040)*	0.931 (0.007)**
18 m LPS-E	-0.913 (0.011)*	0.696 (0.125)
<i>Homer-1b/c</i>		
6 m (all mice)	-0.483 (0.042)*	0.560 (0.016)*
18 m CON	-0.909 (0.012)*	0.887 (0.019)*
18 m LPS	-0.735 (0.096)	0.970 (0.001)*
18 m LPS-E	-0.671 (0.145)	0.825 (0.043)*

* $P < 0.05$; ** $P < 0.01$.

finding indicates that EE treatment postpartum onwards could relieve age-related cognitive impairment accelerated by gestational inflammation.

4.2. Effects of Age, Gestational Inflammation, and EE Postpartum Onwards on Synaptic Proteins. Long-term synaptic plasticity decrease is a feature of aging and dementia, which is coupled with the changes of synaptic protein in mammals. PSD-95, GluA1, and Homer-1b/c are postsynaptic proteins. PSD-95 is a major element of synapses and can interact with glutamate receptors. PSD-95 is involved in aging and neurodegenerative disorders [43]. Increasing evidence shows that expression levels of PSD-95 in the hippocampus are diminished in aged animals and in neurodegenerative disorders such as AD and Huntington's disease [44]. GluA1, GluA2, GluA3, and GluA4 are the constitutive subunits of AMPARs, the major mediators of fast excitatory transmission in the brain. Accumulating evidence suggests that dysregulation of AMPARs can be linked to natural and pathological aging [45]. For instance, the expression of GluA1 and GluA2 subunits is reduced in the hippocampus of the old Wistar rats [46]. Normal 20–22-month-old mice show decreased GluA1 mRNA levels in the hippocampus relative to 4–6-month-old mice [47]. Homer proteins are the scaffolding proteins that play a critical role in maintaining synaptic integrity by organizing synaptic proteins. They act as scaffolding support for group 1 metabotropic glutamate receptors. Homer 1a and Homer 1b/c are isoforms of homer proteins, and they have been associated with aging especially for Homer 1a. Thus far, a couple of studies have revealed a decrease in Homer1 expression in aged rodents [48, 49]. But, only one study has indicated that aged, male Long Evans rats show increased expression levels of Homer1c in the hippocampus [49]. In

the present study, the 18-month-old CON mice had significantly lower hippocampal expressions of these three synaptic proteins (PSD-95, Homer-1b/c, and GluA1) than the 6-month-old CON mice. Our results regarding the levels of PSD-95 and GluA1 in aged mice were in accordance with several previous studies. However, contrary to aged, male Long Evans rats [50], our aged CD-1 mice expressed low levels of Homer-1b/c in the hippocampus. There are many reasons for this inconsistency, such as strain, sex, and experimental methods.

Besides age, other factors such as neuroinflammation and psychosocial environment could also affect the expressions of synaptic proteins in the brain, including PSD-95, GluA1, and Homer-1b/c. For example, prenatal long-term exposure to mobile phone radiation leads to decreased PSD-95 expression in the hippocampus in elderly offspring, which could be alleviated by postnatal EE housing [51]. Repetitive LPS administration during the early life period decreases the expression of subunits of GluN2B in the hippocampus of young rats [52]. To our knowledge, our study has explored for the first time the effects of gestational inflammation induced by LPS administration and/or postpartum EE on the expression levels of PSD-95, GluA1, and Homer-1b/c in the hippocampus during aging. The results showed that the aged (18 months) CD-1 mice, who experienced a mild inflammatory process in late pregnancy, had obviously lower contents of PSD-95, Homer-1b/c, and GluA1 (less than one-half) than the same-age CON. The aged (18 months) LPS-E mice had markedly higher levels of PSD-95, Homer-1b/c, and GluA1 when compared with the identical-age LPS mice (nearly two times), but significantly lower levels when compared with the identical-age controls, with no significant difference among the three 6-month-old groups.

In brief, our results suggested that the hippocampal levels of PSD-95, Homer-1b/c, and GluA1 decreased in older females; late-pregnancy inflammation could significantly accelerate these reductions; and EE could alleviate these changes. However, these effects of age and treatments did not occur in the young (6 months) female mice. Further, it is yet unknown when the effects of these factors on synaptic proteins begin and whether it reaches its peak at the age of 18 months.

4.3. Correlations between Hippocampal Synaptic Proteins with Spatial Ability of Learning and Memory. Synaptic plasticity is the neurobiological basis of learning and memory [53, 54] and involves the integrity of synaptic structures and orchestration of synaptic proteins. Cognitive decline with aging is often because of altered levels of some synaptic plasticity-related protein expression. For instance, the hippocampal reductions of PSD-95 expression in aged female C57BL/6J mice may be linked to the reduced performance of this group of animals in the memory tasks [55]. 3xTg-AD mice show an age-dependent decrease of mRNA levels for the AMPAR subunits (GluA1, GluA3, and GluA4), which has contributed to the decline of spatial learning and memory during aging [56]. The ratio of Homer 1a/Homer 1b/c bound to mGluR5 in the postsynaptic densities (PSD) in the hippocampus was four times lower for memory-unimpaired, aged,

Long Evans rats than memory-impaired rats [28]. Our results also showed that the decreased levels of hippocampal PSD-95, Homer-1b/c, and GluA1 in aged CD-1 control mice were associated with impaired performance in the MWM test.

Aging is correlated with an exaggerated response to peripheral inflammatory challenges together with behavioral and cognitive deficits. LPS could affect neuronal cells via activation of microglia as well as directly by initiating neuroinflammation. LPS has been used to establish animal models of memory loss similar to that in the case of AD. At various developmental time points, LPS administration might have short- and long-term effects on cognitive function in both male and female rodents. For instance, prenatal LPS-treatment causes impaired recognition memory for objects in both sexes, with males being more severely affected at postnatal days 33 and 60 [57]. Gestational LPS exposure exacerbates the decline of spatial learning and memory and hippocampal changes in 15-month-old CD-1 mice [10]. It is important to note that compared to pregnant animals, nonpregnant animals have lower sensitivity to LPS and exhibit much less intense and persistent inflammation when exposed to even a very low dose of LPS [58–61]. One study using normal nulliparous female C57BL/6 mice (2–4 months) showed that LPS-induced acute inflammation did not affect working memory but impaired long-term memory consolidation [62]. In the present study, the levels of PSD-95, GluA1, and Homer-1b/c correlated negatively with the swimming distance in the learning phase and positively with the percentage of the swimming distance in the target quadrant in the memory phase at the age of 18 months for the LPS-treated groups. This suggested that the decreased levels of PSD-95, GluA1, and Homer-1b/c were correlated with cognitive decline induced by gestational LPS exposure in old mice. To date, the exact mechanisms by which neuroinflammation induced during pregnancy leads to cognitive impairment with aging have not been completely understood. Our previous study [10] indicated that gestational LPS exposure intensified age-related hippocampal changes, including increased amyloid- β 42, phosphorylated tau, synaptotagmin-1, and GFAP and decreased syntaxin-1 and H4K8ac, which were associated with impaired ability for spatial learning and memory.

Increasing evidence indicates that EE exposure is a method for alleviating cognitive impairments during normal and pathological aging. However, the cellular mechanisms behind this EE therapy are still unknown. The morphological and biochemical markers of brain plasticity, such as hippocampal neurogenesis, synaptic protein expressions, and synaptic morphology, are involved in the positive impact of EE on cognition. For instance, the beneficial effect of prolonged running wheels (a component of EE) on spatial learning and memory in older mice is coupled with the increased thin spines carrying small synapses expressing PSD-95 in the Schaffer pathway [63]. Postpubertal EE rescues the disrupted spatial discrimination ability induced by LPS injection during midgestation in offspring rats. This is likely because EE inhibits elevations in plasma corticosterone and alters the mRNA expression of several genes associated with resiliency and synaptic plasticity [64]. In the current study, the levels of

three postsynaptic plasticity-related proteins were correlated negatively with the swimming distance in the learning phase and positively with the percentage of the swimming distance in the target quadrant in the memory phase at the age of 18 months in the LPS-E group. This indicates that postpartum EE could alleviate decreased levels of hippocampal PSD-95, Homer-1b/c, and GluA1 induced by gestational inflammation during aging, which likely contributes to the cognitive positive effect of EE.

Therefore, our results indicated that the three postsynaptic plasticity-related proteins detected in this study may be involved in the mechanisms of spatial learning and memory impairment caused by inflammation during late pregnancy in CD-1 mice during aging.

5. Summary

Our findings showed that aging could lead to impaired learning and memory ability and decrease the expression of hippocampal postsynaptic plasticity-related proteins—PSD-95, Homer-1b/c, and GluA1. The neuroinflammation in late pregnancy might aggravate changes in cognition and synaptic plasticity-related proteins, but postpartum EE might ameliorate these effects in the aged CD-1 female mice. Notably, EE could not completely rescue the impairments in the spatial learning and levels of synaptic proteins. The expressions of synaptic plasticity-related proteins in the hippocampus are involved in cognitive dysfunction especially in old mice. Our study has some limitations. First, there are fewer groups of mice for age. Second, this study only determined the expression of individual synaptic plasticity-related proteins but did not further explore the signal pathways that are involved in these synaptic proteins. However, despite these limitations, our study highlights the importance of reducing/avoiding adverse environments during pregnancy and maintaining a lifelong enriched environment for successful aging.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

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.

Conflicts of Interest

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

Authors' Contributions

Shi-Yu Sun, Xue-Yan Li, and He-Hua Ge equally contributed to this work.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (81370444 and 81671316) and University Natural Science Foundation of Anhui Province (KJ2019A0270).

Supplementary Materials

The supplementary material for this article can be found in the supplementary file. (*Supplementary Materials*)

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