Environmental Enrichment Protects Offspring of a Rat Model of Preeclampsia from Cognitive Decline

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Abstract
Preeclampsia affects 5–7% of all pregnancies and contributes to adverse pregnancy and birth outcomes. In addition to the short-term effects of preeclampsia, preeclampsia can exert long-term adverse effects on offspring. Numerous studies have demonstrated that offspring of preeclamptic women exhibit cognitive deficits from childhood to old age. However, effective ways to improve the cognitive abilities of these offspring remain to be investigated. The aim of this study was to explore whether environmental enrichment in early life could restore the cognitive ability of the offspring of a rat model of preeclampsia and to investigate the cellular and molecular mechanisms by which EE improves cognitive ability. L-NAME was used to establish a rat model of preeclampsia. The spatial learning and memory abilities and recognition memory of 56-day-old offspring were evaluated by the Morris water maze and Novel object recognition (NOR) task. Immunofluorescence was performed to evaluate cell proliferation and apoptosis in the DG region of the hippocampus. qRT-PCR was performed to examine the expression levels of neurogenesis-associated genes, pre- and postsynaptic proteins and inflammatory cytokines. An enzyme-linked immune absorbent assay was performed to evaluate the concentration of vascular endothelial growth factor (VEGF) and inflammatory cytokines in the hippocampus. The administration of L-NAME led to increased systolic blood pressure and urine protein levels in pregnant rats. Offspring in the L-NAME group exhibited impaired spatial learning ability and memory as well as NOR memory. Hippocampal neurogenesis and synaptic plasticity were impaired in offspring from the L-NAME group. Furthermore, cell apoptosis in the hippocampus was increased in the L-NAME group. The hippocampus was skewed to a proinflammatory profile, as shown by increased inflammatory cytokine levels. EE improved the cognitive ability of offspring in the L-NAME group and resulted in increased hippocampal neurogenesis and synaptic protein expression levels and decreased apoptosis and inflammatory cytokine levels. Environmental enrichment resolves cognitive impairment in the offspring of a rat model of preeclampsia by improving hippocampal neurogenesis and synaptic plasticity and normalizing the apoptosis level and the inflammatory balance.

Keywords Preeclampsia · Cognition · Environmental enrichment · Neurogenesis · Apoptosis · Inflammation

Abbreviations
EE Environmental enrichment
SH Standard housing
NOR Novel object recognition
DI Discrimination index
BDNF Brain-derived neurotrophic factor
NGF Nerve growth factor
VEGF Vascular endothelial growth factor
L-NAME N(ω)-nitro-l-arginine methyl ester
PE Preeclampsia
GD Gestational day
PND Postnatal day
ELISA Enzyme-linked immunosorbent assay

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Introduction

Preeclampsia has recently received increasing attention due to its long-term adverse effects on offspring. Preeclampsia (PE), a pregnancy-specific disorder, is characterized by de novo hypertension after the 20th gestational week accompanied by associated end organ damage (Brown et al., 2018). This disease affects 5–7% of all pregnancies worldwide and is a major cause of maternal and neonatal morbidity and mortality, such as premature birth and intrauterine growth restriction (Rana et al., 2019). In addition to these short-term adverse effects, long-term adverse effects of preeclampsia on offspring have been reported, including cardiovascular, metabolic and neurological disorders (Lu and Hu, 2019). Nonetheless, ways to improve long-term outcomes remain to be investigated.

In recent years, the impairment in the neurological system of offspring exposed to preeclampsia has become evident. Researchers have found that intrauterine exposure to preeclampsia can impair the cognition of offspring (Gumusoglu et al., 2020). Epidemiological studies revealed an association between PE and impaired cognition from childhood to old age (Pinheiro et al., 2016). In one report, 7- to 10-year-old children exposed to preeclampsia had an impairment in working memory and visual spatial processing (Rätsep et al., 2016a, b). Ehrenstein et al. discovered that 18- to 19-year-old offspring exposed to preeclampsia were at increased risk of low cognitive function, which was defined as IQ < 85 (Ehrenstein et al., 2009). Moreover, Tuovinen et al. revealed that offspring aged 69.3 years who were born after exposure to preeclampsia were at increased risk of impaired memory and cognition compared with those born after a normotensive pregnancy (Tuovinen et al., 2014). Moreover, animal studies have confirmed the causal relationship between preeclampsia and cognitive impairment in offspring (Liu et al., 2016; Ijomone et al., 2019; Shu et al., 2018). Researchers found impaired brain structure and cognitive decline in offspring of a preeclamptic-like rat model induced by L-NAME, a nitric oxide synthase inhibitor, accompanied by decreased hippocampal neurogenesis and increased expression of glucocorticoid receptor (Liu et al., 2016; Zhu et al., 2017). In RUPP (reduced uterine perfusion pressure) rat model of preeclampsia, researchers found increased neuroinflammation in offspring (Giambrone et al., 2019). Notably, it has been reported that prenatal preeclampsia exposure could decrease offspring parietal and occipital cerebral vessel radii (Rätsep et al., 2016a, b). The changes are associated with decreased placental growth factor (PIGF) levels in preeclamptic maternal plasma, which may drive angiogenic and microvascular deficits in offspring (Wang et al., 2009). Therefore, the cognitive deficits observed in preeclampsia offspring may be associated with neuroinflammation, disrupted neurological functions, such as neurogenesis and disrupted cerebrovascular structure (Barron et al., 2021). However, there is no known effective way to optimize cognition in offspring born after exposure to preeclampsia. Therefore, identifying a way to improve the cognition of offspring is of great clinical significance.

Environmental enrichment (EE) is the addition of physical, somatosensory and social stimuli to the animal environment, including running wheels, toys, nesting materials, tubes and larger group housing (Ohline and Abraham, 2019). EE contains three basic components including exercise, novelty and social contact (Crofton et al., 2015). It has been reported that EE can lead to enhancements in hippocampal cognition and neuroplasticity and alleviate hippocampal cognitive deficits associated with neurodegenerative disease and aging (Gríñan-Ferré et al., 2016). We are interested in whether these changes that successfully enhance cognition have similar effects on offspring with cognitive impairment after being born to preeclampsia rats. In the present study, we explored the effects of environmental enrichment on cognitive alterations in the offspring of a preeclamptic rat model and the mechanism by which EE affects cognitive ability.

Materials and Methods

Animals

All procedures in this study were approved by the Animal Care and Use Committee of the University of Fudan and were conducted in accordance with the animal care guidelines of the National Institute of Health. For this study, 15 female and 5 male Sprague–Dawley rats, 8–10 weeks old, were purchased from Shanghai JieSiJie Laboratory Animals Co. LTD company. All animals were habituated for 1 week to the housing room where they were kept under controlled conditions, a temperature of 21 °C, humidity of 50%, a 12-h light/12-h dark cycle (lights on 8 am) and they had access to food and water ad libitum. All efforts were made to minimize animal suffering and to reduce the number of animals used.

The Establishment of the Preeclampsia Rat Model

Female rats were randomly assigned to Control or L-NAME group. At the age of 9 weeks, the rats were mated in groups of 2 females and 1 male. Vaginal plugs were carefully looked for every day at approximately 7 am. When a vaginal plug was observed, the female rats were considered pregnant and placed in an individual cage. This date was defined as gestational day (GD) 0.
To establish the preeclampsia animal model, L-NAME, an NO synthesis inhibitor commonly used to establish a rat model of preeclampsia, was administered. Briefly, pregnant dams either received L-NAME (125 mg/kg subcutaneously, Sigma-Aldrich) or NaCl as vehicle from GD13 to GD21 (Fig. 1). All solutions were freshly prepared each day. The rats’ blood pressure and urine protein were measured to validate the establishment of the preeclampsia model. Blood pressure was detected with a BP-2000 blood pressure analyzer via a tail cuff before and after the administration of L-NAME. The protein level in the urine was quantified using the Bradford method (P0006C, Beyotime). A total of 24 male offspring were randomly selected from 6 dams were analyzed in this study (8 offspring were from Control group, 16 offspring were from L-NAME group). All offspring were weaned at postnatal day 21.

**Experimental Housing**

Between postnatal days (PNDs) 21 and 56, the male offspring were housed in standard housing (SH) conditions or an enriched environment (EE) (Fig. 1). Under standard housing conditions, 4 littersmates were kept together in one rat cage with sawdust bedding material. In the enriched environment, 8 rats were housed in a large cage (100 x 60x90 cm), which included a free-running wheel, two plastic tunnels, a raised platform, a stair case and various colored balls. To increase the novelty, these objects were exchanged for different ones once a week during cage cleaning. These environmental stimuli and the increased number of partners improved environmental complexity compared with SH. Group sizes were NaCl SH: n = 8; L-NAME SH: n = 8; L-NAME EE: n = 8.

**Morris Water Maze**

After 5 weeks of experimental housing, all animals were housed under standard housing conditions for one week before starting the behavioral tests. Cognitive ability was evaluated by the Morris water maze, which is widely used for testing animals’ spatial learning ability and memory. This test involves two stages: the spatial learning stage and the memory test stage. In the spatial learning stage, a circular platform is placed at a specific location away from the edge of the pool. The platform is submerged 1.5 cm below the water surface. The rats were trained in three trials per day. The animals were placed at a certain position and given 60 s to find the platform. If the animal could not find the platform, it was guided to the platform and was allowed to remain on the platform for 20 s. The training procedure lasted for 4 days.

In the memory test stage, the platform was removed, and the trained animals were placed at a specific position in the pool to swim. The time they spent in each quadrant (target quadrant, left quadrant, opposite quadrant and right quadrant), the latency to the platform area and the frequency of reaching the platform area were recorded. All data from the water maze test were collected with a video camera fixed to the ceiling and were connected to a computer and a video-tracking system (Noldus Information Technology, Holland).

**Novel Object Recognition (NOR) Task**

The NOR task was carried out after five weeks of experimental housing. Briefly, offspring habituated in NOR arena for 5 min in five consecutive day. On the sixth day, the offspring explored two identical objects (A and B) which were placed at different corners of the arena for 10 min (Training stage). On the next day, the object B were replaced with a novel object C. Then, the offspring explored a familiar object C. The number of explorations of the familiar object A and the novel object C were recorded.
object A and a novel object C for 10 min (Testing stage). The time offspring spent on exploring the familiar object A and the novel object C was recorded by ANYmaze software. The discrimination index (DI) was calculated \( \frac{\text{time spent exploring the novel object} - \text{time spent exploring the familiar object}}{\text{time spent exploring both objects}} \).

**Sample Preparation**

Experimental rats were anesthetized with chloral hydrate and perfused transcardially with cold phosphate-buffered saline (PBS), followed by 4% paraformaldehyde (PFA). The hippocampus was dissected out and fixed in 4% PFA at 4 °C. Then, the tissues were sliced into 20 μm coronal sections that were placed on glass slides.

**Immunofluorescence and Microscopy**

Immunofluorescence assays and microscopy were performed on cryosections of rat hippocampus as previously described (Pan et al., 2020, 2017). In brief, cryosections were fixed in 4% paraformaldehyde for 30 min and washed three times with PBS, then incubated with 1% glycine in PBS for 30 min, and blocked with 5% BSA at room temperature for 2 h. Then, the slices were incubated with primary antibodies overnight at 4 °C. The unbound antibodies were removed via 5 min × 3 washes with PBST. The slices were incubated with secondary antibodies at room temperature for 1 h. The slices were then rinsed with PBST for 5 times. DAPI (Sigma-Aldrich) was used for staining nuclei. Then, the stained sections were mounted with anti-fade mounting medium, covered by glass coverslips, sealed with nail polish, and kept in -20°C prior to imaging analyses. To evaluate the apoptotic cells, the hippocampal sections were subjected to fluorometric terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL; KGA7073, KeyGEN BioTECH) staining according to the manufacturer’s instruction (Ma et al., 2020). Immunofluorescence was detected using Fluorescent Microscopy (OLYMPUS BX53) in a room temperature. BrdU and TUNEL signal were acquired using a 40× objective. The images were captured by OLYMPUS cellSens Standard software.

**RNA Isolation, Complementary DNA Synthesis and Quantitative PCR**

Total hippocampal RNA was extracted with TRIzol (Invitrogen, 15,596,026, Carlsbad, CA, USA). Complementary DNA (cDNA) synthesis was performed with the PrimeScript™ RT reagent Kit with gDNA Eraser (RR047A Takara) in accordance with the manufacturer’s instructions. Quantitative PCR was performed using a commercial mix (SYBR Green Master Mix; YEASEN) and a thermal cycler (appliedbiosystems; Thermo Fisher Scientific, Waltham, MA, USA) with the primers shown in Table 1. The total volume for qPCR was 10 μl, comprised 0.2 μl of each primer.

| Name  | Sequence (5’–3’) |
|-------|------------------|
| GAPDH | ACCACAGTTCATGCCATCAC TCCACACCTGTGCTGTA |
| Fgf   | CCAAGGGCTTCTTACGCAAGAC TAGAGGAGCCAGGCGTCATC |
| PTN   | AGCGTGGACACTGTGGAGG TCCATGCAATCTCCACAGTCAG |
| EP300 | CTGCTCTGGGACTACCTAT CAGAAGAAGATTCGGTTC |
| Creb  | AGTGGCAGCAACCAAGATGGGGAGAGGAGGCGCATAACA |
| BNDF  | TGGAACTCGCAATGCCAGAACTAC TCTTATGAACCAGCCAGCAATTC |
| NGF   | CCAACAGACTACAGGAGCAGCAAGAC GATGTCCGTGGCTGGTCTTATC |
| IL-1β | AAGCAAGACAAAAATCCCT TGTCGAGACTCAAAACTCCA TCCAGGAGATTGGTGATCC |
| IL-6  | CACCACCAACAGCTCTCATC TGGAGTCAGCTGGTGGGAG |
| TNF-α | CACCACCAACAGCTCTCATC CGGAGACAGCTGTCCTCCT |
| PSD95 | TCCAGTCGTGCGAGAGGATGCGGAGAGGAG |
| synapsin I | AGACGCTCATGCTGGAAACTGTGG TGGAGTCAGCTGGTGGGAG |
| SNAP25 | GAGCAGTGAGGGCCATCATC GTCGACGTGGGTTGGCTCCTAC |
that in the control group (Fig. 2d, p < 0.001). Furthermore, neonates in the L-NAME group was significantly lower than that in the control group (Fig. 2c, p < 0.05). In addition, the body weight of the L-NAME group was also decreased compared with that in the control group (Fig. 2f). The proportion of limb defects in L-NAME group was significantly increased (Fig. 2e, p < 0.05).

**Improvement in Learning and Memory Ability in the Offspring of the L-NAME Group After EE**

To analyze the learning ability and memory of the offspring, the Morris water maze was used. In both stages, the performance of offspring in the EE group was indistinguishable from that of offspring in the control group, in sharp contrast to offspring in the L-NAME group.

In the training stage, the latency to reach the platform progressively decreased in the control group (Fig. 3a, p < 0.005), which indicated that offspring learned the task from day 1 to day 4. However, latency to plateau in the L-NAME group decreased much slower, less efficiently and was longer than that in the control group (Fig. 3a, p < 0.005). EE generally restored the learning ability deficiency, reflected by the decrease in latency to plateau (Fig. 3a, p < 0.005). There was no significant difference in swimming speed among the 3 groups (Fig. 3b, p > 0.05). The performance in the training stage revealed that offspring in the L-NAME group had impaired learning abilities and that EE could improve their learning abilities.

At the memory test stage, the results showed that the frequency of crossing platform quadrant in the L-NAME group was much lower than that in the control group (Fig. 3d, p < 0.005). They also spent significantly less time in the platform quadrant than offspring in the control group (Fig. 3e, p < 0.005). A shorter swimming distance to the target quadrant was found in the L-NAME group (Fig. 3f, p < 0.005). All of these changes could be reversed by EE. Therefore, we have demonstrated that EE protected PE offspring from impaired memory.

**Improvement in Recognition Memory in the Offspring of the L-NAME Group After EE**

The NOR task was used to analyze object recognition memory of offspring in three groups. In the training stage, there was no significant difference of time spent on two identical objects (A and B) among three groups (Fig. 4a). However, in the testing stage, the offspring from L-NAME group showed a significant decline in time exploring the novel object C compared with offspring in the Control group, accompanied by a decrease in DI in L-NAME group (Fig. 4b, p < 0.05). Interestingly, offspring from EE group spent much more time exploring the novel object C and DI was increased in EE group (Fig. 4b, p < 0.05). The results showed that offspring in L-NAME group displayed NOR deficits, which could be reversed by EE intervention in early life.
Increased Neurogenesis in the Hippocampus of Preeclampsia Offspring After EE

To reveal the cellular and molecular mechanisms through which EE improves brain function in the offspring of preeclamptic rats, we investigated hippocampal neurogenesis, which has been reported to be associated with spatial learning ability and memory. The immunofluorescence (IF) results showed that the number of BrdU + cells was significantly decreased in the L-NAME group but increased in the EE group (Fig. 5a,5b), indicating that decreased neurogenesis was resolved by EE. Next, qRT-PCR was used to test the expression level of adult hippocampal neurogenesis-associated genes, including Fgf, PTN, EP300, Creb, BNDF and NGF. However, there were no significant differences in the expression levels of these genes among the three groups (Fig. 5d, p > 0.05). We then tested whether there were changes in VEGF concentration. VEGF has been reported to promote neurogenesis in the adult brain, possibly through enhancement of the vascular niche. The results showed a significant reduction in VEGF in the L-NAME group compared with the control group. However, EE restored hippocampal VEGF to a level similar to that of the control group (Fig. 5c, p < 0.05). Thus, EE prevented decreases in hippocampal VEGF levels in L-NAME offspring, and this is possibly the reason for its positive effect on neurogenesis.

Reduced Apoptosis in the Hippocampus of the Offspring of the L-NAME Group After EE

Neural apoptosis in the DG region of the hippocampus, measured by TUNEL + nuclei staining, was significantly different among the three groups (Fig. 5e). The number of TUNEL + cells was increased in the L-NAME group compared with the control group and the EE group. No significant difference existed between the control and EE groups.
Fig. 3  Effect of Environmental Enrichment on spatial learning ability and memory. 

a Latency to the platform for the offspring in each group in the training stage. N = 8 per group. 

b Mean swimming speed of each group in the training stage. N = 8 per group. 

c Tracks of the tested offspring in each group. 

d Frequency of crossing the platform area for each group. N = 8 per group. 

e Time spent in the platform quadrant of each group. N = 8 per group. 

f Swimming distance in the platform quadrant of each group. N = 8 per group. 

g Typical behavior tracks. Statistical analysis was performed using one-way ANOVA. Data are presented as mean ± SD or median ± interquartile range. ** p < 0.005
Fig. 4 Effect of Environmental Enrichment on novel object recognition. 

**a** Time spent exploring two identical objects A and B in the training stage. N = 8 per group.

**b** Time spent exploring the familiar object A and the novel object C in the testing stage. N = 8 per group.

**c** Discrimination Index in each group. N = 8 per group. Data are presented as the mean ± SD or median ± interquartile range. *p < 0.05

Fig. 5 Analysis of progenitor cell proliferation and neural apoptosis in the hippocampus of offspring.

**a** BrdU immunofluorescence in DG sections of the hippocampus. N = 6 imaging fields per group, collected from three independent experiments.

**b** The number of BrdU + cells in the hippocampus of offspring in each group. N = 6 imaging fields per group, collected from three independent experiments.

**c** VEGF concentration in the hippocampus. N = 6 per group.

**d** mRNA levels of adult hippocampal neurogenesis-related genes. N = 6 per group.

**e** TUNEL immunofluorescence in DG sections of the hippocampus. F TUNEL + cell number in the hippocampus of offspring in each group. N = 6 imaging fields per group, collected from three independent experiments. Statistical analysis was performed using one-way ANOVA. Data are presented as the mean ± SD or median ± interquartile range. *p < 0.05, ***p < 0.001
These results indicate that EE alleviated neural apoptosis in the L-NAME group.

**Increased Synaptic Plasticity in Hippocampus of the Offspring of the L-NAME Group After EE**

To evaluate the synaptic plasticity in the hippocampus, we assessed the mRNA expression levels of the pre- and postsynaptic proteins synapsin I, SNAP25, and PSD95. We found decreased expression levels of synapsin I (Fig. 6c, Control vs L-NAME, $p < 0.005$; L-NAME vs EE, $p < 0.05$) and PSD95 in the L-NAME group (Fig. 6a, $p < 0.05$) compared with the control group. However, the expression levels of these synaptic-related proteins were restored by EE. No significant difference in SNAP25 expression was observed among these groups (Fig. 6b, $p > 0.05$).

**Reduced Inflammation in the Hippocampus of Preeclampsia Offspring After EE**

Considering the strong association between cognitive impairment and inflammation, we examined the inflammatory cytokines in the hippocampus. Both qRT-PCR and ELISA results revealed different inflammatory profiles between these groups. The results showed that both the mRNA and protein levels of proinflammatory cytokines, including IL-1β (Fig. 7a, Control vs L-NAME, $p < 0.005$; L-NAME vs EE, $p < 0.05$; Figure 7d, Control vs L-NAME, $p < 0.005$; L-NAME vs EE, $p < 0.05$) and IL-6 were increased in the L-NAME group compared with the control and EE groups (Fig. 7b, Control vs L-NAME, $p < 0.001$; L-NAME vs EE, $p < 0.005$. Figure 7e, Control vs L-NAME, $p < 0.005$; L-NAME vs EE, $p < 0.05$). Nonetheless, no significant differences were found in TNF-α levels among the three groups (Fig. 7c, f, $p > 0.05$). These results suggest that EE could reverse the excessive hippocampal inflammation in L-NAME offspring.

**Discussion**

In this study, we examined the effects of an EE intervention in early life on the prevention of preeclampsia-related cognitive decline in adolescent offspring in an L-NAME-treated rat model. Previous epidemiological studies have demonstrated that maternal preeclampsia is strongly associated with poor cognitive performance of their children. Consistent with this, we found that the administration of L-NAME to pregnant rats induced cognitive deficits in offspring. Offspring in the L-NAME group exhibited pathological changes, including impaired neurogenesis and synaptic plasticity, increased neural apoptosis, and increased levels of inflammatory cytokines in the hippocampus compared with their counterparts in the control group. Notably, rearing offspring in the L-NAME group in an enriched environment for five weeks prevented hippocampus-dependent learning ability and spatial memory decline, NOR deficits as well as pathological changes in the hippocampus. Altogether, these results indicated that EE might effectively reverse cognitive changes caused by an adverse intrauterine environment.

We used an L-NAME rat model of preeclampsia, in which L-NAME (an inhibitor of NOS) was administered to pregnant rats during gestational days 13 to 21. This model...
is effective in exploring preventive strategies for cognitive decline in offspring who were born after preeclampsia due to its ability to recapitulate the clinical features of preeclampsia, including increased blood pressure and urine protein. In women with preeclampsia, NO production is reduced. The L-NAME rat model could mimic the NO deficiency. Moreover, since gestational days 13 to 21 are a critical stage for brain development, this model is effective in studying the neurodevelopment of preeclampsia offspring. A previous study from our laboratory reported that the spatial learning ability and general learning ability decline and there is impaired neurological development among adolescent offspring of L-NAME rats, which supports the effectiveness of this model.

Few studies have explored ways to improve the cognitive ability of preeclampsia offspring and they have mainly focused on gestational diet interventions. To our knowledge, the present study represents the first examination of whether EE could protect preeclampsia offspring against cognitive decline and thus provides novel insights for early intervention. We showed that EE in early life was sufficient to prevent cognitive deficits in adolescent offspring from the L-NAME group. There are various ways to provide an enriched environment; thus, the protocols lack consistency.

Fig. 7 Effect of environmental enrichment on the inflammatory status of the hippocampus. a The mRNA level of IL-1β in the hippocampus. N=6 per group. b The mRNA level of IL-6 in the hippocampus. N=6 per group. c The mRNA level of TNF-α in the hippocampus. N=6 per group. d The concentration of IL-1β in hippocampus. N=6 per group. e The concentration of IL-6 in the hippocampus. N=6 per group. f The concentration of TNF-α in the hippocampus. N=6 per group. Statistical analysis was performed using one-way ANOVA. Data are presented as median±interquartile range. * p<0.05. ** p<0.005. *** p<0.001
However, the most common procedure includes rearing the rats in a large cage and providing them with novel subjects and social contact for at least 30 days starting immediately after weaning. This procedure provides all of the key factors of EE, including social contacts, novelty and exercise, all of which have been reported to be rewarding. The offspring were weaned at postnatal day 21. Therefore, they were reared in an enriched environment from postnatal day 21 to day 56, which lasted for five weeks.

In this study, the Morris water maze and NOR task were used to assess cognitive function in offspring. Our data showed that offspring in the L-NAME group exhibited a clear decline in spatial learning ability, reflected by an increased “latency to platform”, which was prevented by EE. With regard to spatial memory, L-NAME induced a spatial memory decline reflected by a shorter swimming distance, less time in the target quadrant and a lower “frequency of crossing the platform” in the test stage. Impaired spatial memory was also revoked by EE. These results showed impaired hippocampus-dependent learning ability and spatial memory in the L-NAME group. However, after 5 weeks of the EE intervention, the performance of offspring in the Morris water maze was dramatically improved. Moreover, offspring in L-NAME group showed an impaired NOR performance which were reflected by the reduced Discrimination index. Five weeks of EE could prevent NOR deficits, the DI of which was similar to the control group.

In an attempt to discern the biological underpinnings of the observed cognitive changes, we focused on the structural and molecular plasticity of the hippocampus. The results showed that EE could improve neurogenesis, attenuate neural apoptosis, and improve synaptic plasticity. Moreover, EE normalized the inflammatory balance in the hippocampus by decreasing the expression of the proinflammatory cytokines IL-1β and IL-6.

The hippocampus is a key structure involved in learning and memory. The adult hippocampus can continuously generate new neurons that are integrated into hippocampal circuits. These newly generated neurons are thought to play an important role in hippocampal-dependent spatial learning and memory (Bruel-Jungerman et al., 2007; Shors et al., 2001). The process of hippocampal neurogenesis has been reported to be influenced by various factors, including physiological conditions and environmental stimuli. Therefore, we investigated neurogenesis in the DG, where new neurons are added to the mature circuit. Our study showed that exposure to an adverse uterine environment exerted a negative effect on hippocampal neurogenesis, reflected by a decreased number of BrdU + cells in the DG region of the hippocampus of offspring, while five weeks of EE intervention in early life could alleviate these changes. This suggests that impaired cognitive function in L-NAME offspring may be associated with reduced neurogenesis, which is attenuated by EE.

Alterations in hippocampal growth factors might be functionally linked with neurogenesis changes. BDNF has been widely studied as a candidate mediator of changes in hippocampal neurogenesis induced by environmental stimuli. For instance, the deletion of TrkB (BDNF receptor) reduced the effects of exercise on adult neurogenesis. In addition, NGF impacts the survival of neuronal progenitor cells. Amy et al. found that cognitive decline with aging was associated with a reduction in NGF (Birch and Kelly, 2019). Moreover, FGF signaling pathways play a role in regulating neurogenesis. The deletion of FGF receptor genes could result in a dramatic loss of neurogenesis in the DG, while enhancing FGF receptor activity in neurogenic cells could increase their numbers (Kang and Hébert, 2015).

VEGF has also been reported to enhance neurogenesis (Fabel et al., 2003; Gao et al., 2009). It can promote the proliferation of not only endothelial cells but also NPCs (Jin et al., 2002). Gao et al. reported that reduced VEGF expression with aging might exert an impact on the angiogenic niche within the DG and reduce neurogenesis (Gao et al., 2009). Jin et al. reported that intracerebroventricular administration of VEGF into rat brain increased BrdUrd labeling of cells in the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) through the Flk-1 receptor. Moreover, VEGF is capable of inducing a long-lasting neurogenic enhancement (Licht et al., 2016). VEGF could enhance the angiogenic niche in the subgranular zone (SGZ) of the DG. In support of these studies, blockade of VEGF eliminates exercise-induced improvements in neurogenesis, indicating that VEGF may play a significant role in the stimulation of neurogenesis (Fabel et al., 2003).

These studies indicate the essential roles of these growth factors in the regulation of neurogenesis. Accompanying the changes in neurogenesis, we found decreased expression levels of VEGF, while there were no changes in BDNF, NGF, FGF or other neurogenesis-related genes. Interestingly, the reduction in VEGF was attenuated in the EE group. Furthermore, an increased neural apoptosis level was observed in the L-NAME group, which was attenuated by EE. Therefore, the improved neurogenesis mediated by EE may be partly attributed to increased expression of VEGF and the inhibition of apoptosis.

Previous studies have shown a substantial link between neuropsychological disorders and neuroinflammation. McAffose et al. demonstrated that elevated or prolonged exposure to inflammatory mediators could have detrimental effects on cognitive function. Moreover, recent evidence has implicated dysregulated inflammation in the development of ASD (Matta et al., 2019). Masi et al. reported that a number of cytokines were dysregulated in ASD and were correlated with the severity of the ASD symptoms (Masi et al., 2017). Similar to these findings, in this study, we found increased inflammatory cytokines in preeclampsia offspring compared
with normal pregnant offspring. The data in our study indicated a proinflammatory phenotype in the hippocampus of offspring in the L-NAME group, reflected by increased levels of IL-1β and IL-6, which was attenuated by EE. IL-1β has been widely reported to have memory-modulating effects and it is increased in many neurodegenerative diseases and normal aging (Frank et al., 2006; Lynch, 2010); thus, the EE-induced modulation of IL-1 expression could help preserve cognitive function. Additionally, EE-induced improvement in cognitive ability could be partly attributed to the attenuation of the increase in IL-6, which could impact synaptic plasticity and neurodegeneration. Indeed, in this study, we found decreased expression levels of the pre- and postsynaptic proteins synapsin I and PSD95 in the L-NAME group compared with the control group, while SNAP25 expression was not changed. EE could rescue this reduction. Notably, recent studies have demonstrated that neuroinflammation is a potent inhibitor of hippocampal neurogenesis (Zonis et al., 2015). A large number of studies have demonstrated the role of cytokines in regulating neurogenesis (Kohman and Rhodes, 2013; Borsini et al., 2015; Goshen and Yirmiya, 2009). For example, in vivo studies showed that IL-1β have inhibitory influence on hippocampal neurogenesis, whereas co-treatment with IL-1β receptor antagonist could prevent the negative effect of IL-1β (Ryan et al., 2013; Boehme et al., 2014). Zonis et al. reported that IL-6 could induce the expression of p21 in hippocampus-derived murine NPC, which arrested the proliferation of progenitors of neuronal lineage (Zonis et al., 2015). Meanwhile, high levels of cytokines could induce the apoptosis of newborn neurons and damage developing neurons through triggering oxidative stress (Monje et al., 2003; Ben-Hur et al., 2003). In addition, there is an indirect mechanism. Inflammatory cytokines could activate HPA axis and high levels of glucocorticoid strongly suppress neurogenesis (Cameron and Glover, 2015). In our previous study, we found increased glucocorticoid receptor expression in L-NAME offspring hippocampus (Zhu et al., 2017), thus the impaired neurogenesis may be partly attributed to HPA hyperexcitability induced by increased pro-inflammatory cytokines. Thus, we speculated that EE attenuated cognitive deficits by reducing inflammation, which improving hippocampal neurogenesis and synaptic plasticity.

In addition to the protective effects of EE on neurological diseases, previous studies have reported that EE could also enhance cognitive performance and cellular plasticity in normal conditions (van Praag et al., 2000; Hannan, 2014). EE has been shown to enhance learning and memory function in various learning task (Renner and Rosenzweig, 1987). Several studies reported that mice living in enriched environment did better on the water maze task (a test of spatial memory) than those in standard housing (Kempermann et al., 1997, 1998). Key aspects of EE’s effects on cellular plasticity includes enhanced synaptic plasticity, adult hippocampal neurogenesis and synaptogenesis (Pang and Hannan, 2013). Various studies have validated that EE could enhance the number of new neurons in hippocampus (Kempermann et al., 1998). Furthermore, synaptogenesis has also been found to be increased in animals raised in enriched environment. Researchers reported that EE increased dendritic spines, synapse-to-neuron ratios, synaptic disc diameter and subsynaptic-plate perforations (Greenough et al., 1978). An increased number of dendritic spines and enhanced density of non-perforated synapses was also found in CA1 area after EE (Rampon et al., 2000). Thus, we speculate that the control offspring would exert improved cognition after EE than those housed in normal condition via enhanced hippocampal neurogenesis and synaptic function.

Notably, spatial learning and memory and NOR tasks are not only relying on hippocampal circuits, but also associated with cortex and basal ganglia. Previous studies showed that prenatal exposure to preeclampsia led to decreased connectivity between the mPFC and left occipital fusiform gyrus (Mak et al., 2018). In RUPP rat model, researchers found increased micro-hemorrhages in the cortical parenchyma and ventricles of offspring. In addition, both L-NAME and RUPP could lead to increased activation of microglia in offspring cortex (Ijomone et al., 2019; Clayton et al., 2018). Moreover, offspring of rat exposed to L-NAME showed decreased number of oligodendrocytes and exhibited higher levels of oxidative stress markers in the cortex (Scott et al., 2018; Phillips et al., 2017). Impaired umbilical circulation and placental hypoxemia in other models caused deficits in cortical gliosis, myelination and neuronal process (Mallard et al., 1998). However, no study examined the effects of preeclampsia on offspring basal ganglia. Therefore, future studies are required to investigate if preeclampsia could exert effects on basal ganglia and whether EE improve the cognitive ability through regulating the structure and function of cortex and basal ganglia.

Conclusion

In this study, we demonstrated that the administration of L-NAME during pregnancy affected the offspring, leading to an impairment in hippocampus-dependent learning ability and spatial memory, accompanied by pathological changes, including decreased neurogenesis and VEGF expression, lower levels of synaptic proteins, and increased apoptosis as well as proinflammatory cytokines. However, providing EE to the offspring in early life for 5 weeks could improve their learning ability and spatial memory and alleviate these changes.
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**Author contributions** XL, RH and HL conceived the study, designed the experiments and wrote this manuscript. HL performed the experiments with the help of LG, H, HZ and SW. QZ contributed to data analysis. All authors read and approved the final manuscript.

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**Data Availability** The data supporting the main findings and conclusions of this article are included within the article. All datasets and analyses used in this study are available from the corresponding author on reasonable request.

**Declarations**

**Conflict of interest** The authors declare that they have no competing interests.

**Ethical Approval** All procedures in this study were approved by the Animal Care and Use Committee of the University of Fudan and were conducted in accordance with the animal care guidelines of the National Institute of Health.

**Consent to Participate** Not applicable.

**Consent for Publication** This manuscript has been approved for publication by all authors.

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