Impaired Spatial Learning and Memory is Linked to Neurochemical Indicators of Brain Aging in the Middle-Aged CD-1 Mice with Maternal Exposure to LPS

Jing-Jing Tong[1], Gui-Hai Chen[1,2,*], Qi-Gang Yang[1], Fang Wang[1] and Wen-Jing Bian[1]

[1]*Department of Neurology, the First Affiliated Hospital of Anhui Medical University, Hefei 230022, Anhui Province, PR China*

[2]*Department of Neurology, the Affiliated Chaohu Hospital of Anhui Medical University, and the Center in Psychologic Medicine of Anhui Province, Chaohu, Hefei 238000, Anhui Province, P. R. China*

Abstract: *Aim:* Prenatal exposure to a disadvantageous circumstance may produce accelerated brain aging. Previously, our middle-aged model of CD-1 mice with maternal exposure to low-dose lipopolysaccharide (LPS) showed accelerated memory aging at a behavioral level. Here we investigated whether there was a corresponding pathophysiological alteration in the brain.

Materials and Methods: The mothers in the LPS group were administered a low dose (i.p. 50μg/kg) of LPS daily for 3 days during late gestation to simulate an inflammatory condition in maternal infection.

Results: The treatment accelerated the age-related decline of spatial learning and memory in the Morris water maze in the middle-aged offspring. Compared to control mice (n = 12), these mice (n = 12) exhibited elevated malondialdehyde contents ($P = 0.042$), decreased activities of superoxide dismutase ($P < 0.001$) and glutathione peroxidase ($P = 0.010$) in the brain, and elevated levels of amyloid beta ($Ps < 0.005$) and synaptotagmin-1 ($Ps < 0.037$) in several hippocampal layers. These age-related indicators correlated with a decline in spatial learning and memory ($Ps < 0.05$).

Conclusions: During gestation, maternal illness in mice might be an initiator of accelerated brain aging in offspring, as indicated by behavioral-cognitive and neurochemical measures.

Keywords: Aging, Amyloid beta, Lipopolysaccharide, Prenatal, Oxidative stress, Synaptotagmin-1, Animal model, Mice.

1. INTRODUCTION

Normal aging in human is manifested by several chemical changes in the brain as well as declines in cognitive functions, especially in hippocampal-dependent learning and memory, which seriously reduces quality of life for older individuals [1]. However, the mechanism of brain aging is not fully understood. Exploring the mechanisms underlying brain aging may hold promise in developing novel interventions for preventing age-related cognitive decline. It is proposed that the early life environment can affect brain function in adulthood, and prenatal exposure to a disadvantageous circumstance may cause unsuccessful brain aging [2, 3]. For instance, prenatal chronic intermittent hypoxia aggravates memory deficit and AD-related neuropathologies in transgenic AD mice [4]. Our middle-aged mice model with maternal exposure to LPS also shows accelerated aged-related memory impairment [5]. In humans, the adults with maternal exposure to famine exhibit an accelerated cognitive decline [2].

In the theories about the mechanisms of brain aging, oxidative-stress theory is one of the most widely acknowledged [6]. It holds that excessive oxidative stress that derived from the overproduction of reactive oxygen species and free radicals is responsible for brain aging [6]. Given the rich unsaturated fatty acids, which produce large amounts of lipid peroxides, and the limited antioxidant capacity, the brain is susceptible to oxidative damage [7]. A reduction in brain antioxidants, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), is correlated with age-related cognitive impairment [8]. In addition, increased oxidative damage in brain during aging might lead to amyloid-beta (Aβ) deposition [9]. Aβ, the main component of amyloid plaques (a characteristic pathological feature of Alzheimer's disease), is also present in at least 20% of normal adults [10]. As the Aβ level in the brain is positively correlated with age-related cognitive impairment, it is a strong predictor of unsuccessful brain aging [10].

Experimental findings have indicated that impaired synaptic plasticity is associated with age-related

*Address correspondence to this author at the Department of Neurology, the Affiliated Chaohu Hospital of Anhui Medical University, and the Center in Psychologic Medicine of Anhui Province, Chaohu, Hefei 238000, Anhui Province, P. R. China; Tel: +86 551 82324252; Fax: +86 551 82324252; E-mail: doctorcgh@163.com

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memory impairment [11]. Synaptotagmin-1 (Syt-1) is a major Ca^{2+} sensor in the pre-synaptic active zone, triggers the fast synchronous fusion of synaptic vesicles and neurotransmitter release, and thus plays an important role in synaptic plasticity [12].

Previously, we treated CD-1 mice with a low dose of lipopolysaccharide (LPS ; i.p. 50μg/kg) for 3 days during late gestation to simulate an inflammatory condition that frequently occurs in pregnant women suffering from cold, influenza, or intestinal infections [5]. The middle-aged (13-month-old) mice offspring had more severely impaired spatial and non-spatial learning and memory abilities after normal growth and development [5]. However, whether corresponding alterations of aging-related neurochemical indicators in the brain exist in this model is unknown. Therefore, we used a middle-aged model of CD-1 mice with maternal exposure to low-dose of LPS, to investigate whether spatial learning and memory abilities associate with several neurochemical indicators of brain aging.

2. METHODS AND MATERIALS

2.1. Animals

The CD-1 colony (12 male, 12 female) purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. Was maintained at 23–25°C and 55 ± 5% humidity on a 12-h light/dark cycle with food and water freely available. Males and females were paired (1:1), and the day that vaginal plug was exhibited was designated as gestational day (gd) 0. Pregnant female mice randomly received an intraperitoneal injection of LPS (50 μg/kg, Escherichia coli LPS, serotype 0127:B8; Sigma-aldrich.) or saline daily during gd 15–17. On postnatal day 21, 3–4 pups of the same sex were housed in polypropylene cages. One male and one female middle-aged (400 day old) offspring of each mice from both groups were randomly selected, *i.e*., 12 mice (males = females) in each group. All animals were treated in compliance with the guidelines for humane treatment set by the Association of Laboratory Animal Sciences.

2.2. Morris Water Maze (*MWM*)

A circular black tank (150 cm in diameter, 30 cm in height) was filled daily with freshwater (22–24 °C) and contained a black escape platform (10 cm in diameter, 24 cm in height) that submerged 1.0-cm below the water surface. The tank was surrounded by a white cloth curtain with three black cardboards of different shapes hung equidistantly served as visual cues.

Experimental Procedures

In the place navigation trial, the mouse was released into the water from different starting positions and underwent four successive trials daily for 10 days. The mouse was allowed a maximum of 60-s to find the platform and upon locating the platform was maintained there for 30-s or be guided and maintained there for 30-s if the mouse failed the locating. The latency (s), swim distance (m) and velocity (m/s) were acquired from the video imported to the computer. The latency and swim distance before locating the platform were considered the indicator of the spatial learning ability. On the last day, 2-h after the last navigation trial, the mouse completed a probe trial that allowed it to swim for 60-s without the platform. The percentage of time the mouse spent in the target quadrant where the platform was previously located was considered a measure of spatial memory. Mice that possess better spatial Memory would spend longer percentage of time in the target quadrant.

2.3. Tissue Preparation

3 days after finishing the behavioral tests the mice were sacrificed, and the brains were rapidly removed and bisected. The left hemisphere was stored for detection of oxidative stress indicators, and the right hemisphere was paraffin-embedded for immunohistochemistry. Distinct hippocampal samples from different tissue blocks were collected, transferred by a needle (10-mm diameter) and relocalized to a new tissue microarray paraffin block. Coronal sections were cut at 6-μm thickness.

2.4. Malondialdehyde (MDA), SOD and GPx

The protein concentration in the brain tissue homogenates was measured by a Bradford assay and then solubilized at the same concentration. The MDA Assay Kit (Biovision technologies, USA) and Activity Assay Kits for SOD and GPx (both from Wuhan Boster biological technology, China) were used following the manufacturer's instructions. The MDA content was expressed as nmol/mg of protein. The activities of SOD and GPx were expressed in U/mg and μmol/min/g of protein, respectively.

2.5. Immunohistochemistry Staining

The streptavidin-biotin-peroxidase complex (SABC) method was used as described [13]. Briefly, antigen retrieval was performed incitrate buffer (pH 6.0) for 20 min in a microwave. After incubating in 0.3 % H_2O_2

solution and blocking with 5 % bovine serum albumin solution, the sections were incubated with anti-$A\beta_{1\text{-}42}$ (Chemicon Millipore co. USA, 1 : 50) or anti-syt-1 (Sigma–Aldrich Inc., 1 : 400) antibodies overnight at 4 °C. Then, the sections were treated with biotin labeled goat anti-rabbit antibody and SABC (Wuhan Boster Bioengineering Limited co., China) and visualized by diaminobenzidine.

The images of the hippocampus were captured by a digital camera mounted on an optical microscope (Olympus). Low magnification (4×) images were obtained for general observation, and high magnification (20×) images of each layer in different subregions were obtained for analysis in the following order: hilus (HL), granule cell layer (GL) and molecular layer (ML) in the dentate gyrus (DG) and ML, radiation layer (RL), pyramidal cell layer (PL) and original layer (OL) in the CA1 subregion. Most CA3 subregions of these samples were missed in the punching process and therefore were not analyzed. The average optical densities of immunoreactive products were measured following the protocol as previously described by Xavier *et al*. [14] using Image-Pro Plus 6.0.

2.6. Statistical Analysis

The data were normally distributed and the results are expressed as the mean ± SEM. The data from the place navigation trial in the MWM was analyzed using repeated measures analysis of variance. The treatment effect in other data and the sex effect were analyzed by independent-samples t test. Pearson correlation test was used to analyze the correlation between MWM performance and neurochemical indicators. All analyses were conducted with SPSS 13.0. The significance level was set at $P < 0.05$.

3. RESULTS

3.1. Performance in the MWM

In the place navigation trial (Figure **1A-C**), the swimming velocity was not affected by the treatment or day (*Ps* > 0.05). The latency and swimming distances progressively shortened with each day for all mice combined [$F_{(9,180)}$ = 4.166 and 4.413, *Ps* < 0.001; respectively]. The LPS-treated mice showed longer latencies [$F_{(1,20)}$ =7.078, P = 0.015] and distances [$F_{(1,20)}$ = 6.445, P = 0.020] than the controls. Sex and the

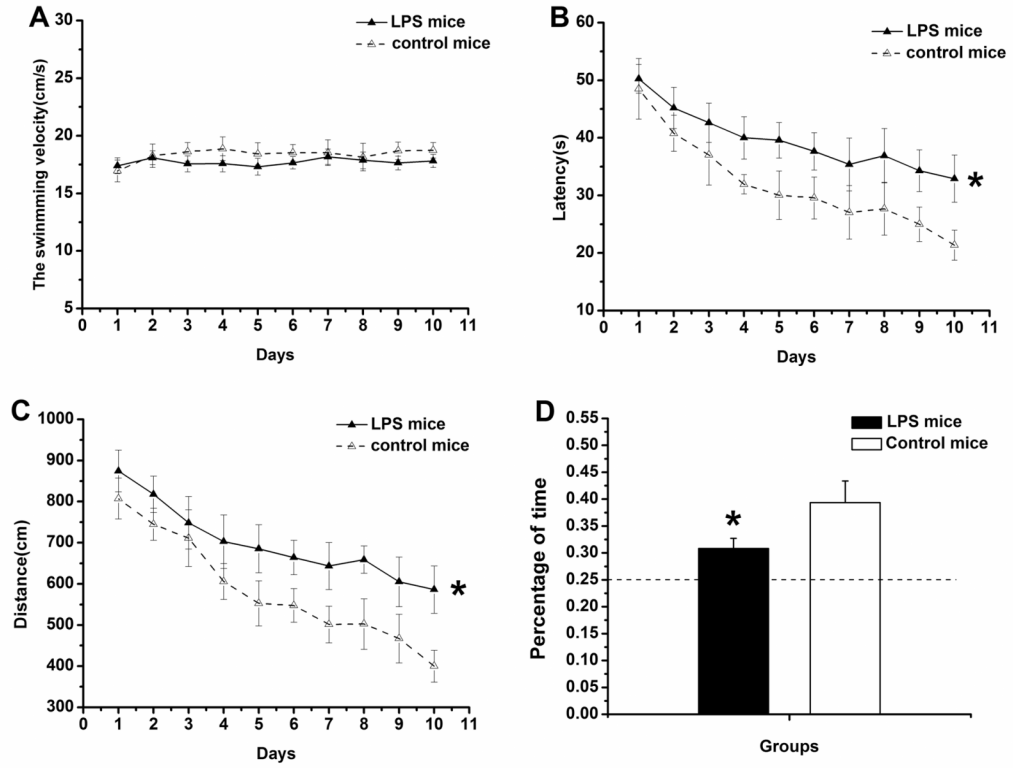

Figure 1: Performance of middle-aged CD-1 mice in the MWM. (**A**) The swimming velocity was similar between LPS group and control group. (**B-C**) The LPS mice exhibited longer latencies and swimming distance than the controls in the place navigation trial. (**D**) The LPS miceexhibited significantly lower percentage of time in the target quadrant than the controls during the probe trial. All values are means ± S.E.M. The bars standing represent for S.E.M. * denotes significant difference compared to the control group ($P < 0.05$).

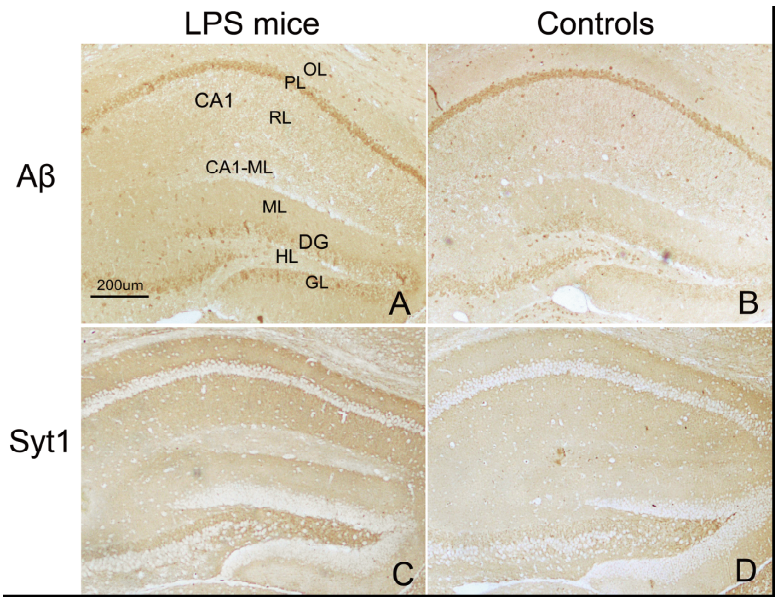

Figure 2: Effect of maternal exposure to LPS on the Aβ and Syt 1 immunohistochemical staining in the hippocampus of middle-aged offspring. Representative low- magnification microphotographs of the immunohistochemical staining in hippocampus of LPS mice (**A** and **C**) and controls (**B** and **D**) were shown. LPS mice had higher immunoreactivity of Aβ (**A** and **B**) and Syt 1 (**C** and **D**) in most layers of hippocampus than the controls (see high-magnification photos in supplementary material). DG, dentate gyrus; GL, granule cell layer; HL, Hilus; ML, molecular layer; OL, original layer; PL, pyramidal cell layer; RL, radiation layer.

interactions of group × day displayed no significant effect (*Ps*> 0.05). In the probe trial, the LPS mice exhibited a significantly lower percentage of time spent in the target quadrant than the controls (t = −2.118, P=0.046; Figure **1D**), indicating impaired spatial memory in the LPS mice. The treatment effect on the MWM performance was not significant when analyzing the sexes separately (*Ps* > 0.05).

3.2. Level of MDA Content and Activities of SOD and GPx

Sex had no significant effect on these indicators (*Ps*> 0.05). The LPS mice exhibited an elevated MDA content than the controls (t = 2.168, P = 0.042), but the treatment effect disappeared when the sexes were analyzed separately (*Ps* > 0.05; Table **1**). The LPS mice also showed decreased SOD activity (t = −5.258, P < 0.001) (in both males and females) and GPx activity (t = −2.986, P = 0.010) (mainly in the females).

3.3. Levels of Aβ and Syt-1 in Different Layers of the Hippocampus

The effect of sex was not significant (*Ps* > 0.05). Compared to the controls (Table **2**), the LPS mice showed significantly increased $A\beta_{1\text{-}42}$ levels in the DG-HL (t = 3.148, P = 0.005), DG-ML (t = 3.227, P = 0.004), CA1-ML (t =3.655, P < 0.001), CA1-RL (t = 5.902, P< 0.001) and CA1-OL (t = 3.598, P < 0.001). This treatment effect on $A\beta_{1\text{-}42}$ in these layers was significant when the sexes were analyzed separately (*Ps* < 0.05). The LPS mice showed significantly increased Syt 1 levels in the DG-HL (t = 2.362, P=0.027), DG-GL (t = 2.289, P= 0.032), DG-ML (t = 2.215, P = 0.037), CA1-ML (t = 2.424, P = 0.024) and CA1-RL (t = 2.317, P = 0.030) than the controls. When

Table 1: Level of MDA Content and Activity of SOD and GPx in Brain

Groups	LPS mice			Controls		
	Total (n=12)	Males (n=6)	Females (n=6)	Total (n=12)	Males (n=6)	Females (n=6)
MDA	61.5 ± 3.1*	61.6 ± 6.4	60.2 ± 7.1	50.3 ± 3.6	57.0 ± 7.3	47.8 ± 6.6
SOD	8.3±0.6**	8.8± 0.7*	8.0 ±0.7*	11.2 ± 0.6	10.8 ± 0.6	11.4 ± 0.5
Gpx	299.0 ±35.7*	302.5 ± 43.2	295.2 ±45.9*	410.7 ±32.8	379.0 ± 44.5	430.1 ± 40.3

The content of MDA is expressed as nmol/mg of protein; the activity of GPxis expressed innmol/min/mg of protein; the activity of SOD is expressed in U/mg of protein. *P < 0.05, **P< 0.01 when compared to the control group.

Table 2: The Levels of Aβ and Syt-1 Indifferent Hippocampal Layers in the Middle-aged CD-1 Mice

	Treatment	DG				CA1			
		HL	GL	ML		ML	RL	PL	OL
Aβ	LPS (n=12)	0.043 ±0.003**	0.119±0.004	0.042±0.003**		0.048±0.003**	0.036±0.002**	0.092±0.008	0.036±0.003**
	Con (n=12)	0.028±0.004	0.111±0.005	0.028±0.003		0.028±0.004	0.018±0.003	0.088±0.009	0.018±0.003
Syt-1	LPS (n=12)	0.361±0.007*	0.253±0.004*	0.349±0.005*		0.317±0.004*	0.360±0.006*	0.266±0.005	0.364±0.005
	Con (n=12)	0.334±0.010	0.237±0.005	0.323±0.009		0.292±0.008	0.333±0.009	0.250±0.007	0.342±0.011

*P < 0.05, **P< 0.01 when compared to the control group. CA1: cornuammonis 1; DG: dentate gyrus; HI:Hilus; GL: granule cell layer; ML: molecular layer; RL: radiation layer; PL: pyramidal cell layer; OL: original layer.

the sexes were analyzed separately, the treatment effect on the Syt-1 level was absent (*Ps* > 0.05).

3.4. Correlations between the MWM Performance and Indicators of Oxidative Stress in the Brain

When all mice were combined, the activities of SOD and GPx were negatively correlated with the latency, and the MDA content was positively correlated with both the latency and distance during place navigation trial (*Ps* < 0.05). The MDA content was also negatively correlated with the percentage of time in the target quadrant during the probe trial (*P* < 0.05). For the LPS mice, the SOD activity was significantly negatively correlated and the MDA level was marginally positively correlated with the place navigation latency (supplementary Table **1**).

SUPPLEMENTARY MATERIAL FOR METHODS

Image Analysis

For the test of each protein, two sections from each animal were chosen. All sections were stained in one formal test under the same experimental conditions and the images were obtained under the same exposure setting. Image analysis was performed using Image-Pro Plus 6.0 image analysis software. Prior to analysis, the subtraction of background staining was performed by the intensity calibration function. For the images of each sample, the intensity of the background staining was measured and the mean value was calculated and used to calibrate the incident light. Areas of interest (AOIs) were selected randomly in each layer in hippocampus subregions and the optical density values of immunoreactive products were measured. The average value of mean optical density (optical density/AOI) was calculated as the average optical density (AOD) in this layer, which represented the relative level of protein. Image analysis was performed follow the blind principle by an investigator unaware to the original number and grouping of the images.

The $A\beta_{1-42}$ levels in the DG-HL, DG-ML, CA1-RL and CA1-OLcorrelated positively with the place navigation latency and distance, and the Aβ level in the DG-HL correlated negatively with the percentage of time in the target quadrant in the probe trial (*Ps* < 0.05). When each group was analyzed separately, significant correlations only existed in the LPS mice.

Supplementary Table 1: The Correlation between Indicators of Oxidative Stress in the Brain and MWM Performances [Sig. (2-Tailed)]

Groups	Trials	Measure	SOD Correlation (P)	GSH-PX Correlation (P)	MDA Correlation (P)
All mice	Place navigation	Distance	-0.243(0.205)	-0.365(0.052)	0.442(0.016)*
		Latency	-0.377(0.044)*	-0.506(0.005)**	0.410(0.027)*
	Probe	% of time in the target quadrant	0.201(0.297)	0.124(0.521)	-0.465(0.011)*
LPS mice	Place navigation	Distance	-0.312(0.277)	-0.033(0.911)	-0.075(0.799)
		Latency	-0.556(0.039)*	-0.103(0.725)	0.489(0.076)
	Probe	% of time in the target quadrant	-0.042(0.886)	-0.099(0.737)	-0.194(0.505)

*P < 0.05.

For Syt-1, the levels in DG-HL, DG-GL, DG-ML, CA1-ML and CA1-PL were positively correlated with the navigation distance, and the DG-HL Syt-1 level was correlated with the navigation latency for all mice combined (*Ps* < 0.05). The Syt-1 levels in the DG-HL and CA1-ML were also negatively correlated with the percentage of time in the target quadrant (*Ps* < 0.05). When the groups were separated, the DG-HL Syt-1 level correlated positively with the probe trial performance in the LPS mice (*P* > 0.05; supplementary Table **2**).

4. DISCUSSION

In this study, the middle-aged mice offspring with maternal exposure to low-dose LPS during the late embryonic phase displayed poorer performance on the MWM, indicating an accelerated age-related declination of spatial learning and memory, which is consistent with our previous study [5]. In addition, the middle-aged LPS mice exhibited increased oxidative stress and elevated $A\beta_{1-42}$ and Syt-1 levels.

SOD and GPx are the two most important enzymes for the antioxidant defense in the brain [15]. During brain aging, the reduced activities of these two enzymes are responsible for age-related cognitive impairments [8]. MDA, a biomarker of oxidative membrane damage under oxidative stress, is also mutagenic and atherogenic due to its interactions with DNA and proteins [16]. In the present study, maternal exposure to LPS elevated the MDA content and decreased the activities of SOD and GPx in the brains of mice offspring during midlife, indicating that maternal exposure to LPS aggravated age-related oxidative stress in the offspring. Furthermore, this enhanced oxidative stress in the brain was closely correlated with impairments in spatial learning and memory, *i.e*., the MDA content in the mice brain was correlated with poorer performance in both place navigation and probe trials of the MWM, and the decreased activities of SOD and GPx were correlated with poorer performance in the place navigation trial. These findings are supported by previous reports showing that chronic exposure in mice to D-galactose induces enhanced MDA in the

Supplementary Table 2: The Correlation between Levels of Proteins in Different Layers of Hippocampus and MWM Performances [Sig. (2-Tailed)]

Proteins	Groups	Trials	Measure	DG			CA1			
				HL	GL	ML	ML	RL	PL	OL
				Correlation (P)	Correlation (P)	Correlation (*P*)	Correlation (*P*)	Correlation (*P*)	Correlation (*P*)	Correlation (*P*)
Aβ	All mice	Place navigation	Latency	.654**(.002)	.101(.673)	.620**(.004)	.443(.057)	.601**(.005)	.397(.128)	.676*(.011)
			Distance	.604**(.006)	.070(.768)	.589**(.006)	.395(.094)	.620**(.004)	.374(.154)	.616*(.025)
		Probe	% of time in the target quadrant	−.468*(.043)	−.061(.798)	−.383(.096)	−.393(.096)	−.330(.156)	−.375(.152)	−.026(.934)
	LPS mice	Place navigation	Latency	.802**(.003)	−.107(.740)	.524(.080)	.299(.372)	.553(.062)	.672*(.048)	.449(.312)
			Distance	.656*(.028)	−.216(.500)	.458(.134)	.110(.748)	.558(.059)	.451(.223)	.290(.528)
		Probe	% of time in the target quadrant	−.637*(.035)	.241(.450)	−.299(.345)	−.564(.071)	−.345(.273)	−.422(.258)	.191(.681)
Syt 1	All mice	Place navigation	Latency	.394*(.031)	.311(.094)	.288(.123)	.377(.053)	.244(.210)	.264(.213)	.185(.398)
			Distance	.518**(.003)	.498**(.005)	.410*(.024)	.486*(.010)	.284(.143)	.437*(.033)	.220(.313)
		Probe	% of time in the target quadrant	-.405*(.026)	-.224(.233)	-.203(.281)	-.419*(.030)	-.317(.100)	-.136(.526)	-.169(.440)
	LPS mice	Place navigation	Latency	.052(.843)	.078(.767)	.031(.907)	.247(.394)	-.057(.841)	-.146(.635)	-.207(.497)
			Distance	.401(.111)	.442(.076)	.281(.274)	.384(.175)	.136(.629)	.269(.374)	-.014(.963)
		Probe	% of time in the target quadrant	-.493*(.044)	-.460(.063)	-.301(.241)	-.511(.062)	-.234(.402)	-.271(.370)	-.060(.846)

*P < 0.05, **P < 0.01.

hippocampus, reduction of SOD activity and accelerated age-related impairment of spatial cognition [17]. Additionally, physical exercise in rats has been shown to upregulate hippocampal SOD and GPx levels and to protect memory ability [18].

Studies have found that exposure to adverse environmental factors during early life could lead to abnormal metabolism of Aβ in aged mice [19, 20]. Using polyriboinosinic-polyribocytidilic acid to mimic a prenatal viral infection can induce accumulation of amyloid precursor protein in aged C57BL/6 mice [19]. Aged monkeys that are exposed to lead as infants have shown increased intracellular Aβ load and extracellular amyloid plaques in the frontal association cortex [20]. Similarly, our results showed that LPS mice had increased hippocampal $A\beta_{1\text{-}42}$ levels, which were closely correlated with the MWM performance. The prenatal adverse environment might epigenetically reprogram the metabolism of Aβ by some unknown mechanism, and Aβ could trigger a pathological cascade in the aging brain, including inflammation, oxidative stress, neuron apoptosis, intracellular calcium overload, and over-phosphorylation of tau, which could contribute to synaptic damage, neuronal death, and eventually learning and memory deficits [21, 22].

Syt-1, a neuronal calcium sensor that induces synchronous neurotransmitter release by promoting synaptic vesicle fusion [23], may be a marker of brain aging. Our previous studies of SAMP8 mice found that the hippocampal Syt-1 level in aged mice increased with impaired learning and memory [24]. Certain prenatal pathological statuses may increase the Syt-1 level in the hippocampus of aged mice. For instance, adverse maternal stress during gestation has been reported to cause overexpression of hippocampal Syt-1 in the offspring, which is associated with learning and memory deficits [25, 26]. In the current study, the LPS-induced maternal inflammatory environment may be such a prenatal pathological situation that leads to an aggravated age-related increase in Syt-1 in most hippocampal layers. The expression of Syt-1 or other genes involved in Syt-1 metabolism in the fetus might be epigenetically affected. Moreover, the Syt-1 level in the hippocampus was closely correlated with MWM performance, indicating that elevated Syt-1 might be linked to the decline in spatial learning and memory abilities in the LPS offspring.

The present study helps better understanding of the mechanisms underlying brain aging and suggests that maternal illness might be an initiator of accelerated aging in the offspring. Therefore, we should pay more attention to the health of pregnant women and to the offspring with prenatal exposure to a disadvantage circumstance. If necessary, early intervention should be given to prevent the accelerated brain aging of the offspring.

5. CONCLUSION

The middle-aged CD-1 mice offspring, whose mothers received a low dose of LPS during late gestation, exhibited accelerated age-related spatial learning and memory impairment. The model also displayed corresponding alterations of aging-related neurochemical indicators in the brain. What's more, the correlation analysis indicated that the increased oxidative stress in the brain (elevated MDA, decreased SOD and GPx) and elevated Aβ and Syt-1 levels in the hippocampus might be responsible for the accelerated age-related decline of learning and memory in the middle-aged LPS mice. In this study, the immunohistochemical data only indicated the relative levels of the proteins. Whether maternal exposure to LPS mediates its effects via alterations in epigenetic regulation is unknown. Further research is needed to explore the precise mechanism of how maternal LPS exposure leads to accelerated brain aging using more accurate and advanced methods.

INTEREST

The authors report no conflicts of interest.

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REFERENCES

[1] Grady CL, Craik FI. Changes in memory processing with age. Curr Opin Neurobiol. 2000;10:224-31. http://dx.doi.org/10.1016/S0959-4388(00)00073-8

[2] de Rooij SR, Wouters H, Yonker JE, *et al*. Prenatal undernutrition and cognitive function in late adulthood. Proc Natl Acad Sci U S A. 2010;107:16881-6. http://dx.doi.org/10.1073/pnas.1009459107

[3] Akbarian S, Beeri MS, Haroutunian V. Epigenetic determinants of healthy and diseased brain aging and cognition. JAMA Neurol. 2013;70:711-8. http://dx.doi.org/10.1001/jamaneurol.2013.1459

[4] Zhang X, Li LX, Zhang XJ, ,*et al*. Prenatal hypoxia may aggravate the cognitive impairment and Alzheimer's disease neuropathology in APPSwe/PS1A246E transgenic mice.

Neurobiology of Aging. 2013;34:663–78. http://dx.doi.org/10.1016/j.neurobiolaging.2012.06.012

[5] Chen GH, Wang H, Yang QG, *et al*. Acceleration of age-related learning and memory decline in middle-aged CD-1 mice due to maternal exposure to lipopolysaccharide during late pregnancy. Behav Brain Res. 2011;218:267-79. http://dx.doi.org/10.1016/j.bbr.2010.11.001

[6] Venkateshappa C, Harish G, Mahadevan A, *et al*. Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer's disease. Neurochem Res. 2012;37:1601-14. http://dx.doi.org/10.1007/s11064-012-0755-8

[7] Emerit J, Edeas M, Bricaire F. Neurodegenerative diseases and oxidative stress. Biomed Pharmacother. 2004;58:39-46. http://dx.doi.org/10.1016/j.biopha.2003.11.004

[8] Droge W, Schipper HM. Oxidative stress and aberrant signaling in aging and cognitive decline. Aging Cell. 2007;6:361-70. http://dx.doi.org/10.1111/j.1474-9726.2007.00294.x

[9] Sutherland GT, Chami B, Youssef P, *et al* . Oxidative stress in Alzheimer's disease: Primary villain or physiological by-product? Redox Rep. 2013;18:134-41. http://dx.doi.org/10.1179/1351000213Y.0000000052

[10] Rodrigue KM, Kennedy KM, Park DC. Beta-amyloid deposition and the aging brain. Neuropsychol Rev. 2009;19:436-50. http://dx.doi.org/10.1007/s11065-009-9118-x

[11] Vanguilder HD, Freeman WM. The hippocampal neuroproteome with aging and cognitive decline: past progress and future directions. Front Aging Neurosci. 2011;3:8. http://dx.doi.org/10.3389/fnagi.2011.00008

[12] Xu J, Pang ZP, Shin OH, *et al*. Synaptotagmin-1 functions as a Ca2+ sensor for spontaneous release. Nat Neurosci. 2009;12:759-66. http://dx.doi.org/10.1038/nn.2320

[13] Nagashima K, Zabriskie JB, Lyons MJ. Virus-induced obesity in mice: association with a hypothalamic lesion. J Neuropathol Exp Neurol. 1992;51:101-9. http://dx.doi.org/10.1097/00005072-199201000-00012

[14] Xavier LL, Viola GG, Ferraz AC, *et al* . A simple and fast densitometric method for the analysis of tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta and in the ventral tegmental area. Brain Res Brain Res Protoc. 2005;16:58-64. http://dx.doi.org/10.1016/j.brainresprot.2005.10.002

[15] Milton VJ, Sweeney ST. Oxidative stress in synapse development and function. Dev Neurobiol. 2012;72:100-10. http://dx.doi.org/10.1002/dneu.20957

[16] Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis. 2005;15:316-28. http://dx.doi.org/10.1016/j.numecd.2005.05.003

[17] Wang W, Li S, Dong HP, *et al*. Differential impairment of spatial and nonspatial cognition in a mouse model of brain aging. Life Sci. 2009;85:127-35. http://dx.doi.org/10.1016/j.lfs.2009.05.003

[18] Yu F, Xu B, Song C, *et al* . Treadmill exercise slows cognitive deficits in aging rats by antioxidation and inhibition of amyloid production. Neuroreport. 2013;24:342-7. http://dx.doi.org/10.1097/WNR.0b013e3283606c5e

[19] Krstic D, Madhusudan A, Doehner J, *et al*. Systemic immune challenges trigger and drive Alzheimer-like neuropathology in mice. J Neuroinflammation. 2012;9:151. http://dx.doi.org/10.1186/1742-2094-9-151

[20] Wu J, Basha MR, Brock B, *et al*. Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (Pb): evidence for a developmental origin and environmental link for AD. J Neurosci. 2008;28:3-9. http://dx.doi.org/10.1523/JNEUROSCI.4405-07.2008

[21] Poling A, Morgan-Paisley K, Panos JJ, *et al*. Oligomers of the amyloid-beta protein disrupt working memory: confirmation with two behavioral procedures. Behav Brain Res. 2008;193:230-4. http://dx.doi.org/10.1016/j.bbr.2008.06.001

[22] Armstrong RA. The pathogenesis of Alzheimer's disease: a reevaluation of the "amyloid cascade hypothesis". Int J Alzheimers Dis. 2011;2011:630865. http://dx.doi.org/10.4061/2011/630865

[23] Fernandez-Chacon R, Konigstorfer A, Gerber SH, *et al* . Synaptotagmin I functions as a calcium regulator of release probability. Nature. 2001;410:41-9. http://dx.doi.org/10.1038/35065004

[24] Chen GH, Wang YJ, Qin S, *et al* . Age-related spatial cognitive impairment is correlated with increase of synaptotagmin 1 in dorsal hippocampus in SAMP8 mice. Neurobiol Aging. 2007;28:611-8. http://dx.doi.org/10.1016/j.neurobiolaging.2006.03.001

[25] Jia N, Yang K, Sun Q, *et al* . Prenatal stress causes dendritic atrophy of pyramidal neurons in hippocampal CA3 region by glutamate in offspring rats. Dev Neurobiol. 2010;70:114-25.

[26] Thome J, Pesold B, Baader M, *et al* . Stress differentially regulates synaptophysin and synaptotagmin expression in hippocampus. Biol Psychiatry. 2001;50:809-12. http://dx.doi.org/10.1016/S0006-3223(01)01229-X

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