Lipoic Acid Facilitates Learning and Memory in Aged Mice

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Abstract Lipoic acid (LA) is identified as an antioxidant to scavenge and inactivate free radicals. Although LA has been used as a dietary supplement for a long time, the effect of LA supplement on preventing aged-memory loss haven't been studied yet. This study aimed to determine whether LA could promote the ability of learning and memory in the aged mice model. There were four groups in this study: aged control group (20 months old C57BL / 6J mice without any dosage), 0.1% LA dosage group (20 months old C57BL / 6J mice), 0.2% LA dosage group (20 months old C57BL / 6J mice), and young control group(3 months old C57BL / 6J mice without any dosage). Cognitive abilities were tested through behavioral tests, and factors related to synaptic plasticity (BDNF, PSD95, p-NMDAR1, p-CAMK II) were detected by Western Blot. In behavioral test, young control group shown a greater capacity in learning and memory than aged control group. Comparing with aged control group, the abilities of passive avoidance and spatial memory and learning in two LA dosage groups were significantly enhanced by using step-down test and Morris water maze respectively. Up-regulated expression of BDNF and higher levels of PSD95, p-NMDAR1, p-CAMK II were observed in both two LA dosage groups and young control group compared with aged control group. Our findings suggest that long-term LA intervention can effectively delay or prevent the decline of learning and memory associated with aging via altered synaptic plasticity.

Keywords: Lipoic acid, learning and memory, aging, synaptic plasticity

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1. Introduction

With the age distribution of the global population steadily increasing, more and more aging people are suffering from age-related cognitive decline [1]. Late-life cognitive decline ranges from normal age-related change at its mildest, through mild cognitive impairment, to dementia at its most severe, Cognitive decline of the elderly, including learning impairment and delayed amnesia can generate significant levels of functional dependence and reduce quality of life [2,3].

Mounting evidence suggests that dietary intervention can effectively prevent or delay the age-induced decline in learning and memory [4,5]. Since oxidative stress plays an important role in the pathogenesis of cognitive decline, the potential of antioxidant supplements to prevent neurodegenerative diseases have gained much interest [6].

Lipoic acid (LA), the chemical name 1,2-dithiolane-3-pentanoic acid, is the first natural product isolated from porcine liver by Reed in 1951 [7]. In the past, LA was considered to be a vitamin-like substance that must be supplemented by food. However, studies have shown that mitochondria in mammals and higher plants are capable of synthesizing LA, and it can be synthesized in the mitochondria of the liver and other tissues, while LA synthesized is not sufficient to meet needs [8]. LA is easily absorbed by the digestive tract and can be distributed to various parts of the body since it can dissolve in both aqueous and lipid phase. It can also pass through the blood-brain barrier to protect the nervous system from oxidative damage [9].

LA can directly remove oxygen radicals [10]. It can also act as a blocking agent in oxidation-reduction reaction chain. LA also has the function of regeneration of endogenous antioxidants, such as direct regeneration of glutathione, indirect regeneration VitC, VitE and other antioxidants. A large number of experiments have confirmed that LA is a more powerful antioxidant compared with VitC and VitE [9,11]. As the strongest anti-oxidant small molecule chemical to date, LA is widely used in the treatment and prevention of various diseases [12,13,14]. Some animal studies have shown that LA may have effect on Alzheimer disease [15]. However, at present, there is no reports about whether LA treatment can prevent or delay the decline of learning and memory caused by aging.

Formation and persistence of memory are complex processes. Synaptic plastic mechanisms are necessary for the information processing and storage (the underlying cognitive). Learning is believed to initially occur via altered synaptic strength by processes such as long-term potentiation (LTP) and long-term depression. In the mid-twentieth century, the idea that changes in the efficacy of synapses within diverse neural circuits could mediate the storage of information was first proposed [16]. Nearly 40 years of studies have shown that synaptic plasticity is the necessary and sufficient condition for learning and memory [17,18,19]. In this study, 20-month-old C57BL/6J mice were used as models to explore the effect of LA treatment on age-related cognitive decline and its effect on synaptic plasticity.

2. Materials and Methods

2.1. Animals

Female C57BL/6J mice of SPF level were obtained from the Animal Service of Health Science Center, Peking University. The animals were maintained under controlled ambient temperature conditions (22±1°C) and humidity (55±5%).

Sixty female C57BL / 6J mice of 20 months old and C57BL / 6J mice of 3 months old raised in SPF animal room were subjected in this study. The aged mice were divided into three groups: aged control group and two LA experimental groups. Two LA groups were fed with special processed feed supplemented with 0.1% and 0.2% LA respectively. The aged control group and the young control group were fed with normal fodder. All animals ate freely. Behavioral tests were performed 6 months after intervention, including the Morris water maze, platform tests and shuttle-box tests.

2.2. Step-down Test

A step-down passive avoidance was examined by using apparatus consisted of a box (25×25×40cm), a floor with stainless-steel grids 2 mm in diameter at 8 mm intervals, and a rubber platform (4 cm diameter, 4 cm height) set on the grid in one corner. Electric stimulation was given through the grid connected with a scrambled shock generator. After 6 months of treatment, an acquisition trial was performed. In this trial, each mouse was placed gently on the platform and allowed to habituate freely for 3 min, and then electric shocks (0.4 mA) were delivered to the grid. If the mouse stepped down from the platform, the electric shock was delivered to the mouse on the grid floor. The cutoff time was 5 min. A retention trial was performed 24 h after the acquisition trial. Each mouse was again placed on the platform. The time (step-down latency) that elapsed until the mouse stepped down from the platform was recorded. If the mouse did not step down from the platform within 300 s, the retention trial was terminated and the maximal step down latency of 300 s was recorded. Each error was counted whenever the mouse stepped down from the platform and the number of errors made within 5 min was recorded [20].

2.3. Shuttle-box Test

The shuttle box escape test was used to measure escape learning. The procedure was modified from Gonzales [21]. The shuttle box (60×20×20 cm) consisted of two equally-sized compartments separated by an open door. The chamber was enclosed in a sound-attenuated box and illuminated by a white light. Electric shocks were delivered via a floor of mental bars spaced 1.5 cm apart. Eight sets of infrared beams located 2 cm above the grid floor allowed for motion detection and tracking of position of each dam during the testing period. In the test, sound was used as conditioned stimulus and electric shock as unconditioned stimulus. The mouse was put into the chamber to adapt for 3 min, and then it was given buzzing stimulation for 5S (60dB). If the animal did not cross to the other chamber, it was given electric shock (10mA). The mice required to make a crossing from one side of the chamber to the other to terminate the shock. The electric shock had a maximum duration of 10s. After 60 s interval, acoustic stimulation was given again. Each test was conducted for 30 trials, lasting for 6 days. Active avoidance reaction (AAR) was the one that can complete the shuttle action after sound stimulation, and passive avoidance reaction (PAR) was the one that can only complete the shuttle action after electrical stimulation. Failure is the one that cannot escape.

2.4. Morris Water Maze

The procedures of Morris water maze (MWM) were modified from Morris [22]. The experimental apparatus which used in the Morris water maze test consisted of a circular water tank (120 cm in diameter, 35 cm in height), containing water (24 ± 1°C) to a depth of 20 cm, which was rendered opaque by adding milk. A platform (4.5 cm in diameter, 19 cm in height) was submerged 1 cm below the water surface and placed at the midpoint of one quadrant. The pool was located in a test room, which contained various prominent visual cues. Each mouse received four training periods per day for seven consecutive days. Before the first trail, each mouse was put on the platform for 10 s, and then it was given 30 s free swim and then was assisted to the platform where it was remained for another 10 s rest. For each trial, mouse was placed in the water facing the wall at one of four starting positions, and the time required for the released mouse to find the hidden platform was recorded. Mouse that found the platform was allowed to remain on the platform for 10 s and then returned to its cage for the inter-trial interval. A mouse failed to find the platform within 90 s was placed on the platform for 10 s at the end of the trial. Latency to escape from the water maze (finding the submerged escape platform), swimming distance and average speed to reach the platform were recorded for each trial. To measure the strength of the spatial memory, on day 7, a "probe test" was performed, during which, mice were swimming freely for 60 s in the pool without platform. In the ''probe test" calculated were: (1) the time (in seconds) spent by mice in the target quadrant in which the platform was hidden during acquisition trails, and (2) the number of times exactly

crossing over the previous position of the platform. Swimming behavior during the test was video-recorded using a commercial VCR and analysed by a PC computer.

2.5. Western Blot

Mice were deeply anesthetised and their hippocampuses were rapidly removed and stored at - 80°C until use. The tissue samples were pooled and lysed in a buffer (50mM Tris–HCl, pH 6.8, 10% glycerol, 2% sodium dodecyl sulfate, and 5% b-mercaptoethanol). 50μg protein lysate were separated by 8% SDS–PAGE and transferred onto polyvinylidene difluoride (PVDF) membrane. After blocked in a 5% non-fat dry milk solution in washing buffer, containing 10 mM Tris (pH7.5), 150 mM NaCl, and 0.05% Tween-20, membranes were incubated overnight at 4°C with primary antibody (monoclonal anti-BDNF, 1:1000, Santa Cruz Biotechnology, USA; monoclonal anti-PSD95, 1:250, Chemicon, Temecula, CA; monoclonal anti-phospho-CaMK□, Neuromics, USA; monoclonal anti-phospho-NMDAR1, Neuromics, USA). After washing three times with TBS, membranes were incubated for 1.5 h with horseradish peroxidase-coupled secondary antibody at roomtemperature. Following the post-secondary washes, the resulting antigen–antibody-peroxidase complex was detected by using the ECL kit (Amersham Pharmacia Biotech) and visualised by exposures of various lengths to Kodak film.

2.6. Statistical Analyses

Data were expressed as mean ± SEM. Effects on escape latency and swimming distance in Morris water maze between groups were evaluated by using repeated-measures two-way ANOVA followed by a least significant difference test as a post hoc test. To evaluate other effects of LA, difference between all groups were compared using one-way ANOVA followed by a least significant difference test as a post hoc test or Dunnett's test. For all statistical tests, p < 0.05 was considered significant. All statistical analyses were performed with the Statistical Package for Social Sciences for Windows version 11.0 (SPSS Inc. Chicago, IL. USA).

3. Results

3.1. Impact on the Latency and Number of Errors in Step-down Test

As shown in Table 1, the latency reduced significantly in aged control compared with young control group, which indicated that the learning and memory ability of the aged mice was decreased. The latency of 0.1% and 0.2% LA experimental group prolonged significantly compared with aged control group, and there was no significant latency difference between young group and LA experimental groups. Although number of errors in 0.1% or 0.2% LA experimental group and young control group showed a declining trend compared with aged control group, no significant difference was found.

Table 1. The impact of LA on the latency and number of errors during the step-down test

Group	n	latency(s)	Errors(times/5min)
Aged control	15	90.93±28.80[††]	1.67±0.81
0.1%LA	16	107.25±33.85	1.31±0.87
0.2%LA	16	129.25±36.23[*]	1.44±0.72
Young control	16	153.00±30.25	1.38±0.61

*$p < 0.05$ compared to aged control mice;
††$p < 0.01$ compared to young control mice.

3.2. Effect on the Active Avoidance Response

The effect of LA on the active avoidance response of mice was determined by using Shuttle-box Test (Table 2). With the increase of training days, the level of active avoidance response in each group gradually increased. No significant difference was found in active avoidance response rate between the two groups on the first and second day of the experiment. From the third day to the sixth day, the active avoidance response rate of the young control group mice was significantly higher than that of the aged control group. On the third and fifth day of the experiment, the AAR (active avoidance response) level of 0.2% LA experimental group was significantly higher than that of the aged control group. On the fifth day, the active avoidance response rate of 0.1% LA experimental group was significantly higher than the aged control group.

3.3. Effect on the Spatial Memory Behavior

The effect of LA on the spatial memory behavior of mice was examined by using Morris water maze test (Figure 1 A). With the increase of training days, the escape latency in each group gradually reduced [F (5,295) = 119.95, p<0.01]. Post hoc test revealed a significant increase in escape latency in aged control mice compared with young control mice and LA-treated mice ($p < 0.01$). The latency of the LA experimental group was significantly longer than that of the young control group (p<0.05). The results of swimming distance analysis (Figure 1 B) showed that the swimming distance of the mice in each group was significantly shortened [F (5,295) = 173.78, p <0.01]. Compared with the old control group, the swimming distance of LA experimental groups and young control group was markly shortened (p <0.01). Swimming distance of 0.1% or 0.2% LA experimental group was significantly longer than that of young control group (p <0.05). There were no differences between LA treated and control mice in the swimming speed (data not shown).

During the probe test, mice were swimming in the vicinity of the place that contained escape platform during the acquisition trial. Young mice showed higher precision of the search for the platform as revealed by more time spent at the former platform position (Table 3). LA seemed to restore the lost procedural subcomponent of spatial memory in aged mice, but only the 0.2% LA had significantly increased on the time spent at target quadrant than that of aged control mice (p <0.05).

Table 2. The effects of LA on the active avoidance response of the aged mice in the shuttle box test

Group	n	AAR level (%)					
		Day1	Day2	Day3	Day4	Day5	Day6
Aged control	15	15.33±10.60	20.67±8.83	28.67±11.26[††]	29.33±14.37[††]	30.67±14.37[††]	31.33±15.05[†]
0.1%LA	16	15.63±9.64	21.25±11.48	35.00±13.17	33.75±13.60	40.63±7.71[*]	38.13±13.27
0.2%LA	16	18.75±8.85	25.03±11.55	39.06±14.05[*]	39.38±11.81	41.88±14.71[*]	41.25±16.28
Young control	16	19.38±8.54	28.13±12.23	43.13±11.96	44.38±17.50	46.25±15.44	44.37±15.90

[*]*p* < 0.05 compared to aged control;
[†]*p* <0.05 compared to young control mice;
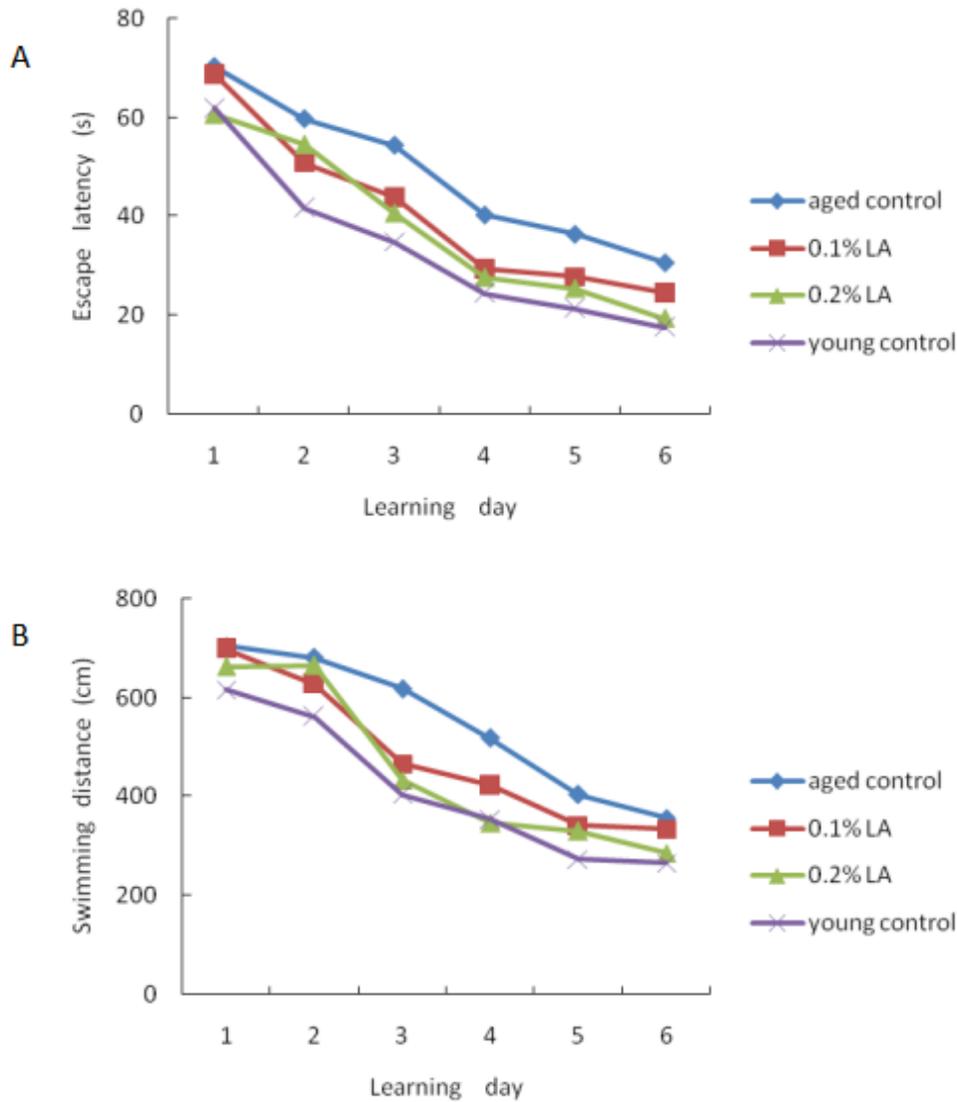[††]*p* < 0.01 compared to young control mice.

Figure 1. Effects of LA on spatial learning and memory of mice in Morris water maze test

Table 3. The effects of LA on time spent in quadrant and number of crossing in Morris water maze test

Group	n	Time spent in quadrant (sec)	Number of crossing
Aged control	15	15.35±2.69[††]	3.07±1.16
0.1% LA	16	16.65±2.48	3.25±1.06
0.2% LA	16	17.23±2.59[*]	3.38±1.3
Young control	16	17.97±2.31[**]	3.44±1.21

[*]*p* < 0.05 compared to aged control;
[**]*p* < 0.01 compared to aged control;
[†]*p* <0.05 compared to young control mice;
[††]*p* < 0.01 compared to young control mice.

3.4. Effect on BDNF Expression

The expressions of BDNF mRNA and protein in the hippocampus were detected by RT-PCR and Western blot. As shown in Figure 2, Western blot and RT-PCR from the aged group showed weak hippocampal BDNF compared with the young mice. The expression of hippocampal BDNF was significantly up-regulated by 25% and 35% in aged mice feed with 0.1% and 0.2% LA for 6 months, respectively. There was no significant difference between the two LA experimental groups and the young control group.
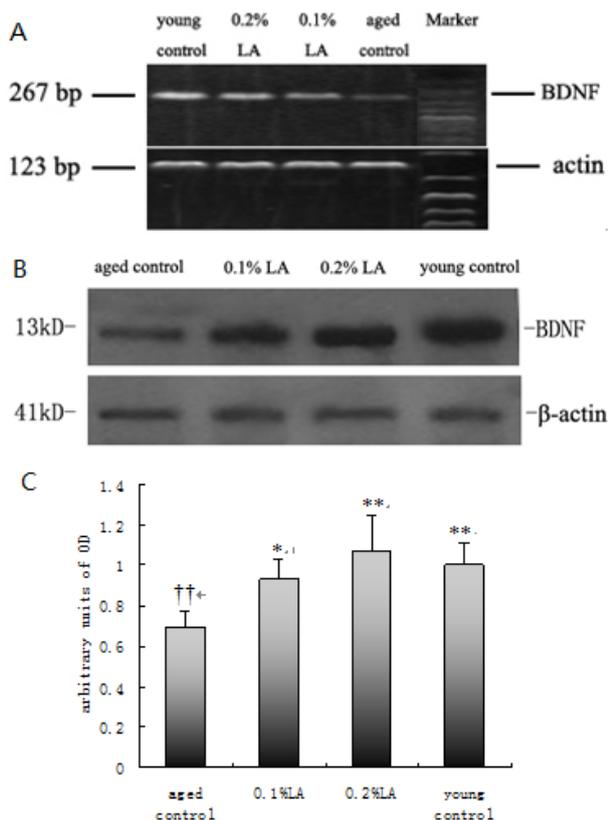

Figure 2. Immunoblot analysis of BDNF in the hippocampus of the young, aged and LA-treated mice (*$p < 0.05$, **$p < 0.01$ vs. the aged control group; †$p<0.05$, ††$p < 0.01$ vs. the young control group)

3.5. Effect on Synaptic Plasticity

The expression of Postsynaptic density 95 (PSD95), N-methyl-d-aspartic acid receptors(p-NMDAR1) and Ca2+/calmodulin-dependent protein kinase II (p-CAMKII) in the hippocampus of the four groups were showed in Figure 3. Compared with the young control group, the expression of PSD95, p-NMDAR1 and p-CAMKII in hippocampus of aged mice decreased by 34%, 42% and 44% respectively. The expression of PSD95 in hippocampus increased by 41% after 6 months of intervention with 0.2% LA. The expression of p-NMDAR1 was increased by 39% respectively in 0.2% LA experimental group, compared with the aged control group. The expression of P-CAMKII increased by 42% and 53% respectively in 0.1% and 0.2% LA groups compared with aged mice. Compared with young control group, the expression of PSD95, p-NMDAR1 and

p-CAMK II in hippocampus of two LA treated mouse groups had no significant difference.
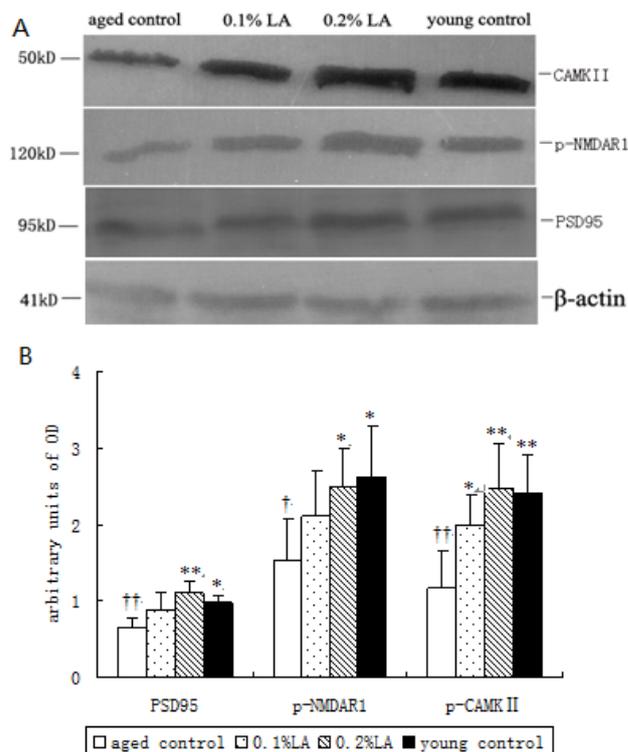

Figure 3. Immunoblot analysis of PSD95, NMDAR and CAMKII in the hippocampus of the young, aged and LA-treated mice (*$p < 0.05$, **$p < 0.01$ vs. the aged control group; †$p<0.05$, ††$p < 0.01$ vs. the young control group)

4. Discussion

Our study found the positive effect of LA on memory in the C57BL / 6Jmice. Behavioral tests, such as step-down test and Morris water maze test, indicated that LA could improve the learning and memory ability of aged mice. Meanwhile, the expression of BDNF, PSD95, p-NMDAR1 and p-CAMK II in the hippocampus of 0.1%, 0.2% LA treated mice were all higher than that of the aged control group, and this indicated that LA could improve the synaptic plasticity of aged mice which might attribute to the repairment of learning and memory in aged mice.

Previous studies concentrated on the effect of LA on diabetes, obesity, and Alzheimer disease [12,13,15,23]. LA supplementation was found to be a potential treatment for the observed loss of cellular energetics in Alzheimer disease and it could potentiate the antioxidant defense system to prevent or delay the oxidative stress in the progression of this devastating dementing disorder [15,23]. However, few studies were focused on the effect of LA on age-related cognitive decline. In the only one related study, Susan *et al* gave 40 18-month-old SAMP8 mice either LA or vehicle for 4 weeks subcutaneously, then tested the mice in object recognition or Barnes maze. They found that LA improved memory and reversed indices of oxidative stress in extremely old SAMP8 mice [24]. In our study, we used C57BL / 6J mice which were fed with LA for 6 months, and got similar findings to Susan's study.

There were various hypotheses about the underlying mechanisms of aged-cognitive decline, among which decreasing synaptic plasticity related to memory loss has aroused interest in recent years [25,26]. PSD95, p-NMDAR1 and p-CAMKII are highly relevant for synaptic plasticity-related events in hippocampus [27]. In our study, we found that the expression of PSD95, p-NMDAR1 and p-CAMK II in the hippocampus of aged control group was lower than that of the young control group. Additionally, up-regulated expression BDNF can enhance synaptic plasticity which is crucial for long-time memory [28]. BDNF is an important nutrient factor in neurons and glial cells, and it is considered to be one of the key proteins in the process of memory formation [29,30]. Declining memory performance during normal aging is associated with reduced BDNF levels [31,32,33]. In the present study, lower level of BDNF was observed in aged control group compared with young control group.

Previous studies on LA were focused on its anti-oxidative function, while there are few researches about the effect of LA on synaptic plasticity. The present study found that LA can promote the expression of BDNF in hippocampus, consistent with the previous studies [34,35]. LA supplementation could lead to stimulating an insulin-like effect and reversing the impaired synaptic plasticity in the old mice [36]. Liu found that LA treatment in mice prevented several high-fat diet-induced metabolic changes and preserved synaptic plasticity [37]. Aforementioned studies used electrophysiology to assess synaptic plasticity. While in our study, we chose proteins (PSD95, p-NMDAR1 and p-CAMKII) highly relevant to synaptic plasticity to find the mechanism of LA acting on synaptic plasticity. The expression of PSD95, p-NMDAR1 and p-CAMKII in the hippocampus of 0.1%, 0.2% LA treated mice both were higher than that of the aged control group, which suggests that LA may reverse the impaired synaptic plasticity to delay or prevent the decline of learning and memory associated with aging.

Synaptic plasticity describes the process by which synapses change in strength depending on the pattern of neuronal activity, and PSD95, NMDAR and CAMKII play an important role in synaptic plasticity [28,38]. The decline of synaptic plasticity is the main reason for the decline of aged-related learning and memory ability [39]. PSD95 is highly abundant in the postsynaptic density, a major constituent of dendritic spines, and it can be regarded as key molecules in neuronal information storage-related processes [40]. The activation of NMDAR and CAMKII is key process of the formation of LTP. BDNF, as a neurotrophic factor, contributes to the learning and memory process by acting on synaptic plasticity [41]. LTP is an activity-induced long-lasting increase in the excitatory synaptic strength identified as the prime candidate to be the cellular correlate of learning and memory, BDNF is a major regulator of LTP in the hippocampus [42,43]. Therefore, the present study used PSD95-NMDAR-CaMKII pathway and BDNF as synaptic plasticity markers to examine the mechanism of LA preventing aged-related memory loss.

However, further examinations are needed to elucidate the detailed mechanisms on how LA affects the expression of PSD95-NMDAR-CaMKII pathway and BDNF in brain.

5. Conclusions

In conclusion, the present study demonstrates that LA can facilitate learning and memory in aged mice through increasing BDNF, PSD95, NMDAR and CAMKII expression level. Future studies should also investigate whether LA can prevent aged-related cognitive decline in human study. It may be a candidate for functional food or drugs to manage/reduce memory deficits associated with aging.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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