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Downregulation of caveolin-1 contributes to the synaptic plasticity deficit in the hippocampus of aged rats

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Research Highlights

(1) The present study demonstrated for the first time the differences in caveolin-1 protein expression in various brain regions across different ages. Caveolin-1 protein expression changed in an age-dependent manner.

(2) Caveolin-1 and synaptophysin declined with age in the hippocampus. Synaptophysin levels were strongly associated with age-related memory impairment.

Abstract

Caveolin-1 is involved in the regulation of synaptic plasticity, but the relationship between its expression and cognitive function during aging remains controversial. To explore the relationship between synaptic plasticity in the aging process and changes in learning and memory, we examined caveolin-1 expression in the hippocampus, cortex and cerebellum of rats at different ages. We also examined the relationship between the expression of caveolin-1 and synaptophysin, a marker of synaptic plasticity. Hippocampal caveolin-1 and synaptophysin expression in aged (22–24 month old) rats was significantly lower than that in young (1 month old) and adult (4 months old) rats. Expression levels of both proteins were significantly greater in the cortex of aged rats than in that of young or adult rats, and levels were similar between the three age groups in the cerebellum. Linear regression analysis revealed that hippocampal expression of synaptophysin was associated with memory and learning abilities. Moreover, synaptophysin expression correlated positively with caveolin-1 expression in the hippocampus, cortex and cerebellum. These results confirm that caveolin-1 has a regulatory effect on synaptic plasticity, and suggest that the downregulation of hippocampal caveolin-1 expression causes a decrease in synaptic plasticity during physiological aging.

Key Words

neural regeneration; cognitive function; aging; caveolin-1; synaptic plasticity; hippocampus; synaptophysin; grants-supported paper; neuroregeneration

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INTRODUCTION

Cognitive function is known to change during normal aging and in age-related disease such as Alzheimer's disease. Although the exact cause for age-related cognitive decline is not clear, recent studies have implicated a contribution of disturbed mechanisms of plasticity in cognitive dysfunction during the normal aging process^[1]. Synaptic plasticity is a fundamental feature of the central nervous system that allows synapses to "remember" previous activity and bring about plastic changes to fine-tune synaptic action^[2], which is reduced with aging. A decrease in synaptic plasticity may change the dynamic interactions between cells in hippocampal networks, causing deficits in the storage and retrieval of information^[3]. Subtle alterations in hippocampal synaptic efficacy often precede neuronal degeneration^[4]. This disturbed plasticity may contribute to age-related cognitive dysfunction. Deficits in synaptic plasticity have been identified in aged animals^[5-7]; however, the factors affecting synaptic plasticity remain unclear. Mounting evidence implicates caveolin-1 as playing an important role in the modulation of synaptic plasticity^[8-9].

Caveolin-1 is the principal structural component of caveolae and participates in cellular cholesterol homeostasis, molecular transport and transmembrane signaling events^[10-12]. Recent work has shown that caveolin-1 may act as a modulator of compensatory synaptic plasticity in Alzheimer's disease^[8]. Another study has also demonstrated that alterations in caveolin-1 expression coincide with the onset of reactive synaptogenesis during injury-induced synaptic remodeling^[9]. Therefore, it is speculated that caveolin-1 may have a lifelong modulatory role in synaptic plasticity and contribute to neurodegenerative disease.

To our knowledge, no studies of caveolin-1 modulation of age-dependent synaptic plasticity have been reported, and studies of age-related changes in hippocampal caveolin-1 are inconclusive. Whereas some studies report upregulated caveolin-1 expression

in the hippocampus of aged mice^[9], others have found the inverse^[13]. This prompted us to investigate caveolin-1 expression in different brain regions and the regulatory function of caveolin-1 on synaptic plasticity during the aging process. We also examined the expression of synaptophysin, a marker of synaptic plasticity^[14], in the hippocampus, cortex and cerebellum of rats at different ages and analyzed its relationship with learning ability in the Y-maze. Furthermore, we investigated caveolin-1 protein levels in the hippocampus, cortex and cerebellum of aging rats and their correlation with synaptophysin levels, with the aim of elucidating the mechanism by which caveolin-1 regulates synaptic plasticity during development and aging.

RESULTS

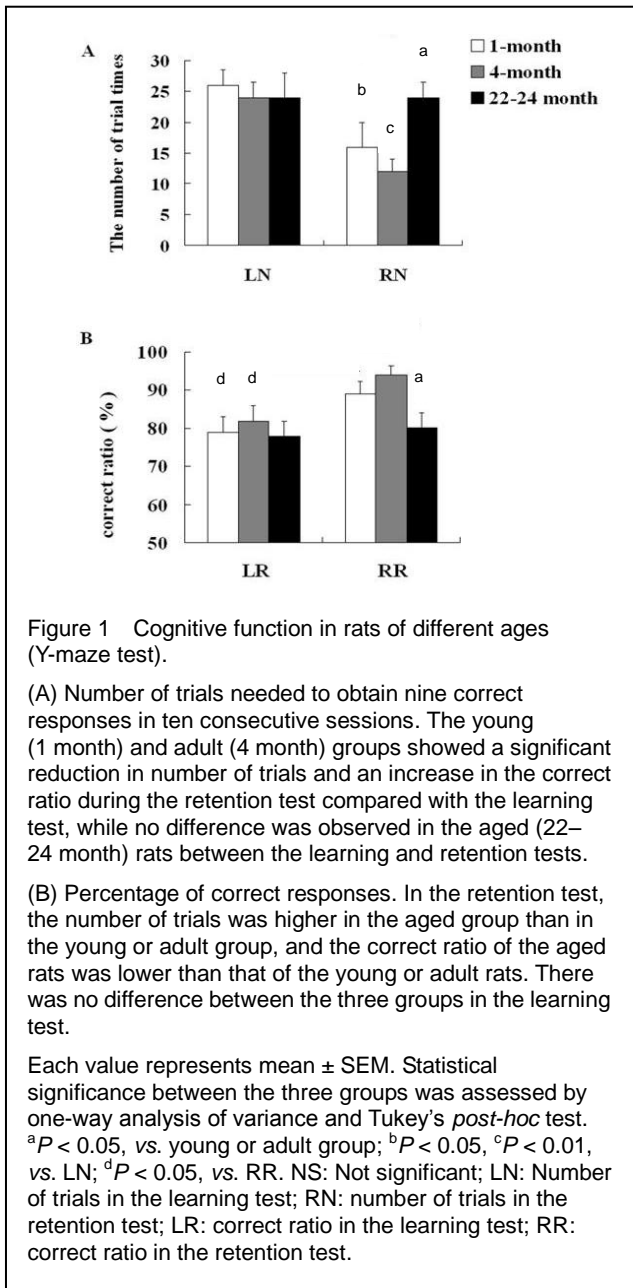
Quantitative analysis of experimental animals

We selected five healthy male Sprague-Dawley (SD) rats in each of three age groups (young, 1 month; adult, 4 months; aged, 22–24 months). All 15 rats were included in the final analysis.

Learning and memory retention performance decrease with age

We examined learning and memory using a forced alternation paradigm in a Y-maze. The 1-month and 4-month groups showed a significant reduction in number of trials and an increase in the correct ratio during the retention test compared with the learning test, while no difference was observed in the aged rats between the learning and retention tests, indicating that the aged rats have a learning deficit. In the learning test, no differences were observed among the three groups in the percentage of correct responses ("correct ratio") or number of trials needed to obtain nine correct responses in 10 consecutive sessions ($P > 0.05$). However, in the retention test, performed under the same conditions but 24 hours later, the number of trials was significantly higher in the aged rats than in the young and adult groups ($P < 0.05$; Figure 1A), and the correct ratio was significantly

lower ($P < 0.05$; Figure 1B).



Moreover, there was no difference in the number of trials or correct ratio between the young and adult groups ($P > 0.05$). These results indicate that the memory of aged rats is impaired.

Altered caveolin-1 and synaptophysin expression in hippocampus and cortex of aged rats

Western blot assay showed that hippocampal caveolin-1 and synaptophysin levels in aged rats were significantly lower than those in young and adult rats. In the cerebral cortex, caveolin-1 and synaptophysin levels in aged rats were significantly higher than those in young and adult rats. However, there were no differences in caveolin-1 or synaptophysin expression in the hippocampus or cortex

between young and adult rats. No significant differences were observed in the cerebellar expression of caveolin-1 and synaptophysin among the three groups (Figure 2).

Synaptophysin expression correlated negatively with memory ability

Synaptophysin expression in the hippocampus had no relationship with the number of trials ($r = 0.112$, $P > 0.05$) or correct ratio ($r = 0.160$, $P > 0.05$) in the learning test. However, in the retention test, the levels of synaptophysin in the hippocampus were negatively correlated with the number of trials ($r = -0.603$, $P = 0.017$) and positively correlated with the correct ratio ($r = -0.578$, $P = 0.024$; Figure 3). The results showed that synaptophysin expression in the hippocampus was correlated with memory ability, but had no relation to learning ability. In addition, synaptophysin expression in the cortex and cerebellum was not found to be correlated with memory or learning abilities (data not shown).

Synaptophysin and caveolin-1 expression correlated positively in the hippocampus, cortex and cerebellum

Expression levels of caveolin-1 and synaptophysin showed age-dependent trends in the hippocampus ($r = 0.744$, $P < 0.01$; Figure 4A), cortex ($r = 0.528$, $P < 0.05$; Figure 4C), and cerebellum ($r = 0.792$, $P < 0.01$; Figure 4E), and were significantly correlated with each other in the three brain regions studied (Figure 4B, D, F).

DISCUSSION

Learning and memory is a complex process, involving not only synaptic transmission but also plasticity. Studies have shown that the changes in synaptic plasticity of neurons can directly influence age-dependent changes in learning and memory ability^[15]. Many studies have demonstrated cognitive impairment and a decline in brain plasticity during the aging process^[16-17], in particular, the impaired synaptic plasticity that occurs in the hippocampus^[18].

Synaptophysin, also known as p38, is a glycosylated polypeptide located in the synaptic vesicle membrane. Synaptophysin plays an important role in the regulation of neurotransmitter release. The expression of synaptophysin responds to synaptic formation during embryonic development, making the protein a marker for synaptic plasticity^[19] and a useful tool in the investigation of physiological and pathological aging, and trauma or ischemia. Previous studies have confirmed that hippocampal

synaptophysin levels decline with aging and show an age-dependent trend^[20].

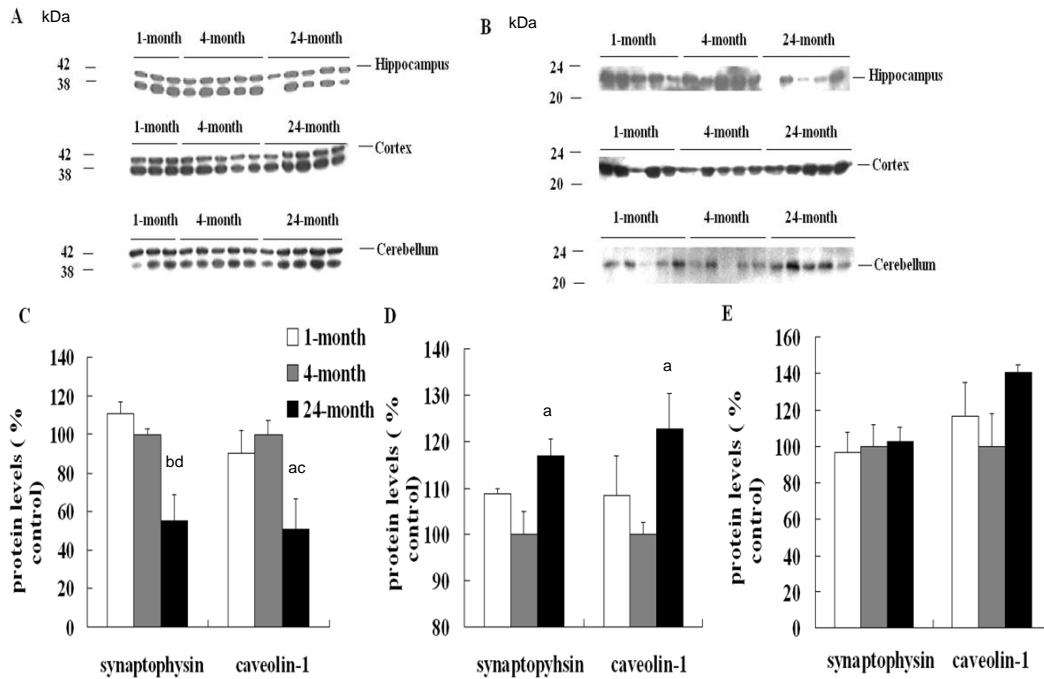


Figure 2 Expression of synaptophysin and caveolin-1 in the hippocampus, cortex and cerebellum of young (1 month), adult (4 month) and aged (22–24 month) rats.

(A) Western blot showing lower expression of synaptophysin (38 kDa) in the cortex and cerebellum of aged rats compared with young and adult rats; 42 kDa band is the internal control, β -actin. (B) Western blot showing greater expression of synaptophysin in the cortex and cerebellum of aged rats compared with young and adult rats.

Quantitative analyses of protein levels in the hippocampus (C), cortex (D) and cerebellum (E). Each value represents mean \pm SEM. Statistical significance between the three age groups was analyzed by one-way analysis of variance and Tukey's *post-hoc* tests. ^a $P < 0.05$, ^b $P < 0.01$, vs. adult group; ^c $P < 0.05$, ^d $P < 0.01$, vs. young group.

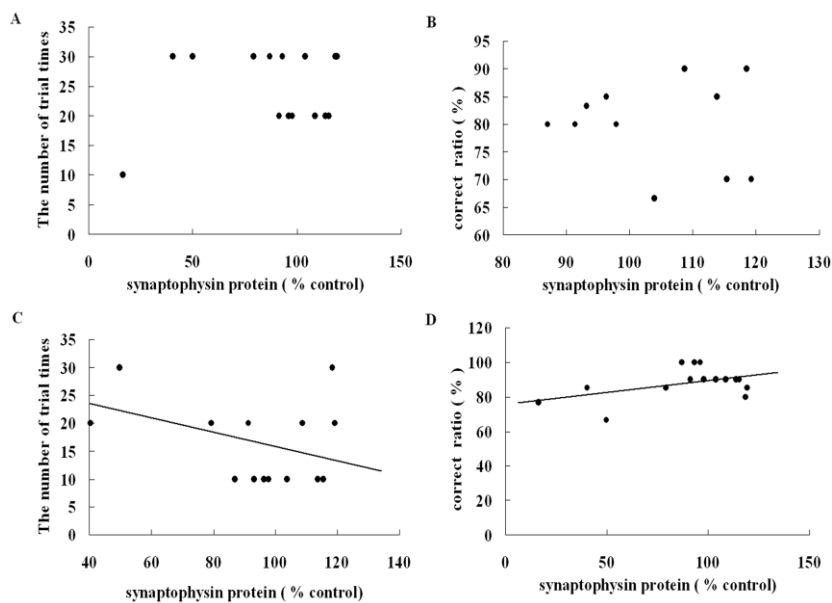


Figure 3 Linear regression analysis of synaptophysin expression in the hippocampus and cognitive function.

Synaptophysin expression was not correlated with the number of trials (A) or correct ratio (B) in the Y-maze learning test. In the retention test, synaptophysin expression was negatively correlated with the number of trials (C) and positively correlated with the correct ratio (D).

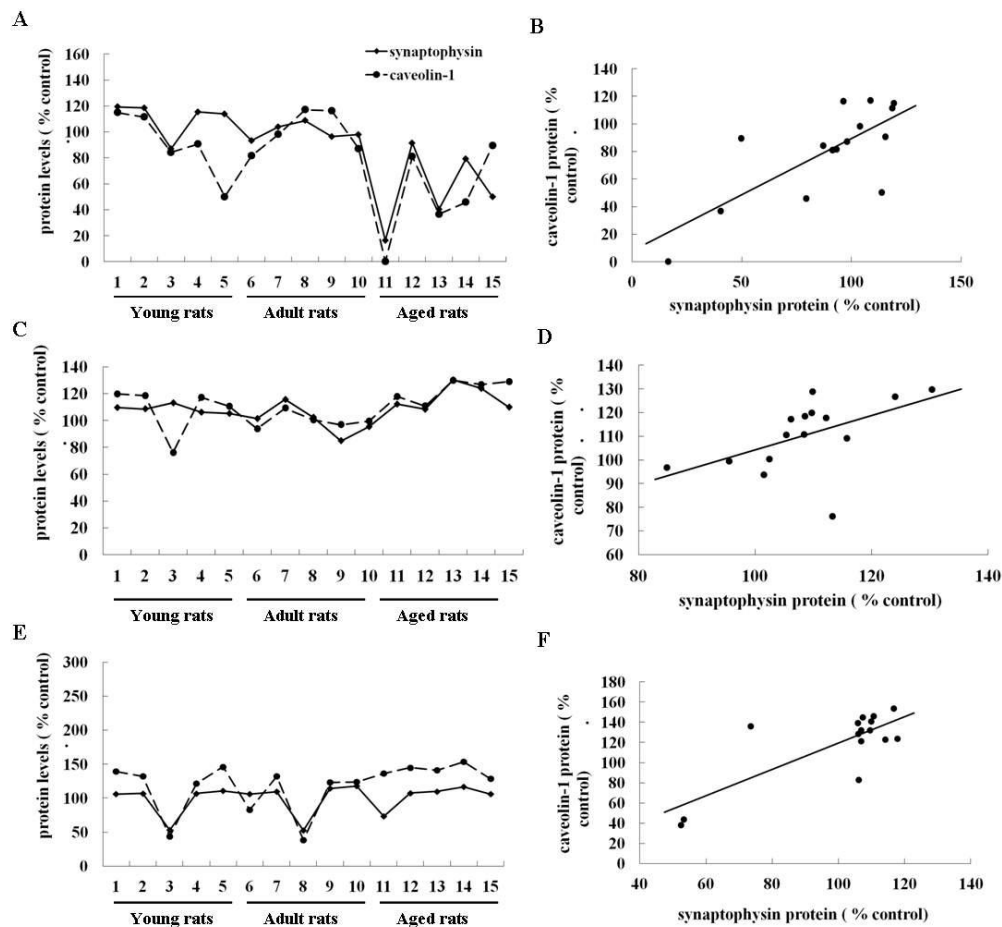


Figure 4 Relationship between age and synaptophysin and caveolin-1 expression in the hippocampus, cortex and cerebellum. Caveolin-1 and synaptophysin protein expression changed in an age-dependent manner in the hippocampus (A), cortex (C) and cerebellum (E). Significant positive correlation was found between synaptophysin and caveolin-1 in the hippocampus (B), cortex (D) and cerebellum (F). X axis: 1–5, young rats; 6–10, adult rats; 11–15, aged rats. Linear regression analysis was performed on the individual samples to evaluate the association between different variables.

In the ischemic model, synaptophysin protein expression is also downregulated in the hippocampal CA1 region, suggesting that presynaptic degenerative changes in CA1 precede delayed neuronal death, and that presynaptic damage may be an important cause of the pathological changes after cerebral ischemia^[21].

Western blot assay of hippocampal caveolin-1 showed significantly lower expression in aged rats compared with adult and young rats, while hippocampal synaptophysin expression was greater in the aged group than the other two groups. Expression levels of the two proteins were highly correlated. These data are consistent with previous reports^[9, 13]. In the cortex, caveolin-1 levels were higher in aged rats than in adult rats, paralleling synaptophysin, while in the cerebellum there were no significant differences in caveolin-1 or synaptophysin expression among the three age groups. The present results

indicate that the distribution of caveolin-1 might differ across developmental stages and brain regions. Park *et al*^[22] have reported that the expression of caveolin-1 in cerebellum and cortex of aged (27 months) female SD rats was remarkably greater than that of young (2 months) rats. Our results of caveolin-1 expression in the cortex are consistent with those of Park *et al*^[22], the discrepancy observed in the cerebellum may be due to the gender difference between the animals used.

We found differences in the hippocampal expression of synaptophysin between the three age groups, being significantly lower in aged rats than in young or adult rats. Furthermore, synaptophysin expression in adult rats was slightly lower than that in young rats, although this did not reach statistical significance. Our results are consistent with those of previous studies that report lower synaptophysin expression in aging brains^[23-24], suggesting a

close relationship between the learning and memory deficits of the aging process and changes in hippocampal synaptophysin. Synaptophysin is a synaptic vesicle-specific membrane channel and is involved in vesicle transport and neurotransmitter release. We hypothesize that a reduction in synaptophysin results in impaired synaptic vesicle transport capacity, which blocks synaptic transmission and neural information transfer, thus impairing information processing and storage.

Some other studies have suggested that hippocampal synaptophysin expression remains unchanged in aged animals^[25]; these conflicting results may be due to differences in research methods or animal species used, and individual differences in aging. To eliminate this impact on the experimental results, we screened aged rats based on international standards^[26-27] to select healthy animals, thus allowing better investigation of synaptic plasticity and learning and memory abilities in the normal aging process.

Previous studies have shown that caveolin-1 protein expression changes individually in different organs during aging^[28-29]. The variations of caveolin-1 expression in different organs may be related to the different regulatory roles of caveolin-1 during the aging process. With this in mind, it is not difficult to understand that caveolin-1 may show opposing expression patterns in different brain regions as aging progresses.

We further studied the relationship between synaptophysin levels and learning and memory performance in the Y-maze, and found that hippocampal synaptophysin protein expression levels correlated positively with memory retention performance. Previous studies have shown that hippocampal synaptophysin immunoreactivity in the transgenic Tg2576 mouse is correlated with spatial reference memory in the Morris water maze^[30]. Our results demonstrate that the learning ability of aged rats is not different to that of young and adult rats but that their memory is impaired, coupled with a downregulation of synaptophysin expression, indicating that age-dependent decline in hippocampal synaptic plasticity may be a reason for memory impairment.

In the present study, caveolin-1 protein expression changed in an age-dependent manner, and synaptophysin revealed a similar expression pattern compared with caveolin-1 in the hippocampus, cortex and cerebellum. Moreover, caveolin-1 levels were also positively correlated with those of synaptophysin in the hippocampus, cortex and cerebellum. These data, together with pre-

vious findings, suggest that caveolin-1 may play an important role in the regulation of synaptic plasticity and that age-related downregulation of caveolin-1 expression contributes to the decrease in synaptic plasticity in the hippocampus of aged rats.

However, caveolin-1 expression in hippocampal tissue is increased in patients with Alzheimer's disease^[8], which seems incongruous with our results and others^[13]. Although the reason for these conflicting results remains unclear, it might be related to a change in regulatory function of caveolin-1 in hippocampal plasticity under physiological or pathological states. In fact, Gaudreault et al also postulated that upregulated caveolin-1 levels in Alzheimer's disease might be responsible for the misdirected reactive sprouting in the disease pathology^[8]. We selected healthy aged rats as our subjects to investigate hippocampal caveolin-1 expression in physiological aging, and the conflicting results in Alzheimer's disease brain suggest that caveolin-1 shows opposite expression patterns under physiological and pathological states. Together with previous studies, we propose that downregulated caveolin-1 might be responsible for the impairment in hippocampal synaptic plasticity in physiological aging, whereas it might show overexpression to accelerate the process of neurodegeneration in a pathological state. Indeed, a previous study^[31] investigated the different regulatory functions of caveolin-1 in the cell under depleted or overexpressed conditions. The level of caveolin-1 is strictly regulated to maintain cellular integrity, leading to cellular transformation if depleted, and to the senescent phenotype if overexpressed^[31]. One study has demonstrated that loss of adipogenic differentiation potential in senescent human mesenchymal stem cells is mediated by the overexpression of caveolin-1^[32]. The regulative function of caveolin-1 should be related to cholesterol metabolism. Both remodeling and plasticity in adult brain require cholesterol redistribution and synthesis for the formation of new membrane components. Studies have indicated that the links between synaptic plasticity, neuronal lipid metabolism, hippocampal cholesterol efflux and lipid synthesis are the critical phenomena for proper synaptic function and plasticity^[2].

In conclusion, we have demonstrated a downregulation of caveolin-1 expression in the hippocampus, which parallels reduced synaptophysin expression in the normal aging process. Furthermore, we have provided the first direct demonstration that caveolin-1 protein expression changes in an age-dependent manner. Synaptophysin revealed a similar expression pattern to that of caveolin-1 in the hippocampus, cortex and cerebellum. Our results

suggest that caveolin-1 may play an important role in modulating synaptic plasticity throughout life, and downregulation of caveolin-1 in the hippocampus may contribute to the impairment in synaptic plasticity in the physiological aging process. This new notion might be helpful in strategic decision-making and preventing aging-induced loss of synaptic plasticity.

MATERIALS AND METHODS

Design

A randomized, controlled, animal experiment.

Time and setting

This study was performed at the Molecular Cell Biology Laboratory, Dalian Medical University, China from September 2011 to January 2013.

Materials

Fifteen male SD rats (five aged 1 month, weighing 100–150 g; five aged 4 months, weighing 300–350 g; and five aged 24 months, weighing 400–650 g) were provided from the Experimental Animal Center of Dalian Medical University (license No. SCXK (Liao) 2008-0002). All methods conformed to the guidelines on animal care established by the Chinese Ministry of Public Health. Rats were housed in a controlled environment at $21 \pm 1^\circ\text{C}$ under a 12-hour dark/light cycle with free access to food and water and allowed three days to acclimate before any behavioral testing. The protocols were conducted in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China^[33].

Methods

Selection of healthy aged rats by step-down passive avoidance test

The apparatus comprised an acrylic box with a stainless-steel grid floor and a fixed platform at one end of the box. Electric shocks (30 V) were delivered to the grid floor with an isolated pulse stimulator. At the beginning of the training trial, rats were placed in the box to acclimate for 3 minutes. When electric shocks were delivered, rats jumped onto the platform. The shocks were maintained for 5 minutes and the latency to step down from the platform was recorded. To eliminate aged rats with dementia, we selected those within the 95% confidence intervals based on the mean \pm 1.96 standard deviations of results from 3-month-old adult SD male rats^[26].

Y-maze behavioral test for learning and memory

The Y-maze test can be used to study foot shock-motivated discrimination learning and memory in rats^[34].

The Y-maze consisted of three equal arms ($45 \times 14 \times 18 \text{ cm}^3$) with a stainless steel grid floor and black plastic walls. A 15 W incandescent lamp was located at the end of each arm. One arm was lit and designated the safe zone while the other two were unsafe zones. The apparatus was placed on the floor of the experimental room. Each rat was placed in one of the arms and was allowed to move freely for 5 minutes. Entry into an arm of the Y-maze was defined as the body of a rat completely entering an arm except for its tail. Foot shock-motivated spatial alternation learning then began.

At the beginning of the training session, a foot shock (30 V, 0.7–1.5 mA, depending on individual sensitivity) was given in the start arm and the animal had to escape into the lit arm (correct response, no foot shock), whereas entry into the dark arm (error) was punished by further foot shock (30 V, 0.7 mA). The rats learned to escape from the dark arms to the lit arm to avoid the foot shocks.

In the learning test, a response was considered to be correct when a rat ran directly to the bright arm within 10 seconds after the onset of foot shocks. The number of trials was defined as the number of shocks needed to obtain nine correct responses in 10 consecutive sessions, up to a maximum of 30 trials^[35]. The retention test was used to evaluate memory by submitting the animals to the same protocol after 24 hours. The number of trials was recorded. The correct ratio was calculated as number of correct responses/total number of sessions \times 100%. The number of trials and the correct ratio were regarded as the indicators for the learning and retention tests.

Tissue preparation

Rats were deeply anesthetized by intraperitoneal injection of ketamine and xylazine until the tail and paw withdrawal reflex were lost. The rats were perfused transcardially with heparinized saline followed by 4% paraformaldehyde^[36]. Brains were removed carefully and placed into cold physiological saline. Hippocampus, cerebral cortex and cerebellum were rapidly dissected, frozen and preserved at -70°C ^[37].

Western blot assay for caveolin-1 and synaptophysin

Hippocampal, cortical and cerebellar tissues were immersed in lysis buffer (0.15 mol/L NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulfate-poly-

acrylamide, 10 mmol/L Tris-HCl pH 7.4, 1% phenylmethyl sulfonyl fluoride) to lyse the cells, then homogenized with ice-cold saline and centrifuged at $179 \times g$ for 10 minutes. The supernatant was frozen at -20°C for western blot assay. 100 μg of total protein was separated using 12% sodium dodecyl sulfate gel electrophoresis for 2 hours and then transferred to a polyvinylidene difluoride membrane (Hybond, NJ, USA) at room temperature, 220 V for 60 minutes. Equal protein loading was verified by Coomassie blue staining. Blots were incubated with rabbit anti-Caveolin-1 polyclonal antibody (1:500, Santa Cruz Biotechnology, Dallas, TX, USA), or mouse anti-rat synaptophysin monoclonal antibody (1:400; Boster Biotechnology, Wuhan, China) at 4°C overnight, then in goat anti-mouse or anti-rabbit IgG (1:2 000; Sigma, St. Louis, MO, USA) at 37°C for 45 minutes. After washing in PBS, protein bands were detected using the Enhanced Chemiluminescence kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) and exposed to Kodak XAR5 films. Detection of mouse anti-rat β -actin monoclonal antibody (1:500, Boster Biotechnology) was used as an internal control. Each tissue was measured three times. Immunoreactive bands were visualized by chemiluminescence. Quantitative measurement of protein level was performed using ImageJ software (version 1.61, NIH).

Statistical analysis

All results are expressed as mean \pm SEM. Statistical analysis was performed using SPSS 10.0 (SPSS, Chicago, IL, USA) and $P < 0.05$ was considered significant. The three age groups were compared using one-way analysis of variance with Tukey's *post-hoc* test. Linear regression analysis was performed on the individual samples to evaluate the association between different variables.

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