Increased Acetylated SNAP25 in the Hippocampus Correlated with Age-Related Deficits in the SAMP8 Mice

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Abstract: Acetylation is an important post-translational modification, which modulates function and localization of cytoplasmic proteins. Synaptosomal-associated protein-25 (SNAP-25) is a presynaptic neurotransmission-regulating protein that can be acetylated. Whether the acetylation level of SNAP25 is affected by aging is unknown. We explored the relative levels of SNAP25 and acetylated SNAP25 in the SAMP8 mice with different ages, and their correlation with spatial cognitive performance in radial six-arm water maze. The SAMP8 mice exhibited decline of spatial learning and memory abilities with aging. The higher hippocampal levels of SNAP25 were found in the 6- and 10-month SAMP8 mice compared to the 2-month mice. The hippocampal level of acetylated SNAP25 in the 10-month mice was higher than those in the 2- and 6-month mice. Positive correlations were found between the age-related increase of SNAP25 and the impairment of spatial learning and memory, and between acetylated SNAP25 level and memory deficits. The results suggested that elevated acetylated SNAP25 during aging might be involved in the age-related memory impairment.

Keywords: SNAP25, Acetylation, Cognition, Brain aging, Post-translational modification.

1. INTRODUCTION

Aging is manifested by declines in motor and cognitive abilities, social activities, as well as an increased incident of age-related multi-domain disorders [1]. During aging, a progressive decline of cognitive ability exists in many domains, including calculation, attention, learning and memory, among which the impairment in episodic memory and spatial memory are the most prominent [2]. These cognitive impairments during aging seriously affected the quality of life in the elderly.

However, the mechanisms underlying declined ability of learning and memory, a common symptom of brain aging, remains to be cleared. Reduced efficiency of synaptic transmission and changed synaptic structure may be involved [3]. The level or function of specific synaptic proteins in the hippocampus and neocortex links to synaptic plasticity that plays an important role in the learning and memory process [4]. Multiple studies have found that various proteins that participate in the process of synaptic transmission are able to change their expression levels during aging, which may contribute to cognitive decline [5]. For instance, our previous studies indicated that aged mice in senescence accelerated prone mouse 8 (SAMP8) had expression of reduced syntaxin-1 and increased synaptotagmin-1 in dorsal hippocampi, which were tightly correlated with the impairment of spatial learning and memory [6, 7].

Besides the expression level, the function and subcellular localization of these proteins maybe responsible for the regulation of synaptic transmission. Post-translational modification, such as protein phosphorylation, glycosylation, ubiquitination, nitrosylation, is an important process that regulates protein stability, subcellular localization and protein interactions [8]. Acetylation is a post-translational modification of introducing an acetyl group into a protein. The acetylation and deacetylation of histone is most studied, as it is a vital epigenetic modification that regulates gene transcription in neurological disorders, cancer and other diseases [9]. Beside the histone, acetylation can also be seen in transcription factors in

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the nucleus and in proteins reside outside of nucleus [10]. Despite the research of cytoplasmic protein acetylation begins much later, a huge number of acetylated proteins have been identified [11, 12]. The proteins reside in cytoplasm and nucleus are the majority of acetylated proteins, both account for 30% of all the acetylated proteins, while those in mitochondria and membrane both account for 15% [10]. The protein acetylation in various cellular compartments potentially mediates many different types of acetylation-dependent protein activities.

Synaptosomal-associated protein-25 (SNAP-25) is a presynaptic membrane protein that can be acetylated. SNAP-25 and syntaxin-1 (target-SNARE) interact with synaptobrevin/VAMP2 (vesicle-SNARE) and form the SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors) complex. The SNARE complex mediates the docking and fusion of synaptic vesicles with the presynaptic membrane, and subsequent neurotransmitter release [13]. SNAP-25 also interacts with synaptotagmin-1, a major calcium sensor on the synaptic vesicle, triggering calcium-dependent membrane fusion [14]. Thus, the SNAP-25 is an essential component of synaptic transmission.

The acetylation of SNAP-25 may have an effect on its function, and therefore influence the docking and fusion of synaptic vesicles. Whether the level of acetylated SNAP-25 (ac-SNAP-25) is affected by aging is unknown. Therefore, we used different-age SAMP8 mice to explore the relative levels of SNAP-25 and ac-SNAP-25. The SAMP8 strain is a commonly used model for the study of brain aging. This mouse strain has a relatively normal phase of growth and development, but manifests various pathobiological phenotypes with aging, including early-onset age-related alteration of protein levels and deficits in various behavioral tests for sensorimotor ability and spatial learning and memory [15]. Our previous study reported that the SAMP8 mice have exhibited deficiencies of spatial learning and memory in radial six-arm water maze (RAWM) test in early life, such as at 3-month-old [16]. Hippocampus is a most susceptible region in brain aging. The morphological and functional changes in the hippocampus is at least responsible to the age-related cognitive decline in part.

In the present study, our purpose was to explore whether the contents of SNAP-25 and ac-SNAP-25 in the hippocampus have age-related changes, and if this is case, whether these changes correlates with the age-related decline of spatial learning and memory in the SAMP8 mice.

2. MATERIALS AND METHODS

2.1. Animals

The SAMP8 mice were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing). The female mice were housed in groups (4–5 per cage) and the male mice were housed alone (to avoid forming a strong hierarchy among the mice, which may affect cognitive performance in the following behavioral task). The mice were reared in a constant environment with standard rodent diet and tap water, a constant temperature of 23-25°C, and a humidity level of 45−55% under a 12-h light–dark cycle (lights on at 7:30 a.m.). Animals that had tumors, or motor incapacitation or other gross defects were removed. The mice of three different-age groups were used, including 14 young mice (7 females and 7 males) aged 2 months, 12 middle-aged mice (6 females and 6 males) aged 6 months, and 12 old mice (6 females and 6 males) aged 10 months. All procedures involving the care of animals were carried out in accordance with the guidelines of Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.2. RAWM

The procedures were done as previously described to evaluate the spatial learning and memory [16]. The apparatus (see Figure **1**) was a black circular tank with six swimming alleys that filled with 20–21°C water. One of the alleys contained a black escape platform submerged 1.0 cm below the water surface near the distant end. A white cloth curtain (75-cm from the tank wall) rounded the tank from the ceiling to the ground with three different black cardboards hung equidistantly served as spatial cues. Four acquisition trials (trials 1-4) and one memory retention trial were carried out each day during ten consecutive days. The mouse was released into water from four random entry alleys in each acquisition trial, except for the alley with the platform and its opposite alley. In each trial, the mouse was allowed to locate the platform for a maximum of 60 s and left on the platform for 30 s upon locating it. If the mouse entered a wrong alley with its whole body, or failed to select any alley within 10 s, the mouse was gently dragged to the start alley and the incorrect attempt was recorded as an error. The number of errors and the latency (the time before locating the

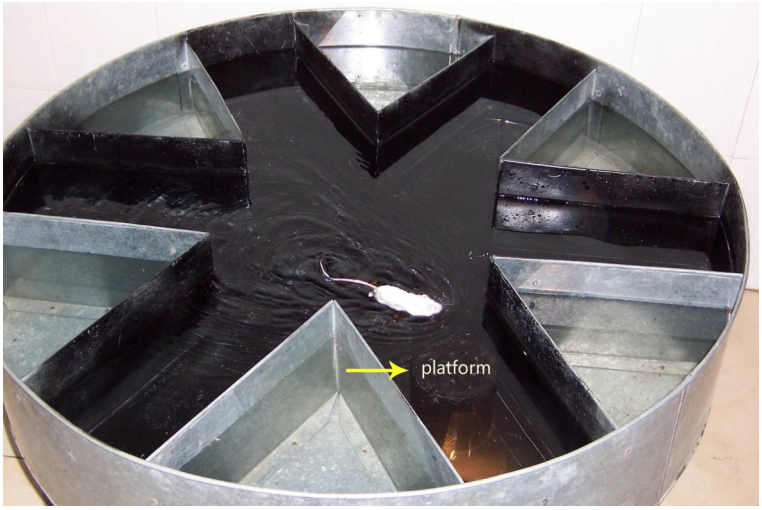

Figure 1: The apparatus of radial six-arm water maze (RAWM) test. A black circular tank with six swimming alleys was filled with 20–21°C water and a black escape platform submerged 1.0 cm below the water surface was located at an alley near the distant end.

platform) in the daily learning period (trials 1 to 4) were averaged for statistical analysis. 30 min after the acquisition trials, to take trial 5, the mouse was released from the start alley identical to that in the trial 4, the number of errors and the latencies were also recorded.

2.3. Tissue Preparation

To avoid the impact of behavioral tests, 10 days after the behavioral testing, the mice were anesthetized with halothane and sacrificed. Their brains were swiftly removed from the skull on dry ice and bisected in the mid-sagittal plane. The bilateral hippocampi were quickly isolated and stored in -20 °C for western blotting.

2.4. Western Blotting for SNAP-25

The left hippocampus was homogenized in a protein lysis buffer with a complete protease inhibitor cocktail (Mini, EDTA-free, Roche) and trichostatin A (TSA). After the homogenates were centrifuged, the protein concentrations in the supernatant were measured by bicinchoninic acid assay kit (Pierce Biotechnology, USA). The samples were adjusted to the same protein concentrations and stored at -20°C. To analyze the level of SNAP-25, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard. The samples were fractionated by sodium dodecyl sulfate, polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to polyvinylidenefluoride membranes (Milipore, USA). The membranes were blocked with 5% bovine serum albumin (BSA) in Tris-buffer saline (TBS) at 4°C overnight, and then incubated with rabbit anti-SNAP-25 polyclonal antibody (Abcam, USA; 1:1000 dilution) and anti-GAPDH monoclonal antibody (Abcam, USA, 1:2000) at room temperature for 2 h. After being rinsed, the membranes were incubated with horse-radish peroxidase (HRP)-conjugated anti-rabbit IgG (1:100,000) and HRP-conjugated anti-mouse IgG (1:20,000) (Zhongshan-Golden Bridge, Beijing, China, 1:100,000 and 1:20,000, respectively) for 1 h at room temperature. The electro-chemi-luminescencereagent (Pierce, USA) revealed an immunoreactive protein band corresponding to SNAP-25 (25 kDa, lane 1) and a band of GAPDH (35 kDa, lane 2). The chemiluminescence intensity was analyzed with Image-Pro Plus 6.0 software. The intensity for the immunoreactive band of SNAP-25 was normalized to GAPDH protein loading control.

2.5. Immunoprecipitation and Western Blotting for ac-SNAP-25

For the detection of the ac-SNAP-25, the sample was purified by immunoprecipitation with anti-SNAP-25 antibody. Briefly, the protein G agarose beads was washed before using, and then incubated with the antigen sample and anti SNAP-25 antibody (Abcam, USA) at 4°C overnight with gentle mixing. We then added immunoprecipitation buffer, centrifuged and discarded supernatant. The complex-bound resin was incubated with electrophoresis loading buffer for 5 min at 95°C for subsequent western blotting. Briefly, the membranes were blocked with 5% BSA, incubated with anti-acetylated proteins antibody (Cell Signaling;

diluted to 1:1000 in a TBS-T with 5% BSA) at 4 °C overnight, followed by incubation with HRP-conjugated anti-rabbit antibody (1:1000) for 2 h at room temperature. In the final step, we analyzed the intensity of immunoreactive band corresponding to ac-SNAP-25 and that to SNAP-25, and calculated the ratios, which represented the acetylated levels of SNAP-25.

2.6. Statistical Analysis

Data were expressed as means ± means of standard errors. For the analysis of the performances in the RAWM, a three-way repeated-measure analysis of variance (3w-rm ANOVAs) was used with day, age and sex as independent variables. Fisher's least-significant difference (LSD) test was used in post hoc comparison to reveal any difference among the different ages or sexes. For the concentrations of SNAP-25 and ac-SNAP-25, the age and sex effects were analyzed using two-way ANOVA with LSD test for post hoc analysis. The number of errors and the latency improved rapidly in the first 5 days in both the learning and memory phases in the RAWM. Since the performance reached a more stable level since day 6, the number of errors and latency in trials of RAWM in the last 5 days were averaged for the correlation analysis. The correlations among the average performance in day 6-10 in the RAWM task and the levels of SNAP-25 and ac-SNAP-25 in the hippocampus were analyzed employing Pearson's correlation test. Significance was assumed when $P<0.05$. All analyses were performed using SPSS® 13.0 software.

3. RESULTS

3.1. Performance in RAWM

3.1.1. Learning Phase

The latency and the number of errors progressively decreased daily for all mice combined [$F_{(9,288)}$ = 17.975 and 16.099, $Ps < 0.001$], suggesting that the mice were able to learn this task. The 3w-rm-ANOVA results revealed a significant age effect on the latency [$F_{(2,32)}$= 7.726, P=0.002] and the number of errors [$F_{(2,32)}$= 6.629, P=0.004], see Figure **1A** and **B**. Further analysis indicated that the latency and errors in the 10-month and 6-month mice were significantly longer or more than those in the 2-month mice (Ps < 0.05). The performance of the 10-month and 6-month mice was insignificant difference (Ps > 0.05). The effect of sex was insignificant on the latency [$F_{(1,32)}$ = 1.526, P = 0.226] and errors [$F_{(1,32)}$ = 1.933, P = 0.174], neither was the effect of interactions of age × sex, age × day, sex × day, and group × sex × day (Ps > 0.05).

3.1.2. Memory Phase

The latency and number of errors progressively decreased daily for all mice combined either [$F_{(9,288)}$ = 4.567 and 4.579, $Ps< 0.001$, respectively]. The age effect was significant on the latency [$F_{(2,32)}$ = 5.614, P = 0.008] and number of errors [$F_{(2,32)}$ = 5.047, P = 0.012], see Figure **1C** and **D**. The post hoc analysis revealed that the latency and errors were significantly longer or more in the 10-month and 6-month mice compared to the 2-month mice (Ps < 0.05). There was no significant difference in the memory performance between the 10-month and 6-month mice (Ps > 0.05). Neither sex nor interactions of age × sex, age × day, sex × day, and group × sex × day had a significant effect on the latency and errors (Ps > 0.05).

3.2. Levels of SNAP-25

Figure **2A** shows the relative levels of SNAP-25 in hippocampus in different-age mice. The two-way ANOVA showed that age significantly impacted on the hippocampal level of SNAP-25 [$F_{(2,32)}$ = 9.904; $P<$ 0.001]. The post hoc analysis indicated that the hippocampal level of SNAP-25 in the young group was significantly lower than those in the middle-aged ($P<$ 0.001) and the old (P = 0.012) groups, with an insignificant difference between the 6-month and 10-month mice (Ps > 0.05). Neither sex nor interaction of age × sex significantly affected the hippocampal level of SNAP-25 (Ps > 0.05).

3.3. Levels of ac-SNAP-25

The age exerted an significant effect on the relative hippocampal level of ac-SNAP-25 [$F_{(2,32)}$ = 3.403, P = 0.046; Figure **2B**]. Further analysis showed that the old mice had higher ratio of ac-SNAP-25 to SNAP-25 in their hippocampi than the young group and middle-aged group(Ps = 0.030), with no significant difference between the young and middle-aged mice (Ps > 0.05). Sex or interaction of age × sex had no significant effect on the ratio of ac-SNAP-25 to SNAP-25 either (Ps > 0.05).

3.4. Correlations between Spatial Learning and Memory Performance and Protein Levels

For all mice combined, the positive correlations were found between the latency (r =0.335, P = 0.040)

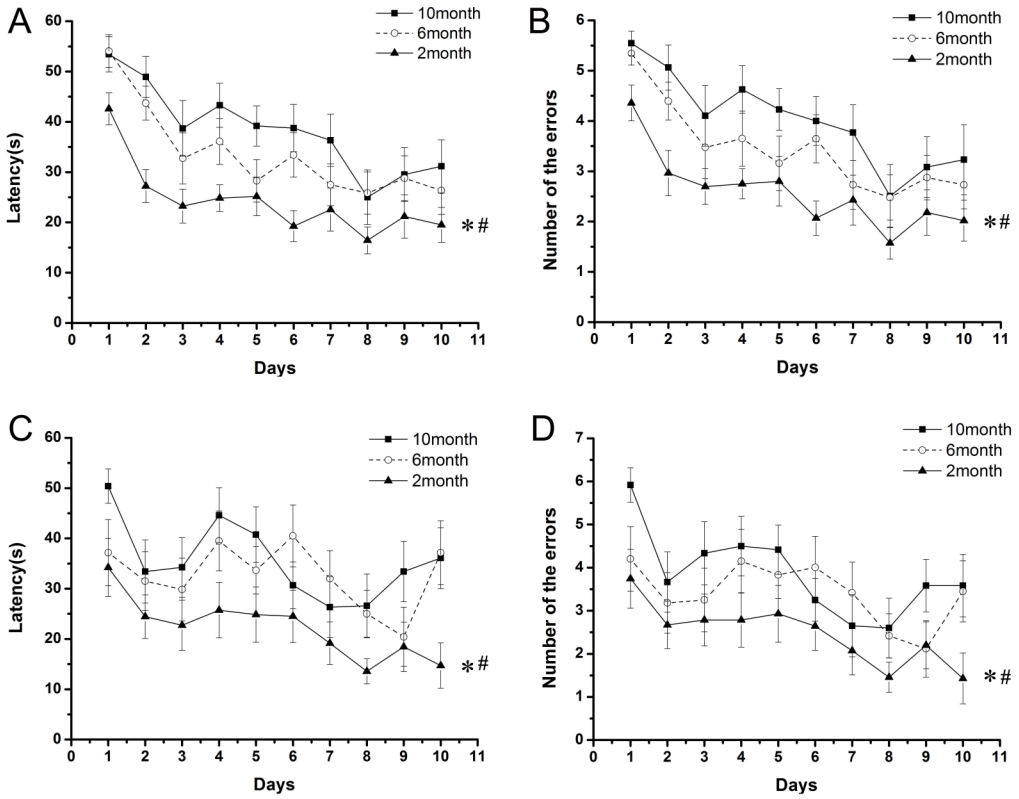

Figure 2: Performances of the 2-, 6- and 10-month-old SAMP8 mice in the RAWM (n = 14, 12 and 12 mice/group, respectively). Latency (**A**) and number of the errors (**B**) during learning phase; and latency (**C**) and number of the errors (**D**) during memory phase are shown. The latency and error number in the 10-month and 6-month mice were significantly longer or more than those in the 2-month mice in both the learning and the memory phase. All values are means ± S.E.M. *Denotes significant difference compared to the 6-month-old mice (P < 0.05). #Denotes significant difference compared to the 10-month-old mice (P < 0.05).

and number of errors (r = 0.340, P = 0.037) in the learning phase of the RAWM and the relative level of hippocampal SNAP-25 (see Figure **3A** and **B**). The latency in the memory phase of the RAWM positively correlated with the hippocampal SNAP-25 level (r = 0.321, P = 0.049; Figure **3C**). Only the latency (r =0.333, P = 0.041; Figure **3D**) and number of errors (r = 0.338, P = 0.038; Figure **3E**) in the memory phase, but not the performance in the learning phase (Ps > 0.05), positively correlated with the hippocampal ac-SNAP-25 level. No significant correlation can be seen between behavioral performance and protein levels when mice in different groups were analyzed respectively (Ps > 0.05).

4. DISCUSSION

4.1. Age-Related deficits of Learning and Memory in the RAWM in SAMP8 Mice

During aging, various species of murine models exhibit declined ability of learning and memory [17, 18]. Behavioral results have shown an age-related deficit of learning and memory capacity start as early as 6 months and further deteriorated with aging in the SAMP8 mice [19]. In the present study, the effect of normal aging on the performance in the RAWM was evaluated in the SAMP8 mice. RAWM was originally designed to detect spatial learning and memory in transgenic mice of Alzheimer disease, and is now a commonly used test for the hippocampus-dependent spatial learning and memory ability in rodents [20]. RAWM exhibits greater sensitivity for detecting mildly impaired spatial learning and memory in mice. It was also proved in our previous study that RAWM is more efficient for detecting the impairment of learning and memory in SAMP8 mice when compared to the Morris water maze [16]. The spatial learning deficits occur at as early as 3 months old and memory deficits display at 5 months old in SAMP8 mice in the RAWM task [16].

In the present study, the SAMP8 mice also exhibited age-related deficits in the RAWM task. The 6-month and 10-month mice showed significantly longer latency and more number of errors than the young mice in the learning phase, indicated age-related

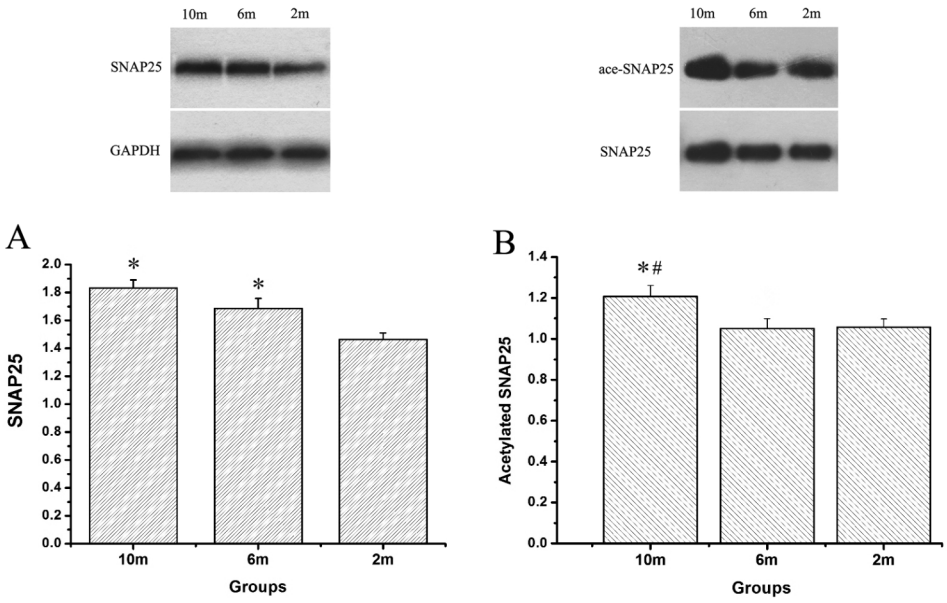

Figure 3: Upper: representative gel pattern showing SNAP-25, ac-SNAP25 protein bands in hippocampus in 2-, 6- and 10-month-old SAMP8 mice. Lower: relative levels (mean ± S.E.M.) of SNAP-25(**A**) and ac-SNAP25(**B**) in hippocampus in mice of different ages. The relative level of SNAP-25 was expressed as the ratio of the optical density of SNAP-25 protein band to the optical density of GAPDH protein band. The level of ac-SNAP25 was expressed as the ratio of the optical density of its protein band to that of SNAP-25 protein band. *Denotes a significant difference compared to the 2-month-old mice ($P < 0.05$). #Denotes a significant difference compared to the 6-month-old mice ($P < 0.05$).

decline of spatial learning ability. The declined memory ability in the old and middle-aged mice was also indicated by the longer latency and more errors in the memory phase compared to the young mice. These indicated age-related decline of spatial learning and memory occur at least in the midlife in the SAMP8 mice. Interestingly, there was no significant difference in the performance between the 6-month and 10-month mice in both learning and memory phases, suggested similar ability of learning and memory between the two age groups. This phenomenon has not been reported so far. The similar performance between these two-age SAMP8 mice may be that the 10-month mice did not have a significant decline of learning and memory ability since the middle age. An alternative reason may be that the RAWM is a relatively difficult task for both 6-month and 10-month mice, thus the 6-month mice nearly reached a plateau of a rather terrible performance.

4.2. Increased Hippocampal SNAP-25 Correlated with Poor Memory and Learning

SNAP-25 that presents at the presynaptic terminals is capable of interacting with syntaxin and synaptobrevin [21]. SNAP-25, syntaxin and synaptobrevin together assemble the machinery, known as the SNAREs, which are considered the core components of the fusion machinery, facilitating vesicle trafficking and neurotransmitter release [21]. SNAP-25 also interacts with synaptotagmin-1 for the regulation of calcium-triggered membrane fusion and controlling of the fusion pore. Thus, SNAP-25 is essential for neural transmission. Knockout SNAP-25 abolishes calcium-dependent neurotransmitters release [22].

In the present study, we detected the contents of SNAP-25 in the hippocampus by western blot or immunoprecipitation. Our results showed significantly higher content of SNAP-25 in hippocampus in the mice aged 6-month and 10-month than that in the mice aged 2-month, and a similar SNAP-25 level between 6-month and 10-month mice. Other group's findings demonstrating increased SNAP-25 in the aged Fischer female rats [23], is in agreement with our results. Our previous studies also reported increased SNAP-25 in dorsal hippocampus in aged Kunming mice and in middle-aged CD-1 mice [24, 25]. In contrast, some studies have shown a progressive decline in SNAP-25 expression during aging [26, 27]. The protein level of SNAP-25 in hippocampal nerve terminals was higher at 2-month old, and then decreased in Wistar rat at 18–24 months [26]. Previous study on the Fischer 344×Brown Norway (F1) hybrid rats also showed decreased SNAP-

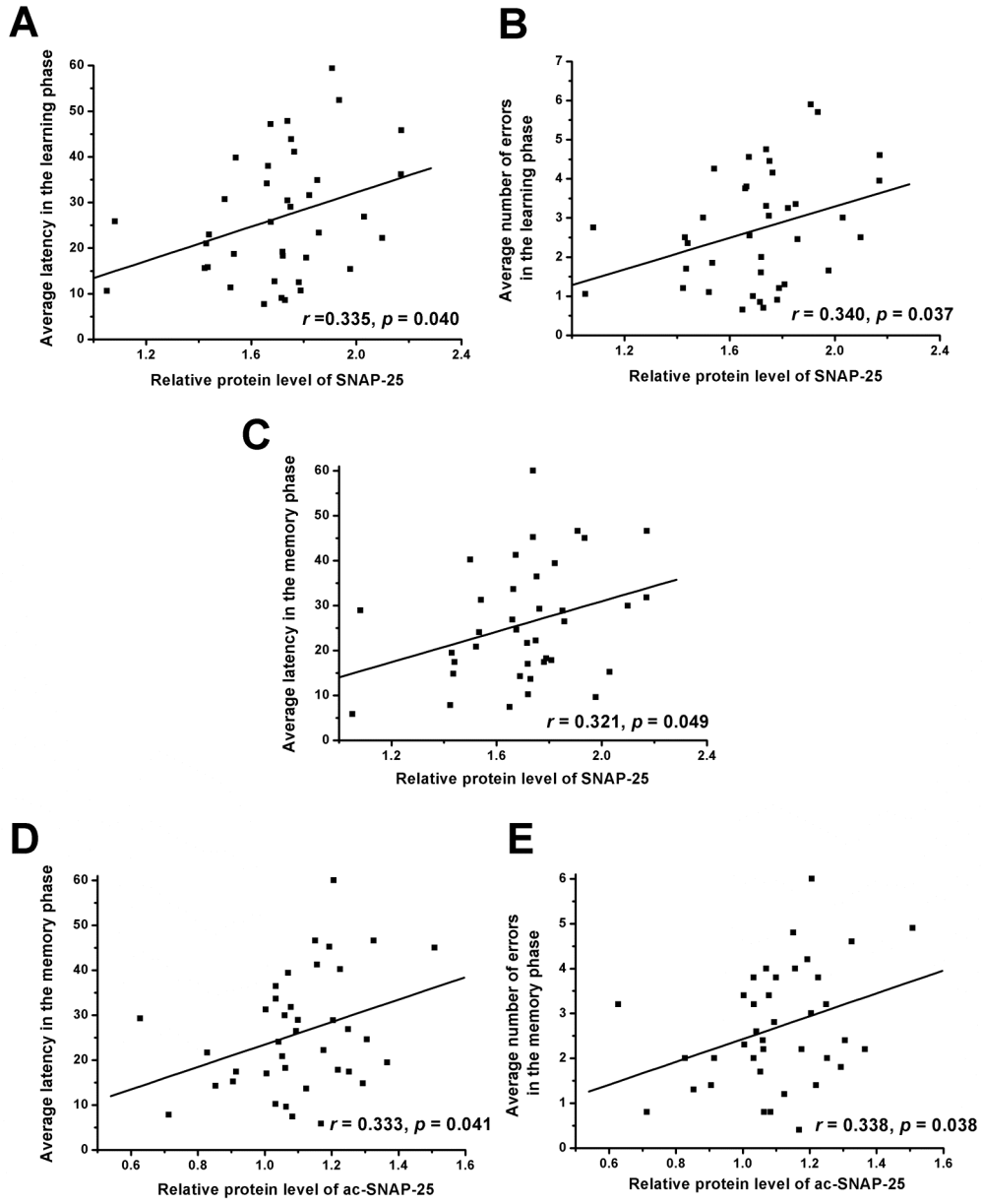

Figure 4: Correlations between behavior performances in the learning and memory phase and the relative level of hippocampal SNAP-25 and ac-SNAP-25 in all mice. The level of SNAP-25 positively correlates with the latency (**A**) and number of errors (**B**) in the learning phase, and with the latency in the memory phase (**C**). There were significantly positive correlations between the latency (**D**) and number of errors (**E**) in the memory phase and the ac-SNAP-25 level.

25 level in the hippocampus synaptosomes [27]. There are also inconsistent findings that show no significant effect of age on the SNAP 25 level in the Long-Evans rats [28].

The correlation analysis demonstrated that the SNAP-25 level in hippocampus was positive correlated with the number of errors and latency in the learning phase and the latency in the memory phases. Studies have pointed to a role of SNAP-25 in neuronal function and neurodegenerative diseases. In transgenic mouse models of Alzheimer's disease, the content of SNAP-25 is reduced in the hippocampus [27]. In a study of human individuals with dementia, their brains showed significantly lower levels of synaptic protein regardless of age [29]. Reductions in synaptic markers, such as SNAP-25 and syntaxin, was associated with dementia rather than with aging [29]. However, in VanGuilder's study of aged rat, the SNAP-25 expression analyzed according to cognitive performance-based sub groupings showed no difference [30]. In our SAMP8 mice, one speculation of the increased SNAP-25 in

middle-aged and old mice may be a compensatory mechanism to cope with the changed level or dysfunction of other synaptic proteins and to relieve the age-related degeneration of neuronal network. Another possible reason may be that some SNAP-25 are dysfunctional or the degradation process is abnormal, leading to accumulated SNAP-25 in hippocampus during aging. Although its high level, the increased SNAP-25 may cannot fulfill its physiological role in neural transmission, thus correlated with poor cognitive performance.

4.3. Elevated Ac-Snap-25 Level in the Hippocampus Correlated with the Age-Related Spatial Memory Decline

It has been elucidated that the fundamental requirements for normal cognitive function included the following: sufficient neurons with axons and dendrites able to maintain and modify their architecture; synapses that transmit electrochemical signals; and activity-dependent neurotrophic factor-mediated modification of synaptic plasticity [31]. To manage the structure and function of neuronal networks, the expression, locating, and removal of proteins, as well as rapid modulation of protein functions (activity, interaction with other proteins, conformational change) have to be strictly controlled. Reversible posttranslational modification, such as phosphorylation, glycosylation and acetylation may be the key to control the functions of synaptic proteins. Acetylation is a reversible post-translational modification, regulating protein function in diverse ways. In the nucleus, histone and non-histone proteins, including transcription factors, non-histone structural chromosomal proteins, can be acetylated by HATs. As for cytoplasmic protein, only a limited number of acetylation targets have been identified, for example the α-tubulin and tau.

The results of current study showed a significant effect of aging on the acetylation level of SNAP-25 in the hippocampus. The mice in both the 2-month and 6-month groups had less ac-SNAP-25 in their hippocampus than 10-month mice. The ac-SNAP-25 level of the 2-month mice showed no significant difference with that of the 6-month mice. A positive correlation between the ac-SNAP-25 level and impairment of spatial memory could be observed.

The role of acetylation of SNAP-25 in neuronal function is unknown. Studies have found that the addition of an acetyl group on lysines neutralizes positive charges on the amino group, thus exert a significant impact on the electrostatic properties of the protein [32]. For acetylated histone, the changed electrostatic property reduces their affinity with DNA, resulting in an opened chromatin conformation. How acetylation affects the property of non-histone proteins and their interaction with other proteins needs to be characterized. For microtubule-associated protein tau, the acetylation of its lysine prevents its ubiquitin-mediated degradation, leading to accumulation of pathogenic tau [33]. The acetylation of non-nuclear tubulin protects the stabilization of microtubules, which play critical roles in the neuronal transport of mRNA and mitochondria, etc [34].

In aged rodents, deficiencies in learning and memory abilities were considered the results of the morphological and functional changes in the hippocampus. The release of neurotransmitters is a highly regulated process that can be affected by changes of pre-synaptic proteins. Available studies have indicated that the expression, function, or location of many synaptic proteins in hippocampus or cortex undergoes significant changes during the period from adulthood to advanced age [30]. The disorders of synaptic proteins during aging may disturb neurotransmission by altering the stabilization or reconstruction of synaptic structure, as well as disrupting synaptic function, thus ultimately contribute to the cognitive decline [35].

Our results found that the ac-SNAP-25 was elevated in the hippocampus in old mice and correlated with the spatial memory decline. This finding provides evidence that the acetylation of SNAP-25 is likely to play a crucial role in the brain. Altered level of SNAP-25 acetylation may leads to errors in transport or function of SNAP-25, and involved in the disorders of neurotransmission and vesicle replenishment during prolonged stimulation, which are necessary for the memory formation and perseverance. In our old SAMP8 mice, the elevated ac-SNAP-25 may be a factor contributing to the age-related memory decline. As the increased ac-SNAP-25 level correlated with the learning and memory decline, and both groups of the 6-month and 10-month mice have worse performance in the RAWM, one would expect the 6-month mice would also have increased ac-SNAP-25 similarly as 10-month mice, but they had not. In the correlation analysis, the differences in performance and ac-SNAP-25 levels between the 2-month and 10-month mice may contributed the most to the correlation. However, the correlation does not mean causality. To determine the causal relationship, further study is needed. It is also possible that the increased ac-SNAP-25 level is the

result of brain aging and cognitive aging. The specific role of acetylation of SNAP-25 is unclear and need further exploration in future studies.

In summary, the present study is the first to explore the effect of age on the ac-SNAP-25 level in the hippocampus. The study proposed the potential contribution of elevated ac-SNAP-25 level in the process of brain aging and age-related memory impairment. Further research should address the pathophysiological function of the SNAP-25 acetylation in the aging brain.

CONFLICT OF INTEREST

The authors have indicated no conflicts of interest.

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REFERENCES

[1] Harman D. Aging: phenomena and theories. Ann N Y Acad Sci 1998; 854: 1-7. https://doi.org/10.1111/j.1749-6632.1998.tb09886.x

[2] Foster TC. Dissecting the age-related decline on spatial learning and memory tasks in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca2+ channels in senescent synaptic plasticity. Prog Neurobiol 2012; 96: 283-303. https://doi.org/10.1016/j.pneurobio.2012.01.007

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

[4] Deak F, Sonntag WE. Aging, synaptic dysfunction, and insulin-like growth factor (IGF)-1. J Gerontol A Biol Sci Med Sci 2012; 67: 611-625. https://doi.org/10.1093/gerona/gls118

[5] VanGuilder HD, Yan H, Farley JA, Sonntag WE, Freeman WM. Aging alters the expression of neurotransmission-regulating proteins in the hippocampal synaptoproteome. J Neurochem 2010; 113: 1577-1588. https://doi.org/10.1111/j.1471-4159.2010.06719.x

[6] Chen GH, Wang YJ, Qin S, Yang QG, Zhou JN, Liu RY. Age-related spatial cognitive impairment is correlated with increase of synaptotagmin 1 in dorsal hippocampus in SAMP8 mice. Neurobiol Aging 2007; 28: 611-618. https://doi.org/10.1016/j.neurobiolaging.2006.03.001

[7] Tong JJ, Chen GH, Wang F, Li XW, Cao L, Sui X *et al.* Chronic acarbose treatment alleviates age-related behavioral and biochemical changes in SAMP8 mice. Behav Brain Res 2015; 284: 138-152. https://doi.org/10.1016/j.bbr.2015.01.052

[8] Sunyer B, Diao W, Lubec G. The role of post-translational modifications for learning and memory formation. Electrophoresis 2008; 29: 2593-2602. https://doi.org/10.1002/elps.200700791

[9] Penney J, Tsai LH. Histone deacetylases in memory and cognition. Sci Signal 2014; 7: re12. https://doi.org/10.1126/scisignal.aaa0069

[10] Lundby A, Lage K, Weinert BT, Bekker-Jensen DB, Secher A, Skovgaard T *et al.* Proteomic analysis of lysine acetylation sites in rat tissues reveals organ specificity and subcellular patterns. Cell Rep 2012; 2: 419-431. https://doi.org/10.1016/j.celrep.2012.07.006

[11] Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, *et al.* Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science 2009; 325: 834-840. https://doi.org/10.1126/science.1175371

[12] Kim SC, Sprung R, Chen Y, Xu Y, Ball H, Pei J, Cheng T *et al.* Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mol Cell 2006; 23: 607-618. https://doi.org/10.1016/j.molcel.2006.06.026

[13] Alabi AA, Tsien RW. Perspectives on kiss-and-run: role in exocytosis, endocytosis, and neurotransmission. Annu Rev Physiol 2013; 75: 393-422. https://doi.org/10.1146/annurev-physiol-020911-153305

[14] Pozzi D, Condliffe S, Bozzi Y, Chikhladze M, Grumelli C, Proux-Gillardeaux V *et al.* Activity-dependent phosphorylation of Ser187 is required for SNAP-25-negative modulation of neuronal voltage-gated calcium channels. Proc Natl Acad Sci U S A 2008; 105: 323-328. https://doi.org/10.1073/pnas.0706211105

[15] Takeda T. Senescence-accelerated mouse (SAM) with special references to neurodegeneration models, SAMP8 and SAMP10 mice. Neurochem Res 2009; 34: 639-659. https://doi.org/10.1007/s11064-009-9922-y

[16] Chen GH, Wang YJ, Wang XM, Zhou JN. Accelerated senescence prone mouse-8 shows early onset of deficits in spatial learning and memory in the radial six-arm water maze. Physiol Behav 2004; 82: 883-890 https://doi.org/10.1016/S0031-9384(04)00309-9

[17] Barreto G, Huang TT, Giffard RG. Age-related defects in sensorimotor activity, spatial learning, and memory in C57BL/6 mice. J Neurosurg Anesthesiol 2010; 22: 214-219 https://doi.org/10.1097/ANA.0b013e3181d56c98

[18] Navarro A, Sanchez Del Pino MJ, Gomez C, Peralta JL, Boveris A. Behavioral dysfunction, brain oxidative stress, and impaired mitochondrial electron transfer in aging mice. Am J Physiol Regul Integr Comp Physiol 2002; 282: R985-992. https://doi.org/10.1152/ajpregu.00537.2001

[19] Pallas M, Camins A, Smith MA, Perry G, Lee HG, Casadesus G. From aging to Alzheimer's disease: unveiling "the switch" with the senescence-accelerated mouse model (SAMP8). J Alzheimers Dis 2008; 15: 615-624. https://doi.org/10.3233/JAD-2008-15408

[20] Arendash GW, King DL, Gordon MN, Morgan D, Hatcher JM, Hope CE, Diamond DM. Progressive, age-related behavioral impairments in transgenic mice carrying both mutant amyloid precursor protein and presenilin-1 transgenes. Brain Res 2001; 891: 42-53. https://doi.org/10.1016/S0006-8993(00)03186-3

[21] Rizo J, Sudhof TC. Snares and Munc18 in synaptic vesicle fusion. Nat Rev Neurosci 2002; 3: 641-653. https://doi.org/10.1038/nrn898

[22] Washbourne P, Thompson PM, Carta M, Costa ET, Mathews JR, Lopez-Bendito G *et al.* Genetic ablation of the t-SNARE SNAP-25 distinguishes mechanisms of neuroexocytosis. Nat Neurosci 2002; 5: 19-26. https://doi.org/10.1038/nn783

[23] Sato Y, Yamanaka H, Toda T, Shinohara Y, Endo T. Comparison of hippocampal synaptosome proteins in young-adult and aged rats. Neurosci Lett 2005; 382: 22-26. https://doi.org/10.1016/j.neulet.2005.02.053

[24] Cao L, Wang F, Yang QG, Jiang W, Wang C, Chen YP, Chen GH. Reduced thyroid hormones with increased hippocampal SNAP-25 and Munc18-1 might involve cognitive impairment during aging. Behav Brain Res 2012; 229: 131-137. https://doi.org/10.1016/j.bbr.2012.01.014

[25] Cao L, Jiang W, Wang F, Yang QG, Wang C, Chen YP, Chen GH. The reduced serum free triiodothyronine and increased dorsal hippocampal SNAP-25 and Munc18-1 had existed in middle-aged CD-1 mice with mild spatial cognitive impairment. Brain Res 2013; 1540: 9-20. https://doi.org/10.1016/j.brainres.2013.09.034

[26] Canas PM, Duarte JM, Rodrigues RJ, Kofalvi A, Cunha RA. Modification upon aging of the density of presynaptic modulation systems in the hippocampus. Neurobiol Aging 2009; 30: 1877-1884. https://doi.org/10.1016/j.neurobiolaging.2008.01.003

[27] Wirths O, Bayer TA. Neuron loss in transgenic mouse models of Alzheimer's disease. Int J Alzheimers Dis 2010. https://doi.org/10.4061/2010/723782

[28] Nicolle MM, Gallagher M, McKinney M. No loss of synaptic proteins in the hippocampus of aged, behaviorally impaired rats. Neurobiol Aging 1999; 20: 343-348.

[29] Beeri MS, Haroutunian V, Schmeidler J, Sano M, Fam P, Kavanaugh A *et al*. Synaptic protein deficits are associated with dementia irrespective of extreme old age. Neurobiol Aging 2012; 33: 1125 e1121-1128.

[30] Van Guilder HD, Farley JA, Yan H, Van Kirk CA, Mitschelen M, Sonntag WE, Freeman WM. Hippocampal dysregulation of synaptic plasticity-associated proteins with age-related cognitive decline. Neurobiol Dis 2011; 43: 201-212 https://doi.org/10.1016/j.nbd.2011.03.012

[31] Mattson MP. Acetylation unleashes protein demons of dementia. Neuron 2010; 67: 900-902. https://doi.org/10.1016/j.neuron.2010.09.010

[32] Glozak MA, Sengupta N, Zhang X, Seto E. Acetylation and deacetylation of non-histone proteins. Gene 2005; 363: 15-23. https://doi.org/10.1016/j.gene.2005.09.010

[33] Min SW, Cho SH, Zhou Y, Schroeder S, Haroutunian V, Seeley WW. Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron 2010; 67: 953-966. https://doi.org/10.1016/j.neuron.2010.08.044

[34] Gold WA, Lacina TA, Cantrill LC, Christodoulou J. MeCP2 deficiency is associated with reduced levels of tubulin acetylation and can be restored using HDAC6 inhibitors. J Mol Med (Berl) 2015; 93: 63-72. https://doi.org/10.1007/s00109-014-1202-x

[35] Ramakrishnan NA, Drescher MJ, Drescher DG. The SNARE complex in neuronal and sensory cells. Mol Cell Neurosci 2012; 50: 58-69. https://doi.org/10.1016/j.mcn.2012.03.009

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