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Age-related spatial cognitive impairment is correlated with increase of synaptotagmin 1 in dorsal hippocampus in SAMP8 mice

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Abstract

The age-related decline of learning and memory is a common phenomenon in humans and animals, even though the underlying mechanism is not yet known. In the present study, we propose that synaptotagmin 1 (Syt 1) might be a synaptic protein involved in the loss of learning and memory with aging. To test this hypothesis, the age-related spatial cognitive ability of 36 P8 mice (15 mice aged 4 months, 11 mice aged 8 months and 10 mice aged 13 months) was measured in a Morris water maze. After the behavioral test, both the protein and mRNA levels of Syt 1 were determined in the dorsal hippocampus by means of immunocytochemistry and reverse transcriptase polymerase chain reaction (RT-PCR), respectively. In the Morris water maze, the latency of the 4-month mice to find the submerged platform was significantly shorter than that of the older mice, while there were no significant differences between the 8- and 13-month-old mice in this respect. Compared to the 4-month-old mice, the Syt 1 protein in the 13-month-old mice was significantly increased in almost all layers of each subfield of the hippocampus. The average level of Syt 1 mRNA in the dorsal hippocampus of the P8 mice had not changed with aging. The latency of the 13-month-old P8 mice tested in the Morris water maze was positively correlated with the Syt 1 immunoreactivity in four circuit-specific regions in the dorsal hippocampus. Interestingly, the latency in the Morris water maze was also positively correlated with the level of Syt 1 mRNA in the dorsal hippocampus in individual aged P8 mouse. These results suggest that increased Syt 1 in the dorsal hippocampus in aged mice might be responsible for the age-related impairment of learning and memory.

Keywords: Aging; Learning and memory; Morris water maze; SAM; Synaptotagmin

1. Introduction

The gradual decline of learning and memory with age is a common phenomenon observed in humans and animals [31] and is often associated with dysfunction of the hippocampus [25], an area that is selectively vulnerable to aging [30]. In the aged human hippocampus, neuronal loss might result in volume loss [40,42], whereas in aged mice, rats and monkeys the total number of dentate gyrus (DG) granule cells and pyramidal neurons in subfields CA1 and CA3 remains stable by means of unbiased stereological techniques [5,31]. In old mice, synapse loss was reported in the outer molecular layer of the DG, where the perforant path terminates [13]. However, synaptic loss was not found in the aged monkeys [47]. These inconsistent results do not support the hypothesis that a substantial synaptic loss in the hippocampus underlies the impairment of learning and memory in normal aging. Based on the available evidence, it was proposed to focus on the integrity of the specific hippocampal circuits within a given subregion and other parameters influencing the synaptic connectivity, such as synaptic proteins, for studying the effects of aging [13,35,44].

Synaptotagmin 1 (Syt 1), an integral membrane protein unique to both small synaptic vesicles and large dense-core vesicles in the brain [38], may act as a calcium sensor to

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mediate stimulus-coupled fast chemical synaptic transmission [14,20], and it could thus be a candidate protein involved in the age-related decline of learning and memory. Using Western blotting technology, one study reported no difference in protein amount of Syt 1 in the hippocampus among the aged Long-Evans rats with or without severe cognitive impairment [34]. However, another group demonstrated that the Syt protein in the hippocampus of Wistar rats (no subtypes were distinguished) gradually increased in development and aging from birth to 96 weeks of age, which corresponds to 70–80 years of age in humans [41]. These inconsistent results may be due to the different strains and observed structures of the hippocampus. The electrophysiological data and selective lesions have demonstrated that the dorsal hippocampus plays a crucial role in spatial information processing [3,19,32,33], whereas the ventral hippocampus is more associated with anxiety [2,3,24]. So far the correlations between levels of Syt 1 in the dorsal or ventral hippocampus and spatial cognitive ability have not been established.

The senescence-accelerated mouse (SAM), which shows early onset and accelerated effects of senescence after normal development and maturation, is an accelerated aging model established in 1981, including nine major senescence-accelerated prone mouse (SAMP) strains and three major senescence-accelerated resistant mouse (SAMR) strains [46]. The senescence-accelerated prone mouse 8 (SAMP8, P8) has been proposed as an excellent model for the study of brain aging [10,29]. Under conventional conditions the mean life span of P8 mice is about 10 months, whereas this is 17 months under specific-pathogen free conditions [10,23,46]. P8 developed a remarkable age-related deficiency in non-spatial learning and memory abilities in a number of tasks, including the active or passive avoidance task, the multiple choice and lever press task, the fear conditioning task and the inferential task [10,27,36,37]. This strain also experienced an early-onset age-related decline of spatial learning and memory in hippocampal-dependent tasks such as the water-filled or dry multiple T-maze, the Morris water maze, and the radial-arm water-filled or dry maze [6,10,27]. The aged P8 mice show altered emotion, abnormality of circadian rhythm, and increased oxidative stress, impaired immune system [4]. Many alterations of the gene expression and protein abnormalities (such as deposition of amyloid β-peptide) with relevance to age-related cognitive decline have been found in P8 brain. Therefore, P8 receives an attention in the neurobiology of aging and dementia [4].

We have recently found that the level of the neural cell adhesion molecule, a synaptic active zone protein as Syt 1, containing variable alternatively spliced exons (NCAM-VASE$^+$), was significantly increased in dorsal but not ventral hippocampus in aged P8 mice, and that the up-regulation of NCAM-VASE$^+$ could be involved in the impairment of their spatial learning and memory [39]. It is therefore of great interest to examine age-related alterations of Syt 1 in different layers of the dorsal hippocampus of P8 mice after having measured spatial learning and memory. In the present study, the protein level of Syt 1 was quantified in various layers in all subregions of the dorsal hippocampus in the P8 mice. Also, the mRNA level of Syt 1 was quantified in the whole dorsal hippocampus, along with assessing spatial learning and memory ability in the Morris water maze.

2. Materials and methods

2.1. Animals and general protocol

Virgin P8 mice were generously provided by Prof. Takeda of Kyoto University, Japan. Our breeding colony was maintained as an inbred strain under specific pathogen-free conditions and was transferred to conventional conditions at 2 months of age. The mice were housed in groups (4–6 same-sex mice per cage) in plastic cages (25.5 cm × 15 cm × 14 cm) with wood shaving bedding. All mice received standard rodent diet and tap water ad lib under a 12 h light–dark cycle (lights turning on at 7:30 a.m.), and a constant temperature of 21–22 °C and humidity of 55 ± 5%. Any animals with gross defects (tumors outside trunk, motor incapacitation, and overt blindness) were excluded prior to starting the behavioral examination. A total of 36 P8 mice were stratified into three groups: 15 young mice (eight males and seven females) aged 4 months, 11 middle-aged mice (six males and five females) aged 8 months, and 10 old mice (five males and five females) aged 13 months. The experimenter was blind to the groups. One hour before the test, the mice were transferred to the experimentation room for acclimatization. The animals were treated according to the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Behavioral test

The apparatus and the procedure were as previously described [6]. Briefly, a black circular tank (150 cm in diameter, 30 cm in height) filled with 20–21 °C water was circled by a white cloth curtain with three differently shaped (circle, triangle and square) black cardboards hung equidistantly. A black platform (diameter 10 cm, height 24 cm) was submerged 1.0 cm below the surface of the water. Each mouse underwent four successive trials a day for 10 days. The sequence of water-entering points differed per day, but the location of the platform was constant. Latency to find the platform was measured up to a maximum of 90 s. On locating the platform, the mouse was left there for 30 s prior to the next trial. If the mouse failed to locate the platform within 90 s, it was guided to the platform and allowed to stay there for 30 s.

2.3. Tissue preparation

Approximately 15 days after the behavioral testing, the mice were anaesthetized with halothane and decapitated and

their brains were rapidly removed from the skull. On dry ice, the whole right-side of the brain was dissected, followed by 7 days of fixation in 4% paraformaldehyde at 4 °C, and the hippocampus on the left side was quickly isolated. The isolated hippocampus was then evenly dissected into three parts along the longitudinal axis, and then stored at −80 °C. The upper third part of the entire hippocampus was considered to be the dorsal hippocampus.

2.4. Immunofluorescent histochemistry

The fixed right side of the brain was dehydrated and embedded in paraffin. Serial 6 μm coronal sections were cut on a Leica Microtome (Leica RM 2135). Of each mouse five sections (1 out of every 20 serial sections) containing the dorsal hippocampus were selected to be mounted on polylysine-coated slides.

The main principle and procedure of histochemistry and measurement have been extensively described before [15,50]. The sections were hydrated, rinsed in TBS (Tris buffered saline: 0.05 M Tris, 0.9% NaCl, pH 7.6) for 10 min, and treated with 0.3% hydrogen peroxide in TBS for 30 min to quench endogenous peroxidase activity. After washing in TBS (3× 10 min), the sections were treated with microwave (700 W) in 0.05 M citrate buffer saline (pH 6.0) for 10 min for antigen retrieval and left to cool for 20 min at room temperature. The sections were subsequently washed in TBS (3× 10 min) and (a) incubated in 5% normal goat serum (Vector Laboratories, Burlingame, CA) in TBS for 1 h at 37 °C to block non-specific staining; (b) incubated with Sudan Black B solution for 2 h to quench endogenous fluorescence; (c) incubated with the primary polyclonal antibody, rabbit anti-Syt 1 IgG using a synthetic peptide corresponding to the N-terminus of Syt 1 of rat origin (Sigma Corporation, S2177) for 1 h at 37 °C and overnight at 4 °C, diluted 1:800 in TBS containing 5% normal goat serum, then washed in TBS (3× 10 min); (d) incubated with the biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) diluted 1:200 in Triton–TBS for 1 h at 37 °C, then washed in TBS (3× 10 min); (e) incubated with Cy^{TM}3 labeled streptavidin (Kirkegaard and Perry Laboratories) diluted in TBS (1:800) for 45 min at room temperature; (f) incubated with 0.05 M glycine–HCl buffer saline (pH 2.2) for 2 h at room temperature to quench additional antibodies. Subsequently, the sections were coverslipped with glycerin.

An image analysis system was used for quantitative analysis. The system includes MetaMorph image acquisition and processing software (Universal Imaging, USA), a Spotcooled color digital camera (Diagnostic Instruments, USA), a Nikon E800u microscope (Nikon, Japan) equipped with a Prior scanning stage (Prior Scientific Instruments, England) and a HP computer. The layers analyzed in the different subfields of the P8 dorsal hippocampus include the stratum oriens (SO), stratum radiatum (SR) and stratum lacunosum-moleculare (SLM) in the CA1; the polymorphic layer (PL), stratum lucidum (SL) and SR in the CA3; and the hilus or PL, outer and middle molecular layer (OMML) and inner molecular layer (IML) in the DG. First, a picture of complete hippocampal formation was obtained at low magnification (4× objective). Then, pictures of high magnification (40× objective) in various subfields of the hippocampus were acquired according to area of each subfield: three pictures in CA1 for SO and SR; one picture in CA3 for PL, SL and SR; two pictures in DG for DG-OMML, DG-IML and CA1-SLM. Digital data were exported into MetaMorph software for measurement. The gray values, ranged from dark to bright of 256 gray scale values ("brightest" represents maximum of protein expression), represented the intensity of Syt 1 immunofluorescence staining. The gray values for threshold were established before measurement and were identical for each lamina to ensure that all measurements were objective. The analyzed region of each layer in each subfield was manually outlined. According to the uniform threshold values the gray value was automatically determined after identifying the positive puncta. Image analysis was performed using a blinded procedure without knowledge of the experimental treatment.

2.5. Reverse transcriptase polymerase chain reaction (RT-PCR)

The main procedure was similar to our previous description [39]. Briefly, total RNA was extracted from the frozen dorsal hippocampus of the P8 brain using the Trizol (Invitrogen, USA) method and determined by spectrophotometric measurements at 260 nm and non-denatured agarose gel electrophoresis. For Syt 1, the sense primer was 5′-GTG AGT GCC AGT CGT CCT GAG-3′ and antisense primer was 5′-TTC TTC TCC ATC AGT CAG TCC-3′ to yield a 393 bp product of Syt 1 cDNA. The endogenously expressed mouse glyceroaldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control. Synthetic sense primer 5′-TGA AGG TCG GAG TCA ACG GAT TTG GT-3′ and the antisense primer 5′-CAT GTG GGC CAT GAG GTC CAC CAC-3′ were used to detect the GAPDH mRNA, which yielded a cDNA product of 983 bp. By using the manufacturer's protocol of RevertAid^{TM} First Strand cDNA Synthesis kit (MBI Fermentas, USA), cDNAs were synthesized in 20 μl reactions containing 5 μg of total RNA, 0.5 μg oligo (dT) primer, 5× reaction buffer, 10 mM dNTPs mix, 20 units of ribonuclease inhibitor, 200 units RevertAid^{TM} M-MuLV reverse transcriptase. For PCR amplification, different PCR cycle numbers and different amounts of synthesized cDNA were first examined to evaluate the linearity of the reaction. The polymerase reaction was carried out in a 25 μl solution that contained 2.5 μl cDNA, 10× polymerase reaction buffer, 0.2 mM dNTP mixture, 0.1 μM primers, and 0.25 U Taq polymerase (Takara, Japan). Amplification was carried out by the following cycle parameters after 95 °C for 6 min: for Syt 1, 94 °C for 60 s, 64 °C for 45 s, and 72 °C for 45 s; for GAPDH, 94 °C for 1 min, 58 °C for 45 s and 72 °C for 45 s. After 30 cycles (for Syt 1) and 27 cycles (for GAPDH) of amplification, a

final extension step was performed at 72 °C for 10 min. The PCR products of Syt 1 and GAPDH were electrophoretically separated on 8.5% polyacrylamide and 1.2% agarose gels, respectively. By staining with ethidium bromide, the cDNA bands were visualized and analyzed with Eaglesight software (Stratagene, USA). The relative amount of Syt 1 mRNA was expressed as the ratio of the optical density (OD) of Syt 1 to GAPDH cDNA band.

2.6. Statistical analysis

The results were expressed as mean ± means of standard error (S.E.M). For the latency in the Morris water maze, analysis was performed using a repeated-measure analysis of variance with ages as independent variables. Post hoc analysis using the Fisher's least-significant difference (LSD) test was used to compare results for the different days and ages. For Syt 1 protein and mRNA the age effect was detected by one-way analysis of variance using LSD for post hoc analysis. The Pearson correlation test was used to analyze the correlation between the average latency of the cognitive task in all trials for 10 days and the relative amount of Syt 1 protein or mRNA. $P < 0.05$ was considered significant. All analyses were conducted by statistical software, SPSS 10.0 for Windows.

3. Results

3.1. Performance in water maze

The learning curves in each group in the Morris water maze are presented in Fig. 1. The latency to find the submerged platform significantly declined every day, but only in the 4-month-old P8 mouse [$F_{(9, 99)} = 17.94$, $P < 0.001$], not in the other two groups. The post hoc analysis indicated that 2 days after the start of the learning task the performance in the 4-month-old P8 mice was significantly better than that in the 8- and the 13-month-old mice (P's < 0.05). There was no significant difference between the 8- and the 13-month-old mice ($P = 0.614$), suggesting that the younger mice were better able to learn this task and that the learning function of older mice had declined.

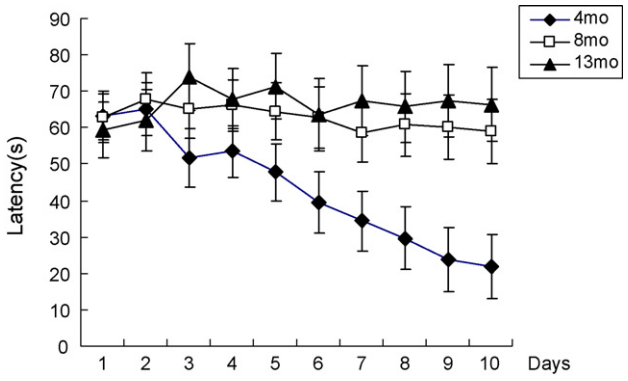

Fig. 1. Latencies of the P8 mice to find the submerged platform in the Morris water maze. The latency in the 4-month-old mice ($n = 15$) was significantly shorter than that in the 8- and the 13-month-old mice ($n = 11$ and 10, respectively), but there was no significant difference between the 8- and the 13-month-old mice. Data (mean ± S.E.M.) are expressed as the average of all four trials of every 1 of the 10 days, and the analysis was performed by a two-way (age × day) ANOVA with repeated measure design.

3.2. Levels of Syt 1 protein

Table 1 shows the Syt 1 immunoreactivity in the different layers of each subfield in the dorsal hippocampus of P8 mice of different ages. Compared to the 4-month-old group, the 13-month-old group had significantly increased immunoreactivity of Syt 1 in all layers of each subfield except DG-IML. In the 8-month-old mice, the increased immunoreactivity occurred in three layers of DG (PL, OMML and IML) and two layers of CA1 (SR and SLM).

3.3. Levels of Syt 1 mRNA

The relative levels of Syt 1 mRNA in the P8 dorsal hippocampus is shown in Fig. 2. There was no

Table 1
Immunoreactivity of synaptotagmin-1 in the different layers of each subfield in the P8 dorsal hippocampus

Subfields	Stratum	4 months ($n = 15$)	8 months ($n = 11$)	13 months ($n = 10$)
CA1	SO	46.31 ± 2.84	54.62 ± 3.63	56.98 ± 3.81*
	SR	42.08 ± 2.75	51.08 ± 3.28*	51.85 ± 3.59*
	SLM	40.33 ± 3.12	52.40 ± 3.56*	51.72 ± 3.56*
CA3	SL	43.79 ± 2.82	50.87 ± 3.46	55.62 ± 3.79*
	SR	42.68 ± 2.98	50.37 ± 3.65	56.63 ± 3.99*
	PL	46.04 ± 3.19	52.45 ± 3.68	59.49 ± 4.04*
DG	PL	44.11 ± 2.74	57.80 ± 3.47*	58.00 ± 3.65*
	OMML	39.89 ± 2.98	50.16 ± 3.88*	56.07 ± 4.64*
	IML	37.28 ± 2.56	47.05 ± 2.89*	43.96 ± 3.02

Data (mean ± S.E.M.) are expressed as the relative protein level of synaptotagmin-1 with gray value of immunofluorescence after 15 days of completed behavioral tests. Asterisks (*) show significant differences between the groups. DG, dentate gyrus; IML, inner molecular layer; OMML, outer and middle molecular layer; PL, polymorphic layer; SL, stratum lucidum; SLM, stratum lacunosum-moleculare; SO, stratum oriens; SR, stratum radiatum.

* $P < 0.05$ vs. 4 months of age; a one-way ANOVA was used.

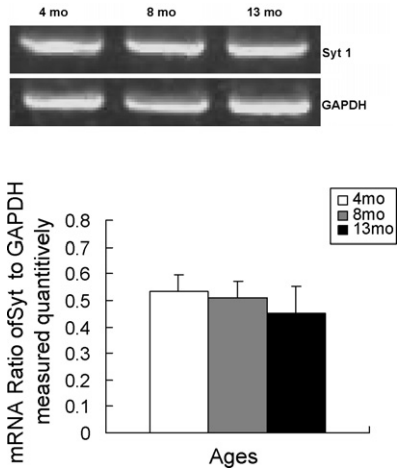

Fig. 2. Upper: representative gel pattern showing Syt 1 as well as glyceroaldehyde-3-phosphate dehydrogenase (GAPDH) cDNA bands. There was no significant difference in Syt 1 mRNA levels in the dorsal hippocampus of P8 mice at any age. Lower: relative amount (mean ± S.E.M.) of Syt 1 mRNA in the dorsal hippocampus of P8 mice after all the tests had been completed (15 days). Data are expressed as the ratio of the optical density of Syt 1 cDNA band to the optical density of GAPDH cDNA band, and are analyzed using one-way ANOVA. There was no significant difference between groups at 4 ($n = 15$), 8 ($n = 11$) and 13 ($n = 10$) months of age, respectively.

significant difference in this respect in all three age groups.

3.4. Relationships between cognitive ability and levels of Syt 1 protein and mRNA

Between the latency in the Morris water maze in all mice combined and the Syt 1 immunoreactivity, a positive correlation only occurred in four circuit-specific regions in two subfields in the dorsal hippocampus, i.e. CA3-SL ($r = 0.330$, $P < 0.05$), CA3-SR ($r = 0.393$, $P < 0.05$), DG-PL ($r = 0.351$, $P < 0.05$) and DG-OMML ($r = 0.398$, $P < 0.05$). When this was analyzed according to the individual age groups, the positive correlation only occurred in the 13-month-old P8 mice: CA3-SL ($r = 0.672$, $P < 0.05$), CA3-SR ($r = 0.645$, $P < 0.05$), DG-PL ($r = 0.685$, $P < 0.05$) and DG-OMML ($r = 0.715$, $P < 0.05$), see respectively Fig. 3A–D in detail. The level of Syt 1 mRNA in the dorsal hippocampuses in all P8 mice combined was also positively related to the latency in the Morris water maze ($r = 0.335$, $P < 0.05$, Fig. 4A), and this positive correlation only took place in 13-month-old P8 mice ($r = 0.888$, $P < 0.01$, Fig. 4B).

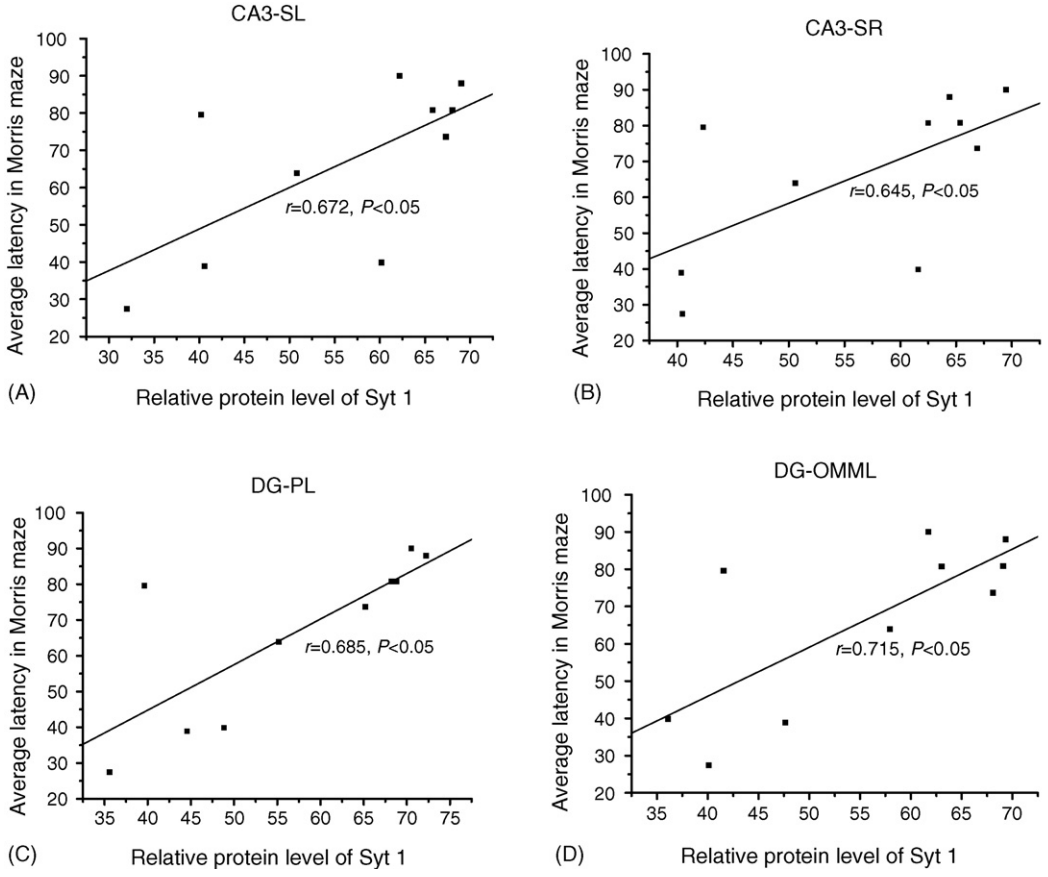

Fig. 3. Correlations between the cognitive abilities and levels of synaptotagmin 1 (Syt 1) protein (A–D) in 13-month P8 mice ($n = 10$). The cognitive abilities were indicated by the average latencies in all trials for 10 days to find the submerged platform in the Morris water maze. The analysis was performed by the Pearson correlation test and the correlation coefficients (r) were respectively shown in each figure. CA3-SL, stratum lucidum of CA3 subfield in hippocampus; CA3-SR, stratum radiatum of CA3 subfield; DG-PL, polymorphic layer of dentate gyrus in hippocampus; DG-OMML, outer and middle molecular layer of dentate gyrus.

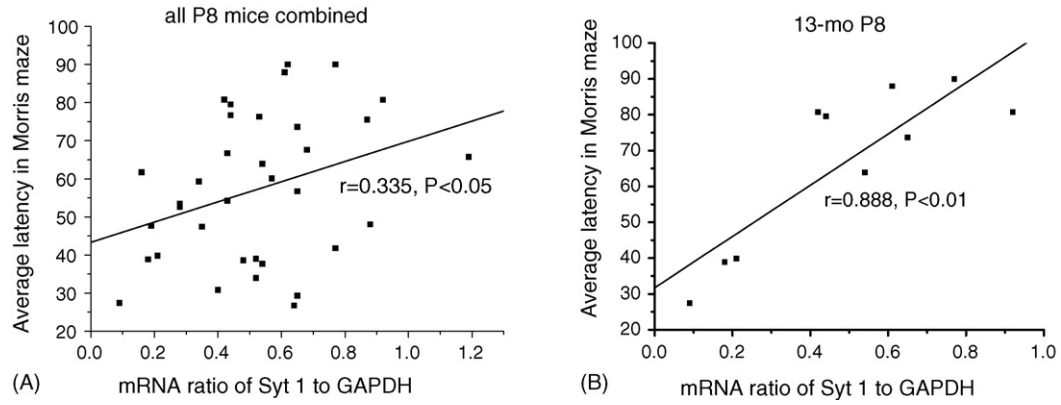

Fig. 4. Correlations between the cognitive abilities and mRNA levels of synaptotagmin 1 (Syt 1) in all P8 mice combined (A, $n = 36$) and in 13-month P8 mice (B, $n = 10$), respectively. The cognitive abilities were indicated by the average latencies in all trials for 10 days to find the submerged platform in the Morris water maze. The level of Syt 1 mRNA was expressed with the ratio of the optical density of Syt 1 cDNA band to the optical density of glyceroaldehyde-3-phosphate dehydrogenase (GAPDH) cDNA band. The analysis was performed by the Pearson correlation test and the correlation coefficients (r) were respectively shown in each figure.

3.5. Relationships between Syt 1 protein and mRNA in the 13-month-old P8 mice

There is no correlation between the levels of proteins in any of the regions measured and that of total mRNAs in the dorsal hippocampuses of the 13 month animals, all Ps > 0.05.

4. Discussion

In the present study, we found a marked impairment of spatial learning and memory in the 8- and the 13-month-old P8 mice. Consistently, an increased Syt 1 immunoreactivity occurred in five circuit-specific regions in the dorsal hippocampus of 8-month mice. This increased Syt 1 immunoreactivity was found in more regions of the hippocampus in 13-month-old mice. The impairment of spatial cognitive performance was positively correlated with the protein level of Syt 1 in a number of circuit-specific regions in the dorsal hippocampus, especially in 13-month-old P8 mice. Furthermore, the impairment of cognitive ability was also positively correlated with the mRNA level in the aged mice, although no significantly age-related difference at the average mRNA level of the Syt 1. Our behavioral results are consistent with previous results [6,23,26]. In the Morris water maze, the decrement of spatial learning ability did not take place in 3-month-old and 5-month-old P8 mice [6], but it emerged at 8 months of age and deteriorated from then on [23,26]. In the present study we show, for the first time, that the age-related impairment of learning and memory is associated with the increased circuit-specific protein level of Syt 1 in the dorsal hippocampus of P8 mice. However, it is worthwhile to note that even correlations at the level of 0.6, this accounts for only about 1/3 of the variance in our data. Thus, in addition to Syt 1, there must be other factors that determine behavioral capacity, such as α-spectrin and protein kinase C-γ [4].

In the few previous published studies, there have been inconsistent findings of the effects of aging on Syt1 expression in different regions of the brain. Throughout the entire cerebral cortex the mRNA level of Syt 1 was decreased in aged (22 months) BALB/c mice [17], but the protein level of Syt 1 did not change in aged (27 months) Wistar rats [16]. Throughout the whole hippocampus, the protein level of Syt gradually increased during aging in the Wistar rats (96-weeks old) [41], but there was no change in the protein level of Syt 1 in the aged (26–27-months old) Long-Evans rats even with severe cognitive impairment [34]. Discrepancies in published findings may relate to what region(s) of the hippocampus were studied, as it is becoming clear that the dorsal and ventral regions are functionally different.

According to the available data, Syt 1 is a promoter of the synaptic vesicle fusion, mainly functioning as a fast calcium sensor for synchronous neurotransmitter release via facilitating exocytosis and endocytosis (reviewed in [21,45]). Therefore, it is difficult to understand the increased protein level of Syt 1 in hippocampus and the impaired spatial cognitive performance in aging. For other synaptic proteins, such as *N*-methyl-D-aspartate subtype of glutamate receptors, synaptophysin, protein kinase C, a number of studies have found an age-related decline throughout the entire or dorsal hippocampus, and in some selectively hippocampal circuits [1,11,12,22,43]. Consistent with our findings, some other studies indicated that the increased Syt 1 protein in the dorsal hippocampus in older P8 mice may be a response to certain pathological statuses. In the apolipoprotein E-deficient C57BL/6J mice at 15 months of age (only corresponding to the middle-aged wild-type C57BL/6J mice whose lifespan is about 26–28 months [18]), the Syt increased by 23.5% in the neocortex, and an increased trend occurs in the hippocampus accompanied by a cognitive impairment [49]. In the amyloid precursor protein + presenilin-1 transgenic mice with a β-amyloid deposition in their brain [28], the Syt 5

mRNA remained stable in the entire hippocampus at the age of 17–18 months [9]. Another reason for the elevated protein level of Syt 1 in the old P8 hippocampus might stem from the compensatory mechanisms. A possible cause is that during aging the decrease of expression in some Syt isoforms such as Syt 7, a possible calcium sensor in the active zone of the synapse [45], might result in the presynaptic increase of Syt 1. Alternatively, the increased Syt 1 in the hippocampus during aging could also be assumed to be the result of increased calcium concentration in neurons [48] or decreased axonal transport, especially retrograde transport [8] in aging. Increased intracellular calcium may bind synaptic proteins involved in localization of the vesicle to the presynaptic terminal, the docking of the vesicle at the release site, and the fusion of the vesicle to the plasma membrane. Syt 1 is a calcium sensor to mediate fast neurotransmitter release [20]. Therefore, the increased Syt 1 in the aged dorsal hippocampus might be concomitant with the increased intracellular calcium levels. Available evidence indicated that the ability of retrograde axonal transport declined with age [8]. It appears to be reasonable to speculate that due to decreased retrograde transport, Syt 1 cannot be removed after acting, which results in a gradual accumulation at the presynaptic terminal during aging.

Our study showed that the protein level of Syt 1 was elevated while its mRNA level was not significantly changed in the dorsal hippocampus of aged P8 mice. Moreover, there is no correlation between the levels of proteins in any of the regions measured and total mRNAs in dorsal hippocampus. This may be explained as a difference between whole hippocampal numbers (mRNA) and levels in discrete hippocampal regions (protein). Alternative, our previous study had shown there was discordance between the protein and the mRNA of arginine vasopressin in patients with depression [51]. This discordance between the patterns of translation and transcription in Syt 1 during aging has also been found in developmental primary cultures of embryonic hippocampal neurons [7]. During development, although Syt 1 is synthesized at a nearly constant rate of the gene transcription in the hippocampal neurons, the half-life of Syt 1 protein is progressively increased so that the protein level of Syt 1 is elevated with time [7]. Consequently, the increased protein of Syt 1 in the aged dorsal hippocampus may be due to the asynchronous prolongation of half-life with increasing age between the protein and the mRNA.

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