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Tube Feeding in Aged SAMP 1 Mice Decreases the Number of Dendritic Spines in the Hippocampus

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We examined the effect of tube feeding on the number of dendritic spines of hippocampal pyramidal cells and spatial performance in a water maze task in SAMP1 mice. Tube feeding decreased learning ability in association with a decrease in the number of dendritic spines in CA1 pyramidal cells in aged SAMP1 mice. The results suggest that tube feeding attenuates input activity in hippocampal synapses.

Key words: Tube feeding, Spine, Learning ability, Hippocampus, SAMP 1

INTRODUCTION

Tube feeding due to a decreased ability to ingest food orally or deterioration of general conditions, results in lower levels of motivation, activities of daily living, and memory in elderly patients. Moreover, appropriate dental care in the elderly stages of dementia can increase the ability to ingest food orally, which results in higher levels of motivation and activity[1,2)], suggesting the importance of masticatory function in the prevention of dementia. In addition, soft-diet feeding during development in rats[3)] or after weaning in mice[4)] reduces synapse formation in the cerebral cortex and impairs spatial learning in adulthood. Previous studies using senescence-accelerated (SAMP 8) mice demonstrated that dysfunctional masticatory conditions in aged mice induce deficits in spatial memory[5)], with various pathologic changes, such as degeneration of hippocampal CA1 pyramidal cells[6)], decreased Fos induction in the hippocampal CA1 region[5)], and decreased number of dendritic spines in hippocampal CA1 pyramidal cells[7)]. These findings suggest that dysfunctional mastication in aged mice and rats reduces input activities in the central nervous system, especially in the hippocampus, thereby leading to deficiencies in spatial memory and neural degeneration.

Decreased input activities in the central nervous system result in the degeneration and neuronal loss of target cells[7,8)]. The number of dendritic spines, which are the main postsynaptic target organ[9)], is reduced by decreased information input[10,11)]. Dysfunctional mastication due to extraction of the upper molars decreases the number of dendritic spines of CA1 pyramidal cells, which is linked to impaired spatial memory in aged mice[7)]. Indeed, in our previous studies of SAMP 1 mice, tube-fed aged mice had decreased spatial memory, Fos induction, and neuron density in the hippocampal CA1 region[12,13)], suggesting decreased input activity to the hippocampus.

We therefore hypothesized that impaired spatial memory might result from tube feeding conditions, possibly leading to a decrease in the number of dendritic spines in hippocampal pyramidal cells. In this study, to examine this hypothesis, we examined the effect of tube feeding on the number of dendritic spines in hippocampal pyramidal cells using Golgi-Cox staining and spatial performance in the Morris water maze in SAMP1 mice.

METHODS

Male SAMP 1 mice (15 and 35-wk-old, n＝15, respectively) were used in this study. The SAMP1 mouse is an experimental murine model of aging[14)]. Male pups were weaned at 3 wk after birth and housed in groups of 5 in standard plastic cages under temperature- and humidity-controlled conditions (23±1℃, 55 ± 2%) with free access to food and water, with a 12-h light cycle. The animals were treated in accordance with the principles approved by the Council of the Japanese Neuroscience Society, and bred and maintained under conventional conditions. Mice from each age group (15 wk and 35 wk) were divided into three groups: mice that were bred under conditions with free access to food and water (control condition), mice that were fed under the same conditions and given water (1.2m*l*) into the stomach with a catheter (4 Fr, catheter made with polybutadiene, outer diameter 1.35mm, Atom Medical Co., Ltd., Tokyo, Japan) three times a day (10：00, 16：00, and 22：00；water condition), and mice that were fed under conditions with free access to water and given a mixture of water (1.2g) and powdered chow (0.7g, 36 ～50mesh) into the stomach with a catheter three times a day (10：00, 16：00, and 22：00；tube feeding condition).

The Morris water maze test is a sensitive assay for abnormalities in the hippocampus[15)]. This maze test was performed as described previously[7)] from 8 d after starting the feeding regimen. Briefly, a stainless steel tank (90cm in diameter, 30cm deep), was filled with water (approximately 28℃) to a height of 22cm and the water surface was covered with floating polystyrene foam granules (approximately 2mm in diameter). A platform was submerged 1cm under the water surface and located at a constant position near the center of one of the four quadrants of the pool.

Eight days after starting one of the above feeding regimens, the mice were placed into the water from one of four points around the perimeter of the tank and given 28 trials over 7 consecutive days (four trials per day). The sequence of starting po-

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sitions was changed randomly every day.

After the Morris water maze test, the mice were killed with an overdose of sodium pentobarbital. The brains were quickly removed and immersed in 100m*l* of Golgi-Cox solution[16] in the dark for 2 d. The tissue blocks were rinsed with distilled water and then immersed in the same solution in the dark for 4 wk. The blocks were dehydrated in a graded ethanol series, and embedded in celloidin. Coronal sections (90μm) were cut on a sliding microtome (ERMA Inc, Tokyo, Japan) and treated according to the method of Glaser et al.[16]. The sections were immersed in 14.0% NH_4OH, immersed in 0.5% p-phenylene diamine, immersed in 1.0% photographic developer (Kodak, Tokyo, Japan), and immersed in .05% photographic fix (Fuji, Tokyo, Japan). The sections were then dehydrated in a graded ethanol series, cleared in xylene, and coverslipped. Cells with clearly stained basal dendrites and dendritic spines of pyramidal cells were examined under 1000x magnification and digitized using a computer and FLv-Fs software (Flovel Co., Ltd, Tokyo, Japan).

Previous studies indicated that learning affects the dendritic spine density of hippocampus CA1 basal dendrites, but not that of apical dendrites[17~20]. In these previous experiments, there were no obvious changes in the number of apical dendritic spines between the molarless and control groups. Thus, at least 10 Golgi impregnated cells in the hippocampal CA1 subfield were randomly chosen for quantitative analysis of dendritic spines from each animal. One of the basal dendrites of the chosen cells stained and impregnated without breaks in staining along the dendrites was used for analysis. Each basal dendrite was divided into 30-μm segments for observation according to the method of Sugiyama et al.[21]. Dendrites and dendritic spines in each segment were followed for up to 120μm. Each group of animals was analyzed with the investigator blind to their experimental grouping. The data were statistically analyzed using ANOVA. If a significant effect was found in the ANOVA, individual group comparisons were made with Fisher's PLSD post hoc test. A P value of less than 0.05 was considered to be statistically significant.

RESULTS

In 35-wk-old control, water, and tube fed mice, the latencies to reach the platform were significantly longer compared to 15-wk-old mice under the same conditions [$F(5, 30) = 1.125$, control, $P = 0.0180$; water, $P = 0.0031$ and tube feeding, $P < 0.0001$, respectively], when trained over 7 consecutive days (Fig.1 A, B). In the 15-wk-old mice, there was no significant difference in the latencies to reach the platform among mice under the three conditions [$F(2) = 0.140$, $P = 0.9531$] (Fig.1 A). On the other hand, in the 35-wk-old mice, the latencies of tube-fed mice were significantly longer than those of control ($P = 0.0073$) and water-fed mice ($P = 0.0200$); there was no significant difference between control and water-fed mice ($P = 0.5983$) (Fig.1 B).

The dendritic spines in the hippocampal CA1 region were morphologically assessed using Golgi-Cox staining after the final maze testing. In light microscopic analysis, pyramidal cells with both apical and basal dendrites were clearly observed in all mice (Fig.2 A and B). Consistent with the Morris water maze test results, the number of dendritic spines decreased in aged tube-fed mice, but there was no clear decrease in the control and water mice. Furthermore, the effects of tube feeding on the num-

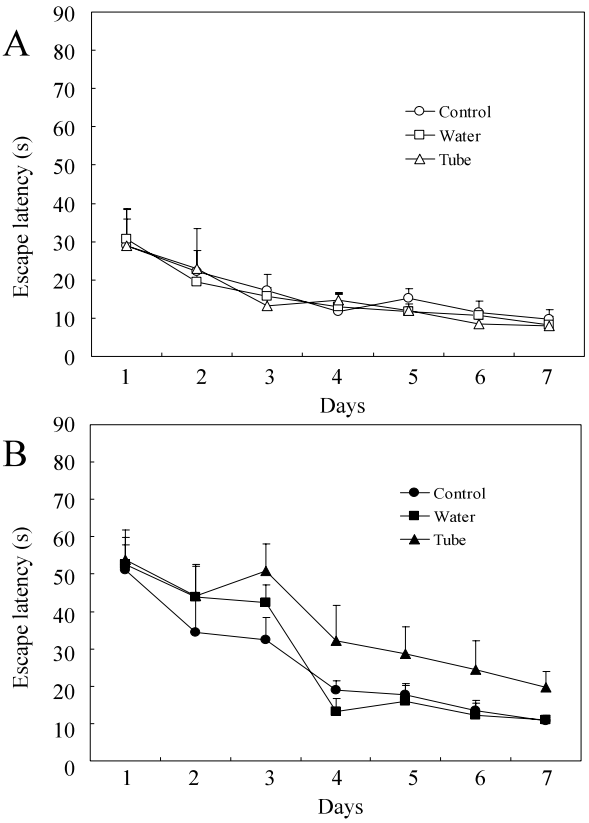

Fig.1. Spatial learning in a Morris water maze test. The results are expressed as the mean score (± standard error, SE, n=5 for each group) of four trials per day. Note that in 35-wk-old mice, the tube-fed group required significantly more time to reach the platform than control ($P < 0.0073$) and water groups ($P < 0.020$). A: 15-wk-old mice, B: 35-wk-old-mice.

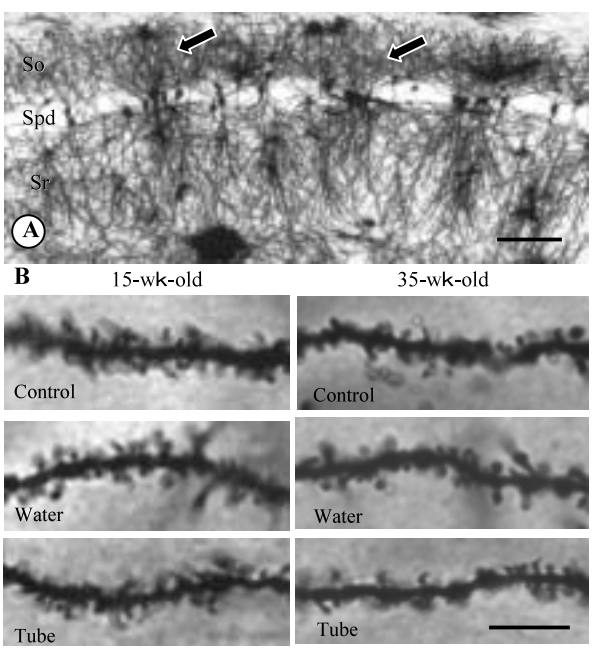

Fig.2. Photomicrographs showing the hippocampal CA1 pyramidal cells (A) and the dendritic spines (B). So: stratum oriens; Spd: stratum pyramidale; Sr: stratum radiatum. Scale bar: 100μm (A) and 10 μm (B). Arrows: basal dendrites.

ber of dendritic spines were limited to segments 30-60, 60-90, and 90-120μm. Dendritic spine number was significantly decreased in 35-wk-old control-, water- and tube-fed mice compared with 15-wk-old control [segments 30-60μm ($P=0.0003$), 60-90μm ($P=0.0025$), and 90-120μm ($P<0.0001$)], 15-wk-old water-fed mice [segments 30-60μm ($P=0.0035$), 60-90μm ($P=0.0061$), and 90-120μm ($P=0.0001$)] and 15-wk-old tube-fed mice [segments 30-60μm ($P<0.0001$), 60-90μm ($P<0.0001$), and 90-120μm ($P<0.0001$); Fig. 3], respectively. When the effect of the tube feeding on dendritic spine number was statistically analyzed across the three conditions, there was no significant difference between all segments in 15-wk-old mice, but in 35-wk-old mice, in which tube feeding strongly impaired learning, there was a marked decrease in segments 30 to 60 [control: $P<0.0001$, water condition: $P<0.0001$], 60-90μm [control: $P<0.0001$, water condition: $P<0.0001$] and 90-120μm [control: $P<0.0001$, water condition: $P<0.0001$] (Fig. 3).

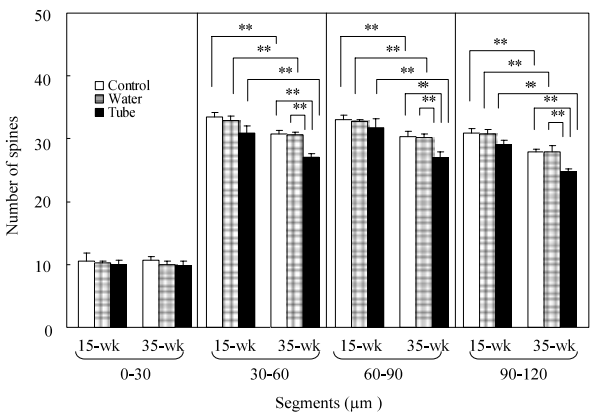

Fig. 3. Effect of tube feeding on the number of dendritic spines in hippocampal CA1 pyramidal cells. The results are expressed as the mean number of spines/ 30μm. Note the greater reduction in the number of spines in CA1 regions in 35-wk-old tube-fed mice.
**: $p<0.01$

DISCUSSION

The present study revealed no significant difference in escape latencies to reach the platform between the water-fed and control groups in both young and aged mice, indicating that mice might not be seriously influenced by catheter insertion into the stomach.

In our previous study, under tube feeding conditions, there was a decline in spatial learning and a degeneration of CA1 pyramidal cells in aged mice[12,13]. Furthermore, in the present experiment, the mean number of pyramidal cell dendritic spines, when exposed to tube feeding, declined in aged mice. In previous reports, the molarless condition enhanced the age-dependent decline in learning ability[5], the number of neurons in the hippocampal CA1 subfield[6], and the number of dendritic spines in the hippocampus CA1 pyramidal cells[7]. SAMP1 TA/ Ngs mice have impaired learning at 3 mo and memory disturbance at 7 mo, but no particular abnormality at 5 mo, suggesting that the number of dendritic spines in the hippocampus in the 3 -and 7 -mo-old mice was significantly lower than that in 5 -mo-old mice[18]. Thus, the variation of spines in hippocampal pyramidal cells might be closely related with learning ability and spatial memory.

There are some studies to suggest that learning influences the number of dendritic spines in the hippocampus. O' Malley et al[22] reported a transient increase in dendritic spine density in the dentate gyrus after spatial learning, whereas several studies report a variation of dendritic spines in the CA1 after similar training procedures[19~23]. Kawaguchi et al[18] and Sugiyama et al.[21] suggested that the decreased number of dendritic spines in hippocampal CA1 pyramidal cells contributes to the decreased learning ability in SAMP 1 TA/ Ngs and SAMP8/ Ta mice. In our previous studies, the dysfunctional mastication decreased number of dendritic spines in hippocampal CA1 pyramidal cells linked to the decreased learning ability in SAMP8 mice[7]. In the present study, the number of dendritic spines in hippocampal CA 1 pyramidal cells decreased in aged mice under tube feeding conditions. These findings suggest that dendritic spine density in CA1 pyramidal cells in the hippocampus might be affected by learning ability.

The mechanism by which tube feeding accelerates senile impairment of spatial learning and decreases the number of dendritic spines in aged SAMP 1 mice is not known. One possibility is that tube feeding reduces input activity to the hippocampus, thereby leading to a reduction in dendritic spine density in the CA1 subfield. The hippocampus receives information via the perforant path from the entorhinal cortex, which is a region of extraordinary convergence of inputs from the association cortex[24,25]. This is the most vulnerable circuit in the cerebral cortex[26] and is devastated by aging-related neuropathology[27,28]. Decreased information inputs in the central nervous system induce degeneration and a decrease in the number of target cells[7,8]. The number of dendritic spines is reduced by decreased input activity from the presynaptic neuronal cells[10,18]. Indeed, our previous findings suggest that tube feeding induces a reduction in the number of Fos-positive cells in the hippocampal CA1 region, which is linked to decreased spatial performance in the Morris water maze test and a decreased neuronal density in the hippocampal CA1 subfield[12,13]. Therefore, the decrease in spine density might be induced by a reduction of input activities to the hippocampus due to tube feeding conditions.

The hippocampus receives cholinergic fibers from the medial septal nucleus and the vertical limb of the diagonal band of Broca[29,30]. The septohippocampal system is thought to have an important role in spatial memory[31,32]. A previous report suggested that dysfunctional mastication enhances the age-related decline in the septohippocampal cholinergic system[33]. The decreased dendritic spine density in hippocampal pyramidal cells in this study might be affected by destruction of the cholinergic system.

In conclusion, we suggest that tube feeding-induced pathologic changes of hippocampal spines might advance the age-related decline in learning and memory.

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経管栄養は老齢マウスの海馬スパイン数を減少させる

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経管栄養が空間認知能や海馬錐体細胞のスパイン数に与える影響を老化促進モデルマウス（SAMP 1）を用いて検討した．経管栄養は老化マウスにおいて学習能力を低下させるとともに海馬錐体細胞のスパイン数を減少させた．これらの結果から，経管栄養は海馬シナプスへの入力を減少させることが示唆された．

キーワード：経管栄養，スパイン，学習能力，海馬，SAMP 1

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