Influences of reduced masticatory sensory input from soft-diet feeding upon spatial memory/learning ability in mice

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ABSTRACT

It has been reported that reduction of masticatory afferent stimulation might influence learning and memory function. In order to clarify the influences of reduced masticatory sensory input on spatial memory/learning ability and neuropathological changes, we conducted the Morris water maze experiment and investigated the number of hippocampal neurons in association with the differences in masticatory afferent stimuli from hard- and soft-diet feeding in mice. The water maze experiment showed no significant difference in learning ability between 180-day-old solid- and powder-diet groups. Meanwhile, the ability was significantly reduced in the 360-day-old powder-diet group as compared with the age-matched solid-diet group. The total number of pyramidal cells in the hippocampal CA1 and CA3 regions was significantly smaller in 360-day-old powder-diet group than in the remaining groups. These results demonstrate that reduction of masticatory afferent stimuli due to long-term soft-diet feeding may induce neuron loss in the hippocampus and reduced memory/learning ability.

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder, which was first reported by Alzheimer in 1907 (1, 18). It is, in general, divided into early and late-onset types, but both have similar clinical and pathological features. It has long been suggested that there is a certain relationship between loss of teeth and dementia. Kondo et al. (6) reported that tooth loss was one of the risk factors of AD. Shigetomi et al. (17) reported that the risk of AD onset increased significantly in a group with less-number teeth than in the age-matched control with more-number teeth. Animal experiments revealed that tooth loss or long-term soft-diet feeding caused a decrease of learning and memory ability. Yama-moto and Hirayama showed that the SAMR1 and SAMP8 mice fed on a solid diet were superior in an eight-arm radial maze to the powder-diet group (21). It was also reported that the aged SAMP8 mice, after the molar extraction, showed a decrease in both learning ability and neuron density in the hippocampal CA1 region compared with the controls (14). Furthermore, it is suggested that afferent sensory input is highly dependent on masticatory function or hardness of the diet (13). Ishizuka (5) demonstrated that the action potential of the masseter muscle in rats was significantly lower in the powder-diet group than in the solid-diet group.

It is reasonably assumed from these findings that tooth loss and the relevant reduction of masticatory afferent stimuli may influence the structure and function of the central nervous system (CNS). However, little information is available for the relation-
ship between pathological changes of CNS and reduced masticatory afferent stimulation. The purpose of this study was to clarify the influences of the reduction of masticatory afferent stimulation from powder-diet feeding in mice on the memory/learning ability evaluated by Morris water maze and neuropathological changes in the CNS such as decrease in the number of hippocampal neurons.

MATERIALS AND METHODS

Animals. B6C3Fe-a/a male mice (n = 109) of 180- and 360-day-old were used in this study. They were divided into four groups 20 days after birth. The first- (group A, n = 24) and second-group mice (group B, n = 26) were fed solid and powder diets (CE-2, Clea Japan, Tokyo, Japan) for 180 days, respectively. The third- (group C, n = 30) and fourth-group mice (group D, n = 29) were fed a solid or power diet for 360 days. The animals were treated under ethical regulations defined by the Ethics Committee, Hiroshima University Faculty of Dentistry.

Water maze experiment. To examine memory/learning ability, the animals were subjected to the Morris water maze (11). In this study, various conditions for the water maze experiment were defined based on a previous report of Onozuka et al. (14) as follows. The maze is consisted of a circular plastic tank (60 cm diameter), of which the inner wall is painted white. Large cues with differing geometric symbols were placed 1 cm above the water surface at four locations between quadrants. Circular transparent escape platform (5 cm diameter) was located 1 cm beneath the water surface near the center of four quadrants in the maze as the exit from the maze. The water temperature was adjusted to be approximately 23°C. During the trial period, the start and goal points were decided one place, respectively. The mice were released into the water at a constant starting point facing the pool wall, and after reaching the goal, point which was able to be geo-stationary on the goal for five seconds was assumed to be a trial end. If a mouse fails to reach the goal within 120 s, the time is regarded as 120 s. Four trials were repeated per day for each mouse, and an enough interval was given between the trials. Image analysis system MZ-40 (Finescs, Toyama, Japan) was used with color CCD camera XC-711 (Sony, Tokyo, Japan), color monitors PC-TM151 (NEC Corporation, Tokyo, Japan), and PC-9821V16 (NEC Corporation, Tokyo, Japan) as image processing devices for the measurement and analytical software MSM (Finescs, Toyama, Japan). The mice were identified by using the color contrast from the reflection input to the color monitor with the color CCD camera, pursued automatically, and the information was transmitted to the computer. The time to reach the goal was measured automatically and then five seconds for the stationary time on the goal was subtracted.

Each mouse was tested for consecutive eight days. The average time of four trials was assumed to be the time on the experiment day and the average of the escape latency (the time required to swim to the platform) on each trial day was calculated. After an analysis of variance, difference in the average escape latency was examined by a Scheffe’s multiple comparison test between solid- and powder-diet groups on each day.

Quantification of hippocampal neurons. The brains of mice were removed under deep anesthesia (n = 10 in each). They were fixed in 4% paraformaldehyde and rinsed in 0.1 M phosphate-buffered saline. Specimens were then embedded in paraffin, and cut into frontal sections of 10 μm thickness. The specimens were subjected to Nissl staining. The samples were selected every 200 μm of hippocampus from Bregma − 1.46 mm to − 2.30 mm, with a reference to the atlas of mouse brain (16). All pyramidal cells in CA1 and CA3 regions of the left hippocampus were counted on each section according to a previously reported method (7, 22). In order to investigate differences in the numbers of pyramidal cells among the four groups, a Scheffe’s multiple comparison test was performed.

RESULTS

As a result of the water maze experiment, the escape latency became shorter in each group with a lapse of the trials. No significant difference in the escape latency was found between the groups A (180-day-old solid-diet group) and B (180-day-old soft-diet group) (Fig. 1). On the other hand, during the experimental period, there was a tendency to prolong the escape latency in the group D (360-day-old soft-diet group) as compared with the group C (360-day-old solid-diet group), showing a significant difference between both groups on the second and third trial days. After four days from the beginning of experiment, the difference in the escape latency became small without any significant difference between both groups (Fig. 2).

In the CA1 and CA3 regions, hippocampal neu-
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Central cholinergic system. Aged SAMP8 mice with extraction of their upper molar teeth also exhibited decreased acetylcholine release and choline acetyltransferase (ChAT) activity in the hippocampus and reduced ChAT-immunopositive neurons in the medial septal nucleus compared to the age-matched controls (15). Thus, it may be suggested that the reduced number of cholinergic neurons in the medial septal nucleus is highly related to impaired spatial memory and/or learning ability. In the present study, no significant differences in the escape latency were found between 180-day-old solid- and powder-diet feeding groups. Meanwhile, it was significantly reduced in the 360-day-old powder-diet group compared with the age-matched solid-diet group on the 2nd and 3rd days after initiating the maze experiment. These results showed that, similarly to molarless or soft-diet feeding aged SAM mice, the influence of the decrease in masticatory afferent stimulation on learning ability was observed in normal powder-diet feeding mice. However, the effects were not so severe in normal mice as compared with aged SAM mice.

DISCUSSION

In a previous study, with an eight-arm radial maze, learning ability was significantly disturbed in the senescence-accelerated mice (SAMR1 and SAMP8) with soft-diet feeding when compared with the hard-diet feeding controls (21). The results also demonstrated that the synaptic formation was reduced in the cerebral cortex of mice fed on a soft-diet. From these findings, it is hypothesized that diminished activity of the mechanoreceptors in the oro-facial regions is highly associated with these neuropathological changes. A reduction of afferent input from sensory receptors was due to decreased masticatory function, which reduced the synaptic density in the cerebral cortex. Onozuka et al. (14) reported that aged SAMP8 mice with molar extraction showed decreases in both spatial memories in water maze and hippocampal neuron density in the CA1 region when compared with the controls. The mechanism of neuron loss was explained by reduction in masticatory sensory input from toothless condition on the cephalic nerve loss was clearly observed in the group D as compared with the remaining groups (Figs. 3 and 4). In the CA1 region, the number of pyramidal cells in the group D was less than 86% of other groups with a significant difference between group D and each of other groups. In the CA3 region, 10% of the pyramidal cells were reduced in the group D compared with other groups, showing significant differences between group D and each of the remaining groups (Table 1).

Fig. 1 Spatial learning in the water maze test. The mice in groups A (n = 24) and B (n = 26) were subjected to the maze test. The results are expressed as the mean of escape latency (mean ± S.E.) for four trials on each day. No significant difference in the time to reach the goal was found between both groups. Group A: 180-day-old solid-diet mice; Group B: 180-day-old powder-diet mice

Fig. 2 Spatial learning in the water maze test. The mice in groups C (n = 30) and D (n = 29) were subjected to the maze test. On days for the second and third trials, group D mice took significant longer times to reach the platform than group C. Group C: 360-day-old solid-diet mice; Group D: 360-day-old powder-diet mice *p < 0.05

central cholinergic system. Aged SAMP8 mice with extraction of their upper molar teeth also exhibited decreased acetylcholine release and choline acetyltransferase (ChAT) activity in the hippocampus and reduced ChAT-immunopositive neurons in the medial septal nucleus compared to the age-matched controls (15). Thus, it may be suggested that the reduced number of cholinergic neurons in the medial septal nucleus is highly related to impaired spatial memory and/or learning ability. In the present study, no significant differences in the escape latency were found between 180-day-old solid- and powder-diet feeding groups. Meanwhile, it was significantly reduced in the 360-day-old powder-diet group compared with the age-matched solid-diet group on the 2nd and 3rd days after initiating the maze experiment. These results showed that, similarly to molarless or soft-diet feeding aged SAM mice, the influence of the decrease in masticatory afferent stimulation on learning ability was observed in normal powder-diet feeding mice. However, the effects were not so severe in normal mice as compared with aged SAM mice.

Neuronal reduction in CNS is a symptom of cerebral aging. Brody (4) demonstrated a reduction of neurons in the cerebral cortex with aging. Many investigators (3, 9, 10, 12) also reported the relationship between aging and decrease of hippocampal neurons. In aged or soft-diet feeding SAMP8 mice with molar extraction, the neuron density in the hippocampus was significantly reduced compared with the controls (14, 21). In this study, significant reduction of the total number of pyramidal cells in the hippocampal CA1 and CA3 regions was similarly
Fig. 3  Nissl staining of pyramidal cells in the hippocampal CA1 and CA3 regions (180-day-old). (a) CA1 region of solid-diet group (low magnification), (b) high magnification of (a), (c) CA1 region of powder-diet group (low magnification), (d) high magnification of (c), (e) CA3 region of solid-diet group (low magnification), (f) high magnification of (e), (g) CA3 region of powder-diet group (low magnification), (h) high magnification of (g) (a, c, e and g: bar = 50 µm, b, d, f and h: bar = 10 µm).
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Fig. 4 Nissl staining of pyramidal cells in the hippocampal CA1 and CA3 regions (360-day-old). (a) CA1 region of solid-diet group (low magnification), (b) high magnification of (a), (c) CA1 region of powder-diet group (low magnification), (d) high magnification of (c), (e) CA3 region of solid-diet group (low magnification), (f) high magnification of (e), (g) CA3 region of powder-diet group (low magnification), (h) high magnification of (g) (a, c, e and g: bar = 50 µm, b, d, f and h: bar = 10 µm).
revealed in the 360-day-old powder-diet mice. In general, it is supposed that higher action potential is exerted during hard-diet mastication compared to the soft diet one (2, 19, 20). Ishizuka (5) demonstrated that the action potential of the masseter muscle was less substantial during mastication and the total duration of muscle activity was significantly shorter in powder-diet rats compared with the solid-diet ones. This was explained by the delayed growth of the masseter muscle spindle in the powder-diet group (8). Since reduced afferent stimuli occur by the decrease in stimulation from the muscle spindle, it is considered that long term soft-diet intake may seriously influence a decrease in the number of neurons.

Given the results in this study, it is highly emphasized that a long-term reduction or defect in masticatory afferent stimulation may cause a decrease in the neurons, and neuronal decrease induces impairment of memory and learning function.

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REFERENCES
