Effects of early tooth loss on the hippocampus in senescence-accelerated mice

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Abstract We evaluated whether long-term tooth loss induces functional and morphologic changes in the hippocampus in senescence-accelerated mice (SAMP8) maintained until old age after tooth extraction shortly after tooth eruption. First, to examine whether early tooth loss acts as a stressor, plasma concentration was measured as an index of stress. Plasma corticosterone concentration was significantly higher in old or mature mice with tooth extraction than in the age-matched controls. Plasma corticosterone concentration did not differ between the young tooth extraction and their age-matched control groups. Next, hippocampal function was assessed by evaluating spatial memory performance in the Morris water maze. In the Morris water maze learning and memory trials was significantly slower in the mature or old tooth extraction groups compared with the age-matched controls. There was no significant difference, however, between the young tooth extraction and control groups. Finally, hippocampal neuronal morphology was assessed by counting Nissl-stained cells. The number of hippocampal neurons was significantly reduced in the CA3 region in the mature and old tooth extraction groups compared with their age-matched controls, but there was no significant difference in the CA1-region or dentate gyrus between the mature or old tooth extraction groups and their age-matched controls. In young mice, there was no significant difference in the number of neurons in CA1, CA3, or dentate gyrus region between the tooth extraction and control groups. The findings indicated that tooth extraction after tooth eruption enhances the effects of aging on the hippocampus in mice.

Key words

Corticosterone,
Early tooth loss,
Hippocampus,
Learning ability,
Senescence-accelerated mice
(SAMP8)

Introduction

Tooth loss impairs mastication and is related to Alzheimer's disease¹⁾ and dementia²⁾, as well as to changes in oral structures such as the mandible and temporomandibular joint³⁾. Further, tooth extraction in young mice accelerates aging and decreases life span^{4,5)}. Tooth extraction in aged mice increase plasma corticosterone concentrations⁶⁾. As the plasma glucocorticoid concentration increases when animals are

exposed to chronic stress⁷⁾, tooth loss is thought to act as a chronic stressor. Chronic stress impairs spatial memory⁸⁾, and leads to a decrease in neurons⁹⁾, an increase in glial fibrillary acidic protein-positive cells¹⁰⁾, a decrease in acetylcholine release in the higher centers of the brain¹¹⁾, particularly the hippocampus, and a decrease in Fos-positive cells in areas of the hippocampus considered important for learning¹²⁾. In all of these studies, however, the teeth were extracted at young, mature, and old ages and soon induces functional and morphologic changes. In the present study, we extracted the teeth soon after eruption, maintained the animals until maturation

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and old age, and evaluated whether the long-term absence of teeth induces functional and morphologic changes in the hippocampus. First, to examine whether early tooth loss acts as a stressor, we measured the plasma corticosterone concentration as an index of stress.

We then tested the mice in the Morris water maze to evaluate the functional effects of early tooth loss on spatial memory. Finally, we examined the effect of early tooth loss on the number of hippocampal neurons.

Materials and Methods

Male senescence-accelerated mice (SAMP8; $n = 60$) were used in the study. The strain was kindly donated by the Institute of Frontier Medical Sciences, Kyoto University. We bred brother-sister in the animal facility of the Central Research Division at the School of Dentistry, Asahi University. The SAMP8 mouse has an average life span of 12 months (about half life span of normal mice) and begins to show deficits in learning and memory and aging at 5 months. The characteristics of this strain were described in detail by Takeda *et al.*^{13,14}. The animals were bred and maintained under conventional conditions; housed in groups of 5 in plastic cages (175W $\times$ 245D $\times$ 125H mm) under temperature ($23 \pm 1^\circ\text{C}$), humidity ($55 \pm 2\%$), and light (12 h; light period, 6:00–18:00; dark period, 18:00–6:00) controlled conditions; and water was available *ad libitum*. This experiment was conducted according to the Animal Experiment Guidelines established by Asahi University.

Tooth extraction procedure

The mice were separated from the dams 4 weeks after birth. At 1 month after birth, 30 mice were anesthetized with pentobarbital (Nembutal®, Dainihonsei-yaku Co., Ltd., Osaka, 30 mg/kg intraperitoneal injection), fixed in the supine position, and the upper molar teeth on both sides were extracted using an excavator as carefully as possible to minimize bleeding (extraction group). The other 30 mice underwent the same anesthesia without surgery and served as controls (control group). Following surgery solid food (CE-2®, Japan Clea Co., Ltd.) was given and the mice were maintained for 1 week (young group), 4 months (mature group), and 8 months (old group).

General conditions

Body weight and food intake of the young, mature, and old mice was measured for 9 days, 4 months, and 8 months, respectively, after surgery.

Corticosterone concentrations

At 1 week (young group), 4 months (mature group), and 8 months (old group) after surgery, plasma corticosterone concentrations were assayed in the control and molarless mice ($n = 5$ for each group). Corticosterone concentrations have a circadian variation, peaking at the beginning of the dark period, when activity is generally greatest, and reaching the lowest levels near the end of the dark period or at the beginning of the light period, when the mice are least active¹⁵. Therefore at 20:00, the beginning of the dark period, blood was sampled in 2.0 ml/microcentrifuge tubes containing no anticoagulant. The blood samples were immediately centrifuged at $3,500 \times G$ for 10 min at 4°C , and the serum was stored at -80°C until the assay was performed. Corticosterone was measured by radioimmunoassay by SRL Biochemistry Lab (Tokyo, Japan).

Morris water maze test

The Morris water maze test is a sensitive behavioral assay for hippocampal abnormalities^{16,17}. At 1 week (young group), 4 months (mature group), and 8 months (old group) after surgery, Morris water maze test was performed as described previously^{8–10}. Briefly, a stainless steel tank (90 cm in diameter, 30 cm deep) was filled with water (approximately 28°C) to a height of 22 cm and the water surface was covered with floating polystyrene foam granules (approximately 2 mm in diameter). A platform (12 cm in diameter) was submerged 1 cm under the water surface and located at a constant position near the center of one of the four quadrants of the pool. Training consisted of 28 trials over 7 days (4 trials per day, approximately 5-min intertrial interval). For each trial, the mice were placed into the water from one of four points at the perimeter of the tank. The sequence of the starting positions was randomized daily. A CCD video camera linked to a computer system (Move-tr/2D, Library Co., Ltd., Tokyo, Japan) was used to measure the latency of each mouse to reach the platform.

Hippocampal neuron number

After the Morris water maze 5 mice of each

Fig. 1 Effects of the extraction condition on body weight and food intake

group were anesthetized with pentobarbital sodium (40 mg/kg) and perfused transcardially with 30 ml saline at 37°C, followed by 100 ml neutral buffered formalin. The brain was carefully removed and post-fixed in the same fixative overnight at 4°C. Serial sections (15 μ m) were cut and mounted on glass slides, and stained with Cresyl Violet. Quantitative analysis of hippocampal pyramidal cells was performed as described previously¹⁸. Briefly, round, clear, medium, and large cells were counted in the left dorsal hippocampus (Bregma: -2.46 mm using the atlas of Franklin and Paxinos¹⁹). Ten sections per mouse were prepared. At 3 points in each of the CA1, CA3, and dentate gyrus (DG) regions of each section, the cells in a 100 μ m $\times$ 100 μ m square area were counted, and the cell count per mm² was calculated.

Statistics

The data were statistically processed by analysis of variance and, then, by Scheffe's or Tukey's multiple comparison procedure. A P -value of less than 0.05 was considered statistically significant.

Fig. 2 Effects of the extraction condition on plasma corticosterone levels

Results

Body weight and food intake

The changes in body weight and food intake are shown in Fig. 1. For the first week after the operation, body weight and food intake decreased slightly in all of the molarless mice. After 1 week, however,

Fig. 3 (A) Spatial learning in the water maze test. The results are expressed as the mean score (mean $\pm$ SE, $n = 5$ for each group) of four trials per day. (B) Visible probe test

these effects were completely restored and body weight and food intake were equivalent to those of control mice.

Plasma corticosterone concentration

The changes in plasma corticosterone concentration due to early tooth loss are shown in Fig. 2. The plasma corticosterone concentration in old mice or mature mice in the tooth extraction group was approximately 141%, 191% that in the age-matched controls, indicating a significant increase [$F(2,24) = 5.940646$, $P < 0.01$]. In the young, on the other hand, there was no significant difference in the plasma corticosterone levels between the tooth extraction and control groups.

Morris water maze test

The results of the Morris water maze test for the tooth extraction and control groups are shown in Fig. 3. The time required to reach the platform decreased with repeated trials regardless of age or group [$F(144,6) = 27.939$, $P < 0.0001$], and significantly increased with an increase in age [$F(144,2) = 23.057$, $P < 0.0001$]. The decrease in the time to reach the platform was significantly smaller in the mature or old tooth extraction groups than in their age-matched controls [$F(6,144) = 27.886$, $P < 0.05$], but there was no significant difference between the young tooth extraction and control groups.

Fig. 4 Photomicrographs showing Nissl staining of hippocampal CA1, CA3 and DG subfield of control and extraction mice. Bars 200 μ m.

Hippocampal neuron number

Nissl-stained pyramidal cells in CA1, CA3, and DG regions of the hippocampus are shown in Fig. 4. The results of the quantitative analysis of neurons in the three regions of the hippocampus are shown in Fig. 5. The number of neurons in the three regions decreased with age in both the tooth extraction

and control groups [CA1: $F(2,294) = 62.62458$, $P < 0.0001$, CA3: $F(2,294) = 15.69082$, $P < 0.0001$, DG: $F(2,294) = 82.20859$, $P < 0.0001$]. Tukey's multiple comparisons, revealed that the number of neurons in the CA3 region was significantly lower in the mature and old tooth extraction groups than in the age-matched controls (mature, 85%; old, 88% that of age-matched controls; $P < 0.01$). In the CA1 and DG

Fig. 5 Effects of the extraction condition on neuronal number in the hippocampal CA1, CA3 and DG subfield

regions, on the other hand, there was no significant difference in the number of neurons in the mature or old tooth extraction and control groups. In the young mice, there was no significant difference in the number of neurons in the three hippocampal regions between the tooth extraction and control groups.

Discussion

In the present study, we extracted only the upper molars because mice die when their incisors are extracted⁵⁾ and there was no difference in the life span or degree of aging according to the site of tooth extraction.

In both the tooth extraction and control groups in the present study, the decrease in the time to reach the platform in the Morris water maze with repeated trials was slower with aging. This finding is consistent with previous reports that spatial memory declines with aging^{20,21)}. The time needed to reach the platform did not differ between the young tooth extraction and control groups, but was significantly prolonged in the old tooth extraction group compared to their age-matched controls. Similar results are observed in bite-raised and tube-fed animals^{22–24)}. Based on the findings of the present study, early tooth loss also accelerates the age-associated decline in spatial memory.

Typical age-associated changes in the hippocampus are a decline in spatial memory and neuronal

death^{21,25)}. Because a decline in spatial memory was observed in the mature or old tooth extraction groups, we examined the effect of early tooth loss on the number of hippocampal neurons. The number of pyramidal cells was markedly reduced in the CA3 region in the mature or old tooth extraction groups compared with the age-matched control groups. The number of neurons in the CA1 and DG regions, however, did not significantly differ between the tooth extraction and control groups. In the young mice, there was no significant difference in the number of neurons in all three areas (CA1, CA3, and DG) of the hippocampus between the tooth extraction and control groups. These results suggest that early tooth loss accelerates both functional and organic age-associated changes.

The neurotoxicity of stress-related agents such as corticosterone and a decrease in signal input may cause neuronal death. Mice have an innate physiologic requirement to grind their teeth²⁶⁾. The inability of a mouse to grind their teeth due to tooth loss is considered to induce stress in the animals. Hagiwara *et al.*²⁷⁾, Clark *et al.*²⁸⁾, and Takeda²⁹⁾ reported that impaired mastication due to occlusal interference induces sleep disorders, changes in autonomic activities, including neuroendocrine responses, and emotional stress. On the other hand, decreases in the incidence of gastric ulcer, which is a stress-related disorder induced by excessive mastication, decreases in ulcer size, and suppression of brain neurotransmitters have also been reported²⁹⁾. In the body

defense system, the hypothalamus-pituitary-adrenal cortex system acts as a mediator between the immune and endocrine systems, and the circulating concentration of cortisol, which is a hormone secreted from the adrenal cortex that controls defense responses, is considered an index of stress³⁰. Therefore, tooth extraction is considered to cause stress.

Because the declines in spatial memory observed in the mature or old tooth extraction groups closely resembled the functional and morphological changes of animals under chronic stress or long-term glucocorticoid administration^{25,31}, we evaluated the changes in the plasma corticosterone concentration in the mice. The plasma corticosterone concentration was significantly elevated in the mature or old tooth extraction group compared with age-matched controls. In the young mice, however, the plasma corticosterone concentration in the tooth extraction groups was not significantly different from that of the controls. In mature mice there is possibility to adapt to their tooth-extracted oral condition, but in old mice it is difficult to adapt. These results suggest that tooth loss acted as a chronic stressor in the mature or old SAMP8 mice.

Luine *et al.*²⁵ reported that 21-day restraint stress impairs spatial perception in rats and induced atrophy of the dendritic processes in the CA3 region of the hippocampus. Furthermore, Sapolsky *et al.*³¹ demonstrated that the administration of corticosterone over 12 weeks induced pyramidal cell death in the CA3 region. Neuronal degeneration in the CA3 region is not observed in rats administered a glucocorticoid synthesis inhibitor, even under chronic stress³². Indeed, Onozuka *et al.*⁶ reported that tooth-extracted mice administered metyrapone, a corticosterone synthesis inhibitor, did not differ from controls in the decline in spatial perception and hippocampal neuronal death. Based on these observations, an elevated plasma corticosterone concentration appears to be closely associated with a decrease in pyramidal cells.

Another cause of cell death is considered to be a decrease in stimulation of the central nervous system such as by reduced periodontal sensibility due to the loss of teeth. Tooth loss and loss of the periodontal membrane inhibit the periodontal-masseter reflex, with a consequent inability to increase the occlusal force³³, inability to bite with a sufficient force due to weak jaw-closing muscle activity resulting from masticatory muscle hypoplasia^{34,35}, and weakening of the mastication force

due to an insufficient number of motor neurons supplying the jaw-closing muscles³⁶. These disorders may lead to a decrease in the mastication force and less stimulation of the central nervous system. Conversely, activities such as mastication are reported to increase temperature³⁷ and blood flow³⁸ over wide areas of the brain and to enhance the secretion of brain-gut hormones and cell activity⁸. Further, a decrease in the number of Fos-positive cells in the CA3 region in association with impaired performance in the Morris water maze has been reported in aged SAMP8 with early tooth loss. As soon as neurons receive input, the immediate early gene *c-fos* gene, is expressed, and Fos protein is produced¹². This protein is expressed in the hippocampus in association with learning activity^{39,40}. Therefore, the decrease in Fos protein production in aged mice with early tooth loss suggests that signal input to the hippocampus was decreased due to tooth loss. External information transmitted to the hippocampus is coded as it is transmitted to the DG→CA3→CA1 pathway, and transferred to the association areas to establish long-term memory⁴¹. Therefore, the loss of teeth may reduce information input into the association areas thereby impairing memory formation.

The effect of tooth extraction on neuron number was observed only in the CA3 region and not in the CA1 and DG regions. Glutamic acid, an excitatory neurotransmitter released from mossy fiber terminals projecting from DG to CA3, is reportedly involved in changes in the neurons in the CA3 region⁴². Glucocorticoid promotes the release of glutamic acid. Increases in glucocorticoid levels and in the release of excitatory amino acids are speculated to act synergistically as neurotoxins to induce neuronal death in the CA3 region⁴³.

Accelerated aging was observed in mature or old mice due to the early loss of teeth, but not in young mice. Because the plasma glucocorticoid levels generally increase with aging⁴⁴, the increase in glucocorticoid levels in mature or old mice is intensified by the stress of early tooth loss, glucocorticoid receptor mRNA and glucocorticoid receptors are down-regulated in the hippocampus, and not only are glucocorticoid receptors reduced, but neurons are also damaged by glucocorticoid. As a result, the negative feedback mechanism from the hippocampus to the hypothalamus is suppressed, and the elevated glucocorticoid level is considered to aggravate the damage to hippocampal neurons. In young mice, however, the glucocorticoid level is initially low,

so that downregulation of glucocorticoid receptors or glucocorticoid receptors mRNA is less likely to occur, and negative feedback from the hippocampus to the hippocampal-pituitary-adrenal-axis functions normally to prevent an increase in glucocorticoid. This likely helps to prevent neuronal death in young mice, even under the stress of tooth loss.

Also, the effects of tooth extraction on the central nervous system may not appear over a short period. In studies of spatial memory⁴⁵⁾, performance is improved with the number of trials 1 week after tooth extraction, and there is no difference between the tooth extraction and control groups. Performance is impaired in tooth extracted mice compared to controls 7 weeks after tooth extraction, indicating effects on brain function. Kubota *et al.*⁴⁶⁾ reported that, in tree shrews and monkeys, the alveolar bone nerve degenerates after tooth extraction, but the infraorbital nerve takes longer to degenerate, and histologic effects of tooth extraction on the central nervous system are also considerably delayed. In addition, Kato *et al.*¹¹⁾ reported that long-term tooth loss in rats impairs cholinergic neuron function, leading to a decline in learning and memory abilities. Watanabe *et al.*⁴⁷⁾ reported that the experimental long-term administration of glucocorticoid induces not only a decline in spatial perception but also the degeneration and death of hippocampal neurons, and that hippocampal function becomes more impaired with increased exposure to glucocorticoid. In the present study, the effects of glucocorticoid administration were observed in the mature or old animals but not in young mice, and this is probably because of the prolonged stress after tooth extraction and because the glucocorticoid neurotoxicity became more evident with the increase in the exposure period and was not just a result of aging alone.

Conclusion

The effects of early tooth loss on hippocampal function were evaluated in senescence-accelerated mice. The plasma corticosterone concentration was increased in mature or old animals, indicating that tooth loss acted as a stressor. Spatial memory declined and the number of hippocampal neurons decreased in both mature or old animals, suggesting that hippocampal function is impaired by early tooth loss.

These results suggest that early tooth loss induces impairment of the hippocampal function.

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