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Tooth loss early in life suppresses neurogenesis and synaptophysin expression in the hippocampus and impairs learning in mice

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ABSTRACT

Objective: Tooth loss induced neurological alterations through activation of a stress hormone, corticosterone. Age-related hippocampal morphological and functional changes were accelerated by early tooth loss in senescence-accelerated mouse prone 8 (SAMP8). In order to explore the mechanism underlying the impaired hippocampal function resulting from early masticatory dysfunction due to tooth loss, we investigated the effects of early tooth loss on plasma corticosterone levels, learning ability, neurogenesis, and synaptophysin expression in the hippocampus later in life of SAMP8 mice.
Design: We examined the effects of tooth loss soon after tooth eruption (1 month of age) on plasma corticosterone levels, learning ability in the Morris water maze, newborn cell proliferation, survival and differentiation in the hippocampal dentate gyrus, and synaptophysin expression in the hippocampus of aged (8 months of age) SAMP8 mice.
Results: Aged mice with early tooth loss exhibited increased plasma corticosterone levels, hippocampus-dependent learning deficits in the Morris water maze, decreased cell proliferation, and cell survival in the dentate gyrus, and suppressed synaptophysin expression in the hippocampus. Newborn cell differentiation in the hippocampal dentate gyrus, however, was not affected by early tooth loss.
Conclusion: These findings suggest that learning deficits in aged SAMP8 mice with tooth loss soon after tooth eruption are associated with suppressed neurogenesis and decreased synaptophysin expression resulting from increased plasma corticosterone levels, and that long-term tooth loss leads to impaired cognitive function in older age.

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1. Introduction

Learning and memory critically depend on the hippocampus, a bilateral brain structure located in the temporal lobe. New cells are generated throughout life in the subgranular cell layer of the hippocampal dentate gyrus (DG) (Gage, 2002), where they mature into functional neurons with axons extending into the hippocampal CA3 region (Hastings, 1999; Stanfield & Trice, 1988). Neuronal cell proliferation, differentiation, and survival are regulated at several levels (Lee et al., 2006), and are likely related to hippocampus-mediated learning ability (Gould, Beylin, Tanapet, Reeves, & Shors, 1999). Neurogenesis in the DG decreases with aging (Gould, Reeves et al., 1999), and the age-dependent cognitive impairment is likely related to the age-related decline in neurogenesis (Bondolfi, Ermini, Long, Ingram, & Jucker, 2004). Newborn cell generation is affected by various factors, including psychologic stress and environmental complexity (Czéh et al., 2002; Torner et al., 2009; Kempermann, Brandon, & Gage, 1998; van Praag, Kempermann, & Gage, 1999). Synaptophysin is a major integral protein of the synaptic vesicle membrane that is involved in the regulation of neurotransmitter release (Bähler, Benfenati, Valtorta, & Greengard, 1990). Synaptophysin-positive synaptic boutons are a

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sensitive correlate of cognitive deficits (Calhoun et al., 1998). Exposure to stress leads to reduced expression of synaptophysin in the hippocampus (Thome et al., 2001).

Impaired mastication is an epidemiologic risk factor for learning and memory dysfunction, such as dementia and Alzheimer's disease, as well as for mortality (Shimazaki et al., 2001; Shigetomi, 1998; Kondo, Niino, & Shido, 1994). Recent studies using senescence-accelerated mouse prone 8 (SAMP8) reported that tooth loss soon after tooth eruption accelerated age-related increases in plasma corticosterone levels, spatial learning deficits, neuronal loss, and increase in astroglial responsiveness in the hippocampus (Kondo et al., 2016; Kubo et al., 2010). These findings suggest that long-term tooth loss and reduced mastication impair hippocampus-related cognitive function later in adulthood. The mechanisms underlying the effect of early tooth loss on neurogenesis in the hippocampal DG, however, are not well understood.

In the present study, we examined whether tooth removal soon after tooth eruption affects cell proliferation, cell survival, and newborn cell differentiation in the DG; synaptophysin expression in the hippocampus; and learning ability in aged SAMP8 mice.

2. Materials and methods

2.1. Animals and experimental protocol

We used male 1-month-old SAMP8 mice (n = 66) in the present study. Mice of this strain mature normally up to 6 months of age and then exhibit features of accelerated aging with a median lifespan of only 12 months compared with the 2–3 year median lifespan of the parent strains. The characteristics of the SAMP8 strain are reported elsewhere (Flood & Morley, 1998). The mice were bred under conventional conditions and then housed in plastic cages in groups of five under controlled temperature ($23 \pm 1\,^{\circ}C$), humidity ($55 \pm 2\%$), and light (12 h; light period, 0600–1800; dark period, 1800–0600). Food and water were available *ad libitum*. The experiment was undertaken in accordance with the guidelines for laboratory animal care and use of Asahi University. The ethics committee of Asahi University School of Dentistry approved the study.

Removal of the upper molar teeth was performed as described previously (Kondo et al., 2016). Briefly, mice at 1 month of age were anesthetized with sodium pentobarbital (35 mg/kg) and the upper molar teeth were extracted using tweezers. As tooth development in mice continues for 25 days after birth (Hamashima, 1963), the molars were extracted at one month of age to ensure complete tooth removal. Control animals underwent the same anesthetic procedures, but their molars were not removed. The mice were maintained under the same controlled conditions after surgery for 8 months. Body weight was measured immediate before surgery, and at the end of 1st, 4th, and 8th months after surgery.

2.2. Plasma corticosterone levels

To examine the effects of early tooth loss on plasma corticosterone levels in aged mice, plasma corticosterone levels were measured in experimental and control mice 8 months after molar removal (n = 5/group). Blood was sampled at the beginning of the dark period (2000 h), when corticosterone levels are typically highest (Olariu, Cleaver, & Cameron, 2007). Mice were then decapitated and blood was collected in 2.0-ml microcentrifuge tubes without anticoagulant and centrifuged at $3500 \times g$ for 10 min at 4 °C. The serum was stored at −80 °C until measurement of the corticosterone levels by radioimmunoassay at the SRL Laboratories in Tokyo, Japan.

2.3. Morris water maze test

The Morris water maze test is commonly used for examining hippocampus-related learning and memory (Morris, 2007). The Morris water maze test was performed for both experimental and control mice (n = 8/group) as described previously (Kondo et al., 2016). Briefly, a stainless steel tank (90 cm in diameter and 30 cm in depth) was filled with water (~25 °C) to a height of 22 cm and the water surface was covered with floating polystyrene foam granules with a ~2-mm diameter. A platform was submerged 1 cm under the water surface at a constant position near the center of one of the four quadrants of the pool. The mice were placed into the water from 1 of 4 points around the perimeter of the tank and given 4 learning trials daily for 7 consecutive days (a total of 28 trials). The sequence of the starting positions for each of the four trials was randomly changed every day. The latency to swim to the platform was monitored using a CCD video camera linked to a computer system (Move-tr/2D, Library Co., Ltd, Tokyo, Japan). On the last day of training, all animals underwent a visible probe test 2 h after the last training trial.

2.4. Immunohistochemistry

We examined the cell proliferation and survival following intraperitoneal injection of bromodeoxyuridine (BrdU; 50 mg/kg; Sigma-Aldrich, St, Louis, MO; 10 mg/ml dissolved in 0.9% NaCl solution) into the mice (n = 10/group) 5 times with 3-h intervals at 8 months after the molar removal procedure, as described previously (Takagi et al., 1999). Either the next day (n = 5/group) or 22 d (n = 5/group) after the BrdU injection, the mice were anesthetized with pentobarbital sodium (40 mg/kg) and transcardially perfused with 30 ml of saline at 37 °C, followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4 °C. The brains were removed and fixed in 2% paraformaldehyde fixative overnight at 4 °C.

Brain sections with a thickness of 25 μm were prepared using a cryostat (CM1850, LEICA, Wetzlar, Germany). The DNA was denatured by incubating the sections for 2 h in 50% formamide/2 x SSC (0.3 M sodium chloride and 0.03 M sodium citrate) at 65 °C, for 30 min in 2N HCl at 37 °C, and then neutralizing them for 10 min in 0.05 M Tris-buffered (TBS, pH 8.5). A standard immunohistochemical procedure was used to process floating sections with the ABC method. First, brain slices were rinsed with phosphate-buffered saline (PBS), incubated with 1% H_2O_2 for 10 min at room temperature, rinsed again with PBS, and incubated for 60 min at room temperature with 5% normal goat serum. After the third rinse with PBS, the sections were incubated with rabbit polyclonal anti-BrdU antiserum (Abcam PLC, Cambridge, UK) diluted 1:200 in PBS containing 0.3% Triton X-100 for 48 h at 4 °C, rinsed with PBS, incubated with biotinylated goat anti-rabbit IgG (Dako Cytomation, Glostrup, Denmark) diluted 1:500 in PBS for 2 h at room temperature, rinsed again with PBS and 0.05 M Tris-HCl buffer (pH7.6), and incubated with peroxidase-conjugated streptavidin (Dako Cytomation) diluted 1:500 with PBS for 1 h at room temperature. Bound complex was visualized using 3,3′-diaminobenzidine (0.5 mg/ml) and hydrogen peroxide (0.01%) in TBS. Control sections were treated with non-immature rabbit immunoglobulin instead of primary antibody.

To investigate the effects of early tooth loss on newborn cell differentiation in the hippocampal DG, double-immunofluorescence with markers for mature granule neurons (NeuN) and astroglial glial fibrillary acidic protein (GFAP) was used to detect colocalization with BrdU. BrdU (50 mg/kg) was injected intraperitoneally into mice (n = 5/group) 5 times with 3-h intervals and, the brains of the mice were collected 22 days after BrdU injection, as described above.

The DNA was denatured, and the brain sections were incubated with sheep polyclonal anti-BrdU antibody (1/200; Abcam) and rabbit polyclonal anti-GFAP antibody (1/1000; Millipore Corp, Billerica, MA) or with mouse monoclonal anti-NeuN antibody (1/100; Millipore). Donkey anti-sheep IgG rhodamine isothiocyanate conjugate, donkey anti-rabbit IgG fluorescein isothiocyanate conjugate or donkey anti-mouse IgG fluorescein isothiocyanate conjugate (all 1/100; Santa Cruz Biotechnology, Dallas, TX) was used to visualize bound anti-BrdU and anti-GFAP or anti-NeuN antibodies.

Quantification of BrdU-positive cells was performed by immunohistochemically processing, every sixth section (125 μm apart) and then examining; 8 sections from each sample [bregma −2.12 mm to −6.30 mm (Franklin & Paxinos, 1996)] under a microscope with a 4 x objective (Olympus BX-50, Japan) using an unbiased stereologic method as reported previously (Cheng et al., 2008). An investigator blinded to the treatment group counted all BrdU-positive cells with visible nuclei in the DG using software (Lumina Vision, Mitani Co., Ltd, Fukui, Japan). At least 50 BrdU-labeled cells were measured per brain, and the number of double-labeled cells was expressed as the percentage of the total number of BrdU-labeled cells (Lee et al., 2006).

2.5. Synaptophysin assay

To measure synaptophysin expression, the animals (n = 5/group) were quickly decapitated following cervical dislocation. After removing the brains, the hippocampus of both hemispheres was rapidly sampled, weighed, frozen in liquid nitrogen and stored at −80 °C until use. For immunoblot analysis, approximately 50 mg wet-weight samples were minced with a knife and lysed by brief sonication in Laemmli's sample buffer. After centrifugation at 15000 rpm for 30 min at 4 °C, the supernatant was collected and adjusted to 2 μg/μL with the same buffer. Lysates of 10 μl (20 μg protein/lane) were heated at 100 °C for 5 min and separated by sodium dodecylsulfate – polyacrylamide gel electrophoresis (SDS-PAGE) with 10% polyacrylamide gel (ATTO Corp., Tokyo, Japan) at a constant current of 15 mA. After SDS-PAGE, proteins in gels were transferred to a polyvinylidene difluoride membranes (Millipore Corp., Billerica, MA, USA). Blotting membranes were blocked with 0.5% casein (Sigma–Aldrich, St. Louis, MO, USA) for 30 min at room temperature to block non-specific binding. The membranes were then reacted with 3000 times diluted rabbit anti-synaptophysin monoclonal antibody (Abcam, Cambridge, UK) was performed for 1 h at room temperature. After rinsing in TBS-T (0.1% (V/V) Tween-20 containing (0.0075 mol/L phosphate-buffered salin (T-PBS, pH7.4))) three times for 5 min each, the membranes were incubated with 1000 times diluted horseradish peroxidase-conjugated rabbit anti-rabbit immunoglobulin G (Dako, Glostrup, Denmark) for 30 min at room temperature. After rinsing in TBS-T, as described above, reaction with Immobilon Western HRP reagent (Millipore Corp) was carried out according to the manufacturer's instructions. Finally, the signal intensities were analyzed using Cool Saver AE-6955 (ATTO Corp., Japan).

2.6. Statistical analysis

Results are presented as means ± SD. Statistical analysis was performed by an unpaired *t*-test and repeated measures analysis of variance. Probability values less than 0.05 were considered statistically significant.

3. Results

The time course of body weight changes in both control and early toothless mice is shown in Fig. 1. Body weights of the control and early tooth loss groups decreased significantly for a few days after the procedure and then returned to the pre-procedure level; thereafter, the body weight of the early tooth loss group tended to decrease compared with that of the control group, but there was no significant difference between the control and early tooth loss groups at any time during the experimental period.

Plasma corticosterone levels were significantly higher in the early tooth loss group than that of the control group ($P < 0.05$; Fig. 2A).

In the Morris water maze test, early tooth loss mice required significantly more time to reach the platform than control mice [$F(1,84) = 15.246$, $P = 0.016$; Fig. 2B]. The time to reach the platform decreased over time [$F(1,6) = 26.014$, $P < 0.001$]. The groups did not differ significantly in their performance in the visible probe test (Fig. 2C).

The number of BrdU-positive cells in the DG of early tooth loss mice was ~38% of that in control mice ($P < 0.01$; Fig. 3A, B). These

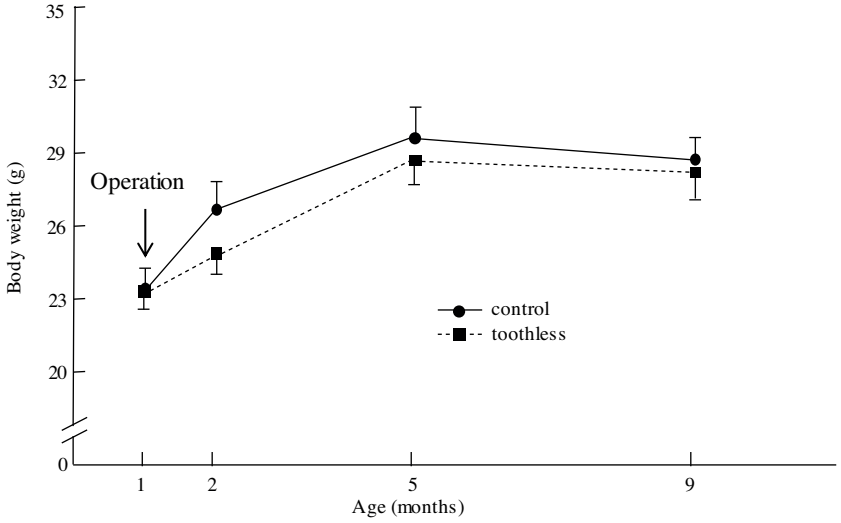

Fig. 1. Body weight changes in control and early toothless mice. Each value represents the mean ± SD (n = 33/group). There were no significant differences between the control and early toothless groups.

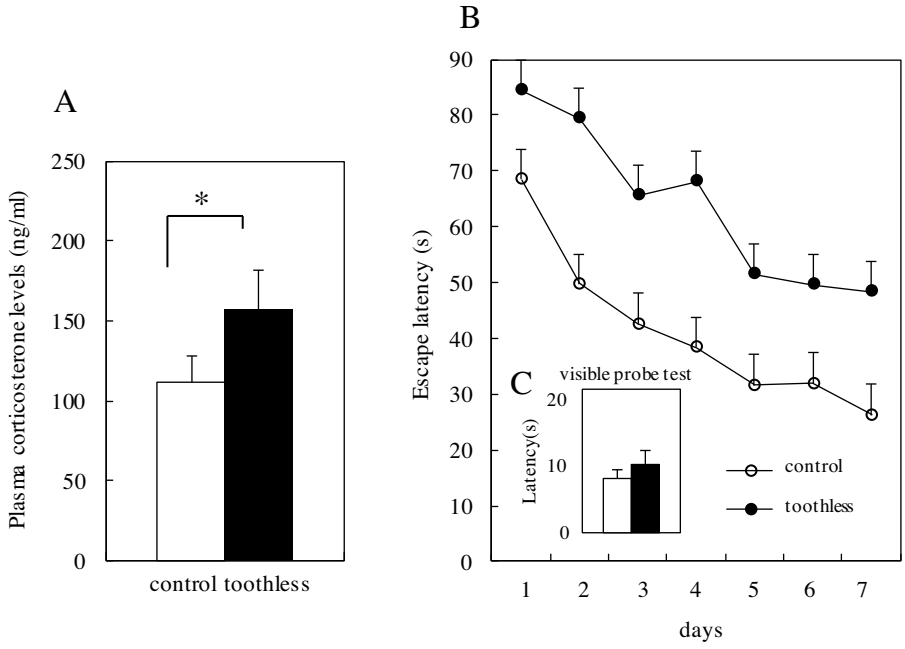

Fig. 2. Effects of early tooth loss on plasma corticosterone levels and spatial learning ability.
Effects of early toothless on plasma corticosterone levels (n = 5/group) (A). The mean score (mean ± SD, n = 8/group) of daily trials in the Morris water maze (B) and visible probe test (mean ± SD, n = 8/group, C). Note the increased plasma corticosterone levels in early toothless mice compared to control mice. *: $P < 0.05$. The escape latency of early toothless mice was longer than that of control mice.

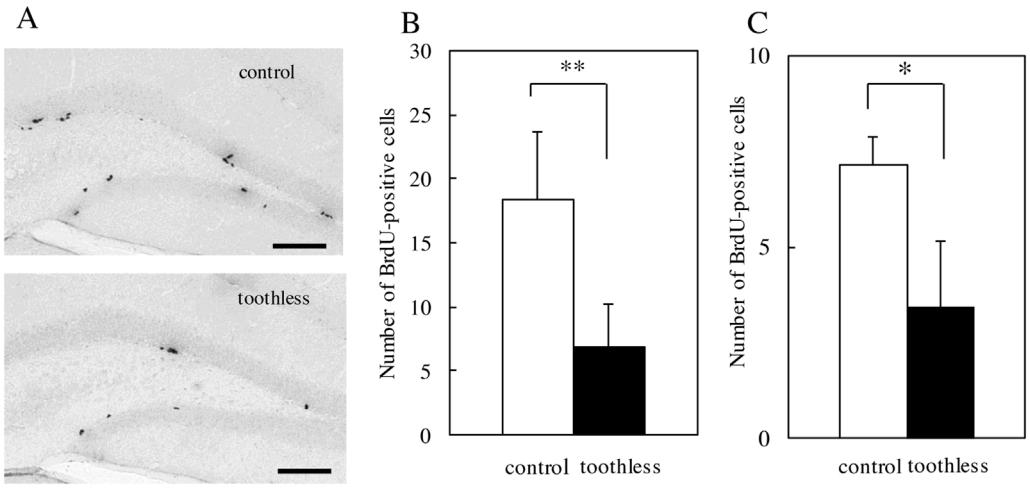

Fig. 3. Effects of early tooth loss on newborn cell proliferation and survival in the hippocampal DG.
Photomicrographs showing BrdU-positive cells in the hippocampal DG (A) and the number of BrdU-positive cells in control and early tooth loss mice (B: cell proliferation; C: survival). Early tooth loss mice exhibited significantly decreased cell proliferation and survival of newborn cells in the hippocampal DG. Bars: 100 μm. Data are presented as means ± SD (n = 5/group). *: $P < 0.05$, **: $P < 0.01$.

findings suggest that the early tooth loss condition impairs cell proliferation in the hippocampal DG.

Newborn cell survival was significantly lower in early tooth loss mice than in control mice ($P < 0.05$; Fig. 3C). These findings suggest that the early tooth loss condition impairs newborn cell survival in the DG of aged mice (Fig. 3).

The phenotype of mature BrdU-positive cells was determined based on BrdU double-labeling with either NeuN or GFAP (Fig. 4A, B). The majority of BrdU-positive cells were immunoreactive for NeuN and GFAP in both the control (NeuN, 68.1%; GFAP, 33.1%) and early tooth loss mice (NeuN, 65.5%; GFAP, 29.3%). The amount of NeuN and GFAP immunoreactivity did not differ between the two groups. These findings suggest that the early tooth loss condition does not affect cell differentiation into neurons and astrocytes.

Synaptophysin expression levels in the hippocampus of the early tooth loss mice was ~66% of that in control mice ($P < 0.05$; Fig. 5).

4. Discussion

The present study showed that tooth loss soon after tooth eruption resulted in increased plasma corticosterone levels, reduced cell proliferation and cell survival in the hippocampal DG, suppressed synaptophysin expression in the hippocampus,

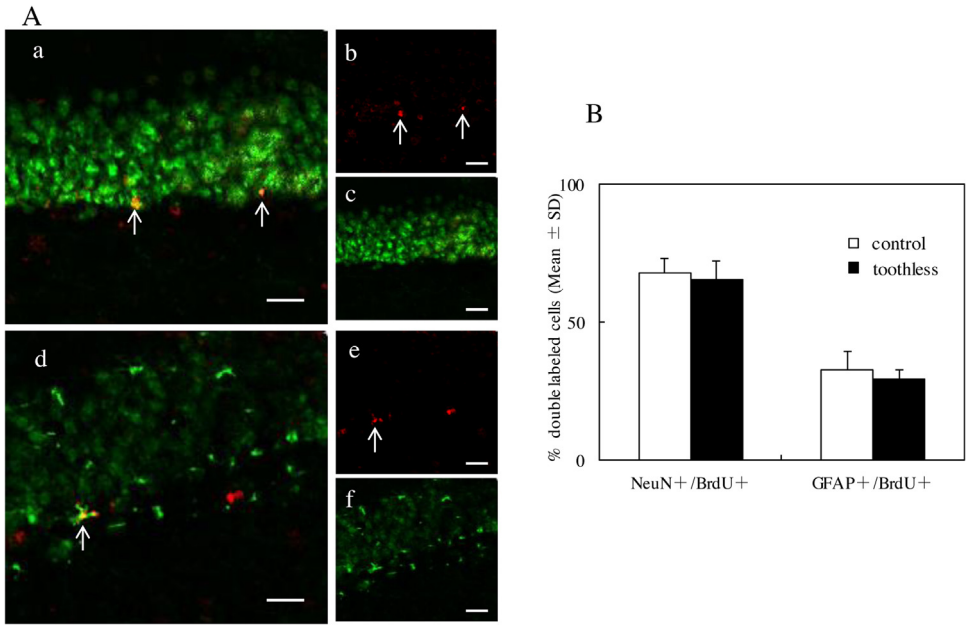

Fig. 4. Effects of early tooth loss on newborn cell differentiation in the hippocampal DG.
Photomicrographs (A) of brain slices showing cells double-labeled with BrdU and NeuN (a), or GFAP (d), and BrdU (b, e), NeuN (c), GFAP (f) in control mice. Bars: 50 μm. Percentage of BrdU-labeled cells (mean ± SD, n = 5/group) immunoreactive for NeuN and GFAP in the hippocampal DG (B). Percentage of BrdU+/NeuN+ and BrdU+/GFAP+ cells did not differ significantly between control and early tooth loss mice.

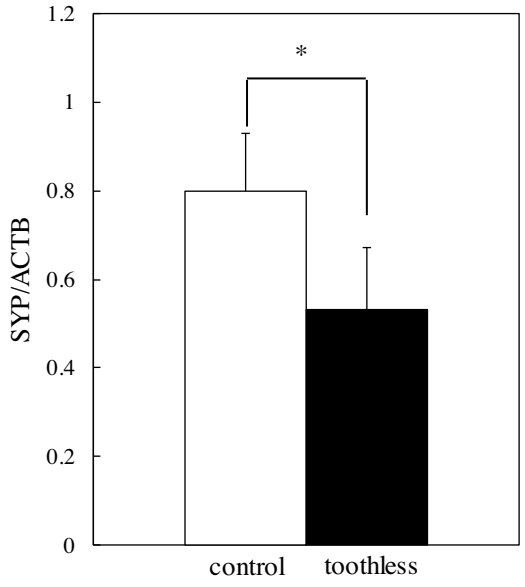

Fig. 5. Effects of early tooth loss on synaptophysin expression in the hippocampus.
Synaptophysin expression (SYP/ACTB) level was significantly lower in the early tooth loss group than that of the control mice (n = 5/group). *: $P < 0.05$. Data are presented as means ± SD.

and impaired hippocampus-dependent cognition in aged SAMP8 mice. The decrease in synaptophysin expression is consistent with previous reports that removing molars in aged SAMP8 mice leads to decreased Fos induction associated with impaired water maze performance and decreased spine density in the hippocampal CA1 region (Kubo, Iwaku, Watanabe, Fujita, & Onozuka, 2005; Onozuka, Watanabe, Fujita, Tomida, & Ozono, 2002). The hippocampal DG has a critical role in memory processes and spatial representation (Eichenbaum, 2000). Neurogenesis is enhanced by an enriched environment (Kondo et al., 2016; van Praag et al., 1999; Kempermann et al., 1998), and impaired by chronic stress (Czéh et al., 2002). It was reported that spatial learning increases newborn neuron survival (Gould, Beylin et al., 1999). Newborn neurons extend their axons into the CA3 region of the hippocampus, and functionally integrate into the existing DG circuitry within 3 weeks (Schmidt-Hieber, Jonas, & Bischofberger, 2004). The amount of the presynaptic vesicle protein synaptophysin in the hippocampus correlates with cognitive function (Calhoun et al., 1998). The present results together with previous findings suggest that learning deficits resulting from decreased mastication due to early tooth loss are closely associated with suppressed neurogenesis in the hippocampal DG, and decreased synaptophysin expression in the hippocampus.

It may be that input from sensory receptors on the periodontal ligaments is reduced due to long-term dysfunctional mastication following tooth extraction, leading to alterations of their neuronal pathways. Chen et al. suggested that prolonged physical training modifies the age-related decrease in the expression of growth associated protein 43, in associations with decreased nerve growth and plasticity, and decreased synaptophysin expression in the hippocampus, indicating that synaptophysin is associated with sprouting and synaptogenesis (Chen, Chen, Lei, & Wang, 1998). Voluntary exercise enhances neurogenesis in the hippocampal DG (van Praag et al., 1999). Tooth extraction or tooth pulp extirpation results in degenerative changes in both nerve fibers and the trigeminal ganglion cell bodies of the primary sensory neurons innervating the teeth, or secondary neurons in the trigeminal spinal tract nucleus (Gobel, 1984; Kubota et al., 1988). Therefore, early tooth loss-induced behavioral and morphologic alterations may be significantly affected by the degeneration of neural pathways due to tooth extraction. Further studies are needed to determine the precise relation between early tooth loss and biologic and/or morphologic changes in the central nervous system associated with the pathways innervating the tooth.

There is no consensus on the effects of tooth loss on body weight. Kawahata et al. extracted molar teeth at 8 weeks of SAMP8 mice and found the body weights decreased significantly after surgery (Kawahata et al., 2014). Several other studies, however,

demonstrated that tooth loss in SAMP8 mice may lead to temporary weight reduction immediately after tooth loss, with no significant change in body weight thereafter (Onozuka, Watanabe, Fujita, Tomida et al., 2002; Hioki et al., 2009; Jiang et al., 2011). In the present study, we observed that after surgery, body weight of the early tooth loss group tended to decrease compared with that of the control group. However, there were no significant differences between the two groups throughout the study.

Plasma corticosterone levels were significantly higher in the aged SAMP8 mice with early tooth loss than in the controls. Early tooth loss enhances age-dependent increases in plasma corticosterone levels, neuron loss in hippocampal CA3 region, and learning impairments (Kondo et al., 2016; Kubo et al., 2010). Morphologic and functional alterations caused by tooth loss early in life closely resemble the changes induced by chronic stress (Luine, Villegas, Martinez, & McEwen, 1994). Moreover, the adrenal glands of aged molarless mice are heavier than those of the molar-intact control mice (Onozuka et al., 2000), and aged molarless mice have increased plasma corticosterone levels (Onozuka, Watanabe, Fujita, Tonosaki, & Saito, 2002). Reduced oral sensory stimulation and malnutrition induced by tooth extraction leads to a sustained increase in circulating corticosterone concentrations (Onozuka, Watanabe, Fujita, Tonosaki et al., 2002). The present study demonstrated that tooth loss early in life may be a chronic stressor that accelerates hippocampus-dependent cognitive impairment. Dysfunctional mastication induced by soft-food diet feeding enhances oxidative stress (Yoshino et al., 2012) and reduces the response of dopamine neurons in the hippocampus, leading to impaired spatial learning (Kushida et al., 2008). Aged rats often exhibit increased glucocorticoid levels in association with age-related memory and learning deficits (Sapolsky, 1999). Increased stress and corticosterone levels also decrease adult progenitor cell proliferation, and could contribute to the declining neurogenesis observed during aging (Kempermann, Gast, & Gage, 2002). Supporting this notion, adrenalectomy in aged rodents leads to increased neurogenesis (Montaron et al., 1999). Chronic immobilization stress reduces synaptophysin expression in the hippocampus (Thome et al., 2001). Together, these findings suggest that increased corticosterone levels contribute to the suppression of neurogenesis and synaptophysin expression, leading to learning deficits in aged SAMP8 mice with early toothlessness.

The dorsal raphe nucleus in the pons provides substantial serotonergic (5-hydroxytryptamine, 5HT) afferents to the cortex, hippocampus, hypothalamus, and other forebrain and midbrain areas (Vertes, 1991). High concentrations of the 5HT1A receptor are present in the hippocampal DG (Azmitia, Gannon, Kheck, & Whitaker, 1996). Serotonergic innervation of the hippocampal DG originates from the median raphe nucleus in the brainstem (Patel, Azmitia, & Zhou, 1996). Serotonin enhances hippocampal DG neuron production via 5HT1A receptor activation (Gould, 1999). Exposure to restraint stress for 14 d decreases 5HT fiber density and the number of 5HT1A receptors, and inhibits 5HT release in the DG (Watanabe, Sakai, McEwen, & Mendelson, 1993). Indeed, removing the molars of aged SAMP8 mice decreases 5HT immunoreactivity in the dorsal raphe nucleus (Kubo et al., 2009). Moreover, molar removal in aged mice enhances the decline in the septohippocampal cholinergic system (Kato et al., 1997; Onozuka, Watanabe, Fujita, Tomida et al., 2002; Onozuka, Watanabe, Fujita, Tonosaki et al., 2002). The hippocampal cholinergic system has an important role in spatial cognition and undergoes several age-dependent changes (Amaral et al., 1991 Amaral, Collier, & Zaccheo, 1991). Mohapel et al. reported that forebrain acetylcholine promotes hippocampal neurogenesis and learning (Mohapel, Leanza, Kokaia, & Lindvall, 2005). Thus, the suppressed cell proliferation in the hippocampal DG and learning deficits of early toothless mice may be due to changes in serotonin expression and/or the cholinergic system. Further investigation is needed to examine the precise relation between early tooth loss and its effects on serotonin secretion and the cholinergic system in the central nervous system.

Animal studies demonstrated that recovery of occlusal support by restoring the molars with artificial crowns, dentures, or dental implant replacement, ameliorates cognitive impairment induced by tooth cutting or tooth extraction (Avivi-Arber et al., 2015; Sakamoto et al., 2014; Watanabe et al., 2002). Interestingly, enriched environmental conditions, such as a large cage containing running wheels for voluntary physical exercise, and tunnels of different colors and shapes, attenuates spatial memory impairment induced by molar tooth loss (Kondo et al., 2016). These animal studies clearly indicate that the restoration of removed teeth could overcome the detrimental effects of tooth loss on cognitive function. On the other hand, many longitudinal cohort studies suggest that tooth loss is associated with physical and cognitive decline (Tsakos et al., 2015; Zukuaga, Montoya, Contreras, & Herrera , 2012). In a prospective cohort study of older Japanese adults, the hazard ratio of those with few teeth and without dentures was ~1.85 compared with that for adults with at least 20 teeth or those with few teeth having dentures (Yamamoto et al., 2012). These findings underline the importance of oral prosthetic treatments, such as dentures and prosthetic implants, for patients missing several teeth. Mastication during childhood may enhance cognitive function in later adult stages, whereas untreated early tooth loss may adversely affect later cognitive function.

5. Conclusion

Aged SAMP8 mice with tooth loss soon after tooth eruption exhibited suppressed cell proliferation and survival in the hippocampal DG, and synaptophysin expression in the hippocampus, with no marked changes in newborn cell differentiation of the DG. In addition, early tooth loss results in impaired learning in the Morris water maze later in life. Early tooth loss may be a risk factor for cognitive impairment later in life.

Ethical approval

This study was approved by the animal care and use ethical committee of Asahi University School of Dentistry (10-018).

Conflict of interest

No conflict of interest declared.

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