Archives of Oral Biology 61 (2016) 1–7

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Hippocampus-dependent spatial memory impairment due to molar tooth loss is ameliorated by an enriched environment

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ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form 11 September 2015
Accepted 5 October 2015

Keywords:
Tooth loss
Enriched environment
Learning ability
Hippocampus
Neurogenesis

ABSTRACT

Background and objective: Teeth are crucial, not only for mastication, but for overall nutrition and general health, including cognitive function. Aged mice with chronic stress due to tooth loss exhibit impaired hippocampus-dependent learning and memory. Exposure to an enriched environment restores the reduced hippocampal function. Here, we explored the effects of an enriched environment on learning deficits and hippocampal morphologic changes in aged senescence-accelerated mouse strain P8 (SAMP8) mice with tooth loss.

Design: Eight-month-old male aged SAMP8 mice with molar intact or with molars removed were housed in either a standard environment or enriched environment for 3 weeks. The Morris water maze was performed for spatial memory test. The newborn cell proliferation, survival, and differentiation in the hippocampus were analyzed using 5-Bromodeoxyuridine (BrdU) immunohistochemical method. The hippocampal brain-derived neurotrophic factor (BDNF) levels were also measured.

Results: Mice with upper molars removed (molarless) exhibited a significant decline in the proliferation and survival of newborn cells in the dentate gyrus (DG) as well as in hippocampal BDNF levels. In addition, neuronal differentiation of newly generated cells was suppressed and hippocampus-dependent spatial memory was impaired. Exposure of molarless mice to an enriched environment attenuated the reductions in the hippocampal BDNF levels and neuronal differentiation, and partially improved the proliferation and survival of newborn cells, as well as the spatial memory ability.

Conclusion: These findings indicated that an enriched environment could ameliorate the hippocampus-dependent spatial memory impairment induced by molar tooth loss.

1. Introduction

Teeth are not only crucial for mastication, but play an essential role in overall nutrition and general health (Zhang, Du, Zhou, & Yu, 2014). Aging, poor oral care, and injury may lead to tooth loss. The prevalence of tooth loss increases with age, peaking at 65 years of age. A recent study reported that the mean number of teeth of American adults over 65 years of age is only 19 (Kassebaum et al., 2014). Tooth loss in adults is associated with an increased risk for obesity, diabetes, cardiovascular diseases, certain types of cancer, and Alzheimer's disease (Hung et al., 2004). Due to the aging population worldwide, Alzheimer's disease has become an increasingly important health and socioeconomic issue.

In mice and rats, molar extraction decreases the masticatory function. Chronic stress due to decreased mastication resulting from tooth loss inhibits hippocampal neurogenesis and impairs learning and memory (Kubo, Iwata, Watanabe, Fujita, & Onozuka, 2005; Onozuka et al., 1999). The hippocampus is involved in learning and memory (Amenta, Collier, & Zaccheo, 1991). The dentate gyrus (DG) of aging hippocampus exhibits alterations in neurogenesis (Ono, Yamamoto, Kubo, Onozuka, 2010). Granule neurons in the DG are produced throughout adulthood in animals and play a significant role in retention of hippocampus-dependent learning and memory (Hastings & Gould, 1999). Neurogenesis in the hippocampal DG is susceptible to various hormonal and environmental stimuli, such as glucocorticoids and stress (Fuchs &

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http://dx.doi.org/10.1016/j.archoralbio.2015.10.006
0003-9969/

Flugge, 1998), physical activity (van Praag, Kempermann, & Gage, 1999), and learning (Kempermann, Brandon, & Gage, 1998).

Epidemiologic data indicate that physical and intellectual activity may delay the onset of dementia and Alzheimer's disease (Larson et al., 2006). The molecular mechanisms underlying the effects of physical and intellectual activity in maintaining cognitive health, however, are unclear, despite a vast literature on experience-dependent changes in the brain at the subcellular, biochemical, and molecular levels (Rosenweig & Berrett, 1996). The hippocampus is of particular interest in this context due to its importance for higher cognitive functions, especially learning and memory, and its pivotal involvement in degenerative disorders of the aging brain. An enriched Environment refers to housing conditions, in which animals are exposed to higher levels of sensory, motor, social, and cognitive stimuli compared to a normal cage environment (Veena et al., 2009). An enriched environment affects rodent brains at the molecular, cellular, and behavioral levels. Brain-derived neurotrophic factor (BDNF) is widely expressed in both the developing and adult brain and is essential for the survival of various populations of neurons during development (Kernie, Liebel, & Parada, 2000; Linnarsson, Willson, & Ernfors, 2000). Hippocampal BDNF levels are increased by enriched environment are associated with neurogenesis (Falkenberg et al., 1992). In addition, exposure to an enriched environment attenuates stress-induced neuronal degeneration and impaired cell proliferation in the hippocampus (Veena et al., 2009).

As the functional morphology of the hippocampus is influenced by tooth loss and enriched environment, we hypothesized that an enriched environment would improve the impaired hippocampal function induced by the molarless condition in aged SAMP8 mice.

2. Materials and methods

2.1. Animals and experimental protocol

SAMP8 mice (8-months old; $n = 80$) were used in this study. Senescence-accelerated mice have a mean lifespan of 12 months (Takeda et al., 1999) and, in addition to accelerated senescence, exhibit age-associated pathologies such as learning and memory deficits and brain atrophy (Takeda et al., 1999), making them good animal model for human cognitive diseases associated with age (Takeda et al., 1999). The mice were group-housed with a 12:12 h light/dark cycle (light period, 6:00–18:00) under controlled temperature ($23 \pm 1\,^{\circ}C$) and humidity ($55 \pm 25\%$). All experiments were performed according to the guidelines for care and use of laboratory animals at Asahi University. The ethics committee of Asahi University School of Dentistry approved the study.

Animals were randomly divided into four groups: either molar-intact or molarless housed in either a standard environment or an enriched environment for 3 weeks after the molar-removing procedure. For the standard rearing conditions, a $33 \times 22 \times 13$ cm cage was used to house 5 animals. For the enriched environment rearing conditions, a large cage ($60 \times 40 \times 17$ cm) containing running wheels for voluntary physical exercise, and tunnels of different colors and shapes was used. The arrangement of the items in the enriched environment was changed randomly every day.

The upper molar teeth of 8 mo-old mice were removed as described previously (Kubo et al., 2007). Briefly, mice were anesthetized with sodium pentobarbital (35.0 mg/kg; Somnopentyl®, Kyoritsuseiyaku Co., Ltd., Tokyo, Japan) and all upper (maxillary) molar teeth were extracted using dental tweezers. The molar-intact control animals were similarly manipulated but the molar teeth were not extracted. Following the procedure, the mice were provided pellet chow (CE-2, CLEA Japan, Inc., Tokyo, Japan) and water ad libitum, and housed separately with respect to molarless or molar-intact mice in either a standard or enriched environment for 3 weeks.

2.2. Morris water maze test

The Morris water maze test was performed as described previously (Kubo et al., 2005) after exposure to the enriched environment for 3 weeks. Briefly, a stainless steel circular pool (diameter, 90 cm; height, 30 cm) was filled to 23 cm with water at room temperature (25 °C). Mice were placed into the water were from one of four randomly selected positions around the pool and had to swim to escape to a platform (12×12 cm) hidden 1 cm under the water surface in the center of one of the four quadrants of the maze. If the animal was unable to find the hidden platform within 90 s was gently guided to the platform by hand, where it was allowed to remain for 20 s between trials. Each mouse received four acquisition trials per day for 7 days. The time from placing the animal into the water until it found the platform was measured as the escape latency. A CCD camera linked to a computer system (Move-er/2D, Library Co., Ltd., Tokyo, Japan) was used to measure the swim path and latency to reach the platform. All animals underwent a visible probe test, in which the platform was visible, 2 h after the last training trial on the last day of training.

2.3. 5-Bromodeoxyuridine (BrdU) treatment

Newborn cell proliferation, survival, and differentiation in the hippocampal DG were analyzed using BrdU (Sigma–Aldrich, St. Louis, MO, USA). BrdU dissolved in 0.9% saline (50 mg/kg) was injected into the mice 5 times a day at 3-h intervals after their 3-weeks exposure to the enriched environment, as described previously (Takagi et al., 1999). The mice were maintained in either the standard or enriched environments for 3 weeks after the molar-removing procedure. Twenty-four hours (for proliferation of newborn cells) or 21 days (for survival and differentiation of newborn cells) after the BrdU injections, the mice were deeply anesthetized and transcardially perfused with 30 ml 0.9% NaCl solution, followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 at 4 °C. The brains were carefully removed and fixed in 2% paraformaldehyde fixative overnight at 4 °C.

2.4. Immunohistochemistry for visualization of BrdU-immunoreactive cells

Serial sections with a thickness of 40 μm (for survival and proliferation) or 20 μm (for differentiation) were prepared on a cryostat (CM1850, Leica, Wetzlar, Germany) for analysis of the neurogenesis of newborn cells. BrdU-immunohistochemistry and quantification of BrdU-immunoreactive cells within the hippocampal DG region were performed as described earlier (Veena et al., 2009). We used a monoclonal antibody to BrdU (Abcam PLC, Cambridge, UK) and avidin–biotin complex method, as described previously (Onishi, Iinuma, Tamura, & Kubo, 2014). DNA was denatured by incubating the sections in 50% formamide/2x SSC (1x SSC = 0.3 M sodium chloride and 0.03 M sodium citrate) at 65 °C for 2 h, in 2 N HCl at 37 °C for 30 min, and then neutralized in 0.05 M Tris-buffered saline (TBS), pH 8.5 for 10 min. Free-floating sections were processed by a standard immunohistochemical procedure using the ABC method. The sections were rinsed in phosphate-buffered saline (PBS), placed in 1% H_2O_2 for 10 min at room temperature, rinsed with PBS, and then placed in 5% normal goat serum for 60 min at room temperature. The sections were rinsed with PBS and incubated with rabbit polyclonal anti-BrdU antiserum (1:200 in PBS containing 0.3% Triton X-100; Abcam PLC, Cambridge, UK) for 48 h at 4 °C, rinsed with PBS, placed in biotinylated goat anti-rabbit IgG (1:500 in PBS; Dako Cytomation,