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**Original Article**

## **Soft-diet Feeding Inhibits Adult Neurogenesis in Hippocampus of Mice**

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### **Abstract**

Our previous study showed that mice fed on a soft diet after weaning had reduced levels of brain-derived neurotrophic factor (BDNF) protein in the hippocampus after 3 months of age compared with mice fed on a hard diet. BDNF is one of the most effective promoters of neurogenesis in the hippocampus, and enhancement of BDNF production has been shown to enhance neural precursor cell proliferation in the dentate gyrus. We hypothesized that soft-diet feeding during development would reduce the proliferation rate of precursor cells, resulting in lower production of new neurons in the hippocampus. Male C57BL/6 mice pups were fed either a solid (hard-diet group) or powdered (soft-diet group) diet starting at weaning. Three and six months after birth, mice of each group received intraperitoneal injections of bromodeoxyuridine (BrdU, 50 mg/kg body weight), twice a day for 3 consecutive days. After survival time of 1 day, 1 week, or 4 weeks, the mice were anesthetized and perfused transcardially. Newborn cells in the dentate gyrus were examined by immunohistochemistry using anti-BrdU antibody. In addition, phenotypically neuronal cells among the newborn cells were detected by immunofluorescent double labeling for BrdU and mature neuron-specific nuclear protein (NeuN) using anti-BrdU and anti-NeuN antibodies. Total number of BrdU-positive cells in the dentate gyrus was fewer in the 6-month-old mice than in the 3-month-old mice at any survival time investigated, and fewer in the soft-diet group than in the hard-diet group at 3 and 6 months of age. Neither soft-diet feeding nor aging affected ratio of phenotypically neuronal cells among newborn cells. These results indicate that insufficient mastication activity during development as well as aging restrains hippocampal neurogenesis in adulthood.

**Key words:** Mastication—Neurogenesis—Neurotrophin

## Introduction

Brain-derived neurotrophic factor (BDNF) plays important roles in the development, plasticity and survival of neurons in the central nervous system<sup>10,19</sup>. Widely distributed throughout the brain, BDNF is expressed at highest levels in the hippocampus and cerebral cortex<sup>5,16</sup>. Our previous study showed that mice fed a powdered diet after weaning had reduced levels of BDNF protein in the hippocampus compared to control mice at 3 months of age<sup>25</sup>, suggesting that insufficient masticatory work during development produces lower levels of BDNF protein in the hippocampus. In addition, reduced secretion of BDNF in such mice has been suggested to reduce synaptic densities in this region<sup>24</sup>.

In adult hippocampus, new neurons are generated throughout life and added to the granule cell layer of the dentate gyrus<sup>3,8,11</sup>. BDNF exerts trophic effects on progenitor cells in the granule cell layer and facilitates neurogenesis in the hippocampus<sup>18</sup>. Therefore, it is supposed that soft-diet feeding during development also affects hippocampal neurogenesis in adulthood. This hypothesis has been investigated by Mitome *et al.*<sup>14</sup> using powdered-diet feeding in 4 week-old mice for 10 weeks, with an injection protocol of bromodeoxyuridine (BrdU), the exogenous marker of cell proliferation<sup>4</sup>, for 12 consecutive days. Their immunostaining for BrdU-positive cells in the hippocampus showed that soft-diet feeding had no effect on the proliferation rate of progenitor cells in the dentate gyrus. However, in their BrdU injection protocol, the proliferation rate of granule cells appears not to have been accurately estimated. Once S-phase cells in the dentate gyrus are labeled with BrdU, each newly born cell may continue to divide. So the number of BrdU-labeled cells counted, even at 1 day after the last injection, is a summation of daily generated cells which survive for up to 12 days. Furthermore, BrdU-labeled cells dilute the label to below detectable limits for immunohistochemistry at around 1 week, and subsequently either migrate out of the proliferative

zone or die<sup>3,8,17</sup>. Therefore, shorter daily injection protocols (less than 1 week) are required to estimate the proliferation rate of progenitor cells<sup>9,17</sup>. Moreover, hippocampal neurogenesis in mice quickly decreases with age, reaching a peak in early adulthood (6–8 weeks)<sup>2,12</sup>. Thus, the effects of soft-diet feeding on hippocampal neurogenesis would vary with the age of the animals.

In the present study, we tested the hypothesis that a reduction in masticatory activity during development would reduce the number of newly generated cells in the dentate gyrus in 3- and 6-month-old mice, and further investigated the ability of such cells to differentiate into mature neurons and survive in the hippocampus.

## Materials and Methods

### 1. Animals

Male C57BL/6 mice pups were weaned at 3 weeks after birth, housed in groups of 4–5 in standard Plexiglas cages (30 × 20 × 14 cm) with a bedding of wood shavings and placed in an air-conditioned room (22 ± 1°C) under a constant light-dark cycle (12:12 hr) with lights on at 06:00. Each group was fed either pelleted (hard-diet group) or powdered (soft-diet group) chow containing the same ingredients (Certified diet, MF, Oriental Yeast, Tokyo) and allowed free access to water. Cages and bedding were changed twice a week. The animals were treated in accordance with the principles approved by the Council of the Physiological Society of Japan and in compliance with the guidelines of the Japanese Government.

### 2. BrdU administration

At 3 and 6 months of age, mice in both the hard-diet and soft-diet groups received an intraperitoneal injection of BrdU (50 mg/kg body weight, Sigma, St. Louis, MO) every 12 hr for 3 consecutive days.

### 3. Immunohistochemistry

At 1 day, 1 week and 3 weeks after the last

injection of BrdU, the mice ( $n = 10$  in each group) were deeply anesthetized and perfused transcardially with heparinized 0.1M phosphate buffered saline (PBS, pH7.4), followed by cold 4% paraformaldehyde in PBS. Their brains were removed, post-fixed in the same fixative at 4°C overnight and cryoprotected in 30% sucrose in PBS. The brains were then frozen, sectioned serially at 40  $\mu\text{m}$  in the coronal plane using a cryostat-microtome and processed for BrdU immunohistochemistry according to the method of Kempermann *et al.*<sup>11)</sup>. In brief, free-floating sections were treated with 0.6% H<sub>2</sub>O<sub>2</sub> in Tris-buffered saline (TBS, pH7.5) for 30 min, incubated in 2 $\times$ SSC (0.3M NaCl and 0.03M sodium citrate) containing 50% formamide for 2 hr at 65°C, the DNA denatured in 2N HCl for 30 min at 37°C, and then the sections washed in 0.1M borate buffer acid (pH8.5) for 10 min at room temperature. The sections were blocked in TBS containing 0.3% Triton X-100 and 0.3% normal goat serum for 1 hr, and then incubated with mouse monoclonal anti-BrdU antibody (1:400, Millipore, Billerica, MA) at 4°C overnight. After rinsing in TBS, the sections were incubated with biotinylated anti-mouse antibody (1:200, Vector Laboratories, Burlingame, CA), followed by avidin-biotin-peroxidase complex (1:100, ABC kit, Vector Laboratories, Burlingame, CA). Sections were mounted on slides and observed under a light microscope. For quantification of BrdU-positive cells a series of every fifth section throughout the entire rostro-caudal extent of the hippocampus was examined.

#### 4. Immunofluorescent staining

To determine the cell phenotype of BrdU-labeled cells, immunofluorescent double-labeling for BrdU and mature neuron-specific nuclear protein (NeuN) was performed as described by Lee *et al.*<sup>13)</sup>. Briefly, sections were treated for DNA denaturation as described above, followed by blocking in TBS containing 0.1% Triton X-100 and 5% normal goat serum for 1 hr. Primary antibodies were then added and the sections incubated overnight at 4°C. Primary antibodies used were rat mono-

clonal anti-BrdU antibody (1:200, Accurate Chemicals, Westbury, NY) and mouse monoclonal anti-NeuN antibody (1:500, Chemicon/Millipore, Temecula, CA). After washing in TBS, sections were incubated for 1 hr in the presence of anti-rat IgG labeled with AlexaFluor-488 (1:500, Molecular Probes, Eugene, OR) and anti-mouse IgG labeled with AlexaFluor-568 (1:500, Molecular Probes). The immunolabeled sections were transferred to glass slides and mounted with an anti-fading medium (FluoroGuard, Bio-Rad, Hercules, CA). Confocal images were acquired using a microscope (Nikon Eclipse TE300, Nikon, Tokyo) with an attached laser confocal scanning system (MRC-1024, Bio-Rad). In the dentate gyrus, 50 BrdU-positive cells per brain were analyzed for co-labeling with NeuN.

#### 5. Statistics

All values represented the mean  $\pm$  SEM. The data were analyzed with an analysis of variance (ANOVA) and the Fisher's PLSD tests for post-hoc comparisons. All statistical analyses were performed with the Stat-View software (SAS Institute, Cary, NC). A value of  $p < 0.05$  was considered statistically significant.

## Results

The distribution of BrdU-immunoreactive structures in the hippocampus was examined using microscopy. BrdU-positive cells were detected in the dentate gyrus in both the hard-diet (Fig. 1A, C) and soft-diet groups (B, D) at 3 (A, B) and 6 months after birth (C, D). In the dentate gyrus, BrdU-positive cells were located predominantly within the subgranular zone along the border between the granule cell layer and the hilus. The number of BrdU-positive cells per dentate gyrus was quantified using every fifth serial section of the hippocampus. In the hard-diet group, BrdU-positive cells in the dentate gyrus diminished with either the age of the animals or survival time after the last injection of BrdU (Fig. 2A, B). A two-way ANOVA on the number of BrdU-positive cells revealed significant effects

for age ( $F_{1,54} = 122.4$ ,  $p < 0.001$ ) and survival time ( $F_{2,54} = 81.4$ ,  $p < 0.001$ ). A reduction in the number of BrdU-positive cells due to soft-diet feeding was recognized in the dentate

gyrus at 3 (Fig. 2A) and 6 months of age (B). In 3-month-old mice, a two-way ANOVA for the effects of soft-diet feeding and survival time after BrdU injection indicated that both

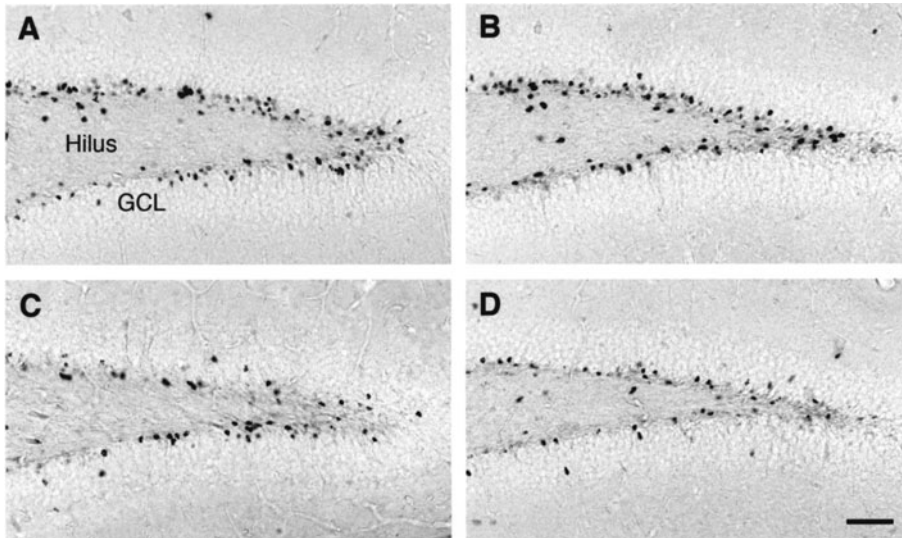


Fig. 1 BrdU immunoreactivity in dentate gyrus of hippocampus in 3- (A, B) and 6-month-old mice (C, D) one week after last injection of BrdU. In both hard- (A, C) and soft-diet groups (B, D), BrdU-positive cells were dominantly distributed in subgranular zone at border between granule cell layer (GCL) and hilus. There were fewer BrdU-positive cells in soft-diet mice than in hard-diet mice, and also fewer in 6-month-old mice than in 3-month-old mice. Scale bar: 50  $\mu$ m.

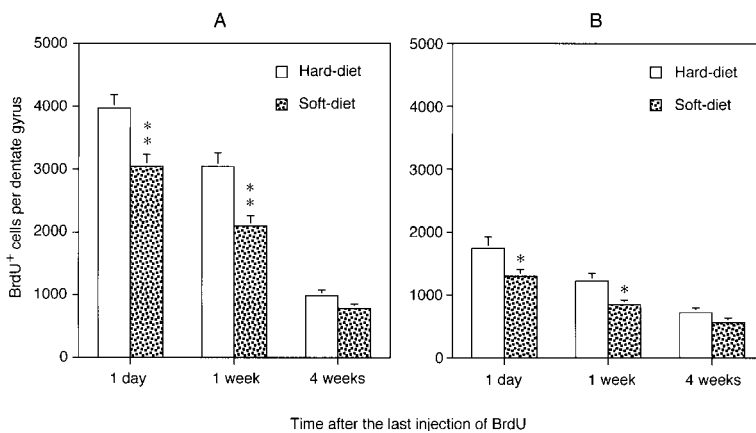


Fig. 2 Quantification of BrdU-positive (BrdU<sup>+</sup>) cells in dentate gyrus of 3- (A) and 6-month-old mice (B) at 1 day, 1 week and 4 weeks after last injection of BrdU. Soft-diet feeding from weaning significantly diminished number of BrdU-positive cells in dentate gyrus at both 3 and 6 months after birth. Numbers represent total per granule cell layer expressed as mean  $\pm$  SEM ( $n = 10$  mice for each group). \* $p < 0.05$ , \*\* $p < 0.005$  compared to hard-diet group (ANOVA with Fisher's PLSD tests for post-hoc comparisons).

soft-diet ( $F_{1,54}=25.8$ ,  $p<0.001$ ) and survival time ( $F_{2,54}=126.3$ ,  $p<0.001$ ) reached statistical significance. A similar analysis for 6-month-old mice also revealed significant effects for soft-diet ( $F_{1,54}=12.9$ ,  $p<0.001$ ) and survival time ( $F_{2,54}=31.6$ ,  $p<0.001$ ). Post-hoc comparisons (Fisher's PLSD) in 3-month-old mice showed a significant reduction in BrdU-positive cells at 1 day ( $p<0.005$ ) and 1 week ( $p<0.005$ ) after the last BrdU injection. In 6-month-old mice, a significant reduction in BrdU-positive cells was also detected at 1 day ( $p<0.05$ ) and 1 week ( $p<0.05$ ) after injection.

In order to determine the phenotypes of the newly generated cells, double-labeling confocal immunohistochemical analysis was performed using an antibody against NeuN in combination with the BrdU antibody. Under a confocal laser scanning microscope, BrdU-

labeled cells co-labeled with NeuN were detected in the granule cell layer and hilus of the dentate gyrus in both the hard-diet (Fig. 3A, C) and soft-diet groups (B, D) at 3 (A, B) and 6 months after birth (C, D). The percentage of cells double-labeled for BrdU and NeuN (BrdU/NeuN-positive cells) per BrdU-positive cells was calculated using a total of 50 BrdU-positive cells per animal. In both 3- (Fig. 4A) and 6-month-old mice (B), the proportion of BrdU/NeuN-positive cells increased with survival time after the last injection of BrdU. Soft-diet feeding slightly reduced the mean percentage of BrdU/NeuN-positive cells (Fig. 4A, B). However, no statistical difference was detected in percentage of neuronal phenotypes between the hard-diet and soft-diet groups at any survival time investigated.

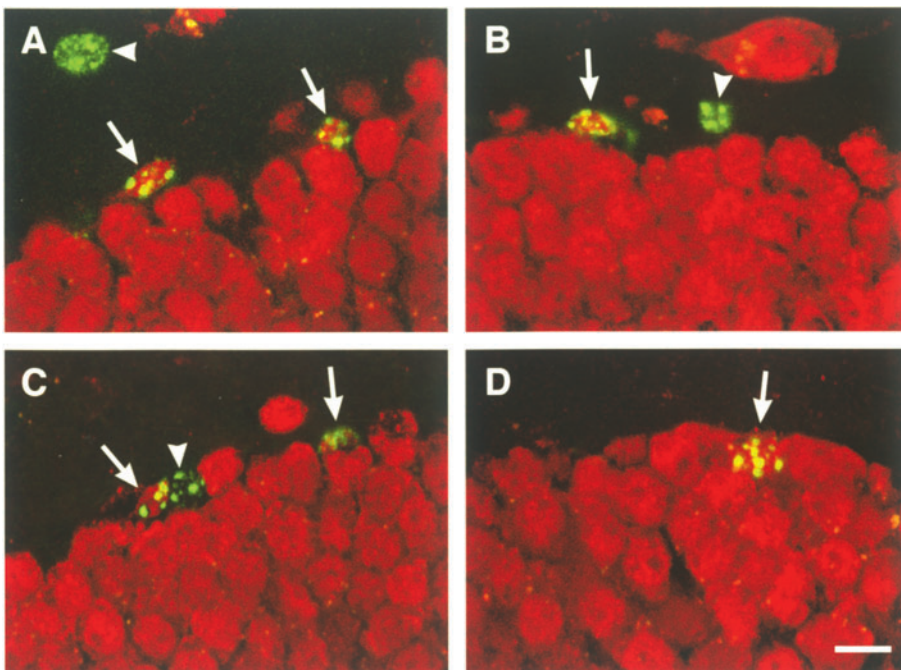


Fig. 3 Confocal laser scanning images showing neuronal phenotype of newly generated cells in dentate gyrus of hard-diet (A, C) and soft-diet (B, D) mice at 3 (A, B) and 6 months after birth (C, D). Sections were reacted with antibodies against BrdU (green, marker of DNA synthesis) and NeuN (red, marker of mature neurons). BrdU-positive cells double-labeled for NeuN (BrdU/NeuN-positive cells) were mainly located within subgranular zone. Arrows indicate BrdU/NeuN-positive cells, while arrowheads show BrdU-positive cells not labeled for NeuN. Scale bar: 50  $\mu$ m.

## Discussion

In the present study, we demonstrated that neurogenesis in the adult hippocampus was restrained by aging and soft-diet feeding from the weaning period. In the hard-diet group, the number of BrdU-positive cells in the dentate gyrus was fewer in 6-month-old mice than 3-month-old mice at any survival period investigated. With BrdU injections limited to 3 days, the number of BrdU-positive cells at 1 day and 4 weeks after the last BrdU injection would indicate the proliferation rate of precursor cells and survival rate of newly born cells for 4 weeks, respectively. Since there was no significant age-dependent reduction in the ratio of BrdU-positive cells exhibiting the neuronal phenotype, the total number of mature granule cells added to the hippocampus would decrease with increasing age. Soft-diet feeding from weaning significantly reduced the proliferation rate of precursor cells in the dentate gyrus in both 3- and 6-month-old mice. On the other hand, no distinct effect of soft-diet feeding was detected in the survival rate of newly born cells. This may have been due to several factors such as dilution of BrdU

and migration of newborn neurons, which would have influenced the number of BrdU-positive cells at 4 weeks after the last BrdU injection.

One of the most effective promoters of neurogenesis in the dentate gyrus is BDNF, which is strongly expressed in the adult hippocampus<sup>10,19</sup>. An enhancement of BDNF production has been shown to enhance neural precursor cell proliferation in the sub-granular zone<sup>10</sup>, and a shortage of BDNF content has been linked to a drop in neurogenesis<sup>18</sup>. The action of BDNF is mediated by the specific tyrosine kinase receptor TrkB, which is also widely expressed in the dentate gyrus<sup>23</sup>. Age-dependent decline in adult hippocampal neurogenesis is a consequence of chronically elevated circulating levels of corticosteroids<sup>1</sup>, whose levels are elevated in aged animals<sup>2</sup>. Corticosterone, in particular, has been identified as a common downregulator of neurogenesis<sup>1,2</sup>. Treatment of adult rats with corticosterone inhibits the proliferation of granule cell precursors<sup>1</sup>. Conversely, adrenalectomy restores hippocampal neurogenesis in aged rats to levels corresponding to those in younger rats<sup>2,6</sup>. Although the

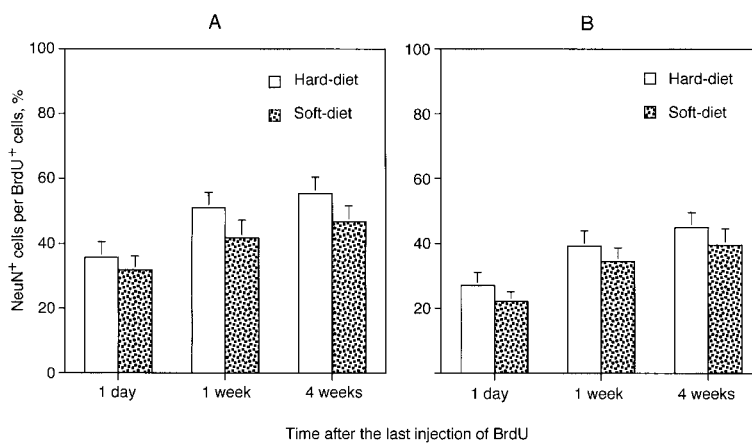


Fig. 4 Percentages of BrdU/NeuN-positive cells per total BrdU-positive cells in dentate gyrus of 3- (A) and 6-month-old mice (B) 1 day, 1 week and 4 weeks after last injection of BrdU. A total of 50 BrdU-positive cells per animal were analyzed. Mean percentage of BrdU/NeuN-positive cells decreased slightly upon soft-diet feeding. However, no statistically significant difference was detected between hard-diet and soft-diet mice at any time point after BrdU injection. Data represent mean  $\pm$  SEM ( $n = 8$  mice for each group).

molecular mechanisms underlying the actions of corticosterone on neurogenesis are not fully known, their effects appear to downregulate BDNF mRNA levels in the hippocampus through intracellular glucocorticoid receptors that regulate BDNF transcription<sup>7,20</sup>. Expression of glucocorticoid receptors in the dentate gyrus increases with age, possibly increasing the sensitivity of the immature progenitor cells to glucocorticoids<sup>7</sup>.

The effects of soft-diet feeding during development on adult hippocampal neurogenesis appear to be the result of chronic emotional stress and/or reduced activity of masticatory muscles. Functional disorders of the mouth and dentition, including missing teeth, not only cause problems during eating, but also act as a source of psychological discomfort. Mice fed a powdered diet after weaning are unable to perform sufficient mastication activity throughout their lifetime. Accordingly, they show signs of emotional stress, such as overeating, biting non-edible substances, and loss of hair glossiness<sup>24</sup>. One explanation is that stress-induced increase in circulating levels of corticosterone reduces BDNF expression in the hippocampus, thereby restraining proliferation of precursor cells in the dentate gyrus.

Adult neurogenesis is thought to be enhanced by exercise-induced neurotrophic factors, which would diminish under conditions of less activity. Our previous work demonstrated that mice fed a soft diet after weaning had significantly lower levels of BDNF protein in the hippocampus compared to those fed a hard diet<sup>25</sup>. It has been reported that exercise in rats in the form of voluntary running increases levels of BDNF mRNA in various brain regions, including the dentate gyrus<sup>15,22</sup>. In contrast, an abrupt deprivation of habitual running in rats leads to long-lasting decreases in BDNF mRNA expression in the hippocampus<sup>22</sup>. Lack of exercise in rats induced by hindlimb suspension, involving elevation of their tails, reduced levels of BDNF protein in the hippocampus and suppressed neurogenesis in the dentate gyrus<sup>26</sup>. Likewise, it is thought that a decline in mas-

tatory activity caused by soft-diet feeding during development leads to decreased levels of BDNF protein in the hippocampus in adulthood, thus restraining hippocampal neurogenesis. Although the underlying mechanisms are far from clear, voluntary physical activity may maintain BDNF levels in the brain *via* an action of the cAMP response element binding protein<sup>21</sup>.

In conclusion, mice fed on a soft diet after weaning exhibited a reduction in neurogenesis in the adult hippocampus compared with mice fed on a hard diet. This suggests that insufficient masticatory activity in childhood restrains hippocampal function later on.

## References

- 1) Cameron HA, Gould E (1994) Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neuroscience* 61:203–209.
- 2) Cameron HA, McKay RD (1999) Restoring production of hippocampal neurons in old age. *Nat Neurosci* 2:894–897.
- 3) Cameron HA, Woolley CS, McEwen BS, Gould E (1993) Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience* 56:337–344.
- 4) del Rio JA, Soriano E (1989) Immunocytochemical detection of 5'-bromodeoxyuridine incorporation in the central nervous system of the mouse. *Brain Res Dev Brain Res* 49:311–317.
- 5) Ernfors P, Wetmore C, Olson L, Persson H (1990) Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. *Neuron* 5:511–526.
- 6) Fabel K, Kempermann G (2008) Physical activity and the regulation of neurogenesis in the adult and aging brain. *Neuromolecular Med* 10:59–66.
- 7) Garcia A, Steiner B, Kronenberg G, Bick-Sander A, Kempermann G (2004) Age-dependent expression of glucocorticoid- and mineralocorticoid receptors on neural precursor cell populations in the adult murine hippocampus. *Aging Cell* 3:363–371.
- 8) Gould E, Tanapat P, Hastings NB, Shors TJ (1999) Neurogenesis in adulthood: A possible role in learning. *Trends Cogn Sci* 3:186–192.
- 9) Hayes NL, Nowakowski RS (2002) Dynamics of cell proliferation in the adult dentate gyrus

- of two inbred strains of mice. *Brain Res Dev Brain Res* 134:77–85.
- 10) Katoh-Semba R, Asano T, Ueda H, Morishita R, Takeuchi IK, Inaguma Y, Kato K (2002) Riluzole enhances expression of brain-derived neurotrophic factor with consequent proliferation of granule precursor cells in the rat hippocampus. *FASEB J* 16:1328–1330.
  - 11) Kempermann G, Kuhn HG, Gage FH (1997) Genetic influence on neurogenesis in the dentate gyrus of adult mice. *Proc Natl Acad Sci USA* 94:10409–10414.
  - 12) Kuhn HG, Dickinson-Anson H, Gage FH (1996) Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation. *J Neurosci* 16:2027–2033.
  - 13) Lee SH, Kim YH, Kim YJ, Yoon BW (2008) Enforced physical training promotes neurogenesis in the subgranular zone after focal cerebral ischemia. *J Neurol Sci* 269:54–61.
  - 14) Mitome M, Hasegawa T, Shirakawa T (2005) Mastication influences the survival of newly generated cells in mouse dentate gyrus. *Neuroreport* 16:249–252.
  - 15) Neeper SA, Gómez-Pinilla F, Choi J, Cotman CW (1996) Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res* 726:49–56.
  - 16) Phillips HS, Hains JM, Laramee GR, Rosenthal A, Winslow JW (1990) Widespread expression of BDNF but not NT3 by target areas of basal forebrain cholinergic neurons. *Science* 250:290–294.
  - 17) Prickaerts J, Koopmans G, Blokland A, Scheepens A (2004) Learning and adult neurogenesis: Survival with or without proliferation? *Neurobiol Learn Mem* 81:1–11.
  - 18) Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, Fabbri ME, Tessarollo L, Maffei L, Berardi N, Caleo M (2006) Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *Eur J Neurosci* 24:1850–1856.
  - 19) Sairanen M, Lucas G, Ernfors P, Castrén M, Castrén E (2005) Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci* 25:1089–1094.
  - 20) Schaaf MJ, De Jong J, De Kloet ER, Vreugdenhil E (1998) Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. *Brain Res* 813:112–120.
  - 21) Vaynman S, Ying Z, Gómez-Pinilla F (2003) Interplay between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. *Neuroscience* 122:647–657.
  - 22) Widenfalk J, Olson L, Thorén P (1999) Deprived of habitual running, rats down-regulate BDNF and TrkB messages in the brain. *Neurosci Res* 34:125–132.
  - 23) Wu CW, Chang YT, Yu L, Chen HI, Jen CJ, Wu SY, Lo CP, Kuo YM (2008) Exercise enhances the proliferation of neural stem cells and neurite growth and survival of neuronal progenitor cells in dentate gyrus of middle-aged mice. *J Appl Physiol* 105:1585–1594.
  - 24) Yamamoto T, Hirayama A (2001) Effects of soft-diet feeding on synaptic density in the hippocampus and parietal cortex of senescence-accelerated mice. *Brain Res* 902:255–263.
  - 25) Yamamoto T, Hirayama A, Hosoe N, Furube M, Hirano S (2008) Effects of soft-diet feeding on BDNF expression in hippocampus of mice. *Bull Tokyo Dent Coll* 49:185–190.
  - 26) Yasuhara T, Hara K, Maki M, Matsukawa N, Fujino H, Date I, Borlongan CV (2007) Lack of exercise, *via* hindlimb suspension, impedes endogenous neurogenesis. *Neuroscience* 149:182–191.

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