Original article

Reduced brain-derived neurotrophic factor expression in cortex and hippocampus involved in the learning and memory deficit in molarless SAMP8 mice

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Keywords: *molarless; learning and memory; water maze; brain-derived neurotrophic factor; TrkB*

Background The molarless condition has been reported to compromise learning and memory functions. However, it remains unclear how the molarless condition directly affects the central nervous system, and the functional consequences on the brain cortex and hippocampus have not been described in detail. The aim of this study was to find the molecular mechanism related with learning and memory deficit after a bilateral molarless condition having been surgically induced in senescence-accelerated mice/prone8 (SAMP8) mice, which may ultimately provide an experimental basis for clinical prevention of senile dementia.

Methods Mice were either sham-operated or subjected to complete molar removal. The animals' body weights were monitored every day. Learning ability and memory were measured in a water maze test at the end of the 1st, 2nd, and 3rd months after surgery. As soon as significantly prolonged escape latency in the molarless group was detected, the locomotor activity was examined in an open field test. Subsequently, the animals were decapitated and the cortex and hippocampus were dissected for Western blotting to measure the expression levels of brain-derived neurotrophic factor (BDNF) and the tropomyosin related kinase B (TrkB), the high affinity receptor of BDNF.

Results Slightly lower weights were consistently observed in the molarless group, but there was no significant difference in weights between the two groups (P >0.05). Compared with the sham group, the molarless group exhibited lengthened escape latency in the water maze test three months after surgery, whereas no difference in locomotor activity was observed. Meanwhile, in the cortex and hippocampus, BDNF levels were significantly decreased in the molarless group (P <0.05); but the expression of its receptor, TrkB, was not significantly affected.

Conclusion These results suggested that the molarless condition impaired learning and memory abilities in SAMP8 mice three months after teeth extraction, and this effect was accompanied by significantly reduced BDNF expression in the cortex and hippocampus.

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Previous studies have reported that the molarless condition can compromise learning and memory functions in mice. For example, the molarless condition has been shown to impair learning and memory abilities in SAMP8 mice, accompanied by concomitant reductions in c-fos expression,[1] the release of hippocampal acetylcholine (Ach), acetylcholine transferase (ChAT) activity,[2] and the number of spines on pyramidal cells in the CA1 region.[3,4] In addition, chewing activity can activate histamine H_1 receptor (H_1R), which in turn relieves the inhibition of hippocampal synaptic plasticity by stress response or repairs damage in N-methyl-D-aspartate (NMDA) receptor to improve hippocampal memory function.[5] However, it remains unclear how the molarless condition directly affects the central nervous system (CNS), and the functional consequences on the brain cortex and hippocampus have not been characterized in detail. Therefore, studies on the effects and mechanism of the molarless condition on learning and memory may shed important insights on the prevention and intervention of learn and memory disorders.

Brain-derived neurotrophic factor (BDNF) is an important member of the neurotrophic factor family, playing a pivotal role in neuronal development of the CNS. More recently, BDNF has also emerged as an

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important regulator of synaptogenesis and synaptic plasticity mechanisms underlying learning and memory in the adult CNS. While a physiological amount of BDNF in the normal brain has been demonstrated to have positive effects on learning and memory, both an increased level of BDNF, and a decreased level of BDNF may disrupt the equilibrium between inhibitory and excitatory neurotransmission in the brain, leading to a loss of synaptic refinement and consequently impairing long-term potentiation (LTP), learning and memory. The activity can regulate the release of BDNF. Its release will regulate both short- and long-term synaptic plasticity.[6] Therefore, in the present work, we hypothesized that BDNF may participate in the molarless-induced impairment of learning and memory.

METHODS

Experimental animals

Male, specific pathogen-free SAMP8 mice were obtained from Tianjin University of Traditional Chinese Medicine.

Reagents

BDNF antibody (sc-20981), TrkB antibody (sc-8316), β-actin antibody (sc-1616-R), and goat anti-rabbit horseradish peroxidase secondary IgG (ZB-2301) were purchased from Santa Cruz Biotech., Inc., USA. Non-fat milk (P1600), ECL reagent (P1010) and total protein extraction kit (P1250) were obtained from Beijing Applygen Gene Technology Co.

Instruments

The water maze apparatus was manufactured by Institute of Materia Medica, Chinese Academy of Medical Sciences. The open field apparatus was made by Shanghai Yishu Information Technology Co. (RD1413). In addition, the following equipments were also used: microplate reader (BioTek, MQX200); centrifuge: Sigma (3-18K); LAS-3000 ECL colorimetric system; vertical electrophoretic and transfer apparatus, Shanghai Tianneng (VE-180 and VE-186, respectively).

Establishment of the animal model

SAMP8 mice of 22 months old were provided by Tianjin University of Traditional Chinese Medicine and acclimated in the laboratory for 1 week. They were randomly divided into the sham group (n=7) and the molarless group (n=13). All mice were anesthetized via intraperitoneal (i.p.) injection of 10% chloral hydrate (400 mg/kg). In the sham group, small amounts of alveolar bone were removed with Rongeur from the toothless gap region between molars and canines in the superior alveolar ridge on the left. In the molarless group, all the bilateral maxillary molars were removed, if there was root fracture, all the tooth structure visible on the gum was removed to eliminate occlusal contacts.[4] Animals were fed with routine pellet diet with free access to water, and observed for general conditions and body weight in the following surgery.

Water maze experiment

The water maze apparatus was a bi-layer, non-transparent rectangular plastic box (80 cm×50 cm×20 cm), consisted of 4 non-exits and an end-point exit where mice could take the steps to climb out of water. The starting platform can be placed at various locations corresponding to different numbers of non-exits.[7] We measured the number of errors entering non-exits and the time required to reach the end-point steps to evaluate their short-term memory and spatial learning and memory at the end of each month after surgery. During the experiment, mice were placed on the end-point steps for 10 seconds, and subsequently moved to the starting platform in water. The number of errors and the time required for reaching end-point steps (escape latency) were recorded. The maximal escape time was restricted to 2 minutes; if the mouse did not find the steps within 2 minutes, the experimenter would lead it to end-point exit, and the latency was recorded as 2 minutes. The mice were trained 3 times, using routes with non-exits 1, 3 and 4, respectively, and tested for 6 consecutive days.

Open field test

Once the significant prolonged escape latency in molarless group was detected, the locomotor activity was examined in open field test. After a pre-adaptation period of 2 minutes, the two groups of animals were placed sequentially into the RD1413 open field apparatus. The mice were video recorded and analyzed by the system-equipped analytical software to calculate the total distance of each mouse activity over 10 minutes.[8]

Western blotting analysis

After open field test, the mice were decapitated. Brain cortex and hippocampus of each mouse were dissected, weighed, and snap frozen in liquid nitrogen. Subsequently, the samples were minced and homogenized with glass homogenizer for 15 times, and total proteins were extracted with Applygen total protein extraction kit. Protein concentration was normalized using Coomassie brilliant blue G-250 staining. Equal amounts of proteins were loaded onto the gel; following electrophoresis, the proteins were transferred to polyvinylidene fluoride (PVDF) membrane. After blocking with 0.1% Tris-HCl buffer solution and Tween (TBST) containing 5% non-fat milk at room temperature (RT) for 2 hours, primary antibody was added for overnight incubation. The membrane was washed with 0.1% TBST three times, 10 minutes each, and incubated with secondary antibody at RT for 2 hours. The membrane was washed as above, and color development was performed using the ECL kit. Images were acquired using the Fuji Digital Science Imager (Fuji Co., Japan), and analyzed with Gelpro Analyzer (version 4.0, Fuji Co., Japan) to measure the integrated optimal density

(IOD) values of specific bands.[9]

Statistical analysis

Data were shown as mean ± standard deviation (SD) or mean ± standard error (SE) and analyzed by *t* test. Statistical analysis was carried out using SPSS 13.0 (SPSS Inc., Chicago, USA). P <0.05 was considered statistically significant.

RESULTS

Body weight changes following complete molarless in SAMP8 mice

Animals of the two groups showed comparable weights prior to surgery. The average weight was 26.5 g. After surgery, the weight was monitored for one week, and significantly decreased in both groups. Relative to the sham group, the molarless group lost greater weight. Five days after surgery, body weight started to recover, and essentially reached pre-surgery levels on the 7th day after surgery (Figure 1). Slight weight loss in the molarless group were observed; however, the differences were not statistically significant. Body weight was monitored for five days at the end of 1st and 3rd months after surgery, respectively. Slightly lower weights were consistently observed in the molarless group. There was no significant difference between the two groups (P >0.05).

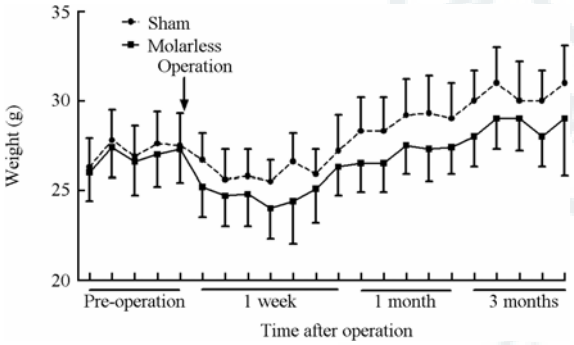

Figure 1. Weight changes in SAMP8 mice before and after surgery. The weight was monitored for five days pre-operation, 7 days after operation, then continuous observation for five days at the end of 1st and 3rd months after surgery, respectively. The data were represented as mean±SD. Sham group, n=7; Molarless group, n=13.

Learning and memory function of SAMP8 mice

Water maze test is routinely conducted to evaluate the short-term memory and spatial learning functions of animals by measuring number of errors entering non-exits and the escape latency duration. In the current study, SAMP8 mice were subjected to monthly water maze tests after surgery. The results indicated that SAMP8 mice did not display any significant deficits in learning and memory in the first two months after complete molarless (Figure 2). However, after three months, as the latency period became shorter due to repeated training, the molarless group started to exhibit significantly longer latency than the sham group (P <0.05), indicating significantly impairment in learning and memory functions under the molarless condition. These observations suggested that the effects of molarless on learning and memory may become more pronounced over an extended period.

Open field test to measure effect of molarless on locomotor activities

The above water maze tests indicated that complete molarless significantly affected the escape latency. However, the difference in the duration of latency may be caused by molarless-induced reduction in food intake and consequently in locomotor activities. As mentioned above, the weights of the two groups of animals showed no significant differences; however, the molarless group animals were consistently lighter in body weight relative to the control group, suggesting a possible effect of complete molarless on food intake. To further investigate this possibility, we next conducted open field tests to evaluate their locomotor functions. Using an open field analyzer, we observed that the molarless animals were indistinguishable from their control counterparts in locomotor functions (Table).

Table. Effect of molarless on mouse locomotor function in open field test

Groups	Distance (cm)	Average velocity (cm/s)
Sham (n=7)	4377±1783	7.30±2.97
Molarless (n=13)	4099±1015	6.83±1.69

Data were shown as mean±SE.

Western blotting analysis

A reciprocal enhancement of long-term potentiation (LTP) and learning and memory has been previously demonstrated. In addition, blocking LTP induction appeared to significantly affect acquisition of hippocampus-dependent learning behavior. Previously, expression of the brain-derived neurotrophic factor (BDNF) gene has been shown to play an important role in LTP formation and memory consolidation.[6] Using Gelpro32 software, we examined expressions of BDNF and TrkB in the brain cortex and hippocampus of SAMP8 mice subjected to three months of molarless condition to quantitatively measure IOD values corresponding to their expressions. The results indicated that after 3 months of complete molarless, BDNF expression was markedly down-regulated in mouse cortex and hippocampus, whereas expression of TrkB was not significantly affected (Figure 3).

DISCUSSION

Social and economic development has allowed increasing awareness on dental health. Other than chewing and digestion, dental health is important for various additional reasons. For example, recent research demonstrated that following molarless, the masticatory dysfunction might increase the risk of senile dementia. It is possible that after tooth loss, the diminished chewing

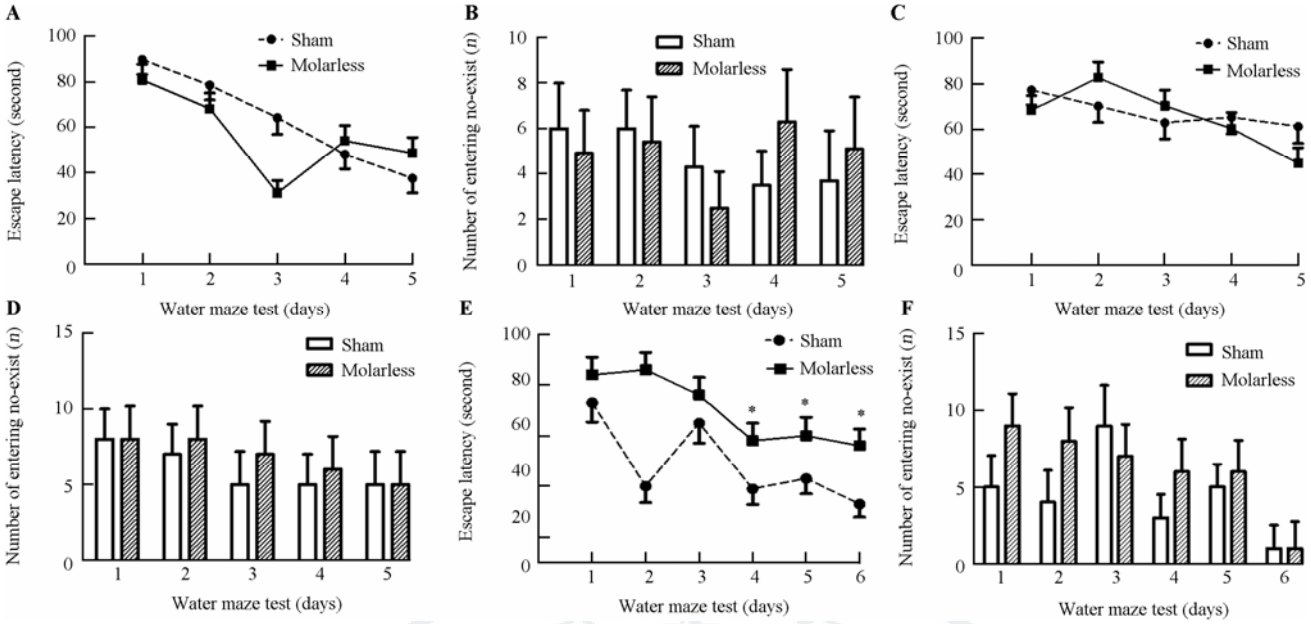

Figure 2. Effect of complete molarless on learning and memory abilities of SAMP8 mice evaluated by water maze test. Water maze tests were performed at the end of 1st (**A, B**), 2nd (**C, D**), and 3rd (**E, F**) months after surgery, respectively. **A**, **C**, and **E**: Escape latency. **B**, **D**, and **F**: The number of errors entering non-exits. Data were shown as mean±SE. Compare to the sham group, $^{*}P$ <0.05, $^{\dagger}P$ <0.01.

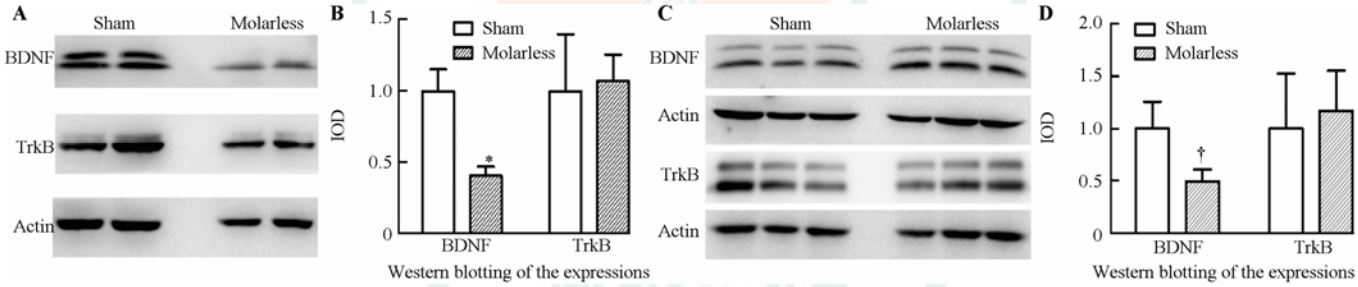

Figure 3. Western blotting analysis of BDNF and TrkB expressions in brain of SAMP8 mice subjected to three months of molarless condition. Each group has 3 mice. Data were shown as mean±SD. **A**, **B**: Results from the cortex. **C**, **D**: Results from the hippocampus. $^{*}P$ <0.01, $^{\dagger}P$ <0.05, Sham group vs. molarless group. IOD values of BDNF and TrkB were normalized by that of β actin, and the normalized values were used to represent the relative levels of BDNF and TrkB.

activity around the toothless area will compromise the stimulation of specific brain cortex neurons associated with chewing, which may ultimately lead to pathological changes.[10] Furthermore, chewing can alleviate stress response-induced damage; for example, when under intense stress, we often clench teeth as a spontaneous response. Therefore, molarless will exacerbate the adverse effects of environmental stress on the body, which possibly accumulates over time.

In the current study, we observed adverse effects of complete molarless on learning and memory functions in SAMP8 mice, which appeared to depend on the duration under the molarless condition. As the data shown, animals of the molarless group had significantly longer escape latency and greater number of errors entering non-exits compared to the sham control. In our previous studies, we conducted similar studies using Kunming mice to examine species difference and got similar results. Enzyme linked immunosorbent assay (ELISA) analysis of plasma corticosterone level in Kunming mice indicated slight changes in plasma corticosterone level, but the difference was not statistically significant. Therefore, complete molarless may diminish learning and memory abilities, but a strict correlation appears unlikely. The statistical analysis of clinical evidence also indicated that though did not strictly correlate with Alzheimer's disease, the molarless condition increased the risk of Alzheimer's disease.

BDNF is an important member of the neurotrophic factor family, playing a pivotal role in neuronal development of the central nervous system. BDNF binds and activates, both pre-and postsynaptically, two different transmembrane receptor proteins: the tropomyosin related kinase TrkB receptor with high affinity, and the pan neurotrophin receptor p75NTR with low affinity.[6] Previous research indicated that BDNF participates in the formation of LTP and synaptic plasticity. It is commonly believed that these two processes are cellular models of long term memory (LTM).[11] Furthermore, BDNF release will regulate both short- and long-term synaptic plasticity;[6] if abnormally expressed, BDNF will induce pathological changes or

diminish plasticity in particular brain regions. We also found reduced markedly brain cortex and hippocampus BDNF expression in the complete molarless mice. It may be partly due to the masticatory dysfunction that the cerebral blood flow decreased. Our results indicated that the reduced BDNF may participate in the molarless-induced impairment of learning and memory. Aoki et al[12] found that molarless condition suppressed proliferation but not differentiation rates into neurons in the rat dentate gyrus.

In conclusion, we found that the molarless condition after three months in SAMP8 mice impaired learning and memory abilities, which accompanied with significantly reduced BDNF expressions in the cortex and hippocampus.

REFERENCES

1. Watanabe K, Ozono S, Nishiyama K, Saito S, Tonosaki K, Fujita M, et al. The molarless condition in aged SAMP8 mice attenuates hippocampal Fos induction linked to water maze performance. Behav Brain Res 2002; 128: 19-25.
2. Onozuka M, Watanabe K, Fujita M, Tomida M, Ozono S. Changes in the septohippocampal cholinergic system following removal of molar teeth in the aged SAMP8 mouse. Behav Brain Res 2002; 133: 197-204.
3. Kubo KY, Kojo A, Yamamoto T, Onozuka M. The bite-raised condition in aged SAMP8 mice induces dendritic spine changes in the hippocampal region. Neurosci Lett 2008; 441: 141-144.
4. Kubo KY, Iwaku F, Watanabe K, Fujita M, Onozuka M. Molarless induced changes of spines in hippocampal region of SAMP8 mice. Brain Res 2005; 1057: 191-195.
5. Ono Y, Kataoka T, Miyake S, Sasaguri K, Sato S, Onozuka M. Chewing rescues stress-suppressed hippocampal long-term potentiation via activation of histamine H_1 receptor. Neurosci Res 2009; 64: 385-390.
6. Cunha C, Brambilla R, Thomas KL. A simple role for BDNF in learning and memory? Front Mol Neurosci 2010; 3: 1-14.
7. Zhao WH, Yu JC, Ji XC, Feng N, Cao DX, Xu SF, et al. Learning and memory deficits in SAMP8 and their biochemical mechanisms. J Xi'an Jiaotong Univ (Med Sci) 2008; 29: 289-293.
8. Barros HM, Tannhauser MA, Tannhauser SL, Tannhauser M. Enhanced detection of hyperactivity after drug withdrawal with a simple modification of the open-field apparatus. J Pharmacol Methods 1991; 26: 269-275.
9. Pang PT, Lu B. Regulation of late-phase LTP and long-term memory in normal and aging hippocampus: role of secreted proteins tPA and BDNF. Ageing Res Rev 2004; 3: 407-430.
10. Bartoletti A, Cancedda L, Reid SW, Tessarollo L, Porciatti V, Pizzorusso T, et al. Heterozygous knock-out mice for brain-derived neurotrophic factor show a pathway specific impairment of long-term potentiation but normal critical period for monocular deprivation. J Neurosci 2002; 22: 10072-10077.
11. Tsai CC, Fondell JD. Nuclear receptor recruitment of histone-modifying enzymes to target gene promoters. Vitam Horm 2004; 68: 93-122.
12. Aoki H, Kimoto K, Hori N, Hoshi N, Yamamoto T, Onozuka M. Molarless condition suppresses proliferation but not differentiation rates into neurons in the rat dentate gyrus. Neurosci Lett 2010; 469: 44-48.

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