development of the mandible (Beecher and Corruccini, 1981; Kiliaridis et al., 1999; Luca et al., 2003; Maki et al., 2003). Our previous studies in mice fed a soft diet indicated that mastication after weaning influences gene expression in mandibular condylar cartilage (Watahiki et al., 2004) and produces major differences in mandibular morphology (Enomoto et al., 2010). Mastication has recently been reported to influence not only craniofacial development, but also brain function. For example, mastication has been reported to be involved in improving memory and learning in adults (Wilkinson et al., 2002). In addition, studies using functional magnetic resonance imaging (fMRI) suggested that mastication may simultaneously activate the prefrontal cortex and parietal cortex and may contribute to higher cognitive function (Takada and Miyamoto, 2004; Hirano, 2008).

Furthermore, human studies showed that reduced bite force and the number of teeth lost are related to the onset and progression of dementia and Alzheimer's disease (Okimoto et al., 1991; Shigetomi et al., 1998; Okamoto et al., 2010).

In an animal study in which senescence-accelerated model SAMP8 mice were fed a long-term soft diet, significant declines were observed in behavioral tests of memory and learning ability (Yamamoto and Hirayama, 2001). Yamazaki et al. (2008) also reported that tooth-extracted rats showed significantly reduced performance on behavioral tests that evaluate spatial memory (Yamazaki et al., 2008). In addition, it has been reported that rats from which the molar teeth have been removed show accumulation of amyloid-β in the hippocampus, which is related to decreased hippocampal neurogenesis (Ekuni et al., 2011a,b).

The studies mentioned above clearly demonstrated that a long-term soft diet and tooth loss influence brain functions related to memory and learning, and may also be related to the onset of dementia and Alzheimer's disease.

Previous work has focused primarily on the relationship between mastication and brain function in senescence. However, there have been no reports regarding the relationship between mastication and brain function during growth and development, particularly the relationship between masticatory alterations and mental disorders.

Mental disorders, including schizophrenia, are multifactorial diseases that result from the interaction of complex genetic and environmental factors. All periods of CNS formation, including the fetal stage, the perinatal period, and infancy, are crucial periods in the onset of schizophrenia (Weinberger, 1987; Lillrank et al., 1995). Epidemiological studies have revealed many environmental factors that exacerbate the incidence rate of schizophrenia (Lewis et al., 1992; Mortensen et al., 1999; Cannon et al., 2002). Many aspects of the relationship between environmental factors and the prodromal stage lasting from infancy to adolescence remain unclear. Therefore, many studies using animal models have been conducted to examine the relationships between environmental factors and onset of mental disorders (Deminière et al., 1992; Eyles et al., 2003).

As behaviors are assumed to express internal mental activity, behavioral assessment is likely to be an effective means of studying mental disorders in animal models. Prepulse inhibition (PPI) is a behavioral test procedure that is widely used to assess sensorimotor gating (a type of information processing). PPI is typically observed in healthy subjects, while a decrease in PPI reflects deficits in sensorimotor gating that may indicate impaired information processing in schizophrenia and other mental disorders (Braff et al., 1978; Braff, 2001; Ludgewig, 2003; Geyer, 2006). In addition, decreased PPI has often been reported in behavioral analyses of genetically modified animals related to schizophrenia and other mental disorders (Swerdlow et al., 1994; Lipska and Weinberger, 2000; Powell et al., 2009).

Along with the behavioral measurements, anatomical and molecular analyses of the nervous system are also essential to understand mental disorders. Reduced hippocampal volume and cell count are frequently observed in MRI and histological analyses of the brains of patients with mental disorders (schizophrenia, bipolar disorder, depression, and post-traumatic stress disorder [PTSD]; Saddath, 1990; Bremmner, 2008). Hippocampal neurogenesis has also been reported to occur in adulthood in both humans and animals (Altman and Das, 1965; Cameron et al., 1993; Eriksson et al., 1998; Gage, 2000). Strong relationships between hippocampal neurogenesis and vulnerability to mental disorders have been reported in animal models (Weinberger, 1987; Lillrank et al., 1995; Harrison, 2004; Watanabe et al., 2007; Maekawa et al., 2009). In addition, decreased levels of neurotrophin brain-derived neurotrophic factor (BDNF) were reported to be related to decreased hippocampal neurogenesis and may be involved in dementia (Durany et al., 2001; Szeszko et al., 2005; Tan et al., 2005). Other studies have also suggested that long-term soft-diet feeding may be related to decreased hippocampal neurogenesis (Mitome et al., 2004; Tsutsui et al., 2007). Furthermore, mastication ability is related to BDNF levels in adult mice or senescence-accelerated mice fed a soft diet (Yamamoto and Hirayama, 2001; Aoki et al., 2005; Yamamoto et al., 2008; Yamazaki et al., 2008).

We focused on the observation that the period of masticatory acquisition coincides with that of brain development related to the onset of mental disorders. We hypothesized that masticatory alterations after weaning may affect emotional development and potentially increase vulnerability to mental disorders. To verify this hypothesis, we examined the relationships between mastication after weaning and mental disorders in mice fed either a hard or soft diet.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL6/J mice (CLEA Japan Inc., Tokyo, Japan) were used in this study, and the experiment was started

with animals at the age of 21 days. All animal experiments were approved by the Animal Experiment Committee of the RIKEN Brain Science Institute (approval No. H22-ER082), and the mice were maintained at the Experimental Animal Facility of the institute. The mice were housed three to a cage after weaning. The mice were kept under a 12-h light/dark cycle, with lights off from 20:00 to 08:00. Food and water were freely available except during experiments. The hard diet was comprised of ordinary laboratory chow for mice in typical hard pellet form (Nosan Corp., Kanagawa, Japan), while the soft diet consisted of the same nutritional components mixed with water in standardized proportions (Enomoto et al., 2010).

Behavioral tests

For behavioral analysis, at the time of weaning (21 days of age), mice were randomly divided into the following three groups (n = 7 or 10 per group): (1) male mice fed a hard diet (HDM), (2) male mice fed a soft diet (SDM), and (3) male mice changed to a hard diet from a soft diet at the beginning of the behavioral analysis (SHDM; Fig. 1). Mice were housed individually in cages from one week before commencement of behavioral analyses. The behavioral tests were started when the mice were 7 weeks old and completed before they reached the age of 14 weeks.

Two sets of behavioral tests were conducted independently. Each test was performed with a 1-day to 1-week interval. In the first batch (n = 7 per group), the following basic tests were conducted: home cage activity test (1st to 2nd week), open field test, light/dark box test, and elevated plus maze test (3rd week), Y-maze test, and auditory startle response and PPI test (4th week), Morris water maze test (5th week), rotarod test (6th week), and classical fear conditioning (7th week). Replication of some basic tests in batch 1 and two optional tests were conducted in the second batch (n = 10 per group). We replicated the basic behavioral tests and some optional tasks to assess the emotionality of mice, because those in the SDM group showed abnormal open field behavior and PPI in the first batch: home cage activity test (1st to 2nd week), open field test (2nd week), auditory startle response and PPI test (3rd week), social interaction test (4th week), and tail suspension test (5th week). The detailed procedures were described previously (Sakatani et al., 2009; Katayama et al., 2010; Takashima et al., 2011; Hattori et al., 2012).

These tests were performed in sequence to keep the general principle of the arrangement to avoid a more stressful task before a less stressful task and to minimize carryover effects.

Dimensions of the experimental apparatus are represented as (width × length × height). After each trial (except the auditory startle response test and the water maze test), the apparatus was wiped and cleaned with 80% alcohol and a damp towel. In the auditory startle response test, holding chambers were washed with tap water, wiped with a paper towel, and dried after each trial. All experimental protocols were approved by the RIKEN Institutional Animal Care and Use Committee.

Home cage activity (1st to 2nd week)

Spontaneous activity of each mouse in its home cage was measured using a 24-channel activity monitoring system (O'Hara & Co., Tokyo, Japan). Cages were individually set into compartments made of stainless steel in a negative breeding rack (JCL, Tokyo, Japan).

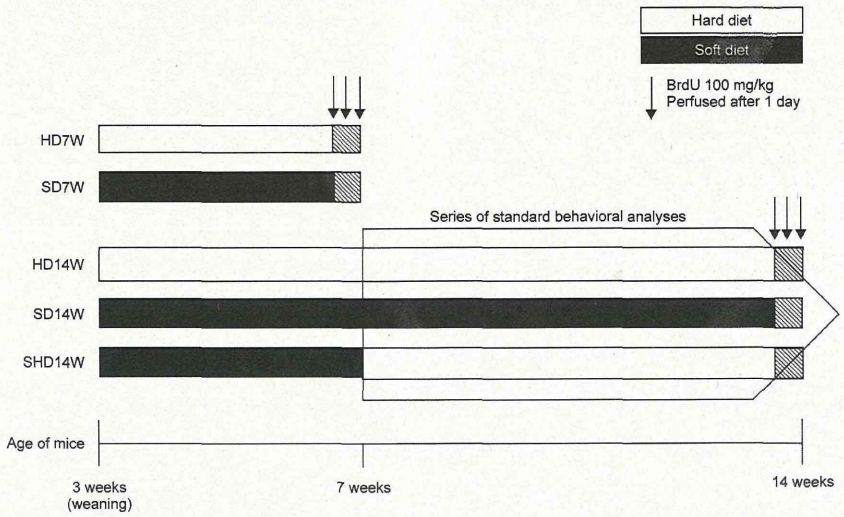

Fig. 1. Time schedule of the experiment. At the time of weaning (21 days of age), male mice were randomly divided into the following three groups (n = 7 or 10 per group): (1) male mice fed a hard diet (HDM); (2) male mice fed a soft diet (SDM); (3) male mice changed to a hard diet from a soft diet at 7 weeks of age (SHDM). Behavioral tests began when mice were 7 weeks old. In addition, at the time of weaning, male mice were randomly divided into the following five groups: (4) male mice fed a hard diet for 4 weeks (HD7W); (5) male mice fed a soft diet for 4 weeks (SD7W); (6) male mice fed a hard diet for 11 weeks (HD14W); (7) male mice changed to a hard diet at 7 weeks of age from a soft diet for 4 weeks (SHD14W); and (8) male mice fed a soft diet for 11 weeks (SD14W).

A piezoelectric sensor was equipped on the ceiling of each compartment to detect the movements of mice. Activity counts represent the number of active time bins (approximately 0.20–0.25 s each) in which spontaneous activities, including locomotor activity, rearing, and other voluntary stereotypic movements, were detected. Home cage activity was measured for 6 consecutive days during which bedding materials were not changed.

Open field test (2nd week)

The open field test apparatus was placed in a small soundproof room (185 × 185 × 225 cm). The apparatus consisted of four white plastic boxes (50 × 50 × 40 cm), two electric fans for ventilation and background noise (35 dB), and a white LED light source (70 lux at the center of the field), which served as the sole source of illumination during the experiment. For each box, a charge-coupled device camera (CCD) was attached to the ceiling to monitor the mice. Mice were individually introduced into the center of the arena and were allowed to move freely for 15 min. Distance traveled (cm) and % duration remaining at the center area of the field (defined as the central 36% of the field) were adopted as the test indices, and they were collected for each 1-min time bin.

Auditory startle response (3rd week)

Each mouse was placed into a small cage (30 or 35 mm in diameter, 12 cm in length) that was set on a sensor block within a soundproof chamber (60 × 50 × 67 cm, Mouse startle; O'Hara & Co.). A dim light was affixed to the ceiling of the soundproof chamber (10 lux at the center of the sensor block), and 65-dB white noise was presented as background noise.

In the acoustic startle response (ASR) test, each mouse was acclimatized to the experimental conditions for 5 min, and the experimental session began immediately after this acclimation period. In the first session, a 120-dB startle stimulus (40 ms) was presented to the mouse 10 times at random intertrial intervals (10–20 s). In the second session, the startle response to stimuli at various intensities was assessed. Five repetitions of 70–120-dB white noise stimuli (70, 75, 80, 85, 90, 95, 100, 110, and 120 dB) were presented for 40 ms in a quasi-random order and random intertrial intervals (10–20 s). In the PPI session, the mouse experienced five types of trial: (1) no stimulus, (2) startle stimulus only (120 dB, 40 ms), (3) 70-dB prepulse (20 ms, lead time 100 ms) followed by a 120-dB pulse, (4) 75-dB prepulse (20 ms, lead time 100 ms) followed by a 120-dB pulse, and (5) 80-dB prepulse (20 ms, lead time 100 ms) followed by a 120-dB pulse. Each trial was repeated 10 times in quasi-random order at random intertrial intervals (10–20 s). In the final session, a 120-dB startle stimulus (40 ms) was again presented to the mouse 10 times at random intertrial intervals (10–20 s). The total duration of an ASR test was about 35–40 min.

Tail suspension test (5th week)

As C57BL/6 mice have a tendency to climb their tail and/or the wire from which they are hung in the tail-suspension test, we used a metal blade (about 15 mm in width and 1 mm in thickness) in place of a wire to hang the mice to prevent their climbing behavior. Mice were individually taped by the tail to a metal blade held horizontally 45 cm above the bench, and the trial was started. Typically, mice would initially struggle to climb up the blade, but soon they showed increased bouts of immobility. A trained observer who was blind to the experimental conditions measured the duration of immobility over a 5-min period. Statistical analysis was carried out using the immobility scores.

5-Bromo-2′-deoxyuridine labeling analysis. Bromodeoxyuridine (Brd-U) analysis was conducted using another set of mice. At the time of weaning (21 days of age), mice were randomly divided into the following five groups: (1) male mice fed a hard diet for 4 weeks (HD7W, $n = 8$), (2) male mice fed a soft diet for 4 weeks (SD7W, $n = 10$), (3) male mice fed a hard diet for 11 weeks (HD14W, $n = 10$), (4) male mice changed to a hard diet at 7 weeks of age from a soft diet for 4 weeks (SHD14W, $n = 9$), and (5) male mice fed a soft diet for 11 weeks (SD14W, $n = 7$; Fig. 1).

Mice in the HD7W, SD7W, HD14W, SD14W, and SHD14W groups received three intraperitoneal injections of 5-bromo-2′-deoxyuridine (BrdU; Sigma, St. Louis, MO) at 100 mg/kg body weight, spaced 2 h apart and were sacrificed one day after BrdU injection.

After deeply anesthetizing the mice with 100 mg/kg sodium pentobarbital (Kyoritsu Pharmaceuticals, Tokyo, Japan), the mice were transcardially perfused with 4% paraformaldehyde containing 0.5% picric acid in 0.1 M phosphate-buffered saline (PBS). The brains were then removed and immersion-fixed for 24 h at 4 °C in the same fixative. After washing in PBS, the brains were successively equilibrated in 5%, 10%, and 20% sucrose in PBS, embedded in Tissue-Tek optimal cutting temperature (OCT) compound (Sakura, Tokyo, Japan), and frozen on dry ice. The frozen brains were coronally sliced into sections 14-μm thick using a cryostat (CM-3000; Leica, Nussloch, Germany) and mounted on MAS-coated glass slides (SUPERFROST; Matsunami, Osaka, Japan). Next, the 14-μm frozen sections were boiled in 0.01 M citric acid, incubated in 2 N HCl for 10 min at 37 °C, and washed in 0.01 M PBS.

BrdU-positive cells were identified throughout the dentate gyrus (DG) in its rostrocaudal extension. Of each set of 12 sections, three consecutive sections (14 μm each) were used for counting, and the total number of positive cells was determined by multiplying the value by 4. Roughly, 20 sets of counts were performed per organism (Maekawa et al., 2005).

Real-time semiquantitative polymerase chain reaction

BDNF, TrkB, and Akt1 expression levels in the hippocampus and frontal cortex were compared by

real-time polymerase chain reaction (RT-PCR) analysis. This analysis was conducted using another set of mice divided into the following two groups (n = 5 per group): HD14W and SD14W.

The mice were decapitated, and two brain regions (the frontal cortex and hippocampus) were quickly dissected on ice. RNA was extracted from the tissue exposed to RNA stabilizing treatment (RNAlater; Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA was extracted with an RNeasy Universal Mini Kit (Qiagen) according to the manufacturer's instructions. After RNA extraction, a reverse transcriptase (RT) kit (Omniscript Reverse Transcriptase Kit; Qiagen) was used to make cDNA. The mixture was heated (2400 GeneAmp PCR System; PerkinElmer Japan, Tokyo, Japan) for 60 min at 37 °C.

Real-time semiquantitative polymerase chain reaction (PCR) was performed on the hippocampus and frontal cortex using an ABI prism 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Real-time PCR was performed for 50 cycles of 95 °C for 15 s and 60 °C for 1 min, followed by 50 °C for 2 min and 95 °C for 10 min using *BDNF* primers (Mm04230667_s1; Applied Biosystems), *TrkB* primers (Mm00435422_m1; Applied Biosystems), and *Akt1* primers (Mm01331626_m1; Applied Biosystems). The *BDNF*, *TrkB*, and *Akt1* gene expression levels were determined after normalization to those of the housekeeping gene, *GAPDH* (Mm99999915_ml; Applied Biosystems). Three repeatedly measured independent samples were examined for each genotype, and their average values were compared.

Statistical analysis

Statistical analyses were conducted using SPSS ver. 16.0 and 21.0 (IBM Japan Inc., Tokyo, Japan). Parametric data were analyzed by the Student's t test, and nonparametric data were analyzed by the Mann–Whitney U-test. Reported P-values refer to the Student's t test unless otherwise indicated. The effects of factors were analyzed by a one-way analysis of variance (ANOVA), two-way ANOVA with *post hoc* tests, and generalized linear model. All values are expressed as the means ± SEM. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

RESULTS

Body weight

Mice were weighed at the ages of 7 and 14 weeks. At the time of weaning (21 days of age), the mice were randomly divided into the following five groups: (1) male mice fed a hard diet for 4 weeks (HD7W), (2) male mice fed a soft diet for 4 weeks (SD7W), (3) male mice fed a hard diet for 11 weeks (HD14W), (4) male mice fed a soft diet for 11 weeks (SD14W), and (5) male mice changed to a hard diet at 7 weeks of age after receiving a soft diet for 4 weeks (SHD14W). There were significant differences in body weight between the HD7W and SD7W groups (P = 0.029). There were significant differences among the HD14W, SHD14W, and SD14W groups by a one-way ANOVA (P = 0.001). Therefore, *post hoc* analysis with Tukey's test for all pairwise comparisons was performed, and significant differences were detected between the HD14W and SD14W groups (P = 0.001), and between the SHD14W and SD14W groups (P = 0.011). However, there were no significant differences between the HD14W and SHD14W groups (P = 0.722, n.s.; Table 1).

Behavioral tests

The light/dark box test, elevated plus maze test, social interaction test, Y-maze test, Morris water maze test, rotarod test, and classical fear conditioning test did not show any abnormalities in the SDM and SHDM groups as compared to the HDM group (Table 2).

Home cage activity test. The levels of activity in the daily environment were measured. As the room illumination was controlled with a 12/12-h light/dark cycle, the animals' activity rhythm was modulated according to this test. SDM showed significantly lower activity levels per day than HDM on all except the sixth day (Fig. 2A). SHDM also exhibited lower activity levels than HDM during the first 2 days (*post hoc* analysis after ANOVA; Fig. 2A). In addition, during the 6 days of continuous monitoring, the activity level was significantly lower in SDM than in HDM (two-way ANOVA with repeated measurements, $F(1, 36) = 4.230$, $P = 0.0116$; Fig. 2B).

Open field test. In the open field test, the total distance of locomotion in 15 min was significantly greater in SDM than HDM (HDM vs. SDM: 6571.1 ± 259.1 cm, SDM 8059.7 ± 454.1 cm [mean ± SE], $P < 0.05$, Mann–Whitney U-test; Fig. 3A). There were no significant differences between HDM and SHDM (P = 0.154, Mann–Whitney U-test) or SHDM and SDM (P = 0.782, Mann–Whitney U-test). On the other hand, the total time spent in the center of the arena was not significantly different between the three groups ($F(2, 19) = 2.166$, P = 0.145, n.s.; Fig. 3B). These results indicated that

Table 1. Mean body weight

	HD	SHD	SD	HD versus SD	HD versus SHD	SHD versus SD
7w	21.29 ± 0.42 (n = 10)	/	22.51 ± 0.31 (n = 10)	*	/	/
14w	25.45 ± 0.47 (n = 10)	25.84 ± 0.27 (n = 10)	27.43 ± 0.30 (n = 10)	**	–	*

There were significant differences in body weight between the SD7W and HD7W groups (P = 0.029), but not between the HD14W and SHD14W groups (P = 0.722, not significant). However, SD14W mice were significantly heavier than HD14W or SHD14W mice (P = 0.001 and P = 0.011, respectively by Tukey's multiple comparison as *post hoc* test).

Table 2. Summary of behavioral results (mean ± SEM)

Test	Age of weeks	Batch (*n*)	HDM	SHDM	SDM
Homecage activity	7w (1st to 2nd week)	2 (*n* = 10)			
Whole day			846.163 ± 82.528	701.697 ± 78.852*	526.011 ± 41.330***
Open field	8w (2nd week)	1 (*n* = 7)			
Total distance (cm)			6571.1 ± 259.131	7689.757 ± 417.470	8059.671 ± 454.113*
Moving speed			10.183 ± 0.280	11.186 ± 0.398	12.043 ± 0.327**
Time in center area (%)			217.333 ± 22.215	206.571 ± 16.161	172.214 ± 8.877
Light–dark box	9w (3rd week)	1 (*n* = 7)			
Total distance (cm)			2509.75 ± 130.158	2893.5 ± 174.303	2655.914 ± 144.064
Number of transitions			53.5 ± 3.490	64.571 ± 5.140	57.286 ± 5.862
Latency to transition to dark box			21.333 ± 2.917	25.571 ± 6.715	36.286 ± 15.731
Distance traveled in light box (%)			43.444 ± 1.610	44.995 ± 0.161	43.536 ± 1.694
Time spent in light box (%)			45.986 ± 2.048	49.023 ± 0.861	44.810 ± 2.353
Elevated plus maze	9w (3rd week)	1 (*n* = 7)			
Total distance (cm)			852.583 ± 116.939	1020.014 ± 95.691	1107.3 ± 140.844
Entry number (open arm)			5.833 ± 2.330	4.429 ± 1.587	7.714 ± 2.201
Time in open arm (%)			19.728 ± 10.862	11.357 ± 4.932	28.238 ± 6.975
Number of entry to open arm (%)			19.753 ± 8.724	17.504 ± 4.730	28.439 ± 5.813
Startle response	9w (3rd week)	2 (*n* = 10)			
startle response			1.352 ± 0.282	1.788 ± 0.226	2.078 ± 0.170
Initial/final			1.41 ± 0.131/ 0.08 ± 0.011	2.048 ± 0.289/ 0.07 ± 0.004	2.164 ± 0.182/ 0.074 ± 0.008
Prepulse inhibition for 80 dB (%)			48.358 ± 6.116	31.994 ± 4.508	18.445 ± 5.513*
Social interaction	10w (4th week)	2 (*n* = 10)			
Number of contacts			1.627 ± 0.051	1.585 ± 0.025	1.605 ± 0.026
Y maze	10w (4th week)	1 (*n* = 7)			
Entry number			19.167 ± 1.759	20.714 ± 0.892	21.000 ± 2.012
Tail suspension	11w (5th week)	2(*n* = 10)			
Immobility time (%)			56.524 ± 5.989	47.259 ± 6.724	32.339 ± 4.498*
Morris water maze	11w (5th week)	1 (*n* = 7)			
Total distance (cm)			1126.867 ± 49.996	1161.286 ± 92.054	1275.043 ± 44.489
Movement time (s)			51.50 ± 1.389	49.0 ± 3.429	55.429 ± 1.259
Latency to platform (s)			26.464 ± 5.152	27.013 ± 3.396	27.612 ± 4.655
Rota-rod test	12w (6th week)	1 (*n* = 7)			
Rotation			119.573 ± 16.547	143.741 ± 10.622	145.491 ± 8.858
Fear conditioning	13w (7th week)	1 (*n* = 7)			
Context test – immobility (%)			48.053 ± 9.104	47.943 ± 6.582	55.0 ± 7.396
Cued test – immobility (%)			36.80 ± 9.276	54.464 ± 9.075	49.882 ± 10.404

* $P < 0.05$ compared with HDM.
** $P < 0.01$ compared with HDM.
*** $P < 0.001$ compared with HDM.

SDM showed more locomotor activity and less anxiety response in the new environment than HDM, suggesting that changes in mastication may influence adaptation to new environments.

Auditory startle response. Although SDM mice showed slightly higher startle responses at 110- and 120-dB white noise, there were no significant differences among HDM, SHDM, and SDM groups (Fig. 4A). Similarly, there was no significant difference between 70-dB and 75-dB prepulses; however, the suppression rate of SDM was significantly decreased in the 80-dB prepulse trials compared to HDM ($P = 0.518$ for 70 dB, $P = 0.079$ for 75 dB, $P = 0.016$ for 80 dB; Fig. 4B). These observations suggested slight impairment of sensorimotor gating/information processing in SDM.

Tail suspension test. The immobility time was significantly shorter in SDM than HDM ($F(1, 36) = 2.3490$, $P = 0.03478$, Tukey's test; Fig. 5). The duration of immobility in this test may reflect the depressive state of the mice (Steru et al., 1985; Cryan et al., 2005). Therefore, SDM was not in a depressive state at least in this experiment.

Quantitative analysis of BrdU-positive cells in the DG

Hippocampal neural progenitor proliferation was evaluated using BrdU. At the age of 7 weeks, there was no significant difference between HD7W and SD7W

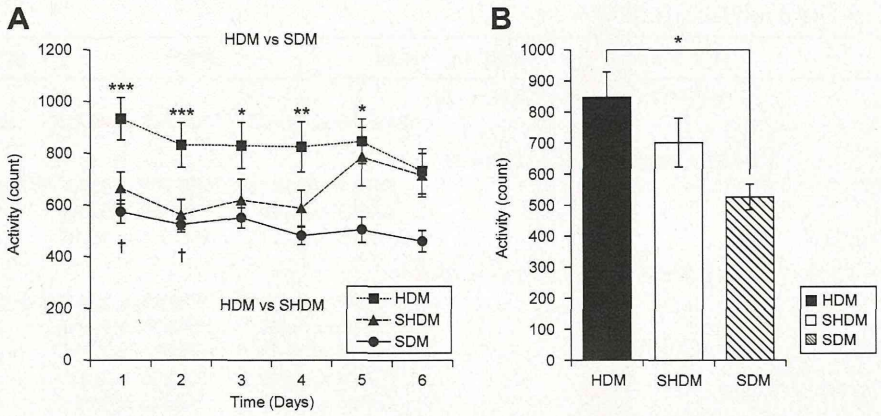

Fig. 2. Home cage activity test. Group comparison of home cage activity in the whole day. SDM had significantly lower activity levels per day than HDM on all except the 6th day (A). SHDM also exhibited lower activity levels than HDM during the first 2 days (A). In addition, during the 6 days of continuous monitoring, the activity level was significantly lower in SDM than HDM (B). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{\dagger}P < 0.05$.

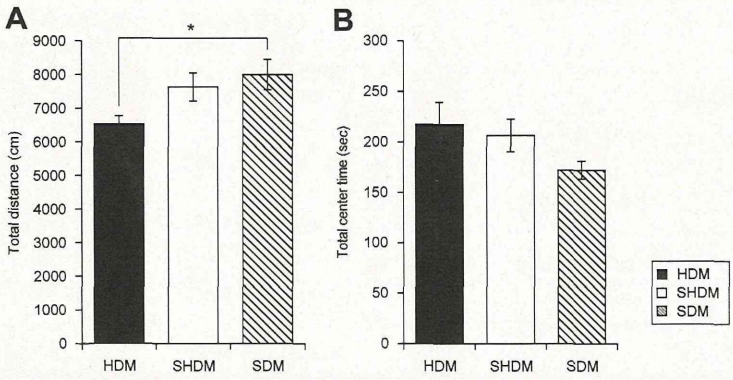

Fig. 3. Open field test. In the open field test, the total distance of locomotion in 15 min was significantly longer in SDM than HDM (HDM vs. SDM: 6571.1 ± 259.1 cm, SDM 8059.7 ± 454.1 cm [mean ± SE], $P < 0.05$, A). The total time spent in the center of the arena showed no significant difference between SDM and HDM ($F(2, 19) = 2.166$, $P = 0.145$, n.s.; B).

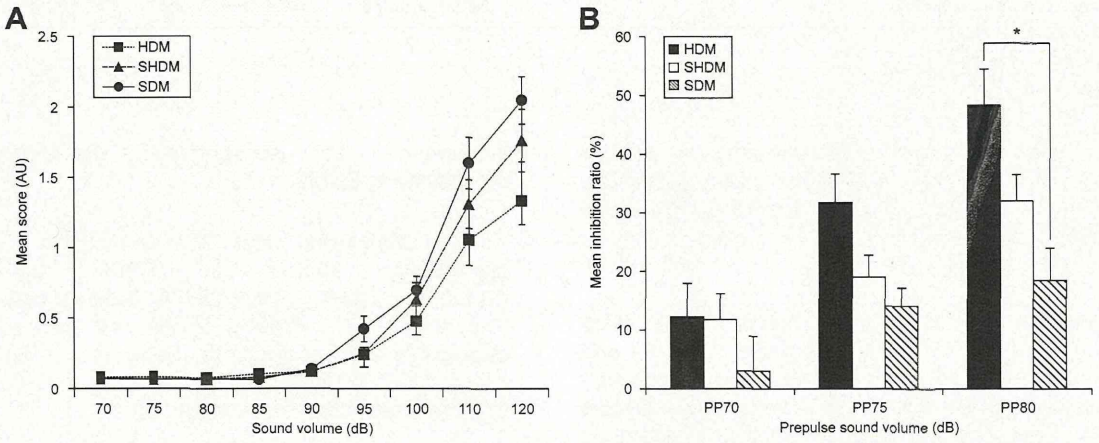

Fig. 4. Auditory Startle response. Auditory startle response. No significant differences were observed in the startle responses induced by auditory signals among HDM, SHDM, and SDM groups (A). Prepulse inhibition. PPI showed a clear difference between HDM and SDM (B). Although no significant difference was observed with 70-dB and 75-dB prepulse tones, the suppression rate was impaired in SDM for 80-dB prepulse tones compared to HDM ($P = 0.518$ for 70 dB, $P = 0.079$ for 75 dB, $P = 0.016$ for 80 dB; B).

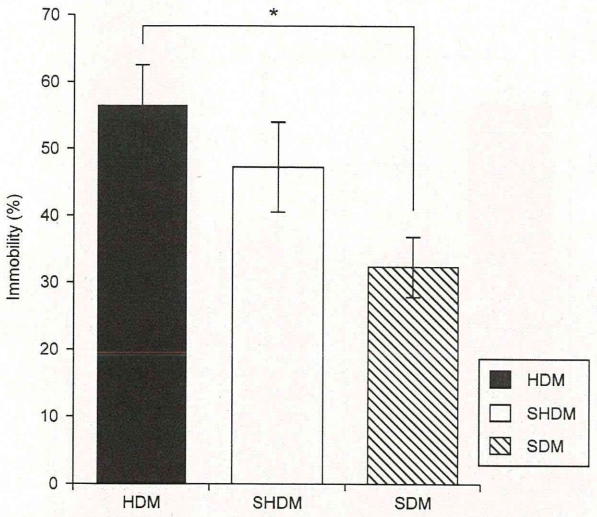

Fig. 5. Tail suspension test. The immobility time was significantly shorter in SDM than HDM ($F(1, 36) = 2.3490$, $P = 0.03478$, Tukey's test).

($P = 0.119$, n.s.). At the age of 14 weeks, however, the level of hippocampal neural progenitor proliferation was significantly lower in SD14W than in HD14W ($P < 0.001$, Kruskal–Wallis test). The number of BrdU-positive cells in the DG was decreased by 32.1% in SD14W compared to HD14W. In mice initially given the soft diet and later changed to a hard diet (SHD14W), the number of BrdU-positive cells was decreased by 16.4% compared to HD14W, but this decrease was not significant ($P > 0.05$, Kruskal–Wallis test; Fig. 6A).

Real-time semiquantitative polymerase chain reaction

Real-time PCR analysis was used to assess changes in the *BDNF*, *TrkB*, and *Akt1* gene expression patterns in the hippocampus and frontal cortex. The non-paired *t* test with Welch's approximation was used for comparison of gene expression between HD14W and SD14W groups. Hippocampal *BDNF* gene expression in SD14W was significantly decreased compared with that in HD14W ($P = 0.009$), but no significant difference was observed in the frontal cortex ($P = 0.535$, n.s.; Fig. 7A). There were no significant differences in *TrkB* expression between HD14W and SD14W groups in the hippocampus ($P = 0.105$, n.s.) or the frontal cortex ($P = 0.095$, n.s.; Fig. 7B). The level of *Akt1* gene expression in SD14W was significantly decreased compared to that in HD14W in the hippocampus ($P = 0.001$) but not in the frontal cortex ($P = 0.362$, n.s.; Fig. 7C).

DISCUSSION

The postnatal period, particularly the first several weeks after weaning, is an extremely important period for acquisition of proper mastication ability, which is related to various processes, such as craniofacial development, CNS maturation, peripheral sensory nerve input, and motor learning (Iriki et al., 1988; Morris, 1989; Gisel, 1991; Westneat and Hall, 1992; Huang et al., 1994; Fucile et al., 2005). Furthermore, brain function development and structural changes in infancy, which coincide with the period of mastication acquisition, are strongly involved in vulnerability to mental disorders (Suzuki et al., 2005; Brewer et al., 2006).

Therefore, we focused on the fact that the masticatory acquisition period after weaning coincides with the period of brain development related to the onset of mental disorders. We hypothesized that masticatory alterations after weaning may affect emotional development and vulnerability to mental disorders. To test our hypothesis, we examined the relationships between mastication after weaning and behavioral changes related to mental disorders in mice that were fed either hard or soft diets. We investigated these relationships with a series of standard behavioral tests, as well as analyses of hippocampal neurogenesis and *BDNF*, *TrkB*, and *Akt1* gene expression, which are related to mastication.

Body weight was compared among groups at 7 and 14 weeks of age to investigate the influence of changes in mastication ability after weaning on body weight. Body weight was significantly higher in SD7W than HD7W, and in SD14W compared to HD14W and SHD14W. However, there were no significant differences in body weight between HD14W and SHD14W (Table 1). These findings were consistent with previous reports indicating that mice fed a powder diet developed obesity, and body weight was significantly altered regardless of nutrition (Desmarchelier et al., 2013). Furthermore, a previous study indicated that rats fed a soft diet after weaning had lower body temperature and higher body weight than those fed a hard diet (Oka et al., 2003). In the present study, SDM showed a significant decrease in home cage activity (Fig. 2A, B). These results suggested that the increased body weight of SD7W relative to HD7W, and SD14W relative to HD14W and SHD14W may have been caused by low activity and decreased basal metabolism due to long-term soft-diet feeding.

In the behavioral tests, we first conducted a home cage activity test to analyze daily activity. This study indicated significantly lower activity in SDM than HDM (Fig. 2A, B). SHDM also showed significantly less activity than HDM for the first 2 days (Fig. 2A). These results suggest that a habitual soft diet after weaning may affect daily activity levels of mice. However, there have been no detailed reports of circadian rhythms or daily activity in mice fed a soft diet. This study suggested that a habitual soft diet after weaning may be related to decreased daily activity and increased body weight. Additional detailed studies are necessary to investigate the effects of soft-diet feeding and basal metabolism during growth and development on the whole body.

In the open field test, the total distance of locomotion was significantly higher for SDM than HDM (Fig. 3A). These results suggest that a habitual soft diet after weaning may affect responsiveness to novel

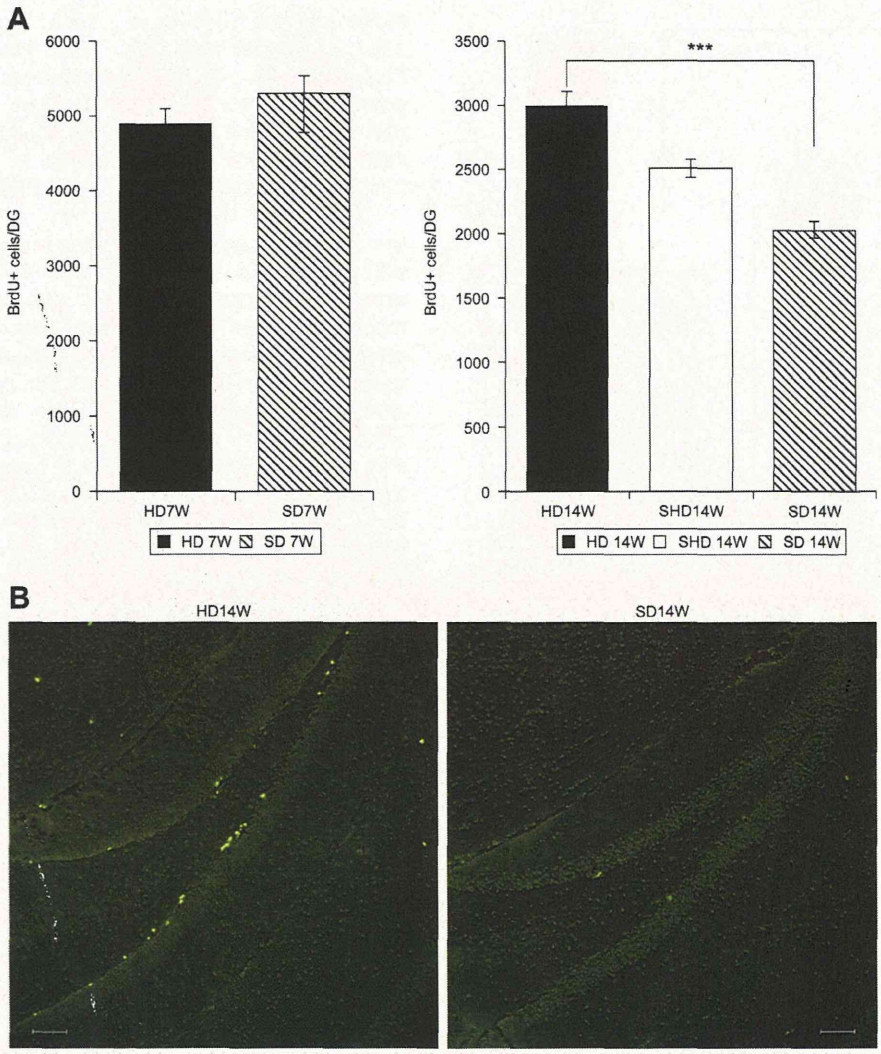

Fig. 6. Cell proliferation analysis. The results of quantitative analysis did not differ between SD7W and HD7W ($P = 0.119$, n.s.). However, proliferation was significantly reduced in SD14W compared to HD14W ($P < 0.001$, Kruskal–Wallis test). The number of BrdU-positive cells in the DG was decreased by 32.1% in SD14W compared to HD14W. In SHD14W, the number was decreased by 16.4% compared to HD14W, but this decrease was not significant ($P > 0.05$, Kruskal–Wallis test; A). BrdU immunoreactivity in DG at 14 weeks of age. BrdU-positive cells were dominantly distributed in the subgranular zone at the border between the granule cell layer (GCL) and the hilus. There were fewer BrdU-positive cells in SD14W than HD14W. Scale bar = 50 μm (B).

environments, and SDM may have more difficulty in adapting to novel environments than HDM.

A light/dark box test and elevated plus maze test were used to evaluate anxiety behaviors. These tests indicated no significant differences between HDM, SHDM, and SDM (Table 2). In addition, there were no significant differences between the three groups in total time spent in the central area in the open field test, which was also used to evaluate anxiety behaviors (Fig. 3B). Furthermore, SDM showed a significant decrease in immobility in the tail suspension test (Fig. 5). Therefore, differences in mastication after weaning may influence neither anxiety behaviors nor depressiveness, at least under our experimental conditions.

PPI was significantly lower in SDM than HDM (Fig. 4B). PPI is a phenomenon observed in both humans and animals in which a weak stimulus is added to suppress the startle response evoked by a sudden auditory stimulus (Hoffman, 1968). Decreased PPI is believed to reflect a sensorimotor gating disorder, indicating impairment of the ability to exclude unnecessary surrounding sensory stimuli. Braff et al. reported that PPI is either absent or weakened in schizophrenia patients (Braff et al., 1978; Braff, 2001). Impaired PPI has been observed in schizophrenic patients with no medication history and in individuals with a family history of schizophrenia who have not developed schizophrenia themselves (Ludgewig, 2003). Decreased PPI has also been observed in several mental disorders (schizophrenia, bipolar disorder, PTSD, attention deficit hyperactivity disorder) and serves as an indicator of impaired information processing in schizophrenia and other mental disorders (Geyer, 2006; Powell et al., 2009). Furthermore,

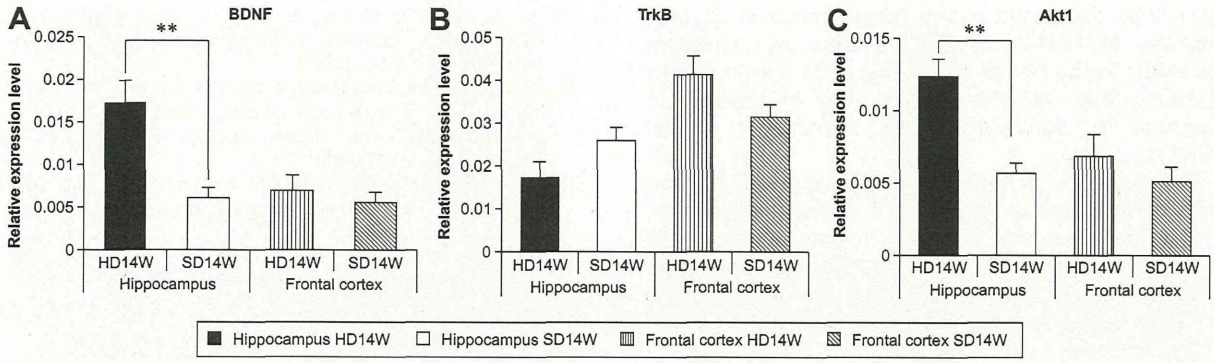

Fig. 7. Real-time PCR analysis was applied to assess the changes in *BDNF*, *TrkB*, and *Akt1* gene expression in the hippocampus and frontal cortex. The non-paired *t* test with Welch's approximation was adopted for comparison of *BDNF* gene expression between HD14W and SD14W. There was a significant difference between HD14W and SD14W groups in the hippocampus ($P = 0.009$) but not in the frontal cortex ($P = 0.535$, n.s.; A). There were no significant differences in *TrkB* expression between HD14W and SD14W groups in the hippocampus ($P = 0.105$, n.s.) or frontal cortex ($P = 0.095$, n.s.; B). *Akt1* gene expression was significantly decreased in SD14W compared to HD14W in the hippocampus ($P = 0.001$) but not in the frontal cortex ($P = 0.362$, n.s.; C).

decreased PPI has frequently been reported in behavioral analyses of genetically modified animals related to schizophrenia and other mental disorders (Swerdlow et al., 1994; Powell et al., 2009). Based on studies such as those cited above, decreased PPI is used as an indicator of vulnerability to mental disorders. The results of the present study suggest that a habitual soft diet after weaning may impair PPI and increase vulnerability to schizophrenia and other mental disorders.

In contrast, the Y-maze test, Morris water maze test, and the classical fear-conditioning test were used to evaluate memory and learning in this study. There were no statistically significant differences in the results of these tests among the three groups (Table 2). Previous studies showed that long-term soft-diet feeding and tooth extraction reduce performance on cognitive tasks (Yamamoto and Hirayama, 2001; Yamazaki et al., 2008; Ekuni et al., 2011a,b). However, in the present study, the soft-diet period was relatively short compared to previous studies, which may be one reason why the results reported here were inconsistent with those of previous studies. Our results suggest that a habitual soft diet influences some basic brain functions, such as responsiveness to novel environments and sensory information processing, but not cognitive function.

As soft-diet feeding caused some behavioral abnormalities, we evaluated proliferation of hippocampal neural progenitors. No statistically significant differences were observed between HD7W and SD7W in the evaluation of hippocampal cell proliferation. However, SD14W showed significantly reduced proliferation compared to HD14W (Fig. 6A, B). In previous animal experiments, long-term (6–12 months) feeding with soft diet or powdered diet resulted in decreased hippocampal neuron proliferation (Mitome et al., 2004; Tsutsui et al., 2007). In the present study, a similar decrease was observed in hippocampal cell proliferation in mice with a shorter period of soft-diet feeding (11 weeks).Weinberger et al. reported that impaired hippocampal neurogenesis in the perinatal period may increase vulnerability to mental disorders in adulthood (Weinberger, 1987; Lillrank et al., 1995). Decreased hippocampal neurogenesis and decreased PPI have also been reported in genetically modified mice related to schizophrenia (Weinberger, 1987; Lillrank et al., 1995; Harrison, 2004; Watanabe et al., 2007; Maekawa et al., 2009). In the present study, we demonstrated that a habitual soft diet after weaning significantly decreased hippocampal neural progenitor proliferation in a shorter period than in previous studies. This may influence hippocampal neurogenesis, and may be one of the causes of abnormal activity and impaired PPI.

Furthermore, BDNF, a member of the neurotrophin family, is related to the survival and maintenance of neurons and the plasticity of neural circuits (Segal and Greenberg, 1996; Huang and Reichardt, 2003). Decreased BDNF expression in the hippocampus and decreased hippocampal volume have been reported in schizophrenic patients (Durany et al., 2001; Szeszko et al., 2005; Tan et al., 2005). Okayasu et al. (2004) investigated the relationship between BDNF expression and mastication by evaluating mandibular movement and mastication muscle activity in BDNF-deficient mice. BDNF was suggested to be involved in masticatory movement control. In addition, decreased BDNF expression has been reported in mice that were fed a soft diet (Yamamoto et al., 2008; Yamazaki et al., 2008). Similar to previous studies, our results also indicated significantly decreased *BDNF* expression in the hippocampus in SD14W compared to HD14W (Fig. 7A). These results suggested that changes in mastication due to soft-diet feeding may result in decreased *BDNF* expression. Further, we showed that decreased *BDNF* expression in the hippocampus in SD14W may coincide with decreased hippocampal neurogenesis and behavioral abnormalities. We measured the expression level of the gene encoding TrkB, a specific receptor of BDNF. There were no significant differences in *TrkB* gene expression in the hippocampus or frontal cortex between HD14W and SD14W (Fig. 7B). In addition, the level of *Akt1* gene expression, which has been suggested to be related to schizophrenia in genetically modified mice and human studies (Emamian et al., 2004; Balu et al., 2012), was

significantly decreased in the hippocampus in SD14W compared to HD14W, but there were no significant differences in the frontal cortex (Fig. 7C). These results suggested that soft-diet feeding may be related to expression of candidate genes involved in mental disorders.

The acquisition period of mastication overlaps with the period of rapid brain development and neural maturation, and this period is related to the prodromal stage of mental disorder onset. Soft-diet feeding after weaning may cause histological and molecular changes in the hippocampus and result in altered performance of behaviors related to mental disorders.

CONCLUSION

Our observations suggested that soft-diet feeding may affect behavior after weaning, and alter brain function at the molecular level. Further detailed molecular and behavioral analyses may be helpful to clarify the underlying mechanisms of the vulnerability to mental disorders.

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