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Original Paper

Influence of soft diet feeding on development of masticatory function

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Abstract: Stomatognathic function is developed in steps from the latter half of infancy up to childhood. This period is thought to be critical for stomatognathic function acquisition. In this study, we investigated how the consistency of the daily diet after weaning affects the development of stomatognathic function in mice. C3H mice were divided into liquid- and solid-diet group. Three-dimensional jaw-movement tracking and jaw-muscle electromyography (EMG) were recorded simultaneously during chewing of pellet and bread at 11 weeks of age. As a result, we found that (1) The masseter activity was larger in the liquid-diet group than the solid-diet group. (2) Total cycle duration indicating chewing rhythm and gape size indicating burst pattern were significantly longer and larger during pellet chewing than bread chewing in the solid-diet group. On the other hand, there was no significant difference in both parameters in the liquid-diet group. These results suggest that greater EMG bursts are necessary, since the masticatory muscles are weaker in mice fed with liquid diet, and that the capacity to perceive the consistency of diet is reduced in these mice. The development of the mechanism to regulate chewing rhythm and pattern is considered to be impeded when raised on soft diet.

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Ⅰ. Introduction

The masticatory function is indisputably one of the essential functions for life conservation in mammals. Similar to other primitive functions such as respiration and locomotion, the rhythmical movements of mastication are controlled by specific groups of neurons in the central nervous system[1,2]. These groups of neurons, collectively called the central pattern generator (CPG) for mastication, are only capable of producing the most basic pattern of jaw movements that merely resemble mastication-like behavior. To produce the complicated and variable patterns of mastication observed in living animals, the CPG is dependent on inputs from peripheral afferent as well as from inputs for higher centers such as the cortical masticatory area in the motor control hierarchy[3]. Such inputs are responsible for initiating and refining the CPG output to continuously meet the requirements of normal masticatory function.

The masticatory function goes through a series of developmental steps early in life. After weaning, the development of the masticatory function is most likely a result of the growth and maturation of the peripheral and central nervous systems, as well as learning processes triggered by environmental demands. It has been suggested that experiences of chewing various kinds of food with different consistency contribute significantly to the development of the human masticatory function[4]. Nevertheless, the modern human diet consists more and more of thoroughly processed and cooked foods, which dramatically reduce the demand on the stomatognathic system. The consequences of such a change in alimentary habits are not fully understood. From research on experimental animals, it has been reported that the consistency of the daily diet may affect the craniofacial morphology [5~7], differentiation and development of muscle fibers of masticatory muscles[8,9], and synaptic formation in the hippocampus and parietal cortex[10]. Also in functional aspects, a previous study showed that the electromyographic (EMG) activity of jaw muscles was affected by dietary consistency[11]; however, so far there have been no studies that analyzed jaw-movements in animals raised on a diet different from the habitual. Therefore, in this study we used three-dimensional jaw-movement tracking and jaw-muscle EMG to analyze changes in masticatory function in mice fed liquid or solid diets after weaning, and the hypothesis that the daily diet of soft food affects the development of masticatory function was examined.

Ⅱ. Materials and Methods

The procedures described here are in agreement with international guidelines for animal welfare. All possible measures were taken to minimize animal suffering, and the number of animals used in this research was kept to the minimum necessary to produce reliable scientific data. The experimental protocol was reviewed and approved beforehand by the Nagasaki University Intramural Animal Care and Use Committee.

1. Experimental animals

C3H mice were divided into two groups, the liquid-diet group (n=7) and the solid-diet group (n=7). After weaning, mice in the liquid-diet group were fed only pellet powder (less than 20μm in diameter) mixed with water at a ratio of 1:4 by weight and mice in the solid-diet group were fed chow pellet and water until 11 weeks of age.

2. Surgical preparation

At 11 weeks of age, in order to install the measurement device, the mice received general (sodium pentobarbital, 25 mg/kg i.p.) and local (lidocaine 2%) anesthesia. For EMG, a pair of bipolar electrodes was placed bilaterally in the superficial masseter muscle, and unilaterally in the anterior belly of digastric muscle. All signals from the electrode were led with copper wires subcutaneously to the connector fixed on the parietal bones. A sensor unit, which consisted of four small magnetic sensors with a plastic frame, was mounted on the nasal bone and a small magnet was fixed to the mandibular bone just anterior to the insertion of the anterior digastric muscle. Jaw-movement trajectories were traced by the four magnetic sensors. A block diagram of the recording system is shown in Fig. 1. A detailed methodology for making recordings from freely moving animals has been previously published[12~14].

3. Recordings

After surgery, the animals were allowed to recover for approximately 3 days before recordings. Recordings were carried out while animals were chewing food. Test foods were pellets and bread. Mice in the both groups were deprived of food, water for 10-12 hr before experiment. It was the first chance for mice in the liquid-diet group to eat solid foodstuffs. Jaw movement and EMG signals were recorded simultaneously.

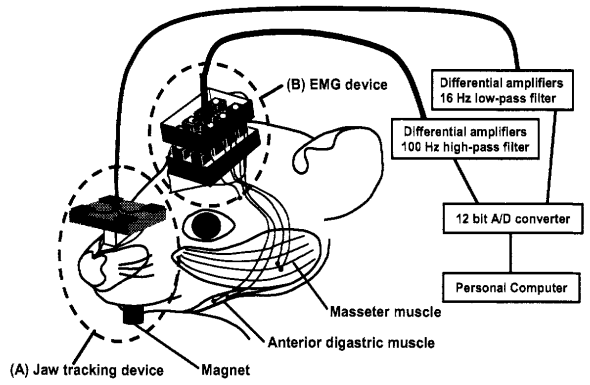

Fig. 1 Block diagram of the recording system. The recording system consists of (A) jaw tracking device and (B) EMG device. The jaw movement and EMG signals are stored simultaneously in computer memory.

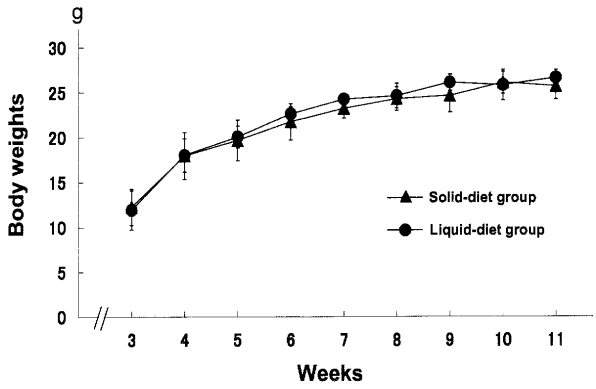

Fig. 2 Mean body weights in the solid- and liquid-diet groups. The vertical lines at each point indicate one standard deviation. There was no significant difference between the two groups by unpaired t-test and EMG signals are stored simultaneously in computer memory.

Jaw movement and EMG signals were amplified with AC amplifiers (jaw movement: low-pass filter 16 Hz, EMG: high-pass filter 100 Hz). These signals were stored in a computer memory through a 12-bit analog/digital (A/D) converter. Sampling rate of recordings was fixed at 1800 Hz.

4. Data analysis

Measured parameters were jaw displacement, the area of EMG burst, phase and total cycle durations. Mean values were obtained for gape size (distance between the minimum and maximum jaw-opening position), lateral excursion (distance between the minimum jaw-opening position and the most lateral jaw position) and anterior excursion (distance between the minimum jaw-opening position and the most protrusive jaw position). Total cycle duration was the time between minimum jaw opening to the next minimum jaw opening. EMG waveforms were first rectified. The area of the EMG burst was characterized in the following way. The mean±S.D. of the EMG at rest was calculated for the masseter and digastric muscles. Individual bursts were then identified when the rectified EMG exceeded the mean level by 3 S.D. Accordingly, the EMG area was calculated at each burst. The average and standard error of the data from all the animals tested were obtained from the average of 10 masticatory cycles. Parametric data were analyzed using paired or unpaired t-tests. A p value of less than 0.05 was considered to be statistically significant.

Ⅲ. Results

There was no significant difference in the mean body weight of two groups for each week (Fig. 2). Both groups were housed in plastic cages with normal wood-wool bedding material.

1. Properties of masticatory sequences

Typical masticatory sequence during pellet chewing in both groups is shown in Fig. 3 and 4. In both groups, on the basis of jaw movements and muscle activity, masticatory sequence could be divided into two functionally different masticatory periods: food intake and mastication. The pattern of jaw movement were irregular and inverse in the direction observed in the mastication phase during food intake. The food intake were followed by the mastication configured with larger movements in each of the three components, i.e.,vertical, horizontal, antero-posterior components. Cycles in mastication were characterized by large anterior movements and regular jaw-movement trajectories.

2. Properties of jaw movements

Figures 5 and 6 show examples of jaw movement patterns during chewing of pellet and bread in liquid-diet group (Fig. 5) and solid-diet group (Fig. 6). Anterior and lateral jaw movements were observed in the sagittal and frontal planes. According to the characteristic of jaw trajectories in the sagittal plane, a chewing cycle was divided as follows; closing (CL) phase, protruding (PR) phase and opening (OP)

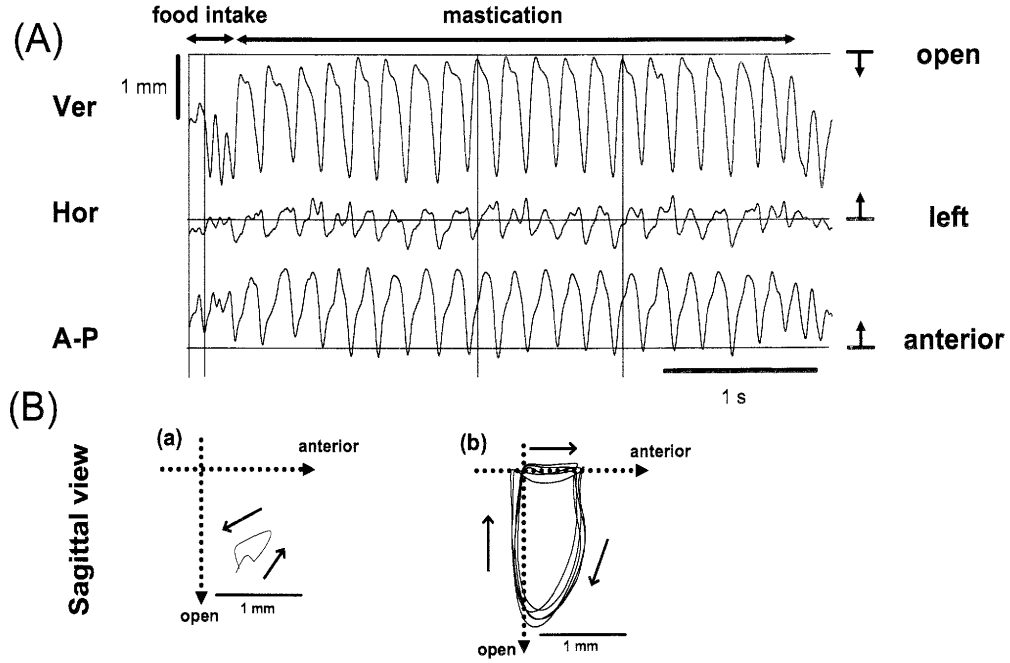

Fig. 3 A typical masticatory sequence from the solid-diet group when chewing pellets. (A) Vertical (Ver), horizontal (Hor), and antero-posterior (A-P) components of the jaw movement are shown in a time sequence. (B) jaw movement trajectories were reconstructed in the sagittal plane during (a) food intake and (b) mastication.

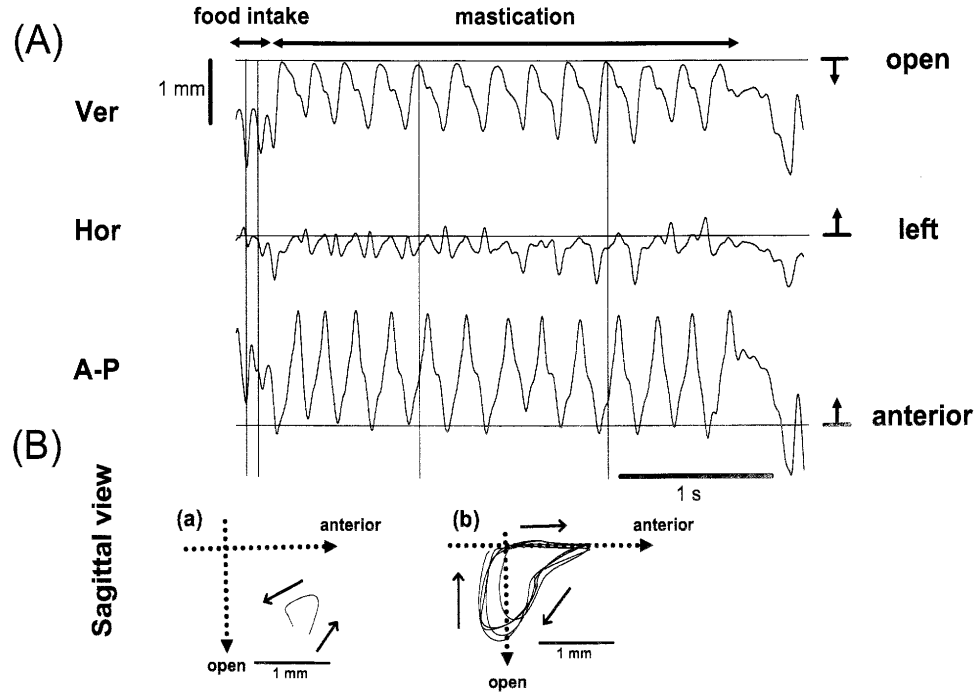

Fig. 4 A typical masticatory sequence from the liquid-diet group when chewing pellets. See previous

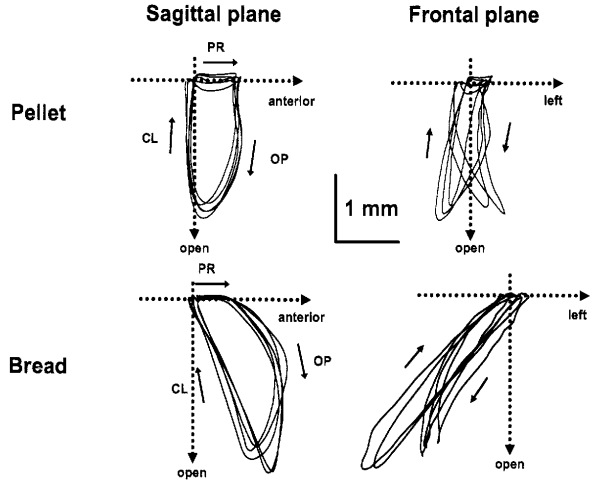

Fig. 5 Movement orbits of five successive chewing cycles in the sagittal and frontal planes when chewing pellet and bread in the solid-diet group. The movement orbit in a sagittal plane coud be divided into three phases, i.e., opening (OP), closing (CL) and protruding (PR) phases.

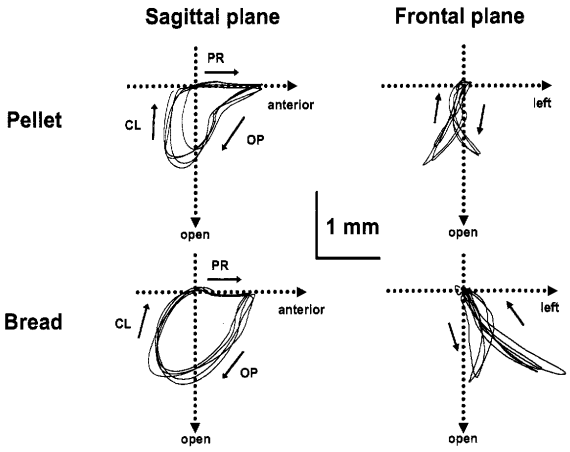

Fig. 6 Movement orbits of five successive chewing cycles in the sagittal and frontal planes when chewing pellet and bread in the liquid diet group. See previous legend (Fig. 5) for details.

phase in both groups. The gape size in the solid-diet group was significantly larger than that in the liquid-diet group during both pellet and bread chewing. In the solid-diet group, the lateral excursion when chewing pellet was significantly smaller than when chewing bread (Table 1). There was no significant difference in the parameter of lateral excursion and anterior excursion between the groups (Table 2).

3. Properties of muscle activity

In both groups, rhythmical activity of masseter and digastric was observed synchronizing with the closing and opening movements of the jaw, respectively (Figs. 7 and 8). The masseter burst began at the middle of CL phase, reached its peak in the early part of PR phase, and ceased its activity in the latter half of PR phase. There were two peaks of burst activity in the digastric muscle. The one burst activity began at the end of PR phase, peaked in the early part of CL phase. The other burst activity almost accorded with the masseter activity. The area of the masseter burst in the liquid-diet group was larger than in the solid-diet group both when chewing pellet and bread although there was no significant difference. Also, the area of the masseter bursts during pellet chewing was larger than that during bread chewing in both groups, but there was no significant difference. When the integrated activity of masseter during bread chewing was taken as 100%, the incremental rates of integrated activity of pellet chewing were 136.2 ± 57.9% and 154.6 ± 60.5% in the liquid-diet group and solid-diet group, respectively. The incremental rate was higher in the solid-diet group than in the liquid-diet group, but there was no significant difference. Regarding the area of the digastric burst, there was no significant difference between groups as well as foods (Table 1 and 2).

4. Properties of chewing rhythms

In the solid-diet group, protruding phase and total cycle duration were significantly longer during pellet chewing than during bread chewing. However, in the liquid-diet group, there was no significant difference in the total cycle duration and individual phase durations between the food (Table 1). There was no significant difference in the total cycle duration and individual phase durations between the groups (Table 2).

Ⅳ. Discussion

All mammals go through a phase where the stomatognathic function is converted from sucking to chewing. In normal rats, it has been shown that this conversion occurs concomitantly with the maturation of peripheral sensory receptors in the orofacial area [15], which suggests a causal relationship between receptor maturation and stomatognathic function development. This relationship is further supported by a neuroanatomical study in mice[16], which showed that facial nerve elements related to sucking exceed the trigeminal nerve elements related to chewing at birth, and that the trigeminal

Table 1 Comparison of jaw movements, muscle activity and cycle durations between foods in Solid-diet group and Liquid-diet group

	Jaw movements (μm)			EMG area (mV· ms)		Phase and cycle duration (ms)			
	Gape size	Anterior excursion	Lateral excursion	MAS	DIG	CL	PR	OP	Total cycle
Solid-diet group (n=7)									
Pellet chewing	2060±528	1090±347	610±185	0.0114±0.0043	0.0122±0.0048	69±11	65±16	95±20	229±34
Bread chewing	2450±450	870±303	990±360	0.0076±0.0021	0.0118±0.0043	60±8	43±18	86±7	189±21
t-test	N.S.	N.S.	p<0.05	N.S.	N.S.	N.S.	p<0.05	N.S.	p<0.05
Liquid-diet group (n=7)									
Pellet chewing	1350±393	1140±365	670±374	0.0155±0.0070	0.0120±0.0059	59±12	76±16	77±23	213±45
Bread chewing	1660±612	880±359	730±282	0.0121±0.0049	0.0141±0.0078	64±10	60±18	78±18	201±26
t-test	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Values are presented as mean's mean and S.E.M. of each group. MAS, masseter muscle; DIG, anterior digastric muscle; CL, closing phase; PR, protruding phase; OP, opening phase; N.S.,not significant. Degree freedom=6. Indivisual differences were tested using paired *t*-test.

Table 2 Comparison of jaw movements, muscle activity and cycle durations between Solid-diet group and Liquid-diet group

	Jaw movements (μm)			EMG area (mV· ms)		Phase and cycle duration (ms)			
	Gape size	Anterior excursion	Lateral excursion	MAS	DIG	CL	PR	OP	Total cycle
Pellet chewing									
Solid diet group (n=7)	2060±528	1090±347	610±185	0.0114±0.0043	0.0122±0.0048	69±11	65±16	95±20	229±34
Liquid-diet group (n=7)	1350±393	1140±365	670±374	0.0155±0.0070	0.0120±0.0059	59±12	76±16	77±23	213±45
t-test	p<0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Bread chewing									
Solid-diet group (n=7)	2450±450	870±303	990±360	0.0076±0.0021	0.0118±0.0043	60±8	43±18	86±7	189±21
Liquid-diet group (n=7)	1660±612	880±359	730±282	0.0121±0.0049	0.0141±0.0078	64±10	60±18	78±18	201±26
t-test	p<0.05	N.S.	N.S.	p<0.05	N.S.	N.S.	N.S.	N.S.	N.S.

Values are presented as mean's mean and S.E.M. of each group. MAS, masseter muscle; CL, closing phase; PR, protruding phase; OP, opening phase; N.S.,not significant. Degree freedom=6. Indivisual differences were tested using unpaired *t*-test.

nerve was rapidly promoted after birth. On the other hand, the conversion from sucking to mastication was also found on osteopetrotic mice, which lack sensation from the periodontal ligaments[17]. Likewise, in this study, we found that the conversion to mastication occurred in mice regardless of whether the animals were fed liquid or solid diets. Therefore, the conversion to mastication may not be dependent on peripheral sensory stimuli.

The masticatory pattern in liquid-diet mice was different from that of solid-diet mice. As in other studies of jaw movement[14], we were also able to distinguish movements associated with food intake and mastication. Masticatory movements were further divided in three phases, namely, opening phase (OP), closing phase (CL), and protruding phase (PR). There were small differences in the amount of anterior excursion, but significant differences in gape size were observed between the groups. One other study that compared liquid-diet and solid-diet mice found an impaired development of the mandibular condyle[18]. According to that study, the shape of the mandibular condyle in normal mice develops successively into bar, tear-drop, ellipse, and long-ellipse. Liquid-diet mice, however, remained at the tear-drop stage for as long as followed up in that study, i.e. up to 60 weeks of age. It is conceivable that the underdeveloped state of the condyles could have been a reflection of the mandibular function in liquid-diet animals; however, further studies would be necessary to determine whether the tear-drop shape is actually related to the masticatory pattern found in liquid-diet animals.

From the results of EMG recording, we observed that the masseter activity was greater in the liquid-diet group than the solid-diet group. This difference occurred independently of the food consistency (bread or hard pellet). When the recordings

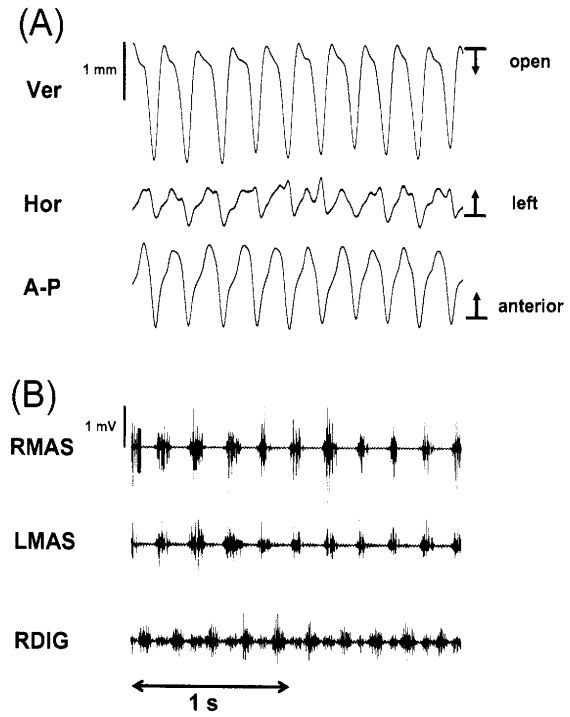

Fig. 7 Jaw movements and jaw muscle activity during pellet chewing in the solid-diet group. (A) Vertical (Ver), horizontal (Hor), and antero-posterior (A-P) components of the jaw movement are shown in a time sequence. (B) EMGs of right masseter muscle (RMAS), left masseter muscle (LMAS) and right anterior digastric muscle (RDIG).

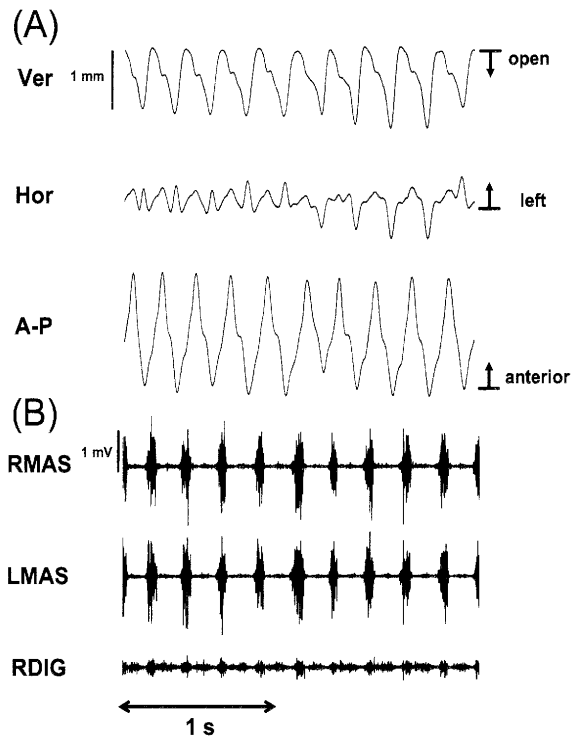

Fig. 8 Jaw movements and jaw muscle activity during pellet chewing in the liquid-diet group. See previous legend (Fig. 7) for details.

were carried out, it was the first chance for mice in the liquid-diet group to eat solid foodstuffs. This result suggests that they could not properly control the masticatory force, since the liquid-diet mice were not used to eating solid foodstuffs. Previous studies have shown that the masticatory muscles are weaker in animals raised on a soft diet. For example, using trains of electrical stimulation, Killiaridis[18] found that the tetanic tension was weaker in rats on soft diet than those on solid diet. Therefore, greater EMG bursts would be necessary to produce a similar muscle tension in animals raised on a soft diet. Interestingly, we found that liquid-diet mice did not show any significant difference in masseter activity when chewing either bread or hard pellet. Solid-diet mice, as expected, showed greater masseter activity when chewing hard pellet than bread. This result suggests that the control of masticatory force might be more difficult in mice raised on a soft diet.

The results from the liquid-diet group indicate an incomplete or underdeveloped control of the masticatory function. The total cycle duration was significantly longer when eating hard pellet than bread in the solid-diet group but there was no significant difference in the liquid-diet group. Likewise, there was a difference in lateral excursion of the mandible (bigger when eating bread than hard pellet) that was observed in the solid-diet group, but not in the liquid-diet group. Therefore, solid-diet mice showed a different masticatory pattern and rhythm when chewing on bread or hard pellet; liquid-diet mice, on the other hand, showed a similar masticatory pattern and rhythm regardless of food consistency. It has been previously reported that the masticatory cycle duration would be longer in normal mice when eating softer foods[14]. This difference may be explained by the fact the previous study was conducted on fully grown, adult mice, while the present study used relatively younger (11 weeks old) mice. It is possible that the masticatory function was not completely developed in either liquid- or solid-diet mice at this age. Further studies might be needed to determine if there is such thing as a critical period of masticatory

function development. Nevertheless, according to the current theory of masticatory control, peripheral inputs may modulate centrally generated rhythm and the pattern of jaw, tongue and facial muscles activity. Analysis of our results suggests either the masticatory CPG or its peripheral feedback mechanisms to be a partially impaired or under- developed in young animals raised on a liquid diet and deprived of solid foodstuffs.

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