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原著論文

Possible effects of periodontal inputs on the masticatory function

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Abstract: This study tested the hypothesis of whether the lack of tooth eruption would affect the masticatory function. For this purpose, we recorded the jaw movement trajectory in the three dimensions and electromyographic (EMG) activity of MAS and DIG muscles in freely behaving osteopetrotic (op/op) and normal mice. A masticatory sequence was divided into food intake and mastication periods, and 10 cycles in the latter stage were selected for analysis. Mean values of total cycle duration, closing phase duration and opening phase duration were obtained from the jaw movement and those of onset time, time duration and amplitude were obtained from the EMGs of the MAS and DIG muscles. The protruding phase coincided with the power stroke lacked in the op/op mouse. The total cycle duration and opening phase duration were longer in the op/op mouse than normal mouse. The pattern of MAS muscle activity in op/op mice was similar to that in normal mice while the duration of the DIG muscle was longer in op/op mice than normal mice. Our results indicate that the periodontal receptors play an important role in the completion of masticatory function.

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Ⅰ. Introduction

Mastication is the first stage in the process of digestive behavior. Basic patterns of masticatory rhythm are generated by the intrinsic neuronal network, so-called masticatory central pattern generator in the brain stem, and are fine-tuned in response to both peripheral feedback and central afferent inputs[1-6]. It is well-known that peripheral receptors responsible for the regulation of masticatory rhythm are distributed in the periodontium, lips, oral mucosa, jaw muscles and temporomandibular joint, and they play an important role in adjusting the movements of the jaw and head to the physical conditions of foodstuffs[3,7].

The acquisition of masticatory function takes place in the early stage of life, and coincides with two important events: tooth eruption and weaning[8]. It can be hypothesized that periodontal mechanoreceptors play a critical role in the acquisition of masticatory function[9,10]. To address this issue, Kobayashi et al.11) recorded the electromyographic (EMG) activity of jaw muscles and trajectory of jaw movements in the osteopetrotic (op/op) mouse which is a mutant with a metabolic bone disease that prevents the eruption of teeth[12] and lacks inputs from periodontal mechanoreceptors throughout life. The authors indicated that conversion from sucking to mastication occurred in the op/op mouse and the masticatory rhythm developed almost normally without tooth eruption. However, it has not yet been clarified how the periodontal mechanoreceptors play a role in the functional regulation of masticatory movement. Therefore, in the present study, we attempted to analyze the differences in the mastication between op/op and normal mice.

Ⅱ. Materials and methods

The methods described here were reviewed and approved by the Animal Welfare Committee of Nagasaki University based on the Animal Care Standards of this institution. Every possible effort was taken to minimize animal suffering and the number of animals used in this study.

1. Experimental animals

We used 12 male B6C3 mice acquired from Jackson Laboratory (Bar Harbor, ME, USA), of which 6 were op/op mutants, weighing 26.7-30.3g, and 6 were normal littermates, weighing 30.5-31.7 g. Mice were considered op/op if no teeth had erupted ten days after birth. The animals were brought into our animal housing facility, where they were kept in acrylic cages with soft bedding and food and water ad libitum. The room was temperature controlled (21-23℃), with a light/dark cycle of 12 hours (lights on at 8:00). Normal and mutant mice were fed on the same material (DE-2, Clea Japan, Tokyo, Japan), except that normal mice received standard feed in the form of solid pellets while mutant mice received powdered feed.

2. Animal preparation

The jaw-tracking device used here is described in further detail elsewhere[13]. The animals were anesthetized with an intra-peritoneal injection of pentobarbital sodium (Nembutal, Dainippon Sumitomo Pharma, Osaka, Japan) at an initial dose of 25 mg/kg. Supplemental doses of 5 mg/kg were administered when necessary. The level of anesthesia was assessed firmly pinching the skin between the digits of the hindpaw -the lack withdrawal reflex denoted medium to deep surgical anesthesia. The body temperature was maintained at 37-38℃ by a heating pad. Local anesthetics were infiltrated in the areas to be incised (Lidocaine 2%, AstraZeneca, Osaka, Japan). An incision was made on the ventral aspect of the neck to expose the lower jaw and jaw musculature, and the periosteum at the anterior portion of the mandibular bone was reflected. Then, two cylindrical samarium magnets (each 4 mm in diameter and 1 mm in thickness, NE080, Niroku Industry, Ootsu, Japan) were piled up and bonded over the midline to the exposed bone. For EMG recordings, bipolar electrodes consisting of Teflon coated stainless steel wires with 2-mm exposed tips and 1-mm interpolar distance were implanted into the left and right masseter (MAS) and right anterior digastric (DIG) muscles. The electrode wires were passed under the facial skin towards the top of the head, where another incision was made on the midline to expose the parietal, frontal and nasal bones. Here, the periosteum was also reflected and the magnet sensors were bonded to the nasal bones after calibration. Sensor and electrode wires were fixed to a connector, which was bonded to the parietal bones. The bonding agent used throughout was the Superbond system (Sun Medical, Moriyama, Japan).

3. Recordings

A detailed methodology for making recordings from freely moving animals has been previously published[13,14]. In brief, after surgery, the animals were allowed to recover for three days before recording. On the day of recording, the animal was brought from the colony room into the recording room and was allowed to acclimatize for a few minutes. Then, the animals were connected by flexible cables via the head connector to the recording device. EMGs and jaw movements were recorded while animals were eating foods, which consisted of a paste prepared from powdered feed mixed with water and were rolled in the shape of balls with about 5mm in diameter. Those signals were amplified with AC amplifiers and were stored in a computer memory through a 12-bit A/D converter. Sampling rate of recordings was fixed at 2000 Hz.

4. Date analysis

A masticatory sequence was first divided into food intake and mastication periods[15]. Further analyses were carried out on the mastication period of rhythmical 10 cycles. Mean values of the following parameters were calculated from ten raw data in each animal. The parameters used to analyze jaw movements were (1) total cycle duration (TC, time between one point of maximum jaw-opening to the next), (2) closing phase duration (CL, time between maximum opening and subsequent minimum opening), (3) opening phase duration (OP, time between minimum opening and subsequent maximum opening). In the present study, the protruding phase, which the opening phase included in the present study, was not analyzed although they could be visibly detectable from the jaw movement orbit in the sagittal plane.

Jaw-muscle activity was analyzed in terms of (1) Onset (onset time of EMG activity), (2) Duration (time duration of EMG activity), (3) Amplitude (mean amplitude of EMG activity). To analyze the temporal relationship between jaw movement and jaw-muscle activity in each cycle, Onset and Duration of each muscle were normalized to TC and were presented as a percentage of TC.

The software Spike2 (Cambridge Electronic Design, Cambridge, UK) aided in the process of data analysis. The Mann-Whitney U-test was performed to determine the significant difference between op/op and normal mice, where a $P<0.05$ was considered significant. All values are displayed as means ± S.E.M.

Ⅲ. Results

In the present study, we did not distinguish between the working and non-working sides for analysis but distinguished the right and left sides in the MAS muscle because, in our preliminary experiment, it was confirmed that there were no significant difference in the values of any parameters between the sides.

1. Jaw movements

Rhythmical masticatory movements were observed in both op/op and normal mice (Figs. 1 and 2). Likewise, both groups showed distinguishable food-intake and mastication periods. In the beginning of sequence, i.e., food-intake period, the pattern of jaw movement was somewhat irregular and the level of EMG amplitude in the MAS muscle was low. Once the mastication period started, large and regular activity was observed of the EMGs in the MAS and DIG muscles.

Regarding the lateral jaw movement pattern, deviation was less and the jaw orbit was composed of two phases, up and down in both groups (Fig. 3, upper tracing). According to jaw orbits in the sagittal plane (Fig. 3, lower tracings), in the normal mice, masticatory movements were divided into closing phase, protruding phase and opening phase. On the other hand, in op/op mice, the protruding phase was obscure or not observable.

A few differences in the duration of jaw movements were observed between op/op and normal mice (Table 1). The TC was significantly longer in op/op mice than normal mice. The OP was also significantly longer in op/op mice than normal mice. It was noted that op/op mice showed significantly shorter CL than that in normal mice (33.4 ±6.1% for op/op mouse and 39.8 ±2.5% for normal mouse) (Fig. 4).

2. Jaw-muscle activity

The rhythmical movements of the jaw were accompanied by synchronized activities of the MAS and DIG muscles (Figs 1 and 2). In both op/op and normal mice, the MAS muscles were active early after maximum opening until the point of minimum opening. Conversely, the DIG muscle was active immediately after the point of minimum closing until the point of maximum opening. The onset of the MAS muscle activity coincided with the switch from posterior to anterior movement in CL (Fig. 4).

There were no significant differences in Amplitude of the MAS and DIG muscles between the groups. The Duration of the DIG muscle was significantly longer in op/op mice than normal mice while no significant difference was noted in the MAS muscle between the groups (Table 2).

Ⅳ. Discussion

1. Jaw movement pattern

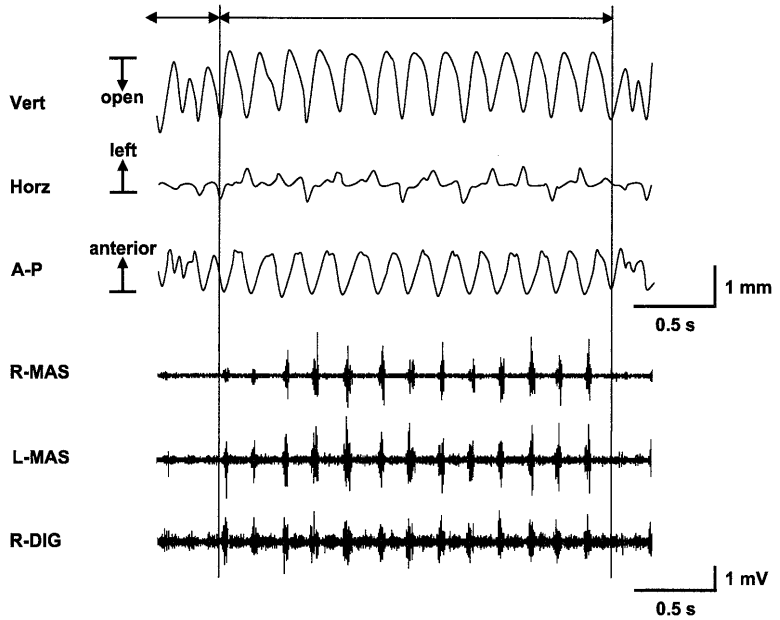

Fig. 1 A typical masticatory sequence from normal mice when chewing paste food. Upper three traces illustrate jaw movements in the vertical (Vert), horizontal (Horz), and anterio-posterior (A-P) direction. Lower three traces show electromyographies(EMGs) of the right masseter (R-MAS), left masseter (L-MAS) and right digastric (R-DIG) muscles. The sequence was divided into food intake and mastication periods. Horizontaland vertical calibrations of jaw movement are common for all tracings as with EMGs.

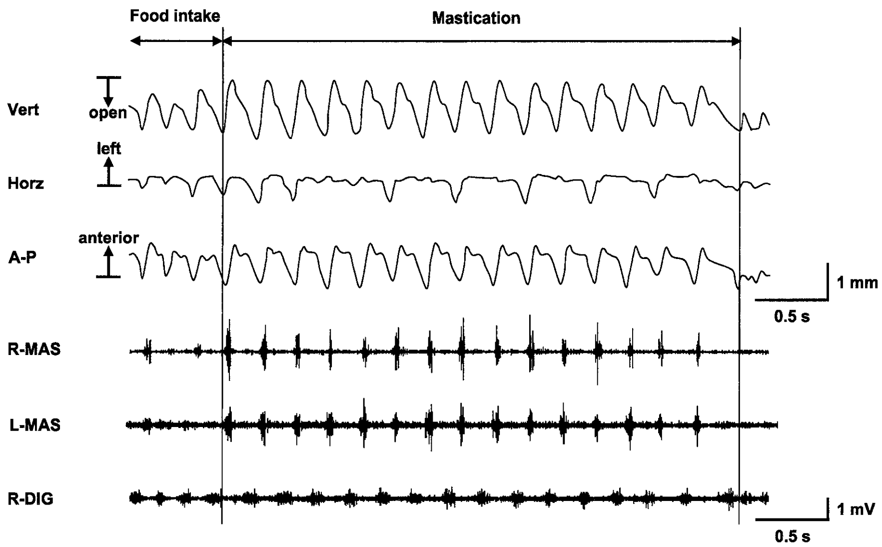

Fig. 2 A typical masticatory sequence from op/op mice when chewing paste food. See previous legend (Fig. 1) for details.

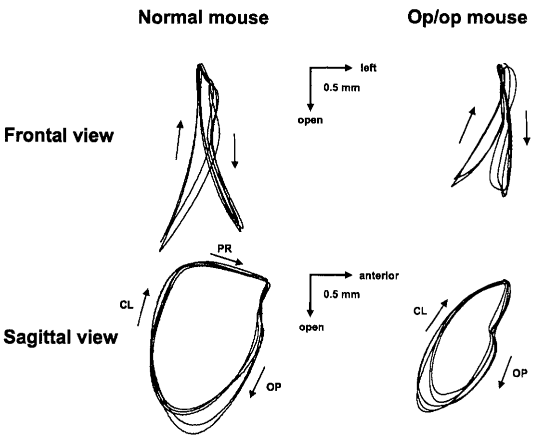

Fig. 3 Jaw movement orbits in normal (left) and op/op (right) mice when chewing food. Tracings show five consecutive cycles superimposed. Upper tracings: frontal plane; lower tracings: sagittal plane; CL: closing phase; PR: protruding phase; OP: opening phase. Horizontal and vertical calibrations are common for all orbits.

The masticatory sequence in normal and op/op mice could be divided into two periods, i.e. food intake and mastication periods[15]. This classification was consistent with previous findings[11]. In the present study, there were considerable differences between normal and op/op mice in the jaw-movement pattern during the mastication period; in op/op mice, protruding phase was not observed clearly. The differences in the jaw-movement pattern may be due to the three possible reasons; 1) the growth of the MAS muscle and related organs, 2) eating habits, and 3) sensory inputs from the periodontal ligaments. For the fist, it is generally believed that MAS muscles have an important role in bone formation and remodeling of the condylar head[16]. Maeda et al., demonstrated that MAS muscles of op/op mice were significantly underdeveloped[17]. In the present study, however, there were no significant differences in the Amplitude and Duration of the MAS muscle between the groups. The paste food might not be hard enough to make difference in the activity pattern of the muscles between the groups. Kawata et al. demonstrated that the condylar heads of op/op mice were underdeveloped than that of normal mice[18]. It may be possible that the underdeveloped state of the condyles could reflect the jaw-movement in op/op mice although we do not have direct evidence. For the second, the masticatory system, as an apparatus which satisfies these functions may be modified by dietary foods[19, 20]. Normal mice may always have protruding phase as an adaptation for grinding hard pellet. On the other hand, op/op feeds soft food which indicates that when less force is required to reduce the bolus, the closing position of the jaw is shifted forward. It may be possible that op/op mice were adapted to eat softer foods only. For the third, sensory inputs from periodontal ligaments may be one of the most important factors to inform the status of the bolus in the process of chewing and to modulate the masticatory patterns in -related muscle activity according to the situation, which lead to changing the jaw movement pattern. This will be discussed below.

2. Jaw muscle activity

In the present study, the activity pattern of MAS muscle in op/op mice was similar to that in normal mice. It is generally believed that the masticatory central pattern generator (CPG) determines the basic pattern of masticatory rhythm[1-5], and that sensory inputs modulate the movements[3, 7]. In addition, sensory inputs may influence development and final output of the masticatory CPG[3, 7]. According to Appenteng et al., the activity of mechanoreceptors in the orofacial area was often modulated during cyclic movements of the jaw, probably sending information on the jaw movements, biting force or position of ingested food in the mouth[7, 21]. It is also well-known that periodontal mechanoreceptors and jaw-muscle spindles provide positive feedback[23]. For example, the activity in the MAS muscle was markedly reduced when inputs from periodontal mechanoreceptors were abolished (e.g. after maxillary and inferior alveolar denervation) [22]. For another study showed that when the mesencephalic trigeminal nucleus where the primary ganglion cells of muscle spindle afferents from jaw-closing muscles was also destroyed, the response of the MAS muscle was completely abolished[23]. These experiments convincingly demonstrated that peripheral receptors, especially the periodontal mechanoreceptors and jaw-muscle spindles, contribute to modulating the pattern of jaw-closing muscle activity in masticatory movements. In this study, the pattern of MAS muscle activity in op/op mice was similar to that in normal mice. As mention above, the sensory inputs may not be strong enough to modulate the jaw muscle activity, especially the jaw closing muscle such as the MAS muscle. However, one may notice that the opening phase duration as with the Duration of DIG muscle were significantly longer in the op/op mouse than normal mouse. The result may be attributed to the fact that op/op mice lack

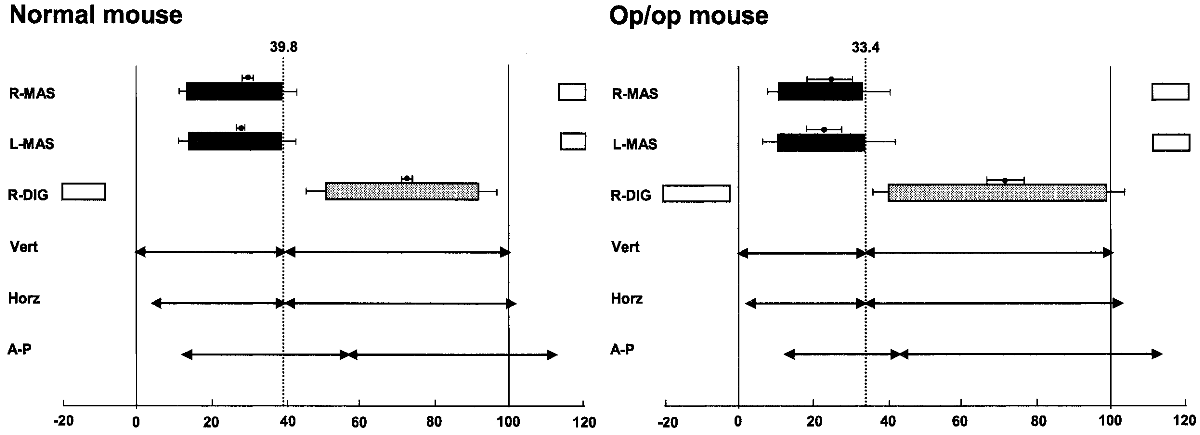

Fig. 4 Timing of muscle activity in relation to jaw movement. The data were obtained from six normal and six op/op mice. Thetotal cycle duration in each animal was normalized to 100%. The onset of each masticatory cycle was defined as the 0% time point, and the end of cycle was defined as the 100% time point. Vertical dotted line represents minimum opening position and vertical solid lines represent maximum opening position. The start of the bars (left end) represent the mean onset, and the end of the bars (right end) represent the mean offset of EMG bursts in relation to the normalized masticatory cycle. The length of each bar represents the mean duration of EMGburst as a proportion of the total cycle. The dot and whiskers above eachbar represent the mean time and S.E.M. of the peak EMG activity. Horizontal arrows represent jaw movements. The point where the arrowheads encounter represent inversion in the direction of movement. Hor: both arrows represent the jaw deviation from the minimum to maximum opening movement in the horizontal direction, A-P: the left arrow represents anterior jaw movement and the right arrow represents posterior jaw movement. See previous legend (Fig. 1) for details..

Table. 1 Comparison of jaw movements between normal and op/op mice

	TC(ms)	CL(ms)	OP(ms)
normal (n=6)	185.9±17.4	74.1±9.1	108.1±11.5
op/op (n=6)	270.1±58.1	89.1±18.5	187.9±49.1
Mann-Whitney U-test	P< 0.01	N.S.	P< 0.01

Values are presented as S.E.M. of each group. TC: total cycle duration, CL: closing phase duration, OP: opening phase duration, N.S.: not significant. Degree freedom=6. Individual differences were tested using Mann-Whitney U-test.

Table. 2 Comparison of muscle activety between normal and op/op mice

	EMG duration (ms)			mean amplitude (mV)		
	R-MAS	L-MAS	R-DIG	R-MAS	L-MAS	R-DIG
normal (n=6)	46.2±4.2	45.1±4.9	76.5±13.3	0.42±0.18	0.44±0.38	0.11±0.07
op/op (n=6)	62.6±23.9	70.6±24.2	150.4±27.0	0.43±0.41	0.52±0.35	0.14±0.18
Mann-Whitney U-test	N.S.	N.S.	P< 0.01	N.S.	N.S.	N.S.

Values are presented as S.E.M. of each group. R-MAS: right masseter, L-MAS: left masseter, R-DIG: right digastric, N.S.: not significant. Degree freedom=6. Individual differences were tested using Mann-Whitney U-test.

periodontal inputs from birth, which may have allowed enough time for partial adaptation to this condition.

In conclusion, analysis of the masticatory behavior in the op/op mouse indicates that the acquisition of masticatory function involves learning processes, and that it can only be fully developed through the appropriate sensory experience.

Acknowledgments

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