Original Article

Functional Changes of Temporomandibular Joint Mechanoreceptors Induced by Reduced Masseter Muscle Activity in Growing Rats

Takayoshi Ishida[a]; Tadachika Yabushita[b]; Kunimichi Soma[c]

ABSTRACT

Objective: To determine the influence of masseter muscle activity during growth on the functional characteristics of temporomandibular joint (TMJ) mechanoreceptors.

Materials and Methods: Sixty-six 3-week-old male Wistar rats were divided into an experimental group, in which the masseter muscles were bilaterally resected at 3 weeks of age, and a control group. Single-unit activities of the TMJ mechanoreceptors were evoked by indirect stimulation of passive jaw movement. Electrophysiologic recordings of TMJ units were made at 5, 7, and 9 weeks of age.

Results: During this period, the firing threshold of the TMJ units was significantly lower and the maximum instantaneous frequency of the TMJ units was significantly higher in the experimental group than in the control group.

Conclusion: Reduced masseter activity during the growth period alters the response properties of TMJ mechanoreceptors. (*Angle Orthod.* 2009;79:978–983.)

KEY WORDS: Temporomandibular joint; Mechanoreceptor; Masseter muscle activity; Primary afferent; Mandible; Rat

INTRODUCTION

Loading of masticatory muscle activity is an important factor that affects craniofacial growth. Clinical studies have shown a correlation between craniofacial morphology and muscular function. Cross-sectional studies in humans have demonstrated a clear correlation between high electromyographic activity of the jaw muscles and uniform characteristic facial morphology.[1–5] Moreover, it has been reported that adults with weak muscles have greater variation in facial morphology than those with strong muscles.[2,5] In vitro, bilateral resection of the masseter muscles in rats results in a skeletal pattern with an open bite and a decrease in the level of chondrocytes.[6,7] It has been shown that bilateral masseter resection at the prepubertal stage in rats leads to reduced mandibular bone formation in adults.[8] Investigators concluded that ideal mandibular growth requires optimal compressive forces on the condyle and gonial region, which are provided by occlusion. However, few studies have investigated the functional changes in the temporomandibular joint (TMJ) that occur under conditions of low masticatory function in growing rats.

Clinical studies have shown that the prevalence of temporomandibular disorder (TMD) in anterior openbite patients, who have less bite force than normal individuals,[9,10] increases with age.[11] Severe divergence of differential skeletal vs dental problems in childhood can lead to the development of abnormal TMJ morphology and functional disorders such as TMD in adults.[12,13] Therefore, we assume that the oral environment, for example, masticatory muscle activity, affects the TMJ. The TMJ is a typical diarthrodial joint, both morphologically and functionally. It connects the cranium with the mandibular bone and plays an important

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role in controlling jaw movement. Some of the sensory receptors in the TMJ transmit information to the brain about mandibular position and movement of the joint and are considered to be proprioceptors.[14–17]

We investigated the effects of decreased temporomandibular loading from masticatory muscles on the functional characteristics of TMJ mechanoreceptors during early growth in rats. We used a bilateral masseter muscle resection model to weaken the masticatory muscle activity. We hypothesized that decreased temporomandibular loading affects many of the functional characteristics of the TMJ receptors, in particular, the mechanoreceptors that play a role in regulating mandibular position.[16]

MATERIALS AND METHODS

The experimental procedures that are described here were approved by the Animal Welfare Committee and were performed in accordance with the Animal Care Standards of Tokyo Medical and Dental University.

Animal Preparation

Sixty-six 3-week-old male albino Wistar rats (Sankyo Lab Service Corporation, Inc, Tokyo, Japan) were divided randomly into an experimental group ($n = 33$) in which the masseter muscles were resected bilaterally, using the same model as described in previous studies,[6,8,18] and a control group ($n = 33$).

Before surgery, all animals were anesthetized deeply with diethyl ether and an intraperitoneal injection of 8% chloral hydrate (1 mL/200 g body weight). After the area around the proposed incision was shaved, the rats were cut open and the masticatory muscles were observed. All superficial and deep portions of the masseter muscles were cut off bilaterally at the end of each muscle and were removed without damage to any of the surrounding major blood vessels and nerves. Then, the incision was sutured. At the end of the operation, amoxicillin (ICN Biomedicals Inc, Aurora, Ohio, USA) was injected to prevent infection at a dose of 9 mg/60 g body weight.

All rats were fed pellets and were given water ad libitum throughout the experimental period, and their body weight increased with no significant differences between groups. No difference was noted in the pattern of jaw movement between rats that had undergone masseter resection and control rats, while they were eating pellets.

Stimulation and Recording

For electrophysiologic recordings, the animals were anesthetized lightly with thiamylal sodium (Isozol; Yoshitomi Pharmaceutical, Osaka, Japan) at 60 mg/kg intraperitoneally. The depth of anesthesia was monitored by checking pupil size, flexor and corneal reflexes, and heart rate. A supplemental injection of thiamylal sodium at 5 mg/kg was given intraperitoneally when a firm pinch to the tail resulted in increased respiration and heart rate. Electrophysiologic recordings were obtained from the trigeminal ganglion, which contains the cell bodies of the trigeminal sensory neurons from the TMJ mechanoreceptors, when the rats were 5, 7, and 9 weeks of age.

The rats were placed in a prone position in a stereotaxic apparatus (models SN-2 and SM-15M; Narishige Scientific Instrument Lab, Tokyo, Japan) (Figure 1). For indirect stimulation of the TMJ mechanoreceptors during passive jaw movement, one end of a cotton thread was fixed to the mandibular symphysis and the other to an automatic pulling machine.[17,19,20] The maximum jaw-opening distance was set to 5.0 mm, which was within the physiologic range, with a ramp duration of 5.0 seconds and a hold duration of 5.0 seconds.[17]

To allow introduction of the recording electrode, the scalp was incised along the midline, and a small aperture, about 3.0 mm wide, was formed in the skull with the use of a stereotaxic microengine. Monopolar tungsten microelectrodes (250 μm diameter shaft with a 8.0 degree tapered tip, 5.0 MΩ AC impedance; A-M Systems Inc, Carlsborg, Wash, USA) were inserted into the trigeminal ganglion, following the stereotaxic coordinates that were reported by Paxinos[21] for recording single-unit activities of the TMJ mechanoreceptors.

Spike signals were recorded and amplified with the use of a differential amplifier (DAM-80; WPI, Sarasota, Fla, USA), using $\times$1000 gain, and 300 Hz and 3.0 kHz for the low and high filters, respectively. All data were captured using a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK) and were stored on a computer hard disk. The data later were analyzed offline with the use of Spike2 software for Windows, version 4.02a (Cambridge Electronic Design).

After each unit had been recorded, the electrode position was marked using a negative current of 50 μA for 10 seconds. At the end of the experiment, the rats were killed by an overdose of thiamylal sodium (120 mg/kg), and their brains were removed. Frozen 50 μm sections were prepared and then stained with cresyl violet to confirm histologically the position of the electrode from the electrolytic markings and signs of electrode penetration (Figure 1).

Data and Statistical Analysis

The effects of reduced compressive force, with the use of a bilateral masseter muscle resection model,

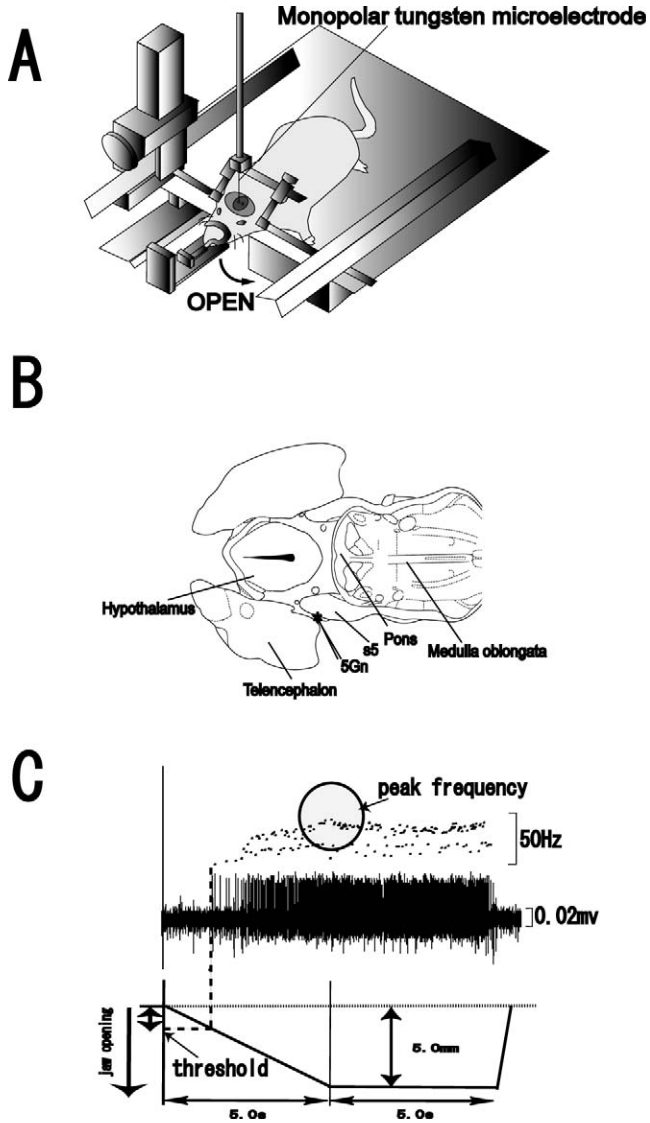

Figure 1. (A) Schematic drawing of the experimental setting. The animal's head was fixed to a stereotaxic frame. A small aperture, approximately 3.0 mm wide, was prepared in the skull, and monopolar tungsten microelectrodes were inserted into the trigeminal ganglion. A string was attached to the mandible. Ramp-and-hold jaw movement was achieved with the use of an automatic pulling machine. (B) Schematic representation of the trigeminal ganglion drawn from a horizontal section of the brain 9.2 mm below the bregma.[21] An asterisk indicates the recording site. s5, sensory root of the trigeminal nerve; 5Gn, trigeminal ganglion. (C) The firing thresholds were calculated as the magnitude of jaw opening that was observed at the first spike. A vertical dashed line indicates the first spike from a temporomandibular joint (TMJ) unit.

on the TMJ units were assessed by analyzing the firing threshold and the maximum instantaneous frequency. The firing threshold was calculated as the magnitude of jaw opening that was observed at the first spike response. The maximum instantaneous frequency was calculated as the minimum firing interval between two spikes (Figure 1).

All data were expressed as the mean ± standard deviation. Differences between control and experimental groups at different ages were evaluated and compared using the Mann-Whitney *U*-test with a 95% significance level. Statview for Windows, version 5.0 (SAS Institute, Cary, NC, USA) was used to perform the statistical analysis.

RESULTS

Unit activities were recorded from 33 TMJ units of the sensory neurons of the trigeminal ganglion in both control and experimental groups. Analyses of the activity of TMJ mechanoreceptors from each unit were performed after three consecutive ramp-and-hold jaw openings. Typical examples of TMJ units that were recorded from the trigeminal ganglion at 5 and 9 weeks in the control and experimental groups are shown in Figure 2. The first spike response of the experimental group was earlier than that of the control group.

Firing Threshold

The firing threshold of the control group was approximately 1.4 mm during the experimental period. In the control group, no significant difference in the firing threshold was noted at 5, 7, and 9 weeks. However, the mean firing threshold in the experimental group was significantly lower than that in the control group at each age that was examined (Figure 3 and Table 1).

Maximum Instantaneous Frequency

The maximum instantaneous frequency of the control group was approximately 50.6 Hz during the experimental period. In the control group, no significant difference in maximum instantaneous frequency was noted at 5, 7, and 9 weeks. By contrast, the maximum instantaneous frequency of the experimental group at 5, 7, and 9 weeks was significantly higher than that of the control group (Figure 4 and Table 1).

DISCUSSION

The purpose of this study was to investigate the consequences of change in the masticatory environment in rats, which was achieved by reducing the masticatory muscles by masseter muscle resection, and to examine the effects of this change on the functional characteristics of the TMJ mechanoreceptors. It has been shown previously that this model does not cause inflammation of the TMJ.[18] In addition, previous studies have suggested that no significant difference occurs as the result of scar tissue.[6,8,18,22]

In the experimental group, in which the temporalis and pterygoid muscles may be compensating for the

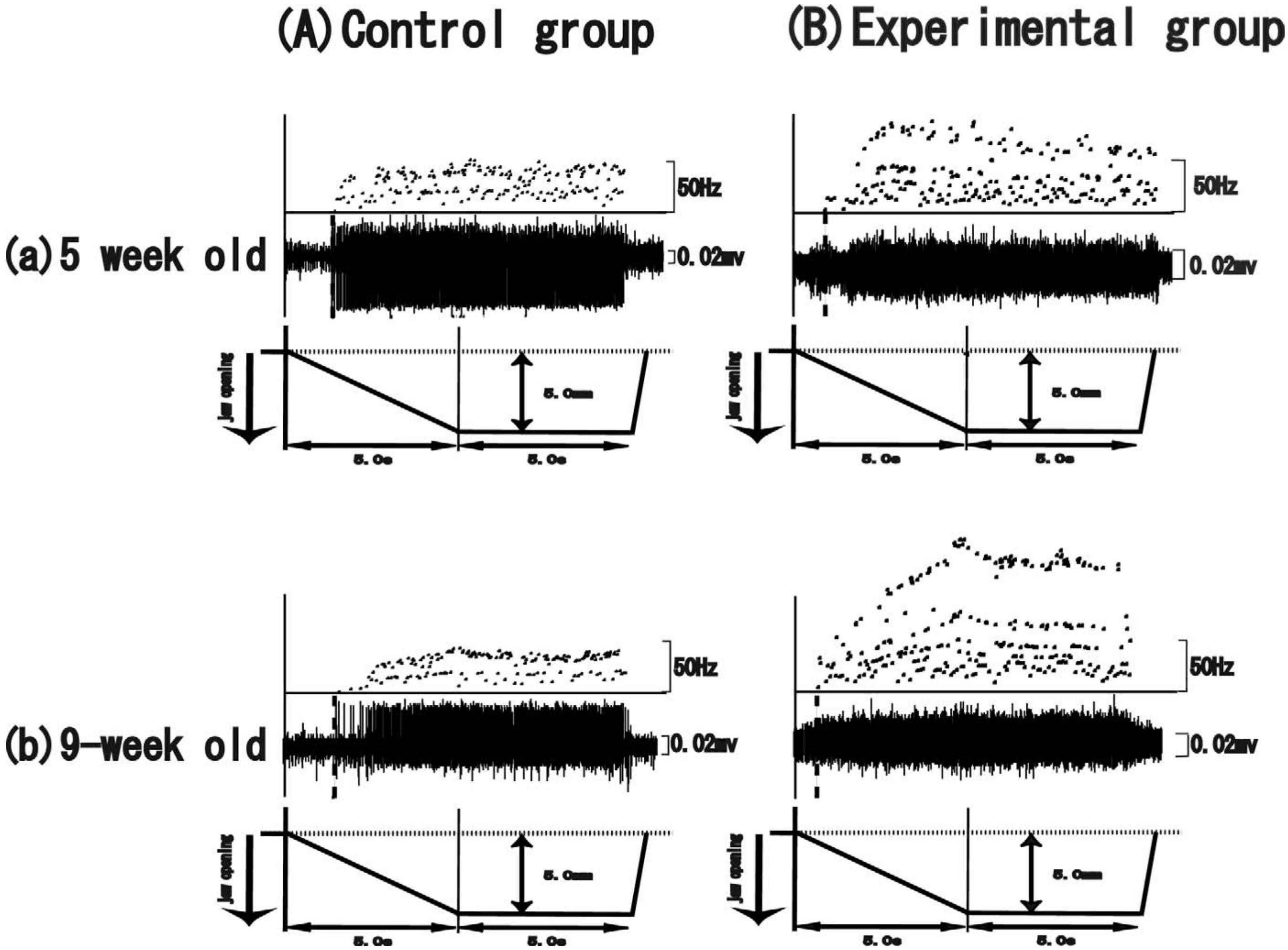

Figure 2. Typical examples of responses from the (A-a) 5-week-old control group; (A-b) 9-week-old control group; (B-a) 5-week-old experimental group; and (B-b) 9-week-old experimental group. Upper plots of all raw data indicate the instantaneous frequency.

action of the masseter muscle to some degree, directional changes in force might cause density alteration and displacement of TMJ mechanoreceptors. However, a previous study mentioned that the thickness of the masseter muscle mainly correlates with bite force,[23] and another study reported that the masseter muscle makes up more than 50% of all the masticatory muscles[24] and is about twice as strong as the temporalis muscle force. Moreover, another study reported that when the bilateral temporalis was resected, total bite force did not change because of compensation of the masseter muscle, whereas when bilateral masticatory muscle was resected, total bite force decreased to 30%.[25] These results explained that the temporalis muscle cannot compensate for the masseter muscle because the masseter muscle is the strongest masticatory muscle. Therefore, we assumed that the resection of masseter muscle force would diminish the masticatory force and reduce the load on the TMJ.

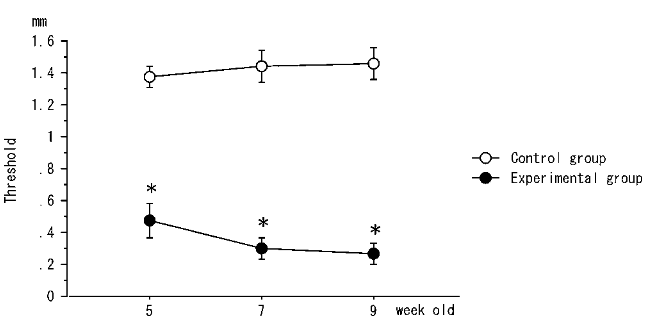

Figure 3. Firing thresholds of temporomandibular joint (TMJ) units in the control (11 units per group) and experimental groups (11 units per group). The firing thresholds of TMJ units from the experimental group were significantly lower than those of the control group. Asterisks indicate significant differences ($P < .05$) between the experimental and control groups.

In the control group, no significant differences were noted at 5, 7, and 9 weeks in the firing threshold value or in the maximum instantaneous frequency. Few studies have investigated functional changes in the TMJ mechanoreceptors in growing rats. The results of

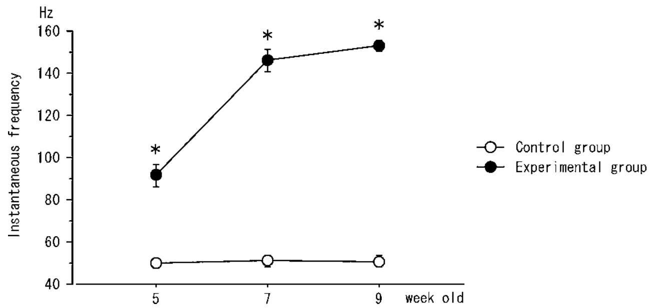

Figure 4. Maximum instantaneous frequencies of temporomandibular joint (TMJ) units in the control (11 units per group) and experimental groups (11 units per group). The maximum instantaneous frequencies of TMJ units of the experimental group were significantly higher than those of the control group. Asterisks indicate significant differences ($P < .05$) between the experimental and the control groups.

previous studies in mice have shown that morphologic development of the trigeminal motor neurons is enhanced at birth and is almost complete by the age of 3 weeks.[26] In addition, a previous study suggested that the response of rat periodontal mechanoreceptors (PMRs) is mature by 5 weeks of age.[27] Both TMJ mechanoreceptors and PMRs are trigeminal nerve endings. The data that are described here indicate that TMJ mechanoreceptors are mature by the age of 5 weeks. Moreover, we believe that early development of orofacial mechanoreceptors, such as TMJ mechanoreceptors, is needed for the maturation of mastication.

In our study, the firing thresholds of the TMJ mechanoreceptors gradually decreased in the experimental group. Many studies have investigated mastication and oral mechanoreceptors after the masticatory environment has been changed,[19,28] but only a few studies have directly investigated the firing thresholds of the TMJ mechanoreceptors.[17] It has been reported that the mechanical thresholds of PMRs in other mechanoreceptors in the oral region are temporarily decreased in response to the loss of occlusal stimuli.[29] It has also been reported that, in this model, changing the masticatory environment through masseter muscle resection reduces the articular forces on the TMJ during biting, thus leading to decreased growth of the TMJ area.[8] These previous studies suggest that the changes in the response of TMJ mechanoreceptors that were observed in the present study are related to alterations in the mechanical stimuli.

We found that the maximum instantaneous frequency of the TMJ units gradually increased in the experimental group. Recently, it was reported that atypical receptors were present and the number of receptors in the anterior cruciate ligament of the knee joint of rats was reduced after a long period of hind limb suspension and relief of the load on the limbs.[30] This is similar to determining the effects of bilateral resection of masseter muscles on the TMJ because the loss of attachment of the masseter muscles reduces the articular forces on the TMJ area while the TMJ is free-moving. It has been shown previously that changing the conditions of various joints, for example, by introducing weightlessness or immobilization, causes morphologic changes in the associated nerve endings.[17,30,31] Our results agree with these studies. Therefore, it may be possible to attribute changes in the functional characteristics of the TMJ mechanoreceptors that were described in the present study to morphologic changes in the TMJ mechanoreceptors.

Table 1. Firing Threshold and Maximum Instantaneous Frequency of TMJ Units in Each Group

	Control Group	Experimental Group	n
Threshold, mm			
5-week old	1.38 ± 0.10	0.48 ± 0.16	11
7-week old	1.41 ± 0.19	0.30 ± 0.10	11
9-week old	1.46 ± 0.15	0.27 ± 0.10	11
Maximum instantaneous frequency, Hz			
5-week old	49.92 ± 3.30	91.6 ± 7.99	11
7-week old	50.95 ± 3.79	146.15 ± 7.80	11
9-week old	50.92 ± 4.50	153.06 ± 3.49	11

n = number of units.

In this study, the functional properties of the TMJ mechanoreceptors changed. The stimulation threshold is defined as the lowest strength of stimulation that is capable of initiating electrical activity. The frequency of firing (action potential) is the magnitude of the postsynaptic potential.[32] Mechanoreceptor excitation is accomplished by opening or closing ion channels that are present in the sensory organs.[33] Consequently, alterations in the masticatory environment might result in changes in the properties and morphology of ion channels, such as Mechano sensitive ion channels.[33–35]

Data from the control group indicate that the development of TMJ mechanoreceptors is advanced by the early development of mastication, as has been described for other oral mechanoreceptors. Data from the experimental group indicate that TMJ mechanoreceptors are unable to sustain their function when mechanical masticatory stimulation is decreased. Therefore, the optimum force that is transmitted to the TMJ from the masticatory muscles is essential to sustain their function.

CONCLUSION

- Masseter activity during the growth period may adversely affect sensory mechanisms that are important for normal masticatory function.

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