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

Takayoshi Ishida; Tadachika Yabushita; Kunimichi Soma

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 open-bite 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...
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 temporo-
mandibular loading from masticatory muscles on the 
functional characteristics of TMJ mechanoreceptors 
during early growth in rats. We used a bilateral mas-
seter muscle resection model to weaken the masti-
catory muscle activity. We hypothesized that de-
creased 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 Univer-
sity.

Animal Preparation

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

Before surgery, all animals were anesthetized deep-
ly 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 mas-
seter 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, Auro-
ra, 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 pat-
tern of jaw movement between rats that had under-
gone 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 intraperitone-
ally when a firm pinch to the tail resulted in increased 
respiration and heart rate. Electrophysiologic record-
ings were obtained from the trigeminal ganglion, which 
contains the cell bodies of the trigeminal sensory neu-
rons 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 ste-
reotaxic apparatus (models SN-2 and SM-15M; Nari-
shige Scientific Instrument Lab, Tokyo, Japan) (Figure 
1). For indirect stimulation of the TMJ mechanorecep-
tors 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 maxi-
imum 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 ap-
erture, 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 Paxinos21 for re-
cording single-unit activities of the TMJ mechanore-
ceptors.

Spike signals were recorded and amplified with the 
use of a differential amplifier (DAM-80; WPI, Sarasota, 
Fla, USA), using ×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 po-

tion 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 thiaramyl 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 elec-
trode from the electrolytic markings and signs of elec-
trode penetration (Figure 1).

Data and Statistical Analysis

The effects of reduced compressive force, with the 
use of a bilateral masseter muscle resection model,
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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...
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, and another study reported that the masseter muscle makes up more than 50% of all the masticatory muscles 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%. 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.

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
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. In addition, a previous study suggested that the response of rat periodontal mechanoreceptors (PMRs) is mature by 5 weeks of age. 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, but only a few studies have directly investigated the firing thresholds of the TMJ mechanoreceptors. 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. 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. 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. 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. 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.

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. Mechanoreceptor excitation is accomplished by opening or closing ion channels that are present in the sensory organs. Consequently, alterations in the masticatory environment might result in changes in the properties and morphology of ion channels, such as Mechanosensitive ion channels.

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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