Original Article

Functional Changes of the Temporomandibular Joint Mechanoreceptors Induced by a Lateral Mandibular Shift in Rats

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ABSTRACT

Objective: To investigate changes in functional characteristics of the temporomandibular joint (TMJ) mechanoreceptors under mandibular lateral shift.

Materials and Methods: Forty 7-week-old male Wistar rats were divided into control and experimental groups. Rats in the experimental group received a modified guiding appliance (MGA) that was designed to shift the mandible to the left side in the occlusal position. Single-unit activities of the TMJ mechanoreceptors were evoked by indirect stimulation of passive jaw movement. Electrophysiologic recordings of TMJ units were obtained 1, 3, 5, 7, and 9 weeks after MGA setting from the gasserian ganglion.

Results: At 1 week after mandibular shift, the firing thresholds were the lowest and the maximum instantaneous frequencies were the highest in TMJ units. At 5 weeks, the firing thresholds in the nonshifted side were significantly lower than those in the shifted side. The maximum instantaneous frequencies in the nonshifted side were significantly higher than those in the shifted side at 1, 5, and 7 weeks.

Conclusion: These results suggest that the functional lateral shift of the mandible could alter the response properties of TMJ mechanoreceptors, particularly on the nonshifted side.

KEY WORDS: Temporomandibular joint; Mechanoreceptor; Lateral shift; Primary afferent; Mandible; Rat

INTRODUCTION

Abnormal occlusal conditions, such as functional mandibular lateral shift or occlusal interference, are often associated with craniomandibular dysfunction.[1–3] A functional mandibular lateral shift, which is caused by a premature contact, has effects on morphologic changes in the mandible, temporal bone, temporomandibular joint (TMJ), and masseter muscle.[4–7]

For instance, the attachment of masseter muscles to the mandible and temporal bone is altered by a mandibular lateral shift, leading to deformity of the temporal bone.[4] A functional mandibular lateral shift affects the structure of the condyle and masseter muscle.[5] Moreover, some histologic studies of rat condylar cartilage have reported that a functional lateral shift of the mandible resulted in increased thickness of the condyle cartilage on the nonshifted side while causing decreased growth on the shifted side.[6,7]

Therefore, it can be assumed that a mandibular lateral shift would affect the characteristics of TMJ receptors, particularly the mechanoreceptors. The TMJ mechanoreceptors exist in the lateral and posterior regions of the capsule of the TMJ and are activated by condyle movements.[8,9] These receptors play a role in regulating mandibular position.[10] This study investigat-

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Accepted: July 2006. Submitted: February 2006.

 DOI: 10.2319/020206-39

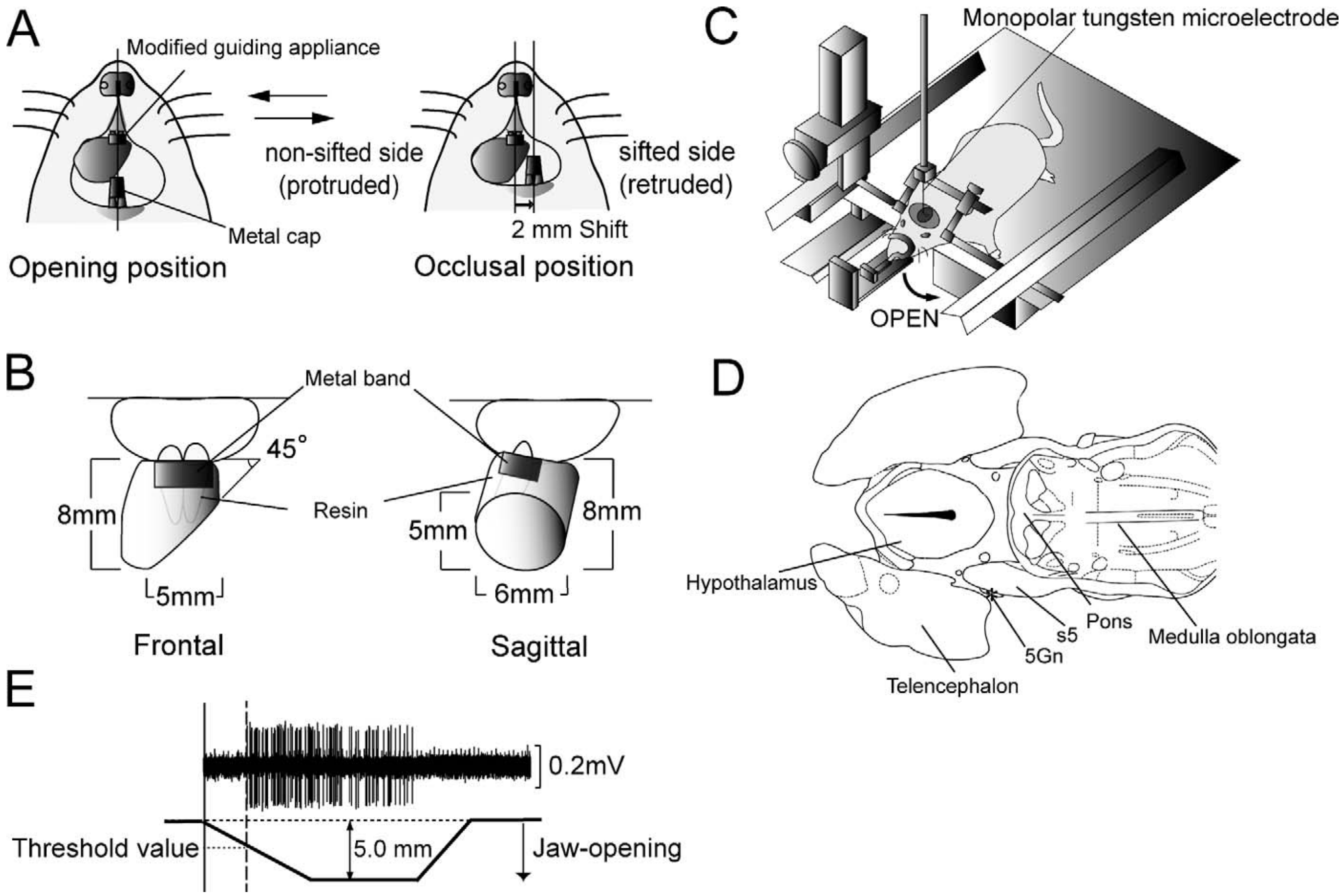

Figure 1. Experimental design. (A) The modified guiding appliance (MGA) in frontal view. The MGA is designed to produce a functional lateral shift of the mandible to the left side at occlusal position. (B) Design of the MGA. The MGA consisted of stainless steel band material and light-curing composite resin, which was attached to the maxillary incisor with light-curing composite resin. (C) Schematic drawing of the experimental setting. The animals had their heads fixed to a stereotaxic frame. A small aperture about 1.0 mm wide was prepared in the skull, and monopolar tungsten microelectrodes were inserted into the gasserian ganglion. A string was attached to the mandible, and ramp-and-hold jaw movement was applied by an automatic pulling machine. (D) Schematic representation of the gasserian ganglion drawn from a horizontal section of the brain over a template from Paxinos and Watson,[12] −9.2 mm below to bregma. An asterisk indicates the recording site. s5, sensory root of the trigeminal nerve; 5Gn, gasserian ganglion. (E) The firing thresholds were calculated as the magnitude of jaw opening observed at the first spike response. Vertical dashed line indicates the first spike of a TMJ mechanoreceptor unit response.

ed the effects of an experimentally-induced mandibular lateral shift on the functional characteristics of the TMJ mechanoreceptors in rats.

MATERIALS AND METHODS

The experimental procedures described here are in agreement with the Animal Care Standards of Tokyo Medical and Dental University and Nagasaki University, and were carried out with approval from their respective Animal Welfare Committees.

Forty male Wistar albino rats (7 weeks old), weighing from 230 to 290 g, were used. All rats were randomly divided into control ($n = 15$) and experimental ($n = 25$) groups.

Animal Preparation for Mandibular Lateral Shift Model

Rats in the experimental group were lightly anesthetized by intraperitoneal injection of thiamylal sodium (Isozol®, Yoshitomi Pharmaceutical, Osaka, Japan; 60 mg/kg). The animals received a modified guiding appliance (MGA), which is designed to produce a functional lateral shift of the mandible to the left side in occlusal position (Figure 1A).

The MGAs (size $5 \times 8 \times 6$ mm, slope angle 45°) consisted of stainless steel band material (0.180×0.005 inch; Rocky Mountain, Denver, Colo) and light-curing composite resin (Clearfil SC, Kuraray, Okayama, Japan) attached to the maxillary incisor with light-curing composite resin (Figure 1B). The mandibular incisors were covered with a metal cap made of the

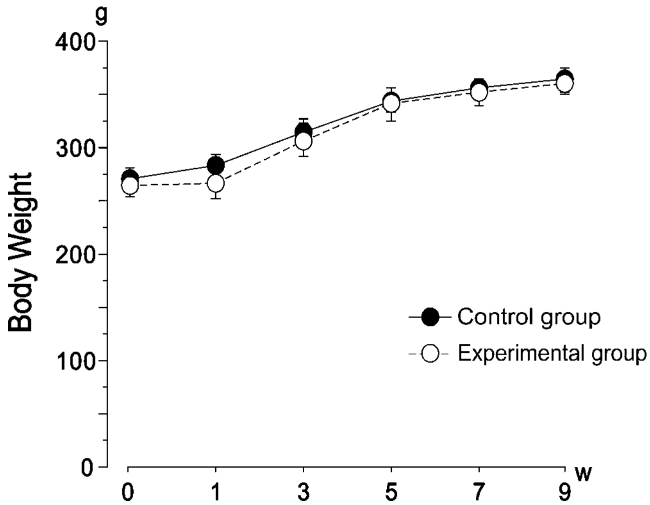

Figure 2. Body weight in control and experimental animals during experimental period. The body weight of animals in the control and experimental groups increased continuously throughout the experimental period, with no statistically significant differences observed between the two groups.

band material to prevent abrasion of the mandibular incisors and maintain the lateral guidance of the mandible. The animals in the control group were also anesthetized but received no MGA. The animals were then returned to their cages and allowed to recover. To clarify the absence of side effects on the development of TMJ mechanoreceptors that could be caused by the growth deceleration as a consequence of the MGA, the body weight of rats in both control and experimental groups was monitored throughout the experimental period (Figure 2). The mean body weights were not significantly different between control and experimental groups.

Recordings

Electrophysiologic recordings were obtained at 1, 3, 5, 7, and 9 weeks after MGA setting in the control and experimental groups. The animals were again anesthetized by intraperitoneal injection of thiamylal sodium (80 mg/kg). The level of anesthesia was monitored by checking pupil size, flexion and corneal reflexes, and heart rate. Additional thiamylal sodium (5.0 mg/kg) was administered by intraperitoneal injection when a firm pinch applied to the tail resulted in increased respiration and heart rate.

The animals were placed into a stereotaxic apparatus (models SN-2 and SM-15M, Narishige Scientific Instruments, Tokyo, Japan) with their bodies in a prone position (Figure 1C). For indirect stimulation of TMJ mechanoreceptors during passive jaw movement, one extreme of cotton thread was fixed to the mandibular symphysis and the other to an automatic pulling machine, as reported elsewhere.[11] The jaw-opening movement was always directed straight downward. The maximum jaw-opening distance was set to 5.0 mm (ramp duration of 5.0 seconds and hold duration of 5.0 seconds). Passive jaw movement was attempted three times per recording unit.

Electrophysiologic recordings were done from the gasserian ganglion, which contains the cell bodies of the trigeminal sensory neuron of TMJ mechanoreceptors. To allow introduction of the recording electrode, the scalp was incised at the midline, and two small apertures about 1.0 mm wide were prepared symmetrically in the skull using a stereotaxic microengine. Monopolar tungsten microelectrodes (250-μm-diameter shaft with 8.0° tapered tip, 5.0 MΩ of AC impedance, A-M Systems Inc, Carlsborg, Washington) were used to record single-unit activities of the TMJ mechanoreceptors. Following stereotaxic coordinates by Paxinos and Watson,[12] recording electrodes were inserted into the gasserian ganglions alternately on the shifted side and the nonshifted side.

Spike signals were recorded and amplified by a differential amplifier (DAM-80, WPI, Sarasota, Florida; $\times$1000 gain, 300 Hz and 3.0 KHz for low and high filters, respectively). All data were captured by means of a CED 1401 interface and stored in a computer hard disk. The data were later analyzed offline with Spike2 software for Windows, Version 4.02a (Cambridge Electronic Design, Cambridge, United Kingdom).

Histologic Identification of the Electrode Position

After each unit recording, the electrode position was marked (50 μA negative current for 10 s). At the end of the experiment, the rats were killed by intraperitoneal injection of thiamylal sodium (120 mg/kg) and the rat brains were removed for histologic sectioning (50 μm frozen sections, cresyl violet staining). The electrode positioning was checked histologically based on the electrolytic markings and signs of electrode penetration (Figure 1D, horizontal section). The electrolytic marking was done for each unit recorded in the 40 rats.

Data and Statistical Analysis

The effects of mandibular lateral shift on TMJ units were assessed by the firing threshold and the maximum instantaneous frequency. The firing thresholds were calculated as the magnitude of jaw opening observed at the first spike response (Figure 1E). The maximum instantaneous frequencies were calculated as the minimum firing interval between two spikes.

All data are expressed as mean $\pm$ SD. The statistical differences between the control group and each experimental group were evaluated by the Mann-Whitney *U*-test with a 95% significance level. The software

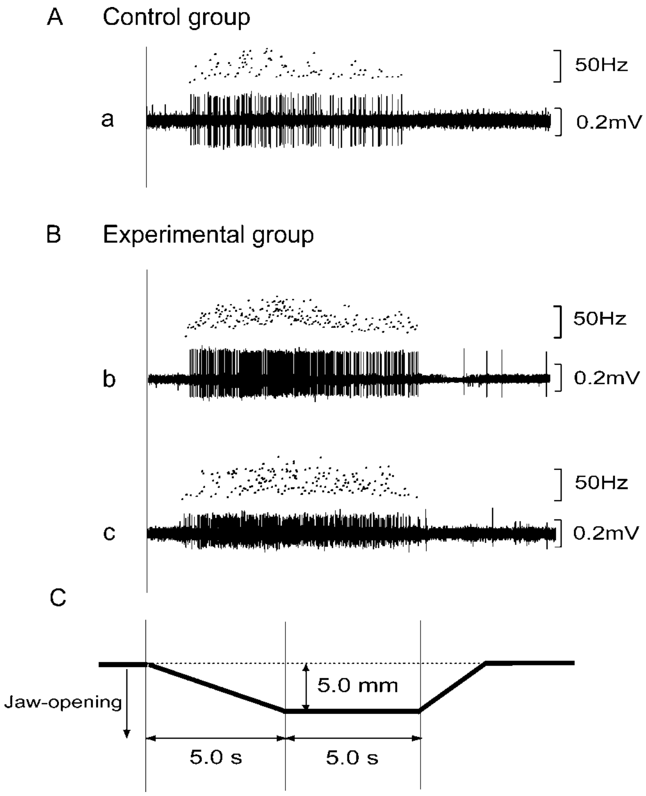

Figure 3. Typical examples of responses from (A) (a) 9-week control group; (B) (b) 9-week shifted group; (c) 9-week nonshifted group. Upper plots of each raw data indicate the instantaneous frequency. (C) Ramp-and-hold jaw opening was applied with maximum opening distance of 5.0 mm; stimulation duration was 10.0 seconds, divided into two phases of 5.0 s each for ramp (open) and hold phases.

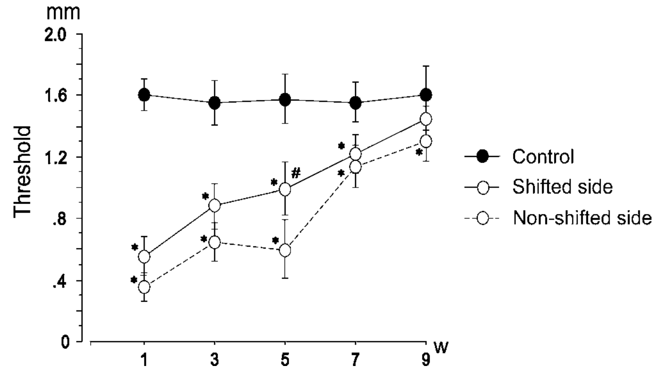

Figure 4. Firing thresholds of TMJ units in control (closed circle) and experimental (open circle) groups. The firing thresholds were significantly lower at 1, 3, 5, and 7 weeks on both sides and at 9 weeks on the nonshifted side versus the control group. At 5 weeks, the firing thresholds on the nonshifted side were significantly lower than those on the shifted side. Asterisks indicate significant difference ($P < .05$) on the shifted side and/or the nonshifted side vs control; number signs indicate significant difference ($P < .05$) between the shifted side and the nonshifted side at that time point.

Statview for Windows, Version 5.0 (SAS Institute, Cary, North Carolina), aided in statistical analysis.

RESULTS

In the experimental group, TMJ unit activities were recorded from 75 units of the sensory neurons of the gasserian ganglion, in both the shifted side and in the nonshifted side; while in the controls, these were recorded from 45 units. Passive jaw movement was attempted three times per recording unit. Analyses of functional changes of the TMJ mechanoreceptor in mandibular lateral shift were performed using three consecutive ramp-and-hold jaw openings from each unit. Typical examples of TMJ units recorded from the gasserian ganglion at 9 weeks in control and experimental groups are shown in Figure 3. It is shown that the first spike response in the nonshifted side was earlier than that in the control group.

Threshold

The firing threshold of the control group was about 1.6 mm during the experimental period.

As for the experimental group, the firing thresholds of the TMJ units were the lowest at 1 week after mandible shift. The firing threshold at 1 week was 0.36 mm in the nonshifted side and 0.55 mm in the shifted side. Over time, the firing thresholds gradually increased in both shifted and nonshifted sides (Figure 4). The firing thresholds were significantly lower in both sides of the experimental group than in the control group at 1, 3, 5, and 7 weeks. At 9 weeks, the nonshifted side of the experimental group was significantly different from the control group. At 5 weeks, the firing thresholds were significantly lower in the nonshifted side than in the shifted side.

Maximum Instantaneous Frequency

In the experimental group, the maximum instantaneous frequencies of TMJ unit discharge had the highest values 1 week after mandible shift and then gradually decreased to baseline in both shifted and nonshifted sides (Figure 5). The maximum instantaneous frequencies were significantly higher in both sides of the experimental group than in the control group at 1, 3, 5, and 7 weeks. At 9 weeks, the values for the nonshifted side of the experimental group were significantly different from those of the control group. In the maximum instantaneous frequencies of the experimental group, the nonshifted side was significantly higher than the shifted side at 1, 5, and 7 weeks.

DISCUSSION

The purpose of this study was to investigate how abnormal occlusal conditions affected the response properties of TMJ mechanoreceptors. The present re-

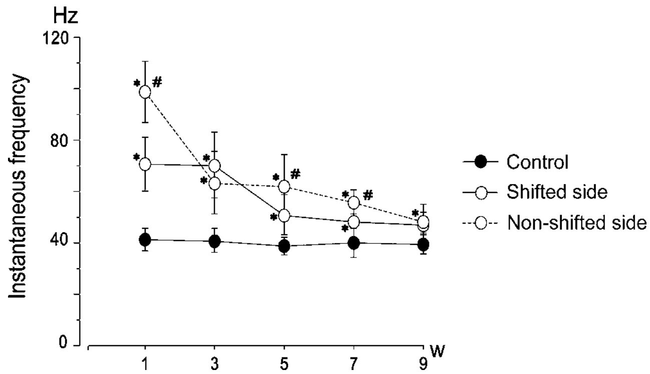

Figure 5. Maximum instantaneous frequencies of TMJ mechanoreceptors in control and experimental groups. The maximum instantaneous frequencies were significantly higher at 1, 3, 5, and 7 weeks on both sides and at 9 weeks on the nonshifted side versus the control group. The maximum instantaneous frequencies on the nonshifted side were significantly higher than those on the shifted side at 1, 5, and 7 weeks. Asterisks indicate significant difference ($P < .05$) on the shifted side and/or the nonshifted side versus controls; number signs indicate significant difference ($P < .05$) between the shifted and nonshifted sides at that time point.

sults suggest that the mandibular lateral shift changed the response properties of the TMJ mechanoreceptors, as measured by firing thresholds and maximum instantaneous frequencies of single-unit activities. It is unlikely that sensory mechanisms were altered by the inhibition of growth, because body weights were not significantly different between the two groups.

In the lateral shift model of this study, the design of the MGA forced the nonshifted-side condyle to become positioned anteriorly (protruded) relative to normal, but the shifted-side condyle was most likely stable in position or even positioned slightly posteriorly (retruded) over time.[6] The tissues around the right and left condyles (capsule, ligaments, and retrodiscal areas) are stretched differently with the lateral shift of the mandible. This could have been one of the factors involved in the different behavior of TMJ mechanoreceptors in the shifted and nonshifted sides in terms of firing frequency during jaw opening.

In a previous study, it was shown that this model of mandibular lateral shift does not cause inflammation of the TMJ.[7] In addition, the maximum jaw-opening distance (5.0 mm) was within the physiologic range of movement of the rats used in this study. Therefore, we believe that our recordings were not from pain receptors and do not reflect pain in the joint.

In the present study, the firing thresholds of TMJ units on the shifted and nonshifted sides were increased by the functional lateral shift of the mandible and gradually recovered during the experimental period. In other mechanoreceptors in the oral region, it has been reported that the mechanical thresholds of periodontal mechanoreceptors were temporarily increased by the loss of occlusal stimuli or tooth movement and recovered during the experimental period.[13,14] This could be caused by several potential factors, such as morphologic alterations or displacement of periodontal mechanoreceptors caused by changes in the periodontium.[14]

Histologic studies have reported that the periodontal mechanoreceptors adapt according to changes in the occlusal conditions.[15,16] The morphology of Ruffini endings, one of the typical mechanoreceptors, is changed by the occlusal interference.[15] Another report has shown that the number of Ruffini endings decreases and their morphology is altered by the loss of occlusal stimuli.[16] Similarly, these explanations might be applied to the changes in the firing thresholds of TMJ units induced by the changes in occlusal conditions.

On the other hand, mandibular lateral shifts have a significant influence on the morphology of the TMJ. A histologic study reported that a functional lateral shift of the mandible resulted in increased thickness of the rat condyle cartilage at 1 week on the nonshifted side and decreased thickness at 2 weeks on the shifted side.[6,7] In addition, the highest level of bone formation of the rat mandibular condyle and glenoid fossa by the anterior displacement of the mandible was observed at 3 weeks.[17,18] The TMJ mechanoreceptors are contained inside the TMJ capsule; therefore, remodeling of TMJ might affect the firing thresholds of TMJ units.

At 5 weeks, the firing threshold of TMJ units was significantly lower on the nonshifted side than on the shifted side. Other studies showed that the density of Ruffini endings decreased significantly 4 weeks after the lumbar facet joints were surgically fixed in a rabbit model.[19] On the other hand, in the case of immobilized rabbit knee joints, the morphologic change occurred in the nerve endings and the number of mechanoreceptors was significantly decreased after 6 weeks.[20] The mandibular condyle movement in a laterally shifted mandible was more restricted on the nonshifted side compared to the shifted side during jaw movement.[21]

Therefore, our findings that the firing thresholds on the nonshifted side were greatly recovered after 5 weeks could be attributed to the morphologic changes in TMJ mechanoreceptors by the restriction of mandibular condyle movement in comparison with the control group. The sensitivity of TMJ units was increased by a reduction in firing thresholds and an increase in maximum instantaneous frequency after mandibular lateral shift.

CONCLUSIONS

a. Functional lateral shift of the mandible altered the response properties of TMJ mechanoreceptors, and these changes were more pronounced in the nonshifted side than in the shifted side.

b. Changes in the sensitivity of TMJ units in the nonshifted side are long-lasting, if not permanent.

c. The present study suggested that a mandibular lateral shift may adversely affect sensory mechanisms that are important for normal masticatory function.

ACKNOWLEDGMENT

This work was partly supported by a Grant-in-aid for Scientific Research (14370688, 2002-2003, and 16209061, 2004-2006) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. A part of this work was presented at the 28th Annual Meeting of the Japan Neuroscience Society, Yokohama, Japan, July 26-28, 2005 (Kokai S, et al. Changes in response properties of TMJ mechanoreceptor by lateral functional shift of the mandible in rats. *Neurosci Res.* 2005;52:S194).

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