Original Article

Functional adaptability of temporomandibular joint mechanoreceptors after an increase in the occlusal vertical dimension in rats

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ABSTRACT

Objective: To investigate the effects of an experimentally-induced increase in the occlusal vertical dimension (iOVD) on the functional characteristics of temporomandibular joint (TMJ) mechanoreceptors in rats.

Materials and Methods: Sixty 13-week-old male albino Wistar rats were divided into control and iOVD groups (30 animals each). The vertical dimension between the maxillary and mandibular molars in the iOVD group was increased by 2.0 mm with a build-up of resin on the maxillary molars. Single-unit activities of TMJ mechanoreceptors were evoked by passive jaw movement. Recording was performed from the gasserian ganglion 1 day and 1, 3, 5, 7, and 9 weeks after the establishment of iOVD.

Results: Compared with the control group, the firing threshold was significantly lower at 1, 3, and 5 weeks after iOVD in the iOVD group. There were no significant differences in the firing threshold at 1 day, or 7 or 9 weeks. The maximum instantaneous firing frequency was significantly higher at 1, 3, and 5 weeks after iOVD in the iOVD group, but there were no significant differences at 1 day, or 7 or 9 weeks. There were no significant differences in the average firing frequency during the experimental period.

Conclusions: The present study findings suggest that TMJ mechanoreceptors in adult rats may ultimately adapt to iOVD. (*Angle Orthod.* 2011;81:453–459.)

KEY WORDS: Temporomandibular joint; Mechanoreceptor; Occlusal vertical dimension

INTRODUCTION

The clinical success of orthodontic/prosthodontic treatment may sometimes be associated with the vertical changes in occlusion.[1–5] Many previous studies have reported that a growing subject with Class II malocclusion enjoys benefits from an intentional increase in the occlusal vertical dimension (OVD) with a functional appliance, which encourages both horizontal and vertical mandibular growth.[6,7] On the other hand, adult subjects may also benefit from an increase in OVD. For instance, it has been reported that an intentional increase in facial height can be produced by orthodontic extrusion of the posterior teeth to correct deep overbite[2] and an Angle Class III molar relationship.[8] Thus, a change in the vertical dimension of occlusion may be an effective treatment choice for functional and/or esthetic purposes.[8] However, the long-term stability after treatment with increased OVD (iOVD) is still controversial on the long-term basis.[9,10] Therefore, a better understanding of the posttreatment stability of iOVD is important in the treatment planning in subjects with occlusal problems in the vertical dimension.

Sensory inputs from low-threshold orofacial proprioceptors such as the muscle spindles and mechanoreceptors in the temporomandibular joint (TMJ) are important afferents in the regulation of OVD.[11,12]

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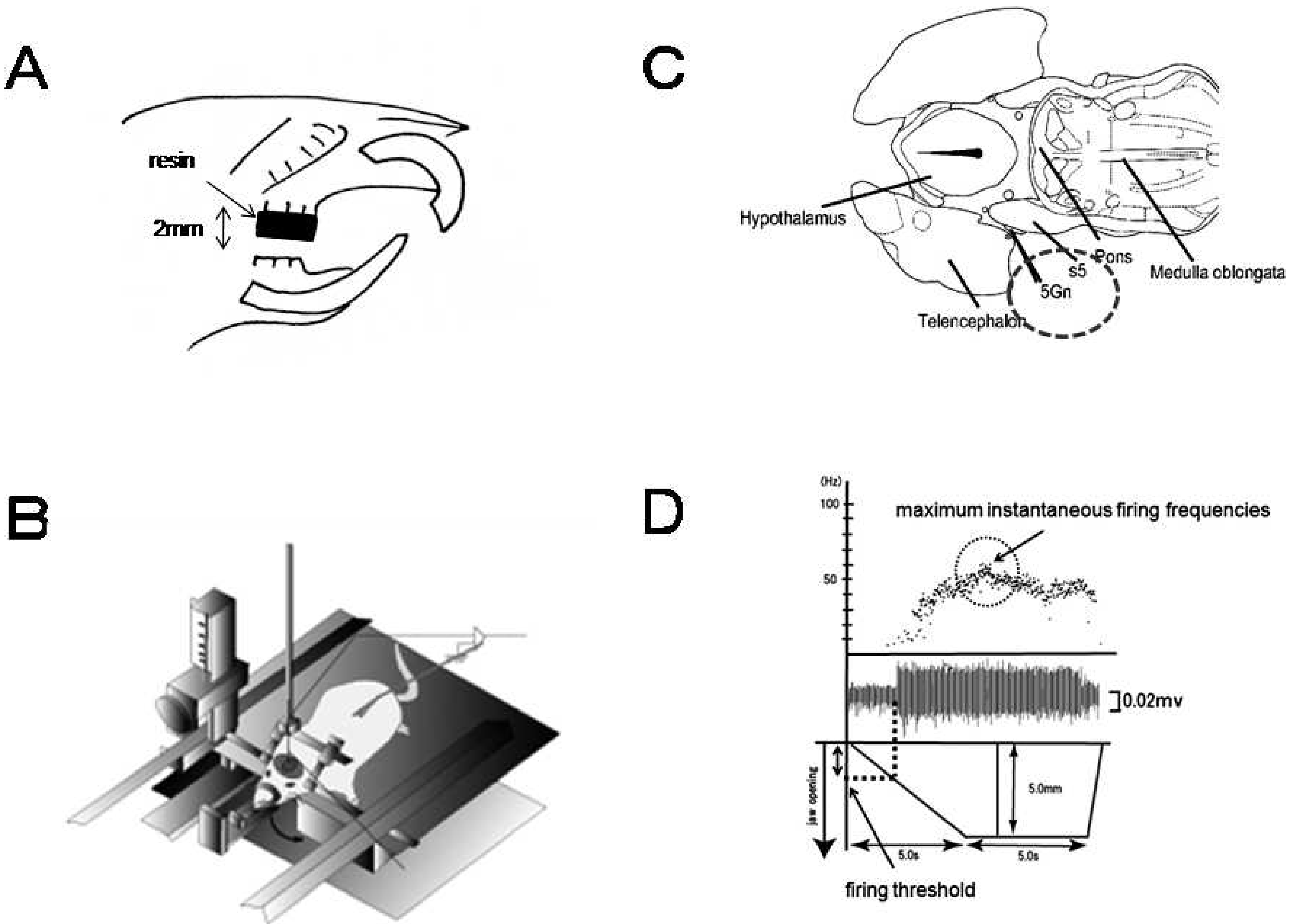

Figure 1. Experimental design. (A) Schematic drawing of preparation of the animal in the iOVD group. The vertical dimension between the maxillary and mandibular molars was increased by 2.0 mm with build-up of resin on the maxillary molars. (B) Schematic drawing of the experimental set-up. The animal's head was fixed to a stereotaxic frame. A small aperture, about 3.0 mm wide, was prepared in the skull, and monopolar tungsten microelectrodes were inserted into the trigeminal ganglion. A thread was attached to the mandible, and ramp-and-hold jaw movement was achieved by means of an automatic pulling machine. (C) Schematic representation of the trigeminal ganglion drawn from a horizontal section of the brain −9.2 mm below the bregma. * indicates the recording site; *s5*, sensory root of the trigeminal nerve; and *5Gn*, trigeminal ganglion. (D) The firing thresholds were calculated as the magnitude of jaw-opening observed at the first spike. A vertical dashed line indicates the first spike from a TMJ unit.

Yabushita and colleagues[13] used an animal model of iOVD to investigate the change in the sensitivity of afferents from muscle spindles of the masseter muscle. They found that there were no significant long-term differences in the firing rate of the units.

However, there have been few studies on the functional characteristics of the TMJ mechanoreceptor after iOVD. Therefore, we tested the possibility of functional adaptability in TMJ mechanoreceptors over the long-term after iOVD.

MATERIALS AND METHODS

The experimental procedures described here were approved by the Animal Welfare Committee (10024) and performed in accordance with the Animal Care Standards of Tokyo Medical and Dental University. Sixty male Wistar albino rats (13 weeks old) were used. They were randomly divided into a control group ($n = 30$) and an iOVD group ($n = 30$).

Animal Preparation

Animals in the iOVD group were lightly anesthetized with thiamylal sodium (Isozol, Yoshitomi Pharmaceutical, Osaka, Japan; 60 mg/kg, intraperitoneally). The vertical dimension between the maxillary and mandibular molars was increased by 2.0 mm with build-up of resin on the maxillary molars. The occlusal surfaces of the mandibular molars were coated with fluid resin to prevent a reduction of the vertical height due to abrasive movement of the mandible (Figure 1A). This model was the same as that used in previous studies.[13] The animals were then returned to their cages and allowed to recover from anesthesia. The body weight of rats in both the control and the iOVD groups was monitored throughout the experimental period.

Stimulation and Recording

Electrophysiologic recordings were performed 1 day and 1, 3, 5, 7, and 9 weeks ($n = 5$ each) after iOVD in the iOVD group and in the corresponding time points in the control group. The animals were again anesthetized by the intraperitoneal injection of thiamylal sodium (80 mg/kg). The level of anesthesia was monitored by checking the pupil size, flexion and corneal reflexes, and the heart rate. Additional thiamylal sodium (5.0 mg/kg) was administered by intraperitoneal injection when a firm pinch applied to the tail resulted in the increased respiratory and heart rates.

The animal was placed in a stereotaxic apparatus (models SN-2 and SM-15M, Narishige Scientific Instruments, Tokyo, Japan) with the body in a prone position (Figure 1B). For the indirect stimulation of TMJ mechanoreceptors during passive jaw movement, one end of a cotton thread was fixed to the mandibular symphysis and the other was attached to an automatic pulling machine.[14–16] 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) from the rest position.

Passive jaw movement was attempted three times per recording session. Stimulation was performed from the position at which the lower jaw was loosened by anesthesia. We confirmed that the mandibular position from which jaw-opening started in the iOVD group was identical to that in the control group by an objective evaluation using cephalometric images.[17]

Recordings of sensory units were performed from the gasserian ganglion, which contains the cell bodies of the trigeminal sensory neurons of the 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, Wash) were used to record single-unit activities of TMJ mechanoreceptors. The recording electrode was inserted into the gasserian ganglion with reference to the stereotaxic coordinates[18] previously reported for the recording of single-unit activities of TMJ mechanoreceptors.[14–16] Electrical stimulation of the auriculotemporal nerve evoked responses with a latency of 0.125 ± 0.01 millisecond in the nucleus of the gasserian ganglion. The conduction distance from the site of stimulation to the recording electrode in the gasserian ganglion was estimated to be 5.0 mm; therefore, the mean conduction velocity recorded in the afferents was 40.5 ± 3.5 m/s, which indicates that these were probably large myelinated (Aβ) fibers.[19] The gasserian ganglion also contains the cell bodies of the trigeminal sensory neurons from periodontal mechanoreceptors.[20] However, we did not stimulate the teeth in the present study, and the recording of sensory units was performed exclusively from TMJ mechanoreceptors.

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

Histologic Identification of the Electrode Position

After each recording, the electrode position was marked (50 μA negative current for 10 seconds). At the end of the experiment, the rats were sacrificed by the intraperitoneal injection of thiamylal sodium (120 mg/kg), and the brains were removed and embedded in paraffin, cut into 5-μm sections, and then stained with cresyl violet. The position of the tip of the electrode was confirmed histologically based on electrolytic markings and signs of electrode penetration (horizontal section) (Figure 1C). Electrolytic marking was performed for each unit recorded in the 60 rats.

Data Analysis

The effects of iOVD on TMJ units were assessed using the firing threshold, the maximum instantaneous firing frequency, and the average firing frequency. The firing threshold was calculated as the amount of jaw opening when the first spike response was observed. The maximum instantaneous firing frequency was calculated as the minimum firing interval between two consecutive spikes (Figure 1D). Average firing frequency was calculated as the average of the instantaneous firing frequency from 5 seconds (maximum jaw-opening) to 6 seconds.

The significance of differences between the control and iOVD groups was evaluated by the Mann-Whitney *U*-test with a 95% significance level. The software Statview for Windows, Version 5.0 (SAS Institute, Cary, NC) was used in the statistical analysis.

RESULTS

There was no significant difference in mean body weight between control and iOVD groups throughout the experiment. In both the iOVD and control groups, firing activities were recorded from 60 TMJ units. Typical examples of TMJ units recorded from the gasserian ganglion at 1 day and 9 weeks in the control groups and at 1 week and 9 weeks in the experimental groups are shown (Figure 2).

Firing Threshold

The firing threshold in the control group was 1.59 ± 0.06 mm during the experimental period. In the iOVD group, the firing thresholds of TMJ units were lowest at 1 week after iOVD (0.98 ± 0.19 mm). Over time, the firing thresholds gradually increased: 0.98 ± 0.19 mm at 1 week, 1.16 ± 0.08 mm at 3 weeks, 1.28 ± 0.08 mm at 5 weeks, 1.51 ± 0.09 mm at 7 weeks, and 1.59 ± 0.04 mm at 9 weeks. The firing thresholds were significantly lower in the iOVD group than in the control group at 1, 3, and 5 weeks after iOVD. At 1 day and 7 weeks after iOVD, there were no significant differences between the two groups (Figure 3).

Maximum Instantaneous Firing Frequency

The maximum instantaneous firing frequency in the control group was 51.41 ± 3.26 Hz during the experimental period. In the iOVD group, the maximum instantaneous firing frequency of TMJ units was higher (95.56 ± 18.31 Hz) than that in the control group at 1 week after iOVD. The values were highest recorded 1 week after iOVD and then gradually decreased to the levels in the control group: 95.56 ± 18.31 Hz at 1week, 73.23 ± 4.70 Hz at 3 weeks, 59.73 ± 3.17 Hz at 5 weeks, 55.68 ± 2.54 Hz at 7 weeks, and 51.98 ± 2.03 Hz at 9 weeks. At 1 day and 7 weeks after iOVD, there were no significant differences between both groups (Figure 4A).

Average Firing Frequency

The average firing frequency in the control group was 51.15 ± 3.26 Hz during the experimental period. The average firing frequency in the iOVD group was 51.70 ± 6.94 Hz during the experimental period. There were no significant differences between both groups (Figure 4B).

DISCUSSION

The present findings indicate that TMJ mechanoreceptors have a high degree of adaptability as measured by firing thresholds, maximum instantaneous firing frequencies, and average firing frequency of single-unit activities. iOVD altered the responses of TMJ mechanoreceptors, as measured by firing thresholds, maximum instantaneous firing frequencies, and average firing frequency of the single-unit activities. Since there was no significant difference in body weight between the two groups, iOVD itself did not affect systemic growth, and it is unlikely that there was a functional development of sensory mechanism due to a difference in systemic growth.

A previous study suggested that masseter muscle spindle function might ultimately adapt to iOVD after 7 weeks of perturbation,[13] which is consistent with our findings. The initial increase in the maximum instantaneous firing frequency in TMJ mechanoreceptors was greater (approximately 86%) than in masseter muscle spindles (approximately 14%).[13] The sensitivity of muscle spindles is centrally controlled by the alpha-gamma system and responds to peripheral stimuli.[21] In contrast, the sensitivity of the TMJ mechanoreceptors is not regulated by the central nervous system. The lack of a central regulatory mechanism may account for the huge increase in the maximum instantaneous firing frequency of TMJ mechanoreceptors during the initial phase after iOVD in the iOVD group.

In the iOVD group, the firing threshold of TMJ units was the lowest at 1 week after iOVD, and then it gradually increased. The response properties of TMJ mechanoreceptors change in association with an altered masticatory environment such as low masticatory function.[16] For instance, a change in the consistency of food to a liquid diet has been reported to increase the physiologic response of the TMJ, resulting in a continuous decrease in the threshold of the TMJ units.[16] In this study, the threshold of TMJ units decreased during the first phase and then increased to the original level during the second phase. Although it appears as though the physiologic function of the TMJ was reduced during the first phase, it recovered to show a normal response during the second phase. Thus, it seems that the amount of iOVD in the present study (ie, 2 mm) was within the tolerance limit for the TMJ mechanoreceptors in the rat.

The maximum instantaneous firing frequency was highest at 1 week after iOVD and then gradually decreased to the levels in the control group. In a previous study, it was reported that remodeling of the condyle was caused by forward mandibular positioning.[22] Therefore, we can speculate that the mechanical stimulation by iOVD could induce remodeling in the TMJ. However, morphologic analyses are necessary to prove this assumption. The maximum instantaneous firing frequency was significantly higher at 1, 3, and 5 weeks after iOVD in the iOVD group than in the control group. But there were no significant differences in the average firing frequency between both groups. This indicates that there was an irregular firing activity in the iOVD group compared with the control group (eg, Figure 2Bc). Neural signals of somatic sensations begin with the excitation of mechanoreceptors in the

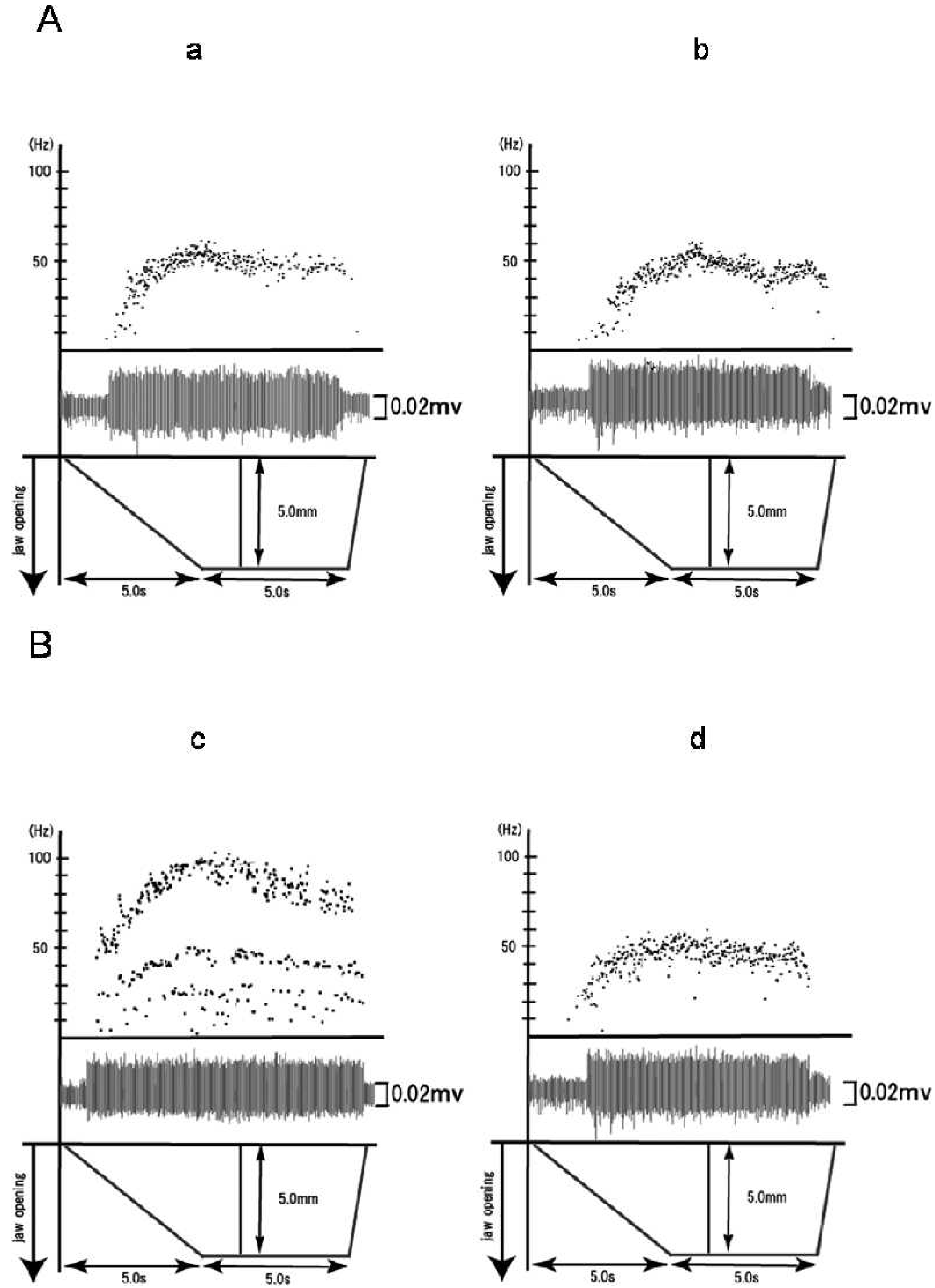

Figure 2. Typical examples of responses from the (A) control and (B) iOVD groups. (a) 1-day control group; (b) 9-week control group; (c) 1-week iOVD group; (d) 9-week iOVD group. Upper plots of raw data show the instantaneous firing frequency. The ramp-and-hold jaw-opening was applied with maximum opening distance of 5.0 mm. Ramp duration was 5.0 seconds and hold duration was 5.0 seconds.

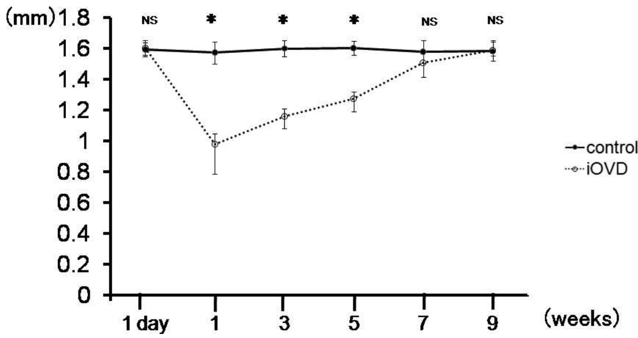

Figure 3. Firing threshold TMJ units in the control (10 units per group) and iOVD groups (10 units per group). Firing thresholds were significantly lower at 1, 3, and 5 weeks after iOVD in the experimental group. There were no significant differences at 1 day, or 7 or 9 weeks. * indicates significant between the iOVD and control groups; NS, not significant between the iOVD and control groups; bars, standard deviation of the means; solid line, control group; and broken line, iOVD group.

sensory nerve, and the excitation of mechanoreceptors is accomplished by the opening or closing of ion channels.[23] Therefore, we speculate that iOVD induces temporary degeneration of TMJ mechanoreceptors, which finally adapts. Moreover, the insignificant temporal change in the average firing frequency between both groups may indicate the final functional adaptation.

It has often been suggested that inputs from the TMJ mechanoreceptors are involved in the physiologic mechanism of OVD regulation. Thus, TMJ mechanoreceptors play a role in regulating mandibular position. In this study, there was no significant difference between the sensitivity of the TMJ mechanoreceptors in the normal rats and those who underwent long-term iOVD. Although the present findings suggest that the TMJ mechanoreceptors in adult rats may ultimately adapt to iOVD, the rat TMJ has a different character compared with the human one. For example, a unilateral condylectomy in rats reduced growth of the mandible and a subsequent lateral shift to the affected side, but the reduced growth and the lateral shift of the mandible were eliminated by a functional appliance, and prominent regeneration of the condyle also occurred.[24] These are not adopted in human condyle. Thus, caution should be exercised when the present findings are applied to humans.

CONCLUSIONS

- iOVD may temporarily alter the properties of TMJ mechanoreceptors.
- Findings in the present study suggest that the TMJ mechanoreceptors in adult subjects may ultimately adapt to iOVD.

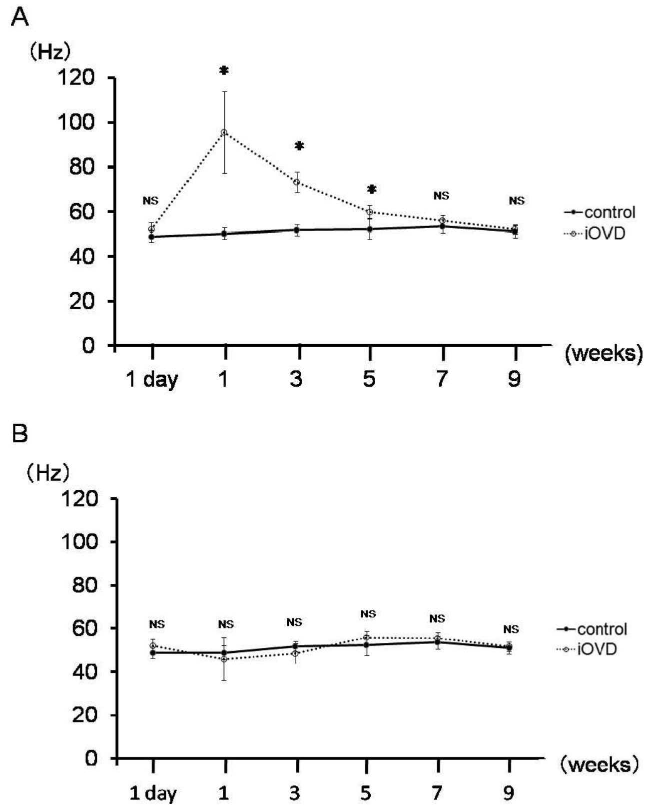

Figure 4. (A) Maximum instantaneous firing frequencies of TMJ units in the control (10 units per group) and iOVD groups (10 units per group). Maximum instantaneous firing frequency was significantly higher at 1, 3, and 5 weeks after iOVD in the experimental group. There were no significant differences at 1 day, or 7 or 9 weeks. * indicates significant between the iOVD and control groups; NS, not significant between the iOVD and control groups; bars, standard deviation of the means; solid line, control group; and broken line, iOVD group. (B) Average firing frequencies of TMJ units in the control (10 units per group) and iOVD groups (10 units per group). There were no significant differences during the experimental period. NS indicates not significant between the iOVD and control groups; bars, standard deviation of the means; solid line, control group; and broken line, iOVD groups.

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