

**Original**

## Temporal change in the occlusal vertical dimension and its involvement in modulation of jaw movement in bite-reduced animals

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**Abstract:** The occlusal vertical dimension (OVD) in guinea pigs is maintained by tooth eruption and grinding. It has been reported that the experimentally raised OVD recovers to the innate OVD within a few days in guinea pigs. However, the mechanisms underlying OVD adjustment are not entirely understood. This study thus aimed to clarify whether the experimentally reduced OVD would recover. Bite-reduced guinea pigs were created by applying bilateral intermaxillary elastics for 10 days. Guinea pigs without elastics were used as a control. The OVD after removal of the elastics in the experimental group was compared with that of the control group. Jaw movement during chewing was also compared between the experimental and control groups. After removal of the elastics, the experimentally reduced OVD did not recover fully and a significant difference was observed between the experimental and control groups for up to 25 days during the recording period. The minimum closed position during chewing was significantly higher in the experimental group than in

the control group, whereas the maximum open position was no different between the groups. The present findings indicated that the experimentally reduced OVD could not be fully recovered, suggesting that reduction of the OVD may have limited influence on jaw movement.

Keywords: occlusal vertical dimension; jaw movement; guinea pig.

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### Introduction

An appropriate occlusal vertical dimension (OVD) is known to have a pivotal role in various oral functions (1). It has been reported that the raised OVD recovered to the innate OVD level within a few days in experimentally bite-raised animals (2,3). Jaw movement analysis revealed that the raised OVD did not affect masticatory rhythm but rather decreased the gape magnitude, and the maximum opening position was maintained. These results suggested a strong relationship between the central program of the masticatory pattern and the innate OVD (2). However, the masticatory muscle activity is altered following bite increase (3), suggesting that the raised OVD may be a risk factor for the impairment of oral function.

In contrast, it has been reported that excessive reduction of the OVD can cause temporomandibular joint disorders, hearing disorders, insomnia, and impaired

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attention (4). However, the pathophysiological mechanisms underlying these dysfunctions remain unknown. A previous study demonstrated that the occlusal phase of the chewing cycle was shortened in bite-reduced rabbits, resulting in a decrease in masseter muscle activity (Morimoto T et al. *Alpha and gamma motor systems*, 409-411, Plenum, New York, 1995).

Imai et al. (5) and Chen et al. (6) used intermaxillary elastic bands unilaterally to create animal models of deviated jaw associated with unilateral shorting of the teeth crowns. In this model animal, the occlusal relationship can be maintained with no pathological changes in the teeth. Thus, in this study, bilateral intermaxillary elastics were employed to develop bite-reduced model animals while maintaining the occlusal relationship without teeth damages.

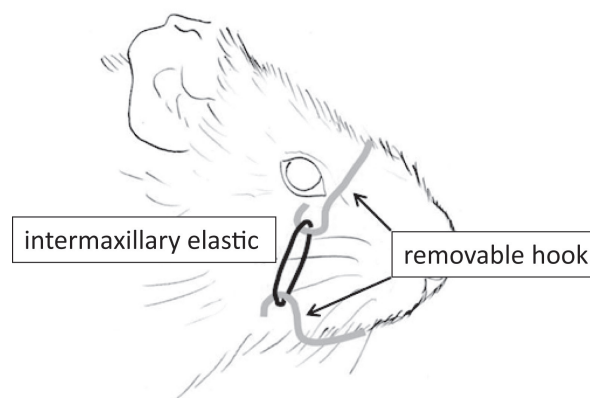
The aim of the present study was to investigate the temporal changes in OVD and evaluate their involvement in the modulation of jaw movement during chewing in bite-reduced model animals.

## Materials and Methods

Four-week-old male Hartley guinea pigs were used in this study. The OVD was measured in bite-reduced model animals, and the change in jaw movement during chewing was investigated. Nine guinea pigs with a reduced OVD were in the experimental group, and seven guinea pigs without reduced bite were in the control group. The animals were kept in an experimental cage under controlled light conditions (from 6 am to 6 pm) at room temperature of 26°C for 1 week to allow them to acclimatize. Two or three animals were housed in one cage, and they had free access to water and solid food. All experimental procedures were approved by the Committee on Animal Research at Matsumoto Dental University (No. 216-13).

### Surgical procedures

Atropine (4 mg/kg, atropine sulfate, Mitsubishi Tanabe Pharma Corporation, Osaka, Japan) and pentobarbital sodium (30 mg/kg, i.p., Nembutal, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) were administered intraperitoneally. The parietal skin was cut, and the periosteum was scraped off to place four small stainless steel screws (1.4 mm in diameter and 3 mm in length) in the frontal and parietal skulls, respectively. An aluminum pipe (7.5 mm in outer diameter and 5.0 mm in inner diameter, Narishige Scientific Instrument Lab, Tokyo, Japan) was fixed in the parietal skull using dental quick cure resin (ADFA, Shofu, Kyoto, Japan) to make the pipe parallel to the vertical line connecting both sides of the eyeballs.

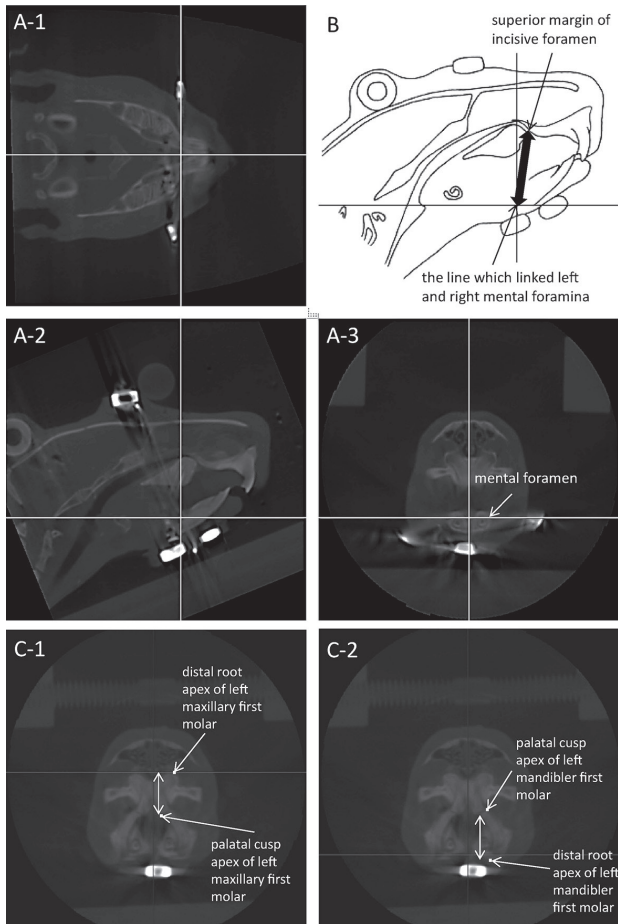


**Fig. 1** During the application of intermaxillary elastics.

A nut was fixed using dental quick cure resin with small stainless steel screws in the frontal skull. The pipe and a modified ear bar (Narishige Scientific Instrument Lab), as well as nuts and fixation screws (3 mm in diameter and 10 mm in length), were used for fixation of the head in a stereotaxic apparatus to make the Frankfurt plane parallel to the floor. The mandibular skin was incised, and the underlying periosteum was scraped off to place the light-emitting diode (LED) attachment in the mandible for jaw-movement recording (combination of nut and magnet, 4 mm in diameter and 2 mm in thickness). Two small stainless steel screws were implanted in the lower surface of the mandible to use as abutments for fixation of the attachment using dental quick cure resin. This method allowed fixation of the animals' head in the stereotaxic apparatus without anesthesia for daily recording of the mandibular position. Co-Cr orthodontic wire (1.0 mm in diameter, Dentsply Sankin, Otawara, Japan) was bent to form hooks at both ends and was implanted into the resin on the head and mandible. An intermaxillary elastic band was attached to the hook at 40 g so that the elastic band was perpendicular to the occlusal plane (Fig. 1).

### OVD measurement using micro-computed tomography (micro-CT)

Three-dimensional micro-CT (R-mCT, Rigaku Inc., Tokyo, Japan) images were taken according to the parameters established by Kanayama et al. (2) to measure the OVD. Radiographic conditions consisted of a tube voltage of 90 kV, tube current of 110 mA, and pixel size of  $100 \times 100 \times 100 \mu\text{m}$ . A head fixation device was used to keep the head position stable during measurement, and micro-CT images were taken before placement of the intermaxillary elastics and on days 0, 1, 4, 7, 11, 14, 18, 21, and 25 after their removal under sodium pentobarbital anesthesia (30 mg/kg, i.p.). The micro-CT volume data were calculated from 1.0-mm-thick slice images



**Fig. 2** A: CT image for OVD measurement. A-1: Horizontal sectional image, A-2: Median sagittal sectional image, A-3: Frontal sectional image. B: Schematic representation of median sagittal sectional image. The length of the arrow indicates the OVD. C: The tooth length measured on a CT image (frontal sectional image). The lengths of the arrows indicate the lengths of the upper (C-1) and lower (C-2) first molars.

at 0.5-mm intervals using image management software (i-View, Morita Inc., Kyoto, Japan). The horizontal plane was set to be parallel to the plate plane, the sagittal plane was set at the upper bilateral incisors, and the palate was in line (Fig. 2A1-3). The plane perpendicular to the horizontal and sagittal axes was defined as the frontal plane, and the reference plane of the measurement was determined. The midpoint of the bottom margin of the left and right mental foramina and the upper margin of the incisive foramen were confirmed on the sagittal plane. The distance between these two points was defined as the OVD (Fig. 2B).

### Measurement of tooth length

The distance between the distal palatal cusp and inferior point of the distal root of the upper and lower left first molars was measured and defined as the tooth length

(Fig. 2C1, 2). The measurement was performed before intermaxillary elastic application and on days 0, 1, 4, 11, 14, 18, 21, and 25 after removal of the elastics.

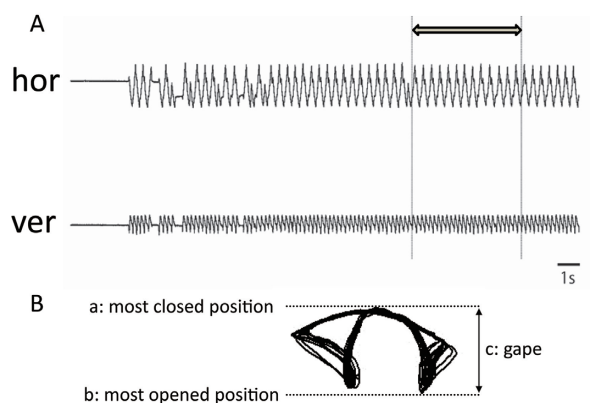
### Measurement of jaw movement

A position sensor system (C5949, Hamamatsu Photonics, Hamamatsu, Japan) was used to record the jaw movement. The animal's head was fixed to the stereotaxic apparatus, and an LED was fixed to the attachment in the mandible. A charge-coupled device (CCD) camera and stereotaxic apparatus were fixed using dental quick cure resin (Tray Resin II, Shofu) to secure their position. Since their heads were fixed to the stereotaxic apparatus, the animals could not ingest food on their own. The food was placed in the mouth with a syringe, and the animals were allowed to chew. The trajectories of jaw movements during chewing were analyzed using waveform management software (Spike 2, Cambridge Electronic Design Limited, Cambridge, UK) with 500-Hz sampling frequency. Jaw-movement was recorded before application of the intermaxillary elastics and on days 0, 1, 4, 7, 11, 14, 18, 21, and 25 after their removal.

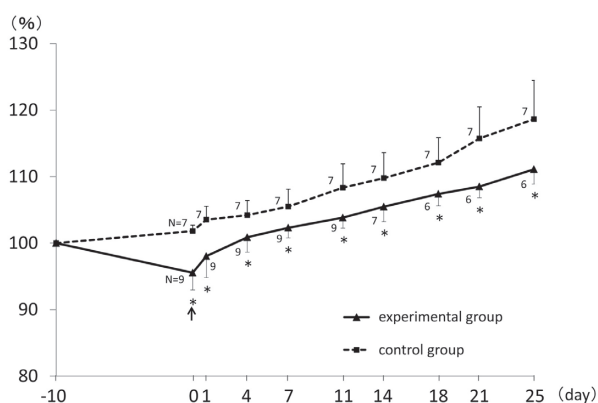
### Analysis of jaw-movement trajectories

In guinea pigs, jaw movements during chewing have been classified into two patterns: movements with large alternative bilateral jaw shifts (figure-eight shape) and those with large unilateral shifts (circle shape) (7). In our preliminary study, the duration of chewing movements was approximately 2 min, and the number of unilateral circle shape chewing cycles decreased while that of bilateral figure-eight shape chewing cycles increased with the progression of each masticatory sequence. To obtain stable recordings of the jaw movements, we analyzed consecutive chewing cycles for 5 s for the bilateral chewing movement within a total record time of 25 s (Fig. 3A).

The following variables were analyzed: 1) the minimum closed position relative to the skull during chewing (Fig. 3Ba), 2) the maximum open position relative to the skull during chewing (Fig. 3Bb), 3) the distance from the minimum closed to the maximum open position in each cycle (the gape magnitude) (Fig. 3Bc), and 4) the time between two consecutive minimum closed positions (total cycle length [TCL]). These analyses were also performed by the waveform management software Spike 2. The averages of the above four variables were calculated, and Mann-Whitney *U* tests were performed to compare these variables between the experimental and control groups using statistical analysis software (SPSS, SPSS Japan Inc., Tokyo, Japan).



**Fig. 3** A: Jaw movement during natural chewing (horizontal and vertical components). The period indicated by the arrows was analyzed. B: Jaw movement trajectory recorded in the frontal plane. a, b, and c indicate analyzed items.



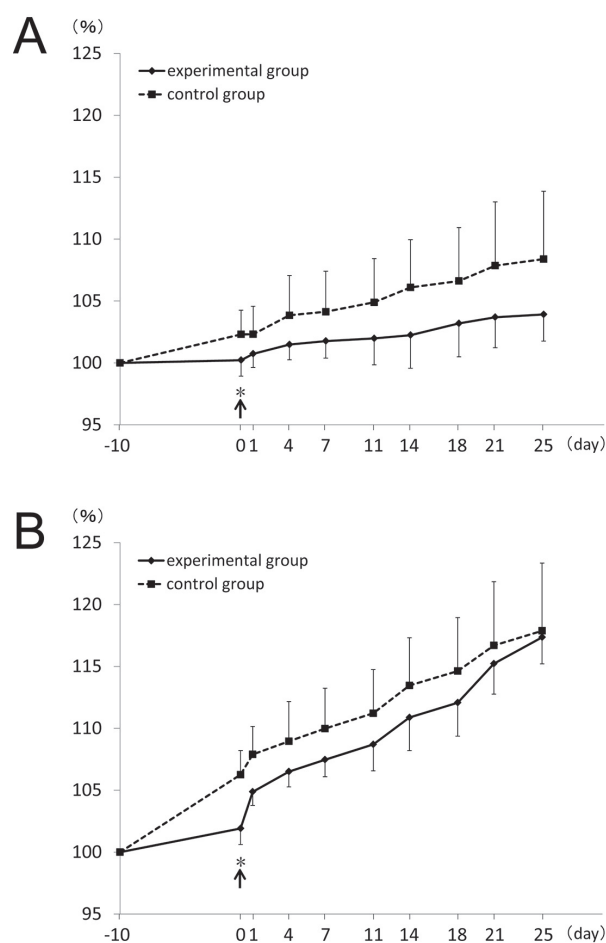
**Fig. 4** Change in OVD. Change in OVD is expressed as the relative value to the OVD before application of elastic (the OVD before application of elastic was defined as 100%). The arrow in the figure indicates the day of elastics removal (day 0). *N*: The number of animals at each recording point, \* $P < 0.05$ , *U* test.

## Results

### Temporal changes in the OVD

Temporal changes in the OVD throughout the experimental periods are shown in Fig. 4. The OVD decreased by approximately 5% (0.56 mm on average) relative to that before application of the intermaxillary elastics, on the day of the removal of elastics (day 0 indicated by the arrow in Fig. 4) in the experimental group. The increment ratio of the OVD was significantly greater on days 1-4 after the removal of elastics than that on days 4-25 ( $P < 0.05$ ). A significant difference was observed in the OVD between the experimental and control groups on days 0-25 after removal of the elastics ( $P < 0.05$ ), indicating that the reduced OVD did not return to the innate OVD during the observation periods.

The OVD of the control animals consistently increased



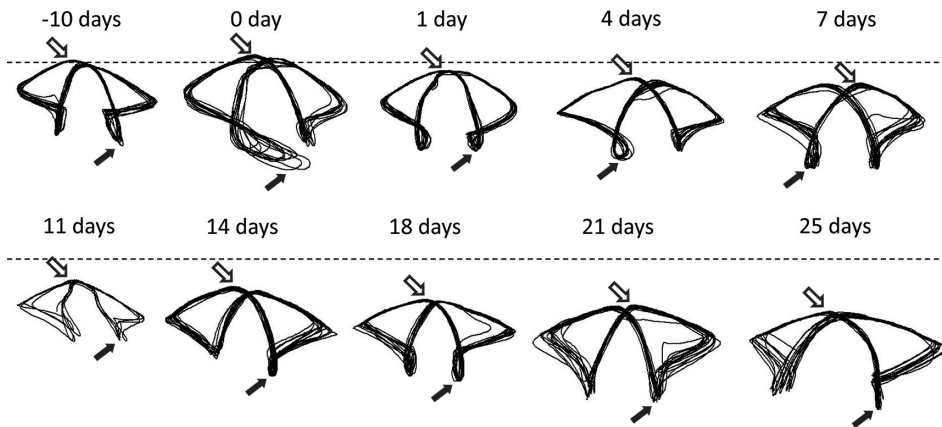
**Fig. 5** Change in tooth length is expressed as the relative value to the tooth length before application of elastic (the tooth length before application of elastic was defined as 100%). The arrow in the figure indicates the day of elastics removal (day 0). A: Upper first molar and B: lower first molar, \* $P < 0.05$ , *U* test.

and reached approximately 120% of that before application of the elastics at the end of the experimental period (day 35), whereas that of the experimental group was about 110%.

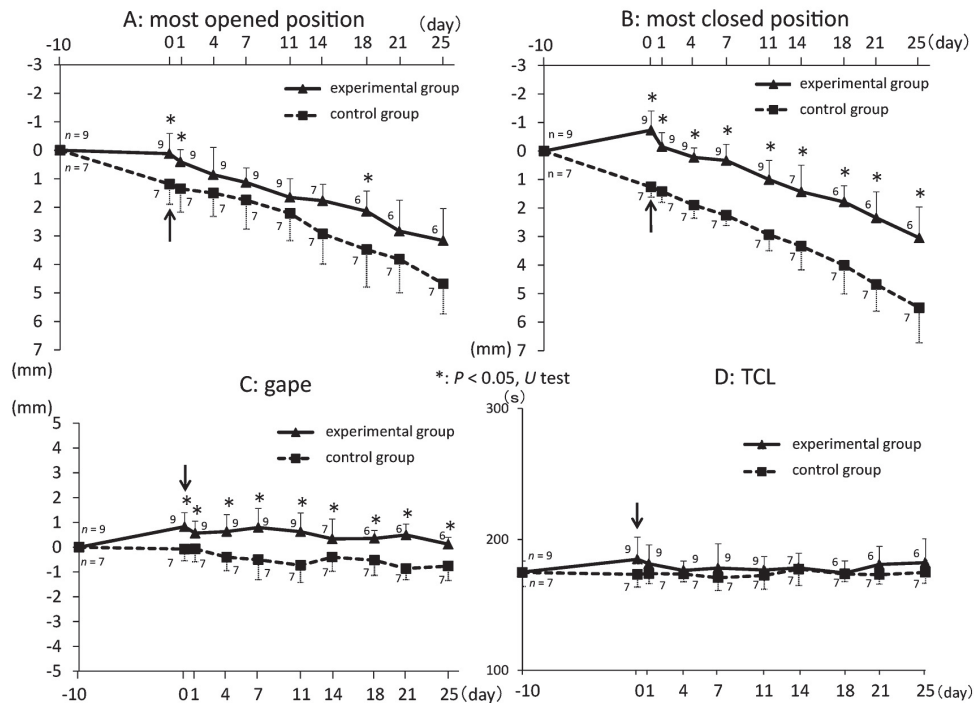
### Temporal change in tooth length

The growth of tooth length in upper and lower molars significantly decreased with the application of elastics in the experimental group in comparison with the control group (day 0 indicated by the arrows in Fig. 5A and B;  $P < 0.05$ ). A significant difference was not observed between experimental and control groups on days 1-25 after removal of the elastics in the upper and lower molars.





**Fig. 6** Temporal change of jaw movement trajectory on the frontal plane in the experimental group with reduced OVD. Horizontal dotted line indicates the most closed position before application of elastic. White and black arrows indicate the most open and closed positions, respectively.



**Fig. 7** Temporal change in most open position (A), most closed position (B), gape magnitude (C), and TCL (D). The differences in the most open position (A), most closed position (B), and gape magnitude (C) are expressed as the relative value to those before application of elastic (the values before application of elastic were defined as 0). The arrows in the figures indicate the day of elastics removal (day 0). *n*: The number of animals at each recording point, \* $P < 0.05$ , *U* test.

### Temporal change in jaw-movement trajectories

Figure 6 shows the typical trajectories of jaw movements on the frontal plane during chewing in the experimental group. The jaw-movement trajectories during chewing showed a similar pattern with large alternative bilateral jaw shifts, and there were no remarkable changes in their trajectories on days 0–25 after removal of the elastics.

The mean minimum closed position was significantly higher in the experimental group than in the control group

on all measurement days after removal of the elastics ( $P < 0.05$ ; Fig. 7B). In contrast, there were no significant differences in the mean maximum open position between the groups except on days 0, 1, and 18 after removal of the elastics (Fig. 7A).

The mean gape magnitude was significantly larger on all measurement days after removal of the elastics in the experimental group compared with the control group ( $P < 0.05$ ; Fig. 7C). There were no significant differences

in TCL after removal of the elastics compared with that before elastic applications in both groups (Fig. 7D).

## Discussion

### Reduced OVD

In the present study, a bite-reduced animal model was developed by applying intermaxillary elastics for 10 days. The rabbit model of deviated mandible was previously developed by unilaterally applying an elastic band (5,6). Since rabbits have continuously erupting teeth, OVD is determined by the balance between eruption and grinding. The mandibular deviation in this model is thought to be caused by attrition of the lower molars on the elastic side. In the present study, guinea pigs, which also have continuously erupting teeth, were used (8-10). Bilateral utilization of intermaxillary elastics did not cause lateral deviation and allowed reduction of the OVD while maintaining the occlusal relationship without tooth damage. The natural increase in OVD due to orofacial growth was interrupted by the application of elastics for 10 days in the experimental group, and ultimately a 5% decrease in OVD was observed after removal of the elastics compared with the control group. The midline of the upper and lower incisors was maintained, indicating that bilateral decrement in the OVD occurred equally. Two factors were associated with the mechanism of OVD reduction in the present experimental model animals. The first factor is tooth intrusion, which follows the same mechanism as orthodontic treatment in humans. However, since guinea pigs have continuously erupting teeth, and the tooth apex is not closed, it is unlikely that tooth intrusion occurs in this model. The second factor is an increase in grinding frequency in the upper and lower molar teeth. Although OVD is maintained by an appropriate balance between eruption and grinding in guinea pigs with continuously erupting teeth, this balance is disrupted by elastics. Based on the present findings, tooth grinding is thought to be accelerated by the presence of elastics. The findings suggest that elastic application causes a change in the balance between eruption and grinding, resulting in reduced OVD. Based on these results, the model appears to be valid for examining the relationship between reduced OVD and oral functions.

### Temporal change in OVD

Yagi et al. (11) and Zhang et al. (12) created bite-raised model animals by applying a bite-raising appliance to the lower incisors in guinea pigs. Although the occlusal relationship of the bite-raised animals was maintained with appropriate tooth contact after removal of the bite-raising appliance, OVD was recovered within 4-7 days after its

removal. This result suggested that the increased OVD was adjusted by grinding of the teeth. In bite-reduced animals, OVD did not recover to the control level even 25 days after removal of the elastics, suggesting that bite-reduction may not affect sensory and motor oral functions. In this study, upper and lower teeth length did not show a significant reduction between experimental and control groups on days 1-25 after removal of the elastics. However, the reduction of OVD may be attributed to the total changes in the upper and lower teeth length. Zhang et al. (12) reported that the tooth-grinding ability of guinea pigs after increasing their bite was impaired by the destruction of the trigeminal mesencephalic nucleus in bite-raised animals. Muscle spindle afferents of the jaw-closing muscles may be closely involved in OVD adjustment. Jaw-closing muscle spindles are known to be involved in muscle extension, and the sensory inputs from bite-raising are continuously delivered to the brainstem. Tooth grinding behavior is then enhanced to adjust the occlusal relationship in guinea pigs. Muscle spindles may not be involved in the adjustment of the occlusal relationship in the bite-reduced animals.

### Effect of reduced OVD on chewing movements

Obvious changes in grinding movement with large alternative bilateral jaw shifts (figure-eight shape) were not observed in our model before application of the elastics and on days 0, 1, 4, 7, 11, 14, 18, 21, and 25 after their removal. There was also no significant change in TCL throughout the experiment. The basic pattern of jaw movements during chewing may be programmed by the central pattern generator (CPG) in the brainstem (13). Together with previous data, our results suggest that reduction of the OVD may not affect the CPG function in our model.

The pathways in the occlusal phase, but not the most open position and jaw-opening pathways, are altered during chewing-like jaw movement induced by cortical stimulation in bite-reduced animals, resulting from manual grinding of the molars (Morimoto T et al. Alpha and gamma motor systems, 409-411, Plenum, New York, 1995). Kanayama et al. (2) reported that there was no change in the most open position in bite-raised animals although the gape magnitude was decreased. These observations were consistent with our findings that the maximum open position was maintained, and the gape magnitude was increased in our model animals. However, the mechanism underlying maintenance of the maximum open position during jaw movements remains unclear. Jaw-closing but not jaw-opening muscle activities are changed during chewing-like jaw movements

by cortical stimulation following placement of elastic materials between upper and lower molars, suggesting that jaw-opening muscle activities may not be modulated by peripheral sensory inputs (14-16). It has also been suggested that CPG activation is involved in maintaining the stability of jaw movements at the opening phase during chewing.

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### Conflict of interest

None declared.

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