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Functional reversibility of temporomandibular joint mechanoreceptors

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ABSTRACT

Objective: Previous studies have reported that the maturation of temporomandibular joint (TMJ) mechanoreceptors occurs during the early stages of mastication, and indicated that TMJ mechanoreceptors lose their function when masticatory loading is decreased. The purpose of the present study was to investigate whether the resumption of proper TMJ loading during the early growth period could restore TMJ mechanoreceptor function.

Designs: Ninety-nine 2-week-old male Wistar rats were divided into two groups and fed either pellets [control group (n = 33)] or a liquid diet [experimental group (n = 66)]. At 5 weeks of age, the experimental group was split into changing-diet (n = 33) and liquid-diet (n = 33) groups; the former was fed pellets instead of a liquid diet. TMJ mechanoreceptor activities were recorded from the trigeminal ganglion at 5, 7 and 9 weeks. The firing threshold and maximum instantaneous firing frequency of single TMJ units were measured in each group.

Results: In the changing-diet group, the firing properties of TMJ units were recovered at 7 weeks.

Conclusions: Proper TMJ loading during the early growth period can lead to the restoration of TMJ mechanoreceptor function.

1. Introduction

Joints play important roles in smooth movement and often endure loading during dynamic movement. The temporomandibular joint (TMJ) afferents are involved in the proprioceptive control of mastication, and allows the effector organ, the mandible, to move efficiently. Sensory receptors in the TMJ, as well as the muscle spindles and cutaneous and periodontal mechanoreceptors, transmit information regarding the position of the mandible and the degree of occlusal contact to the brain to achieve rhythmic masticatory movements[1–5].

The TMJ is a diarthrodial joint both morphologically and functionally. Like many other joints, the TMJ is load-bearing[6]. A previous study reported that TMJ loading is an important factor in the normal structure and function of the joint components[7], as well as those of other related components in the stomatognathic system. Moreover, ithas been reported that TMJ mechanoreceptors, important afferents in regulating mastication, lose their functionfollowing a decrease inmasticatory loading[8,9]. However, it is unknown whether TMJ mechanoreceptors show functional recovery if a proper mechanical load is re-applied. This is an important issue in the field of dentistry, since many patients have orofacial dysfunction caused by temporomandibular disorders and/or abnormal occlusal conditions. However, there are no studies investigating the early correction of the masticatory environment during growth with respect to the functional recovery of

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TMJ mechanoreceptors. Studies have shown that mechanical stimulation might accelerate peripheral nerve regeneration[10]and that periodontal Ruffini endings (oral mechanoreceptors) have high potential for neuroplasticity[11]. Thus, the present study aimed to investigate whether appropriate TMJ re-loading during the early growth period could restore TMJ mechanoreceptor function. We tested the hypothesis that the resumption of proper TMJ loading during the early growth period can restore TMJ mechanoreceptor function.

2. Materials and methods

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

2.1. *Animal preparation*

Ninety-nine 2-week-old male Wistar albino rats were used in this experiment (Fig. 1A). To prevent the experimental group from having any experience of chewing solid food, all rat pups were fed by their mothers and examined every 12 h to observe weaning. Soon after weaning, the rats were randomly divided into control ($n = 33$) and experimental groups ($n = 66$). The control group was fed chow pellets (CE-2, CLEA Inc, Tokyo, Japan) while the experimental group was fed a liquid diet consisting of CE-2 powder mixed with water in a blender at a ratio of 1:4 (w/v) in a graduated feeding tube (Dyets® Inc., Bethlehem, PA, USA). At the age of 5 weeks, the experimental group was then further divided into liquid-diet group and changing-diet groups, where rats in the changing-diet group were fed pellets instead of the liquid diet until the end of the experiment (at 9 weeks of age). Food and water were freely accessible throughout the experiment. The body weight of the rats was measured once per week throughout the experimental period (Fig. 2).

2.2. *Stimulation and recording*

For electrophysiological recordings, rats were lightly anaesthetized with 60 mg/kg thiamylal sodium (Isozol®, Yoshitomi Pharmaceutical, Osaka, Japan) administered intraperitoneally (i.p.). The depth of anaesthesia was monitored by checking pupil size, flexor and corneal reflexes, and heart rate. A

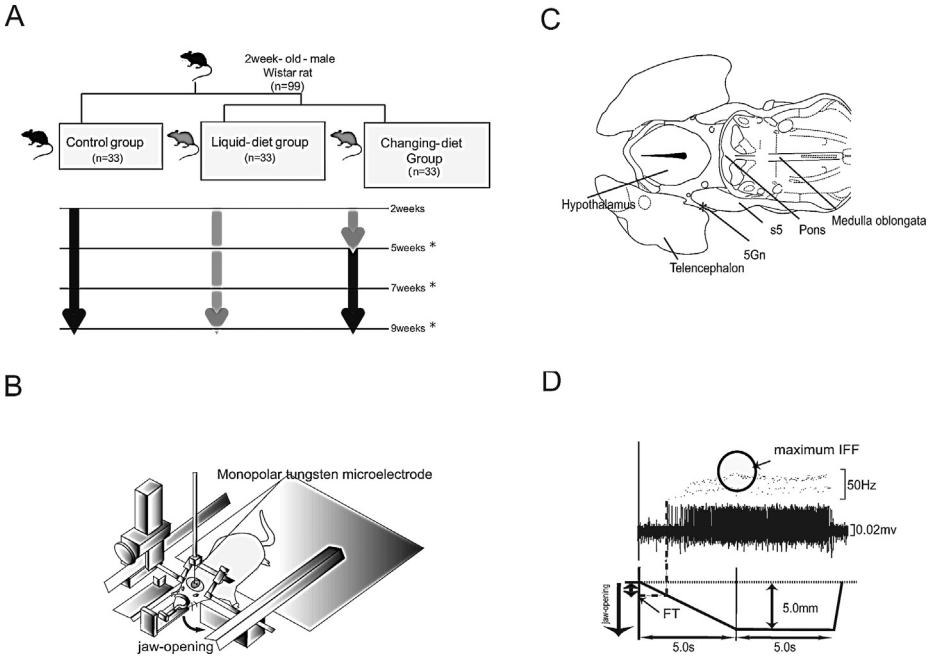

Fig. 1 – Experimental design. (A) Summary of experimental protocol and time schedule. Soon after weaning, rats were divided into control (solid-diet) and experimental (liquid-diet) groups. At 5 weeks of age, the experimental group was further divided into liquid-diet and changing-diet subgroups. Electrophysiological recordings of TMJ mechanoreceptors were obtained in anesthetized rats in all groups at 5, 7 and 9 weeks. An asterisk indicates the recording time. Dark and light arrows indicate feeding periods with pellets and liquid diets, respectively. (B) Schematic drawing of the experimental setting. The rat's head was fixed to a stereotaxic apparatus. A 3.0 mm wide aperture was prepared in the skull, and a monopolar tungsten microelectrode was inserted into the trigeminal ganglion. A string was attached to the mandible, and the ramp-and-hold jaw opening was achieved using 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 (reproduced with permission from Paxinos and Watson, 1998). An asterisk indicates the recording site. (D) The firing threshold (FT) was defined as the magnitude (mm) of jaw opening when the first potential was recorded. The vertical dashed line indicates the timing of the first spike potential. The maximum IFF was calculated as the minimum firing interval (Hz) between two consecutive spikes. Abbreviations: s5, the sensory root of the trigeminal nerve; 5Gn, the trigeminal ganglion; FT, the firing threshold; IFF, the instantaneous firing frequency.

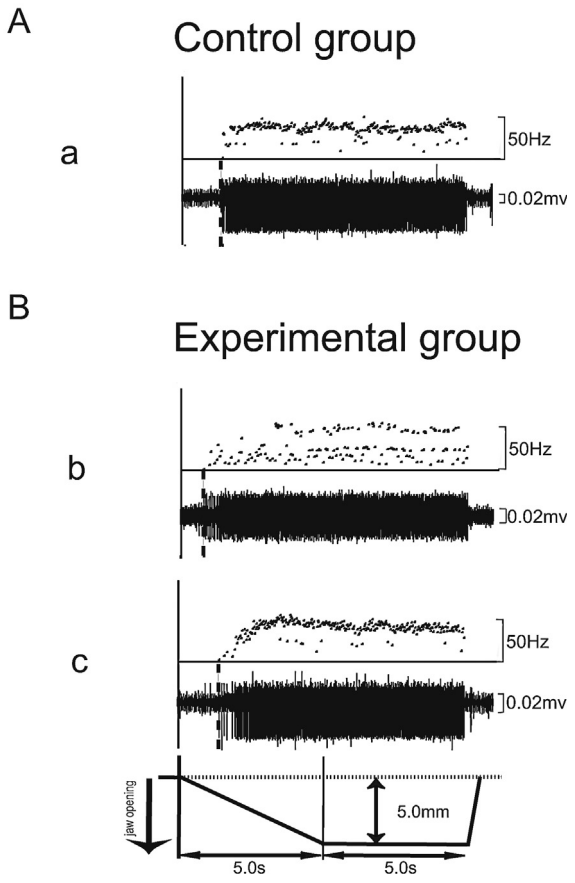

Fig. 2 – Representative recordings of single TMJ units in response to the ramp-and-hold stretch in the 7-week-old (A) control and (B) experimental groups. a, control group; b, liquid-diet group; c, changing-diet. Broken lines in the lower portion of each graph depict the timing of the first spike potentials, with a shorter latency observed in the experimental (b) group.

supplemental i.p. injection of 5 mg/kg of thiamylal sodium was given when a firm pinch applied to the tail resulted in increased respiration and heart rates.

Rats were placed in a prone position in the stereotaxic apparatus (models SN-2 and SM-15 M, Narishige Scientific Instrument, Tokyo, Japan; Fig. 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 to an automatic pulling machine[5,12,13]. Passive jaw-opening was performed in a ramp-and-hold manner for a duration of 5.0 s. The maximum jaw-opening distance was set to 5.0 mm, which is within the physiological range[5,12,13].

At ages 5, 7 and 9 weeks, electrophysiological recordings were obtained from the trigeminal ganglion, which contains the cell bodies of the trigeminal sensory neurons from TMJ mechanoreceptors. To introduce a recording electrode, a midline incision was first made in the scalp, followed by a small aperture in the skull, about 3.0 mm wide, using a stereotaxic microengine. Through this aperture, a monopolar tungsten microelectrode (250 μm diameter shaft with 8.0 degree tapered tip, 5.0 MΩ of AC impedance, A-M Systems Inc, Carlsborg, WA, USA) was inserted into the trigeminal ganglion, as per the previously reported stereotaxic coordinates for recording single-unit activities of TMJ mechanoreceptors[14].

The identification of TMJ afferents was complemented by electrical stimulation of the auriculotemporal nerve. To provide a clear visualization of the mandibular condyle (condylar process) and the TMJ capsule for electrical stimulation, the auriculotemporal nerve was exposed in the TMJ area by removing the skin around the head and detaching the posterior parts of the temporal and masseter muscles[15]. Conduction velocity was estimated using the distance and conduction delay between the stimulating electrode and recording electrodes. The criterion for conduction velocity was based on the classification reported by Koltzenburg and colleagues[16].

Spike potentials were recorded and amplified using a differential amplifier (DAM-80, WPI, Sarasota, FL, USA) of ×1000 gain, using 300 Hz and 3.0 KHz for the low- and high-cut filters, respectively. All data were captured using an interface (CED 1401, Cambridge Electronic Design, Cambridge, UK) and stored on a computer hard disk. The data were later analysed offline using Spike 2 software, version 4.02a (Cambridge Electronic Design, Cambridge, UK).

After the activity of each unit was recorded, the tip position of the electrode was marked by a lesion using 50 μA negative current for 10 s. At the end of each experiment, rats were sacrificed by an overdose of thiamylal sodium (120 mg/kg, i.p) and their brains harvested. Frozen 50 μm sections were prepared and stained with cresyl violet to histologically check the electrode position based on electrolytic lesions and signs of electrode penetration (Fig. 1C).

2.3. *Data analysis*

The effects of a liquid diet on TMJ units were assessed by an analysis of the firing threshold (FT) and the maximum instantaneous firing frequency (IFF) of the spike potentials. The FT was calculated as the magnitude (mm) of jaw-opening observed at the first spike response. The maximum IFF was calculated as the minimum firing interval (Hz) between two consecutive spikes (Fig. 1D). All data were expressed as the mean ± standard deviation (SD). Differences between the control and experimental groups at each age were compared using a Wilcoxon rank sum test with Bonferroni correction (5/3% significance level). Statview, version 5.0 (SAS Institute, Cary, NC, USA) was used for the static analysis.

3. Results

The mean body weight of the rats in the control and two experimental subgroups increased continuously throughout the experimental period, with no significant differences among the three groups at each age. Single unit activities were recorded from 99 TMJ units of the sensory neurons of the trigeminal ganglion in both the control and experimental groups (Table 1). Spike 2 classifies all data (from multiple recording units) into a single unit recording. Thus the unit number was equal to the animal number. We chose to display the data as a single unit per animal to remove contamination

Table 1 – The firing threshold (FT) and maximum instantaneous firing frequency (IFF) in the control and the two experimental groups. The results are presented as the mean ± standard deviation.

	Control ($n = 33$)	Liquid-diet ($n = 33$)	Changing-diet ($n = 33$)
FT (mm)			
5-week old ($n = 11$)	1.38 ± 0.10	0.94 ± 0.13[a]	0.92 ± 0.14[b]
7-week old ($n = 11$)	1.44 ± 0.15	0.74 ± 0.17[a]	1.38 ± 0.12[c]
9-week old ($n = 11$)	1.46 ± 0.15	0.39 ± 0.19[a]	1.40 ± 0.12[c]
Maximum IFF (Hz)			
5-week old ($n = 11$)	49.9 ± 3.30	52.6 ± 2.40	50.8 ± 3.55
7-week old ($n = 11$)	50.9 ± 3.79	58.3 ± 3.57[a]	51.7 ± 2.91[c]
9-week old ($n = 11$)	50.9 ± 4.50	82.1 ± 5.44[a]	50.5 ± 2.02[c]

FT, firing threshold; IFF, instantaneous firing frequency; Control, the control group; Liquid-diet, the liquid-diet group; Changing-diet, the changing-diet group.

[a] $p < 0.05/3$ between the control and liquid-diet.

[b] $p < 0.05/3$ between the control and changing-diet groups.

[c] $p < 0.05/3$ between the liquid and changing-diet groups.

in the data. In the event that contamination was evident in the data from more than one unit, these data were separated from the data of interest (the large amplitude data) using the spike discrimination software Spike 2. The activities of TMJ mechanoreceptors from each unit were analysed after three consecutive ramp-and-hold jaw-openings.

Typical examples of TMJ unit responses to jaw-opening in the control group and the two experimental subgroups are shown in Fig. 2. To calculate the conduction velocity (CV) of the single fibre, electric stimulation was delivered by an electrode (0.1 ms in duration, 1 Hz in frequency and 0.5–3 mA) to the auriculotemporal nerve, and the evoke unit responses in the trigeminal ganglion were calculated from the latency. The CV of the innervating fibres was calculated from the distance (d) between the stimulating and recording electrodes and latency (Δt), as per the methods described in a previous study ($CV = d/\Delta t$)[9]. A unit CV of more than 10.0 m/s indicated Aβ fibres, whereas a unit CV of between 2.0 and 10.0 m/s indicated Aδ fibers[16]. The mean CV we recorded in the afferents was 42.3 ± 2.4 m/s, indicating that these afferents were probably large myelinated (i.e., Aβ) fibers[16].

At 5 weeks, the FT in the liquid-diet and changing-diet group was significantly decreased when compared to the control group. At 7 and 9 weeks, compared to the control group, significantly decreased in the FT was observed in the liquid-diet group. On the other hand, the FT in the changing-diet group were significantly increased when compared to the liquid-diet group and there was no significant difference when compared to the control group. (Fig. 3A)

The maximum IFF had no significantly differences observed between the control and two experimental subgroups at 5 weeks. However, at 7 and 9 weeks, significant increases in IFF were observed in the liquid-diet group when compared to the control. Furthermore, the maximum IFF in the changing-diet group was significantly decreased when compared to the liquid diet group and there was no significant difference when compared to the control group. (Fig. 3B)

4. Discussion

Previous studies have shown that the altered masticatory environment that occurs during growth affects TMJ morphological development and recovery[17–19]. Our previous studies have shown that the functional sensitivity of mechanoreceptors can be modified by changes in the masticatory environment[8,9]. The present study is the first to shed light on the functional recovery of TMJ mechanoreceptors in the nervous system after correcting the masticatory environment during the early growth period. Further, our study clarifies the importance of early functional correction by taking advantage of the plasticity of the sensory neural network of the masticatory system.

We chose a modified dietary consistency model to alter the mechanical load delivered to the TMJ, since such models have frequently been used to achieve a change in loading on the TMJ *in vivo*[8,9,20–22]. In this study, we identified a significantly lower FT in the liquid-diet group at 5, 7 and 9 weeks as compared with the FT in the changing-diet and control groups after the 5-week time point. Previous studies have demonstrated that external perturbation elicits organic changes in various joints, such as morphological changes in the nerve endings associated with weightlessness or immobilization[5,23,24]. Immobilization of the knee joint, for example, induces morphological changes and a decrease in the number of neural endings in the anterior crucial ligament[24]. Yet, when the knee joint was allowed to move freely again, the number of neural endings increased. Our results are consistent with these findings. In Fig. 3A, the analysis at the firing threshold showed that the threshold in the liquid diet group was decreased significantly. The TMJ mechanoreceptors in the liquid diet group did not lose the function but were more sensitive than those in the control group. We suggest that the change observed in this study involves the functional recovery of TMJ mechanoreceptors in association with the resumption of appropriate masticatory loading. Moreover, this functional recovery may be associated with morphological changes in TMJ mechanoreceptors.

In this study, the maximum IFF in the liquid-diet group was significantly greater than that in the control group at 7 and 9 weeks, while there was no statistically significant difference between the changing-diet group and the control group at 5, 7, or 9 weeks. Previous studies have shown that the changes in cartilage thickness, condylar bone volume and alkaline phosphatase activity engendered by a soft diet are restored within 2–4 weeks following resumption of a firm diet[25–27].

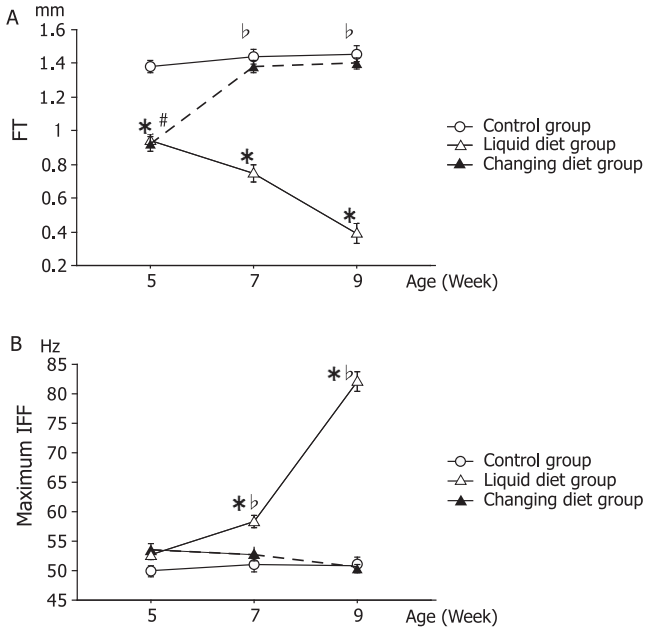

Fig. 3 – The FT and the maximum IFF of the TMJ units in the control, liquid-diet and changing-diet groups. (A) FT was significantly lower at 5, 7, and 9 weeks in the liquid-diet group and at 5 weeks in the changing-diet groups as compared with the control group. (B) The maximum IFF of the liquid-diet group was significantly higher than that of the control group at 7 and 9 weeks. No significant differences were observed between the changing-diet and control groups throughout the experimental period. Error bars indicate SD. $^{*}p < 0.05/3$ between the control and liquid-diet groups, $^{\#}p < 0.05/3$ between the control and changing-diet groups. $^{\flat}p < 0.05/3$ between the liquid and changing-diet groups.

Since TMJ mechanoreceptors are localized inside the TMJ capsule, remodelling of the TMJ might affect the maximum IFF of TMJ units.

The molecular responses of mechanoreceptors to touch, pressure, position, and movement are still poorly understood. Mechanosensitive ion channels, existing in a variety of cells, are thought to open and close in response to the excitation of mechanoreceptors in sensory nerves by neural signals of somatic sensations[28]. The stimulation threshold is defined as the lowest strength of stimulation that is capable of initiating electrical activity, and the frequency of firing (i.e., the action potential) is the magnitude of the postsynaptic potential[29]. Therefore, we consider that alterations in the masticatory environment may lead to changes in cell membrane tension or in the properties and morphology of mechanosensitive ion channels.

Our previous findings regarding both the FT and maximum IFF in the control group indicate that TMJ mechanoreceptor maturation is attained by the age of 5 weeks[9]. Moreover, the TMJ mechanoreceptors cannot sustain their function when mechanical masticatory stimulation is decreased[9]. The present finding is the re-establishment of physiological masticatory stimuli during the early growth period may be able to correct inappropriate maturation and promote the physiological function of TMJ mechanoreceptors. Therefore, we conclude that correction of the masticatory environment during the early growth period is essential and sufficient for maturation of the masticatory system.

5. Funding

None

6. Conflict of interest

There are no conflicts of interest.

7. Ethical approval

Not required

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REFERENCES

1. Greenfield BE, Wyke B. Reflex innervation of the temporomandibular joint. *Nature* 1966;**211**(5052):940–1.
2. Klineberg I, Lillie J. Regional nerve block of the temporomandibular joint capsule: a technique for clinical research and differential diagnosis. *Journal of Dental Research* 1980;**59**(11):1930–5.
3. Lund JP. Mastication and its control by the brain stem. *Critical Reviews in Oral Biology & Medicine* 1991;**2**(1):33–64.
4. Yamamura K, Inoue M, Igarashi N, Takahashi Y, Yamada Y. Effects of food consistency on the modulatory mode of the digastric reflex during chewing in freely behaving rabbits. *Brain Research* 1998;**796**(1–2):257–64.
5. Kokai S, Yabushita T, Zeredo JL, Toda K, Soma K. Functional changes of the temporomandibular joint mechanoreceptors induced by a lateral mandibular shift in rats. *The Angle Orthodontist* 2007;**77**(3):436–41.
6. Tanaka E, van Eijden T. Biomechanical behavior of the temporomandibular joint disc. *Critical Reviews in Oral Biology & Medicine* 2003;**14**(2):138–50.
7. Kato T, Usami T, Noda Y, Hasegawa M, Ueda M, Nabeshima T. The effect of the loss of molar teeth on spatial memory and acetylcholine release from the parietal cortex in aged rats. *Behavioural Brain Research* 1997;**83**(1–2):239–42.

8. Ishida T, Yabushita T, Soma K. Functional changes of temporomandibular joint mechanoreceptors induced by reduced masseter muscle activity in growing rats. *The Angle Orthodontist* 2009;**79**(5):978–83.
9. Ishida T, Yabushita T, Soma K. Effects of a liquid diet on temporomandibular joint mechano-receptors. *Journal of Dental Research* 2009;**88**(2):187–91.
10. Angelov DN, Ceynowa M, Guntinas-Lichius O, Streppel M, Grosheva M, Kiryakova SI, et al. Mechanical stimulation of paralyzed vibrissal muscles following facial nerve injury in adult rat promotes full recovery of whisking. *Neurobiology of Disease* 2007;**26**(1):229–42.
11. Harada F, Hoshino N, Hanada K, Kawano Y, Atsumi Y, Wakisaka S, et al. The involvement of brain-derived neurotrophic factor (BDNF) in the regeneration of periodontal Ruffini endings following transection of the inferior alveolar nerve. *Archives of Histology and Cytology* 2003;**66**(2):183–94.
12. Yabushita T, Zeredo JL, Fujita K, Toda K, Soma K. Functional adaptability of jaw-muscle spindles after bite-raising. *Journal of Dental Research* 2006;**85**(9):849–53.
13. Yabushita T, Zeredo JL, Toda K, Soma K. Role of occlusal vertical dimension in spindle function. *Journal of Dental Research* 2005;**84**(3):245–9.
14. Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. 4th ed. New York: Academic Press; 1998.
15. Kobayashi M, Yabushita T, Zeredo JL, Toda K, Soma K. Splenius muscle activities induced by temporomandibular joint stimulation in rats. *Brain Research Bulletin* 2007;**72**(1): 44–8.
16. Koltzenburg M, Stucky CL, Lewin GR. Receptive properties of mouse sensory neurons innervating hairy skin. *Journal of Neurophysiology* 1997;**78**(4):1841–50.
17. Bouvier M. Effects of age on the ability of the rat temporomandibular joint to respond to changing functional demands. *Journal of Dental Research* 1988;**67**(9):1206–12.
18. Ito G, Mitani S, Kim JH. Effect of soft diets on craniofacial growth in mice. *Anatomischer Anzeiger* 1988;**165**(2–3): 151–66.
19. Yonemitsu I, Muramoto T, Soma K. The influence of masseter activity on rat mandibular growth. *Archives of Oral Biology* 2007;**52**(5):487–93.
20. Kiliaridis S, Engstrom C, Thilander B. Histochemical analysis of masticatory muscle in the growing rat after prolonged alteration in the consistency of the diet. *Archives of Oral Biology* 1988;**33**(3):187–93.
21. Kiliaridis S, Thilander B, Kjellberg H, Topouzelis N, Zafiriadis A. Effect of low masticatory function on condylar growth: a morphometric study in the rat. *American Journal of Orthodontics and Dentofacial Orthopedics* 1999;**116**(2):121–5.
22. Weijs WA, Dantuma R. Electromyography and mechanics of mastication in the albino rat. *Journal of Morphology* 1975;**146**(1):1–33.
23. Kanemura N, Kobayashi R, Kajihara H, Minematu A, Sasaki H, Tanaka S, et al. Changes of mechanoreceptor in anterior cruciate ligament with hindlimb suspension rats. *Journal of Physical Therapy Science* 2002;**14**(1):27–32.
24. Michinaka Y, Yamamoto H, Kawakami T, Morisawa Y, Uemura H. Effect of immobilization of the knee joint on mechanoreceptors in anterior cruciate ligament of the rabbit. *Journal of Orthopeadic Science* 1997;**2**:259–65.
25. Bouvier M. Variation in alkaline-phosphatase activity with changing load on the mandibular condylar cartilage in the rat. *Archives of Oral Biology* 1987;**32**(9):671–5.
26. Bouvier M, Hylander WL. The effect of dietary consistency on gross and histologic morphology in the craniofacial region of young rats. *American Journal of Anatomy* 1984;**170**(1):117–26.
27. Yamada K, Kimmel DB. The effect of dietary consistency on bone mass and turnover in the growing rat mandible. *Archives of Oral Biology* 1991;**36**(2):129–38.
28. Cho H, Shin J, Shin CY, Lee SY, Oh U. Mechanosensitive ion channels in cultured sensory neurons of neonatal rats. *Journal of Neuroscience* 2002;**22**(4):1238–47.
29. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. *Molecular biology of the cell. Membrane transport of small molecules and the electrical properties of membranes*. 4th ed. New York: Garland Science; 2002. 615–658.