

## Masticatory Function and Maturation of the Jaw-Opening Reflex

Chidsanu Changsiripun<sup>a</sup>; Tadachika Yabushita<sup>b</sup>; Kunimichi Soma<sup>c</sup>

### ABSTRACT

**Objective:** To test the null hypothesis that alteration in masticatory function due to liquid-diet feeding during growth does not affect jaw-opening reflex (JOR) maturation.

**Materials and Methods:** Soon after weaning, 70 female Wistar rats were divided into two equal groups and fed either solid (control group) or liquid (experimental group) diets. At 5, 9, and 13 weeks, the rats were anesthetized and the JOR was recorded in the anterior belly of the digastric muscles as evoked by a low-intensity electrical stimulation of the left inferior alveolar nerve.

**Results:** There were similar tendencies at each recording age. Peak-to-peak amplitude of the JOR was significantly smaller, and the latency was significantly longer in the experimental group, although the duration was not significantly different between the two groups.

**Conclusions:** These data suggest that long-term masticatory functional change due to liquid-diet feeding during growth may impede the learning for JOR maturation, and thus may affect the masticatory performance in the adult. (*Angle Orthod.* 2009;79:299–305.)

**KEY WORDS:** Jaw-opening reflex; Masticatory function; Development; Liquid diet

### INTRODUCTION

Temporomandibular disorders and abnormal occlusal conditions such as open bite malocclusion were reported to cause an alteration in masticatory function whether measured as chewing performance, bite force, or muscle activity.<sup>1,2</sup> There are several clinical reports revealing that many kinds of jaw motor function, including jaw reflex, are modulated under these pathophysiologic states.<sup>3,4</sup> A current epidemiologic study showed that these clinical dysfunctions are

found in all age groups and are not rare findings in children and adolescents.<sup>5</sup> However, it has not yet been investigated as to whether children and adolescents who developed with altered jaw motor functions can perform mastication accurately during adulthood.

The jaw reflex is accepted as one of the crucial neuronal functions for controlling jaw movements in various oral situations.<sup>6,7</sup> In the masticatory system, the jaw-opening reflex (JOR) has been widely studied, and a number of studies suggest a relationship between JOR and masticatory function. During mastication, the phase-dependent modulatory mode of the JOR is thought to play an important role in regulating jaw movement.<sup>6,8</sup>

Recently, it is interesting that jaw reflex properties change remarkably with development and involve the learning process. Jaw reflexes are maturing as the child continues to acquire new oral motor skills.<sup>9</sup> In more detailed literature, it was found that the latency of the JOR in neonatal rats was much longer than in adults, and the short-latency digastric reflex was recommended as the necessary precondition for adult oral function.<sup>10</sup>

Mastication is an acquired-learning function, which has the critical period for programming during young ages.<sup>11</sup> Normally, juvenile changes could be directed to achieve optimal masticatory function in an adult.<sup>12</sup> However, some environmental changes during childhood, eg, the type of food consumed, might alter the functional demands of the masticatory system. It has

---

<sup>a</sup> Graduate student, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.

<sup>b</sup> Assistant Professor, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.

<sup>c</sup> Professor and Chairman, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.

Corresponding author: Chidsanu Changsiripun, DDS, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan  
(e-mail: chidorts@tmd.ac.jp)

Accepted: April 2008. Submitted: February 2008.

© 2009 by The EH Angle Education and Research Foundation, Inc.

been reported that in various animals, including humans, electromyographic (EMG) activity of the masticatory muscles and jaw movements is influenced by orofacial sensations arising during the mastication of foods with different consistencies.<sup>13,14</sup> In particular studies concerned with the JOR, the reflex amplitude was significantly larger when hard food was taken than when soft food was taken, although the relationship between this reflex and food properties was not evaluated long term in these reports.<sup>15,16</sup> Moreover, maturation of JOR in grown-up mammals without chewing experience has never been examined.

To clarify this, the present study was undertaken to test the null hypothesis that alteration in masticatory function due to liquid-diet feeding during growth does not affect JOR maturation.

## MATERIALS AND METHODS

### Animal Preparation

Seventy 2-week-old female Wistar albino rats were used in this experiment. To prevent the experimental group from having any experience of chewing a solid diet, all pups were fed by their mother and were examined every half-day to observe weaning. Soon after weaning, the pups were randomly divided into control ( $n = 35$ ) and experimental ( $n = 35$ ) groups. The control group was fed rat chow pellets (CE-2, CLEA Inc, Tokyo, Japan), while the experimental group was fed a liquid diet, which consisted of CE-2 powder mixed with water in a blender at a ratio of 1:4 by weight, as described in a previous study,<sup>17</sup> using a graduated feeding tube. Food and water were freely accessed all of the time. The body weight of the animals was measured throughout the experimental period. At 5, 9, and 13 weeks, a JOR recording was conducted in the anesthetized animals in both groups.

### Stimulation and Recording

The animals were anesthetized with thiamylal sodium (Isozol, Yoshitomi Pharmaceutical, Osaka, Japan; 60 mg/kg, intraperitoneally (IP)). Then, cannulae were inserted into the trachea and the femoral vein. Because differing levels of anesthesia were known to have an effect on the latency of the digastric reflex,<sup>18</sup> the depth of anesthesia was carefully monitored throughout the experiment by checking pupil size, withdrawal, and corneal reflexes and heart rate. Also, in order to keep the same anesthetic level in all rats while performing the JOR recording, a supplemental dose (5 mg/kg, intravenously) was routinely given 15 minutes before the start of a recording process if a withdrawal reflex was elicited by pinching the paws. The rectal temperature was maintained at 37°C with a heating pad. Before an incision was made, the skin

was injected with 2% lidocaine to prevent the noxious stimulation that could have an inhibitory effect on JOR.<sup>19</sup>

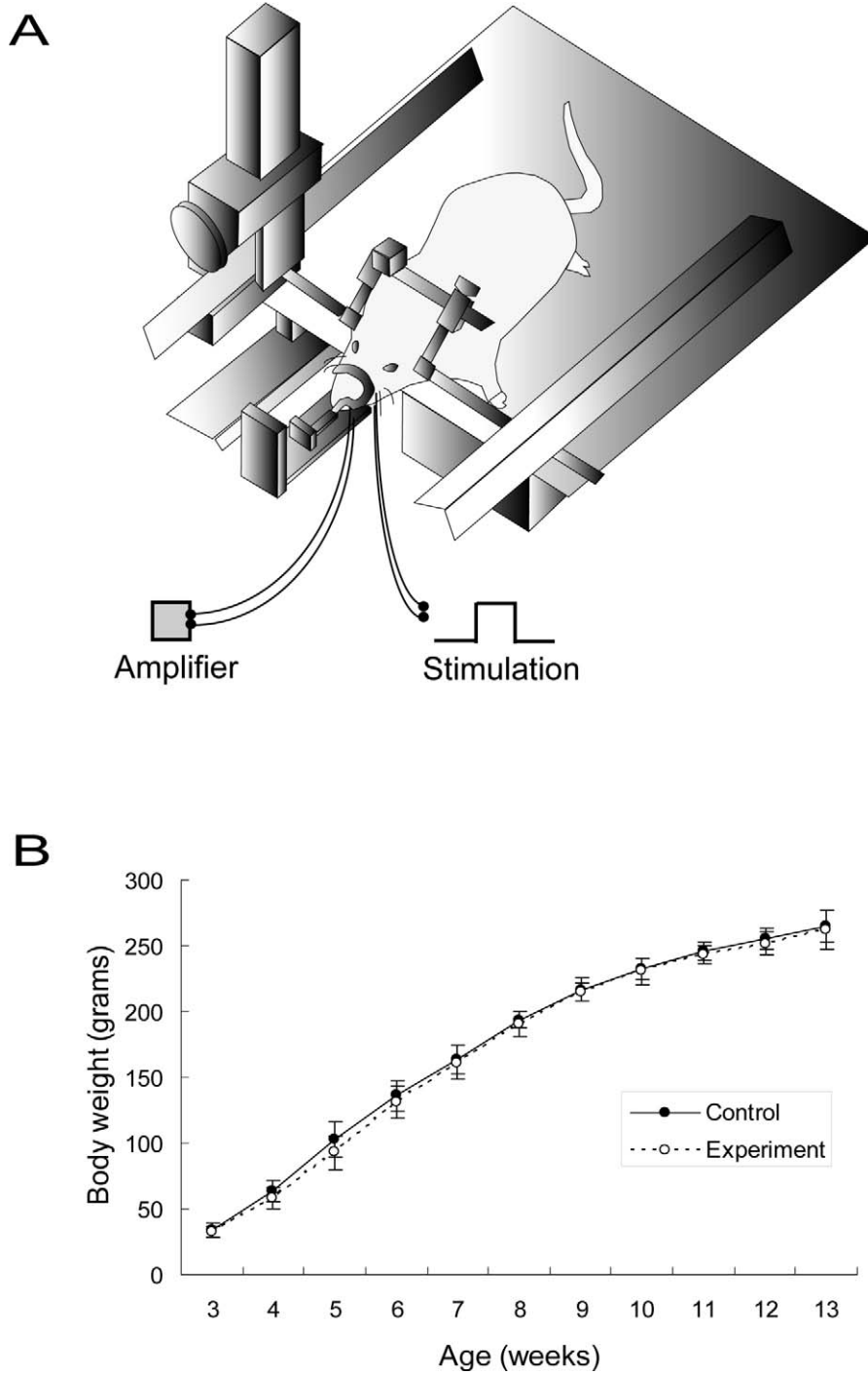
To stimulate the left inferior alveolar nerve (IAN), a pair of stainless-steel wire electrodes (diameter 0.1 mm, tip exposure 0.5 mm) was inserted into the left mandibular canal through the mental foramen at a depth of 1 mm and 3 mm. Then, the bipolar electrode was kept in place by fixing on the adjacent mandibular bone with light-cured dental resin. An expression of the JOR was recorded from the ipsilateral digastric muscle (Dig), using paired stainless-steel wire electrodes with 1-mm exposed tips implanted along the direction of the muscle fibers. The inter-polar distance was set at 3 mm for these recording electrodes. To be able to reproduce this amount every time, a 2-keyhole index, with 3 mm between the holes, made from dental acrylic (UNIFAST II, GC Corporation, Tokyo, Japan) was used for leading paired electrode-inserted needles to leave the electrodes in place with the same inter-polar distance in every recording. The animals were then transferred to a stereotaxic apparatus (models SN-2 and SM-15M, Narishige Scientific Instruments, Tokyo, Japan) with their bodies in a prone position (Figure 1A).

Before the stimulation of the JOR, Dig EMG activity was captured for 1 minute in each rat to be considered as a baseline activity. Then, the left IAN was electrically stimulated (single pulse, 0.2 millisecond duration) once every 5 seconds to evoke the JOR. The stimulus intensity was applied gradually in increasing in steps of 1  $\mu$ A, and the lowest intensity that constantly elicited Dig EMG responses was determined as the JOR threshold (T). To attain the comparable responses, the test stimulation current was adjusted to 1.5 times the threshold (1.5T). Besides, the intensity, as being equal to or less than 2T, can be considered as nonnoxious stimuli,<sup>8</sup> which we expect in this study. EMG activity was amplified by a differential amplifier (DAM-80, WPI, Sarasota, Fla; bandwidth 100 Hz–3 kHz). All signals were fed into a computer by means of CED 1401 interface (sampling rate 5,000/s) and were later analyzed offline with the Spike2 software for Windows, version 4.02 (Cambridge Electronic Design, Cambridge, UK).

After recording, the animals were killed with a thiamylal sodium (IP) overdose. The Dig was then dissected to confirm the electrode-placed location. All surgeries and procedures described above were in agreement with the Animal Care Standards of the Tokyo Medical and Dental University, and had the approval from its Animal Welfare Committee.

### Data Analysis

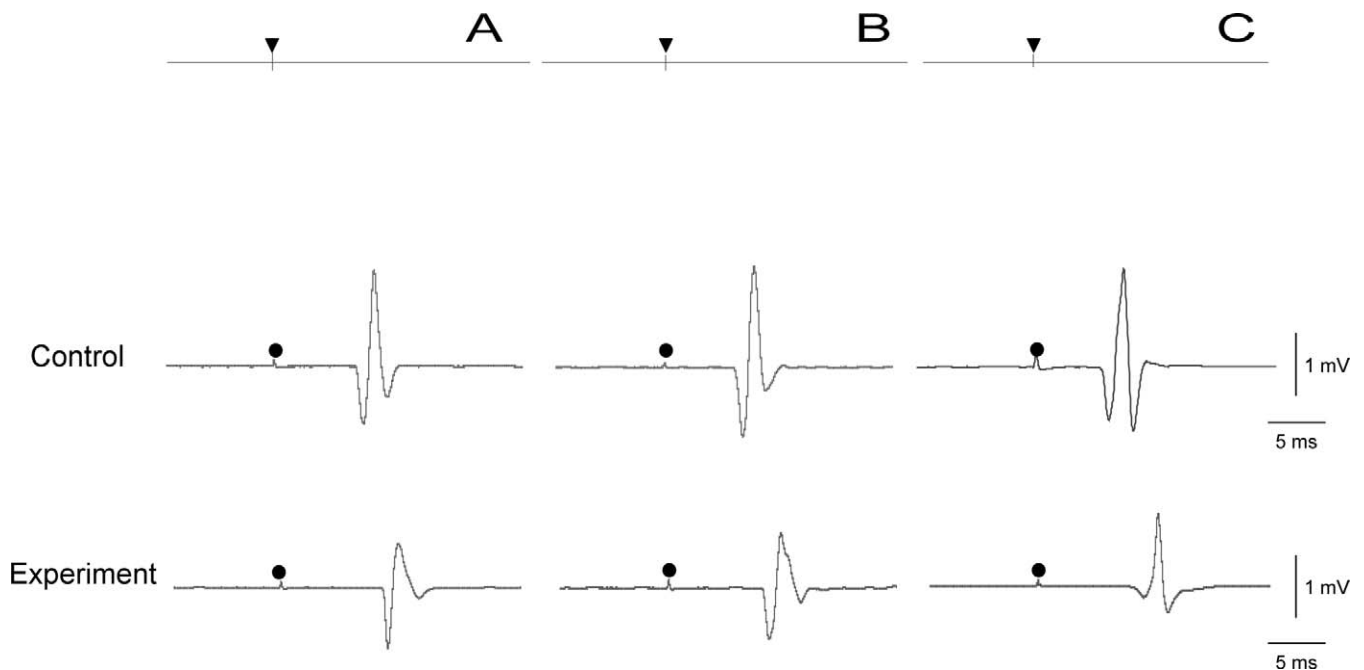
Peak-to-peak amplitude, latency, and duration were measured as the JOR properties for comparison be-



**Figure 1.** (A) Scheme of the experimental setting. Anesthetized rats had their heads fixed to a stereotaxic frame. Jaw-opening reflex (JOR) was evoked by electrical stimulation of the left inferior alveolar nerve and recorded in the anterior belly of ipsilateral digastric muscles. (B) Mean body weight throughout the experimental period. The weight-age curves were similarly increased without statistically significant differences between the two groups.

tween the two groups. The mean values of these parameters were averaged from the reflex responses after 30 consecutive stimuli in each rat. EMG activities were full-wave rectified and smoothed with a time constant of 20 milliseconds. The latency and duration were indicated as time intervals (ms). Latency was de-

finied as between the stimulus and the first point at which digastric EMG activity exceeded 2 standard deviations (SD) of the baseline activity (onset), whereas duration was defined as between the onset and the point of time when the response dropped below 2 SD of the baseline activity (offset). Peak-to-peak ampli-



**Figure 2.** Typical examples of jaw-opening reflex (JOR) responses from control and experimental groups at (A) 5 weeks old, (B) 9 weeks old, and (C) 13 weeks old. The stimulus intensity was adjusted to 1.5 times the threshold (1.5T) in each rat. ▼ and ● indicate stimulus and artifact, respectively.

tude (mV) was calculated using the Spike2 analysis function between the onset and offset in each single sweep.

All data were expressed as mean  $\pm$  SD and unpaired *t*-test was used for statistical comparison at the different ages between two groups. *P* values less than .05 were considered significant. The software SPSS for Windows, version 12.0 (SPSS Inc, Chicago, Ill), aided in statistical analysis.

## RESULTS

The mean body weight of all rats regularly increased throughout the experimental period. There was no significant difference between the two groups (Figure 1B).

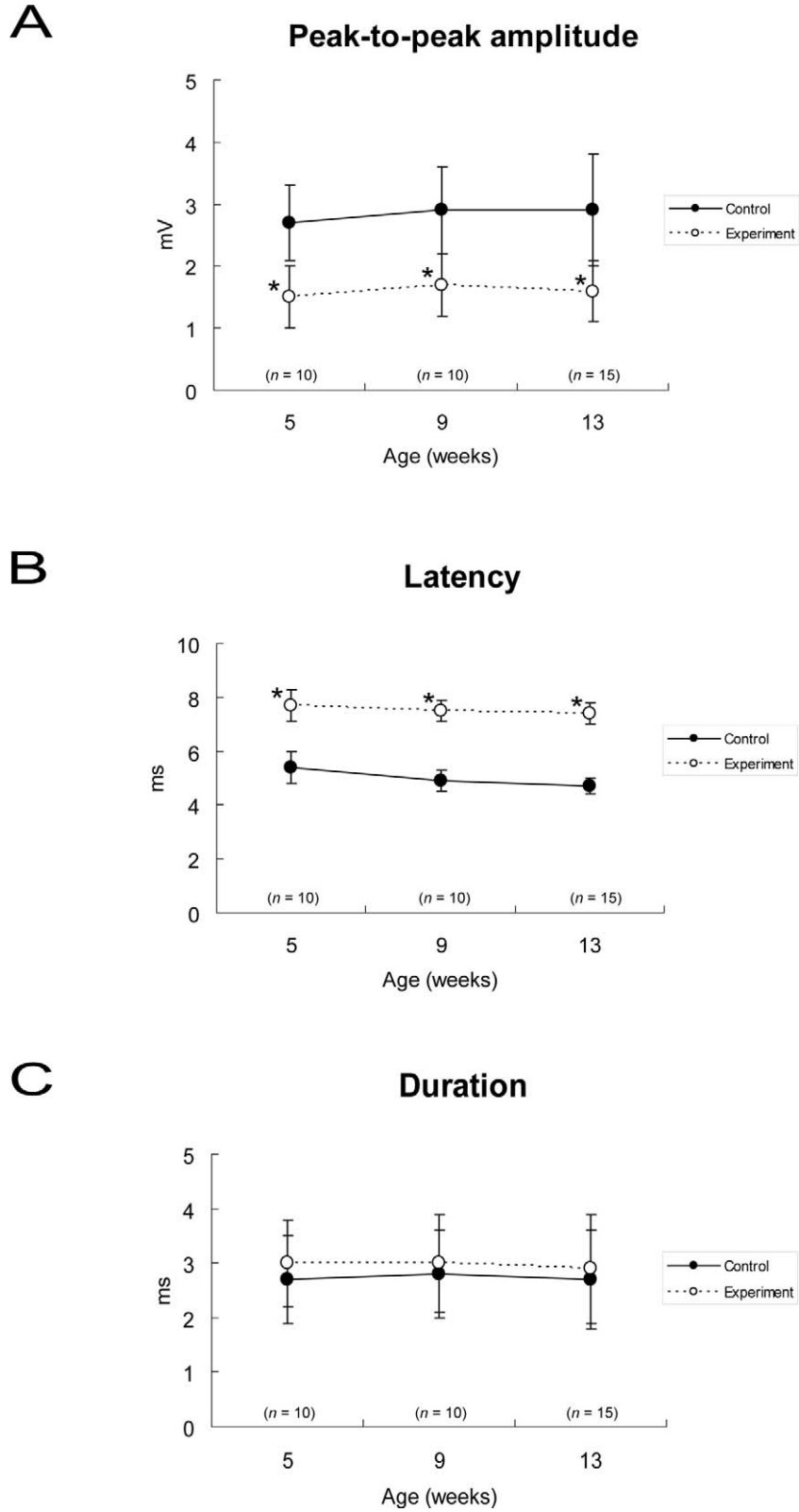
Low-intensity electrical stimulation to the left IAN always elicited JOR responses at the ipsilateral Dig in all animals. Typical examples of both groups recorded at 5, 9, and 13 weeks of age are shown in Figure 2A through C, respectively. The reflex threshold was  $25.9 \pm 11.1 \mu\text{A}$  (mean  $\pm$  SD) in the control and  $30.1 \pm 14.3 \mu\text{A}$  (mean  $\pm$  SD) in the experimental group. The JOR peak-to-peak amplitude, latency, and duration values when the stimulus intensity was set at 1.5T, (Figure 3A through C, respectively). There were no significant differences in JOR properties at 5, 9, and 13 weeks in either group. However, the reflex peak-to-peak amplitude was significantly smaller, and the reflex latency was significantly longer in the experi-

mental group than in the control group ( $P < .05$ ) at each recording age, although the reflex duration was not significantly different between the two groups. For the 13-week-old animals, the peak-to-peak amplitude was  $2.9 \pm 0.9 \text{ mV}$  for the control and  $1.6 \pm 0.5 \text{ mV}$  for the experimental group, and the latency was  $4.6 \pm 0.2 \text{ ms}$  for the control and  $7.4 \pm 0.4 \text{ ms}$  for the experimental group. The duration was  $2.7 \pm 0.9 \text{ ms}$  and  $2.9 \pm 1.0 \text{ ms}$  in the control and experimental animals, respectively. All data are written as mean  $\pm$  SD.

## DISCUSSION

Masticatory functional alteration during growth is widely known to influence the developmental process of the masticatory system morphologically, but little is known concerning its effects on functional maturation. It is generally agreed that muscle activity for the basic pattern of rhythmical alteration of jaw-closing and jaw-opening movements is generated by central pattern generators (CPG) in the brain stem.<sup>6</sup> However, mastication is a behavior that has to adapt to environmental demands through the mediation of inputs to the CPG, which is highly dependent upon orofacial sensory afferents, and this modification can be carried out by interacting via reflex circuits on the motor neurons.<sup>6,20</sup> Therefore, jaw reflex studies can be useful to investigate alterations of sensorimotor processing in masticatory performance.

Our data showed that the altered masticatory envi-



**Figure 3.** (A) Peak-to-peak amplitude (mV), (B) Latency (ms), and (C) Duration (ms) were measured as the jaw-opening reflex (JOR) properties for statistical comparison between the two groups. The data are expressed as mean  $\pm$  SD; n indicates number of rats in each group at each recording age. \* Indicates significant difference from control group ( $P < .05$ , unpaired  $t$ -test).

ronment, consequent upon liquid-diet feeding during growth in rats, affects the JOR maturation as measured by peak-to-peak amplitude and latency properties. Although the differences between threshold values did not reach the statistically significant levels, it shows that the motor pool of the digastric muscle may not be as active in the experimental group due to underusage and reflects a decrease in low-threshold trigeminal sensory transmission to the digastric motor neurons.

Sensory inputs from low-threshold orofacial mechanoreceptors, such as mucosal and periodontal mechanoreceptors, muscle spindles, and temporomandibular receptors are known to be very important in regulating masticatory movement and force by providing sensory feedback along with eliciting the JOR when the mastication is performed.<sup>15,20,21</sup> Thus, modified sensory feedback elements from these mechanoreceptors due to alteration in masticatory function would possibly be one of the factors causing JOR suppression in the experimental rats. Nevertheless, the influences on the central control system, eg, cortical masticatory area, sensorimotor cortex, and other subcortical structures, cannot be excluded because the consistency of food was also suggested to affect the central mechanism controlling masticatory movement. This higher center may automatically reduce the level of attainable function after the decrease of the contributions from orofacial receptors, which would reveal the modulation of the gain for digastric reflex.<sup>16</sup> More practically stated, the suppression in the mean amplitude of JOR indicated a reduction in the sensitivity of the reflex. This implies that the regular pressure applied to the receptors might be subthreshold for evoking JOR.

Functional transition of feeding behavior from sucking to chewing during the early developmental period was reported to happen related to maturation of the JOR, which shows a short latency of digastric reflex as a sign of this maturation.<sup>10</sup> In the present study, this sucking-chewing transition in experimental rats that have been fed a liquid diet since the weaning time may be impeded. Therefore, the long-latency reflex, which is typically found in immature rats,<sup>10</sup> was recorded from adult rats in the experimental group when evoked by low-intensity electrical stimulation of the ipsilateral IAN. It may not be possible to accept totally that only afferent fibers mediating the nonnociceptive JOR were activated in this study. However, we made every effort to stabilize polarization of the stainless-steel stimulating electrodes for consistency of stimulus strength, and all intensities were clearly limited below 2T. Thus, we believe that such stimuli are nonnoxious stimulation and similar to those which naturally could occur while animals are performing mastication.<sup>8</sup> This suggests that the masticatory performance, developed

with a lack of experience in solid-diet mastication during growth, may be different from that with short-latency JOR in normal adult behavior.

This long-latency result in experimental rats agrees with a previous report<sup>22</sup> that showed a significantly slower conduction velocity of nerve fibers after an occlusal hypofunctional condition. This might be first explained by peripheral sensory system alteration. Maturation of peripheral A-fiber transduction mechanism to acquire their full stimulus-response sensitivity was recently shown to coincide with development of myelination.<sup>23</sup> There is no report directly revealing the effect of altered masticatory function on development of myelination or growth of fiber diameter in these nerve fibers, but it was reported to cause structural changes of the microvasculature in periodontal ligaments,<sup>24</sup> which could result in a deficiency of nutrients to the nerves. In undernourished developing rats, peripheral nerves have been reported to have retarded myelination<sup>25</sup> and a deficit in fiber growth.<sup>26</sup> Consequently, experimental rats, raised with a liquid-diet feeding, might have smaller diameter and/or decreased myelination of peripheral sensory nerves. Another possible explanation can be a longer central delay in the experimental rats because the functional sucking-chewing conversion is not only related to the development of the peripheral nervous system, but also to the maturation of the masticatory central pathway.<sup>27</sup> This is demonstrated to have a shift of the cortical projection area from the cortical sucking area to the cortical masticatory area during the maturation.<sup>20</sup>

Apparently, a lower occlusal force due to liquid-diet feeding can be one of the factors in this alteration, but masticatory function that is used to transport food intraorally has been argued to also be the main difference between the intake of a liquid and solid diet.<sup>14</sup> Although the liquid-diet feeding experimental model in rats used in the present study might not exactly resemble the alteration of masticatory function in temporomandibular disorders or malocclusion conditions, patients with poor masticatory function were reported to change the types of food and avoid chewing of hard food.<sup>28</sup> Taking all evidence and our data together, the maturation of the JOR might be impeded in these patients growing up with modulated masticatory function, and thus may affect the masticatory performance during adulthood.

## CONCLUSIONS

- Alteration in masticatory function during growth affects the functional development of mastication by causing the underdevelopment of JOR properties.
- The JOR is not fixed, but matures according to mastication during growth.

- Shortening of the latency, corresponding to the increased amplitude of the JOR, is the necessary sign of maturation for adult oral behavior.

## ACKNOWLEDGMENT

This study was financially supported by Grants-in-Aid for Scientific Research (17592129 and 17592130) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. Part of this study was presented at the 83rd Congress of the European Orthodontic Society, Berlin, Germany, June 20–24, 2007.

## REFERENCES

- Bakke M, Michler L. Temporalis and masseter muscle activity in patients with anterior open bite and craniomandibular disorders. *Scand J Dent Res*. 1991;99:219–228.
- Pereira LJ, Pastore MG, Bonjardim LR, Castelo PM, Gaviao MB. Molar bite force and its correlation with signs of temporomandibular dysfunction in mixed and permanent dentition. *J Oral Rehabil*. 2007;34:759–766.
- Santilakanawong P, Kirimoto H, Seki Y, Soma K. Inhibitory reflex responses of masseter muscle in anterior open bite. *Orthod Waves*. 2003;62:415–421.
- Maillou P, Cadden SW. Characteristics of a jaw reflex in humans with temporomandibular disorders: a preliminary report. *J Oral Rehabil*. 2007;34:329–335.
- Thilander B, Rubio G, Pena L, de Mayorga C. Prevalence of temporomandibular dysfunction and its association with malocclusion in children and adolescents: an epidemiologic study related to specified stages of dental development. *Angle Orthod*. 2002;72:146–154.
- Lund JP, Kolta A, Westberg KG, Scott G. Brainstem mechanisms underlying feeding behaviors. *Curr Opin Neurobiol*. 1998;8:718–724.
- Turker KS. Reflex control of human jaw muscles. *Crit Rev Oral Biol Med*. 2002;13:85–104.
- Haraguchi N, Yamada Y, Furusawa H, Matsuo K, Oi K, Sato T, Mizuno A. Variation of the jaw-opening reflex during spontaneous mastication in rabbits. *Brain Res Bull*. 1994;35:93–95.
- Smith A, Weber CM, Newton J, Denny M. Developmental and age-related changes in reflexes of the human jaw-closing system. *Electroencephalogr Clin Neurophysiol*. 1991;81:118–128.
- Thexton AJ, McGarrick JD. Maturation of brainstem reflex mechanisms in relation to the transition from liquid to solid food ingestion. *Brain Behav Evol*. 1984;25:138–145.
- Herring S. The ontogeny of mammalian mastication. *Am Zool*. 1985;25:339–349.
- Weijts WA, Brugman P, Grimbergen CA. Jaw movements and muscle activity during mastication in growing rabbits. *Anat Rec*. 1989;224:407–416.
- Horio T, Kawamura Y. Effects of texture of food on chewing patterns in the human subject. *J Oral Rehabil*. 1989;16:177–183.
- Thexton AJ, McGarrick JD. The electromyographic activities of jaw and hyoid musculature in different ingestive behaviours in the cat. *Arch Oral Biol*. 1994;39:599–612.
- Yamada Y, Haraguchi N. Reflex changes in the masticatory muscles with load perturbations during chewing hard and soft food. *Brain Res*. 1995;669:86–92.
- 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 Res*. 1998;796:257–264.
- Yoshida R. Study on the differentiation and development of muscle fibers of masticatory muscles in mice fed a liquid diet. *J Jpn Orthod Soc*. 1995;54:52–63.
- Thexton AJ, McGarrick JD, Stone TW. Pharmacologically induced changes in the latency of digastric reflexes in 1–7 day old rabbits. *Comp Biochem Physiol C*. 1988;89:383–387.
- Clarke RW, Matthews B. The effects of anesthetics and remote noxious stimuli on the jaw-opening reflex evoked by tooth-pulp stimulation in the cat. *Brain Res*. 1985;327:105–111.
- Nakamura Y, Katakura N. Generation of masticatory rhythm in the brainstem. *Neurosci Res*. 1995;23:1–19.
- Linden R. Periodontal mechanoreceptors and their functions. In: Taylor A, ed. *Neurophysiology of the Jaw and Teeth*. London, UK: Macmillan; 1990:52–95.
- Seki Y, Ishii N, Toda K, Soma K. Influence of occlusal hypofunction induced by opposed tooth loss on periodontal mechanoreceptors in rat molars. *Jpn J Oral Biol*. 2002;44:66–74.
- Lizarraga I, Chambers JP, Johnson CB. Developmental changes in threshold, conduction velocity, and depressive action of lignocaine on dorsal root potentials from neonatal rats are associated with maturation of myelination. *Can J Physiol Pharmacol*. 2007;85:251–263.
- Tanaka A, Iida J, Soma K. Effect of hypofunction on the microvasculature in the periodontal ligament of the rat molar. *Orthod Waves*. 1998;57:180–188.
- Hedley-Whyte ET. Myelination of rat sciatic nerve: comparison of undernutrition and cholesterol biosynthesis inhibition. *J Neuropathol Exp Neurol*. 1973;32:284–302.
- Sima A. Studies on fibre size in developing sciatic nerve and spinal roots in normal, undernourished, and rehabilitated rats. *Acta Physiol Scand Suppl*. 1974;406:1–55.
- Ikuno S. Development of chewing center at transition period from sucking to chewing [in Japanese]. *Jpn J Ped Dent*. 1989;27:595–606.
- Krall E, Hayes C, Garcia R. How dentition status and masticatory function affect nutrient intake. *J Am Dent Assoc*. 1998;129:1261–1269.