Effects of nasal obstruction on maturation of the jaw-opening reflex in growing rats

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ABSTRACT

Objective: Nasal obstruction during growth changes craniofacial morphology and function. However, the etiological mechanisms of these changes are unknown. The aim of the present study was to investigate the effects of nasal obstruction during growth on the maturation of the jaw-opening reflex (JOR) using an electrophysiological technique. We focused on the oral sensory receptors that regulate the activities and reflexes of the orofacial muscles.

Design: Sixty 6-day-old male Wistar rats were randomly divided into control and experimental groups (n=30 each). The experimental group underwent unilateral nasal obstruction at 8 days of age. The JOR was evoked by bilateral, low-intensity electrical stimulation of the inferior alveolar nerve. The electromyographic responses were recorded bilaterally from the digastric muscles at 5, 7, and 9 weeks of age.

Results: The latency of the JOR was significantly longer and the peak-to-peak amplitude was significantly smaller in the experimental group than in the control group at each age, while the duration was not significantly different. Intragroup comparison of the latency, peak-to-peak amplitude, and duration at 5, 7, and 9 weeks of age revealed no significant differences in either the control or experimental groups.

Conclusions: Unilateral nasal obstruction during growth may have significant effects on maturation of craniofacial function.

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1. Introduction

Normal craniofacial development and occlusion depend on various factors. For example, genetic factors influence the constitution of the facial and occlusal pattern.1 Acquired factors such as caries, oral habits, and nasopharyngeal conditions can also play important roles in craniofacial and occlusal development.2 Mammals, including humans, usually breathe through the nose. Normal respiratory activity favors harmonious craniofacial structures that allow for adequate interaction between mastication and swallowing.3

In orthodontic practice, patients often present with maxillary protrusion or an anterior open bite, problems that are frequently associated with nasal obstruction and mouth breathing.4 Recent studies have indicated that the number of patients with breathing problems caused by obstructive sleep apnea syndrome and allergic rhinitis is increasing, especially during the growth period of preadolescence and adolescence.5,6 A previous study in humans indicated that mouth
breathing may be due to an anatomical predisposition, velopharyngeal insufficiency, habit, or interference of lip closure.\textsuperscript{7} Many clinical studies have also elucidated relationships between nasal obstruction and craniofacial morphology. For example, a lower position of the mandible caused by mouth breathing increases the lower facial height\textsuperscript{8} and adenoidal facies.\textsuperscript{9} Changes in the craniofacial pattern might induce simultaneous changes in occlusion, such as an anterior open bite,\textsuperscript{8,10} maxillary protrusion,\textsuperscript{10} or posterior crossbite.\textsuperscript{10} On the other hand, some studies have elucidated the effects of nasal obstruction on masticatory function, such as inhibited masseter muscle activity\textsuperscript{11} and increased suprahoid muscle activity\textsuperscript{12} during mouth breathing. Moreover, hypotonia of the lips, tongue, and buccinator muscle\textsuperscript{13} has been reported, while mouth breathing was also shown to decrease masticatory activities.\textsuperscript{14,15}

Biochemical studies in rats suggest that nasal obstruction is associated with reduced growth of the masseter muscle and anterior digastic (Dig) muscle.\textsuperscript{16} In addition, fatigue-resistant myosin heavy chain (MHC) fiber types are increased in muscles in association with nasal obstruction.\textsuperscript{16} In terms of morphological changes, nasal obstruction results in a significant reduction in the vertical development of the nasomaxillary complex and in the skull base along the longitudinal axis.\textsuperscript{17} Although alterations of craniofacial morphology and function during nasal obstruction have been thoroughly investigated, little is known about the effects of nasal obstruction on the regulation of jaw movement. It is possible that alterations in jaw movements may modify craniofacial sensory transmission to jaw motoneurons, since the activity of jaw motoneurons is dependent on sensory feedback from craniofacial structures.\textsuperscript{18} The jaw-opening reflex (JOR), a trigeminal brainstem reflex that can be evoked by both nociceptive and non-nociceptive stimulation,\textsuperscript{19} plays an important role in the regulation of jaw movement during mastication.\textsuperscript{19} Thus, the JOR is a useful tool for investigating alterations of sensorimotor processing in masticatory performance.

In the present study, we focused on the oral sensory receptors that regulate the activity and reflexes of orofacial muscles. The aim of this paper was to investigate the effects of unilateral nasal obstruction during growth on JOR maturation using an electrophysiological technique.

2. Materials and Methods

2.1. Animal preparation

Sixty 6-day-old male Wistar albino rats were used in this experiment. The rats were weighed and then randomly divided into the control and experimental groups (n = 30 each). At 8 days of age, all rat pups were first anesthetized by hypothermia (10 min at \( -18 \) °C). Left-sided nasal obstruction was performed in the experimental group.\textsuperscript{17,20} The selected method involved cauterization of the left external nostril, which is the most common and simplest procedure allowing for the establishment of nasal obstruction in neonatal animals. The tissue surrounding the left external nostril was burned by placing a surgical cauterizing instrument (1 mm in diameter) on the nostril, consequently occluding the orifice of the nostril without mechanical or chemical damage to the olfactory mucosa. After cauterization, the nostril was coated with 3% chlorotetracycline (Aureomycin\textsuperscript{16} Ointment; Pola Pharma, Tokyo, Japan) to prevent infection. The pups were kept warm (37 °C) for 30 min and then returned to their mothers. The control group underwent a sham operation in which the cauterizing instrument was placed about 1–2 mm above the left nostril. The body weights were measured throughout the experimental period. The JOR was then recorded from the Dig muscle in control and experimental groups when the rats were 5, 7, and 9 weeks old (n = 10 per group per time point).

2.2. Stimulation and recording

For electrophysiological recordings, all rats were anesthetized with 60 mg/kg of thiamylal sodium (Isovol\textsuperscript{16}; Yoshitomi Pharmaceutical, Osaka, Japan) administered intraperitoneally. Cannulae were then inserted into the tracheal and femoral veins. The depth of anesthesia was carefully monitored throughout the experiment by checking the pupil size, withdrawal and corneal reflexes, and heart rate. To maintain the same anesthetic level in all rats while performing the JOR recording, a supplemental dose (5 mg/kg, intravenously) was routinely given 15 min before the start of the 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 noxious stimulation that could inhibit the JOR.

To bilaterally stimulate the inferior alveolar nerve (IAN), a pair of stainless-steel wire electrodes (0.1 mm in diameter, 0.5 mm in tip exposure) was inserted bilaterally into the mandibular canal through the mental foramen at depths of 1 and 3 mm. The bipolar electrode was kept in place by fixing it onto the adjacent mandibular bone with light-cured dental resin. Expression of the JOR was recorded bilaterally from the Dig muscles using paired stainless-steel wire electrodes with 1-mm exposed tips implanted along the direction of the muscle fibers. The interpolar distance was set at 3 mm for these recording electrodes (Fig. 1A). To ensure consistent reproduction of this distance, a two-keyhole index, with the keyholes 3 mm apart, made of dental acrylic (UNIFAST II; GC Corporation, Tokyo, Japan) was used to lead the paired electrode-inserted needles, thus allowing for placement of the electrodes at the same interpolar distance for every recording. The animals were then transferred to a stereotactic apparatus (models SN-2 and SM-15; Narishige Scientific Instruments, Tokyo, Japan) with their bodies in prone position (Fig. 1B). Before the stimulation, Dig electromyographic (EMG) baseline activity was captured for 1 min in each rat.

Next, the right and left IAN were alternately stimulated electrically (single pulse, 0.2 ms in duration) once every 5 s to evoke the JOR. The stimulus intensity was applied gradually in increasing in steps of 1 mA, and the lowest intensity that constantly elicited a Dig EMG response was determined as the JOR threshold (T). To attain comparable responses, the test stimulation current was adjusted to 1.5 times the threshold (1.5 T).\textsuperscript{21,22} An intensity of 2 T or less can be considered as non-nociceptive stimulation,\textsuperscript{23} which we expected in this
study. Dig responses on the right side were recorded by stimulating the ipsilateral IAN, and the contralateral Dig responses were recorded by stimulating the contralateral IAN.

EMG activity was amplified by a 100-Hz to 3-kHz bandwidth differential amplifier (DAM-80; WPI, Sarasota, FL, USA). All signals were fed into a computer by means of a CED 1401 interface (5000/s sampling rate) and were later analyzed offline with Spike2 software for Windows, version 4.02 (Cambridge Electronic Design, Cambridge, UK). After recording, the animals were euthanized with an intraperitoneal overdose of thiamylal sodium. The Dig muscle was then dissected to confirm the location of the placed electrode.

2.3. Data analysis

The latency, duration, and peak-to-peak amplitude were measured as the JOR properties for comparison between 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 ms. The latency and duration were indicated as time intervals in ms. The latency was defined as the amount of time between the stimulus and the first point at which Dig EMG activity exceeded 2 SD of the baseline activity (i.e., onset), whereas the duration was defined as the amount of time between the onset and the point at which the response dropped below 2 SD of the baseline activity (i.e., offset). The peak-to-peak amplitude in mV was calculated using the Spike2 analysis function between the onset and offset in each single sweep (Fig. 1 C).

2.4. Statistical analysis

All data are expressed as mean ± SD. Unpaired t-test was used for statistical comparison of the weight between the two groups. Repeated measures multivariate analysis of variance
was used for intergroup and intragroup statistical comparison. Simple main effects analysis using the Sidak adjustment was used for multiple comparisons. Statistical analysis was performed using SPSS for Windows software, version 13.0 J (SPSS Inc., Chicago, IL, USA), and statistical significance was established at $p < 0.05$.

### 3. Results

The mean body weight of all rats regularly increased throughout the experimental period. There was no significant difference between the two groups (Fig. 2). Low-intensity electrical stimulation of the right and left IAN always elicited JOR responses of the bilateral Dg muscles in all animals. Typical examples in both groups recorded at 7 weeks of age are shown in Figure 3. The threshold was $39.6 \pm 15.0 \text{ mA (mean \pm SD)}$ in the control group and $41.7 \pm 22.2 \text{ mA}$ in the experimental group.

The latency, duration, and peak-to-peak amplitude when the stimulus intensity was set at 1.5 $\text{T}$ are shown in Figure 4A–C. For the 5-week-old animals, the latency of the left side was $5.1 \pm 0.3 \text{ ms}$ for the control and $6.0 \pm 0.4 \text{ ms}$ for the experimental group, while the right side was $5.3 \pm 0.3 \text{ ms}$ for the control and $6.1 \pm 0.6 \text{ ms}$ for the experimental group. For the 7-week-old animals, the latency of the left side was $4.9 \pm 0.6 \text{ ms}$ for the control and $5.7 \pm 0.3 \text{ ms}$ for the experimental group, while the right side was $5.1 \pm 0.4 \text{ ms}$ for the control and $5.8 \pm 0.5 \text{ ms}$ for the experimental group. For the 9-week-old animals, the latency of the left side was $4.9 \pm 0.5 \text{ ms}$ for the control and $5.6 \pm 0.3 \text{ ms}$ for the experimental group, while the right side was $5.0 \pm 0.5 \text{ ms}$ for the control and $5.8 \pm 0.5 \text{ ms}$ for the experimental group. The latency was significantly longer in the experimental group than in the control group at each recording age (Fig. 4A). Intragroup comparison among 5, 7, and 9 weeks of age revealed no significant difference in either the control or experimental group. There were also no significant differences between the left and right sides in each group at each recording age.

For the 5-week-old animals, the duration of the left side was $6.1 \pm 1.0 \text{ ms}$ for the control and $6.5 \pm 0.5 \text{ ms}$ for the experimental group, while the right side was $6.0 \pm 0.7 \text{ ms}$ for the control and $6.1 \pm 0.7 \text{ ms}$ for the experimental group. For the 7-week-old animals, the duration of the left side was $6.1 \pm 0.5 \text{ ms}$ for the control and $6.3 \pm 0.3 \text{ ms}$ for the experimental group, while the right side was $5.9 \pm 0.4 \text{ ms}$ for the control and $6.2 \pm 0.7 \text{ ms}$ for the experimental group. For the 9-week-old animals, the duration of the left side was $6.0 \pm 0.6 \text{ ms}$ for the control and $6.4 \pm 0.5 \text{ ms}$ for the experimental group, while the right side was $6.0 \pm 0.4 \text{ ms}$ for the control and $6.3 \pm 0.8 \text{ ms}$ for the experimental group. The duration was not significantly different between the two groups (Fig. 4B). Intragroup comparison among 5, 7, and 9 weeks of age revealed no significant difference in either the control or experimental group. There were also no significant differences between left and right sides in each group at each recording age.

For the 5-week-old animals, the peak-to-peak amplitude of the left side was $1.0 \pm 0.3 \text{ mV}$ for the control and $0.7 \pm 0.3 \text{ mV}$ for the experimental group, while the right side was $1.1 \pm 0.2 \text{ mV}$ for the control and $0.8 \pm 0.2 \text{ mV}$ for the experimental group. For the 7-week-old animals, the peak-to-peak amplitude of the left side was $1.2 \pm 0.3 \text{ mV}$ for the control and $0.8 \pm 0.1 \text{ mV}$ for the experimental group, while the right side was $1.2 \pm 0.1 \text{ mV}$ for the control and $0.8 \pm 0.2 \text{ mV}$ for the experimental group. For the 9-week-old animals, the peak-to-peak amplitude of the left side was $1.3 \pm 0.2 \text{ mV}$ for the control and $0.9 \pm 0.2 \text{ mV}$ for the experimental group, while the right side was $1.3 \pm 0.2 \text{ mV}$ for the control and $1.0 \pm 0.3 \text{ mV}$ for the experimental group. The peak-to-peak amplitude was significantly smaller in the experimental group than in the control group at each recording age (Fig. 4C). Intragroup comparison among 5, 7, and 9 weeks of age revealed no significant difference in either the control or experimental group. There were also no significant differences between the left and right sides in each group at each recording age.

### 4. Discussion

The aim of the present study was to examine how nasal obstruction affected the regulation of JOR. The present results indicate that unilateral nasal obstruction impacts oral function by delaying conduction velocity and thereby delaying the JOR.

#### 4.1. Animal models of nasal obstruction

There are numerous studies examining the effects of nasal obstruction on systemic function. Bilateral nasal obstruction
is reportedly associated with reduced muscle growth of the superficial masseter and anterior Dig muscles,\textsuperscript{16} respiratory muscle fiber adaptation,\textsuperscript{24} reduced growth of the olfactory bulbs,\textsuperscript{25} an initial decrease in body weight\textsuperscript{20} and lung growth,\textsuperscript{24} and adrenal hypertrophy.\textsuperscript{24} Bilateral nasal obstruction also affects craniofacial growth and development; increases in both the vertical dimension of the nasomaxillary complex and the anteroposterior dimension of the cranial base were proven to be smaller than those in normal breathing groups.\textsuperscript{25}

On the other hand, several researchers have investigated the effects of unilateral nasal obstruction. For example, the ipsilateral olfactory bulb does not grow to be as large as that on the normal side,\textsuperscript{26} and craniofacial growth is altered; there are reductions in the vertical development of the nasomaxillary
complex and in the longitudinal axis of the skull base. These findings suggest that while bilateral nasal obstruction induces invasive effects in the whole body, unilateral nasal obstruction may also affect whole body systems. Bilateral nasal obstruction was associated with significant reductions in body weight in our pilot study. As such, we chose an animal model of unilateral nasal obstruction for the present study.

With regard to morphology in association with nasal obstruction, previous animal studies have reported a significant reduction in the vertical development of the maxillary complex and in the skull base along the longitudinal axis. Gross also demonstrated a significant decrease in the maxillary width and the intermolar width in rats. A narrow maxilla is one of the craniofacial morphological patterns accompanied by mouth breathing. Asahina reported the effects of nasal obstruction on the growth and development of the craniofacial complex in young golden hamsters. According to Asahina, the mandibular plane (MP) angle was significantly larger than controls, as nasal obstruction caused significant molar extrusion. Therefore, our animals might have had larger MP angles.

In humans, the relationship between masticatory muscle function and craniofacial morphology has been extensively investigated. Findings include a negative correlation between the masseter muscle activity and occlusal force. As our experimental setup limited morphological investigation, these relationships between morphological and functional influences of nasal obstruction should be investigated in future studies.

Tongue pressure during nasal obstruction was reported to be significantly greater. In the rat, nasal obstruction can lead to hypoxia. Further, hypoxia in rats increased genioglossus muscle activity from these findings, we suggest that nasal obstruction increases the tongue-protruding muscle activity, which may cause a occlusal disharmony.

4.2. Changes in JOR associated with nasal obstruction

4.2.1. JOR induced by non-nociceptive stimulation and maturation of JOR

The JOR evoked by high-intensity stimulation is related to pain, and its functional significance is well established. The JOR evoked by low-intensity stimulation is also thought to be important in masticatory movement. Several previous studies examined the JOR evoked by low-intensity stimulation of the periodontal mechanoreceptors, and reported that it is essential in modulation of mastication. In the IAN, there are fibers for sensation of pain, touch, and pressure that originate from the lips, teeth, and intraoral mucosa. In our study, the intensity of IAN stimulation was less than 120 μA. This is lower than the intensity used in awake animals (maximum 130 μA) and could be considered to be non-nociceptive stimulation.

We found that the latency, duration, and peak-to-peak amplitude in both the control and experimental groups remained stable throughout the experimental period. These findings are in line with those of soft-diet feeding. A previous study suggested that the response properties of rat periodontal mechanoreceptors attain maturation by 5 weeks of age. Another study indicated that temporomandibular joint (TMJ) mechanoreceptor maturation is attained by the age of 5 weeks. Both TMJ mechanoreceptors and periodontal mechanoreceptors are trigeminal nerve endings. Our findings indicate that the JOR also attains maturation by 5 weeks of age without a significant influence by nasal obstruction.

4.2.2. Changes in latency

The latency of the JOR decreases in parallel with the aging of rats. In rat pups the long latency of the Dig reflex shortens to the adult value just before the animal starts to explore solid food (at 14 days). This change takes place before weaning, which usually occurs about a week later (i.e., 3 weeks of age). Latency is associated with the conduction velocity of nerve fibers. The conduction velocity rises with increases in myelination and axon diameter.

Periodontal mechanoreceptors are reportedly affected and the conduction velocity of nerve fibers decreases after an occlusal force reduction. The delayed conduction velocity might be explained by peripheral sensory system alteration. Maturation of peripheral A-fibers, which control touch and pressure transduction, was recently shown to coincide with the development of myelination. The prolongation of the latency was supported by the findings in animal models of soft-diet feeding and an anterior open bite. No report has directly revealed the effect of altered masticatory function on the development of myelination or enlargement of fiber diameter in these nerve fibers. However, it can be assumed that nasal obstruction induces a similar condition by reducing occlusal force. In turn, nasal obstruction delays the conduction velocity and increases the latency, resulting in delayed development of reflex regulation.

The interneurons of the JOR are located in the spinal trigeminal subnucleus oralis (SpVo) and in the subnucleus interpolaris (SpVl). Morphological studies have revealed the reciprocal connections between the vestibular nuclei (VN) and spinal trigeminal nuclei in rats. Both the SpVo and SpVl are known to contain premotor neurons projecting to the trigeminal motor nucleus. Moreover, the trigeminal primary afferent fibers project to the VN. A physiological study found that activation of the vestibular afferents elicited excitatory responses in the jaw-opening motoneurons. Thus, it is plausible that the VN may be involved in control of the JOR. Another study revealed that nasal obstruction induced hypoxia in the rat. On the other hand, reported that hypoxia could cause inhibition of postsynaptic components in the VN. Therefore, nasal obstruction may affect a component of the reflex pathway of the JOR via hypoxia.

4.2.3. Changes in duration and peak-to-peak amplitude

We found no significant difference in the reflex duration between the control and experimental groups, which is supported by previous studies of soft-diet feeding and an anterior open bite. The reflex duration is related to the number of muscle fibers contributing to the motor unit. In general, the number of muscle fibers decreases when muscle atrophy occurs. As the reflex duration did not decrease in our study, this suggests that muscle atrophy did not occur. Therefore, it is likely that the number of muscle fibers that contribute to the motor unit activity was not significantly affected by nasal obstruction.
The effects of nasal obstruction on the peak-to-peak amplitude were also similar to those in studies of soft-diet feeding and an anterior open bite, the amplitude of the JOR did not significantly increase with developmental age. Gelhaye and colleagues found that in 21-day-old rats the nasal obstruction was associated with a greater decrease of MHC 2x compared with 2a in the anterior Dig muscle. This indicates that the fatigue-resistant MHC fiber types increased in muscles in association with nasal obstruction. It was reported that the anterior Dig muscle attained its specific adult fiber-type profile by approximately 6 weeks. Therefore, we suggest that the amplitude of the JOR is not affected by aging from 5 to 9 weeks.

The peak-to-peak amplitude is calculated as the voltage difference of the motor unit potential and is determined by the diameter and number of muscle fibers closest to the electrode. The amplitude decreases when the periodontal ligament becomes narrower, and periodontal mechanoreceptors become degenerated. Thus, nasal obstruction may induce the weak occlusal force, triggering a reduction in the stimulation to the periodontal mechanoreceptors, thus affecting the periodontium and decreasing the amplitude. This assumption should be investigated in a further study.

4.2.4. Laterality
The results of the present study showed no significant differences in the latency, duration, or peak-to-peak amplitude between the left and right sides. This may be explained by the fact that the left and right nasal cavities of the rat are anatomically connected, and the open side may compensate for the occluded side. Therefore, we speculate that a unilateral nasal obstruction model can simulate incomplete nasal breathing, which may affect the systemic craniofacial function without laterality.

4.3. Oral environmental changes and masticatory performance
Masticatory efficiency is influenced by many factors, including the occlusion, occlusal contact area, oral tissue, salivary secretion, occlusal force, and mandibular movements. The coordination of oral tissue in cases of nasal obstruction is compromised. The activity of the jaw-closing muscles decreases, as does the occlusal force, and jaw movement is no longer smoothly performed. A decreased occlusal force reportedly affects the craniofacial morphology. Furthermore, a reduced occlusal force in growing rats reportedly affects the morphological maturation of the periodontal mechanoreceptors. Previous studies have shown that soft-diet feeding affects the masticatory function and changes the response characteristics of the oral sensory receptors. Our findings demonstrated similar findings to those studies. Therefore, unilateral nasal obstruction is a factor that influences masticatory efficiency.

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Conflict of interest
None declared.

Ethical approval
The experimental procedures described herein were approved by the Institutional Animal Care and Use Committee (#0130064A) and performed in accordance with the Animal Care Standards of Tokyo Medical and Dental University.

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Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.archoralbio.2014.02.013.

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