Unilateral nasal obstruction induces degeneration of fungiform and circumvallate papillae in rats

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KEYWORDS
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Background: In clinical orthodontic treatment, chronic respiratory disturbance or mouth breathing has been concerned symptoms and screening criteria. In this study, to analyze the relation between nasal obstruction and taste sensing, a unilateral nasal obstruction model was performed to investigate the taste papillae and taste buds in rats.

Methods: Fourteen 6-day-old male Wistar rats were randomly divided into control and experimental groups (n = 7 each). The experimental group underwent unilateral nasal obstruction at 8 days of age. The rats were euthanized at 9-week-old. The morphology of the circumvallate papillae and taste buds were identified by immunohistochemical methods. The fungiform papillae were visualized with 1% methylene blue and sectioned for taste bud observation.

Results: Some defects in the gustatory epithelium were observed after unilateral nasal obstruction. Rats in the experimental group had significantly fewer fungiform papillae and smaller volumes of taste bud. In circumvallate papillae, smaller total taste bud area was found in experiment group.

Conclusion: Findings in the present study suggest that nasal obstruction might have significant influences on the gustatory function via morphologic change in the taste papillae and taste buds in tongue area.

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Introduction

Chronic mouth breathing caused by chronic nasal obstruction has been associated with atypical mobilization of oral muscles, which in turn might affect craniofacial development and lead to a decreased posterior facial height and increased lower facial height. Changes in the craniofacial pattern might induce simultaneous changes in occlusion, such as the development of an anterior open bite, maxillary protrusion, and posterior crossbite. Thus, in clinical orthodontic practice, chronic respiratory disturbances and mouth breathing are concerning symptoms and screening criteria, especially in growing children.

Ingestion and sucking habits are altered in the early stage of chronic nasal obstruction. In fact, a very strong relationship between nasal breathing and oral function exists. During food intake, nasal breathing ensures an ongoing supply of oxygen; furthermore, orthonasal and retronasal olfaction plays an important role in the tasting of food and motivation for food intake. Smell disorders are considered to have a great influence on taste. In one clinical study, approximately two-thirds of patients with olfactory loss reported not only decreased olfactory function but also taste loss. Another study indicated that the incidence of smell and taste disorders in patients with allergic rhinitis was 21.4% and 31.2%, respectively.

Food intake behavior has crucial importance in the nutrition and quality of life of developing children and young adults. Whether other factors besides olfactory deprivation affect food oral processing in patients with chronic nasal obstruction remains unclear. Disruption of the normal nasal breathing route by nasal obstruction may affect not only respiratory function but also all interdependent sensorimotor nasal, oral, and laryngeal functions. However, the effect of chronic nasal obstruction on peripheral taste anatomical structures has not been described.

Taste receptor cells in the oral cavity predominantly reside within multicellular rosette clusters called taste buds. Taste buds are most prevalent on small pegs of epithelium on the tongue called papillae. Fungiform papillae are present on the anterior two-thirds of the tongue, foliate papillae are present on the posterolateral edges of the tongue, and circumvallate papillae are present on the posterior tongue.

Gustatory function has crucial importance in appreciation of food and quality of life. It might adversely affect ingestion of nutrients, which are essential to health, especially for growing children and young adults. In this study, a unilateral nasal obstruction model was established to investigate the taste papillae and taste buds in rats and elucidate the relationship between nasal obstruction and taste sensation.

Material and methods

Animals

All animal use and experimental procedures were approved by the Institutional Animal Care and Use Committee and performed in accordance with the Animal Care Standards of our institution (0170352A). Fourteen 6-day-old male Wistar rats (n = 14) were purchased from Sankyo Labo Service Corporation (Tokyo, Japan) and randomly divided into experimental and control groups (7 rats in each group). At 8 days of age, all rat pups were anesthetized by hypothermia (10 min at −18 °C). Left-sided nasal obstruction was established in the experimental group. The tissue surrounding the left external nostril was coagulated by placing a 1-mm-diameter heat-treated surgical cauterizing instrument 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% chlortetracycline (Aureomycin Ointment; Pola Pharma, Tokyo, Japan) to prevent infection. The pups were kept warm (37.8 °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. Water and food were provided ad libitum during the whole experiment. The body weights of the rats were measured throughout the experimental period.

Number of fungiform papillae

The rats were euthanized 56 days after the surgical procedure (9 weeks of age). Tongue tissues were removed and fixed overnight with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4 °C. The tongues were sectioned in front of the intermolar eminence and then divided into left and right sides along the median sulcus with a surgical blade. Each tongue specimen was stained with 1% methylene blue tetrahydrate (Wako Pure Chemical Industries, Osaka, Japan). The number of fungiform papillae was counted on the left and right sides under a light microscope (Microphot-FXA; Nikon, Tokyo, Japan) equipped with a digital camera (DXM 1200; Nikon).

Taste bud volume of fungiform papillae

The anterior and laryngeal portions of the tongue were embedded in paraffin separately according to a standard protocol using an automatic process machine (RH-12DM; Sakura Finetek Japan, Tokyo, Japan). Serial 5-μm-thick coronal sections were prepared using a microtome (Leica RM 2155; Leica, Nussloch, Germany). The first 1 mm of the anterior portion of the tongue was discarded. The remaining continuous sections were deparaffinized with xylene, rehydrated in a graded ethanol series, and stained with hematoxylin and eosin. Taste bud volume measurements across 1 mm of tongue tissue were performed randomly among 10 fungiform papillae that contained taste buds. The volume of each taste bud of each fungiform papilla in these areas was reconstructed by drawing around the perimeter of each serial section and multiplying the area by a section thickness of 5 μm with the aid of digital imaging software (ImageJ 1.33; NIH, Bethesda, MD, USA).

Immunohistochemical staining and structural measurement of taste papillae and taste buds

The continuous sections of whole circumvallate papillae were prepared and numbered, and the middle number of 10
sections was then selected for histological evaluation because the taste buds were generally <50 μm in diameter, and most were encompassed in 10 5-μm sections.

Immunostaining using a streptavidin-biotin-peroxidase method was performed to evaluate the taste buds in the circumvallate papillae. The sections were deparaffinized with xylene and rehydrated in a graded ethanol series. Endogenous peroxidase was blocked by incubation for 15 min at room temperature in peroxidase-blocking solution (Dako, Carpinteria, CA, USA). Then samples were washed for 3 min each in 0.1% Tween 20 in 0.1 M phosphate-buffered saline (PBS), followed by overnight incubation at 4 °C in 1:100 rabbit anti-rat polyclonal antibody to cytokeratin 8 (Abcam, Cambridge, MA, USA) in 0.1 M PBS. The sections were then incubated with a secondary antibody, biotin-conjugated goat anti-rabbit polyclonal antibody (Histofine Simple Stain Rat MAX PO (MULTI); Nichirei, Tokyo, Japan), for 30 min at room temperature. Three additional washes in PBS preceded incubation with 3,3-diaminobenzidine (Vector Laboratories Inc., Burlingame, CA, USA) for 1 min. After incubation, the sections were rinsed in distilled water, counterstained in hematoxylin, rinsed for 30 min under running tap water, and mounted with mounting reagent (Aqua Poly/Mount Coverslipping Medium; Polysciences Inc., Eppelheim, Germany).

The width of the elevated portion of the circumvallate papillae trench depth and trench area was measured. The width of the circumvallate papillae was defined as the separation between the two trenches at their anteroposterior midpoint. The trench depth was defined as the distance from the top of the trench opening to the bottom of the trench. The trench area was referenced to the epithelium area of the inner and outer trench walls (the fringing epithelium of the circumvallate papilla). The number of taste buds per trench and taste bud area was determined. The taste bud area was defined as the sum of the taste buds per section, which was detected by immunohistochemical staining of cytokeratin 8 according to a previously described technique (Fig. 1). All parameters were measured three times independently from cross-sections of uniformly mounted tongue with the aid of digital software (Image J 1.33; NIH, Bethesda, MD, USA), and the average values were used for statistical analysis.

Statistical analysis

Statistical analysis of the independent samples was performed by an unpaired two-sample t-test using statistical analysis software (JMP; SAS Institute, Cary, NC, USA). A normality test in each group indicated that the sample data were normally distributed. Data are reported as mean ± standard deviation, and differences are considered statistically significant at P < 0.05.

Results

Body weight

The body weights of the rats are shown in Fig. 2A. There were no significant weight differences between the control and experimental groups during the study period.

Tongue lengths and numbers of fungiform papillae

Tongue lengths were measured from the intermolar eminence to the point of transition from the ventral smooth epithelium to the rough dorsal surface. There was no significant difference in tongue length between the experimental and control groups (Fig. 2B). The tongue was separated into left and right sides by the midline. The number of fungiform papillae on half of the tongue from the tip to the intermolar eminence was counted. In the control group, the average number of fungiform papillae on the right and left sides was 64.1 ± 4.5 and 63.9 ± 3.4, respectively. In the experimental group, the average number of fungiform papillae on the right and left sides was 58.0 ± 5.2 and 58.3 ± 4.2, respectively. The mean number of fungiform papillae was significantly lower in the
experimental than control group on both the right ($P = 0.013$) and left sides ($P = 0.015$). There was no significant difference between left and right sides in either group (Figs. 2C and 3).

Changes in taste bud volume in fungiform papillae

The average volume of taste buds in the fungiform papillae in the control and experimental groups was $65605.1 \pm 1358.5$ and $63587.5 \pm 1310.3 \mu m^3$, respectively. There was a significant difference between the two groups ($P = 0.026$) (Figs. 2D and 4).

**Morphological changes in circumvallate papillae**

Morphological changes in the circumvallate papillae were defined by the papillae width and height (trench depth). The trench area and taste bud area were also evaluated.

The average circumvallate papillae width in the control and experimental groups was $641.0 \pm 81.1$ and

![Figure 2](image)

**Figure 2** (A) Weight change throughout the experiment period. (B) Length of the tongue. (C) Numbers of fungiform papillae. (D) Volume of taste buds in fungiform papillae. (E) Width of circumvallate papillae and trench length. (F) Trench wall area and total taste bud area in a trench. The statistical significance in the control and experimental groups is given at the top of the bars (N.S: not significant, *$P < 0.05$).

![Figure 3](image)

**Figure 3** Dorsal surface of tongue stained with 1% methylene blue under microscopic observation. The white spots indicate the position of the fungiform papillae, while the small prominences are the filiform papillae. Scale bar = 500 \mu m. Left: control group. Right: experimental group.
There was no significant difference in the width between the two groups ($P = 0.117$). The average circumvallate papillae trench depth in the control and experimental groups was $514.1 \pm 75.3$ and $501.7 \pm 56.7 \mu$m, respectively. There was no significant difference in the trench depth between the two groups ($P = 0.524$) (Fig. 2E).

The average circumvallate trench area in the control and experimental groups was $66443.1 \pm 7235.0$ and $65678.5 \pm 6148.1 \mu\text{m}^2$, respectively. There was no significant difference in the trench area between the two groups ($P = 0.201$). The average total area of taste bud area in the circumvallate papillae in the control and experimental groups was $20052.3 \pm 1583.4$ and $17270.6 \pm 1231.0 \mu\text{m}^2$, respectively. There was a significant difference in this area between the two groups ($P = 0.034$) (Figs. 2F and 5).

Discussion

No significant difference was observed in the weight of the rats between the two groups, suggesting that differences in growth and development did not influence the differences observed in the peripheral taste organ. In a study involving humans, patients with olfactory loss reported alterations of dietary behaviors; however, the mean body mass index was negatively correlated.$^{17}$

In the present study, a unilateral nasal obstruction model was established to change the breathing pattern in the rats. Indeed, the survival rate of the rats was low after performing the bilateral nasal obstruction because food intake and utilization were affected.$^{15}$ Several studies involving the use of a unilateral naris occlusion model revealed that the change of the jaw-opening reflex$^{37}$ increased the tongue

**Figure 4** Continuous section of fungiform papillae stained by hematoxylin and eosin. Upper: control group. Lower: experimental group. Scale bar = 100 \mu m.

**Figure 5** Taste bud in the trench of a circumvallate papilla immunopositive for cytokeratin 8 (brown) and counterstained with hematoxylin. Left: control group. Right: experimental group. Scale bar = 100 \mu m.
Nasal obstruction alters taste papillae in rats

protrusive force. Another study suggested a decrease in the arterial oxygen saturation, which indicated a change in the breathing pattern. Rats do not readily learn to breathe totally by mouth. In humans, most patients with nasal obstruction breathe through both the mouth and nose; only <5% breathe only through the mouth.

A number of reports have illustrated the consequences of unilateral nasal obstruction on the unilateral olfactory pathway. In animal models, unilateral naris occlusion leads to a substantial decrease in the thickness of the ipsilateral respiratory and olfactory mucosa. Furthermore, unilateral naris occlusion performed in neonate animals prevents the ipsilateral olfactory bulb from reaching its normal adult size. Nerve innervation in the taste system is crucial to taste cell maintenance and turnover. Several studies have reported that fungiform taste buds and circumvallate papillae degenerate after nerve injury or depletion of neurotrophin-dependent innervation. The findings of the present study indicate a possible relationship between nasal obstruction and morphological changes in peripheral taste anatomical structures. The neuroplastic relationship between these two sensory systems seems worthy of further research. In the present study, the peripheral gustatory organ showed no significant ipsilateral change; this is in contrast to previous studies of the nasal mucosa and nose bulb that used the same manipulation. In fact, in a study of unilateral chorda tympani nerve damage, taste perception bulb that used the same manipulation. In fact, in a study of unilateral chorda tympani nerve damage, taste perception

Nasal obstruction also disturbs intake of the mother’s milk. A few hours of deprivation of the mother’s milk is correlated with a significant reduction in thyroid hormones and an increase in plasma corticosterone levels. Thyroid, renal, adrenal, and gonadal hormones play a key role in early development. Thyroid hormones have crucial effects in the maturation of fungiform papillae. Thus, hypothroidism could act as a negative factor in the development of papillae with subsequent reduction in taste perception. Nasal obstruction has been shown to induce changes in the physiology, form, and function in the craniofacial area. The findings in the present study suggest that nasal obstruction might have significant influences on gustatory function via morphologic changes in the taste papillae and taste buds of the tongue. This is the first study to indicate the possible relationship between nasal obstruction and changes in taste anatomical structures. Further studies are needed to specify the modulatory effects of nasal obstruction and the cascade effect of mouth breathing on gustatory function.

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Ethical approval

All procedures involving animals in this study were approved by the Institutional Animal Care and Use Committee and followed the Animal Care Standards of Tokyo Medical and Dental University.

Conflict of interest

All co-authors have agreed with the paper’s contents, and there is no conflict of interest.

References


