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Original Article

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

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## KEYWORDS

Nasal obstruction;  
Oral breathing;  
Taste papillae;  
Taste bud

**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.

**Conclusions:** 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,<sup>1</sup> which in turn might affect craniofacial development and lead to a decreased posterior facial height and increased lower facial height.<sup>2,3</sup> 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.<sup>4</sup> 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.<sup>5</sup> 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.<sup>6</sup> In one clinical study, approximately two-thirds of patients with olfactory loss reported not only decreased olfactory function but also taste loss.<sup>7</sup> Another study indicated that the incidence of smell and taste disorders in patients with allergic rhinitis was 21.4% and 31.2%, respectively.<sup>8</sup>

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.<sup>9</sup> 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.<sup>10,11</sup>

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  $-18^{\circ}\text{C}$ ). Left-sided nasal obstruction was established in the experimental group.<sup>12,13</sup> 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 ( $37.8^{\circ}\text{C}$ ) 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  $4^{\circ}\text{C}$ . 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- $\mu\text{m}$ -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  $\mu\text{m}$  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

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