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Impairment of nasal airway under intermittent hypoxia during growth period in rats

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ABSTRACT

Objective: To clarify the influences of intermittent hypoxia (IH) on the growth and development of the midfacial area, including the nasal cavity, in growing rats.

Design: Seven-week-old male Sprague–Dawley rats were divided into two groups: the experimental group ($n = 5$), which was exposed to IH for 8 h during light periods at a rate of 20 cycles/h (nadir, 4% O_2 to peak, 21% O_2 with 0% CO_2), and the control group ($n = 5$), which was exposed to room air. After 3 weeks, the maxillofacial structures in both groups were evaluated with respect to the height, width, length, surface area, cross-sectional area, and volume of the nasal cavity using soft X-ray and micro-CT.

Results: The experimental group showed a significantly smaller cross-sectional area and volume than did the control group. The surface area exhibited no significant differences between the two groups, although it tended to be smaller in the experimental group than in the control group. The nasal volume divided by the length of the tibia (for comparison with whole-body growth) was significantly smaller in the experimental group than in the control group.

Conclusions: These data suggest that IH exposure suppresses growth and development of the nasal cavity and may result in nasal breathing disturbance.

1. Introduction

Obstructive sleep apnoea (OSA) is defined as partial or complete upper airway obstruction during sleep that is associated with at least one of the following: sleep disruption, hypoxaemia, hypercapnia, or daytime symptoms.[1] Many studies have investigated OSA in adults. However, the pathogenesis of OSA in childhood is unique and different

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from that in adulthood, especially the effects on cognitive function and delayed physical development. Furthermore, the effect of craniofacial morphological development contributes to the onset of adult OSA.[2] Because delayed treatment may cause irreversible developmental disorders, adequate early diagnosis and intervention are needed.

Snoring is a fricative produced by airway narrowing during sleep, and it is an important sign of a potential sleep disorder. In 2005, paediatric OSA (POSA) was independently classified, and diagnostic criteria were specified for the first time.[3] Habitual snoring and breathing cessation were reported in 34.5% and 18.6% of preschool-aged children, respectively. Moreover, pathologic snoring was present in 12.0% of children.[4,5] Habitual snorers have significantly more nighttime symptoms, including observed apnoeas, difficulty breathing, restless sleep, and mouth breathing during sleep, than do nonsnorers.[6] Thus, the presence of POSA may be hidden among these symptoms, and it is currently estimated to affect up to 2% of all children.[7] The influence of respiratory function on the development of the orofacial structures has been widely discussed. The causes of POSA include obesity, adenotonsillar hypertrophy, a small dental arch, and micrognathia. Therefore, POSA and orofacial development are closely related.

Intermittent hypoxia (IH) is one of the main symptoms of OSA.[8] It is a condition in which the human body is temporarily deprived of an adequate oxygen supply to the blood. The causes of hypoxia vary according to growth and gender.[9] IH can be present in both children and adult patients. During IH, periods of normal breathing and oxygen supply, termed normoxia, also take place. Many studies have shown on the systemic level that IH is associated with several conditions, including obesity, high blood pressure, cardiovascular disease, inflammation, and postnatal growth and development.[10,11] On the cellular level, IH increases the levels of reactive oxygen species (ROS) in cells.[12] The sympathetic nervous activation under intermittent hypoxia is widely implicated in the systemic pathophysiology of OSA.[13] ROS signalling in neural cells critically mediates IH-evoked autonomic morbidities including persistent sympathetic activation, hypertension and elevated circulating catecholamines.[14,15] The study using rat IH model suggested that IH suppresses mRNA expression of growth hormone (GH) in the rat anterior pituitary.[16] IH may contribute impairment of facial features in OSA children. However, no study has shown the influence of IH on orofacial development. Therefore, the aim of this study was to clarify the influences of IH on the growth and development of the midfacial area, including the nasal cavity, in growing rats.

2. Materials and methods

2.1. *Animal model*

Seven-week-old male Sprague–Dawley rats were divided into two groups: the experimental group ($n = 5$), which underwent IH at a rate of 20 cycles/h (nadir, 4% O_2 to peak, 21% O_2 with 0% CO_2), and the control group ($n = 5$), which was exposed to room air for breathing. The rats were in the growing stage of maxillofacial region[17] at the start of IH exposure, and we planned to finish the experiment after the age of puberty onset in male rats.[18] Both groups were placed in the same type of plastic cage and placed next to each other. The IH cage was equipped with an IH apparatus that functioned for 8 h during the 12-h "light on" period each day.[19] Both groups of rats were allowed free access to food and water. After 3 weeks, the rats were anaesthetized by an intraperitoneal injection of sodium pentobarbital and euthanized.

All experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85-23, revised 1996). The protocol was approved by the Institutional Animal Care and Use Committee of the University of Tokyo (approval # P12-149).

2.2. *Cephalometric analysis*

Soft X-rays were taken in both the submentovertex and latero-lateral projections.[17,20] Cephalometric analysis was carried out with consideration of the following points as indices of the nasal cavity size (Fig. 1): the intersection between the frontal

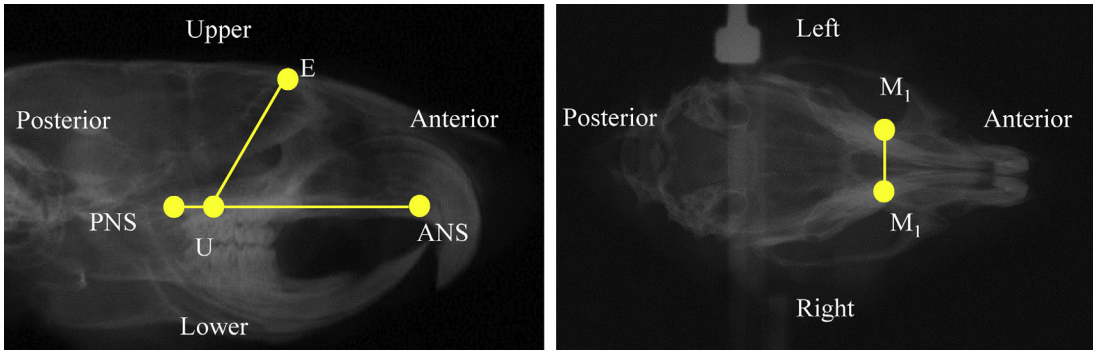

Fig. 1 – Representative soft X-ray images and cephalometric analysis. The left image shows a lateral X-ray image of the rat craniofacial region, and the right image shows an axial X-ray image. *Abbreviations*: E, intersection between the frontal bone and the most superior and anterior point of the ethmoid; U, intersection between the maxillary sinus and the distal surface of the third superior molar tooth; line linking E–U, index of the overall height of the nasomaxillary complex; M_1, upper front molar; line linking right and left M_1, index of the overall width of the nasomaxillary complex; ANS, the point of the anterior nasal spine of the maxilla; PNS, the point of the posterior nasal spine of the maxilla; line linking ANS–PNS, index of the overall length of the nasomaxillary complex.