Distribution of Taste Buds on the Epiglottis of the Rat and House Shrew, with Special Reference to Air and Food Pathways

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Summary: We investigated the positioning of the epiglottis in the pharyngo-laryngeal region and the distribution of taste buds on the epiglottis in the rat and house shrew, animals which have different feeding habits. In the fixed samples of both species, when the mouth was closed or slightly opened, the epiglottis was found to protrude into the nasopharyngeal hiatus above the soft palate. But it retracted from its position when the mouth was widely opened. In omnivorous rats (n=6), the mean number (mean density ± s.d.) of taste buds was 52 (12.6 ± 2.2/mm²) on the laryngeal surface but only 4 (1.3 ± 1.0/mm²) on the oral surface. The three-dimensional view was reconstructed from serial sections. The taste buds were distributed most densely close to the caudal base and became fewer toward the more rostral tip. In insectivorous house shrews (n=2), 4 taste buds on average were found only on the laryngeal surface of the epiglottis. Epiglottal taste buds may work as chemosensory detectors to initiate the reflex reaction to protect the airway from oral substances during swallowing and drinking.

The presence of a large number of taste buds on the laryngeal surface of the epiglottis has been reported in many kinds of mammals with different feeding habits. They include omnivores such as human (Lalonde and Eglitis, 1961), monkey (Khaisman, 1976) and rat (Andrew and Olive, 1951; Travers and Nicklas, 1990), herbivores such as guinea pig (Palmieri et al., 1983), hamster (Belecky and Smith, 1990), goat (Palmieri et al., 1983, Shrestha et al., 1993), sheep (Bradley et al., 1980) and buffalo (Shrestha et al., 1993), and carnivores such as cat (Palmieri et al., 1983; Stedman et al., 1980) and dog (Palmieri et al., 1983). Insectivores have not yet been studied. The functional roles of these taste buds, however, have not been fully understood. Kiesow (1902) hypothesized that the epiglottal taste buds reflect phylogenetic residue. Wilson (1905) rejected this view but suggested that they can detect four kinds of taste sensations. Recently some researchers (Bradley, et al., 1980; Stedman et al., 1983) have suggested that the epiglottal taste buds mediate the reflex response involved in improper swallowing and apnea.

We carried out several morphological investigations of this reflex hypothesis. 1) We investigated precisely the number and distribution of epiglottal taste buds in rats and house shrews. Three-dimensional structures of the rat epiglottis were reconstructed with the aid of computer graphics. 2) We presumed that the spatial relationships of both air and food pathways might be a key to solving the above question. We performed extensive macroscopic observations of the pharyngo-laryngeal region, while asking how the epiglottis is configured. 3) Another area we investigated was whether the density of epiglottal taste buds might differ depending upon the feeding habits of various animals. Here we chose omnivorous rats and insectivorous house shrews. The latter is also a primitive form of mammal.

Comparing the spatial relationships between air and food pathways, and the distributions of taste buds between rats and house shrews, we discuss the importance of taste buds on the laryngeal surface of the epiglottis.
Materials and Methods

Six 13-week-old female Wistar rats of about 200 g, and one male and one female house shrew (Suncus murinus) of about 40 g were used. They were fully anesthetized by peritoneal injection of 0.4 ml sodium pentobarbital solution (30 mg/kg) and perfused initially with 100 ml of normal saline (0.9% NaCl) containing 0.1 ml of heparin, followed by 600 ml of 10% formalin. Then the pharyngolaryngeal region was dissected and postfixed in 10% formalin.

Macroscopic observation of the epiglottis was performed using a binocular dissecting microscope (Nikon TMD). Photographs were taken to study the spatial relationships.

For histological study, tissues were softened in 5% trichloro-acetic acid for 24 hours, rinsed in 90% ethanol, dehydrated and embedded in paraffin (60°C). Serial sections of epiglottis were cut at 6–7 μm in thickness horizontally or longitudinally on a sliding microtome and mounted on slides. They were stained with hematoxylin and eosin, and examined under light microscopes (Olympus Vanox, Zeiss Axiphoto).

Taste buds were counted with great care to prevent double counting. We chose to designate the laryngeal surface of the epiglottis into ten strips of equal width from the tip to the base. (The width differed slightly from rat to rat due to size differences.) We counted the number of taste buds in each strip and averaged it for 6 rats. Densities of taste buds on the laryngeal and oral surfaces of the epiglottis were also calculated after measuring surface areas from camera lucida drawings of serial sections. Three-dimensional reconstruction of the epiglottis was made with an IBAS image analysis system (Krontron) from serial sections to show the location of taste buds on epiglottal surfaces.

Abbreviations used in figures
A: Arytenoid  AS: Anterior surface
E: Epiglottis  ES: Esophagus
L: Lateral food channel  N: Nasal cavity
O: Oral cavity  PS: Posterior surface
RF: Regurgitated food  S: Soft palate
SF: Swallowed food  V: Vocal fold
LC*: Laryngeal cavity

Observations

Macroscopic observations

The rat epiglottis was about 2 mm in length from the rostral tip to the caudal base. The rostral tip was pointed and triangular in shape (Fig. 1A and B).

The caudal base of the epiglottis was attached to the rostral facet of the thyroid cartilage. In the larynx of the present samples fixed by perfusion, the glottis was always closed (Fig. 1C and D).

A crown-shaped projection of epiglottis-arytenoid complex almost always protruded into the nasopharyngeal hiatus (Wood Jones, 1940), which was surrounded by the soft palate and the pharyngeal wall (Fig. 2A and C). Thus the nasal cavity was confluent with the laryngeal cavity, and there was no interference from the food pathway. Both channels on the left and right sides of this projection, corresponding to the recessus piriformis in humans, were thought to be used as food pathways. Thus air and food pathways were separated from each other.

When the mouth was opened to a great extent, this projection slipped out of the soft palate and lay in the oropharynx (Fig. 2B and D). Conversely, when the mouth was closed, the epiglottis fitted into the nasopharyngeal hiatus again. The configuration of intranarial rat epiglottis was demonstrated by camera lucida drawings of sections (Fig. 3).

The house shrew epiglottis had a slightly notched rostral tip and a length of about 1 mm. The rostral portion of epiglottis was found to rest on the soft palate, forming a continuous air passage from nasal cavity to trachea. This portion was able to be separated from the soft palate and returned again when the mouth was widely opened and closed again, respectively. Food channels were also observed at both sides of the epiglottis (Fig. 4). Thus the basic spatial relationships of the pharyngo-laryngeal region were common to the two species.

Microscopic observations

Laryngeal and oral surfaces of the epiglottis were covered with stratified squamous epithelium in rats (Fig. 5A, C, D). A thin keratinized layer was observed on the oral surface. The morphology of epiglottal taste buds was found to be similar to tongue taste buds. While the tongue taste buds were found in groups which were associated with papillae, the epiglottal taste buds were singly present.

Most of the taste buds were observed on the laryngeal surface (Fig. 5A, D), with only a few observed on the oral surface (Fig. 5C). The mean number of epiglottal taste buds in rats was 52 on the laryngeal surface and 4 on the oral surface. The density of taste buds on each total surface was as follows (mean ± standard deviation, n = 6): 12.6 ± 2.2 on the total laryngeal surface and 1.3 ± 1.0 on the total oral surface. Among the ten strips which we designated on the laryngeal surface of the rat epiglottis, the maximal number of taste buds was observed close to the caudal base (strip 8) and fewer taste buds were found toward the rostral tip (Fig. 6).

In contrast, a few taste buds were sporadically observed in the epithelium only on the laryngeal surface of the house shrew epiglottis (Fig. 5B). The
structure and morphology of the epithelium and taste buds were similar to those of the rat. The average number of taste buds \((n = 2)\) was 4. No taste bud was found on the oral surface. Thus there was a noticeable difference in the number of epiglottal taste buds between the rat and the house shrew.

A reconstructed three-dimensional view of a rat epiglottis is shown in Fig. 7, indicating the concave laryngeal surface and convex oral surface. This figure also presents a characteristic distribution of taste buds: numerous on the laryngeal surface, especially on the basal part, but very sparse on the oral one.

**Discussion**

In both the rat and house shrew, the epiglottis, together with the arytenoid, projects into the nasopharyngeal hiatus above the soft palate to interlock the larynx directly to the nasal cavity (Figs. 2, 3 and 4). This configuration keeps the laryngeal air channel open to external nares, keeping the air passage from the nose to the lungs open at all times. Lateral food channels on both sides of the larynx under the palate seem to be normally wide enough to allow passage of food without interfering with the air passage (Figs. 1, 2 and 4).

Travers and Nicklas (1990) reported that the epiglottis was intranasal in the majority \((8/10)\) of rats fixed with perfusion. Nakano and Muto (1985) observed that the epiglottis was intraloral in the mouse fixed with immersion. We think that both configurations are possible, depending on the situation: intranasal when the mouth is closed and also slightly opened but intraloral when the mouth is widely opened.

Epiglottal taste buds are numerous in the omnivorous rat but very few in the insectivorous house shrew. Since the house shrew is thought to be a kind of primitive mammal, the above result suggests that epiglottal taste buds do not reflect phylogenetic residue (Kiesow, 1902).

In general, omnivorous animals are thought to eat much larger amounts of food than insectivorous animals. It may be reasonable to suppose that the lateral food channels in the rat may not be wide enough for the passage of a large food bolus. There is probably more chance of food coming in contact with the laryngeal surface of the epiglottis in the rat than in the house shrew. The small amount of food eaten by a house shrew may rarely come into the laryngeal cavity.

In human infants, the epiglottis is located at the highest portion of the pharynx. The epiglottis makes contact with the soft palate, by which the air pathway is moderately isolated from the food pathway. Thus a baby can drink milk while respirating. However, milk may enter the trachea. Likewise, when a rat tries to engulf such a large amount of food or fluid that the epiglottis-palate junction becomes loose or opens, the ingested substance may enter the trachea.

If the taste buds on the laryngeal surface of the epiglottis detect the ingested substance entering the airway in order to induce reflex reactions, the characteristic numbers of taste buds can be regarded as a functional adaptation of these species. This viewpoint is substantiated by our previous observation (Shrestha, 1993) that even more numerous taste buds than in rats were found on the laryngeal surface of the epiglottis in various herbivorous animals like goats and buffalos. These animals not only eat a great amount of grass but also chew the cud, and thus have a high risk of the bolus entering the airway.

These findings suggest that the greater number of taste buds is responsible for the general chemoreception that initiates the protective reflex to prevent the entry of food by closing the laryngeal cavity or coughing up substances. This is also in agreement with the evidence obtained by other workers (Stedman et al., 1983; Bradley et al., 1983).

The epiglottis seems to be mainly respiratory in function. Negus (1949) considered that the epiglottis is primarily an organ of olfaction, because it was observed to be large and prominent in animals that require more pronounced olfaction for survival. He believed that these animals can feed and swallow while breathing and thus preserve their olfactory function.

Histologically, as shown in Fig. 5, both surfaces of the epiglottis have stratified squamous epithelium (Lewis and Prentice, 1980). This observation indicates that epiglottis, in spite of its location in the nasopharyngeal area, is not only associated with respiratory functions but also acts as an oral apparatus. Put another way, in order to detect substances coming from the oral cavity, the taste buds of oral origin should be located on the wall of the airway.

There are more epiglottal taste buds on the laryngeal surface \((52)\) than on the oral surface \((4)\) in the rat. Lalonde and Eglitis (1961) reported a similar differential distribution in the human infant. Other workers (Andrew and Oliver, 1951; Feidel, 1956; Khaisman, 1975; Palmieri et al., 1983; Travers and Nicklas, 1990) also reported the presence of taste buds on the laryngeal side in various animals, although they seldom provided quantitative descriptions.

In our data (Figs. 6 and 7), the density of taste buds varied according to location from the tip to the base of the epiglottis. Epiglottal taste buds are highly concentrated near the base of the laryngeal surface, while fewer are present toward the more rostral regions.
Thus the taste buds are selectively placed on the laryngeal side and differentially distributed over the laryngeal surface. The location of taste buds at the entrance to the lungs seems to be finely optimized for their function.

The presence of a cartilage core and lack of muscles suggest that the rat epiglottis does not completely fold over the laryngeal cavity, so that some food particles may pass through the aperture of the larynx. Or regurgitated food may come into the laryngeal cavity, as illustrated in Fig. 8. Fluids also may more easily enter the airway. The closing and opening of a vocal slit is performed by abduction and adduction of the vocal folds by laryngeal muscles. Thus vocal folds may help to prevent ingested substances from entering the laryngeal cavity, although their primary functions are air passage and sound production. When food particles come in contact with the laryngeal surface of the epiglottis, the nervous system initiates a reflex reaction to close the laryngeal aperture or cough up the food.

This reflex requires sensory devices as well as motor effectors. Taste buds on the laryngeal surface of the epiglottis have a relevant strategic position for the detection of such emergent signals. The number of taste buds on the laryngeal surface of the epiglottis is correlated with the feeding habits of animals. In addition, the glottis may work as a gate of the air pathway to prevent obstacles from entering. Further investigations are needed for full clarification of the functional significance of epiglottal taste buds.

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References

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Explanation of Figures

Plate I

Fig. 1. Binocular view of the epiglottis-arytenoid complex of the rat. Scale: 1 mm for all.
A. Frontal view. B. Lateral view. The epiglottis and arytenoid form a crown-shaped projection subserving the air passage.
C. Bottom of the laryngeal cavity where vocal folds are seen to be closed. D. The same bottom forced open by the insertion of a black rod between the vocal folds. Thus the position of the laryngeal inlet is indicated.
Fig. 2. Configuration of rat pharyngeal and laryngeal areas viewed under a binocular microscope. Scale: 1 mm for A and B, and 5 mm for C and D.

A. Epiglottis fitting into the nasopharyngeal hiatus. The epiglottal tip lies on the soft palate in the nasal cavity, which is hidden from view. Two grooves run around the basal and lateral surface of the epiglottis toward the esophagus. B. The epiglottis and arytenoid are separated from the nasopharyngeal hiatus. (This separation was created by opening the mouth wide.) C. Lateral view of the nasopharynx and oropharynx showing the lateral food channel with black thread and the respiratory channel through the epiglottis-palate junction. The channels are isolated from each other. D. The same view as above when the epiglottis (arrowhead) is separated from the soft palate. The laryngeal cavity (asterisk) is open to the food channel.
Fig. 3. Camera lucida drawings of longitudinal sections of the rat pharyngolaryngeal region through the midmost plane (A), intermediate plane (B) and most lateral plane (C). Projection of the epiglottis and arytenoid complex fits into the nasopharyngeal hiatus, which is formed by the soft palate and pharyngeal wall. The nasal and laryngeal cavities are interconnected by this projection, and thus isolated from the oral cavity and esophagus.
Fig. 4. Binocular view of house shrew epiglottis. Scale: 1 mm for all.
A. Epiglottis conjoined with soft palate, as most often seen.
B. Epiglottis slipped out of nasal cavity. Laryngeal cavity (asterisk) is open to oral cavity.
C. Crown-shaped projection of epiglottis and arytenoid complex. House shrew epiglottis has a slightly notched tip which is also rounder than the rat's.
Plate V

Fig. 5. Photomicrographs of taste buds (arrowheads) on posterior (PS, laryngeal) and anterior (AS, oral) surfaces of epiglottis at different magnifications. Scale: 100 μm for A and B, 30 μm for C and D.
A. Taste bud on laryngeal surface of rat epiglottis. Note the presence of elastic cartilage (C).
B. House shrew epiglottis bearing taste bud on the laryngeal surface. Elastic cartilage (C) is seen in the connective tissue core.
C. Two taste buds near the edge of the oral surface of rat epiglottis.
D. Taste bud with a taste pore seen on the laryngeal surface of rat epiglottis.
Fig. 6. The number of taste buds increases from the rostral tip toward the caudal base, reaching a peak near the caudal base, and then decreases. The epiglottis surface is divided into 10 levels of equal width from rostral to caudal. Each column represents the mean number of taste buds with the standard deviation (n = 6).
Fig. 7. Three-dimensional reconstruction of the rat epiglottis from 218 serial sections.
A. Posterior view showing numerous taste buds on the concave laryngeal surface, which confronts the air passage (asterisk).
B. Side view showing a small number of taste buds (arrowhead) near the edge of the oral surface.
Plate VIII

Fig. 8. Diagram displaying two possible chances of food contact on the laryngeal surface of the epiglottis. F1 represents the food particle during swallowing. F2 indicates the regurgitated food particle. In addition, fluid may enter the laryngeal cavity during drinking. Taste buds (asterisks) may send messages about the arrival of food and fluid to the nervous system to initiate the airway reflex.