Morphological Investigation of Nucleus Tractus Solitarius Somatotopy

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Taste is one of the most important senses that allows for human survival. Recent electrophysiological investigations have revealed some information about the somatotopy of the rat nucleus tractus solitarius (NTS). The aim of this study was to assess rat NTS somatotopy using morphological techniques.

Each animal was anesthetized and a retrograde axonal tracer, horseradish peroxidase, was injected intra- and sub-mucosally into the anterior portion or the middle and posterior portions of the left half of rat tongue. After 24–48 hr, each rat was anesthetized again and perfusion-fixed. The brainstem was removed and cut in 50 µm-thick sections using a vibrotome. The sections were subjected to a tetramethylbenzidine–peroxidase

reaction, and were then assessed under light-microscopy.

The horseradish peroxidase that was deposited in the anterior third of the tongue labeled neuron cell bodies in the rostrofrontolateral region of the ipsilateral NTS, while that deposited in the middle and posterior thirds of the tongue labeled neuron cell bodies in the caudoposteromedial region of the ipsilateral NTS. There was no cross-projection.

The morphological findings were in accord with the electrophysiological results in the literature. Retrograde labeling revealed a distinct pattern of neural projection that corresponded to the anterior portion, and the middle and posterior portions of the tongue.

Key words: Taste, Nucleus tractus solitarius, Horseradish peroxidase, Somatotopy, Retrograde labeling

I. Introduction

Taste is one of the senses most vital to human survival. Afferent information from the oral cavity is conveyed by the chorda tympani branch of the facial nerve from the anterior two-thirds of the tongue; by the lingual branch of the glossopharyngeal nerve from the posterior third of the tongue; and by the internal branch of the superior laryngeal nerve (a branch of the vagus nerve) from the anterior surface of the epiglottis. All these pathways transmit sensory stimuli to the nucleus tractus solitarius of the brainstem [3, 14].

The entire tongue is sensitive to the four main taste qualities (sweet, sour, salty and bitter) but the tip of the tongue is more sensitive to sweet, the lateral borders to sour,

the lateral areas of the anterior third to salty and the area near the sulcus terminalis to bitter [3, 14, 18]. The “specific pathways” theory suggests that a certain group of neurons carries the stimuli for a specific taste quality [18, 19]. In contrast, the “interfibrillary coding” theory suggests that, although each fiber has a specific sensitivity for a given taste quality, each may also have lesser sensitivity to other taste qualities, and the central neurons analyze the impulses that are transmitted via many different fibrils [4]. Experiments have supported both theories; thus it is likely that mapping the patterns of projection for the taste stimuli in the central nervous system can only be done by comparing the electrophysiological activity in the quality-specific fibers to activity in other nonspecific fibrils [4, 5].

Recent electrophysiological investigations have shown that the somatotopic organization of the nucleus tractus solitarius (NTS) for salty and bitter tastes corresponds to the map of these taste qualities on the tongue, but this has not yet been assessed morphologically [1, 10, 11]. We undertook this study to assess whether the somatotopic organization of

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Fig. 1

Fig. 2. The neuron cell bodies labeled by HRP injection into the anterior third of the tongue are located in the rostral region of the nucleus tractus solitarius on the brainstem section referred to as 5A in Fig. 1. (neutral red, $\times 120$).

nerve cell bodies in the NTS corresponds to the distinct distribution of the taste quality projections to this brainstem nucleus.

II. Materials and Methods

Twelve male Wistar rats weighing 200–260 g were used in this study. The experiment was conducted with strict adherence to the Guide For the Care and Use of Laboratory Animals[®] (National Institutes of Health Publication No: 86-23). Each rat was anesthetized with an intraperitoneal injection of ketamine (44 mg/kg) and xylazine (5 mg/kg).

A total of 10 μ l of 30% horseradish peroxidase (HRP) (Sigma type 6) solution, a retrograde axonal tracer, were then slowly injected into the mucosa on the left side of each rat's tongue via glass micropipettes. In half of the animals ($n=6$), we injected 10 different points in the anterior third of

the tongue, and in the other half ($n=6$) we injected 10 different points in the middle and posterior thirds of the tongue.

After 24 hr, the animals were reanesthetized and perfused with saline, followed by 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Postfixation times ranged from 1 to 2 hr.

We removed the brainstem en bloc and cut 50 μ m-thick serial sections on a vibratome. Free floating sections were processed in the chromogen tetramethylbenzidine. To determine which neuronal cell bodies retrogradely labeled with intracytoplasmic HRP, we counterstained the sections with neutral red. Thus, neurons that did not contain HRP granules could also be morphologically assessed; and neurons that contained intracytoplasmic HRP reaction product could be compared to populations of unlabeled cells [12].

The counterstained brainstem sections were studied under light microscopy and camera lucida tracings were made. Intracellular HRP granules that were detected in the

Fig. 1. Camera lucida drawings of the brainstem sections obtained after HRP injection into the anterior third (left) or middle and posterior thirds (right) of the left half of the rat tongue. Sections are separated by intervals of 0.8 mm and are displayed in rostrocaudal order. The interrupted lines indicate the perimeters of various brainstem nuclei, the continuous line outlines the nucleus tractus solitarius, and the dark dots represent neurons that contain intracellular granules of HRP reaction product.

Abbreviations: mlf, fasciculus longitudinalis medialis; mve, nuc. vestibularis medialis; lve, nuc. vestibularis lateralis; dco, nuc. cochlearis dorsalis; sp5, nuc. spinalis nervi trigeminalis; 7, nuc. nervi facialis; rmg, nuc. reticularis raphe magnus; spve, nuc. spinalis vestibularis; 12, nuc. nervi hypoglossi; amb, nuc. ambiguus; cu, nuc. cuneatus; ecu, nuc. cuneatus externalis; 10, nuc. dorsalis nervi vagi; lrt, nuc. reticularis lateralis medulla oblongata; gr, nuc. gracilis; pmn, nuc. reticularis paramedianus; py, tractus pyramidalis; v4, ventriculus quartus; nst, nuc. tractus solitarius.

Fig. 3. The neuron cell bodies labeled by HRP injection into the middle and posterior thirds of the tongue are located in the nucleus tractus solitarius on the brainstem section referred to as 6A in Fig. 1. (neutral red, $\times 120$).

Fig. 4. Neuron cell bodies labeled by HRP injection into the anterior third of the tongue are located in the anterolateral region of the nucleus tractus solitarius on the brainstem section referred to as 5C in Fig. 1. (neutral red, $\times 120$).

Fig. 5. Neuron cell bodies labeled by HRP injection into the middle and posterior thirds of the tongue are located in the posteromedial region of the nucleus tractus solitarius on the brainstem section referred to as 6C in Fig. 1. (neutral red, $\times 120$).

Fig. 6. Neuron cell bodies labeled by HRP injection into the anterior third of the tongue are situated in the caudal region of the nucleus tractus solitarius on the brainstem section referred to as 5E in Fig. 1. (neutral red, $\times 120$).

Fig. 7. Neuron cell bodies labeled by HRP injection into the middle and posterior thirds of the tongue are located in the caudal region of the nucleus tractus solitarius on the brainstem section referred to as 6E in Fig. 1. (neutral red, $\times 120$).

histological preparations were indicated by dark dots in these camera lucida drawings. The boundaries of the rat NTS were drawn according to widely accepted cytoarchitectonic criteria [9]. Finally, the brainstem section tracings were compared to photographs of normal tissue in a rat brain atlas [17]. Figure 1 shows a representative sample of the drawings presented in rostrocaudal sequence.

III. Results

HRP applied to the anterior third of the tongue labeled the rostral and anterolateral regions of the NTS, while HRP applied to the middle and posterior thirds of the tongue labeled the caudal and posteromedial regions of the ipsilateral nucleus (Fig. 1). There was no cross-projection.

Of the nerve cell bodies that were labeled by HRP, those located in the rostral regions of the nucleus (shown in the upper sections of Fig. 1) were mostly labeled by the tracer that was injected to the anterior third of the tongue (Figs. 2, 3). However, cell bodies located in the middle regions of the nucleus on the rostrocaudal axis (shown in the middle sections of Fig. 1) were labeled by HRP that was injected into both the anterior and posterior parts of the tongue (Figs. 4, 5). Neuron cell bodies located in the caudal regions of the nucleus on the rostrocaudal axis (shown in the lower sections of Fig. 1) were labeled by tracer that was injected into the middle and posterior thirds of the tongue (Figs. 6, 7).

IV. Discussion

The first synapse of the gustatory system is within the taste bud. Since there are significantly more taste buds in the anterior part of the rat tongue than in other areas (two- to five times the numbers found in the posterior region) [6, 8, 13], we chose to compare results of intramucosal HRP injection of the anterior third to results from injections into the middle and posterior thirds of the tongue. The receptive neuroepithelial cells in the taste bud form synapses with the afferent fibers [23, 26]. Since these fibers also innervate the sub-epithelial tactile receptive bodies and epithelium surrounding the taste buds, they include somesthetic afferents from this region as well [16, 21, 22].

Hayama *et al.* [7] showed that most taste sense maps to the rostrocaudal regions of the NTS [7, 16, 21]. Electrophysiological studies have also shown that the NTS is topographically organized into regions that correspond to sweet, salty and bitter tastes similar to the tongue and thalamus [1, 2]. Use of the term "orotopy", which refers to the topographic NTS representation of somatotopic sensibility in the tongue, has led to the adoption of the term "chemotopy" to describe the spatial patterning of the different chemosensitive receptor subgroups in the NTS [4, 5, 10, 15, 20]. Travers and colleagues [23–25] showed that taste-receiving nerve cell bodies in the rostral region of the rat NTS were located anterior and medial to the mechanically stimulated cells. Anteroposterior topography has also been demonstrated

in the mapping of projections from the taste neurons, in that stimulation of the anterior tongue mostly activates cells in the anterorostral region of the NTS, whereas posterior tongue stimulation activates cells in the rostrolateral region [23–25]. In our study, HRP applied to the anterior third of the rat tongue labeled the anterolateral region of the NTS, and HRP applied to the middle and posterior thirds of the tongue labeled the posteromedial region.

In conclusion, our morphological findings support the orotopy that has been demonstrated in electrophysiological experiments. Neural pathways that started in the anterior third of the tongue projected to the rostrofrontolateral region of the NTS; and pathways from the middle and posterior thirds of the tongue projected to the caudoposteromedial region of the nucleus. It remains to be seen whether the pattern of somatotopic organization in the human NTS is similar to that in the rat. One possible investigative approach may be to assess taste perception and orotopy in patients with brainstem lesions.

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VI. References

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