The Centrifugal Influence on Gustatory Neurons in the Nucleus of the Solitary Tract

Young Kyung Cho

Department of Physiology and Neuroscience, College of Dentistry, and Research Institute of Oral Science, Gangneung-Wonju National University, Gangneung, Gangwon, Korea

Neuronal activities of taste-responsive cells in the nucleus of the solitary tract (NST) are affected by various physiological factors, such as blood glucose level or sodium imbalance. These phenomena suggest that NST taste neurons are under the influence of neural substrates that regulate nutritional homeostasis. In this study, we reviewed a series of *in vivo* electrophysiological investigations that demonstrate that forebrain nuclei, such as the lateral hypothalamus or central nucleus of the amygdala, send descending projections and modulate neuronal activity of gustatory neurons in the NST. These centrifugal modulations may mediate plasticity of taste response in the NST under different physiological conditions.

Key words: taste, forebrain, descending, modulation, neuronal activity

*Correspondence to: Young Kyung Cho, Department of Physiology and Neuroscience, College of Dentistry, and Research Institute of Oral Science, Gangneung-Wonju National University, Gangneung, Gangwon 210-702, Korea.
Tel.: +82-33-640-2451, Fax: +82-33-642-6410
E-mail: ykcho@gwnu.ac.kr

Introduction

We prefer palatable food to non-palatable one and avoid aversive food or toxic substance. Even the same tasty food, sometimes it induces more appetite than usual. Often we consume more foods than needed to maintain nutritional homeostasis. These facts suggest that not only feeding behavior but also taste perception is affected by various physiological and psychological factors. Substantial investigations support that taste processing is modulated by various physiological influences. Sweet taste-evoked neuronal firings of taste neurons in the nucleus of the solitary tract (NST), which is the first taste nucleus, were altered by blood glucose, insulin or glucagon level [1-3]. Conditioned taste aversion or preference also changed neuronal firing of gustatory neurons in the NST, in response to conditioning stimuli [4,5]. Sodium appetite is a condition, in which sodium deficiency makes animals indulge in ingestion of high concentration NaCl solution. Sodium appetite also modulated neuronal activity in response to NaCl [6]. These studies suggest that gustatory neurons in the NST receive information regarding nutritional and taste-related learning, although the neural substrate or mechanism underlying such interaction is not clearly elucidated.

The central taste pathway has been well documented anatomically. From the peripheral taste receptors, taste

information is carried to the NST in the medulla via three gustatory nerves, the VIIth, IXth, and Xth nerves [7,8]. The second taste relay, parabrachial nucleus (PbN) in the pons transfers taste information from the NST to the rostral gustatory nuclei in the rodents [9,10]. Taste information goes to the gustatory cortex (GC) via the parvocelluar division of the ventroposteromedial nucleus of the thalamus (VPM) [11,12]. In parallel, taste input also carried to various brain sites, such as the lateral hypothalamus (LH), central nucleus of the amygdala (CeA), and bed nucleus of the stria terminalis (BNST), in the ventral forebrain [11,12]. While the thalamocortical taste pathway contributes the perception of taste input, roles of the ventral forebrain nuclei in taste processing, are not clearly defined. The LH receives visceral input and regulates feeding behavior and thirst [13]. The CeA is involved in the conditioned taste aversion [14]. The BNST may have a role in sodium appetite [15]. Taken together, the neural communications between the NST and the ventral forebrain have some roles in plasticity of gustatory responses of the NST taste neurons.

Whereas taste pathway, in which neurons in one taste nucleus send axons to other nuclei are clearly demonstrated by neural tracing techniques, such methods could not determine whether the projecting neurons are taste-responsive cells or not. On the other hand, the functional relationship between gustatory neurons in the NST and nuclei along the central taste pathway, can be investigated with *in vivo* electrophysiological experiments. *In vivo* recording of taste-evoked neuronal firing in single gustatory neurons clearly demonstrate the modulation of taste-responsiveness. The recording neuronal firings of the NST gustatory neurons while electrical stimulation of the target nucleus in the forebrain, which mimics the activation of the stimulated nucleus, can indicate the influence of ventral forebrain.

In this review, a series of electrophysiological studies investigating the functional relationships between the medullary gustatory neurons with each of the nuclei, rostral to the NST along the central taste pathway, were addressed. The target nuclei include the PbN, VPM, and GC along the thalamocortical pathway, and the LH, CeA, and BNST in the ventral forebrain. In addition, the influence from the shell region of the nucleus accumbens (NAcSh) on the NST taste neurons was also reviewed.

Recording of single unit neuronal activity of gustatory neurons in NST

A series of the experiments described in this manuscript were *in vivo* electrophysiological studies using hamsters anesthetized with urethane. The common experiments schemes were described first. Action potentials from single taste-responsive neurons were recorded extracellularly. Glass micropipettes filled with Chicago Blue dye in 0.5 M sodium acetate were used for recording single unit action potentials from the gustatory NST. Taste-responsive neurons were found mostly in the rostral central and rostral lateral subdivisions in the NST, where gustatory nerves terminate [16]. Taste-responsiveness was confirmed by counting the number of action potentials in response to taste stimuli delivered to the anterior tongue. Taste stimuli presented to the anterior tongue were sweet (sucrose), salty (sodium chloride: NaCl), bitter (quinine hydrochloride: QHCl), and sour (citric acid) taste. After recording taste-evoked neuronal firings, the target nucleus in the forebrain was stimulated bilaterally; brief single electrical pulses (<0.1 mA, 0.5 ms) were applied at 1/3 Hz to the target nucleus and peristimulus time histograms (PSTHs) were accumulated over 100 - 200 stimulus trials to reveal excitatory or inhibitory modulation on spontaneous firings of the NST taste neurons. For a subset of the stimulation-responsive cells, taste-evoked neuronal firings were compared with/without the high frequency (100 Hz, 0.2 ms) electrical stimulation of the target nucleus. Except the experiments in which the target nucleus was the PbN, the stimulating sites were determined on the basis of stereotaxic coordinates. As for the PbN, taste-responsiveness was recorded before locating the stimulating electrode in the PbN. Usually a couple of taste cells were recorded from one animal and the last recording site in the NST and the stimulation sites in the target nuclei were marked electrically. The histological procedures were conducted in order to exclude data from the experimental animals with the incorrect positions of the recording or stimulating electrodes.

Parabrachial nucleus

The PbN is the second taste relay in the rodent [9,10]. Taste-responsive neurons are located in the medial part of the PbN which receives gustatory input from the NST,

whereas the visceral information goes to the lateral regions of the PbN [17,18]. Earlier electrophysiological studies, in which the stimulating sites of the PbN was determined on the basis of stereotaxic coordinates, reported that 30 - 45% of taste-responsive cells project to the ipsilateral PbN in rats [19,20]. In contrast, a neural tracing study reported that 2/3 of projection neurons in the NST send axons to the ipsilateral PbN [21]. The electrophysiological study using hamster, in which the stimulating spot of the ipsilateral PbN was determined after confirming taste-evoked neuronal firings using stimulating/recording electrode combination, demonstrated 81 of 101 NST taste neurons project to the ipsilateral gustatory PbN area [22]. The NST gustatory neurons which project to the ipsilateral gustatory PbN, were antidromically activated when the gustatory PbN was electrically stimulated. The mean antidromic latency was 4.1 ± 0.4 ms. Six cells responded orthodromically, suggesting that some NST taste neurons receive descending input from the ipsilateral gustatory PbN [22]. Another study, in which the contralateral PbN was also stimulated, demonstrated that only four of 119 NST taste neurons were antidromically invaded from the contralateral PbN, whereas 97 of 119 cells projected to the ipsilateral PbN from the NST with a mean latency of 4.7 ± 0.4 ms [23]. In that study, 30 and 45 gustatory neurons in the NST received excitatory descending projections from the ipsilateral and contralateral PbN, respectively. Mean excitatory latencies following the ipsilateral and contralateral PbN stimulation were 11.3 ± 0.7 ms and 36.7 ± 1.5 ms, respectively.

Lateral hypothalamus

Stimulation of the LH increases food ingestion, whereas lesion of the LH decreases food intake [24,25]. Bipolar stimulating electrodes were inserted in the LH and the neuronal firings of 99 gustatory neurons in the NST were examined for their responses to the LH stimulation [26]. Forty nine out of 99 taste-responsive cells were modulated by LH stimulation. Mostly the responses were excitatory; spontaneous firings of taste cells increased. Stimulation of the contralateral LH exerted greater influence than stimulation of the ipsilateral LH, which excited 10 and inhibited 6 cells. In comparison, contralateral stimulation increased neuronal firing in 41 NST taste cells. Ten taste neurons were excited bilaterally. One cell showed an excitatory response to contralateral LH stimulation and an inhibitory response to ipsilateral stimulation. The mean excitatory latency following the ipsilateral LH stimulation was 27.6 ± 3.2 ms and that of the contralateral LH was 19.7 ± 1.4 ms. Interestingly, two contralaterally excited taste neurons also showed antidromic activation in response to the ipsilateral LH stimulation. Fourteen taste neurons were tested for the effects of electrical stimulation of the LH on taste-evoked neuronal firings. Responses to taste stimuli were enhanced more than twice during the LH stimulation.

Electrical stimulation excited not only the cell bodies, but also fibers passing by. In order to verify the effect of LH stimulation is originated from the soma of stimulating site, the effect of microinjection of DL-homocysteic acid (DLH), a glutamate receptor agonist was examined for 13 LH-responsive neurons; 12 excited and 1 inhibited cells. DLH injection mimicked modulatory effect on spontaneous activity of the tested cells whereas saline injection produced no influence, suggesting neurons in the LH send projections to the NST and modulate spontaneous and taste-evoked neuronal activity of gustatory neurons.

Central nucleus of the amygdala

The CeA is involved in conditioned taste aversion learning and sodium appetite [27]. Recorded action potentials were analyzed from 109 NST gustatory neurons with/without electrical stimulation of the CeA [28]. No antidromic activation occurred in response to stimulation of the CeA. An excitatory response was observed in 33 of 109 taste responsive cells. Out of 33 excitatory responsive cells, the ipsilateral and contralateral CeA stimulation excited in 21 and 26 neurons, respectively; 14 were excited bilaterally. The mean excitatory latency following the ipsilateral CeA stimulation was 29.7 ± 2.3 ms and that of the contralateral CeA was 17.2 ± 1.6 ms. A few inhibitory responses were observed. Two and one cell was inhibited by ipsilateral and contralateral CeA stimulation, respectively. High frequency electrical stimulation of the CeA during taste trials enhanced the taste responses of all seven tested CeA-excitatory responsive NST cells. DLH injection into the CeA also increased baseline activity of eight taste neurons: four ipsilaterally excited and the other four contralaterally excited cells. For a taste cell which

was inhibited by contralateral stimulation of CeA, DLH injection eliminated firing activity of this cell.

Bed nucleus of the stria terminalis

The BNST plays a role in sodium appetite [6]. The influence of electrical stimulation of the bilateral BNST on NST taste cells' neuronal activity was examined [29]. Taste-responses of 101 NST neurons were extracellularly recorded. None of the 101 cells tested, were antidromically invaded from the BNST. Inhibitory responses were more dominant than excitatory responses following electrical stimulation of the BNST. Electrical stimulation of the ipsilateral BNST inhibited the activity of 23 and excited 2 cells out of 101 NST taste cells. Stimulation of the contralateral BNST inhibited 10 neurons and excited 5 NST taste neurons. Four cells were inhibited bilaterally and two of contralaterally excited cells were inhibited ipsilaterally. Mean excitatory latencies following stimulation of the BNST were 60.0 ± 13.0 ms for ipsilateral stimulation and 59.2 ± 6.3 ms for contralateral stimulation, respectively. In a subset of seven inhibitory-responsive cells, modulatory effect of high frequency stimulation of the BNST was tested. Taste-evoked neuronal firings of all seven neurons were reduced during the BNST stimulation.

Ventroposteromedial nucleus of the thalamus

The VPM transfers taste information to the GC from the brain stem nuclei [30,31]. Extracellular single unit activities were recorded from 83 NST taste neurons and the VPM was stimulated bilaterally [32]. Thirty seven of 83 neurons were orthodromically activated following VPM stimulation. The results suggest that VPM not only transfer taste neurons but also exerts an influence on the medullary taste neurons. Among the VPM-responsive neurons, Stimulation of the ipsilateral VPM excited 19 and inhibited 3 neurons while the contralateral VPM stimulation excited 18 and inhibited 5 neurons. Seven NST neurons were excited and one cell was inhibited bilaterally. Mean excitatory latencies following the ipsilateral and contralateral VPM stimulation were 35.4 ± 4.3 ms and 37.4 ± 3.0 ms, respectively. Interestingly, four NST taste neurons were antidromically invaded from the ipsilateral VPM, suggesting a direct projection from gustatory neurons in the NST to the VPM although majority takes route via the PbN. The effect of VPM activation on taste responses

was tested on eight out of 30 excitatory-responsive neurons and all seven cells that were inhibited. Taste-driven responses of NST neurons, in response to high frequency stimulation of the VPM, were enhanced in the cells which showed excitation, and suppressed in the cells which were inhibited by single pulse stimulation of the VPM.

Gustatory cortex

The stimulation effect of the GC was also investigated previously. In that study, effects of the electrical and chemical stimulation of the ipsilateral GC on the NST taste neurons were investigated using multibarrel glass micropipette [33]. The baseline activity of 17 of 50 cells was modulated by cortical stimulation: eight cells were inhibited and nine were excited. The mean excitatory latency was 15.5 ± 2.9 ms. After recording neuronal firings in response to the electrical stimulation of the GC, effect of microinjection of DLH was also examined. Neuronal firings of 17 GC-responsive cells were changed by DLH injection in the same direction of electrical stimulation but saline injection did not. Modulatory effect of the GC on taste-evoked responses was not investigated in this study. Instead, GABA$_A$ antagonist, bicucullinemethiodide (BICM) was injected into the vicinity of recorded neurons in the NST. BICM injection blocked the inhibitory effect of GC stimulation but not the excitatory effect on NST taste neurons, suggesting descending projections from the GC make GABAergic inhibitory synapses with gustatory neurons in the NST.

Shell region of the Nucleus Accumbens

The most recent investigation reported the functional relationship between the NST and nucleus accumbens [34]. The nucleus accumbens consists of the shell and core regions. The shell region of the nucleus accumbens (NAcSh) plays a significant role in coordinating the hedonic value of taste stimuli and motivated behavior [35,36]. Neuronal activity of a total of 90 NST taste neurons was analyzed. Electrical stimulation of the NAcSh did not produce antidromic activation but induced action potentials in variable latencies. Stimulation of the ipsilateral NAcSh produced firing from 54 neurons whereas 37 cells evoked neuronal firings by the contralateral NAcSh stimulation. Thirty cells were responded bilaterally. The mean excitatory latency following ipsilateral NAcSh stimulation was $16.0 \pm$

1.0 ms and was 30.1 ± 1.9 ms following contralateral NAcSh stimulation. No inhibitory response was observed following NAcSh activation. In the subset of taste cells tested (*n* =16), high frequency electrical stimulation of the NAcSh during taste delivery enhanced taste-evoked neuronal firings.

Conclusions

This series of experiments demonstrate that neuronal activity of gustatory neurons in the NST is under the extensive descending influence from various forebrain nuclei. Virtually every forebrain relays along the taste pathway send descending projections to the gustatory NST. The NAcSh has not known to be a member of taste system, but nonetheless it plays a role in mediating rewarding value of substance [36]. The LH, CeA, VPM and NAcSh show mostly excitatory effect, whereas the GC and BNST produce more inhibitory influence on medullary taste neurons. These descending inputs could alter taste-evoked neuronal activity of gustatory neurons in the NST. These substantial centrifugal influences on NST taste neurons certainly function as neural substrates underlying the modulation of gustatory responses by physiological and psychological factors in the NST.

Acknowledgements

Conflict of interest

The authors declare that they have no competing interest.

References

1. Giza BK, Deems RO, Vanderweele DA, Scott TR. Pancreatic glucagon suppresses gustatory responsiveness to glucose. Am J Physiol. 1993;265:R1231-1237.

2. Giza BK, Scott TR. Blood glucose selectively affects taste-evoked activity in rat nucleus tractus solitarius. Physiol Behav. 1983;31:643-650.

3. Giza BK, Scott TR. Intravenous insulin infusions in rats decrease gustatory-evoked responses to sugars. Am J Physiol. 1987;252:R994-1002.

4. Chang FC, Scott TR. Conditioned taste aversions modify neural responses in the rat nucleus tractus solitarius. J Neurosci. 1984;4:1850-1862.

5. Giza BK, Ackroff K, McCaughey SA, Sclafani A, Scott TR. Preference conditioning alters taste responses in the nucleus of the solitary tract of the rat. Am J Physiol. 1997;273: R1230-1240.

6. Cho YK, Smith ME, Norgren R. Low-dose furosemide modulates taste responses in the nucleus of the solitary tract of the rat. Am J Physiol Regul Integr Comp Physiol. 2004;287:R706-714.

7. Hamilton RB, Norgren R. Central projections of gustatory nerves in the rat. J Comp Neurol. 1984;222:560-577.

8. Whitehead MC, Frank ME. Anatomy of the gustatory system in the hamster: central projections of the chorda tympani and the lingual nerve. J Comp Neurol. 1983;220:378-395.

9. Norgren R, Leonard CM. Taste pathways in rat brainstem. Science 1971;173:1136-1139.

10. Travers JB. Efferent projections from the anterior nucleus of the solitary tract of the hamster. Brain Res. 1988;457:1-11.

11. Halsell CB. Organization of parabrachial nucleus efferents to the thalamus and amygdala in the golden hamster. J Comp Neurol. 1992;317:57-78.

12. Norgren R. Taste pathways to hypothalamus and amygdala. J Comp Neurol. 1976;166:17-30.

13. Bray GA. Autonomic and endocrine factors in the regulation of food intake. Brain Res Bull 1985;14:505-510.

14. Yamamoto T, Sako N, Sakai N, Iwafune A. Gustatory and visceral inputs to the amygdala of the rat: conditioned taste aversion and induction of c-fos-like immunoreactivity. Neurosci Lett. 1997;226:127-130.

15. Reilly JJ, Maki R, Nardozzi J, Schulkin J. The effects of lesions of the bed nucleus of the stria terminalis on sodium appetite. Acta Neurobiol Exp. (Wars) 1994;54:253-257.

16. McPheeters M, Hettinger TP, Nuding SC, Savoy LD, Whitehead MC, Frank ME. Taste-responsive neurons and their locations in the solitary nucleus of the hamster. Neuroscience 1990;34:745-758.

17. Norgren R, Pfaffmann C. The pontine taste area in the rat. Brain Res. 1975;91:99-117.

18. Van Buskirk RL, Smith DV. Taste sensitivity of hamster parabrachial pontine neurons. J Neurophysiol. 1981;45: 144-171.

19. Monroe S, Di Lorenzo PM. Taste responses in neurons in the nucleus of the solitary tract that do and do not project to the parabrachial pons. J Neurophysiol. 1995;74:249-257.

20. Ogawa H, Imoto T, Hayama T. Taste relay neurons in the solitary tract nucleus of rats. Neurosci Lett. 1980;18: 295-299.

21. Halsell CB, Travers SP, Travers JB. Ascending and descending projections from the rostral nucleus of the solitary tract

originate from separate neuronal populations. Neuroscience 1996;72:185-197.

22. Cho YK, Li CS, Smith DV. Gustatory projections from the nucleus of the solitary tract to the parabrachial nuclei in the hamster. Chem Senses 2002;27:81-90.

23. Cho YK, Li CS. Gustatory neural circuitry in the hamster brain stem. J Neurophysiol. 2008;100:1007-1019. doi: 10.1152/jn.01364.2007.

24. Berridge KC, Valenstein ES. What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? Behav Neurosci. 1991;105:3-14.

25. Clark JM, Clark AJ, Bartle A, Winn P. The regulation of feeding and drinking in rats with lesions of the lateral hypothalamus made by N-methyl-D-aspartate. Neuroscience 1991;45:631-640.

26. Cho YK, Li CS, Smith DV. Taste responses of neurons of the hamster solitary nucleus are enhanced by lateral hypothalamic stimulation. J Neurophysiol. 2002;87:1981-1992.

27. Zardetto-Smith AM, Beltz TG, Johnson AK. Role of the central nucleus of the amygdala and bed nucleus of the stria terminalis in experimentally-induced salt appetite. Brain Res. 1994;645:123-134.

28. Li CS, Cho YK, Smith DV. Taste responses of neurons in the hamster solitary nucleus are modulated by the central nucleus of the amygdala. J Neurophysiol. 2002;88:2979-2992.

29. Smith DV, Ye MK, Li CS. Medullary Taste Responses are Modulated by the Bed Nucleus of the Stria Terminalis. Chem Senses 2005;30:421-434.

30. Beckstead RM, Morse JR, Norgren R. The nucleus of the solitary tract in the monkey: projections to the thalamus and brain stem nuclei. J Comp Neurol. 1980;190:259-282.

31. Halsell CB, Frank ME. Organization of taste-evoked activity in the hamster parabrachial nucleus. Brain Res. 1992;572: 286-290.

32. Cho YK, Mao L, Li CS. Modulation of solitary taste neurons by electrical stimulation of the ventroposteromedial nucleus of the thalamus in the hamster. Brain Res. 2008;1221:67-79. doi: 10.1016/j.brainres.2008.05.006.

33. Smith DV, Li CS. Tonic GABAergic inhibition of taste-responsive neurons in the nucleus of the solitary tract. Chem Senses 1998;23:159-169.

34. Li CS, Lu DP, Cho YK. Descending projections from the nucleus accumbens shell excite activity of taste-responsive neurons in the nucleus of the solitary tract in the hamster. J Neurophysiol. 2015;113:3778-3786. doi: 10.1152/jn.00362.2014.

35. Berridge KC. Food reward: brain substrates of wanting and liking. Neurosci Biobehav Rev. 1996;20:1-25.

36. Norgren R, Hajnal A, Mungarndee SS. Gustatory reward and the nucleus accumbens. Physiol Behav. 2006;89:531-535.