Taste-responsive neurons in the nucleus of the solitary tract receive gustatory information from both sides of the tongue in the hamster

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Li C-S, Mao L, Cho YK. Taste-responsive neurons in the nucleus of the solitary tract receive gustatory information from both sides of the tongue in the hamster. Am J Physiol Regul Integr Comp Physiol 294: R372–R381, 2008. First published December 12, 2007; doi:10.1152/ajpregu.00791.2007.—Taste receptors on the left and right sides of the anterior tongue are innervated by chorda tympani (CT) fibers, which carry taste information to the ipsilateral nucleus of the solitary tract (NST). Although the anterior tongue is essential for taste, patients with unilateral CT nerve damage often report no subjective change in their taste experience. The standing theory that explains the taste constancy is the “release of inhibition”, which hypothesizes that within the NST there are inhibitory interactions between inputs from the CT and glossopharyngeal nerves and that the loss of taste information from the CT is compensated by a release of inhibition on the glossopharyngeal nerve input. However, the possibility of compensation by taste input from the other side of the tongue has never been investigated in rodents. We recorded from 95 taste-responsive neurons in the NST and examined their responsiveness to stimulation of the contralateral CT. Forty-six cells were activated, mostly with excitatory responses (42 cells). Activation of NST cells induced by contralateral CT stimulation was blocked by microinjection of lidocaine into the contralateral NST but was not affected by anesthetization of the contralateral parabrachial nuclei (PbN). In addition, the NST cells that were activated by contralateral CT stimulation showed reduced responsiveness to taste stimulation after microinjection of lidocaine into the contralateral NST. These results demonstrate that nearly half of the taste neurons in the NST receive gustatory information from both sides of the tongue. This “cross talk” between bilateral NST may also contribute to the “taste constancy”.

One of the unique features of the gustatory system is that unlike the olfactory, auditory, and visual sensory systems, which depend on a single cranial nerve, gustatory information is processed by three cranial nerves: the VIIth, IXth, and Xth. Terminals of these nerves are distributed in a topographic fashion with rostrocaudal sequence in the rostral nucleus of the solitary tract (NST) (9, 16, 17, 29, 51). Although taste information is transmitted to the NST by three cranial nerves, taste information from the anterior tongue plays an essential role in gustation. The chorda tympani (CT) branch of the facial (VIIth) nerve innervates the anterior two-thirds of the tongue on the same side. In hamsters, bilateral CT nerve crush abolishes previously learned conditioned taste aversion (CTA) to NaCl, and the animals are able to relearn the CTA 16 wk following the crush, which is the critical time point of CT nerve regeneration (2). In rats, bilateral transection of CT nerve impairs prelearned taste avoidance to NaCl and increases NaCl detection threshold, indicating that the anterior tongue is critical for NaCl detection (43). The anterior tongue is also important for detection of sweet taste in the hamster (13, 14).

Another feature of the gustatory system is the convergence between cranial nerves responsible for taste information processing. Sucrose preference tests in the rat have shown that bilateral CT section marginally attenuates the rats’ responsiveness. Sectioning of the CT nerves combined with lesions of the naso-incisor ducts (NID), which are innervated by the greater superficial petrosal (GSP) branch of the VIIth nerve, had greater effect (44). This synergistic effect likely represents the central convergence of primary afferent fibers from the GSP and CT, since NST neurons are responsive to stimulation of both receptor subpopulations on the anterior tongue and those associated with the NID (48). In rats, some NST neurons responded to taste stimulation of receptor subpopulations innervated by both CT and glossopharyngeal (IXth) nerves (47). In hamsters, 64% of NST neurons are responsive to stimulation of receptor subpopulations located both in anterior tongue and posterior oral cavity (45).

The convergence of gustatory input from different receptor subpopulations on the NST taste-responsive neurons is presented as a mechanism to explain “taste constancy”, in which patients with unilateral CT nerve damage often report no subjective change in their taste experience. Halpern and Nelson (15) hypothesized a “release of inhibition” theory that emphasizes inhibitory interactions between inputs from the CT and IXth nerves, and that the loss of taste information from the CT is compensated by a release of inhibition on the IXth nerve input on NST taste neurons. In contrast, Dinkins and Travers (10) did not observe the compensatory increase in response to the stimulation of the palatal or posterior tongue taste stimulation during the anesthetization of CT nerve in rats.

Whereas the investigations of taste convergence in the NST have been focused on convergence of taste input from different subpopulations of receptors or gustatory afferents, the possibility of convergent input on gustatory neurons in the NST from taste receptor subpopulations of the contralateral side of the tongue has never been investigated in the rodent. The possibility of convergence from the contralateral side was suggested in a clinical study. This study, in which patients with left insula damage experienced impairment of taste quality...
identification on both the left and right sides of the tongue, suggests that gustatory information from both sides of the tongue converges in the brain (32). In a recent tract-tracing study, Whitehead and colleagues (50) demonstrated reciprocal neural connections between bilateral rostral NST, suggesting that taste information from right and left sides of the tongue may converge onto single NST neurons through the NST-NST connectivity. Here, we investigated whether taste neurons in the NST receive neural input from the contralateral CT nerve, and whether this pathway carries taste information.

MATERIALS AND METHODS

Animal and surgery. The experimental procedures were conducted so as to prevent animal suffering and minimize the number of animals used in accordance with the Institutional Animal Care and Use Committee (IACUC) and National Institutes of Health guidelines. All procedures used in this experiment were reviewed and approved by the IACUC of Southern Illinois University at Carbondale. Young adult male Syrian golden hamsters (Mesocricetus auratus, Harlan Sprague Dawley, Indianapolis, IN) weighing between 131 and 182 g were used in this study. For recording from the NST, a recording/injecting pipette assembly for stimulating the tongue was constructed of a single glass recording micropipette, which was glued to a Teflon coating injector (Bak Digital Injector, Bak Electronics, Germantown, MD). The recording/injecting pipette assembly was filled with 2% (wt/vol) solution of Chicago Sky Blue dye (Sigma, St. Louis, MO) in 0.5 M sodium acetate for extracellular single-unit recording of action potentials from the gustatory NST contralateral to the CT nerve-stimulating side. The mean coordinates for the NST recording were 2.01 ± 0.12 mm anterior to the obex, 1.28 ± 0.09 mm lateral to the midline, and 0.56 and 1.12 mm ventral to the surface of the brainstem. Extracellular action potentials were amplified with a band-pass of 15–5,000 Hz (NeuroLog, Digitimer, Hertfordshire, United Kingdom), discriminated with a dual time-amplitude window discriminator (Bak DDIS-1, Bak Electronics, Germantown, MD), displayed on oscilloscopes, and monitored with an audio monitor. A Dell Pentium 4 XPS laptop computer configured with a CED Power1401 interface board and Spike2 software (Cambridge Electronic Design, Cambridge, United Kingdom) controlled taste stimulus delivery and online data acquisition and analysis.

Taste responses of NST and PbN neurons were initially identified by a change in neural activity associated with the application of electrical shock (≤40 μA, 500-ms duration at 1/3 Hz) to the anterior tongue through a parallel platinum electrode (50 μm, 2-mm separation, Fisher Scientific, Pittsburgh, PA). It has been reported that electric current lesions were produced without damaging the NST. After lesion confirmation by its response to chemical stimulation of the anterior tongue. Taste stimuli were 32 mM sodium chloride (NaCl), 32 mM sucrose, 32 mM quinine hydrochloride (QHCl), and 3.2 mM citric acid. These concentrations evoke roughly equal multi-unit responses in the hamster NST (11). These taste solutions were delivered by a gravity-flow system composed of a computer-controlled two-way solenoid-operated valve connected via tubing to a distilled-water rinse reservoir and a stimulus funnel. The stimulation sequence, during which the computer acquired data, was a continuous flow initiated by the delivery of distilled water for 10 s, followed by 10 s of stimulus, followed by 10 s of distilled-water rinse. The flow rate was 2 ml/s. After each stimulus, the tongue was rinsed with distilled water (>50 ml), and individual stimulations were separated by at least 2 min to avoid adaptation effects (37).

Classification of taste neurons by their responsiveness to the contralateral CT stimulation. After each NST neuron was characterized for its taste response profile, rectangular pulses (0.5 ms duration, 0.15 mA intensity, 1/3 Hz) were delivered to the CT nerve through the stimulating electrode from an isolated stimulator (Grass S88, Grass Instruments, QuinCY, MA) to examine whether the neuron recorded was responsive to the CT nerve stimulation. A hook-shaped parallel bipolar electrode, which was constructed with two Teflon-coated Platinum-Iridium wires (bare size 75 μm, with Teflon coating 113 μm diameter, A-M systems, CA), was used to electrically stimulate the CT nerve. The insulation coating had been removed at the tip for 500 μm, and two tips were separated by 400 μm. A peristimulus time histogram (PSTH) with a bin size of 1 ms was created from data acquired on each NST cell in response to 50–200 stimulus pulses delivered to the CT nerve-stimulating electrode.

To test whether local anesthetization of gustatory PbN or NST ipsilateral to the CT nerve-stimulating side affect the CT-evoked activation of NST taste cells, experiments involving microinjection of lidocaine into PbN or NST were performed. The NST or PbN was located by physiological guidance with a combined recording/injecting pipette assembly. The recording/injecting pipette assembly was constructed of a single glass recording micropipette, which was glued to a double-barrel glass micropipette that had been pulled and then broken to a tip diameter of 30 μm. This configuration is constructed so that the tip of the recording micropipette extends 1 mm beyond the tip of the drug injection pipette. The two barrels of the microinjection pipette were loaded with 0.5 μl of 1–4% lidocaine into the medulla or spinal cord spreads 435–500 μm from the cannula tip (35, 36). The mean coordinates for the contralateral PbN recording were 4.0 ± 0.11 mm anterior to the obex and 1.4 ± 0.07 mm lateral to the midline. The mean coordinates for the
contralateral NST recordings were 2.04 ± 0.10 mm anterior to the obex, 1.23 ± 0.11 mm lateral to the midline, and between 0.62 and 0.94 mm ventral to the dorsal surface of the brainstem. In these experiments, after a taste neuron was isolated in the NST or the PbN, the recording/injecting pipette assembly was further advanced 1 mm to place the tip of the injecting pipette to where the NST or PbN neuron was recorded. After confirming that a taste-responsive NST cell was responsive to the contralateral CT nerve stimulation, a dose of 0.5 μl lidocaine or the same volume of physiological saline was injected into the contralateral NST or PbN (i.e., ipsilateral to CT nerve-stimulating side). The lidocaine and physiological saline injections were separated by 90- to 120-min intervals. PSTHs were created from data acquired on each NST cell in response to 50–200 CT nerve stimulus pulses following microinjection of lidocaine or saline into the NST or PbN.

Histology. At the end of each experiment, the last recording site of the day was marked by passing a 10-μA cathodal current through the recording electrode for 10 min (5 s ON-OFF) to deposit a spot of Chicago Sky Blue dye. The hamster was then given a lethal overdose of urethane and perfused through the heart with 4% formalin containing 3% potassium ferrocyanide and ferricyanide. Brains were removed, post-fixed, frozen sectioned (40 μm) in the coronal plane, and stained with Neutral Red. The recording sites were located microscopically and plotted on standard atlas sections (27).

Data analysis. The responses of each cell to taste stimulation of the tongue were accumulated over three consecutive time periods during 1) 10 s of distilled water perineum just prior to the stimulus, 2) 10 s of stimulus flow, and 3) 10 s of distilled water rinse just after the stimulus. The net taste response was calculated as the mean number of action potentials (impulses/s) during the first 5 s of chemical stimulation minus the mean number of discharge spikes during the 5-s distilled water perineum. The size of the time bin used in the analysis of taste responses was 1 s. A taste response was defined as effective if it was ≥2.0 SD above the baseline discharge, which was calculated from the firing activity during the 5-s distilled water perineum before each of four taste stimuli. Responses are reported as means ± SE.

For orthodromic responses of NST cells to electrical stimulation of the contralateral CT nerve, an individual PSTH, which has a time span of 1 s of 1-ms bins, was analyzed. A baseline period was defined as the 200 ms preceding stimulation; the mean ± SD of the number of spikes/1-ms bin during this baseline period was determined. The accumulated spikes during the 800 ms after stimulation were analyzed to determine excitatory or inhibitory epochs. An excitatory effect of the contralateral CT nerve stimulation was defined as an epoch of at least five consecutive bins with a mean value ≥2 SD above the baseline mean, which defines a mean response with a probability of <0.05 (6). The response latency of the excitatory response was defined as the time at which the firing rate became ≥2 SD above the baseline mean after stimulation. Inhibitory responses were defined as those with at least 20 consecutive bins with a mean <50% of baseline firing rate. Because of the slow rates of spontaneous firing of many NST cells and their asynchronous discharge patterns, a criterion for inhibition based on variance is not practical; using 20 bins defines the inhibitory epoch as a relatively sustained decrease in firing rate (6). The duration of excitability of an orthodromic response was determined by the following procedures. After a PSTH was created following the contralateral CT stimulation, the inhibitory or excitatory epoch was determined on individual PSTH. An individual PSTH has 1,000 bins over a 1-s period. The onset and the termination of response epoch was determined in millisecond scale by enlarging the x-axis (time) of a PSTH by means of CED software. The duration of excitability was defined as the time from CT stimulation (0 s) to the termination of excitatory epoch minus the latency.

The entropy (H) of each neuron, which is a measure of its breadth of responsiveness, was calculated via excitatory components of responses to four standard taste stimuli by the following formula:

\[ H = -\frac{k}{\sum_{i=1}^{n} p_i \log_2 p_i} \]

where \( H \) = breadth of responsiveness, 1.661 is a scaling constant, and \( p_i \) is the proportional response to each of the \( n \) component. \( H \) ranges from 0.0 for a cell that responds exclusively to one stimulus to 1.0 for a cell responding equally to all four (40).

Each NST cell was categorized as either a CT-responsive neuron if it was activated by contralateral CT nerve stimulation, or a non-CT-responsive neuron otherwise. Within those two categories, the cells were further characterized by their best taste stimulus, which produced the greatest response.

Univariate ANOVA was used to compare differences in mean firing rates to taste stimuli and in entropies between CT-responsive and non-CT-responsive neurons across taste stimuli and best stimulus. The baseline activities of the CT-responsive and non-CT-responsive groups were compared via \( t \)-tests. Comparison of the number of neurons in each category was made via the chi square test. All means were reported with SE unless noted otherwise.

RESULTS

Histology. A total of 95 taste-responsive NST neurons were recorded from 29 male hamsters, and at least one NST taste cell that responded to the contralateral CT stimulation was recorded from each animal. Neurons that did not meet statistical criterion for taste response were excluded from the analysis. All 29 recording sites were identified histologically, and a representative example of the Chicago Sky Blue dye marking in the NST is shown in Fig. 1. On this coronal section through the hamster medulla, the marking is located medial to the solitary tract, most likely in the rostrocentrul subdivision. Cells were recorded from the NST where the caudal border of the dorsal cochlear nucleus (DC) is first apparent on the dorsolateral margin of the medulla, which is the area of the NST receiving its predominant gustatory input from the VIIth nerve (49, 51).

The locations of the last NST cell to be recorded in each animal were marked with Chicago Sky Blue dye, and the positions of these cells from all animals (\( n = 29 \)) were depicted on a standard atlas section of the medulla at the level of the DC (the figure is not shown). The tips of the recording electrodes were confined to the region of the rostral NST and appeared to

\[ \text{Fig. 1. Photomicrograph of representative recording marking in hamster brainstem. Coronal section through the medulla, showing a recording site in the nucleus of the solitary tract (NST), marked with Chicago Sky Blue dye (arrow). DC, dorsal cochlear nucleus; MVe, medial vestibular nucleus; SpVe, spinal vestibular nucleus; st, solitary tract. Calibration bar = 500 μm.} \]
be in the rostrocentral or rostrolateral subdivision. The distribution of these recording markings was similar to that in our previous recordings (7, 41). There was no relationship between the recording sites and the effect of the CT nerve stimulation (i.e., CT-responsive or non-CT-responsive), or response types (i.e., excitatory response or inhibitory response).

**Taste response characteristics of NST neurons.** A total of 95 taste neurons were isolated from the NST and tested for their responsiveness to the four basic taste stimuli: 46 from CT-responsive and 49 from non-CT-responsive groups. A representative taste trial of one NST cell to gustatory stimuli is shown in Fig. 2.

The overall baseline activity of the 95 taste-responsive NST neurons recorded in the present study ranged from 0 to 25.35 impulses/s with a mean of 2.24 ± 0.39 impulses/s. This mean firing rate was similar to that recorded previously (i.e., 2.12 ± 0.27 impulses/s, t = 0.270, df = 202, p = 0.786; Ref. 22) and significantly lower than that recorded in the PbN in our previous experiments (i.e., 3.63 ± 0.26 impulses/s, t = 3.055, df = 210, P < 0.005; Ref. 21). Comparison of baseline activities between the CT-responsive group (range = 0–16.65 impulses/s, mean = 1.94 ± 0.44 impulses/s, n = 46) and non-CT-responsive group (range = 0–25.35 impulses/s, mean = 2.53 ± 0.64 impulses/s, n = 49) showed no significant difference (t = 0.751, df = 93, P = 0.455; Fig. 4A).

Each of the 95 NST neurons was tested for its responsiveness to the four basic taste stimuli and categorized as NaCl-, sucrose-, citric acid-, or QHCl-best on the basis of its response profile. Of the 95 neurons, 20 were NaCl-best, 23 were sucrose-best, 27 were citric acid-best, and 25 were QHCl-best. Chi-square analysis demonstrated a relatively even distribution of the NST taste cells among the four best-stimulus categories (χ² = 1.126, df = 3, P = 0.771). The 46 CT-responsive neurons were composed of 13 NaCl-best, 10 sucrose-best, 10 citric acid-best, and 13 QHCl-best cells. This distribution among the four best-stimulus groups was not different from that of non-CT-responsive taste neurons (χ² = 9.179, df = 3, P = 0.027). These best-stimulus categories are indicated in Fig. 3. The black and gray bars represent the mean response magnitudes of the CT-responsive neurons, whereas open bars represent those of non-CT-responsive cells.

ANOVA analysis of firing rate to each of four taste stimuli of the CT-responsive (including both excitatory and inhibitory responses) and nonresponsive NST neurons demonstrated that overall taste responses of nonresponsive neurons was greater than CT-responsive neurons (F[1,372] = 5.415, P < 0.05). Four basic taste stimuli also produced responses that differed from one another (F[3,372] = 4.547, P < 0.005), but there was no significant interaction between CT-responsiveness and taste stimulus (F[3,372] = 0.309, P = 0.819; Fig. 4A). A comparison by stimulus, however, did not show a significant difference between the CT-responsive and non-CT-responsive groups; the difference in the response to citric acid was greatest but insignificant (P = 0.06). The baseline activity was not different between these two groups (t = 0.751, df = 93, P = 0.455). The mean firing to each taste stimulus and mean baseline firing of CT-responsive and non-CT-responsive NST neurons is depicted in Fig. 4A.

The breadth of responsiveness (entropy) of each NST neuron was obtained by means of excitatory components of taste response to four taste stimuli. The mean entropies in each category and CT-responsiveness are described in Fig. 4B. The mean entropy (0.644 ± 0.032) of CT-responsive neurons was not different from that (0.672 ± 0.034) of nonresponsive cells (F[1,87] = 0.019, P = 0.889). At these relatively equally effective stimulus concentrations, NaCl-best cells were the most narrowly tuned (H = 0.578 ± 0.066), and citric acid-best cells were the most broadly tuned (H = 0.770 ± 0.025). The differences in entropy among the four best-stimulus groups were statistically significant (F[3,87] = 3.586, P < 0.05).

**Effect of the contralateral CT nerve stimulation on the NST taste neurons.** A total of 95 taste neurons were recorded in the NST, and the effects of the contralateral CT nerve stimulation were examined. Of the 95 neurons recorded, 46 cells were activated; 42 cells were excited and four cells, which were all QHCl-best, were inhibited following contralateral CT nerve stimulation. These neurons were classified as CT-responsive.
neurons and the remaining 49 as non-CT-responsive neurons. Examples of four NST neurons that were activated after the contralateral CT nerve stimulation are shown in Figs. 5 and 6. The superimposed oscilloscope traces from two NST cells in Fig. 5 illustrate the examples of the variable-latency action potentials evoked after the contralateral CT stimulation; the cell in A showed short onset response latency (24 ms), and the cell in B showed long onset response latency (44 ms). The PSTH and corresponding raster plots of the responses of two additional NST cells after the contralateral CT stimulation are depicted in Fig. 6, showing excitatory (A) and inhibitory (B) responses, respectively.

The latencies of excitation of the NST cells following the contralateral CT nerve stimulation varied between 19 and 95 ms with a mean latency of 33.76 ± 2.43 ms. The action potentials of the NST cells induced by the contralateral CT stimulation were spread between a 5- and 176-ms period with a mean duration of excitation of 28.55 ± 4.98 ms. Each CT-responsive neuron (excited neuron) was subcategorized into short and long latency groups based on its response latency. The response latencies of the short latency group varied between 19 and 38 ms (n = 30) with a mean latency of 25.97 ± 0.93 ms (e.g., the cell in Fig. 5A and the cells indicated with black bars in Fig. 7A), while the response latencies of the long latency group varied between 40 and 95 ms (n = 12) with a mean latency of 53.25 ± 4.77 ms (e.g., the cell in Figs. 5B and the cells indicated with open bars in Fig. 7A); the difference between the latencies of these two groups was significant (t = 8.233, df = 40, P < 0.0001). The cells that showed longer response latencies also exhibited prolonged excitability (range = 24–176 ms, mean = 63.75 ± 12.40 ms; open bars in Fig. 7B), while cells that showed shorter response latencies exhibited short duration of excitability (range = 5–28 ms, mean = 14.47 ± 1.38 ms; black bars in Fig. 7B). The duration of the excitability between these two groups was statistically different as well (t = 6.158, df = 40, P < 0.0001). The distribution of the latencies and the excitation durations in response to the contralateral CT stimulation are demonstrated in Fig. 7.

Inhibitory responses to the contralateral CT nerve stimulation were observed in four neurons. The mean latency of the inhibition and the mean duration of the silent period induced by the contralateral CT nerve stimulation were 24.50 ± 2.10 ms and 49.75 ± 9.20 ms, respectively. The numbers of NST neurons in each category are shown in Table 1.

NST cell responses evoked by contralateral CT stimulation were blocked by local anesthetization of contralateral NST. To investigate whether the neural pathway that conveys taste information from one side of the tongue to the other side of the NST is passing through the NST contralateral to the recording side, the following experiments were performed. After identifying a taste neuron that was activated by contralateral CT nerve stimulation, 0.5 µl of lidocaine was injected into the contralateral NST (ipsilateral to CT nerve stimulation side), and the CT nerve was stimulated again. The CT nerve stimulation-evoked activity of the NST neurons was completely blocked following lidocaine injection. An example of this lidocaine effect is shown in Fig. 8. The anesthetization of the contralateral NST completely blocked the activation of the NST neurons evoked by the CT nerve stimulation in all eight
cells tested (Fig. 8, middle). Microinjection of physiological saline at 120 min after lidocaine injection was without effect (Fig. 8, bottom). There was no detectable change in the baseline firing rate of NST neurons after injection of lidocaine into the contralateral NST ($t = 0.564$, $df = 7$, $P = 0.590$). The eight neurons tested in this set of experiments were one sucrose-best, two NaCl-best, two citric acid-best, and three QHCl-best. Among these eight neurons, two cells belonged to the long latency group, and the remaining six cells belonged to the short latency group.

NST cell responses evoked by contralateral CT stimulation were unaffected by local anesthetization of contralateral PbN. To determine whether the course of the input to taste-responsive NST neurons from the contralateral CT nerve is passing through the contralateral PbN, the following experiments were performed. After identifying an NST neuron that was activated by contralateral NST stimulation, the contralateral gustatory PbN was anesthetized by microinjection of 0.5 μl of lidocaine, and then the CT nerve was stimulated again. An example of this experiment is shown in Fig. 9. NST neuron activation by contralateral CT stimulation was not affected by lidocaine injected into the contralateral PbN in any of the six neurons tested. The neurons tested in this set of experiments included one sucrose-best, two NaCl-best, one citric acid-best, and two QHCl-best. Among these six neurons, two cells belonged to the long latency group, and the remaining four cells belonged to the short latency group.

Taste responses of NST cells were reduced by contralateral NST anesthetization. To investigate whether the neural input to the NST cells from the contralateral CT nerve influences gustatory responses, taste trials were conducted before and after anesthetization of the contralateral NST. A total of 19 taste trials, including five NaCl, one sucrose, six citric acid, and seven QHCl responses from eight neurons were compared before and after local anesthetization of the contralateral NST. Exemplary taste trials that show the effect of contralateral NST anesthetization on gustatory responses of a citric acid-best

![Fig. 5. Excitatory responses (arrowhead) of two taste-responsive neurons recorded from NST in response to contralateral CT stimulation. Note that the neuron in A responded with relatively short latency (24 ms) and exhibited short duration of excitability (13 ms), while the neuron in B responded with relatively longer latency (44 ms) and showed longer duration of excitability (34 ms) following contralateral CT stimulation. Electrical stimulation of contralateral CT started at arrow. Each recording is composed of 50 superimposed oscilloscope traces.](image5)

![Fig. 6. PSTHs depicting two NST neurons that showed excitatory (A) and inhibitory (B) responses following contralateral CT nerve stimulation. Raster PSTHs of impulses over 1-s peristimulus period of same neurons were also shown at the top of each of PSTH. Note that stimulus artifact appeared at time = 0 in the PSTH in B and in its raster plot. Electrical pulses were delivered to contralateral CT nerve at time = 0. The PSTHs were accumulated over 60 (A) or 200 (B) stimulus sweeps at 1/3 Hz, respectively.](image6)

![Fig. 7. Frequency distribution of latencies (A) and durations of excitability (B) of the NST cells in response to electrical stimulation of contralateral CT nerve. Note that the NST cells with short response latencies ($\leq 38$ ms, black bars, A) following contralateral CT stimulation also have short durations of excitation ($\leq 28$ ms, black bars, B).](image7)
neuron are shown in Fig. 10A. Overall gustatory response was reduced following the lidocaine injection into the contralateral NST (Fig. 10B). The mean taste responses of the 19 taste trials before and after the local anesthetization of the contralateral NST were 12.06 /\pm/ 3.72 impulses/s and 8.17 /\pm/ 2.61 impulses/s, respectively ($t = 3.205$, $df = 18$, $P = 0.005$). The taste responses of the NST neurons returned to control level 90–120 min after the anesthetization; the mean taste response after recovery (saline injection) was 11.93 /\pm/ 3.57 impulses/s, and it was not different from the control level ($t = 0.027$, $df = 18$, $P = 0.979$).

**DISCUSSION**

The main finding of the present investigation was that gustatory neurons in the NST receive input from the contralateral CT nerve, and that the input from the contralateral side of the CT conveys taste information. We also demonstrated that the contralateral NST but not the PbN is involved in this pathway, which connects the gustatory NST and the anterior portion of the contralateral tongue.

**Bilateral taste convergence on the NST.** In the present study, we demonstrated that taste neurons in the NST also receive gustatory input from the contralateral CT. Stimulation of the contralateral CT activated 46 of 95 NST neurons; 42 cells were excited and four cells were inhibited, indicating that nearly 50% of taste neurons in the NST receive neural input from both sides of the tongue. Whitehead et al. (50) reported that following microinjection of cholera toxin B into the rostral NST, many labeled neurons and axons were identified in the contralateral NST, indicating that there are reciprocal neural connections between the rostral NST. In a recent investigation, we demonstrated that stimulation of the NST activates 70% of taste neurons in the contralateral NST in the hamster (5). These studies support our results that taste neurons in the NST receive gustatory information from both sides of the tongue.

Convergence is a common scheme in sensory pathways. Recent studies have provided evidence of convergence of descending projections from nuclei in the gustatory forebrain, e.g., the lateral hypothalamus (LH) and central nucleus of the
amygdala (CeA) (6, 8, 22), as well as convergence of ascending inputs from subpopulations of taste receptors (45, 47, 48) onto neurons of the NST in rats and hamsters. Here, we have demonstrated the presence of another converging scheme of the gustatory system: the convergence of gustatory information from both sides of the tongue onto NST neurons. The bilateral convergence of taste seems to occur only centrally, because there is no evidence for overlap between the taste buds of the anterior tongue on right and left sides (3).

The possible neural pathway of peripheral gustatory input to the neurons in the contralateral NST. It has been very well established that taste information from either side of the tongue is sent to the ipsilateral NST. The main CT terminals are distributed most densely in the rostral pole of the ipsilateral NST in the rat (16) and in the hamster (51). We hypothesized that contralateral taste information to the NST crosses from the contralateral CT via the contralateral NST. To prove our hypothesis we anesthetized the contralateral NST. Microinjection of lidocaine into the contralateral NST completely blocked the activation of NST neurons induced by contralateral CT stimulation (Fig. 8). These results indicate that taste information from one side of the tongue is sent to the same side of the NST and then to the contralateral NST.

It is known that gustatory information from the NST is further sent to the Pbn (28, 46). Electrophysiological studies have shown that 80% of the NST taste neurons are projected to the ipsilateral Pbn in the hamster (7). Taste neurons in the NST also receive centrifugal input from the Pbn, the CeA, the LH, and the insular cortex (7, 8, 22, 39). There is a possibility that the neural pathway to the NST from the contralateral side of the tongue passes by way of the contralateral CT through the contralateral NST and continues to the contralateral Pbn or beyond before crossing over as centrifugal projection. However, anesthetization of Pbn did not affect the activation of the NST taste neurons induced by contralateral CT stimulation (Fig. 9), indicating that the pathway does not involve the gustatory nuclei beyond the NST. This conclusion has also been supported by other studies. It has been reported that the mean response latency of the rat NST neurons following stimulation of the ipsilateral CT nerve was 7.2 ± 4.3 ms (30). The mean onset latencies of antidromic and orthodromic activation of NST taste neurons following ipsilateral Pbn stimulation were 4.1 ± 0.4 ms or 4.7 ± 0.4 and 11.3 ± 0.7 ms, respectively (5, 7). In addition, the mean orthodromic response latency of NST neurons following the contralateral NST stimulation was 22.6 ± 0.7 ms in the hamster (5). The mean response latency (33.8 ± 2.4 ms) of the NST neurons following the contralateral CT stimulation was shorter than the sum of the total latency, the response latency of NST cells after the CT stimulation, the latencies (antidromic invasion and orthodromic responses) of the NST neurons following the Pbn stimulation, and the response latency of the NST cells after the contralateral NST stimulation. These data also support our conclusion that this neural pathway does not involve the Pbn or other forebrain gustatory nuclei.

The NST neurons that were activated by contralateral CT stimulation were categorized into two groups based upon their response latencies: the neurons with a latency shorter than 40 ms (short latency group), and those with a latency longer than 40 ms (long latency group). The spread of the evoked spikes in the short latency group was significantly narrower than that of the cells in the long latency group. One of the explanations for the latency difference is that it may be due to different CT fiber types. Earlier studies have demonstrated two types of afferent CT fibers in rats (12, 24) and in hamsters (18): the myelinated and unmyelinated fibers, with associated differences in conduction velocities (34). In the hamster, 67% of the fibers within the CT, which is made up of both myelinated and unmyelinated fibers (18), are sensory. However, the difference in conduction velocity (response latency) of different CT fiber types cannot explain the significantly different spike distribution of the NST neurons evoked by contralateral CT stimulation. Therefore, the differences in the response latency may suggest diversity in neural connections between the bilateral NST. The wider distribution of the evoked spikes may suggest there are more synaptic contacts with a more detoured course and/or differences in fiber size between bilateral NST. Further investigations are necessary to determine the underlying mechanisms responsible for these differences.

The neural pathway to NST neurons from the contralateral side of the tongue contributes to gustatory responses. It is known that the CT is not purely gustatory. The CT not only carries gustatory information but also conveys mechanical and thermal information from the anterior tongue. It was reported...
that many single CT fibers respond to both gustatory and thermal stimulation of the tongue, both in rats and hamsters (31). Some single CT nerve fibers also respond to taste, mechanical, or both stimuli in the rat (23). To examine whether this pathway influences taste responses, we compared taste responses of the NST neurons that responded to CT stimulation, before and after anesthetization of the contralateral NST. Lidocaine blockage of the contralateral NST reversibly reduced taste responses. Although we could not exclude some other components, such as thermal and mechanical/tactile inputs, being involved in the reduced taste responses, these data suggest that the projection to the NST from the contralateral CT carries taste information (Fig. 10). The contralateral NST anesthetization procedures reduced, but did not totally block, the taste responses of the NST neurons. This indicates that taste information is also flowing to the NST from the periphery through the unanesthetized side.

Although we could not test taste modulation effect, we observed four NST taste neurons in which activity was suppressed by contralateral CT stimulation. All four were QHCl-best cells. In fact, we observed such inhibition in citric acid-best and sucrose-best cells, which were not included in the analysis because the magnitude of gustatory responses barely missed our criterion for taste response. More data are desired to investigate the inhibitory nature of the NST cells evoked by the contralateral CT stimulation.

**Perspectives**

“Taste constancy” refers to the phenomenon that individuals who have sustained considerable chorda/lingual nerve damage often fail to experience any subjective change in real-world taste experience (3, 4, 19, 20, 25, 26, 33). To explain this phenomenon, researchers have hypothesized a “release of inhibition”, which emphasizes that taste constancy results from removal of the inhibition of the CT nerve on cells that receive peripheral excitatory inputs from other gustatory nerves within the NST. Halpern and Nelson (15) reported that the rat NST responded more to the application of NaCl to the posterior tongue after anesthesia of the ipsilateral CT nerve. In the clinical studies, the taste of QHCl was intensified and the taste of NaCl was diminished at areas innervated by the glossopharyngeal nerve on both sides of the tongue if CT nerves are anesthetized bilaterally (52). However, a recent study reported that average anterior tongue responses were eliminated, but no compensatory increases in palatal or posterior tongue responses were observed following anesthetization of the CT, indicating that other mechanisms may be involved to compensate for the loss of taste input (10).

In a clinical study involving the investigation of interactions between taste input from the right and left sides of the tongue in gustatory cortex, Pritchard et al. (32) reported that humans with damage to the left insula experience a deficit in taste recognition on both the left and right sides of the tongue. This result suggests that taste information from both sides of the tongue converges in the central nervous system, although the site of the convergence in the gustatory pathway was not investigated. In the present study, we have demonstrated that nearly 50% of the taste neurons in the NST receive gustatory input from both sides of the anterior tongue. This cross talk between bilateral NST is important for two reasons. First, it maintains the taste information flow in the bilateral NST even if unilateral taste input is blocked peripherally. Second, it insures sufficient volume of gustatory information that flows to both sides of the NST. Therefore, we propose that, together with the “release of inhibition” mechanism, this cross talk between bilateral NST plays an important role in maintaining “taste constancy”. Further studies are required to determine whether the convergence of taste information from both sides also takes place in more rostral gustatory nuclei.

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