Descending projections from the nucleus accumbens shell excite activity of taste-responsive neurons in the nucleus of the solitary tract in the hamster

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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 113: 3778–3786, 2015. First published March 5, 2015; doi:10.1152/jn.00362.2014.—The nucleus of the solitary tract (NST) and the parabrachial nuclei (PbN) are the first and second relays in the rodent central taste pathway. A series of electrophysiological experiments revealed that spontaneous and taste-evoked activities of brain stem gustatory neurons are altered by descending input from multiple forebrain nuclei in the central taste pathway. The nucleus accumbens shell (NAcSh) is a key neural substrate of reward circuitry, but it has not been verified as a classical gustatory nucleus. A recent in vivo electrophysiological study demonstrated that the NAcSh modulates the spontaneous and gustatory activities of hamster pontine taste neurons. In the present study, we investigated whether activation of the NAcSh modulates gustatory responses of the NST neurons. Extracellular single-unit activity was recorded from medullary neurons in urethane-anesthetized hamsters. After taste response was confirmed by delivery of sucrose, NaCl, citric acid, and quinine hydrochloride to the anterior tongue, the NAcSh was stimulated bilaterally with concentric bipolar stimulating electrodes. Stimulation of the ipsilateral and contralateral NAcSh induced firings from 54 and 37 of 90 medullary taste neurons, respectively. Thirty cells were affected bilaterally. No inhibitory responses or antidromic invasion was observed after NAcSh activation. In the subset of taste cells tested, high-frequency electrical stimulation of the NAcSh during taste delivery enhanced taste-evoked neuronal firing. These results demonstrate that two-thirds of the medullary gustatory neurons are under excitatory descending influence from the NAcSh, which is a strong indication of communication between the gustatory pathway and the mesolimbic reward pathway.

medullary neuron; gustatory neuron; reward; modulation; in vivo electrophysiology

GUSTATORY NEURONS in the nucleus of the solitary tract (NST) in the medulla receive taste information from peripheral taste cells and transfer it to the parabrachial nuclei (PbN) in the pons of rodents (Cho et al. 2002a; Norgren and Leonard 1971, 1973; Travers 1988; Whitehead 1990). In turn, pontine taste-responsive cells transmit taste information to the gustatory thalamus and several nuclei in the ventral forebrain (Halsell 1992; Kosar et al. 1986; Norgren 1974, 1976). The nuclei in the ventral forebrain, which receive monosynaptic projections from the gustatory PbN, such as the lateral hypothalamus (LH), the central nucleus of the amygdala (CeA), and the bed nucleus of the stria terminalis (BNST), send efferent fibers back to the PbN and NST (Moga et al. 1989; van der Kooy et al. 1984; Whitehead et al. 2000). A large number of studies have reported the importance of LH, CeA, and BNST in ingestive behavior, reward, and motivation (Reilly et al. 1994; Rolls 1999; Stellar and Stellar 1985; Zardetto-Smith et al. 1994). Although <10%, sapid taste stimulation evoked firings of neurons in these nuclei (Azuma et al. 1984; Nishijo et al. 1998, 2000; Yamamoto et al. 1989). A series of electrophysiological investigations demonstrated that electrical stimulation of these nuclei in the ventral forebrain modulates spontaneous and taste-evoked activities in a substantial number of gustatory NST neurons (Cho et al. 2002b; Cho and Li 2008; Li et al. 2002; Smith et al. 2005a).

The nucleus accumbens (NAcc) in the ventral forebrain, which consists of core (NAcCo) and shell (NAcSh) regions, is a component of the reward system (Berridge 2003; Norgren et al. 2006). Although the NAcc is not positioned in the central taste pathway, it communicates extensively with various ventral forebrain nuclei, including LH, CeA, and BNST (Brog et al. 1993; Hasue and Shamah-Lagnado 2002; Wood and Swann 2005). A considerable volume of evidence suggests that the NAcSh plays a significant role in mediating the hedonic value of orosensory stimuli and motivated behavior (Di Chiara 2002; Loriaux et al. 2011; Norgren et al. 2006). Recently, we reported that electrical stimulation of the NAcSh inhibits the spontaneous and taste-evoked neuronal activities of hamster pontine cells (Li et al. 2012). In addition to inhibitory modulation of pontine gustatory cells following NAcSh activation, one-fifth of all gustatory neurons recorded in the PbN projected to the NAcSh (Li et al. 2012).

As the first taste relay cells, gustatory neurons in the NST have many features that are comparable to PbN taste neurons. For example, gustatory responses of medullary and pontine neurons are altered by treatments that mimic the nutritional state, such as gastric distension or sodium appetite (Baird et al. 2001; Cho et al. 2004; Glenn and Erickson 1976; Shimura et al. 1997; Tamura and Norgren 1997). Electrical stimulation of LH, CeA, or BNST also modulates pontine and medullary gustatory neurons (Cho et al. 2002b; Cho and Li 2008; Li et al. 2002, 2005; Li and Cho 2006; Lundy and Norgren 2001; Smith et al. 2005a). This study investigated whether the NAcSh exerts a descending influence on medullary taste cells and...
whether activation of the NAcSh modulates gustatory responses in the hamster NST.

METHODS

Animal care and surgery. Experimental procedures were conducted in accordance with the guidelines for the use and care of laboratory animals set by the Institutional Animal Care and Use Committee (IACUC) of Southern Illinois University (SIU) and were reviewed and approved by the SIU IACUC. Young adult male Syrian golden hamsters (Mesocricetus auratus, n = 36) weighing between 138 and 176 g were used in this study. The animal preparation and insertion of stimulating electrodes were previously described (Li et al. 2012). Briefly, each animal was deeply anesthetized with urethane (1.7 g/kg ip) and tracheotomized. The animal was then mounted in a stereotaxic instrument (Narishige SR-6N), and the tissue overlying the frontal portion of the skull covering the brain was removed. After removal of a small part of the frontal bone, a concentric bipolar stimulating electrode was lowered into the NAcSh on each side of the brain (2.65 mm anterior to bregma, 1.1 mm lateral to the midline, and 5.4 mm ventral to the brain surface) and secured in position with dental cement. The stimulating electrodes were constructed from 26-gauge stainless steel tubing and 140-μm-thick stainless steel wire, and they were insulated with Epoxy lite 6001 (Epoxy lite, Irvine, CA) except for the tip area. After the electrodes were positioned in the NAcSh, the head of the animal was positioned downward 27° from the horizontal plane with a nontraumatic head holder. The skin and soft tissue overlying the occipital bone were removed. Part of the occipital bone was removed to reveal the cerebellum. The posterior portion of the cerebellum was aspirated bilaterally 3–4 mm anterior to the obex, allowing direct access to the NST.

Gustatory NST cell characterization and electrical stimulation of NAcSh. Single-barrel glass micropipettes (1- to 2-μm tip diameter, 5- to 7-MΩ resistance) filled with 2% (wt/vol) Chicago Blue dye (Sigma) in 0.5 M sodium acetate were used for the extracellular recording of single-unit action potentials from the gustatory NST. The mean coordinates for the NST recordings were 2.10 ± 0.17 (SD) mm anterior to the obex and 1.26 ± 0.15 mm lateral to the midline. Initially, an electrical shock (≤50 μA, 500-ms duration at 1/3 Hz) was applied to the anterior tongue to single out a gustatory neuron, and then taste stimuli were delivered to the anterior tongue to confirm taste-evoked neuronal activity of the taste cell. The following taste stimuli were presented to the anterior tongue: 32 mM sucrose, sodium chloride (NaCl), quinine hydrochloride (QHCl), and 3.2 mM citric acid in random order. These concentrations of taste solutions evoke approximately equal multiunit taste responses in the hamster NST (Duncan and Smith 1992). The responses of each cell to taste stimulation of the tongue were recorded over a 30-s period consisting of a continuous flow of 10 s of distilled water (dH2O) prerinse, 10 s of taste stimulus, and 10 s of poststimulus dH2O rinse. The net taste response was calculated as the mean number of action potentials (imp/s) during the first 5 s of taste stimulation minus the mean number of spikes during the 5 s of dH2O prior to taste delivery. Responses are reported as means ± SE. A taste response was considered significant if it was ≥2 SD above the baseline discharge, which was calculated from the firing activity during the 5-s dH2O prerinse prior to each of the taste stimuli. Each gustatory neuron was categorized as sucrose-best, NaCl-best, QHCl-best, or citric acid-best based on the stimulus that produced the greatest response (Frank 1973). The entropy (H) of each neuron, which is a measure of its breadth of responsiveness, was calculated with the excitatory components of responses to four standard taste stimuli according to the formula contrived by Drs. Smith and Travers (Smith and Travers 1979).

After the taste response of each NST was recorded, rectangular pulses (0.5 ms, ≤0.1 mA) were delivered manually to the NAcSh through stimulating electrodes with an isolated stimulator (Grass S88, Grass Instruments, Quincy, MA) to determine whether antidromic activation could be observed. After determining the antidromic response status of each neuron, we tested whether taste-responsive NST cells receive descending input from the NAcSh. To this end, the ipsilateral and contralateral NAcSh were stimulated in random order (0.1 mA, 0.5 ms at 1/3 Hz), and we recorded the electrically evoked activity of NST neurons. A peristimulus time histogram (PSTH) was created from the data acquired from each NST cell in response to 50–200 stimulus pulses delivered to the NAcSh. An individual PSTH was analyzed to determine the excitatory or inhibitory epoch in response to electrical stimulation of the NAcSh. A baseline period was defined as 200 ms preceding stimulation, and we determined the mean and SD of the number of spikes per millisecond during the baseline period. The excitatory effect of NAcSh stimulation was defined as an epoch of at least five consecutive 1-ms bins with a mean of >2 SD of the baseline firing rate (Li et al. 2002). An inhibitory effect was defined as at least 40 consecutive 1-ms bins in which the mean was <50% of the baseline firing rate (Mao et al. 2008). In addition to the four best stimulus categories, each NST cell was categorized either as a NAcSh-responsive neuron or a nonresponsive neuron on the basis of its responsiveness to the electrical stimulation of NAcSh. We also investigated the effect of activating the descending input from the NAcSh on the taste responses of NST cells in a subset of NST neurons by comparing taste responses before and during the delivery of high-frequency trains of constant square pulses (0.1 mA, 0.2 ms at 100 Hz) to the NAcSh. Because high-frequency electrical train stimulation (HFES) did not affect firing activity during the dH2O prerinse (Cho et al. 2002b, 2003; Li et al. 2002, 2005; Smith et al. 2005b), HFES was delivered during the 10-s taste stimulation period. To observe recovery, taste trials were repeated without NAcSh activation 20 min after the NAcSh activation trials. A total of 16 NST taste neurons were tested with this experimental protocol.

Histology and data analysis. The last recording sites in the NST and stimulation sites in the NAcSh were marked electrically at the experiment. After intraperitoneal injection of a lethal dose of pentobarbital sodium, the animal was perfused with 4% formalin containing 3% potassium ferrocyanide and ferricyanide. The brain was then removed, postfixed, sectioned (40 μm) in the coronal plane, and stained with Neutral Red. The locations of the recording and stimulation electrodes were examined histologically.

Differences in the mean firing rates between NAcSh-responsive and nonresponsive neurons, as well as taste stimuli, were compared by analysis of variance (ANOVA). The effects of electrical stimulation of the NAcSh on spontaneous activity (latency and duration of activation) and gustatory response (% change) were compared by ANOVA. The number of neurons in each category was compared by the χ²-test. Data represent means ± SE unless otherwise indicated.

RESULTS

Histology. We recorded 113 taste-responsive NST neurons from 36 hamsters. The responses of the cells that failed to meet gustatory response criteria and/or the animals with misplaced stimulating electrodes were excluded from the analysis. Accordingly, a total of 90 gustatory neurons from 31 animals were recorded and analyzed. An example of a stimulating site in the ipsilateral NAcSh and a recording site in the NST is shown in Fig. 1. The arrow in Fig. 1A indicates the location of the tip of the stimulating electrode within the NAcSh, which is located medial to NAcCo; the insertion of the stimulating electrode also damaged the wall of the lateral ventricle. We histologically confirmed and plotted the stimulating sites with the atlas brain section (Morin and Wood 2001) (Fig. 2). The majority of stimulating spots were located between midline and the NAcCo in Fig. 2. Overall, the distribution of NAcSh
stimulating sites was similar to those described in our previous report (Li et al. 2012).

The brief delivery of dye-deposition current through the recording electrode created a small lesion along with a blue-colored perimeter in the NST (arrow in Fig. 1B). The recording sites were confined in the rostral part of the NST at the level of the dorsal cochlear nucleus. The overall distribution of the recording sites in the NST was similar to that of our previous recordings (not shown) (Cho et al. 2008; Li et al. 2008).

**Taste responses of NST gustatory neurons.** Ninety NST neurons that met taste response criteria responded to a single stimulus or multiple taste stimuli. The population consisted of 25 sucrose-, 34 NaCl-, 15 citric acid-, and 16 QHCl-best neurons (Table 1). NaCl-best cells were the most frequently recorded ($\chi^2 = 10.533, df = 3, P = 0.015$). The taste response profiles of the neurons of both the NAcSh-responsive and nonresponsive groups in the best stimulus categories are demonstrated in Fig. 3. The responses of each best-stimulus group are arranged along the $x$-axis in order of their response to the best stimulus for that group. The response pattern of all 90 cells to a single taste is read horizontally and that of a neuron to all tastes is seen vertically. Each bar represents the net response of a cell to a single taste stimulus (see METHODS). The bottom row in Fig. 3 shows the neuronal firing during the prerinse dH$_2$O stimulation.

The difference in taste responses between NAcSh-responsive and nonresponsive groups ($F_{[1,352]} = 0.003, P = 0.955$) and in response to the four taste stimuli was not significant ($F_{[3,352]} = 2.206, P = 0.087$). The interaction between groups and stimulus was not significant, either ($F_{[3,352]} = 2.360, P = 0.071$). The overall baseline activity of 90 taste neurons (mean = 1.44 ± 0.22 imp/s), which ranged between 0 and 13.65 imp/s, was not different between NAcSh-responsive and nonresponsive groups ($F_{[1,88]} = 0.387, P = 0.536$). The mean taste responses of each best-stimulus group and baseline activity of NAcSh-responsive neurons are compared with those of nonresponsive cells in Fig. 4.

The entropies of the 90 taste neurons ranged between 0 and 0.95, but the difference in entropies between NAcSh responsive and nonresponsive was not significant ($F_{[1,82]} = 0.767, P = 0.384$). Differences among four best-stimulus groups was significant ($F_{[3,82]} = 4.321, P = 0.007$), likely because of a small number of citric acid- and QHCl-best cells (3 in each) in the nonresponsive group. The interaction between NAcSh responsiveness and best stimulus was not significant ($F_{[3,82]} = 1.089, P = 0.358$). The mean entropies of each best-stimulus group are addressed in Table 2.

**Effect of NAcSh stimulation on taste neurons in NST.** After the gustatory response of a particular NST neuron was con-

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**Fig. 1.** Photomicrographs of the stimulating and recording sites in the brain. **A:** coronal Neutral Red-stained section through the ventral forebrain showing the position of the ipsilateral stimulating electrode (arrow). An iron deposit from the tip of the stimulating electrode along with tissue damage caused by the electrode penetration indicates the placement of the electrode within the nucleus accumbens shell (NAcSh). **B:** coronal section through the medulla showing a recording site in the nucleus of the solitary tract (NST) marked with Chicago Blue dye (arrow). aca, Anterior commissure, anterior part; CPu, caudate putamen; fmi, forceps minor of the corpus callosum; icp, inferior cerebellar peduncle; LSI, lateral septal nucleus, intermediate part; LV, lateral ventricle; MVe, medial vestibular nucleus; NAcCo, accumbens nucleus, core; sp5, spinal trigeminal tract. Scale bars, 500 μm in both **A** and **B**.

**Fig. 2.** Atlas section of the coronal hamster brain [reproduced from Morin and Wood (2001) with permission] showing the distribution of the tips of stimulating electrodes in the bilateral NAcSh for the 31 experimental animals. Filled circles represent the stimulating sites that induced excitatory responses, whereas open circles indicate the stimulating spots that produced no response. NDB, nucleus of the diagonal band of Broca. Scale bar, 500 μm.
firmed, its responsiveness to electrical stimulation of the bilateral NAcSh was examined. First, we applied single pulses of electrical stimulation to the NAcSh to examine antidromic activation status in order to confirm whether any of the gustatory neurons in the NST project their axons to the NAcSh. We did not observe any antidromic activation but induced action potentials in variable latencies (orthodromic response) in some neurons, which were classified as NAcSh responsive. For the NAcSh-responsive neurons, NAcSh-stimulation-evoked firings were collected and analyzed while electrical stimulation was applied to the NAcSh at 1/3 Hz. Sixty-one of ninety taste cells (67.8%) were activated by NAcSh stimulation either unilaterally or bilaterally. NAcSh-responsive cells outnumbered non-NAcSh-responsive taste neurons ($\chi^2 = 11.378$, df = 1, $P < 0.005$). Of these 61 cells, neuronal activity of 30 neurons was enhanced by bilateral NAcSh stimulation. Among the 31 unilaterally activated cells, more cells were excited by the ipsilateral NAcSh stimulation than by the contralateral NAcSh stimulation. The difference was significant if only the numbers of unilaterally responsive cells were compared (24 vs. 7, $\chi^2 = 9.323$, df = 1, $P < 0.005$) but was not if bilaterally excited cells were also included in the analysis (54 vs. 37, $\chi^2 = 3.176$, df = 1, $P = 0.075$). Table 2 shows the number of cells in each best-stimulus group and the responsiveness to NAcSh stimulation.

All of the NAcSh-stimulation-produced responses of the NST gustatory neurons were excitatory orthodromic responses; no antidromic activation or inhibitory responses were observed. Examples of the excitatory effect of the NAcSh stim-

Table 1. Number of NST gustatory neurons as a function of NAcSh responsiveness and best stimulus

<table>
<thead>
<tr>
<th>Best Stimulus</th>
<th>Sucrose-Best</th>
<th>NaCl-Best</th>
<th>Citric Acid-Best</th>
<th>QHCl-Best</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilaterally excited cells</td>
<td>7</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Ipsilaterally excited cells</td>
<td>4</td>
<td>13</td>
<td>3</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Contraterally excited cells</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Nonresponsive cells</td>
<td>12</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>34</td>
<td>15</td>
<td>16</td>
<td>90</td>
</tr>
</tbody>
</table>

NST, nucleus of the solitary tract; NAcSh, nucleus accumbens shell; QHCl, quinine hydrochloride.

Fig. 3. Taste responses of the 90 NST neurons. Responses are net taste responses during the first 5 s of chemical stimulation [impulses (imp)/s]. Cells are arranged along the x-axis according to their best stimulus. Within each best-stimulus group, cells are arranged according to the magnitude of the response to their best stimulus in A and B. A: NAcSh-responsive cells are arranged as cells 1–13 being sucrose-best, 14–36 NaCl-best, 37–48 citric acid-best, and 49–61 QHCl-best. B: non-NAcSh-responsive NST neurons, which are composed of 12 sucrose-, 11 NaCl-, 3 citric acid-, and 3 QHCl-best cells, are similarly arranged.

Fig. 4. Comparison of the mean firing rate ($\pm$SE) of NST neurons as a function of best-stimulus category (columns) in response to sucrose (S), NaCl (N), citric acid (C), QHCl (Q), and distilled H$_2$O (dH$_2$O) in the NAcSh-responsive (open bars) and nonresponsive (filled bars) groups.
ulation on taste-responsive NST cells are shown in Fig. 5. PSTHs derived from three NST neurons after stimulation of the ipsilateral and contralateral NAcSh are shown. As seen in the PSTHs, repeated single-pulse stimulation of the NAcSh at 1/3 Hz induced firing activity on these taste-responsive NST neurons. Onset latencies and the duration of excitation were measured for responsive neurons. The latencies ranged from 10 to 40 ms and from 15 to 55 ms after ipsilateral ($n = 54$, mean = $16.00 \pm 0.95$) and contralateral ($n = 37$, mean = $30.14 \pm 1.86$) NAcSh stimulation, respectively. The duration of excitation ranged from 12 to 114 ms and from 11 to 113 ms after ipsilateral ($n = 54$, mean = $39.37 \pm 2.92$) and contralateral ($n = 37$, mean = $50.65 \pm 3.75$) NAcSh stimulation, respectively. The latency and response duration of the ipsilateral excitation were shorter than those of the contralateral excitation ($F_{[1,89]} = 54.634$, $P < 0.001$ for latency and $F_{[1,89]} = 5.756$, $P < 0.05$ for duration). If the comparison was restricted to the bilaterally responsive cells ($n = 30$), the mean excitation latency was also significantly shorter after ipsilateral (mean = $16.73 \pm 1.51$) than contralateral (mean = $29.70 \pm 2.04$; $t = 8.437$, df = 29, $P < 0.001$) NAcSh stimulation, but the difference of the excitation duration was not significant (mean = $42.60 \pm 3.31$ vs. mean = $51.67 \pm 4.05$; $t = 1.829$, df = 29, $P = 0.078$).

Effect of electrical stimulation of NAcSh on taste responses of NST neurons. The spontaneous activity of NAcSh-responsive neurons in the NST was enhanced after electrical stimulation of the NAcSh. To test whether activation of the descending input from the NAcSh exerts the same excitatory influence on gustatory responses, we compared taste responses of the NST neurons with or without electrical stimulation of the NAcSh in a subset of NAcSh-responsive cells. We measured taste stimulation-evoked activity from 16 NAcSh-responsive neurons before, during, and 20 min after HFES, which was the delivery of a train of rectangular pulses (100 $\mu$A, 100 Hz, 0.2 ms) to the NAcSh. Nine NST neurons were tested with HFES of the bilateral NAcSh stimulation, and the remaining seven neurons were tested with HFES of the ipsilateral NAcSh stimulation only. The HFES pulses were delivered to the NAcSh during the 10 s of taste stimulation. Recovery of the taste-evoked responses was examined by repeating the same taste trials without NAcSh stimulation at least 20 min after the testing trials. The effect of HFES of the NAcSh on taste responses is demonstrated in Fig. 6. The neuron in Fig. 6A responded to sucrose stimulation only, and HFES enhanced sucrose-evoked firings but did not alter cell firings to the other taste stimuli. Delivery of sucrose at 20 min after the test trial induced sucrose response comparable to response derived from the trial before the ipsilateral NAcSh HFES (recovery). Con-

![PSTHs](http://jn.physiology.org/)

**Fig. 5.** Peristimulus time histograms (PSTHs) depicting the responses of 3 NST taste-responsive neurons after single-pulse electrical stimulation of the ipsilateral or the contralateral NAcSh. Electrical stimulation of the NAcSh evoked neuronal activities with variable latencies. For cell NAc_NST1, the response latencies after the ipsilateral and contralateral NAcSh stimulation were 15 and 33 ms, respectively. The latencies after the ipsilateral and contralateral NAcSh stimulation were 12 and 34 ms for neuron NAc_NST16 and 22 and 32 ms for neuron NAc_NST19, respectively. Electrical pulses were delivered to the NAcSh at time = 0. Note that a stimulus artifact was seen for NAc_NST1 at time = 0 but was filtered out for NAc_NST16 and NAc_NST19. Each PSTH was accumulated over 200 stimulus sweeps.

**Table 2.** Entropy of NST gustatory neurons as a function of NAcSh responsiveness and best stimulus

<table>
<thead>
<tr>
<th>+</th>
<th>NAcSh Responsive ($n = 61$)</th>
<th>Nonresponsive ($n = 29$)</th>
</tr>
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<tbody>
<tr>
<td>Sucrose-best</td>
<td>$0.45 \pm 0.08$</td>
<td>$0.45 \pm 0.10$</td>
</tr>
<tr>
<td>NaCl-best</td>
<td>$0.57 \pm 0.05$</td>
<td>$0.49 \pm 0.06$</td>
</tr>
<tr>
<td>Citric acid-best</td>
<td>$0.71 \pm 0.06$</td>
<td>$0.77 \pm 0.07$</td>
</tr>
<tr>
<td>QHCl-best</td>
<td>$0.58 \pm 0.07$</td>
<td>$0.84 \pm 0.02$</td>
</tr>
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</table>

Values are means $\pm$ SE.
tralateral NAcSh HFES was also effective in increasing sucrose-evoked activity, which returned to the control level after HFES trial (not shown). For the neuron in Fig. 6B, three effective taste stimuli were tested for the bilateral NAcSh HFES. HFES, either ipsilaterally or contralaterally, increased taste-evoked responses regardless of the quality of taste stimulus.

Taste responses of the NST cells were increased by HFES of both ipsilateral and contralateral NAcSh for all tested stimuli. The difference in gustatory responses of NST neurons before and during ipsilateral HFES of the NAcSh barely missed statistical significance ($F_{(1,42)} = 4.041, P = 0.051$). The difference for contralateral NAcSh HFES was not significant ($F_{(1,22)} = 2.758, P = 0.111$). The rates of increment ranged from 22.45% to 213.33% with a mean of 75.23 ± 8.62% and from 16.33% to 93.33% with a mean of 63.10 ± 5.72% in response to ipsilateral and contralateral NAcSh HFES, respectively. The enhancement of the taste-evoked responses of NST neurons was not observed when gustatory responses were measured again 20 min after HFES. The difference in gustatory responses before and after HFES was not significant ($F_{(1,42)} = 0.129, P = 0.722$ for ipsilateral and $F_{(1,22)} = 0.030, P = 0.864$ for contralateral HFES). Although the ipsilateral NAcSh seemed to have a stronger influence on enhancing taste responses of NST neurons than the contralateral NAcSh, the difference was not significant ($F_{(1,38)} = 1.018, P = 0.319$). We did not compare the difference among the four taste stimuli because of the scarcity of citric acid and QHCl trials. The mean taste-evoked neuronal firings of the NST cells before, during, and after HFES of the NAcSh are demonstrated in Fig. 7.

**DISCUSSION**

In the present study, single-pulse electrical stimulation of the NAcSh induced discharges from taste-responsive neurons in the NST (67.8%) and high-frequency stimulation of the NAcSh augmented taste-evoked neuronal activity in a subset of neurons tested with this scheme. These results demonstrate that more than half of the taste-responsive neurons in the NST receive descending input from the NAcSh and activation of the NAcSh possibly enhances the gustatory responses of those neurons. Previously, we demonstrated that pontine gustatory neurons were affected by NAcSh stimulation (Li et al. 2012). In contrast to the PbN-NAcSh relationship, no antidromic activation was observed in this study, indicating that the axons of gustatory NST neurons do not directly project to the NAcSh. Another clear difference is how the NAcSh influences medullary and pontine gustatory neurons: activation of the NAcSh suppresses pontine gustatory neurons (Li et al. 2012), whereas...
stimulation of the NAcSh exclusively excites medullary gustatory neurons.

The NST is the first gustatory relay nucleus in the taste pathway (Lundy and Norgren 2004; Norgren and Leonard 1971; Norgren and Pfaffmann 1975). The majority of taste-responsive neurons in the NST project to the ipsilateral PbN and receive descending input from the bilateral PbN (Cho et al. 2002a; Cho and Li 2008). In a series of in vivo recording studies, we reported that NST gustatory cells receive descending inputs from LH, CeA, BNST, ventral posteromedial nucleus of the thalamus (VPM), and gustatory cortex (Cho et al. 2002b, 2003, 2008; Smith et al. 2005b; Smith and Li 2000). We also demonstrated that a small number of NST gustatory neurons project directly to the ipsilateral LH and VPM but not to the CeA or BNST (Cho et al. 2002b, 2003, 2008; Smith et al. 2005b). The NAcSh is densely connected with a number of the nuclei in the ventral forebrain. For example, the NAcSh receives projections from LH, CeA, and BNST, which are reciprocally connected with the PbN (Kiurocu and Ganguly 1995; Lundy and Norgren 2004). In the present study, the mean latencies following ipsilateral and contralateral NAcSh stimulation were 16.00 ± 0.95 ms and 30.14 ± 1.86 ms, respectively. In the previous study investigating the relationship between the gustatory NST and BNST, which is located closely posterior to the NAcSh, the ipsilateral and contralateral latencies were 60 ± 13.0 ms and 59.2 ± 6.3 ms, respectively, for the excitatory response and 58.1 ± 4.3 ms and 71.1 ± 9.9 ms, respectively, for the inhibitory response (Smith et al. 2005b). Thus the BNST is unlikely to mediate descending circuits from the NAcSh to the NST. Although electrical stimulation of the contralateral LH or CeA displayed shorter excitation latencies than ipsilateral stimulation, those latencies are comparable with the latency data in the present study (Cho et al. 2002b, 2003; Li et al. 2002). Further studies are required to investigate the role of potential pathways and clarify the possible route from the NAcSh to the gustatory NST.

NAcSh is not known to be a gustatory nucleus. Nonetheless, numerous investigations have reported that it is involved in the processing of evaluation of gustatory reward and motivated ingestive behavior (Berridge 2003; Norgren et al. 2006). For example, sham sucrose feeding-induced Fox expression was observed in the NAcSh and other ventral forebrain gustatory nuclei, such as LH and CeA (Mungarndee et al. 2008). Additionally, licking of sucrose increased dopamine release in the NAcSh in sham-feeding rats (Hajnal and Norgren 2005). Although the taste responsiveness of NAcSh neurons was not systematically analyzed, neuronal firing of the NAcSh cells in response to intraoral infusions of sucrose or QHCl (Roitman et al. 2005) and to NaCl (Loriaux et al. 2011) has been recorded in rats. In addition, we recently reported that a substantial number of pontine gustatory neurons project to the NAcSh, mostly ipsilaterally, and PbN gustatory cells receive descending input from the NAcSh (Li et al. 2012).

This study revealed that 61 of 90 NST gustatory neurons were excited by NAcSh stimulation. Similar to our previous investigations, gustatory cells in the NST receive bilateral inputs from the NAcSh; of the 90 total cells, 60% and 41% of them were excited by ipsilateral and contralateral NAcSh stimulation, respectively. We did not observe any measurable difference in the characteristics of NST cells between the NAcSh-responsive and nonresponsive groups, and the magnitudes of gustatory response and entropy were similar between the two groups. To further investigate whether the effect of the NAcSh activation modulates gustatory responses in NST cells, we compared taste responses with or without HFES of the NAcSh. Although we could not test every NAcSh-responsive neuron, taste responses were enhanced during NAcSh HFES in all neurons tested, regardless of taste quality. While we are aware that the sample size (16 cells) was relatively small, the majority of the neurons tested responded to more than two taste qualities, and the experiment consisted of 40 taste trials. In addition, 10 of 16 taste neurons were examined bilaterally. Taken together, these data indicate that the NAcSh plays a role in modulation of gustatory processing in the NST. One might raise a concern with the HFES experiments, which were delivered only during taste stimulation but not during the dH2O prerinse, but we have previously shown that applying HFES during dH2O prerinse did not change baseline activity (Cho et al. 2002b, 2003; Li et al. 2002, 2005; Smith et al. 2005b). One limitation of the present study is that we could not test other sensory modalities. Without verifying the influence of NAcSh activation on thermoreponsive and/or mechanoresponsive cells in the NST, our results cannot speak to the specificity of the NAcSh effect on taste response.

The response of the gustatory NST neurons to NAcSh stimulation was opposite from the response of the gustatory PbN neurons to NAcSh stimulation (Li et al. 2012), making it difficult to interpret the reverse effect of NAcSh activation on pontine and medullary taste neurons. In the previous study, gustatory neurons in the PbN were more heavily influenced by NAcSh (88 of 98 cells, 90%) and the descending influence exclusively inhabited spontaneous and taste-elicted firings (Li et al. 2012). In the previous study, we compared gustatory response with HFES, or with single-pulse stimulation while we delivered taste solution to the anterior tongue if the PbN neurons tested were spontaneously inactive. In addition, 12 pontine taste neurons from 9 animals were isolated and recorded after we assessed gustatory neurons in the NST in our pilot experiment of this project. Indeed, NAcSh stimulation suppressed neuronal activity of taste neurons in the PbN (result not shown), while activation of the NAcSh with the same electrodes excited medullary taste neurons. The connotation of the opposite effect of NAcSh activation on NST and PbN gustatory neurons remains to be elucidated. Considering that the NST is the gateway for gustatory information entering the brain from the peripheral apparatus, the conceivable priority for the first central taste relay is to detect gustatory information coming from the oral cavity. Some information from the periphery might not be as strong, making it difficult to detect. Augmentation of taste information in the NST via descending projection from the NAcSh can amplify taste information, and therefore it may play a role in detecting relatively weak incoming information. In contrast, the PbN plays a role in the integrative process for gustatory and visceral input (Baird et al. 2001; Hajnal et al. 1999; Lundy and Norgren 2004); it may not be desirable for the purpose of integration and shaping information when neuronal activity is too robust. Reducing the
activity of neurons in the PbN through descending input from the NAcSh may help this process. Further behavioral/electrophysiological investigations are necessary to understand the opposite effect of the NAcSh on the NST and PbN. For example, an experiment may be designed to observe whether electrical stimulation of NAcSh can enhance taste detectability in free-moving animals. Thus, while both the NST and PbN relay gustatory information, they may play different roles in taste information processing.

Although the present and previous PbN-NAcSh studies did not elucidate whether the opposite modulatory effect on gustatory responses of cells in the NST and PbN by NAcSh was due to the activation of different cell populations and/or passing fibers within the NAcSh, we consider this to be unlikely. First, we used the same coordinates to locate the NAcSh stimulating sites in both studies. Second, considering the number of animals we used for the two studies, 29 and 31 for the NAcSh-PbN and NAcSh-NST studies, respectively, the stimulating sites might be overlapped substantially. A more parsimonious explanation is that the opposite modulatory effects were due to the presence of different types of neural connections between the NAcSh and the NST and PbN. It is likely that projections from the NAcSh form an inhibitory synapse with pontine gustatory cells, whereas they form an excitatory synaptic contact with medullary taste neurons, which can be composed of interneurons or gustatory neurons. Nonetheless, brain stem gustatory neurons are capable of responding in opposite ways to descending inputs from the NAcSh, similar to the VPMD modulation of gustatory responses in the NST and PbN (Cho et al. 2008; Mao et al. 2008). The significance of altering taste processing in the brain stem is not clear. It was reported that the taste-evoked neuronal activities of NST and/or PbN neurons can be changed by physiological factors such as blood glucose, sodium appetite, or taste aversion (Chang and Scott 1984; Cho et al. 2004; Giza and Scott 1983; Tamura and Norgren 1997). With these results in mind, descending projections from the ventral forebrain on the gustatory brain stem may represent a mechanism by which the brain tailors gustatory processing in accordance with changes in the homeostatic or hedonic value of orosensory stimuli.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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