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Medullary Taste Responses are Modulated by the Bed Nucleus of the Stria Terminalis

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Abstract

Previous studies have shown a modulatory influence of limbic forebrain areas, such as the central nucleus of the amygdala and lateral hypothalamus, on the activity of taste-responsive cells in the nucleus of the solitary tract (NST). The bed nucleus of the stria terminalis (BST), which receives gustatory afferent information, also sends descending axons to the NST. The present studies were designed to investigate the role of the BST in the modulation of NST gustatory activity. Extracellular action potentials were recorded from 101 taste-responsive cells in the NST of urethane-anesthetized hamsters and analyzed for a change in excitability following bilateral electrical stimulation of the BST. The response of NST taste cells to stimulation of the BST was predominately inhibitory. Orthodromic inhibitory responses were observed in 29 of 101 (28.7%) NST taste-responsive cells, with four cells inhibited bilaterally. An increase in excitability was observed in seven of the 101 (6.9%) NST taste cells. Of the 34 cells showing these responses, 25 were modulated by the ipsilateral BST and 15 by the contralateral; four were inhibited bilaterally and two inhibited ipsilaterally and excited contralaterally. The duration of inhibitory responses (mean = 177.9 ms) was significantly longer than that of excitatory responses (35.4 ms). Application of subthreshold electrical stimulation to the BST during taste trials inhibited or excited the taste responses of every BST-responsive NST cell tested with this protocol. NST neurons that were most responsive to sucrose, NaCl, citric acid or quinine hydrochloride were all affected by BST stimulation, although citric acid-best cells were significantly more often modulated and NaCl-best less often modulated than expected by chance. These results combine with excitatory and inhibitory modulation of NST neurons by the insular cortex, lateral hypothalamus and central nucleus of the amygdala to demonstrate extensive centrifugal modulation of brainstem gustatory neurons.

Key words: centrifugal modulation, gustation, solitary nucleus, taste processing

Introduction

The rostral subdivisions of the nucleus of the solitary tract (NST) receive topographically organized input from gustatory and somatosensory afferent fibers of the facial, glossopharyngeal and vagal nerves that terminate in a rostral–caudal sequence (Aström, 1953; Beckstead and Norgren, 1979; Contreras *et al.*, 1982; Hamilton and Norgren, 1984). The chorda tympani (CT) branch of the facial nerve innervates taste receptor cells in the fungiform papillae on the anterior portion of the tongue (Miller and Smith, 1984). Axons of the CT terminate onto second-order neurons in the most rostral portion of the NST (Contreras *et al.*, 1982; Hamilton and Norgren, 1984). In hamsters, 80% of NST cells that respond to taste stimulation of the anterior tongue send their axons to the parabrachial nuclei (PbN) of the pons (Cho *et al.*, 2002a). From the PbN, taste information is carried to the thalamus and insular cortex and to several areas of the ventral forebrain, including the lateral hypothalamus (LH), the central nucleus of the amygdala (CeA), the substantia innominata and the dorsolateral bed nucleus of the stria terminalis (BST) (Norgren, 1976; Lasiter *et al.*, 1982; Halsell, 1992).

The responsiveness of some gustatory neurons in the NST is altered by physiological factors associated with satiety, such as blood insulin and glucose levels (Giza and Scott, 1983,

1987; Giza *et al.*, 1992, 1993) and gastric distension (Glenn and Erickson, 1976), and by taste aversion (Chang and Scott, 1984) or preference (Giza *et al.*, 1997) learning. Although the neural substrates for these effects are not well understood, they likely involve higher-order gustatory nuclei. Indeed, there are centrifugal inputs to both the NST and PbN from several forebrain gustatory areas, including the gustatory neocortex, the LH and the extended amygdala, including both the CeA and the BST (Shipley, 1982; van der Kooy *et al.*, 1984; Moga *et al.*, 1989; Halsell, 1998; Whitehead *et al.*, 2000; De Olmos *et al.*, 2004). Stimulation of the insular cortex (IC) (Di Lorenzo and Monroe, 1995; Smith and Li, 2000), the LH (Cho *et al.*, 2002b, 2003) and the CeA (Cho *et al.*, 2003; Li *et al.*, 2002) modulates taste activity in the NST. Recent studies have shown similar modulation of taste responses in the PbN (Lundy and Norgren, 2001, 2004b; Li *et al.*, 2005). Although a centrifugal projection from the BST to both the PbN and the NST has been shown anatomically in both rats (van der Kooy *et al.*, 1984; Moga *et al.*, 1989; De Olmos *et al.*, 2004) and hamsters (Halsell, 1992; Whitehead *et al.*, 2000), there is no direct evidence for a role of the BST in modulating brainstem gustatory activity.

The purpose of the present study was to determine the descending influence of the BST on the excitability of taste-responsive neurons of the NST and to show how responses to taste stimulation of the anterior tongue are altered by this influence. We recorded both the spontaneous activity and taste-evoked responses of hamster NST cells and stimulated the BST bilaterally to determine the modulatory influence of the BST on NST neuronal activity.

Materials and methods

Animals and surgery

Young adult male hamsters, *Mesocricetus auratus* (124–197 g, $n = 63$) were deeply anesthetized with urethane (1.7 g/kg, i.p.) and additional anesthetic was given as needed during the course of each experiment. The animal was tracheotomized and mounted in a stereotaxic instrument with blunt ear bars and with the incisor bar at the same level as the interaural line. The tissue overlying the parietal bone was removed and a hole was drilled on each side of the skull. A concentric bipolar stimulating electrode, constructed from 26-gauge stainless steel tubing and 140-μm-thick stainless steel wire, was lowered into the lateral BST on each side of the brain (coordinates: 0.8 mm anterior to bregma, 1.65 mm lateral to the midline and 5.2 mm ventral to the brain surface) and secured with dental cement. The electrodes, except for the tip area, were insulated with Epoxylite 6001 (Epoxylite Corp., Irvine, CA). After implanting the stimulating electrodes into the BST, the animal was mounted in a non-traumatic head holder (Erickson, 1966) with the snout angled downward 27° from horizontal to straighten the brainstem and minimize brain movement associated with breathing (Van Buskirk and Smith, 1981). A sagittal skin incision was made along the midline overlying the posterior skull and a portion of the occipital bone just dorsal to the foramen magnum was removed to reveal the cerebellum. The dura covering the cerebellum was excised and the posterior portion of the cerebellum was aspirated to expose the floor of the IVth ventricle for 3–4 mm anterior to the obex, allowing direct access to the dorsal surface of the medulla. Body temperature was monitored and maintained at 37 ± 1°C with an electric heating pad.

Single-unit recording and electrical stimulation

Single-barrel glass micropipettes (tip diameter = 2 μm, resistance = 7–10 MΩ) filled with a 2% (w/v) solution of Chicago Sky Blue dye in 0.5 M sodium acetate were used for the extracellular recording of action potentials from the rostral NST. The mean (± SD) coordinates for the NST recordings were 2.03 ± 0.14 mm anterior to the obex, 1.30 ± 0.11 mm lateral to the midline and between 0.53 and 1.16 mm ventral to the surface of the brainstem. Extracellular potentials were amplified with a bandpass of 16–3000 Hz (NeuroLog, Digitimer Ltd, Hertfordshire, UK), 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 computer configured with a CED Power 1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK) controlled taste stimulus delivery and online data acquisition and analysis.

The taste responsiveness of the NST cells was initially identified by a change in neural activity associated with the application of anodal current pulses (50 μA, 0.5 s, 0.33 Hz) to the anterior tongue (Smith and Bealer, 1975) and confirmed by responses to chemical stimulation of the tongue. The chemical stimuli presented to the anterior tongue were: 32 mM sucrose, 32 mM sodium chloride (NaCl), 3.2 mM citric acid and 32 mM quinine hydrochloride (QHCl). These concentrations evoke approximately equal multiunit taste responses in the hamster NST (Duncan and Smith, 1992). The tastants were delivered by a gravity flow system composed of a 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 10 s of distilled water, followed by 10 s of stimulus and then 10 s of distilled water rinse. The flow rate was 2 ml/s. After each tastant, the tongue was rinsed with distilled water (>50 ml) and individual stimulations were separated by ≥2 min to avoid adaptation effects (Smith and Bealer, 1976).

After each NST cell was characterized for its taste responsiveness, rectangular pulses (0.5 ms, ≤0.1 mA, 0.33 Hz) were delivered to the BST through each bipolar electrode from an isolated stimulator (Grass S88, Grass Instruments, Quincy, MA) to examine the effect of electrical stimulation of the

forebrain on ongoing spontaneous activity of the NST cell. Peristimulus time histograms (PSTHs) were created from data acquired on each NST cell in response to 100–200 stimulus pulses delivered to each of the two BST electrodes. Orthodromic responses were differentiated from antidromic based on the criteria for antidromic activation of constant latency, high-frequency following and collision (see Cho *et al.*, 2002a).

To observe the influence of electrical stimulation of the BST on the responses of NST cells to taste stimuli, the responses of a subset of the NST cells to taste stimulation were recorded before and during trains of constant square pulses (100 Hz, 0.2 ms) to the BST on each side. The electrical stimulation started 5 s prior to taste stimulus delivery and lasted for 15 s. To prevent BST-evoked spikes from contributing to the taste responses, the intensity of the BST stimulation was adjusted to 0.9 × the minimum intensity that would orthodromically activate the NST cell using single pulses at 0.33 Hz. That is, train stimulation of the BST in this testing protocol did not produce orthodromic action potentials in any NST cell (see Results).

Histology

At the end of each experiment, the last recoding 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 Blue dye. The stimulation sites in the BST were also marked by passing a 10 μA anodal current through the inner wire of the stimulating electrode for 20–30 s to deposit a spot of iron. The hamster was then given an overdose of urethane and perfused through the heart with 4% formalin containing 3% potassium ferrocyanide and ferricyanide. Brains were removed, fixed, frozen sectioned (40 μm) in the coronal plane and stained with Neutral Red. The recording and stimulating sites were located microscopically and plotted on standard atlas sections (Morin and Wood, 2001).

Data analysis

The responses of each cell to taste stimulation of the tongue were accumulated over three consecutive time periods: (i) 5 s of water rinse just prior to the stimulus; (ii) 10 s of stimulus flow; and (iii) 5 s of water rinse just after the stimulus. The net response was calculated as the mean number of action potentials (impulses/s) during the first 5 s of chemical stimulation minus the number during the 5 s pre-stimulus water rinse (Vogt and Smith, 1993). Responses are reported as means ± SEM. For orthodromic responses of NST cells to electrical stimulation of the BST, individual PSTHs were analyzed to determine excitatory or inhibitory epochs. A baseline period was defined as the 200 ms preceding stimulation; the mean and SD of the number of spikes/1 ms bin in this baseline period were determined. An excitatory effect of BST 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. Inhibitory responses were defined as at least 20 consecutive bins with a mean of <50% of baseline firing rate. Low rates of spontaneous activity in gustatory neurons make a criterion for inhibition based on variability difficult to achieve; the criterion applied here calls for a large and sustained decrease in activity. Each taste-responsive cell for which an excitatory or inhibitory effect could be defined was categorized as BST-responsive.

Differences in mean firing rates between BST-responsive and non-responsive neurons and among taste stimuli were compared using analysis of variance. The effect of electrical stimulation on the mean firing rate to taste stimuli and the differences in latency and duration of the effects of ipsi- and contralateral BST stimulation were compared using *t*-tests. The numbers of BST-responsive neurons following ipsilateral or contralateral BST stimulation were compared with the chi-square test.

Results

Histology

The recording and stimulating sites were examined histologically and representative examples are shown in Figure 1. A recording site in the NST is shown in Figure 1A, located medial to the solitary tract, most likely in the rostral central subdivision. Cells were recorded from the NST near 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 (Whitehead and Frank, 1983; Whitehead, 1988). We could not unambiguously assign each recorded cell to a nuclear subdivision within the NST, although all of the recorded cells appeared to be in the region of the NST corresponding to the rostral central or rostral lateral subdivisions (Whitehead, 1988). Although only the ipsilateral side of the brain is depicted here, damage from the tip of the stimulating electrode can be seen in the posterior lateral BST (BSTPL; Figure 1B), just medial to the internal capsule (ic); this is the portion of the BST that receives afferent input from the gustatory region of the PbN (Norgren, 1976).

Data were collected from 63 hamsters and the locations of the stimulating electrodes in these animals were reconstructed on standard atlas sections of the hamster brain (Morin and Wood, 2001), shown in Figure 2. The areas encompassing the stimulating sites in the BST are shown schematically in two sections through the hamster forebrain, ranging from 0.5 (Figure 2B) to 0.8 (Figure 2A) mm anterior to bregma. Individual electrode placements are depicted by symbols in the enlarged schematics of the central core of each section. Open circles show the placements that produced inhibitory responses in gustatory cells of the NST, open

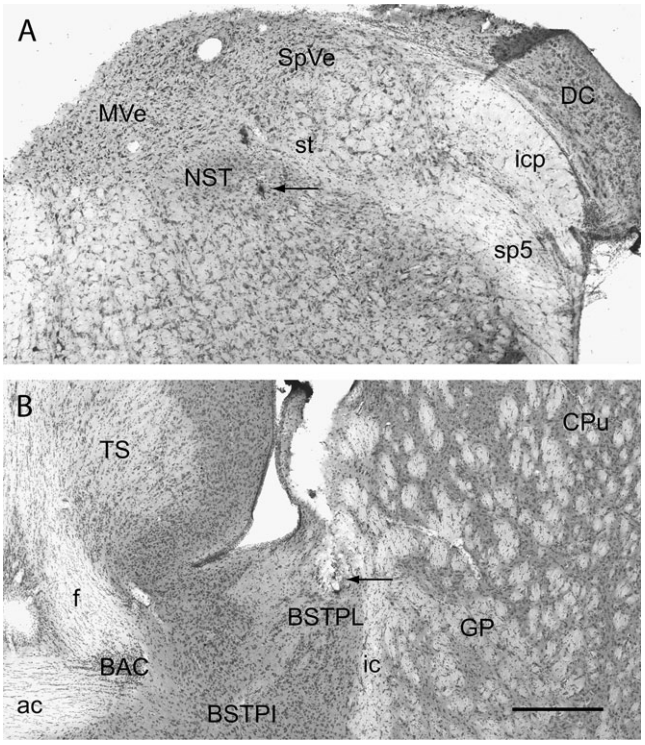

Figure 1 Photomicrographs of stimulating and recording sites in the hamster brain. **(A)** Coronal neutral-red stained section through the medulla, showing a recording site in the NST, marked with Chicago Blue dye (arrow). **(B)** Coronal section through the ventral forebrain of the same hamster, stained with neutral red, showing the position of the ipsilateral stimulating electrode (arrow). Iron deposits and tissue damage indicate a placement within the BST, specifically within the BSTPL. Abbreviations: ac, anterior commissure; BAC, bed nucleus of the anterior commissure; BSTPI, BSTPL, bed nucleus of the stria terminalis, posterointermediate and posterolateral; CPu, caudate putamen; DC, dorsal cochlear nucleus; f, fornix; ic, internal capsule; icp, inferior cerebellar peduncle; GP, globus pallidus; MVe, medial vestibular nucleus; NST, nucleus of the solitary tract; sp5, spinal trigeminal tract; SpVe, spinal vestibular nucleus; st, solitary tract; TS, triangular septal nucleus. Calibration bar = 500 μm in both (A) and (B).

triangles indicate the few sites that induced excitatory responses and filled circles show electrode placements within the lateral BST that did not alter NST activity. In six hamsters, in which the stimulating electrodes were outside the lateral BST (open squares, Figure 2), there was no effect of electrical stimulation; these animals were not included in the analysis. The tips of the stimulating electrodes in the 63 hamsters included in the study were confined to the BST, specifically its anterior and posterior lateral subdivisions (BSTAL, BSTPL; Figure 2). The stimulating sites were distributed from the level of the crossing of the anterior commissure (ac) rostrally (Figure 2A) to the level containing the bed nucleus of the anterior commissure (BAC) caudally Figure 2B, see also Figure 1B). The location of the last NST cell recorded in each animal (n = 63) was marked with Chicago blue dye (as in Figure 1B); the positions of these cells are not shown, but all were within the rostral NST.

Taste response characteristics of NST cells

Each of the 101 NST neurons was tested for its response to the four gustatory stimuli. The responses of one cell are shown in Figure 3, which depicts the extracellularly recorded action potentials occurring 10 s prior to the stimulus (the onset of which is indicated by the first stimulus artifact), the activity of the cell during 10 s of stimulus flow and 10 s of activity following stimulus termination (indicated by the second artifact). This cell had a relatively slow rate of spontaneous discharge (~ 0.5 imp/s), responded to 32 mM NaCl and 32 mM QHCl, and was most responsive to 3.2 mM citric acid. The Spike2 program was used to filter background activity and stimulus artifacts; an example of the filtered response to citric acid is shown. These filtered responses were used to construct a peri-stimulus time histogram (PSTH) for each stimulus presentation, as shown at the bottom of Figure 3 for citric acid. The waveform of the action potential of this cell is also depicted in Figure 3.

The overall spontaneous firing rate of 101 taste-responsive NST cells varied between 0 and 18.6 imp/s and the mean firing rate was 1.79 ± 0.24 imp/s. The effects of BST stimulation on NST neuronal activity are delineated below, but here we describe the gustatory responsiveness of the cells that were and were not responsive to BST stimulation. There was no significant difference in spontaneous firing rate between cells that received modulatory input from the BST (range = 0–4.0 imp/s, mean = 1.31 ± 0.19 imp/s) and the cells that were not modulated by the BST (range = 0–18.6 imp/s, mean = 2.04 ± 0.34 imp/s; t = 1.456, df = 99, P = 0.149). Each of the 101 NST cells was tested for its responsiveness to the four basic taste stimuli and categorized as sucrose-, NaCl-, citric acid- or QHCl-best on the basis of its response profile. Of the 101 cells, 29 were sucrose-best, 36 were NaCl-best, 16 were citric acid-best and 20 were QHCl-best cells. These best-stimulus categories are indicated in Figure 4, where the responses of the cells in each best-stimulus group are arranged along the abscissa in order of their response to the best stimulus for that group. The cells in Figure 4 are further divided into BST-responsive (Figure 4A) and non-responsive groups (Figure 4B), based on the criteria for excitation and inhibition delineated above. Among the 34 NST cells that were modulated by electrical stimulation of the BST, 13 were sucrose-best, 6 were NaCl-best, 11 were citric acid-best and 4 were QHCl-best (Figure 4A). There were 16 sucrose-best, 30 NaCl-best, 5 citric acid-best and 16 QHCl-best neurons among the 67 neurons that were not responsive to BST stimulation (Figure 4B). A comparison of the numbers of cells in each best-stimulus group between the BST-responsive and non-responsive categories demonstrated that these proportions were significantly different (χ^2= 16.768, df = 7, P < 0.02). Individual tests showed that there were significantly more citric-acid-best cells in the BST-responsive group (χ^2= 8.805, df = 1, P < 0.003) and significantly more NaCl-best neurons in the non-responsive

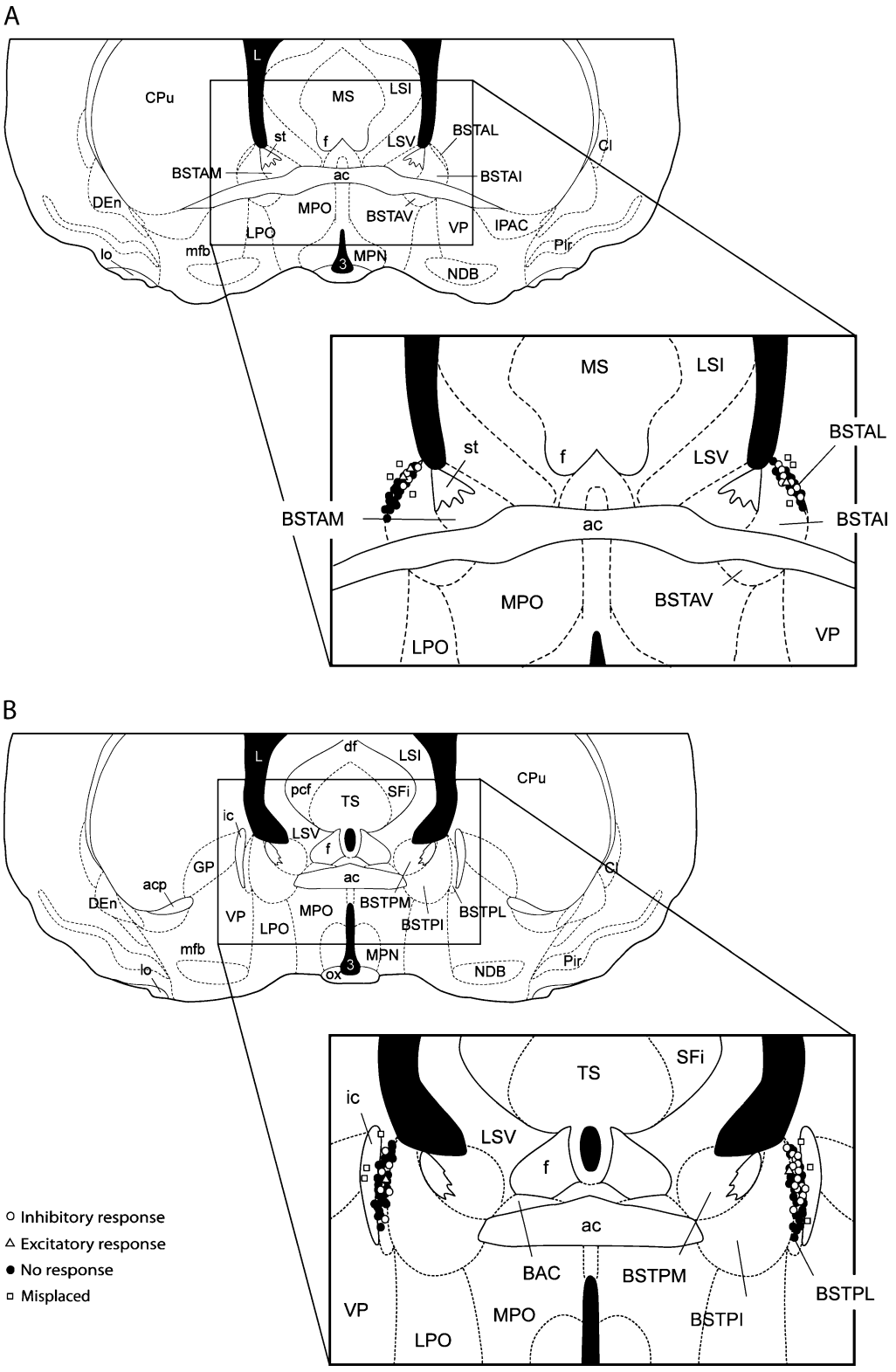

Figure 2 Standard atlas sections of the hamster brain (adapted from Morin and Wood, 2001), showing the distributions of stimulating sites for the 63 experimental animals. **(A)** Section through the ventral forebrain at the level of the crossing of the anterior commissure (ac), showing the distribution of stimulating sites in the anterolateral BST (BSTAL) bilaterally. Sites producing inhibition, excitation, or no effect on the NST are indicated by different symbols. Sites placed outside the lateral BST are shown by open squares; these animals were not included in the analyses. The side contralateral to the recording electrode is on the left. **(B)** A more caudal section through the ventral forebrain at the level of the bed nucleus of the anterior commissure (BAC), showing the distribution of electrode placements within the posterolateral BST (BSTPL). Abbreviations: 3, 3rd ventricle; ac, anterior commissure; acp, anterior commissure, posterior; BAC,

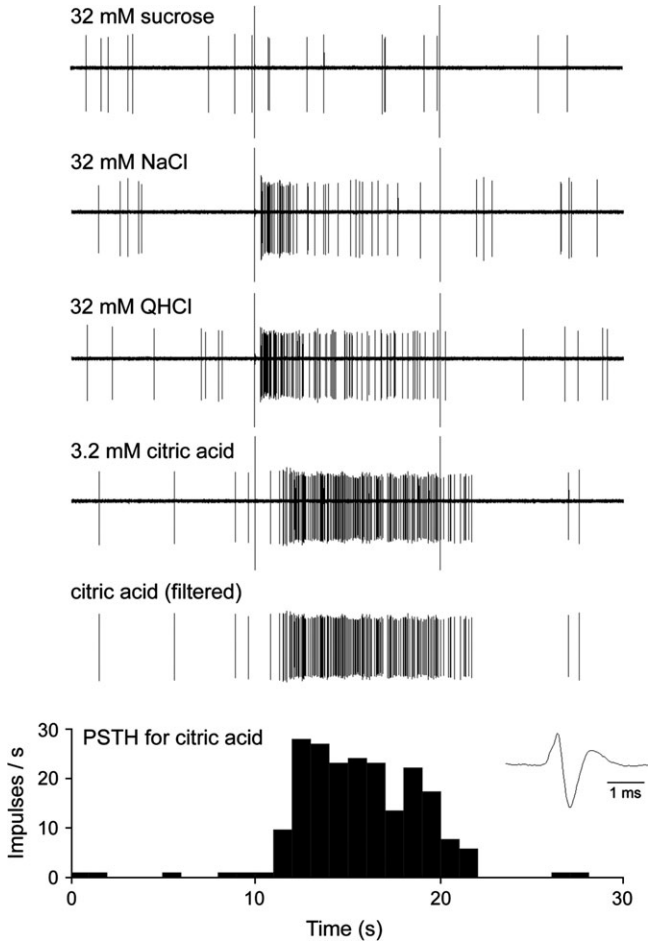

Figure 3 Action potentials recorded from a single NST neuron in response to taste stimulation of the anterior tongue. The first four traces show unfiltered responses to sucrose, NaCl, QHCl and citric acid; records show 30 s of activity. Artifacts before and after each evoked response indicate opening of the solenoids controlling the flow of the stimulus and the subsequent rinse, respectively. This cell responded best to citric acid and the response to citric acid is replotted with the background activity and stimulus artifacts filtered out. Below the last trace, the waveform of the action potential is shown. The PSTH showing the impulse frequency in response to citric acid, derived from the last trace, is shown at the bottom of the figure. Taste responses to all four stimuli were obtained from 101 neurons in the NST.

group of neurons ($\chi^2 = 4.672$, df = 1, $P < 0.05$) than would be expected by chance.

There were no differences between the BST-responsive and non-responsive groups of cells in their sensitivity to gustatory stimulation. The mean responses to each of the four taste stimuli and the mean spontaneous activity of the BST-responsive and non-responsive neurons are shown in Figure 5. The mean net taste response of NST cells that were modulated by BST stimulation was 5.42 ± 0.71 imp/s, whereas the mean response of those cells that did not respond to BST stimulation was 5.68 ± 0.50 imp/s; this difference was not statistically significant [$F(1,396) = 0.090$, $P = 0.647$]. That is, BST-responsive neurons and those that were unaffected by BST stimulation responded equivalently to gustatory stimulation. As may be seen in Figure 5, responses to the four stimuli were relatively similar to one another in both BST- and non-responsive neurons. The mean responses to each of the four taste stimuli did not differ significantly from one another [$F(3,396) = 0.851$, $P = 0.467$], nor was there any interaction between the response to taste stimuli and responsiveness to the BST [$F(3,428) = 0.279$, $P = 0.841$].

BST modulation of NST taste cells

Single-pulse stimulation of the BST resulted in orthodromically activated action potentials or inhibition of ongoing spontaneous activity in about one-third of the cells of the NST. Several examples of BST-evoked changes in activity are provided in Figure 6, in which accumulated PSTHs for six different NST neurons are shown. The PSTHs of the three cells on the left (Figure 6A) were accumulated following stimulation of the ipsilateral BST, whereas those on the right (Figure 6B) were acquired following stimulation of the contralateral BST. In each instance, 200 ms of activity prior to the 0.5 ms stimulus pulse (which occurred at $t = 0$) and 800 ms of post-stimulus activity are shown. The most common effect of BST stimulation was inhibition of the activity of NST neurons. Four different examples of inhibition are shown, two following ipsilateral BST stimulation (BST 6-2 and BST 8-2) and two after contralateral stimulation (BST 27-2 and BST 6-3). The period of reduced activity varied from 41 to 356 ms. Excitatory responses were observed less often and were briefer (20–73 ms) than inhibitory responses. Two examples of BST-evoked excitation are shown in Figure 6, one following ipsilateral BST stimulation (BST 91-2) and one after contralateral stimulation (BST 7-2).

Electrical stimulation of the BST orthodromically modulated 34 of 101 (33.7%) taste-responsive NST cells. None of the 101 cells tested were antidromically invaded from the BST. The responses of NST cells to electrical stimulation of the BST were predominantly inhibitory; 23 of the 34 cells were inhibited by ipsilateral BST stimulation and 10 were inhibited by the contralateral BST; four were bilaterally inhibited. Seven of these 34 cells were excited, two by

bed nucleus of the anterior commissure; BSTAI, BSTAL, BSTAM, bed nucleus of the stria terminalis, anterointermediate, anterolateral and anteromedial; BSTPI, BSTPL, BSTPM, bed nucleus of the stria terminalis, posterointermediate, posterolateral and posteromedial; Cl, claustrum; CPu, caudate putamen; Den, dorsal endopiriform nucleus; df, dorsal fornix; f, fornix; GP, globus pallidus; ic, internal capsule; IPAC, interstitial nucleus of the posterior limb of the anterior commissure; L, lateral ventricle; lo, lateral olfactory tract; LPO, lateral preoptic area; LSI, LSV, lateral septal nucleus, intermediate and ventral; mfb, medial forebrain bundle; MPN, medial preoptic nucleus; MPO, medial preoptic area; MS, medial septal nucleus; NDB, nucleus of the diagonal band of Broca; Pir, piriform cortex; st, stria terminalis; TS, triangular septal nucleus; VP, ventral pallidum.

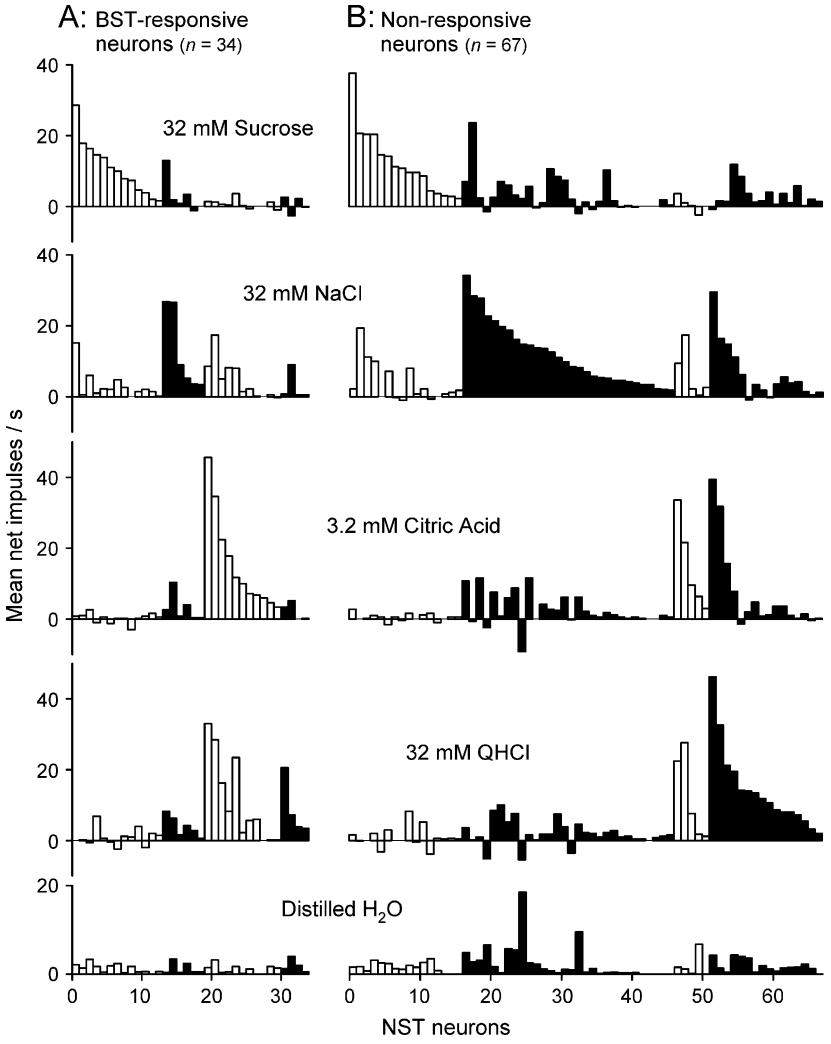

Figure 4 Net responses (mean impulses/s) to gustatory stimulation in 101 hamster NST neurons. Responses of the 34 neurons that were modified by stimulation of the BST are shown in **(A)**, whereas the responses of the 67 neurons not modulated by the BST are depicted in **(B)**. In both groups, neurons are arranged along the abscissa according to their best stimulus. For BST-responsive neurons, cells 1–13 are sucrose-best, 14–19 are NaCl-best, 20–30 are citric-acid-best and 31–34 are QHCl-best. For the non-responsive cells, neurons 1–16 are sucrose-best, 17–46 are NaCl-best, 47–51 are citric-acid-best and 52–67 are QHCl-best. The numbers of neurons in the best-stimulus groups differed significantly between BST-responsive and non-responsive neurons (see text).

ipsilateral and five by contralateral BST stimulation; two of the contralaterally excited cells were inhibited ipsilaterally. There was a significantly greater proportion of NST cells inhibited by the BST than excited (χ^2 = 16.9, df = 1, P < 0.0001). Among those cells inhibited, there were significantly more influenced by the ipsilateral than the contralateral BST (χ^2 = 5.12, df = 1, P < 0.05). Overall, counting cells that were modulated bilaterally, 23 cells were inhibited and two excited by ipsilateral BST stimulation and 10 were inhibited and five excited by contralateral BST stimulation. A summary of these effects is shown in Table 1.

The orthodromic latencies and response durations were determined for each excitatory and inhibitory response to BST stimulation. The response latencies of the 7 cells excited by the BST varied from 41 to 73 ms; mean = 60.0 ± 13.0 ms for ipsilateral stimulation and 59.2 ± 6.3 ms for contralateral stimulation. The difference in excitatory latency between ipsilateral and contralateral BST stimulation was not statistically significant (t = 0.06, df = 5, P = 0.951). Latencies of the inhibitory responses (range = 23–139 ms) were also not significantly different between ipsilateral (58.1 ± 4.3 ms) and contralateral stimulation (71.1 ± 9.9 ms; t = 1.417, df = 31, P = 0.166).

Excitatory responses to single-pulse stimulation of the BST were of briefer duration than inhibitory responses. There was no significant difference between ipsilateral and contralateral stimulation in the duration of either excitatory (23.5 ± 3.5 versus 40.2 ± 6.7 ms; t = 1.477, df = 5, P = 0.199) or inhibitory responses (181.3 ± 20.4 versus 170.1 ± 31.3 ms; t = 0.30, df = 31, P = 0.766). However, the mean duration of inhibitory responses (177.9 ± 16.8 ms) was significantly longer than excitatory (35.4 ± 16.8 ms; t = 3.852, df = 38, P < 0.001).

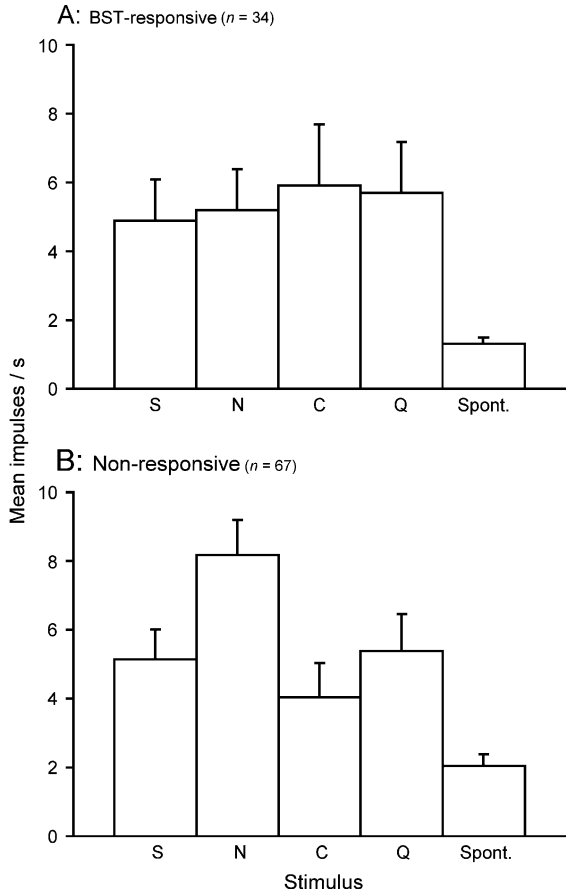

Figure 5 Mean net responses (+ SEM) to each of the four taste stimuli and mean spontaneous rate of the 34 BST-responsive neurons and the 67 non-responsive NST cells. Cells that were modulated by the BST were similar in their responses to all stimuli and similar to those that were unaffected by BST stimulation.

Effects of electrical stimulation of the BST on taste-evoked responses

In a subset of taste-responsive NST neurons, we examined the influence of electrical stimulation of the BST on the responses to taste stimulation of the anterior tongue. This experiment was conducted on a subset (n = 7) of the 34 NST cells that were inhibited by single-pulse (i.e. 0.33 Hz) stimulation of the BST. After confirming a cell's response to single-pulse stimulation, a train of rectangular pulses (100 Hz, 0.2 ms duration) was delivered to the BST during taste stimulation trials. The current delivered to the BST was adjusted to 0.9 × the minimum current intensity necessary to elicit a change in the activity of the NST cell at 0.33 Hz. These electrical stimulus trains did not produce any effect on the spontaneous activity of the NST cells, as evidenced by the failure to see any difference in firing rate during the pre-stimulus water rinse in control versus train stimulation conditions (see Figure 7C); the electrical stimulation was present during the 5 s of pre-stimulus water flow.

Responses of these seven NST cells to taste stimuli presented to the anterior tongue before and during train stimulation of the BST were compared. Five of the tested cells were sucrose-best, one was NaCl-best and one was citric acid-best. Among these seven cells, three were inhibited by ipsilateral and three by contralateral BST stimulation and one was inhibited bilaterally. The effect of electrical stimulation on one of these cells is shown in Figure 7A. The responses of this cell to chemical stimulation of the anterior tongue show that electrical train stimulation (ES) of the BST decreased the responses to taste stimulation (both sucrose and NaCl). In this figure, the cell's activity (imp/s) during each taste stimulus is shown in 1 s bins for the 5 s pre-stimulus period (open bars), the 10 s stimulus period (solid bars) and the first 5 s of the post-stimulus rinse (open bars). This cell was responsive to sucrose and NaCl, but not citric acid or QHCl. When the cell was tested with sucrose (Figure 7A; sucrose + ES) and with NaCl (NaCl + ES) during train stimulation, its response to taste stimulation was dramatically reduced, although there was no effect on the response during the pre-stimulus rinse (open bars prior to taste response), during which time the train stimulation was also present. In Figure 7B are taste responses of another cell, tested during train stimulation with the two most effective taste stimuli (NaCl and citric acid), showing decreased responses to these stimuli during BST stimulation.

The responses of these seven NST cells to taste stimulation before and during train stimulation of the BST are summarized in Figure 7C. The mean net response of these cells to 16 taste trials without electrical stimulation of the BST was 12.53 ± 2.13 imp/s. Application of electrical stimulation to the BST while repeating the same taste trials significantly reduced the net response to the taste stimuli (3.54 ± 0.47 imp/s; paired t = 5.051, df = 15, $P < 0.001$), whereas the baseline activity (i.e. during the pre-stimulus water rinse) of the cells was not significantly different before (1.99 ± 0.42 imp/s) and during (1.96 ± 0.27 imp/s) BST stimulation (paired t = 0.090, df = 15, P = 0.929). In every instance, and in each cell type (sucrose, NaCl-, or citric acid-best), the effects of train stimulation of the BST on taste responses mimicked the effect of single-pulse stimulation on spontaneous activity, i.e. it produced a decrement in stimulus-evoked taste activity.

Discussion

Connections between the BST and the NST

The present investigation has demonstrated for the first time that a subset of taste-responsive NST neurons is subject to a modulatory influence from the lateral BST and that this descending projection affects the responsiveness of these neurons to taste stimulation. One-third of the neurons in the hamster NST that responded to stimulation of the anterior tongue were centrifugally modulated and the vast majority of these were inhibited by BST stimulation.

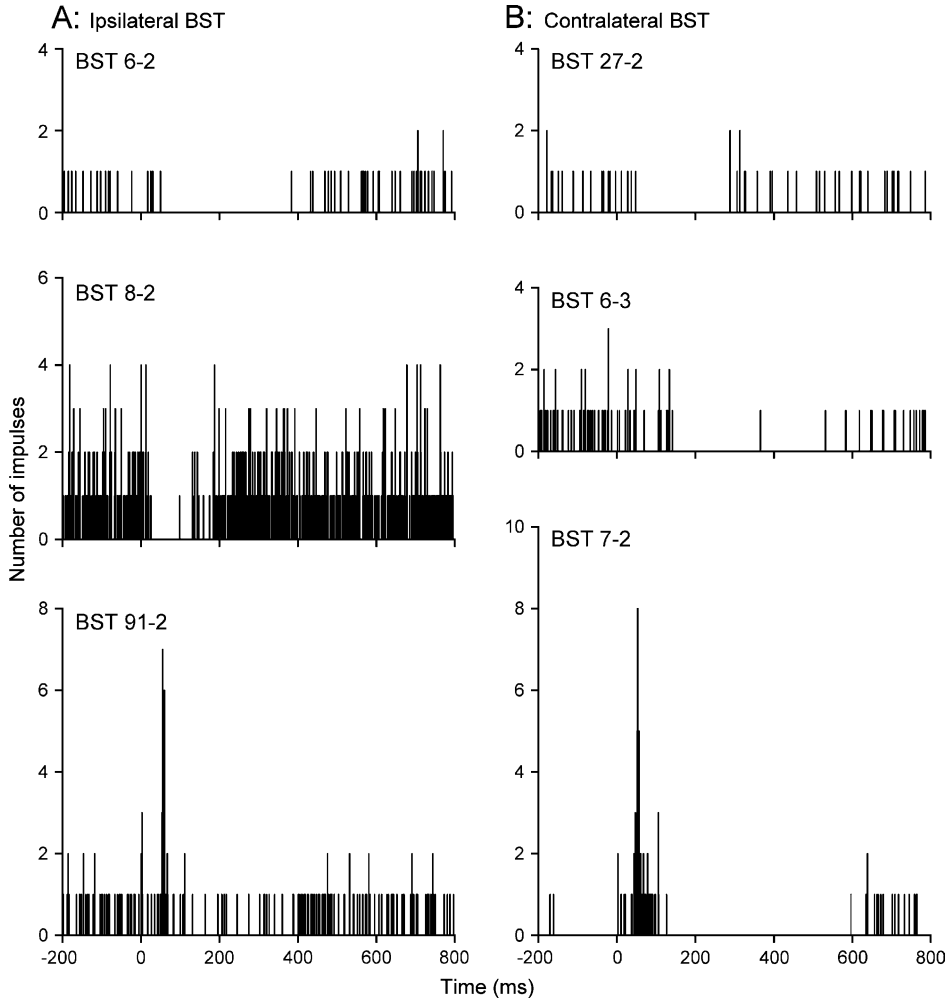

Figure 6 Orthodromic responses of NST neurons to single-pulse electrical stimulation of the BST. **(A)** Peri-stimulus time histogram (PSTH) of the action potentials of three single NST neurons in response to ipsilateral BST stimulation. Two of these cells (BST 6-2 and BST 8-2) were inhibited by the BST and one was excited (BST 91-2). **(B)** PSTHs of three NST neurons modulated by contralateral BST stimulation. Two of these cells (BST 27-2 and BST 6-3) were inhibited and one (BST 7-2) was excited by stimulation of the BST. Each histogram was accumulated over 200 stimulus sweeps.

Neuroanatomical studies in both rats and hamsters have shown that the dorsolateral BST receives afferent projections from taste-responsive areas of the PbN (Norgren, 1976; Halsell, 1992), and that the PbN (Moga *et al.*, 1990; Halsell, 1992) and NST (van der Kooy *et al.*, 1984; Whitehead *et al.*, 2000) both receive descending projections from neurons in the BST.

Neuroanatomical studies have shown that direct descending projections from the BST to the NST are predominantly ipsilateral, although some contralateral distribution of fiber terminals was seen in the NST (van der Kooy *et al.*, 1984; Whitehead *et al.*, 2000). The present data show a bilateral influence on NST neurons from the BST, although these effects were most often ipsilateral. In all, 15 NST cells were activated by contralateral BST stimulation and 25 responded to stimulation of the ipsilateral BST (see Table 1). The mean latencies of orthodromic excitation were no different after contralateral than following ipsilateral BST stimulation. Latencies for orthodromic excitation ranged from 47 to 73 ms, indicative of a multisynaptic pathway for both ipsilateral (mean = 60.0 ms) and contralateral (59.2 ms) influences. Similar long latencies were seen for ipsilateral (58.1 ms) and contralateral (71.1 ms) inhibitory responses to BST stimulation. In contrast, excitatory influences on hamster NST neurons from the LH and CeA are sometimes of very short latency (4–5 ms), suggestive of monosynaptic activation (Cho *et al.*, 2002b, 2003; Li *et al.*, 2002). Longer latencies may involve interneurons within the NST, projections from the opposite NST (Whitehead *et al.*, 2000) or descending connections from the BST through the PbN (Moga *et al.*, 1989; Halsell, 1992), which in turn sends some descending axons to the NST (Bianchi *et al.*, 1998; Karimnamazi and Travers, 1998). Indeed, we have observed a few cells in the hamster NST that exhibit orthodromic impulses following ipsilateral

Table 1 Distribution of BST-responsive and non-responsive NST taste cells

Best stimulus	BST-responsive cells				Non-responsive cells	BST response ratio (%)[c]
	Ipsilateral	Contralateral	Bilateral[b]	Total		
Sucrose	8 (1)[a]	3 (2)	1	13	16	13/29 (44.8%)
NaCl	5	3 (1)	2 (1)	6	30	6/36 (16.7%)
Citric acid	7	3 (2)	(1)	11	5	11/16 (68.8%)
QHCl	3 (1)	1	1	4	16	4/20 (20.0%)
Total	23 (2)	10 (5)	4 (2)	34	67	34/101 (33.7%)

[a]Excited cells are shown in parentheses.
[b]Bilateral cells were counted in the ipsilateral and contralateral columns.
[c]The proportions of cells in different best-stimulus categories were significantly different between BST-responsive and non-responsive groups ($\chi^2 = 16.77$, df = 7, $P < 0.02$).

PbN stimulation (Cho *et al.*, 2002a). Descending connections may also flow from the BST through the medullary reticular formation into the NST (van der Kooy *et al.*, 1984; Travers, 1988; Whitehead *et al.*, 2000). Such indirect connections would be reflected in the longer latencies (47–73 ms) observed in these cells in response to BST stimulation.

Taste response modulation by the BST

Electrical stimulation of the BST orthodromically influenced one-third of the cells in the NST that responded to taste stimulation of the anterior tongue; most of these were inhibited. When a subset of the cells inhibited by single-pulse stimulation of the BST was tested with gustatory stimuli during subthreshold electrical train stimulation of the BST, the taste responses were significantly decreased (see Figure 7C). Similar to what we observed following stimulation of the LH (Cho *et al.*, 2002b) and CeA (Cho *et al.*, 2003; Li *et al.*, 2002), there did not appear to be any selectivity in the modulation of taste responsiveness. Rather, in any given cell that was inhibited by the BST, responses to all stimuli tested after stimulation of the BST were decreased. BST stimulation modulated the response of all cell types (sucrose-best, QHCl-best, etc.), although there were significantly fewer NaCl-best and significantly more citric acid-best cells modulated by the BST than expected by chance. In considering the relative lack of taste specificity of BST stimulation, it is important to remember that electrical train stimulation in no way mimics the natural pattern of activity that undoubtedly determines the effect of the BST or other forebrain areas on brainstem taste activity. This study and others on the LH and CeA (e.g. Cho *et al.*, 2002b, 2003; Li *et al.*, 2002) serve to demonstrate an excitatory or inhibitory influence on the NST, but cannot replicate the specific pattern of that influence. Because the taste response of every BST-responsive cell tested with taste stimulation was modulated by the BST, it is highly likely that all of the cells responsive to single-pulse electrical stimulation (Table 1) would show a similar enhancement or suppression of taste activity. These results suggest that descending input from the BST modulates the processing of taste information in about one-third of the taste-responsive cells of the NST, mostly through inhibitory mechanisms.

Even though there is clearly a reciprocal relationship between the NST and the BST, there is little direct evidence that the BST plays a role in taste-mediated behavior. The only such evidence comes from behavioral studies in rats, where lesions of the BST reduce both need-free and sodium-depletion-induced salt appetite (Reilly *et al.*, 1994; Zardetto-Smith *et al.*, 1994). In the present study, the one class of neurons in which significantly fewer cells were modulated by the BST was the NaCl-best group (see Figure 4), although it is not clear how this would relate to alterations in sodium consumption. Various subnuclei in the BST have been implicated in a number of neural systems, including those involved in responses to stress (Herman and Cullinan, 1997; Cecchi *et al.*, 2002) and in motivation, reward and drug addiction (Walker *et al.*, 2000; Georges and Aston-Jones, 2002; Macey *et al.*, 2003). For example, cells in the ventral lateral BST activate dopaminergic neurons in the ventral tegmental area (VTA; Georges and Aston-Jones, 2002); these neurons in turn are a critical component of a circuit involved in reward and drug abuse (Koob and Le Moal, 2001). Orally administered sucrose produces a dose-dependent release of dopamine from the nucleus accumbens (NAcc), the target of VTA dopaminergic neurons (Hajnal *et al.*, 2004). The NAcc has reciprocal connections with the lateral BST, as does the central nucleus of the amygdala (CeA; De Olmos *et al.*, 2004), which is itself responsive to gustatory input (Nishijo *et al.*, 1998). These various connections within the BST and between the BST and brainstem gustatory nuclei and other limbic forebrain areas such as the CeA provide a potential substrate for the relationship between environmental and/or physiological stress, taste-mediated reward mechanisms and excess consumption of sweets or other palatable foods, including alcohol (see Lemon *et al.*, 2004).

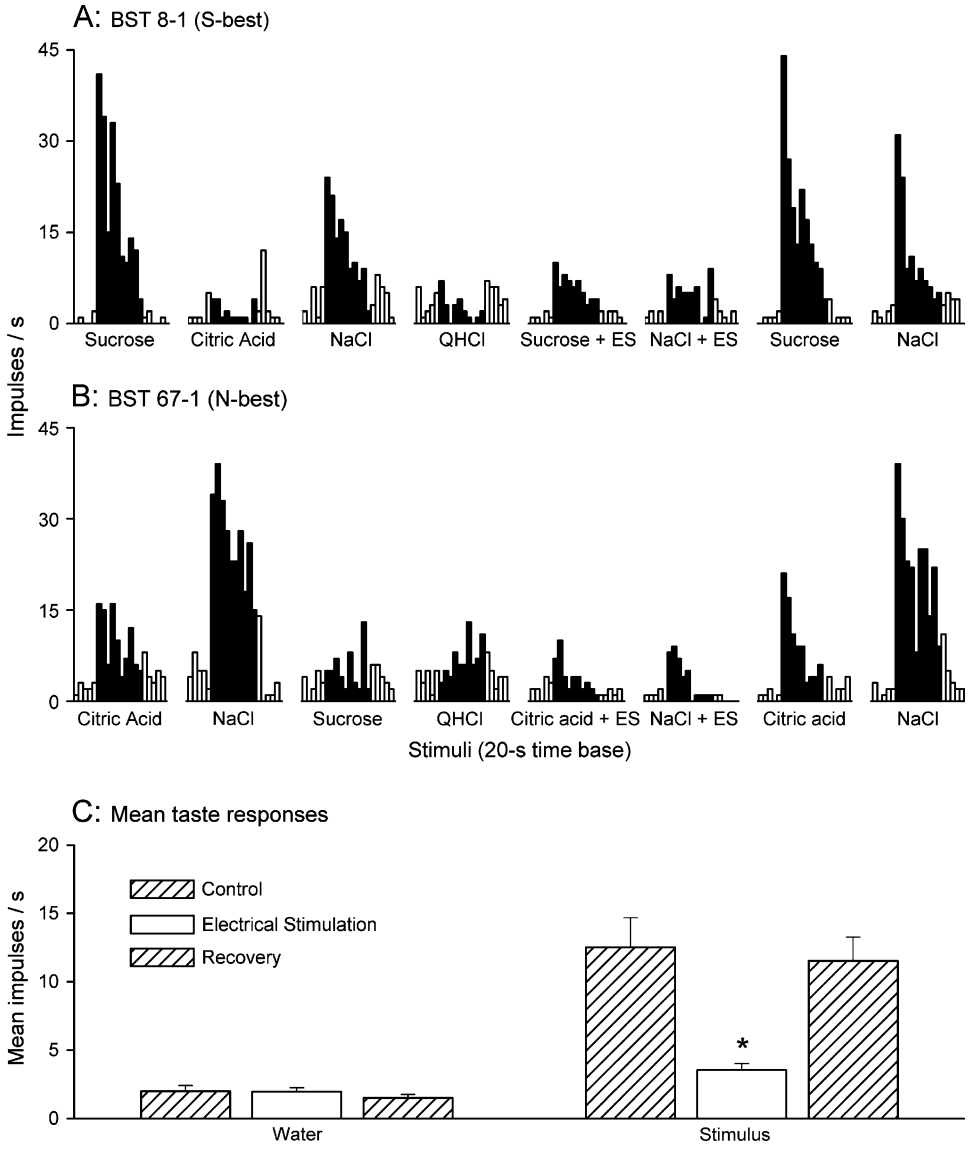

Figure 7 Taste response profiles of NST neurons to taste stimulation before and during subthreshold BST stimulation. **(A)** Responses of a sucrose-best NST neuron. The control response to each of the four basic stimuli (sucrose, citric acid, NaCl and QHCl) are shown first. The responses (impules/s) during the 10 s stimulus are shown as black bars and 5 s pre-stimulus and post-stimulus water rinse periods are shown as open bars; each histogram reflects 20 s of activity. After the four taste stimuli are the responses to the two most effective stimuli in the presence of electrical stimulus (ES) trains delivered to the BST. The responses to both sucrose and NaCl were decreased by BST stimulation; this cell's spontaneous activity was inhibited by the single-pulse stimulus protocol. **(B)** Control responses and responses to the two most effective taste stimuli during ES for another BST-inhibited NST neuron (NaCl-best). For this cell also, subthreshold train stimulation of the BST markedly decreased the response to taste stimulation, but had no effect on the firing rate during the water pre-rinse. **(C)** Mean (+ SEM) firing rates to all taste stimuli tested before (control), during (electrical stimulation) and after (recovery) electrical train stimulation of the BST. Activity during the 5 s pre-rinse period (shaded bars) was unaffected by BST stimulation, whereas the response to taste stimuli (open bars) was significantly (*) decreased; responses returned following BST stimulation (recovery; shaded).

Forebrain modulation of brainstem gustatory activity

Ascending projections and known descending modulatory influences on the rodent gustatory system are depicted schematically in Figure 8. Ascending pathways are shown on the left and descending on the right; contralateral projections are not indicated, but occur at all levels beyond the PbN. Gustatory afferent input to the NST is projected to the PbN, where multiple pathways carry taste information to the ventral posterior medial nucleus of the thalamus (VPMpc), the LH, the CeA and the BST (for a recent review, see Lundy and Norgren, 2004a,). Thalamic neurons, in turn, project to the dysgranular and agranular insular cortex (IC). In addition to these major pathways, there are interconnections (not shown) between some of these areas, such as the between the IC and

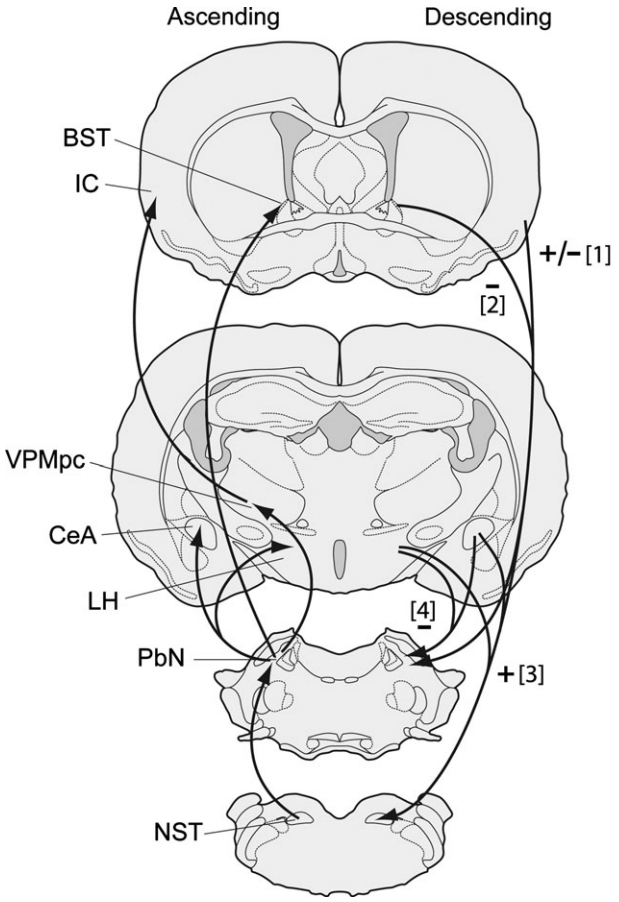

Figure 8 Schematic diagram showing known forebrain influences on gustatory cells of the NST. Ascending pathways from the NST are shown on the left; only ipsilateral connections are shown. On the right are depicted the descending pathways for which physiological evidence demonstrates a modulatory effect on NST or PbN neurons; only ipsilateral connections are shown. Numbers refer to the following studies: [1] (Di Lorenzo and Monroe, 1992, 1995; Smith and Li, 2000); [2] present experiment; [3] (Cho *et al.*, 2002b, 2003; Li *et al.*, 2002); [4] (Lundy and Norgren, 2001, 2004b; Li *et al.*, 2005). Influences that are predominantly excitatory are indicated with a plus sign (+), whereas those that are predominantly inhibitory are depicted with a minus sign (−). IC has a relatively equal distribution of excitatory and inhibitory effects (+/−).

CeA or the CeA and LH or BST. With the exception of the BST, gustatory-responsive neurons have been recorded from each of these areas, e.g. PbN (Van Buskirk and Smith, 1981; Nishijo and Norgren, 1997), VPMpc (Ogawa and Nomura, 1988; Verhagan *et al.*, 2003), LH (Norgren, 1970; Yamamoto *et al.*, 1989a), CeA (Uwano *et al.*, 1995; Nishijo *et al.*, 1998) and IC (Kosar *et al.*, 1986; Yamamoto *et al.*, 1989b).

In addition to the influence of the BST shown in the present experiment, earlier studies have shown that the IC (Di Lorenzo and Monroe, 1995; Smith and Li, 2000), LH (Bereiter *et al.*, 1980; Matsuo *et al.*, 1984; Murzi *et al.*, 1986; Cho *et al.*, 2002b) and CeA (Li *et al.*, 2002; Cho *et al.*, 2003) each modulates the activity of NST cells, including their responses to gustatory stimuli. There is also evidence that the CeA, LH and gustatory cortex can modulate the activity of taste cells in the PbN (Di Lorenzo and Monroe, 1992; Lundy and Norgren, 2001, 2004b; Tokita *et al.*, 2004; Li *et al.*, 2005). These descending influences are depicted schematically on the right side of Figure 8; only ipsilateral influences are shown, although all are bilaterally effective, sometimes stronger on the contralateral side. Blocking neuronal activity of the IC with procaine results in both increased and decreased responses to taste stimulation in cells of the rat NST (Di Lorenzo and Monroe, 1995), as does decerebration (Mark *et al.*, 1988). Direct electrical or pharmacological activation of IC produces excitatory or inhibitory modulation (+/−) of about one-third of the taste-responsive neurons of the hamster NST (Smith and Li, 2000); cortex-induced inhibition is mediated by $GABA_A$ receptors in the NST. After several early reports that stimulation of the LH modulated activity of cells within the rat NST (Bereiter *et al.*, 1980; Matsuo *et al.*, 1984; Murzi *et al.*, 1986), we demonstrated that about half of the taste-responsive cells of the hamster NST are modulated by electrical or chemical stimulation of the LH (Cho *et al.*, 2002b). Further, about one-third of the neurons in the hamster NST that respond to tastants are modulated by the CeA (Li *et al.*, 2002), and both the LH and the CeA influence many of the same cells (Cho *et al.*, 2003). The effects of the LH and CeA are predominantly excitatory on NST taste neurons. On the other hand, these same areas produce mostly an inhibitory effect on taste-responsive cells of the PbN in both rats (Lundy and Norgren, 2001, 2004b) and hamsters (Li *et al.*, 2005).

Taken together, these data demonstrate that activity in brainstem taste neurons is not simply the result of afferent input. This series of experiments demonstrates extensive centrifugal modulation of brainstem gustatory activity. Each of the forebrain targets of the gustatory system that have so far been examined, including the IC, LH, CeA and BST, plays a descending modulatory role in the processing of taste information. This extensive neural substrate no doubt underlies the modulation of taste activity by physiological and experiential factors, and produces a dynamic flux in the responsiveness of these neurons. Further research should be directed toward determining if and how these pathways are engaged by alterations in blood glucose (Giza and Scott, 1983), gastric distension (Glenn and Erickson, 1976), intraduodenal lipids (Hajnal *et al.*, 1999), conditioned taste aversion learning (Chang and Scott, 1984), sodium appetite (Jacobs *et al.*, 1988) and other physiological conditions known to alter taste sensitivity.

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