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Descending Influences from the Lateral Hypothalamus and Amygdala Converge onto Medullary Taste Neurons

Young K. Cho[1], Cheng-Shu Li[2] and David V. Smith[2]

Department of Anatomy & Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore MD 21201, USA

Present addresses: [1]Department of Behavioral Science, Pennsylvania State University, College of Medicine, Hershey PA 17033 and [2]Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN 38163, USA

Correspondence to be sent to: Dr David V. Smith, Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, 855 Monroe Avenue, Suite 515, Memphis, TN 38163, USA. e-mail: dvsmith@utmem.edu

Abstract

The lateral hypothalamus (LH) and the central nucleus of the amygdala (CeA) exert an influence on many aspects of ingestive behavior. These nuclei receive projections from several areas carrying gustatory and viscerosensory information, and send axons to these nuclei as well, including the nucleus of the solitary tract (NST). Gustatory responses of NST neurons are modulated by stimulation of the LH and the CeA, and by several physiological factors related to ingestive behavior. We investigated the effect of both LH and CeA stimulation on the activity of 215 taste-responsive neurons in the hamster NST. More than half of these neurons (113/215) were modulated by electrical stimulation of the LH and/or CeA; of these, 52 cells were influenced by both areas, often bilaterally. The LH influenced more neurons than the CeA (101 versus 64 cells). Contralateral stimulation of these forebrain areas was more often effective (144 responses) than ipsilateral (74). Modulatory effects were mostly excitatory (102 cells); 11 cells were inhibited, mostly by ipsilateral LH stimulation. A subset of these cells ($n = 25$) was examined for the effects of microinjection of DL-homocysteic acid (DLH), a glutamate receptor agonist, into the LH and/or CeA. The effects of electrical stimulation were completely mimicked by DLH, indicating that cell somata in and around the stimulating sites were responsible for these effects. Other cells ($n = 25$) were tested for the effects of electrical stimulation of the LH and/or CeA on the responses to taste stimulation of the tongue (32 mM sucrose, NaCl and quinine hydrochloride, and 3.2 mM citric acid). Responses to taste stimuli were enhanced by the excitatory influence of the LH and/or CeA. These data demonstrate that descending influences from the LH and CeA reach many of the same cells in the gustatory NST and can modulate their responses to taste stimulation.

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

Introduction

In the central gustatory pathway, there are reciprocal connections between the brainstem and several areas of the forebrain. The lateral hypothalamus (LH) and the central nucleus of the amygdala (CeA) are nuclei in the ventral forebrain that contain taste-responsive neurons (Norgren, 1970; Nishijo *et al.*, 1998). Sensory projections to the LH and CeA convey gustatory, gastrointestinal, and cardiovascular information from the peripheral nervous system via the nucleus of the solitary tract (NST) and the parabrachial nuclei (PbN) in the brainstem (Norgren, 1976; Voshart and van der Kooy, 1981; Ter Horst *et al.*, 1989; Halsell, 1992; Bernard *et al.*, 1993; Bester *et al.*, 1997). The LH and CeA also receive input from the cortex, thalamus and other nuclei in the ventral forebrain and there is reciprocal connectivity between the LH and CeA (Ottersen, 1980; van der Kooy *et al.*, 1984; Allen *et al.*, 1991; Turner and Herkenham, 1991; Nakashima *et al.*, 2000). Both of these forebrain areas send descending axons to the PbN and rostral NST in both rats and hamsters (Hosoya and Matsushita, 1981; Berk and Finkelstein, 1982; van der Kooy *et al.*, 1984; Whitehead *et al.*, 2000).

The LH and CeA have important roles in the regulation of homeostasis, ingestive behavior, reward and motivation (Stellar and Stellar, 1985; Rolls, 1999; Aggleton, 2000). Stimulation of the LH enhances food intake (Coons *et al.*, 1965; Frank *et al.*, 1982; Shiraishi, 1991), whereas lesions of this area elicit opposite effects: from inhibition of feeding to aphagia, depending on the extent of the lesion (Grossman *et*

al., 1978; Grossman and Grossman, 1982; Arase *et al.*, 1987; Bray, 1991). LH stimulation also increases blood glucose levels (Atrens *et al.*, 1984), glucose utilization in the PbN (Roberts, 1980) and basal metabolism (Ruffin *et al.*, 1995). The CeA has been implicated in the regulation of sodium intake (Galaverna *et al.*, 1992, 1993; Seeley *et al.*, 1993), aversive taste (Touzani *et al.*, 1997) and, along with the basolateral amygdala, conditioned taste aversion learning (Yamamoto *et al.*, 1994; Tucci *et al.*, 1998). Although many investigators have studied the functions of these areas in relation to feeding, the underlying neural mechanisms relating activity in these forebrain nuclei to gustatory processing are as yet unknown.

Descending projections from forebrain areas could provide a substrate through which physiological or experiential factors alter taste responsiveness of brainstem neurons. The taste-evoked activity of some NST neurons 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. Indeed, previous electrophysiological studies have demonstrated that electrical stimulation of the LH modulates neuronal activity in the NST (Bereiter *et al.*, 1980; Matsuo *et al.*, 1984; Murzi *et al.*, 1986) and we have recently shown that taste responses in half of the gustatory cells in the hamster NST are modulated by stimulation of the LH (Cho *et al.*, 2002b). Stimulation of the CeA alters taste responses in the rat PbN (Lundy and Norgren, 2001) and in the hamster NST (Li *et al.*, 2002). The present study employed bilaterally implanted electrodes in both the LH and CeA to determine whether the descending influences from these areas converge on the same cells or modulate different NST neuronal populations.

Materials and methods

Animals and surgery

Young adult male Syrian golden hamsters (*Mesocricetus auratus*), weighing between 150 and 250 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 a polyethylene tube was inserted into the trachea to allow the animal to breathe freely. Body temperature was maintained at 37°C (± 1°C) with a heating pad. To place the stimulating electrodes, the animal's head was positioned in a stereotaxic instrument (SR-6N, Narishige Scientific Instruments, Tokyo, Japan) with the incisor bar at the same level as the interaural line. The tissue overlying bregma was cut along the midline and separated. Two holes were drilled on each side of the skull to permit access to the LH and CeA. The coordinates for LH and CeA with the brain at this angle, which were determined histologically in previous experiments (Cho *et al.*, 2002b; Li *et al.*, 2002), were 0.5 mm caudal to bregma, 1.8 mm lateral to the midline, and ~7 mm ventral to the surface of the brain for the LH. Those for the CeA were 0.6 mm posterior to bregma, 3.9 mm lateral to the midline and ~5.5 mm ventral to the brain surface.

Stimulating electrodes were implanted bilaterally and fixed in place with dental cement. Each bipolar stimulating electrode was composed of an insulated 140 μm-diameter stainless steel wire inside 26-gauge stainless steel tubing. The components of this concentric electrode, except for the tip area, were insulated with Epoxylite 6001 (Epoxylite Corp., Irvine, CA). For stimulating the LH or CeA chemically, a double-barrel glass micropipette (tip diameter = 35 μm) was glued to each stimulating electrode with its tip positioned 0.2 mm above the inner wire of the electrode. After fixing the stimulating electrodes in place, the animal was placed in a non-traumatic headholder (Erickson, 1966) with the head angled nose-downward 27° below the horizontal to straighten the brainstem and minimize brain movement associated with breathing (Van Buskirk and Smith, 1981). The tissue over the occipital bone was cut along the midline and separated and the occipital bone and underlying dura were excised. The posterior portion of the cerebellum was aspirated to expose the floor of the fourth ventricle for 3–4 mm anterior to the obex, allowing direct access to the NST.

Extracellular recording and taste stimulation

Action potentials of taste-responsive NST neurons were recorded with glass micropipettes (tip diameter = 2 μm; resistance = 7–10 MΩ) filled with a 2% (w/v) solution of Chicago Blue dye in 0.5 M sodium acetate. The taste responsiveness of NST cells was initially determined by a change in neural activity in response to anodal current pulses (50 μA, 0.5 s, 0.33 Hz) applied to the anterior tongue, which drive electrolyte-sensitive taste fibers of the chorda tympani nerve (Smith and Bealer, 1975; Herness, 1987), and then confirmed with chemical stimulation of the tongue. Extracellular action potentials were differentially amplified (NeuroLog System, Digitimer Ltd, Hertfordshire, UK), discriminated with a dual time-amplitude window discriminator (Bak DDIS-1, Bak Electronics Inc., Germantown, MD), displayed on oscilloscopes and monitored with an audio monitor. Analog and digital signals were acquired with a Pentium computer, configured with a CED 1401 *plus* interface board and Spike2 software (Cambridge Electronic Design, Cambridge UK). The mean coordinates of the 215 taste-responsive cells recorded from the NST were 2.0 ± 0.1 (SD) mm anterior to obex and 1.3 ± 0.1 mm lateral to the midline. These cells were encountered from 0.5 to 1.2 mm below the surface of the brainstem.

Four stimuli representative of the sweet, salty, bitter and sour taste qualities: 32 mM sucrose, NaCl and quinine hydrochloride (QHCl), and 3.2 mM citric acid, respectively, were applied to the anterior tongue in random order for each

recorded neuron. These concentrations evoke roughly equal multi-unit responses in the hamster NST (Duncan and Smith, 1992). The stimuli 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 reservoir. The stimulation sequence, during which the response of the neuron was recorded, was a continuous flow (at 2 ml/s) initiated by the delivery of 5 s of distilled water, followed by 10 s of stimulus, and then by 5 s of distilled water. Following each taste stimulus, the tongue was rinsed with distilled water (>50 ml) and individual stimulations were separated by at least 2 min to avoid adaptation effects (Smith and Bealer, 1976). Each cell was categorized as responding best to sucrose, NaCl, citric acid or QHCl on the basis of its taste response profile.

LH and CeA stimulation and classification of forebrain-responsive neurons

After each NST cell was characterized for its taste responsiveness, 50–200 constant-current square pulses (≤0.1 mA, 0.5 ms), generated from an isolated stimulator (Grass S88, Grass Instrument Co., Quincy MA), were delivered through each stimulating electrode (ipsilateral and contralateral) at a frequency of 0.33 Hz to examine the effect on the ongoing spontaneous activity of the cell. The stimulation intensity used for each cell (range = 50–100 μA) was set to the lowest intensity that would produce a discernible orthodromic response. Peri-stimulus time histograms (PSTHs) were created from the acquired data on each NST cell; action potentials associated with single pulse stimulation were accumulated over a 1 s period for 50–200 sweeps. The 200 ms preceding stimulation were defined as the baseline period. The mean and standard deviation of the firing rate (impulses/1 ms bin) during this 200 ms period were determined. The action potentials occurring during a period of 800 ms after LH or CeA stimulation were analyzed to determine the effects of the stimulation on NST activity. An excitatory response was defined as an epoch of at least five consecutive 1 ms bins with a mean value of 2 SD above the baseline mean, which defines a mean response with a probability of <0.05. The onset latency of the excitatory response was defined as the time at which the firing rate became at least twice the average baseline spontaneous rate. Inhibitory responses were defined as at least 20 consecutive bins in which the mean value was <50% of the baseline firing rate. Low rates of spontaneous activity 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 forebrain-responsive. To specify the effective forebrain site, each cell was also classified as an LH- or CeA- or LH/CeA-responsive neuron.

Antidromic activation of an NST neuron was defined by the standard criteria of constant latency, high-frequency (>250 Hz) following and collision (Bishop *et al.*, 1962; Cho *et al.*, 2002a). Antidromically activated action potentials were observed in three cells in response to ipsilateral LH stimulation. Two of these cells also responded orthodromically to contralateral LH stimulation; they were categorized as LH-responsive neurons. The other cell produced only antidromic spikes following stimulation of the ipsilateral LH and did not respond to stimulation of the other three sites. This cell was categorized as a non-responsive cell.

To examine the effects of electrical stimulation (ES) of the LH or CeA on the responses of NST cells to taste stimuli, responses of a subset of the forebrain-responsive neurons were recorded while trains of constant-current square pulses (100 Hz, 0.2 ms; at 0.9 × the lowest intensity that would produce an orthodromic response in each cell) were delivered to the LH or CeA during stimulation of the tongue with tastants (duration = 15 s, starting 5 s prior to taste stimulation).

For chemical stimulation of the LH or CeA, one barrel of the micropipette attached to the stimulating electrode was filled with 10 mM DL-homocysteic acid (DLH; Aldrich Chemical Co., Milwaukee WI) in buffered physiological saline (pH 7.4) and the other with saline. DLH is an excitatory amino acid analog, which presumably excites neuronal somata but not fibers of passage (Goodchild *et al.*, 1982; Yang and Coote, 1998). Pressure pulses (30 psi, 10 ms) from a Picospritzer II (General Valve Co., Fairfield, NJ) were used to trigger the microinjections, which produced an injection volume of ~50 nl (Smith and Li, 2000). Physiological saline served as a control for possible pressure effects of microinjection.

At the end of each experiment, the last recording site in the NST was marked with Chicago Blue dye by passing an intermittent (10 s on/off) 10 μA cathodal current through the recording electrode for 10 min. The stimulation sites in the ventral forebrain were 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% formaldehyde containing 3% potassium ferrocyanide and ferricyanide. The brain was removed and fixed; frozen sections (40 μm) were cut in the coronal plane and stained with Neutral Red.

Data analysis

Responses of NST cells to taste stimuli were quantified by subtracting the 5 s pre-stimulus baseline from the first 5 s of the evoked response to yield a mean net response (mean impulses/s over 5s). Responses are reported as means ± SEM. Differences in mean firing rates between forebrain-responsive and non-responsive neurons and among taste stimuli were compared using ANOVA. For the effect of DLH (or saline) on spontaneous activity of the NST taste cells, the mean firing rate in each cell over a 1 min period

before drug administration was compared with the mean firing rate during a comparable period following the drug administration using *t*-tests. The effect of ES on the mean firing rate to taste stimuli and differences in excitatory latency were also compared using *t*-tests. Comparisons of the number of neurons in each category; forebrain-responsive versus non-responsive, and excitatory versus inhibitory, were made using the chi-square test.

Results

Histology

Recording sites in the NST and stimulating sites in the ventral forebrain were examined histologically in 43 of the 63 animals; representative examples are shown in Figure 1. The iron deposits at the tips of the stimulating electrodes in the ipsilateral LH and CeA are shown in Figure 1A. The electrode in the LH was positioned dorsomedial to the optic tract (ot) and dorsolateral to the anterior hypothalamic (AH) area and ventromedial hypothalamic (VMH) nucleus. The tip of the electrode in CeA was positioned in the central amygdaloid nucleus, capsular division (CeC), which corresponds to the lateral central amygdaloid nucleus in rats, dorsal to the basolateral amygdaloid nucleus, anterior (BLA).

A recording site in the NST is shown in Figure 1B, located medial to the solitary tract (st), most likely in the rostral central (RC) or rostral lateral (RL) subdivision. Cells were recorded from the NST near the rostrocaudal level at which the dorsal cochlear (DC) nucleus is first apparent on the dorsolateral margins of the medulla, which is the area 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 cells appeared to be in the region of the NST corresponding to the RC or RL subdivisions (Whitehead, 1988).

Stimulation sites in these 43 animals are reconstructed on standard atlas sections (Morin and Wood, 2001) in Figure 2. Individual electrode placements in the LH and CeA are depicted in Figure 2A–F, arranged from rostral (A) to caudal (F). Sites evoking excitatory responses in gustatory cells of the NST are shown as filled circles, those producing inhibitory responses as half-filled circles, and those that did not alter NST activity as open circles. The three ipsilateral sites in the LH from which antidromic action potentials were activated are shown as open triangles. The majority of effective sites in the LH were at the level of the VMH, posterior to the AH nucleus (Figure 2D,E). The effective sites in the CeA were most often located in the CeC, rather than the central amygdaloid nucleus, medial (CeM; Figure 2A,B). The position of the last cell to be recorded in each animal was marked with Chicago Blue dye (as in Figure 1B) and the positions of these cells (n = 43) are depicted in Figure 2G on a standard atlas section of the medulla at the level of the DC (Morin and Wood, 2001).

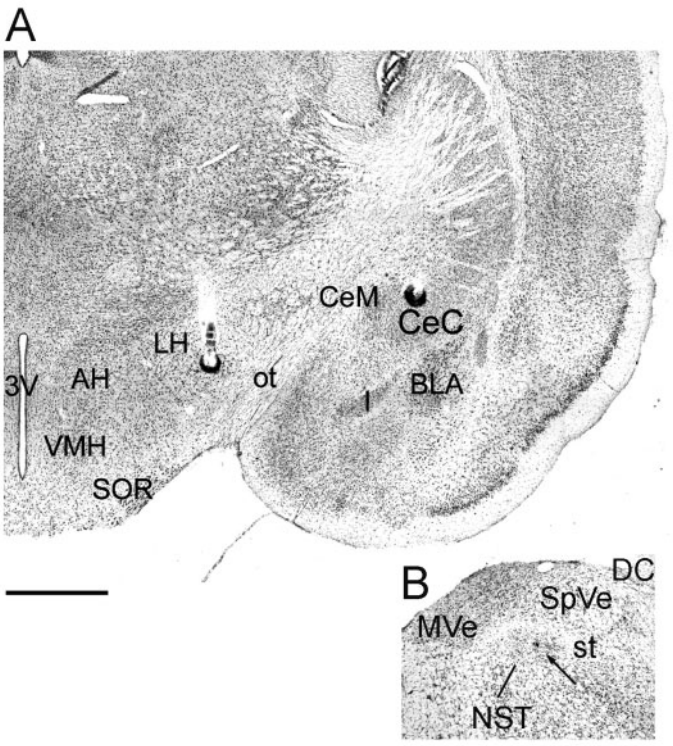

Figure 1 Photomicrographs of stimulating and recording sites in the hamster brain. **(A)** Coronal section through the diencephalon, stained with neutral red, showing the positions of two stimulating electrodes on the ipsilateral side. Iron deposits and tissue damage indicate a placement within the lateral aspects of the LH, where cells projecting to the gustatory NST are located (Whitehead, *et al.*, 2000). The other electrode left iron deposits in the central amygdaloid nucleus, capsular (CeC). **(B)** Coronal neutral-red stained section through the medulla, showing a recording site in the NST, marked with Chicago Blue dye (arrow). Abbreviations: 3V, third ventricle; AH, anterior hypothalamus; BLA, basolateral amygdaloid nucleus, anterior; CeC, central amygdaloid nucleus, capsular; CeM, central amygdaloid nucleus, medial; DC, dorsal cochlear nucleus; I, intercalated nuclei of amygdala; MVe, medial vestibular nucleus; LH, lateral hypothalamic area; NST, nucleus of the solitary tract; ot, optic tract; SOR, supraoptic nucleus, retrochiasmatic; SpVe, spinal vestibular nucleus; st, solitary tract; VMH, ventromedial hypothalamic nucleus. Calibration bar = 1 mm in both A and B.

The LH and CeA orthodromically activate many of the same NST taste neurons

The responses of 215 taste-responsive neurons were recorded from the NST. Of these, the activity of 113 (52.6%) was orthodromically modulated by stimulation of nuclei in the ventral forebrain (forebrain-responsive neurons). Effective sites were one or a combination of the four stimulated nuclei: ipsilateral and contralateral LH and CeA (Table 1). Sixty-one of these 113 neurons (53.9%) were activated by more than one forebrain site and 15 cells (13.3%) were responsive to stimulation of all four sites. The other 102 taste-responsive neurons did not respond to any

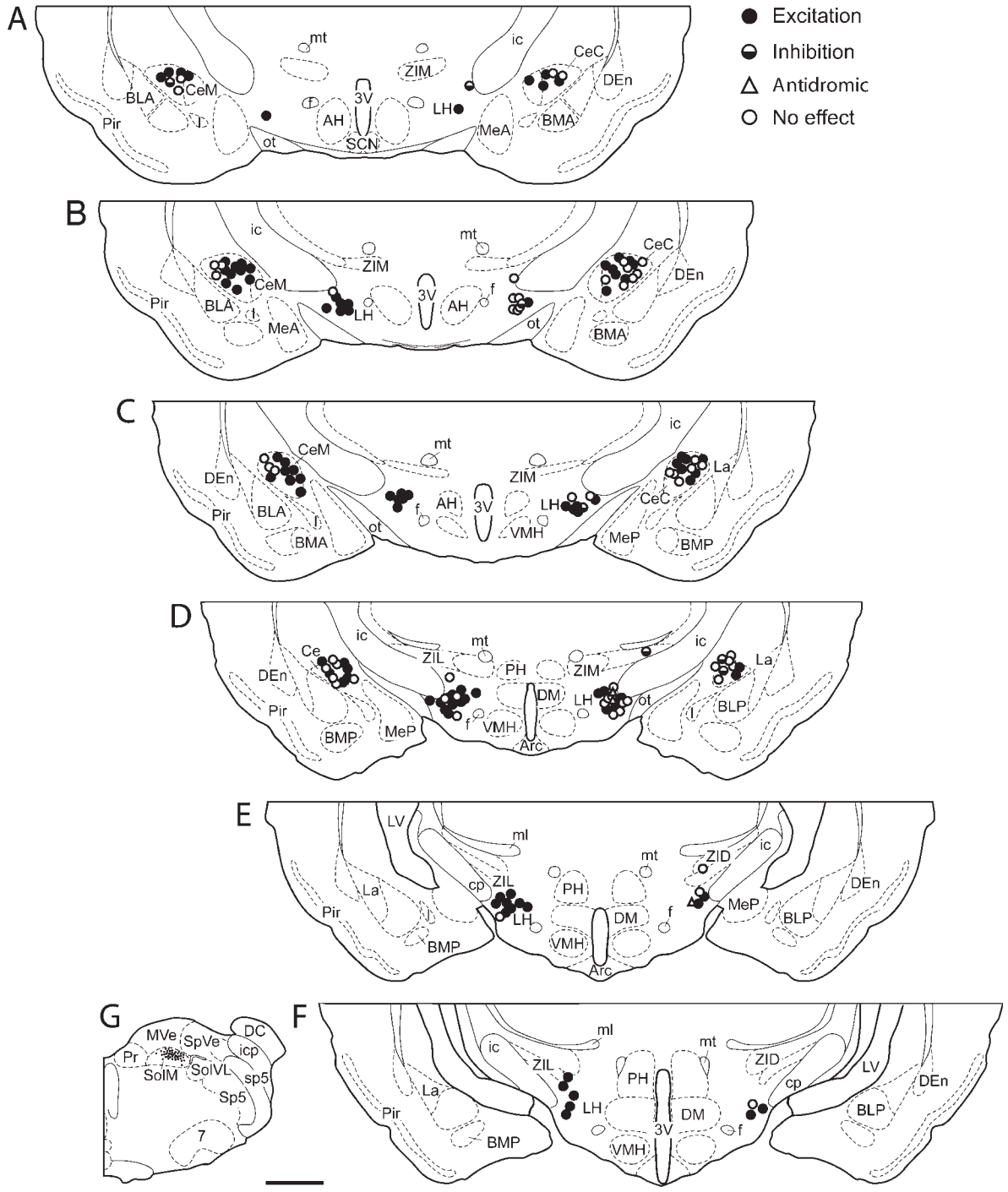

Figure 2 Standard atlas sections of the hamster brain [adapted from (Morin and Wood, 2001)] showing the distributions of stimulating and recording sites of 43 of the experimental animals. **(A–F)** Successive sections through the diencephalon, from the most rostral electrode placement (A) to the most caudal (F). The side contralateral to the recording electrode is on the left. Sites producing excitation, inhibition or no effect on NST neurons are indicated by different symbols; the three open triangles show sites that produced antidromic responses in three NST neurons. **(G)** Section through the medulla at the level of the DC, showing the distribution of the last NST cell recorded from each animal. Abbreviations: 3V, third ventricle; 7, facial nucleus; AH, anterior hypothalamus; Arc, arcuate hypothalamic nucleus; BLA, BLP, basolateral amygdaloid nucleus, anterior and posterior; BMA, BMP, basomedial amygdaloid nucleus, anterior and posterior; Ce, central amygdaloid nucleus; CeC, CeM, central amygdaloid nucleus, capsular and medial; cp, cerebral peduncle; DC, dorsal cochlear nucleus; DEn, dorsal endopiriform nucleus; DM, dorsomedial hypothalamic nucleus; f, fornix; I, intercalated nuclei of amygdala; ic, internal capsule; icp, inferior cerebellar peduncle; La, lateral amygdaloid nucleus; LV, lateral ventricle; MeA, MeP, medial amygdaloid nucleus, anterior and posterior; ml, medial lemniscus; mt, mammillothalamic tract; MVe, medial vestibular nucleus; LH, lateral hypothalamic area; ot, optic tract; PH, posterior hypothalamic area; Pir, piriform cortex; Pr, prepositus nucleus; SCN, suprachiasmatic nucleus; SolM, SolVL, nucleus of the solitary tract, medial and ventrolateral; sox, supraoptic decussation; sp5, spinal trigeminal tract; Sp5, spinal trigeminal nucleus; SpVe, spinal vestibular nucleus; VMH, ventromedial hypothalamic nucleus; ZID, ZIL, ZIM, zona incerta, dorsal, lateral and medial. Calibration bar = 1 mm.

Table 1 Number of NST neurons responsive to LH and/or CeA stimulation

No. of effective sites	Possible combinations	No. of neurons	Totals
1	iLH	3 (5)[a]	46 (6)
	cLH	35	
	iCeA	4 (1)	
	cCeA	4	
2	iLH/cLH	4 (2)	29 (3)
	iLH/iCeA	1	
	iLH/cCeA	0 (1)	
	cLH/iCeA	2	
	cLH/cCeA	19	
	iCeA/cCeA	3	
3	iLH/cLH/iCeA	2 (1)	12 (2)
	iLH/cLH/cCeA	4 (1)	
	iLH/iCeA/cCeA	0	
	cLH/iCeA/cCeA	6	
4	iLH/cLH/iCeA/cCeA	15	15
Total		102 (11)	

[a]Cells that showed an inhibitory response are indicated in parentheses.

Table 2 Number of neurons responding best to each taste stimulus in each forebrain-responsive group

Forebrain responsiveness	S-best	N-best	C-best	Q-best	Total
Responsive					
LH	6	17	20	6	49
CeA	2	1	4	5	12
LH/CeA	4	16	12	20	52
Total	12	34	36	31	113
Non-responsive	25	42	17	18	102
Total	37	76	53	49	215

of these stimulating sites (non-responsive neurons), except one neuron, which was antidromically invaded from the ipsilateral LH and was categorized as a non-responsive neuron.

Each cell was also classified into one of four groups according to its most effective (best) taste stimulus. Although more QHCl- and citric acid-best neurons and fewer sucrose-best cells occurred in the forebrain-responsive category, the proportion of cells in each best-stimulus category was not significantly different between the forebrain-responsive and non-responsive neurons (χ^2 = 7.186, df = 3, P = 0.066). As shown in Table 2, among the 113 forebrain-responsive neurons, 49 (43.4%) responded only to stimulation of the ipsilateral and/or contralateral LH (LH-responsive neurons). In comparison, 12 cells (10.6%) responded only to stimulation of the CeA (CeA-responsive neurons). The activity of the other 52 neurons (46.0%) was altered by stimulation of both the LH and CeA (LH/CeA-responsive neurons).

Electrical stimulation of the ipsilateral LH produced antidromically activated action potentials in three cells, all of which met the three criteria for antidromic activation (Bishop *et al.*, 1962; Cho *et al.*, 2002a). Two of these cells were NaCl-best and also produced orthodromically induced action potentials following contralateral LH stimulation; these two cells were classified as LH-responsive neurons. The latencies of these antidromic spikes were 17 and 23 ms and the thresholds were 13 and 10 μA, respectively. The other neuron was QHCl-best and did not show an orthodromic response to the stimulation of any site; this cell was classified as a non-responsive neuron, with an antidromic latency of 27 ms and a threshold of 75 μA. The mean antidromic latency across these three cells was 23.3 ms.

The activity of significantly more (101) neurons was modulated by LH stimulation than by CeA stimulation (64 neurons) (χ^2 = 8.297, df = 1, P < 0.01). Contralateral stimulation was significantly more often effective than ipsilateral: 74 responses were induced in NST neurons by simulation of the ipsilateral LH and/or CeA, whereas 144 were evoked by contralateral stimulation (χ^2 = 22.477, df = 1, P < 0.001). If the comparison was limited to responses to LH stimulation: 39 responded to ipsilateral stimulation versus 91 to contralateral, this difference was also significant: (χ^2 = 20.800, df = 1, P < 0.001), whereas it was not significant for responses to CeA stimulation: 35 responded to ipsilateral stimulation versus 53 to contralateral (χ^2 = 3.682, df = 1, P = 0.055). Among the 113 forebrain-responsive neurons, only 11 cells demonstrated an inhibitory response to stimulation of the LH and/or CeA (Table 1, parentheses). The other 102 taste-responsive neurons were excited by forebrain stimulation (χ^2 = 73.283, df = 1, P < 0.001).

Electrophysiological features of NST responses to forebrain stimulation

Orthodromically induced action potentials and the corresponding peri-stimulus time histograms (PSTHs) for two cells are illustrated in Figure 3. Action potential traces and PSTHs in Figure 3A,B,E,F are from a cell that produced orthodromic spikes following stimulation of all four sites. The occurrence of action potentials in Figure 3A were concentrated in a period from 14 to 24 ms after ipsilateral LH stimulation (see also the PSTH in Figure 3E), whereas stimulation of the contralateral LH evoked some spikes at ~9 ms, inhibited impulse discharge for a 20 ms period and then induced more orthodromic action potentials over a period of ~50 ms (Figure 3B,F). The PSTH in Figure 3F shows the occurrence of additional evoked spikes more than 100 ms after stimulation.

Figure 3C,D,G,H depicts responses of another cell that responded to both the contralateral LH and CeA. The

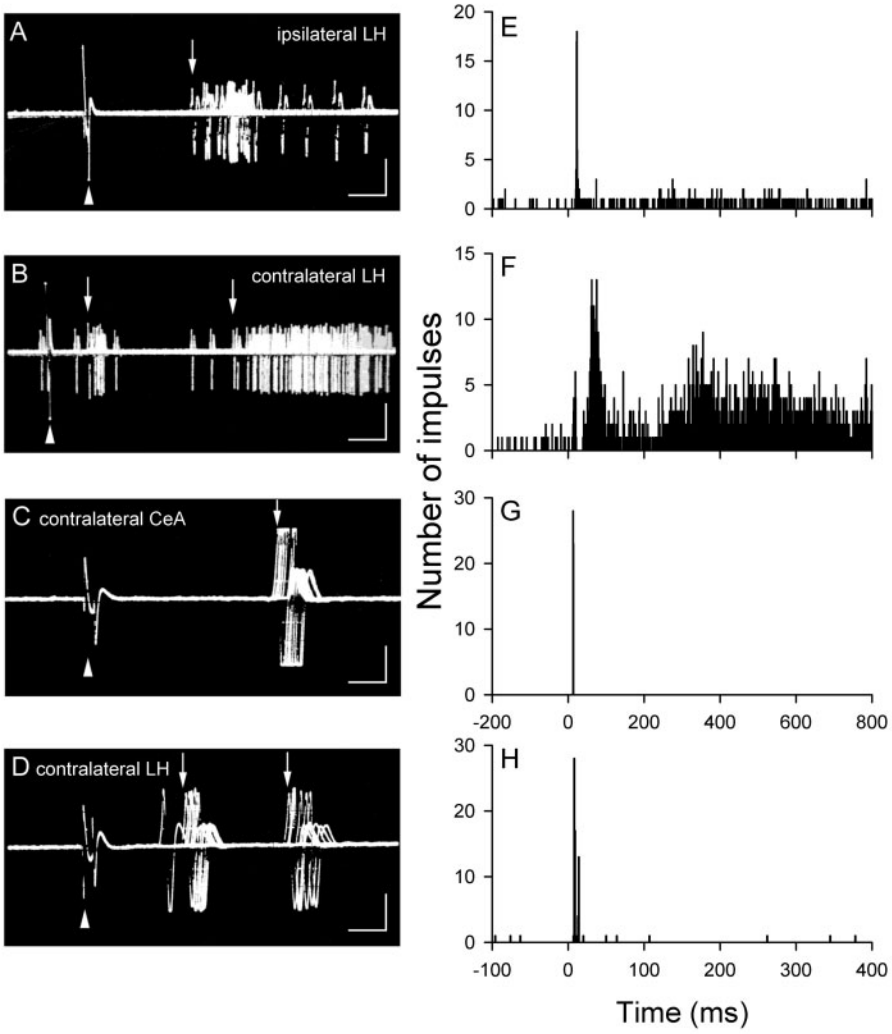

Figure 3 Orthodromic action potentials recorded from two neurons (**A** and **B**, **C** and **D**) in the NST and their corresponding PSTHs in response to LH and/or CeA stimulation. (A) Orthodromically activated impulses, which mostly occurred between 14 and 24 ms after ipsilateral LH stimulation (arrowhead; 20 sweeps). **(E)** Corresponding PSTH, in which evoked action potentials (of cell in A) were accumulated over a 1 s period for 125 sweeps. An excitatory peak occurred around 15 ms after the stimulation (0 ms). (B) Orthodromically induced spikes following contralateral LH stimulation (arrowhead) during 30 sweeps from the same cell in A. Action potentials were generated at ~9 ms (arrow) and more spikes (second arrow) were widely temporally distributed after 50 ms (note different scale than in A). **(F)** Corresponding PSTH (200 sweeps). In addition to evoked spikes indicated with arrows in (B), stimulation produced action potentials above spontaneous rate >300 ms after stimulation. (C) Evoked spikes during 20 sweeps following contralateral CeA stimulation of another NST taste neuron. Action potentials were generated at ~10 ms after the stimulus. **(G)** Corresponding PSTH showed the excitatory peak at 10 ms and no spontaneous firing during 150 sweeps. (D) Evoked action potentials following contralateral LH stimulation of the same neuron in (C) (20 sweeps). Stimulation-induced spikes occurred at ~5 and 10 ms. **(H)** Corresponding PSTH showed two closely located excitatory peaks of impulses. The number of spikes from 100 ms before to 400 ms after the stimulation was shown here to reveal the two peaks (200 sweeps). Calibration bars = 2 mV and 5 ms for (A), 10 ms for B and 2 ms for (C) and (D).

occurrence of evoked spikes following stimulation of the contralateral CeA was limited to a 2 ms span, 10–11 ms after stimulation (Figure 3C,G). The evoked spikes following contralateral LH stimulation were grouped at ~5 and 11 ms after stimulus onset (Figure 3D,H). As in our previous experiments on the LH and CeA (Cho *et al.*, 2002b; Li *et al.*, 2002), orthodromic response durations were bimodally distributed, involving either single spikes on each sweep, distributed over sweeps within <10 ms (as in Figure 3C,G) or more than one spike per sweep, spread over a considerable period (>10 ms, as in Figure 3B). Such long-duration responses were more often observed (n = 124) than single impulses (n = 81), as in Figure 3C (χ^2 = 9.020, df = 1, $P < 0.001$).

Of 13 inhibitory responses from 11 taste neurons, 9 were observed after stimulation of the ipsilateral LH, which was the most effective site for producing inhibitory responses (χ^2 = 13.769, df = 3, $P < 0.001$). The spontaneous activity of

two cells was inhibited by two sites: ipsilateral and contralateral LH and ipsilateral LH and CeA, respectively. Four of these inhibited cells also were excited by stimulation of other sites. No inhibitory response was observed from any of the 15 neurons that responded to all four sites. The inhibition of spontaneous firing in these cells started within 30 ms after stimulation and the duration of inhibition ranged from 28 to 140 ms (mean = 64.92 ± 8.89 ms). An example of an inhibitory response is shown in the PSTH of Figure 4A; starting 26 ms after ipsilateral LH stimulation, spontaneous firing was eliminated for 58 ms.

For five cells that were initially excited by forebrain stimulation, an inhibitory period was observed following orthodromically evoked spikes (as in Figure 3B,F). In the response depicted in Figure 4B, two peaks of evoked action potentials were separated by a brief inhibitory period and another longer period of inhibition followed after the second peak in response to ipsilateral LH stimulation. These five cells were categorized as excited cells because their initial response was excitatory. DLH injection into the ipsilateral LH of the cell depicted in Figure 4B produced only an excitatory response (see Figure 8B below).

In seven cells, the occurrence of evoked action potentials formed two peaks, but without an inhibitory period between them. In three of these cells and in the five cells mentioned above, the evoked spikes following the initial excitatory impulses were more widely temporally distributed than the initial excitation (as in Figures 3B,F and 4B). In the responses of the other four cells, two distinct peaks of spikes were closely separated in time (5–10 ms, as Figure 3D). These four responses were all induced following stimulation of the contralateral LH.

Relationship between gustatory responses and stimulating sites

The taste-evoked responses of the 113 forebrain-responsive and the 102 non-responsive NST neurons are shown in Figure 5A,B, respectively. Within each group, cells are arranged along the abscissa into best-stimulus categories and within a category by the magnitude of the response to their best stimulus. These individual responses show that all best-stimulus neuron types were represented and that the firing rates of the cells were similar in both forebrain-responsive and non-responsive groups. The overall mean firing rates to gustatory stimulation were not significantly different between the forebrain-responsive and non-responsive neurons [$F(1, 852) = 3.007$, $P = 0.083$], but there was a significant interaction between the responses to taste stimuli and forebrain responsiveness [$F(3, 852) = 4.391$, $P < 0.01$].

NST taste cells were categorized into four groups as mentioned above: LH-responsive ($n = 49$), CeA-responsive ($n = 12$), LH/CeA-responsive ($n = 52$), and non-responsive neurons ($n = 102$). The response to each taste stimulus in each of these groups is shown in Figure 6. The mean net

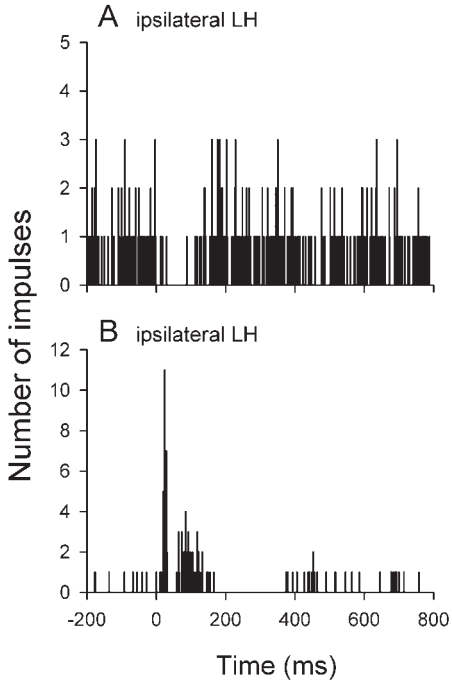

Figure 4 PSTHs showing orthodromic responses of two NST taste cells. **(A)** No spontaneous firing occurred from 26 ms after ipsilateral LH stimulation for 58 ms during 100 sweeps. **(B)** Ipsilateral LH stimulation evoked action potentials and inhibited spontaneous firing. Evoked action potentials formed a peak at ~17 ms and a smaller and wider peak after 70 ms. Spontaneous activity was inhibited during a period of 40 ms between the two excitatory peaks and again for a much longer period following the second peak of excitation.

responses across all four taste stimuli did not differ among the four groups ($F[3, 844] = 1.643$, $P = 0.178$). Taste stimuli produced mean responses that differed from one other [$F(3, 844) = 8.240$, $P < 0.001$] and there was a significant interaction between stimulus and effective site [$F(9, 844) = 2.467$, $P < 0.01$]. NST neurons that were modulated only by the CeA had greater responses to citric acid and QHCl and smaller responses to sucrose and NaCl than did the other groups (Figure 6). The greater number of C-best and Q-best neurons in CeA-responsive group relative to the S- and N-best cells (see Table 2) may have contributed to the significant interaction between the response to taste stimuli and responsive group. There was a smaller response to both sucrose and NaCl in all three forebrain-responsive groups than in the non-responsive neurons.

Latency of excitatory responses

The latency of orthodromic action potentials varied from 4 to 85 ms after stimulation of the LH and/or CeA. The mean latency of excitatory responses was 22.08 ± 0.87 ms. The mean latencies associated with each of the four stimulation sites are shown in Figure 7A, along with the mean latency for the three cells that were antidromically activated from the ipsilateral LH. The mean latency after LH stimulation (21.03 ± 1.04 ms, $n = 120$) did not differ from that following

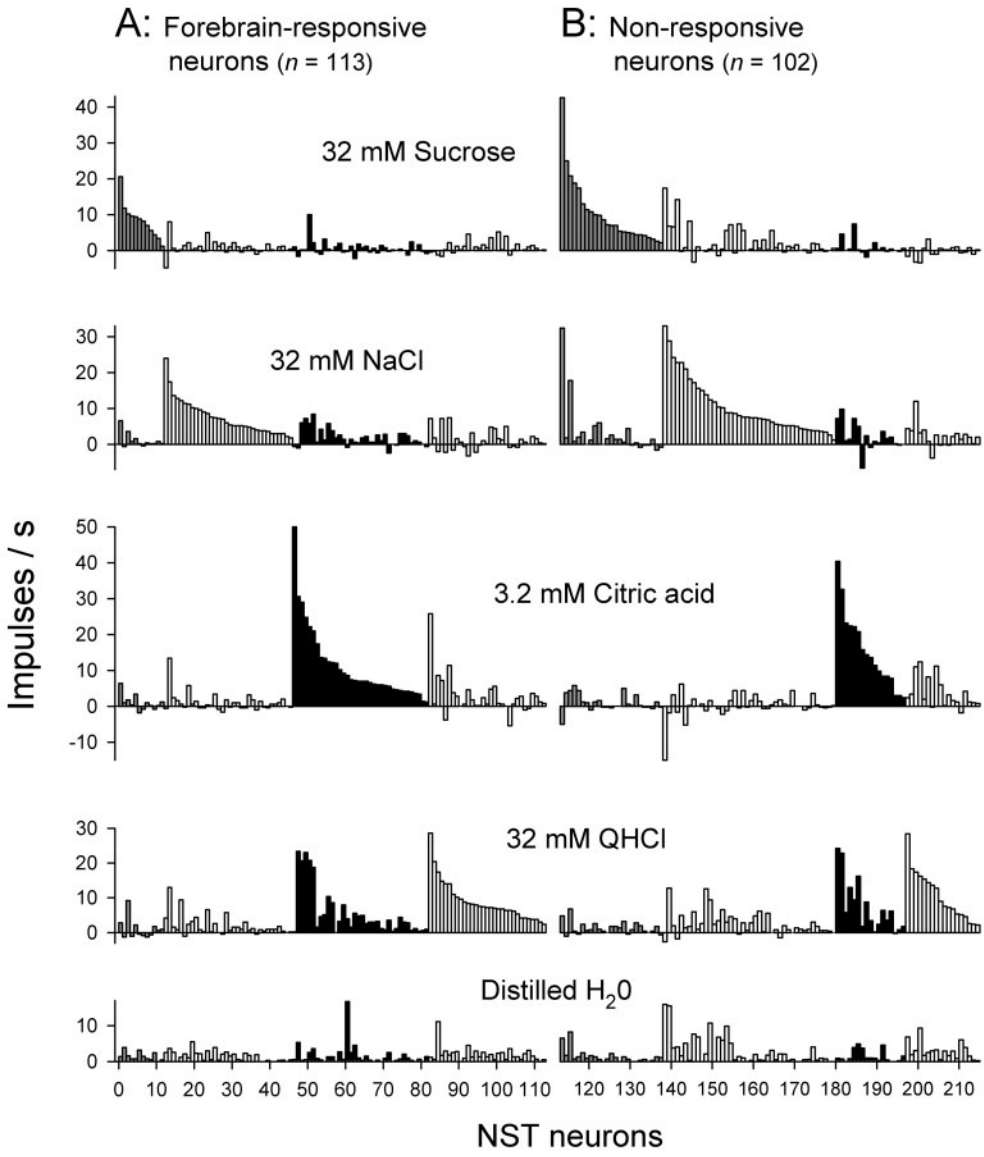

Figure 5 Taste responses (impulses/s) of 113 forebrain-responsive **(A)** and 102 non-responsive **(B)** NST neurons. Forebrain-responsive neurons are arranged along the abscissa according to their best stimulus, with neurons 1–12 being sucrose-best (gray bar), 13–46 NaCl-best (unfilled), 47–82 citric acid-best (black) and 83–113 QHCl-best (unfilled). In the same manner, non-responsive neurons are arranged so that 114–138 are sucrose-best (gray), 139–180 NaCl-best (unfilled), 181–197 citric acid-best (black) and 198–215 QHCl-best (unfilled). Cells are arranged within each best-stimulus group according to the magnitude of the response to their best stimulus. Responses of single NST cells to taste stimuli were quantified by subtracting the 5 s pre-stimulus baseline from the first 5 s of the evoked responses to yield a mean net response (impulses/s). The response profile for any one cell can be read from top to bottom. The spontaneous rate (mean response to distilled water during the 5 s prior to each stimulus) of each cell is shown at the bottom of the figure.

CeA stimulation [23.55 ± 1.47 ms, $n = 85$; $F(1, 201) = 1.391$, $P = 0.240$], whereas there was a significant difference in latency between ipsilateral (mean = 27.49 ± 1.88 ms, $n = 63$) and contralateral stimulation [mean = 19.68 ± 0.86 ms, $n = 142$; $F(1, 201) = 17.815$, $P < 0.001$]; there was no significant interaction [$F(1, 201) = 1.099$, $P = 0.296$]. Thus, stimulation of the contralateral side, either LH or CeA, evoked orthodromic action potentials with a shorter latency than stimulation of the ipsilateral side. The difference between the latencies to stimulation of each side is also seen in a comparison of the latencies from neurons that responded bilaterally to the LH or/and CeA ($t = 2.58$, df = 25, $P < 0.05$ for the LH responses; $t = 4.56$, df = 23, $P < 0.001$ for the CeA responses). For the fifteen neurons that produced excitatory response to all four sites, the difference between the latencies of ipsilateral (mean = 27.77 ± 3.31 ms) and contralateral stimulation (mean = 17.07 ± 2.28 ms) was significant ($t = 3.511$, df = 29, $P < 0.001$), but there was no difference between the latencies for LH (mean = 22.70 ± 2.90 ms) and CeA stimulation (mean = 22.13 ± 3.12; $t = 0.161$, df = 29, $P = 0.874$).

The mean latency of long-duration facilitations (26.54 ±

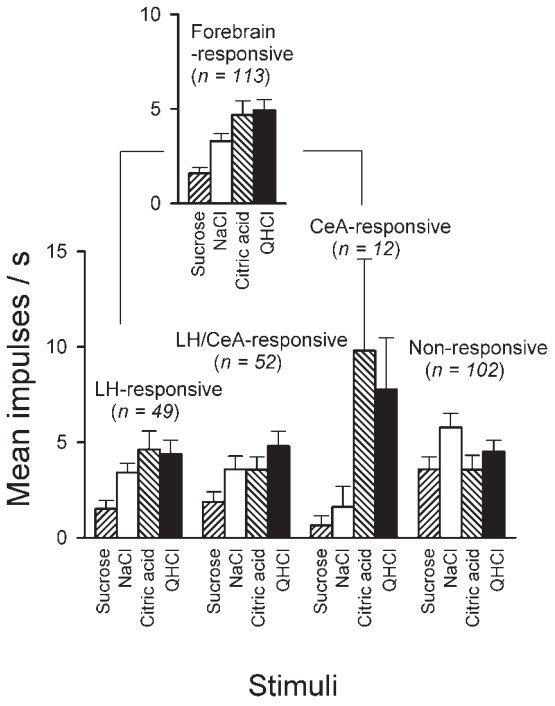

Figure 6 Mean (+ SEM) firing rate of LH-, CeA-, and LH/CeA-responsive and non-responsive neurons of the NST. Responses to the four taste stimuli are mean net responses (above spontaneous rate) across all cells in that category.

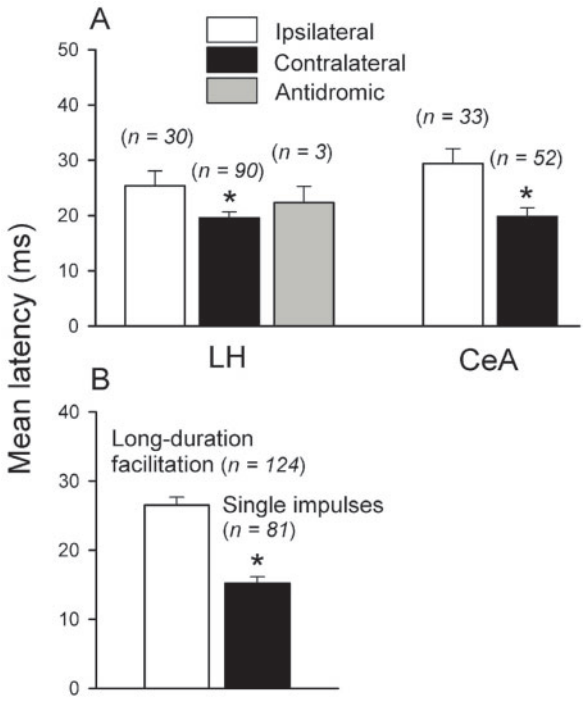

Figure 7 Mean (+ SEM) excitatory orthodromic latencies of forebrain-responsive neurons. **(A)** Comparison of mean latencies among four stimulating sites. The mean latencies following contralateral LH or CeA stimulation were significantly shorter than those following stimulation of the ipsilateral side. No significant difference was observed in orthodromic latencies between LH and CeA stimulation. Mean antidromic latency after ipsilateral LH stimulation is shown for three cells. **(B)** Mean latency of long-duration facilitations was significantly longer than that of single impulses.

1.14 ms, n = 124) was significantly longer than that of single-impulse responses (15.25 ± 0.90 ms, n = 81; t = 7.115, df = 203, $P < 0.001$, Figure 7B). The correlation between latency and duration was significant (r = +0.470, df = 203, $P < 0.01$), showing that short latency responses were more likely to be of short duration. However, there was no significant interaction between the response type (long-duration facilitation versus single impulses) and the effective sites [$F(1, 201) = 0.389$, $P = 0.533$] or effective sides [$F(1, 201) = 1.289$, $P = 0.258$]. Thus, long-duration facilitations were common in the excitatory response to both LH and CeA stimulation, regardless of which side was stimulated.

DLH injection mimics the response to single-pulse stimulation

For 25 of the forebrain-responsive neurons (22 excitatory and three inhibitory neurons; one of three inhibitory neurons also showed excitatory responses after stimulation of other sites as shown in Figure 8A), the effect of 10 mM DLH microinjection into the stimulating sites was compared with the effect of saline injection. For six neurons, DLH injections were made into more than two sites. Figure 8A shows the effects of DLH on the spontaneous activity of such a cell. Electrical stimulation of the contralateral LH induced an excitatory response, whereas contralateral CeA stimulation inhibited the spontaneous firing of this cell. The injection of DLH, but not saline, into the contralateral LH increased the spontaneous firing over a period of ~2 min. In contrast, DLH injection into the contralateral CeA inhibited the spontaneous activity. Saline injection was without effect.

The response to electrical stimulation of the ipsilateral LH of the cell in Figure 8B was demonstrated above (Figure 4B). Although the orthodromic response to single ipsilateral LH shocks showed an inhibitory effect following orthodromically evoked action potentials, the DLH effect was only excitatory (Figure 8B). This cell responded to injections of DLH into all four sites. The effect of DLH (into the ipsilateral LH) lasted ~80 s but was no less than the effects of DLH injection into the other sites; the responses to electrical stimulation of the other three sites were purely excitatory.

For the neurons showing excitatory responses to DLH, the mean firing rate over a 1 min period after DLH injection (9.60 ± 1.41 impulses/s, n = 32) was significantly greater than that of a corresponding period before injection (2.16 ± 0.77 impulses/s; t = 8.358, df = 31, $P < 0.001$, Figure 8C). In contrast, the change in mean firing rate before (2.17 ± 0.82 impulses/s) and after (2.03 ± 0.75 impulses/s) saline injection was not significant (t = 1.822, df = 31, P = 0.078). There was

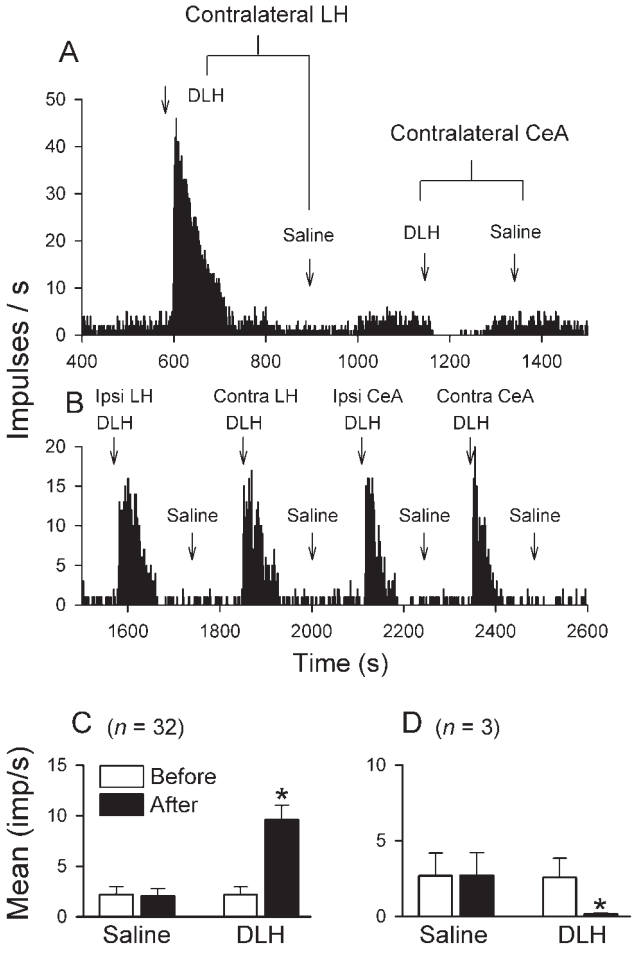

Figure 8 Effects of microinjection of DLH into the LH and CeA on responses of taste cells in the NST. **(A)** PSTH (1 s bins) showing the effect of 10 mM DLH (at arrow) injected into the contralateral LH and contralateral CeA; DLH injection into the LH induced evoked orthodromic potentials whereas that into the CeA inhibited spontaneous firing. Injections of saline had no effect. **(B)** PSTH showing the excitatory effect of DLH injections into all four stimulating sites and no effect of saline injections on a single NST neuron. **(C)** Mean firing rate of NST neurons in response to DLH microinjection. Mean firing rate from 1 min before and after DLH injection was compared with that for saline across 32 responses from 23 forebrain-responsive NST neurons that were excited by single pulse stimulation. DLH produced a significant increase in firing rate (*) after microinjection, but saline did not. **(D)** Mean firing rate of three inhibitory responsive NST neurons before and after DLH injection. DLH, but not saline, produced a significant decrease in firing rate for these neurons.

no case in which DLH injection reduced the spontaneous activity of cells that were excited by electrical stimulation. There was no significant relationship between the increase in firing rate and injection site (F [3, 28) = 1.333, P = 0.284). Three of the 11 neurons that showed an inhibitory response to electrical stimulation were tested for the effect of DLH, which reduced the spontaneous rate in every instance. The reduction of mean spontaneous rate was –95.0, –73.8 and –97.5%, respectively; the effect of DLH was significant compared to saline (t = 56.540, df = 2, P < 0.001) for inhibitory responses (Figure 8D). Thus the injection of DLH mimicked the effect of electrical stimulation, producing either excitation or inhibition as occurred following single-pulse electrical stimulation.

Forebrain stimulation alters gustatory responses

Twenty-five cells were tested for the effects of electrical stimulation (ES) of the LH and/or CeA on the responses to taste solutions. For this experiment, trains of constant-current square pulses (100 Hz, 0.2 ms, 15 s duration) were delivered to the forebrain site. Single-pulse stimulation of the LH and/or CeA induced excitatory orthodromic spikes in 23 of these 25 cells. From one to four taste stimuli were tested on each cell during forebrain stimulation. In all, 56 gustatory responses were recorded from these 23 neurons while ES was delivered to the stimulating electrode in the LH and/or CeA. The intensity of ES was below the threshold necessary to elicit orthodromic action potentials (0.9 × threshold for orthodromic spikes). All 56 gustatory responses were increased during ES.

Several examples of the effect of LH and/or CeA stimulation on gustatory responses are shown in Figure 9. For the three cells in Figure 9A–C, which showed excitatory responses following single-pulse stimulation, responses to taste stimuli were enhanced by ES. Although all four taste stimuli were not tested during ES, the response to the best stimulus, citric acid, was most enhanced by ES in Figure 9A (by 155%). In contrast, the increase in the response to the best stimulus was less than to the other stimuli in Figure 9B,C. For the cell in Figure 9B, although the response to sucrose was the greatest with or without ES, the proportional increase in the response to QHCl, which was the third-best stimulus, was greater (442%) than those to sucrose (74%) or citric acid (215%). Two cells that showed an inhibitory effect on spontaneous activity during electrical stimulation of the ipsilateral LH and CeA were also tested. For these cells, ES reduced the gustatory response to sucrose (by 100%) and QHCl (by 48.3%), which were the best stimuli for each. The taste responses of the Q-best cell are shown in Figure 9D.

For the NST cell in Figure 9E, all four sites were stimulated during the application of NaCl and sucrose, which were best and second-best stimuli, respectively. In this example, the response to sucrose, the second-best stimulus, was enhanced more in each instance than that to NaCl. In Figure 9F, the response to the best stimulus, QHCl, was more enhanced than that to the second-best stimulus, citric acid, by stimulation of both the contralateral LH and the contralateral CeA. The increased responses to taste stimulation did not differ among the four stimuli [F(3, 40) = 2.617, P = 0.064] nor the four stimulating sites [F(3, 40) = 1.999, P = 0.130].

The mean response to taste solutions without ES was 8.79 ± 0.97 impulses/s, which increased significantly to 17.39 ± 1.51 during ES of the LH and/or CeA (t = 9.746,

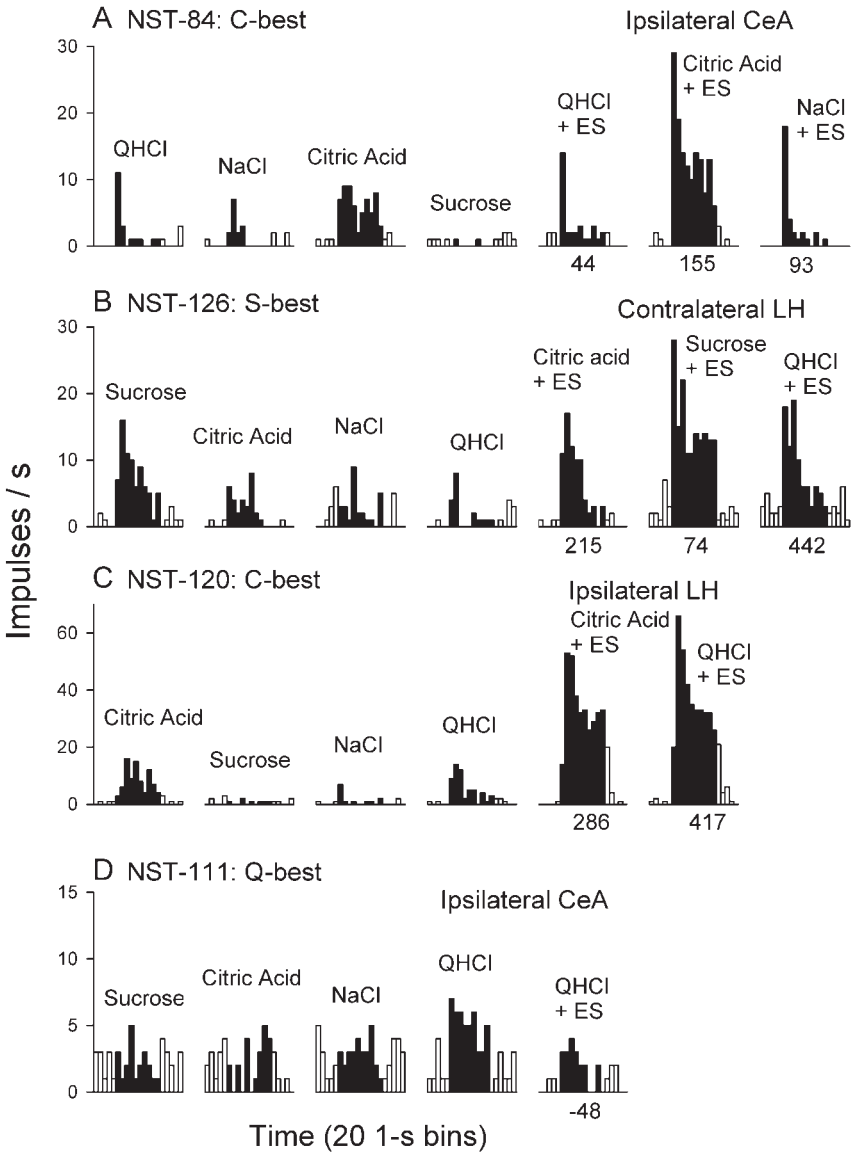

Figure 9(A–D)

df = 55, $P < 0.001$). Mean spontaneous firing during the pre-stimulus period of these 25 cells was 1.02 ± 0.24 imp/s, whereas mean firing during the pre-stimulus period with ES was 1.14 ± 0.26 ($t = 0.367$, df = 24, $P = 0.717$). The increase of each taste response with ES trains varied from 13% to 442%. On average, the ES trains more than doubled the response of cells to taste stimulation (mean = 145%), whereas the firing during the pre-stimulus period was not altered by the ES trains (Figure 9G). The comparison between the responses to best- and second-best stimuli (Figure 9H) showed that the increased rate of response to the second-best stimulus (mean = 195.3%) surpassed that to the best stimulus (mean = 118.0%; $t = 3.292$, df = 17, $P < 0.005$).

Discussion

Convergence of descending influences from the LH and CeA

The main finding in the present study was that more than half of the gustatory neurons in the hamster NST were under the influence of the LH and/or CeA. Many of these NST neurons received descending inputs from more than one stimulating site. Of 113 forebrain-responsive neurons, 61 responded to stimulation of two or more sites. Among them, 52 cells received input from both the LH and CeA and 15 of them responded to stimulation of both of these areas bilaterally. Whereas the LH and CeA have been implicated in different aspects of feeding behavior and reactivity to

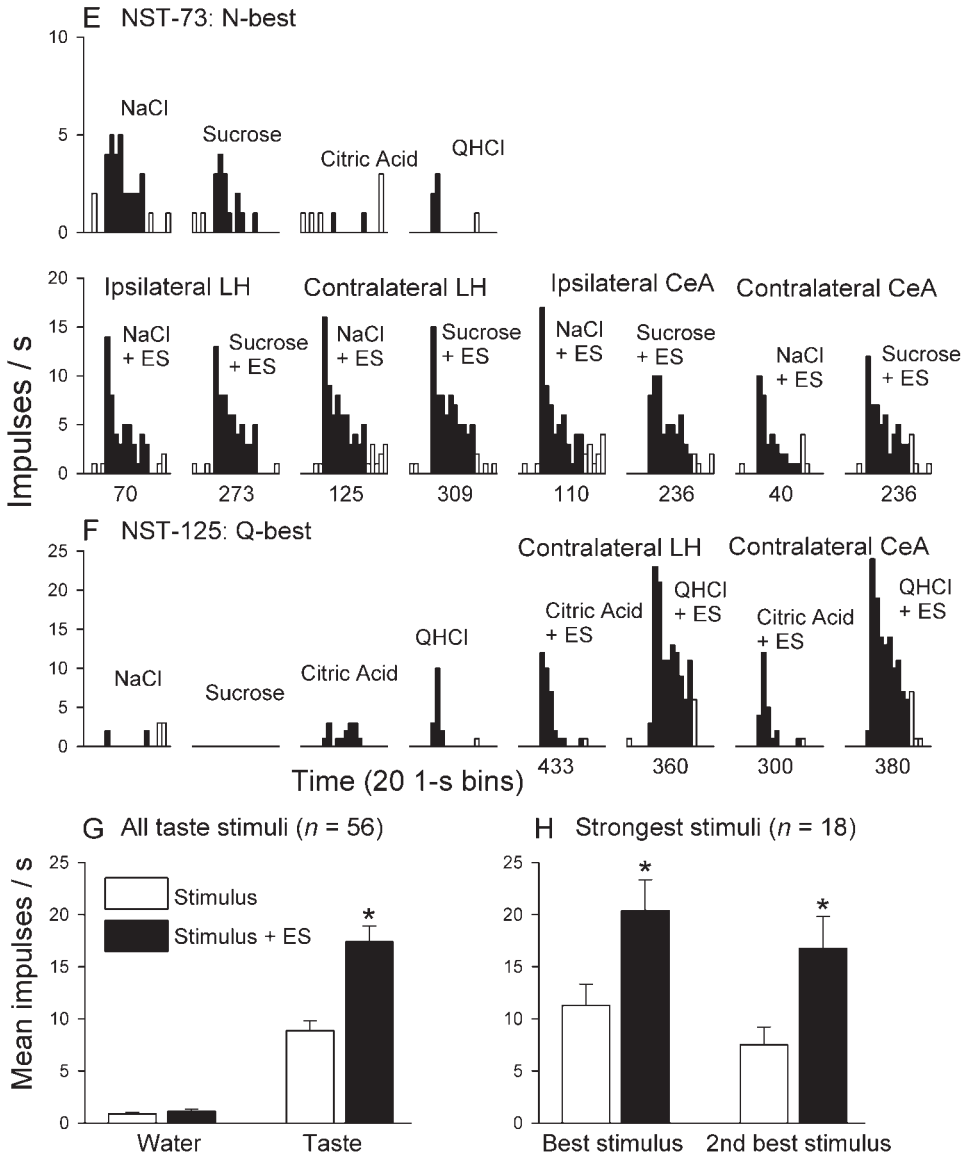

Figure 9 Responses of NST neurons to taste stimulation before and during LH or CeA stimulation. The responses (impulses/s) during the 10 s stimulus are shown as black bars and the 5 s pre-stimulus and post-stimulus water rinse periods are shown as open bars; each histogram reflects 20 s of activity. The number shown below each taste response with ES, indicates the percentage increase (or decrease) in mean firing rate during the first 5 s of the response. **(A–C)** Taste responses of three NST cells, which exhibited excitatory responses following single-pulse stimulation, are shown with and without ES. Taste responses were enhanced during forebrain stimulation, but the responses to pre-stimulus water were not. **(D)** Ipsilateral CeA stimulation inhibited the QHCl response of this cell, the spontaneous activity of which was inhibited by single-pulse stimulation. **(E)** The taste responses to the best and second-best stimulus were enhanced during ES delivered to each of four stimulating sites in the forebrain. **(F)** For this neuron, the contralateral LH and CeA, which were effective sites for single-pulse stimulation, were also stimulated with ES during the response to citric acid and QHCl. The responses to both stimuli were enhanced by forebrain stimulation. **(G)** Mean responses to gustatory stimulation before and during forebrain stimulation, in which taste responses ($n = 56$) were acquired before and during ES of the LH or CeA. Mean firing rate to gustatory stimulation during ES was significantly enhanced (*). **(H)** The comparison of mean responses to the best and second-best stimulus shows that both were enhanced by ES, although the percentage increase in the response to the second-best stimulus was greater than that to the best stimulus (see text).

taste stimuli, we would expect that these two areas would differentially modulate taste processing. The present data show that LH and CeA can exert an influence on many of the same NST neurons, although over half the forebrain-responsive cells were differentially affected by these two areas. Thus it is likely that taste information would be differentially altered by descending influences of the LH and the CeA. Cells that were exclusively modulated by the CeA responded more to aversive stimuli (citric acid and QHCl) than to sucrose and NaCl (Table 2, Figure 6), which may relate to the responsiveness of CeA neurons to the hedonic aspects of taste stimulation (Nishijo *et al.*, 1998)

and its role in conditioned taste aversion (Yamamoto *et al.*, 1994). Determining the exact influence of these forebrain areas on the coding of gustatory quality and hedonic value will require data on a wider array of taste stimuli than that employed in the present experiment.

The LH exerted an influence on more NST gustatory neurons (47%) than did the CeA (30 %), although 24% were modulated by both. The LH receives information about taste and nutritional status and regulates the animal's feeding behavior (Nicolaidis, 1981; Norgren, 1976; Ter Horst *et al.*, 1989). The CeA is involved in the neuronal circuitry underlying sodium intake and conditioned taste aversion, which is dependent on gustatory experience (Galaverna *et al.*, 1992; Seeley *et al.*, 1993; Tucci *et al.*, 1998). Our previous studies on the LH (Cho *et al.*, 2002b) and the CeA (Li *et al.*, 2002) have shown similar proportions of LH- and CeA-responsive NST neurons, although we did not investigate convergence in these earlier studies. The convergence of LH and CeA inputs onto many of the same NST neurons provides a substrate through which these forebrain areas can coordinate a descending modulation of taste information in response to changes in physiological state and experience. On the other hand, neurons influenced solely by one or the other of these areas allow for differential modulation of NST processing by the LH and the CeA.

Modulatory effects of forebrain stimulation on the activity of NST neurons

The modulatory effect of ventral forebrain gustatory nuclei on NST taste neurons was predominantly excitatory. Stimulation of the LH and CeA induced orthodromic excitation in 90% of the forebrain-responsive neurons and inhibitory responses in only 10%. The most effective site for inducing inhibitory responses was the ipsilateral LH, stimulation of which elicited an inhibitory response in nine of 11 cells [see also (Cho *et al.*, 2002b)]. Because the present experiments were conducted on anesthetized animals, there is some possibility that in awake, behaving hamsters there could be differences in the proportions of excitatory and inhibitory influences. Within the subset of taste-responsive neurons (25 cells) that was tested for the effect of electrical stimulation on taste responses, all but two cells were excited by forebrain stimulation. Electrical stimulation of the LH and/or CeA enhanced all taste responses of those cells, whether the taste stimulus was palatable or aversive. Although the response to no particular stimulus was affected more than others, the responses to the second-best stimulus surpassed that to the best stimulus in five cases out of 20, suggesting that the representation of taste information can be altered by the ventral forebrain.

The fact that most modulatory effects were excitatory and spread across the various taste stimuli suggests that the primary function of the descending input from the LH or CeA is to increase the overall sensitivity of a subset of gustatory neurons to taste stimuli. Such an increase in the signal-to-noise ratio should in turn increase the detectability of differences among taste stimuli [see also (Cho *et al.*, 2002b)]. Berridge and Valenstein (Berridge and Valenstein, 1991) reported that electrical stimulation of the LH increased feeding behavior and also enhanced aversive taste reactions in rats. These investigators suggested that LH stimulation influences feeding by potentiating the difference between positive and aversive hedonic evaluations, not by merely evoking pleasant sensations. The increased signal-to-noise ratio in the present results suggest a substrate through which such increased discriminability could occur.

Origin of descending modulation and possible pathways

The present study also demonstrated that the effect of stimulating LH or CeA was due to the excitation of cells in or around these nuclei. Injection of DLH, but not saline, mimicked the effect of electrical stimulation in the 25 forebrain-responsive neurons that were tested with this protocol. This result makes less likely the possibility that the effect of electrical stimulation of the LH or CeA was a result of stimulating fibers of passage. The histologically reconstructed stimulating sites in the LH corresponded to the area where stimulation induced feeding behavior (Frank *et al.*, 1982; Sasaki *et al.*, 1984; Murzi *et al.*, 1986) and where some neurons responded to taste stimuli (Norgren, 1970) in rats. The CeA stimulating sites in the present study corresponded to the area where lesions altered ingestive behavior (Kemble *et al.*, 1979; Galaverna *et al.*, 1992), where stimulation antidromically activated gustatory PbN neurons (Norgren, 1976), and where some neurons responded to taste stimuli (Azuma *et al.*, 1984; Uwano *et al.*, 1995; Nishijo *et al.*, 1998) in rats. Although it is not known whether the neurons in the LH or CeA that modulate taste responses in the NST are also involved in motivating animals to feed and/or are gustatory-responsive neurons themselves, they reside in the same areas within these nuclei.

Another interesting finding was that NST taste neurons were influenced more from the contralateral than the ipsilateral side. In comparison, anatomical studies have demonstrated more descending projections from the ipsilateral LH or CeA to the NST than from the contralateral (van der Kooy *et al.*, 1984; Whitehead *et al.*, 2000). However, such neuronal tracing studies only reveal direct projections. The discrepancy between the anatomical studies and the present data implies that the forebrain influences neuronal activity of NST neurons primarily through multisynaptic pathways. Although the present results did not demonstrate which nuclei participate in the information flow from the ventral forebrain to the NST, the reticular formation (RF) and caudal NST are likely candidates. These areas also receive projections from the LH and CeA (Berk and Finkelstein, 1982; van der Kooy *et al.*, 1984) and the RF sends axons to the motor nuclei involved in orofacial function (Travers and Norgren, 1983; Beckman and Whitehead, 1991). The RF and caudal NST also reciprocally

communicate with the rostral NST, mostly through the ventral (V) and medial (M) subdivisions (Travers, 1988; Beckman and Whitehead, 1991). Although the trajectories of the LH and CeA to the rostral NST terminate more in the V and M subdivisions (van der Kooy *et al.*, 1984; Halsell, 1998) than in the rostral central (RC) or rostral lateral (RL), where most taste neurons were recorded in the present study, subdivisions in the NST are interconnected with one another (Beckman and Whitehead, 1991). It is likely that some information from the LH and CeA is carried to the RF and from there to the NST and also to various motor nuclei involved in feeding behavior. Some descending influence may also occur through the PbN. A previous investigation reported that 6% of gustatory neurons in the NST were orthodromically activated from the ipsilateral PbN (Cho *et al.*, 2002a). This small descending projection has also been seen anatomically (Karimnamazi and Travers, 1998).

Taste information is transferred from the PbN bilaterally to the gustatory nucleus of the thalamus, the parvicellular division of the ventroposteromedial nucleus (VPMpc) (Norgren and Leonard, 1973; Halsell, 1992). Ventral forebrain nuclei send some axons to the NST on the contralateral side (van der Kooy *et al.*, 1984; Whitehead *et al.*, 2000) and there are commissural connections within the NST (Whitehead *et al.*, 2000). Antidromic activation of three NST neurons from the ipsilateral LH suggests that some taste neurons in the NST project directly to the LH, although direct projections to limbic forebrain areas have been shown previously only from caudal NST in rats (Ricardo and Koh, 1978). The mean latency following stimulation of the LH or CeA on the contralateral side was shorter than that on the ipsilateral side, implying that there may typically be fewer synapses between the contralateral LH or CeA and the NST. The present results imply that information from the contralateral side of the forebrain plays an important role in the descending gustatory pathway, although it is unknown where these descending inputs cross the midline en route to the NST.

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