Taste Responses of Neurons of the Hamster Solitary Nucleus Are Enhanced by Lateral Hypothalamic Stimulation

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Cho, Young K., Cheng-Shu Li, and David V. Smith. Taste responses of neurons of the hamster solitary nucleus are enhanced by lateral hypothalamic stimulation. J Neurophysiol 87: 1981–1992, 2002; 10.1152/jn.00765.2001. Gustatory responses in the brain stem are modifiable by several physiological factors, including blood insulin and glucose, intraduodenal lipids, gastric distension, and learning, although the neural substrates for these modulatory effects are not known. Stimulation of the lateral hypothalamus (LH) produces increases in food intake and alterations in taste preference behavior, whereas damage to this area has opposite effects. In the present study, we investigated the effects of LH stimulation on the neural activity of taste-responsive cells in the nucleus of the solitary tract (NST) of the hamster. Bipolar stimulating electrodes were bilaterally implanted in the LH, and the responses of 99 neurons in the NST, which were first characterized for their taste sensitivities, were tested for their response to both ipsilateral and contralateral LH stimulation. Half of the taste-responsive cells in the NST (49/99) were modulated by LH stimulation. Contralateral stimulation was more often effective (41 cells) than ipsilateral (13 cells) and always excitatory; 10 cells were excited bilaterally. Six cells were inhibited by ipsilateral stimulation. A subset of these cells (n = 13) was examined for the effects of microinjection of DL-homocysteic acid (DLH), a glutamate receptor agonist, into the LH. The effects of electrical stimulation were completely mimicked by DLH, indicating that cell somata in and around the LH are responsible for these effects. Other cells (n = 14) were tested for the effects of electrical stimulation of the LH on the responses to stimulation of the tongue with 0.032 M sucrose, NaCl, and quinine hydrochloride, and 0.0032 M citric acid. Responses to taste stimuli were more than doubled by the excitatory influence of the LH. These data show that the LH, in addition to its role in feeding and metabolism, exerts descending control over the processing of gustatory information through the brain stem.

INTRODUCTION

The responsiveness of some gustatory neurons in the nucleus of the solitary tract (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. However, the underlying neural substrates for these modulatory effects are not known. Because the lateral hypothalamus (LH) is involved in the regulation of feeding behavior and autonomic function (Bray 1985; Oomura and Yoshimatsu 1984; Panksepp 1975; Steffens et al. 1988) and reciprocally connected with the brain stem (van der Kooy et al. 1984; Whitehead et al. 2000), this area could be involved in the modulation of brain stem gustatory activity.

Several studies have previously linked the LH with gustatory and/or ingestive function. Electrical stimulation of the LH enhances the responses of rat NST neurons evoked by chorda tympani (CT) nerve stimulation (Bereiter et al. 1980) or by electrical stimulation of the anterior tongue (Matsuo et al. 1984). Stimulation of the LH induces feeding behavior (Frank et al. 1982; Shiraiishi 1991; Sweet et al. 1999) and alters taste preference behavior (Vasudev et al. 1985) in rats. Lesions of the LH reduce food intake and alter plasma glucose levels and taste preferences (Arase et al. 1987; Bernardis et al. 1990; Grossman and Grossman 1982; Touzani and Velley 1990). Some neurons in the LH in awake rats respond to taste stimuli applied to the oral cavity (Norgren 1970; Yamamoto et al. 1989) or alter their activity during food ingestion (Ono et al. 1986; Sasaki et al. 1984).

Some of the mechanisms of LH regulation of metabolism or taste-related behavior may be mediated through neuronal connections between the LH and the brain stem. There are descending projections from the LH to both the parabrachial nuclei (PbN) and NST (Berk and Finkelstein 1982; Hosoya and Matsushita 1981; Moga et al. 1990; van der Kooy et al. 1984; Whitehead et al. 2000). The LH projects bilaterally to the NST (van der Kooy et al. 1984; Whitehead et al. 2000). Because previous investigations of the effects of LH stimulation on NST neurons were limited to the ipsilateral LH and to NST cells responsive to electrical stimulation of either the CT nerve or the tongue, it is not known whether the activity of NST cells can be differentially modulated by the contralateral LH or whether LH stimulation alters the responses of NST neurons to gustatory stimuli.

In the present study, neurons in the NST, which were first characterized for their taste responsiveness, were tested for their response to both ipsilateral and contralateral LH stimulation. A subset of these cells was examined for the effects of microinjection of DL-homocysteic acid (DLH), a glutamate receptor agonist, into the LH and another for the effects of electrical stimulation of the LH on the responses to taste.

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stimulation of the tongue. These experiments tested the hypothesis that the lateral hypothalamus exerts a modulatory influence over gustatory neurons in the NST.

A portion of these results was presented at the 2000 meeting of the Society for Neuroscience, New Orleans, LA, and the 2001 meeting of the Association for Chemoreception Sciences, Sarasota, FL.

METHODS

Animals and surgery

Young adult male Syrian golden hamsters (Mesocricetus auratus), weighing between 150 and 250 g (n = 26), were deeply anesthetized with urethan (1.7 g/kg ip), and additional anesthetic was given as needed during the course of each experiment. A polyethylene tube was inserted into the trachea to allow the animal to breathe freely. Body temperature was maintained at 37 °C with a heating pad. To place the LH stimulating electrodes, the animal’s head was positioned in a stereotaxic instrument with the incisor bar at the same level as the interaural line. The tissue overlying bregma was cut along the midline and examined histologically to locate the LH. The coordinates for LH with the brain at this angle were determined histologically in several hamsters prior to this experiment. Briefly, penetrations were made into the hypothalamic area with a glass micropipette filled with 2% Chicago Blue dye, which was deposited with cathodal current (see following text). Brains were removed and sections were cut at 50 μm, and additional anesthetic was given as needed during the course of each experiment. A polyethylene tube was inserted into the trachea to allow the animal to breathe freely.

Body temperature was maintained at 37 °C with Urethan (1.7 g/kg ip), and additional anesthetic was given as needed during the course of each experiment. A polyethylene tube was inserted into the trachea to allow the animal to breathe freely. Body temperature was maintained at 37 °C with Urethan (1.7 g/kg ip), and additional anesthetic was given as needed during the course of each experiment.

A hole was drilled on each side of the skull to permit access to the LH. The coordinates for LH with the brain at this angle were determined histologically in several hamsters prior to this experiment. Briefly, penetrations were made into the hypothalamic area with a glass micropipette filled with 2% Chicago Blue dye, which was deposited with cathodal current (see following text). Brains were removed and sections were cut at 50 μm, and additional anesthetic was given as needed during the course of each experiment.

LH stimulating electrodes were implanted bilaterally and fixed in place with dental cement. Each bipolar stimulating electrode was composed of an insulated 140-μm-diam stainless steel wire inside 26-gauge stainless steel tubing. The components of this concentric electrode, except for the tip area, were insulated with epoxy 6001 (Epoxyllite, Irvine, CA). For stimulating the LH chemically, a double-barrel glass micropipette (tip diameter = 35 μm) was glued to the LH stimulating electrode with its tip positioned 0.2 mm above the inner wire of the electrode. After fixing the LH electrodes in place, the animal was placed in a nontraumatic headholder (Erickson 1966) with the head angled nose-downward 27° below the horizontal to straighten the brain stem 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% (wt/vol) 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 associated with the application of anodal current pulses (50 μA, 0.5 s, 0.33 Hz) applied to the anterior tongue, which drive taste fibers of the chorda tympani nerve (Smith and Bealer 1975), and then confirmed with chemical stimulation of the tongue. Extracellular action potentials were differentially amplified (NeuroLog System, Digitimer, 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. 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 99 taste-responsive cells recorded from the NST were 2.04 ± 0.11 (SD) mm anterior to obex and 1.35 ± 0.10 mm lateral to the midline. These cells were encountered from 0.5 to 1.0 mm below the surface of the brain stem.

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 ≥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 response profile.

LH stimulation and classification of LH-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, Quincy, MA), were delivered through each LH 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 orthorhomic response. For six cells, the initial 100-μA stimulus produced a decrease in activity; these cells were then examined with this stimulus intensity. Peristimulus time histograms (PSTHs) were created from the acquired data on each NST cell; action potentials associated with the LH pulse were accumulated over a 1-s period for 50–200 sweeps. A baseline period was defined as 200 ms preceding stimulation. The mean firing rate (number of impulses per 1-ms bin) during this 200-ms period was determined. The action potentials occurring during a period of 800 ms after LH stimulation were analyzed to determine the effects of the LH on NST activity. An excitatory response was defined as an epoch of at least five consecutive 1-ms bins with a mean value ≥2 SD above the baseline mean. 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 (which were rare) were defined as ≥20 consecutive bins for which the mean value was <50% of the baseline firing rate. Each taste-responsive cell for which an excitatory or inhibitory effect could be defined was categorized as LH responsive. Antidromically activated action potentials were observed in two cells in response to ipsilateral LH stimulation. Because these two cells also responded orthodromically to contralateral LH stimulation, they were categorized as LH-responsive neurons.

To examine the effects of electrical stimulation (ES) of the LH on the responses of NST cells to taste stimuli, responses of a subset of the LH-responsive neurons were recorded while trains of constant-current square pulses (100 Hz, 0.2 ms; at 0.9 times the lowest intensity that would produce an orthorhodmic response in each cell) were delivered to the LH during stimulation of the tongue with tastsants (duration = 15 s, starting 5 s prior to taste stimulation). For chemical stimulation of the LH, one barrel of the micropipette attached to the stimulating electrode was filled with 10 mM DLH (Aldrich Chemical, Milwaukee, WI) in buffered physiological saline (pH = 7.4) and the other with saline. DLH is an excitatory amino acid analogue, which presumably excites neuronal somata but not fibers of passage (Goodchild et al.
were compared using ANOVA. The effect of DLH (or saline) on LH-responsive and nonresponsive neurons and among taste stimuli reported as means responses to yield a net response (mean imp/s over 5 s). Responses are subtracting the 5-s prestimulus baseline from the /H9262 ring rates between LH-responsive and nonresponsive neurons and among taste stimuli were compared using ANOVA. The effect of DLH (or saline) on spontaneous activity, the effect of ES on the mean firing rate to taste stimuli, and the difference of excitatory latency of ipsi- and contralateral LH stimulation were compared using t-tests. The numbers of LH-responsive neurons following ipsilateral or contralateral LH stimulation were compared using the χ² test.

RESULTS

Histology

The recording and stimulating sites were examined histologically and representative examples are shown in Fig. 1. A Bilateral electrode tracks ending in iron deposits at the tips of the stimulating electrodes in the LH area are shown in Fig. 1A. The electrodes were positioned dorsal to the optic tract, ventral to zona incerta and dorsolateral to the ventromedial hypothalamic (VMH) nucleus. Data were obtained from 26 hamsters, and the locations of the stimulating electrodes in 24 of these animals are reconstructed on standard atlas sections (Morin and Wood 2001) in Fig. 2 (the 2 animals not depicted had cells that were excited bilaterally, although the tissue from these animals was not successfully recovered). The areas encompassing the effective stimulating sites in the LH are shown schematically in a mid-level section in Fig. 2A (same level as 2F), and the individual electrode placements are depicted in Fig. 2, C–H, arranged from rostral (2C) to caudal (2H). Sites evoking excitatory responses in gustatory cells of the NST are shown as ●, those producing inhibitory responses (restricted to the ipsilateral side) as ◊, and those which did not alter NST activity as ○. The two ipsilateral sites from which antidromic action potentials were activated are shown as △. The majority of effective sites were at the level of the VMH, posterior to the anterior hypothalamic (AH) nucleus (Fig. 2, F–H).

A recording site in the NST is shown in Fig. 1B, located medial to the solitary tract, most likely in the rostral central 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 1988; Whitehead and Frank 1983). 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). The position of the last cell to be recorded in each animal was marked with Chicago Blue (as in Fig. 1B), and the positions of these cells (n = 24) are depicted in Fig. 2B on a standard atlas section of the medulla at the level of the DC (Morin and Wood 2001).


LH responsiveness of NST taste cells

A total of 99 NST neurons were recorded that responded to taste stimulation of the anterior portion of the tongue. Of these, the activity of 49 was modulated by stimulation of the LH (see following text). Figure 3 shows the taste-evoked responses of 49 LH-responsive and 50 nonresponsive neurons in the NST. Within each group, cells are arranged along the abscissa into best-stimulus categories and within a category by the magnitude of their response to the best stimulus. Among the 49 LH-responsive neurons (Fig. 3A), there were 5 sucrose-best (cells 1–5), 15 NaCl-best (6–20), 15 citric-acid-best (21–35), and 14 QHCl-best (36–49) neurons. There were 12 sucrose-best (cells 50–61), 18 NaCl-best (62–79), 6 citric-acid-best (80–85), and 14 QHCl-best (86–99) cells among the nonresponsive neurons (Fig. 3B). The proportion of cells in each best-stimulus category was not significantly different between the LH-responsive and nonresponsive neurons (χ² = 3.54, df = 3, P = 0.316).
The firing rates (imp/s) of NST neurons in response to gustatory stimulation were not significantly different between the LH-responsive and nonresponsive neurons. Responses to the four taste solutions (means ± SE) averaged across all cells in each category were 3.82 ± 0.40 for the LH-responsive and 4.02 ± 0.44 for the nonresponsive neurons. Mean net responses to each of the four taste stimuli did not differ between the two groups \( F(1,388) = 0.113, P = 0.737 \). Although taste stimuli produced mean responses that differed from one another \( F(3,388) = 5.481, P < 0.01 \), there was no interaction between stimulus and LH responsiveness \( F(3,388) = 0.318, P = 0.813 \). Thus in terms of their basic responsiveness to gustatory stimulation, neurons that are modulated by descending projections from the LH do not differ from those that are not influenced by the LH.

**Electrophysiological features of LH-responsive neurons**

Stimulation of the LH resulted in the orthodromic excitation of 44 taste-responsive neurons in the NST as illustrated by the

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**FIG. 2.** Standard atlas sections of the hamster brain (adapted from Morin and Wood 2001), showing the distributions of stimulating and recording sites for 24 of the 26 experimental animals. A: section through the diencephalon at the level of the VMH (same section as in F), showing the composite distribution of effective stimulating sites in the LH. B: section through the medulla at the level of the DC, showing the distribution of the last NST cell recorded from each animal. C–F: successive sections through the hypothalamus, from the most rostral electrode placement (C) to the most caudal (H). 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; ⌂, sites that produced antidromic responses in NST neurons. 3V, 3rd ventricle; 7, facial nucleus; AH, anterior hypothalamus; Arc, arcuate hypothalamic nucleus; cp, cerebral peduncle; DM, dorsomedial hypothalamic nucleus; cp, inferior cerebellar peduncle; ml, medial lemniscus; MGP, medial globus pallidus; PH, posterior hypothalamic area; 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; Subl, subnucleus of the superior laminar tract; VMHL, VMHM, lateral and medial VMH; ZID and ZIL, dorsal and lateral zona incerta. Calibration bar = 1 mm in A, 500 μm in B–H.

**FIG. 3.** Taste responses (impulses/s) of 49 LH-responsive (A) and 50 nonresponsive (B) NST neurons. LH-responsive neurons are arranged along the abscissa according to their best stimulus, with neurons 1–5 being sucrose-best (●), neurons 6–20 NaCl-best (●), neurons 21–35 citric acid-best (●), and neurons 36–49 QHCl-best (●). In the same manner, nonresponsive neurons are arranged so that neurons 50–61 are sucrose-best (●), neurons 62–79 NaCl-best (●), neurons 80–85 citric acid-best (●), and neurons 86–99 QHCl-best (●). 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 mean 5-s prestimulus baseline from the mean of the first 5 s of the evoked responses to yield a net response (imp/s). The response profile for any 1 cell can be read from top to bottom. The spontaneous rate (mean response to distilled H2O during the 5 s prior to each stimulus) of each cell is shown at the bottom of the figure.
variable-latency action potentials of one cell in Fig. 4A. Each neuron was tested for 50–200 sweeps with an LH stimulus between 50 and 100 μA. A raster plot of the responses of one such neuron is depicted in Fig. 5A, showing the impulses occurring during 125 sweeps, from 200 ms before to 800 ms after the LH stimulus pulse. Also shown is the PSTH accumulated over these trials, indicating the occurrence of an evoked spike at ~18 ms after stimulus onset (time 0).

Additional examples of orthodromic responses in cells of the NST are shown as PSTHs in Fig. 5, B–E. The orthodromic responses, which were spread over a longer period, evoked in another cell are shown in Fig. 5B. Such longer-duration facilitations, which often involved more than one spike per sweep, were more common (41 of 54 responses) than only single impulses (as in Fig. 5A). Some excitatory responses were temporally complex. In Fig. 5A, for example, a small peak of impulses (at 100–150 ms) followed the initial response. The PSTHs of Fig. 5, D and E, are from the same cell, one evoked by ipsilateral (D) and the other by contralateral (E) LH stimulation. The ipsilateral response (D) showed an initial brief peak followed by a longer-duration response and then by a short period of inhibition, whereas the contralateral response (E) was only a longer-duration response. The PSTH in Fig. 5C shows an inhibitory response of an NST cell evoked by ipsilateral LH stimulation. Starting at 16 ms after LH stimulation, there was a period of 79 ms during which no action potentials were generated. Five additional cells were inhibited by ipsilateral LH stimulation over periods ranging from 40 to 80 ms.

Influence of LH stimulation on the spontaneous activity of gustatory NST neurons

LH stimulation modulated the ongoing activity of 49 of the 99 taste-responsive cells (LH responsive, 49.5%), contralaterally, ipsilaterally, or bilaterally (Table 1). Electrical stimulation of the LH excited orthodromic action potentials in 44 cells and inhibited spontaneous firing in 6 cells. One cell that showed an excitatory response to contralateral LH stimulation and an inhibitory response to ipsilateral stimulation was included in both counts. The remaining 50 taste-responsive neurons were not responsive to LH stimulation. With only one exception, every nonresponsive cell was recorded from an animal in which there were also LH-responsive neurons. Combined with the overlapping distribution of effective and noneffective stimulation sites (Fig. 2), this result suggests that it is unlikely that the failure of these 50 cells to respond to LH stimulation resulted from misplacement of the stimulating electrodes.

There were significantly more cells excited by LH stimulation than inhibited ($\chi^2 = 28.88, \text{df} = 1, P < 0.001$). Descending input from the contralateral LH (41 cells) more often had an excitatory influence on NST neurons than the input from the ipsilateral LH (13 cells; $\chi^2 = 14.52, \text{df} = 1, P < 0.001$). Among the 13 LH-responsive neurons that produced action potentials in response to ipsilateral LH stimulation, 10 were also excited by the contralateral LH. The inhibitory effect observed in 6 cells was produced only by stimulation of the ipsilateral LH.

Latency of excitatory responses to LH stimulation

The excitatory latency of 13 responses to ipsilateral LH stimulation ranged from 7 to 51 ms and that of 41 responses to contralateral stimulation from 8 to 42 ms. The mean excitatory latency of the ipsilateral responses was 27.62 ± 3.24 ms and that of the contralateral responses was 19.71 ± 1.43 ms. The response latency after contralateral LH stimulation was significantly shorter than that after ipsilateral LH stimulation ($t = 2.531, \text{df} = 52, P < 0.05$).

Twelve LH-responsive neurons generated single impulses following LH stimulation (as in Fig. 5A). The remaining LH-responsive cells, including those that were bilaterally responsive, produced longer-duration facilitation following LH stimulation (as in Fig. 5B). The mean latency of the longer-duration facilitation (23.76 ± 2.18 ms) was greater than that of the short-duration responses (15.00 ± 1.56 ms; $t = 2.891, \text{df} = 52, P < 0.01$). Among 10 neurons that produced bilaterally evoked action potentials, the mean latency after contralateral LH stimulation (22.60 ± 4.00 ms) was not significantly different from that after ipsilateral stimulation (29.10 ± 3.65 ms; $t = 1.315, \text{df} = 9, P = 0.221$).

Effects of DLH injection into the LH

For 13 neurons that were modulated by electrical stimulation of the LH, the effect of DLH (10 mM) microinjection into the LH on their spontaneous activity was compared with the effect of saline injection. Figure 6A shows that injection of DLH, but not saline, into the ipsilateral LH enhanced the spontaneous firing of an NST neuron over a period of ~2 min. Figure 6B shows an inhibitory effect on the spontaneous firing of another NST neuron following DLH microinjection. For 12 LH-responsive NST neurons that showed excitatory responses, firing rates before and after DLH microinjection were compared with those for saline injection (Fig. 6C). The mean firing rate before DLH was 1.61 ± 0.45 imp/s and was 6.16 ± 0.95 imp/s.
following injection. In comparison, the mean firing rates before and after saline were 1.40 ± 0.39 and 1.32 ± 0.40 imp/s, respectively. DLH microinjection into the LH mimicked the effect of electrical stimulation in all 13 cells tested: an excitatory influence in 12 cells and an inhibitory effect in the other. For these 13 cells, there was no effect of saline microinjection. This result suggests that the modulation of NST activity by electrical stimulation of the LH is likely due to its effects on cells within that area rather than on fibers of passage (Goodchild et al. 1982; Yang and Coote 1998).

Modulation of gustatory responses by electrical stimulation of the LH

The gustatory responses of 14 LH-responsive neurons to taste solutions were examined with electrical stimulation (ES) of the LH. Trains of constant-current square pulses were delivered to the LH stimulating electrode (100 Hz, 0.2 ms for 15 s) to investigate the modulatory effect on responses to sucrose, NaCl, citric acid, and QHCl. The intensity of the LH pulses was 0.9 times that of the weakest current that would evoke an orthodromic action potential; the ES train alone did not alter the firing of the NST neurons (see Fig. 8A, following text). Among these 14 cells, the spontaneous activity of 1 cell was suppressed and that of the other 13 was enhanced following single-pulse LH stimulation. The taste responses of five of these cells are shown in Fig. 7. For each of these neurons, the control response to each of the four stimuli is shown (Fig. 7A), including activity occurring 5 s before and after (D) the 10-s stimulus presentation (E). For the first four cells, the enhancement of the responses to several of the stimuli is shown during LH stimulation (Fig. 7B). For two of these cells (NST-41 and NST-37), all four stimuli were tested, and for two others (NST-28 and NST-43), all but the least-effective stimulus were tested during LH stimulation. The magnitudes of the gustatory responses were increased, whereas the spontaneous response (to distilled water) was not affected by the ES trains (which

| Table 1. Excitatory and inhibitory effects of LH stimulation on NST neurons |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Excitation*     | Inhibition      |
|                 | Ipsilateral     | Contra-lateral  | Bilateral       | Ipsilateral     | Contra-lateral  | Total          |
| Sucrose         | 1/17            | 3/17            | 1/17            | 2/17            | 0/17            | 5/17           |
| NaCl            | 4/33            | 12/33           | 2/33            | 2/33            | 0/33            | 15/33          |
| Citric acid     | 4/21            | 13/21           | 3/21            | 1/21            | 0/21            | 15/21          |
| QHCl            | 4/28            | 13/28           | 4/28            | 1/28            | 0/28            | 14/28          |

LH, lateral hypothalamus; NST, nucleus of the solitary tract. * A total of 44 cells was excited, but 1 cell excited by contralateral stimulation was also inhibited by ipsilateral stimulation.

FIG. 5. Orthodromic responses of NST neurons to electrical stimulation of the LH. A: raster plot and peristimulus time histogram (PSTH, 1-ms bins) of the impulses in an NST cell before and after contralateral LH stimulation. The 125 sweeps depicted in the raster plot were accumulated to produce the PSTH. Stimulus-evoked action potentials were elicited at ~18 ms after LH stimulation. B: PSTH of the firing of another NST cell following contralateral LH stimulation in which the evoked action potentials were more widely temporally distributed than those in A (200 sweeps). C: PSTH of a cell that was inhibited by ipsilateral LH stimulation (110 sweeps). D and E: PSTHs of activity in 1 NST cell, in which action potentials were evoked by both ipsilateral (D) and contralateral (E) LH stimulation (200 sweeps).
Gustatory responses of NST neurons can be inhibited by physiological factors associated with satiety, including blood insulin and glucose levels and gastric distension (Giza and Scott 1984; Whitehead et al. 2000), it is in a position to influence gustatory afferent activity. LH stimulation induces feeding behavior and metabolic homeostasis (Grossman and Krasne 1983, 1987; Giza et al. 1992, 1993; Glenn and Erickson 1976). These data suggest that taste activity may vary between states of hunger and satiety. Because the LH is involved in both physiological factors associated with satiety, including blood insulin and glucose levels and gastric distension (Giza and Scott 1984; Whitehead et al. 2000), it is in a position to influence gustatory afferent activity. LH stimulation induces feeding.

**DISCUSSION**

We found that half of the gustatory neurons in the hamster NST are subject to a potent descending influence from the LH. Gustatory responses of NST neurons can be inhibited by physiological factors associated with satiety, including blood insulin and glucose levels and gastric distension (Giza and Scott 1983, 1987; Giza et al. 1992, 1993; Glenn and Erickson 1976). These data suggest that taste activity may vary between states of hunger and satiety. Because the LH is involved in both feeding behavior and metabolic homeostasis (Grossman and Grossman 1982; Nicolaides 1981) and reciprocally connected to both pontine and medullary taste nuclei (van der Kooy et al. 1984; Whitehead et al. 2000), it is in a position to influence gustatory afferent activity. LH stimulation induces feeding.

**Antidromic response to LH stimulation**

Electrical stimulation of the LH produced antidromically activated action potentials in two NaCl-best neurons. Antidromic action potentials were evoked in these two cells by ipsilateral LH stimulation, although they both produced orthodromic action potentials in response to stimulation of the contralateral LH. Both cells met the three standard criteria for antidromic activation (Bishop et al. 1962) as depicted for one cell in Fig. 4B. The latencies of the antidromic spikes in these two cells were 17 and 23 ms and the thresholds were 13 and 10 μA, respectively. In addition to constant latency following ipsilateral LH stimulation (as depicted in Fig. 4B), these cells produced action potentials with a constant latency following paired-pulse stimulation at high frequency (>200 Hz at 1.2 times threshold; Fig. 4C). Collision tests were conducted at the same intensity as the paired-pulse stimulation, and the first evoked action potential of each pair was cancelled as it met the spontaneously generated action potential, which was used to trigger the paired-pulse stimulation (Fig. 4D).

**FIG. 6.** Effects of microinjection of DL-homocysteic acid (DLH) into the LH on responses of taste cells in the NST. A: PSTH (1-s bins) showing the effect of 10 mM DLH (at ↓) injected into the contralateral LH; injection of saline had no effect. B: PSTH showing the inhibitory effect of DLH injection into the ipsilateral LH (at ↓) and the failure of saline injection to produce inhibition. C: mean firing rate of NST neurons to DLH microinjection. Mean firing rate from 1 min before and after DLH injection was compared with that for saline injection in 12 LH-responsive NST neurons that were excited by LH stimulation. DLH produced a significant increase in firing rate (*) after microinjection but saline did not.

The percent increase in mean firing during the first 5 s of each response is indicated in Fig. 7B. In some instances, these increases were considerable (>100% enhancement) and in others quite small (e.g., 9%). The net effect was to alter the relative effectiveness of the four stimuli, enhancing the responses to some more than others, and to increase the signal-to-noise ratio. The last cell in Fig. 7 (NST-27) shows inhibition of the response to the cell’s best stimulus; in this case, the spontaneous activity was also reduced (but LH stimulus intensity was 100 μA, which reduces spontaneous firing—see METHODS). This cell was not tested for the effects of LH stimulation on the three ineffective stimuli. The direction of the effects of ES trains on gustatory responses in these 14 cells (enhancement or inhibition) was the same as seen in the single-pulse trials described in the preceding text.

The magnitudes of the responses to gustatory stimulation were compared with and without LH stimulation (Fig. 8). Mean responses to gustatory stimulation during ES from the 13 NST neurons that showed excitatory effects are depicted in Fig. 8A. The mean response to taste solutions (a total of 31 taste trials) without ES was 10.07 ± 1.44 imp/s, which increased significantly to 20.41 ± 2.28 during ES of the LH (t = 6.950, df = 30, P < 0.001). Mean spontaneous firing during the prestimulus period with and without ES was 2.00 ± 0.43 and 1.88 ± 0.31 imp/s, respectively (t = 0.257, df = 30, P = 0.799). Thus LH stimulation doubled the response of cells in the NST to taste stimulation, whereas the ES trains alone were without effect on the cells’ spontaneous activity (Fig. 8A), effectively doubling the signal-to-noise ratio. A comparison of the response of 12 cells that were tested with both their best and second-best stimuli during ES stimulation is shown in Fig. 8B. ES produced a significant increase in the response of both the best stimulus (t = 4.257, df = 11, P < 0.01) and the second-best stimulus (t = 3.787, df = 11, P < 0.01). Proportionately, the effect on the second-best stimulus (175.8% increase) was significantly greater (t = 3.667, df = 11, P < 0.01) than the increase in the response to the best stimulus (102.6%).
behavior and lesions of the LH reduce food intake (Arase et al. 1987; Frank et al. 1982; Grossman and Grossman 1982; Morgan 1961), and these effects have been shown to interact with taste-guided behavior (Conover and Shizgal 1994; Murzi et al. 1986; Touzani and Sclafani 2001; Touzani and Velley 1990; Vasudev et al. 1985). Whereas previous experiments in the rat have shown that LH stimulation activates NST neurons known to receive input from tongue afferent neurons (Bereiter et al. 1980; Matsuo et al. 1984; Murzi et al. 1986; Touzani and Velley 1990; Vasudev et al. 1985), the present investigation physiologically confirms a bilateral influence of the LH on the responses of NST neurons to gustatory stimulation of the tongue.

**Orthodromic responses of NST gustatory neurons to LH stimulation**

Ipsilateral LH stimulation enhances the spontaneous activity of some NST neurons in rats that respond to CT nerve stimulation (Bereiter et al. 1980) and to taste stimulation (Matsuo et al. 1984; Murzi et al. 1986). The response to electrical stimulation of the anterior tongue or CT nerve is also increased by LH stimulation in rats (Bereiter et al. 1980; Matsuo et al. 1984). However, none of these earlier experiments investigated the effects of LH stimulation on the gustatory responses of NST neurons or demonstrated any effect of contralateral stimulation of the LH. We observed that 49.5% of gustatory neurons in the hamster NST responded to LH stimulation with a greater influence from the contralateral LH. Forty-four neurons produced orthodromic action potentials in response to LH stimulation (41 of these were activated contralaterally and 10 bilaterally), whereas the spontaneous activity of 6 cells was suppressed, exclusively by ipsilateral stimulation (Table 1).

**FIG. 7.** Taste response profiles of several NST neurons to taste stimulation. A: the control response to each of the 4 basic stimuli (sucrose, NaCl, citric acid, and QHCl) are shown first for each of 5 NST neurons. B: the stimuli were repeated where possible during electrical stimulation of the LH, and these responses are shown on the right of the figure; those stimuli that were not tested during LH stimulation are indicated. For each of the 1st 4 neurons, LH stimulation markedly enhanced the response to taste stimulation but had no effect on the firing rate during the water rinse period. The percentage increase in mean firing rate during the first 5 s of each response is indicated in B. The last neuron (NST-27) was inhibited by electrical stimulation of the LH, and this produced a complete inhibition of both the response to sucrose and the water rinse (due to the LH stimulus strength, see METHODS).

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**Table 1.**

<table>
<thead>
<tr>
<th>Stimuli (20 1-s bins)</th>
<th>NST-41</th>
<th>NST-37</th>
<th>NST-28</th>
<th>NST-43</th>
<th>NST-27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>108</td>
<td>102</td>
<td>32</td>
<td>183</td>
<td>-100</td>
</tr>
<tr>
<td>NaCl</td>
<td>25</td>
<td>593</td>
<td>148</td>
<td>75</td>
<td>Not tested</td>
</tr>
<tr>
<td>Citric acid</td>
<td>9</td>
<td>46</td>
<td>265</td>
<td>Not tested</td>
<td></td>
</tr>
<tr>
<td>QHCl</td>
<td>Not tested</td>
<td>173</td>
<td>Not tested</td>
<td>Not tested</td>
<td></td>
</tr>
</tbody>
</table>

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**Orthodromic responses of NST gustatory neurons to LH stimulation**

Ipsilateral LH stimulation enhances the spontaneous activity of some NST neurons in rats that respond to CT nerve stimulation (Bereiter et al. 1980) and to taste stimulation (Matsuo et al. 1984; Murzi et al. 1986). The response to electrical stimulation of the anterior tongue or CT nerve is also increased by LH stimulation in rats (Bereiter et al. 1980; Matsuo et al. 1984). However, none of these earlier experiments investigated the effects of LH stimulation on the gustatory responses of NST neurons or demonstrated any effect of contralateral stimulation of the LH. We observed that 49.5% of gustatory neurons in the hamster NST responded to LH stimulation with a greater influence from the contralateral LH. Forty-four neurons produced orthodromic action potentials in response to LH stimulation (41 of these were activated contralaterally and 10 bilaterally), whereas the spontaneous activity of 6 cells was suppressed, exclusively by ipsilateral stimulation (Table 1). Matsuo et al. (1984) observed ipsilateral LH modulation of 62% of NST cells responding to gustatory, mechanical, or thermal stimulation; 12/17 (70.5%) of the taste-responsive cells were excited by the LH. In contrast, the number of
rat also found that this area of the hypothalamus was effective
were more rostral where the AH occurs. Previous studies in the
located in the LH at the level of the VMH, although a few sites
caudally to the midbrain tegmentum (Morin and Wood 2001). In the present experiment were most often
stimulating sites in the present experiment were most often
injections of DLH in
1982; van der Kooy et al. 1984), such strong stimulating
projections from other forebrain nuclei. Injections of DLH in
31) were acquired before and during
ES of the LH. Mean firing rate to gustatory stimulation during ES was significantly enhanced (\(^*\)), whereas the response to prestimulus water application was unaffected. B: in 12 neurons, both the best and second-best stimuli were tested after LH stimulation. Both were significantly enhanced (\(^*\)) by stimulation of the LH, although the percent increase of the response to the second-best stimulus was significantly greater than that for the best stimulus.

Connections between the LH and the rostral NST

The LH sends axons bilaterally to the NST, including the rostral gustatory portion, although the ipsilateral projection is heavier (Hosoya and Matsushita 1981; van der Kooy et al. 1984; Whitehead et al. 2000). There is a minor reciprocal ipsilateral projection from the rostral NST to the LH (Norgren 1978; Ter Horst et al. 1989). In the present study, antidromically evoked action potentials were observed in two NST cells after stimulation of the ipsilateral LH. These same two cells produced orthodromically activated impulses in response to contralateral LH stimulation. The latencies of the antidromic action potentials were 17 and 23 ms, which are shorter than the mean orthodromic latency following ipsilateral stimulation but longer than the orthodromic latencies (13 and 15 ms) in the same cells after contralateral LH stimulation. Over all the LH-responsive cells, the contralaterally evoked orthodromic responses had a mean latency (19.7 ms) that was significantly shorter than that produced by ipsilateral LH stimulation (27.6 ms). Such a result suggests that the descending inputs from the contralateral LH may be more direct than the ipsilateral ones. Although there are more cells with direct projections from the ipsilateral LH to the NST in hamsters (Whitehead et al. 2000), the latencies of the LH effects in the present experiment suggest that these descending effects are predominantly multisynaptic.

Some axons from the LH may synapse directly on taste-responsive neurons of the NST as suggested by the shorter latencies observed for orthodromic activation of some cells (7–8 ms), although without independent estimates of conduction velocity, this conclusion is only tentative. Other inputs may involve interneurons within the NST, projections from the opposite NST (Whitehead et al. 2000), or descending connections from the LH through the PbN (Hosoya and Matsushita 1981), which in turn sends some descending axons to the NST (Bianchi et al. 1998; Karimnamazi and Travers 1998). Indeed, we have recently observed a few cells that exhibit orthodromic impulses in the hamster NST following ipsilateral PbN stimulation (Cho et al. 2002). Descending connections may also flow from the LH through the medullary reticular formation into the NST (Travers 1988). Such indirect connections would be reflected in the longer latencies (42–51 ms) observed in many cells. The shorter mean latency for contralateral stimulation may reflect a less complex descending pathway to the NST than occurs ipsilaterally.

Taste response modulation by the LH

The ability of LH stimulation to evoke orthodromic responses in or inhibit the spontaneous activity of gustatory NST neurons does not directly demonstrate that the LH can modify gustatory afferent processing. Thus we tested a subset of taste-responsive neurons (\(n = 14\); Figs. 7 and 8) for the effects of electrical stimulation on the responses to taste stimuli applied to the anterior tongue. For these experiments, we kept the intensity of the LH stimulating pulses below threshold for the activation of orthodromic impulses or for the enhancement of

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**FIG. 8.** Mean responses to gustatory stimulation before and during LH stimulation. A: mean responses of 13 NST neurons that were excited by LH stimulation in which taste responses (\(n = 31\)) were acquired before and during ES of the LH. Mean firing rate to gustatory stimulation during ES was significantly enhanced (\(^*\)), whereas the response to prestimulus water application was unaffected. B: in 12 neurons, both the best and second-best stimuli were tested after LH stimulation. Both were significantly enhanced (\(^*\)) by stimulation of the LH, although the percent increase of the response to the second-best stimulus was significantly greater than that for the best stimulus.

![Graph A](https://example.com/graph-a.png)

![Graph B](https://example.com/graph-b.png)
spontaneous activity (see METHODS) and delivered the stimuli in a continuous pulse train during the presentation of taste stimuli. We found that electrical stimulation of the LH often produced potent excitation, and sometimes inhibition, of the taste responses evoked in these NST cells. The effects in each case were the same as observed on the cells’ spontaneous activity with higher stimulus intensities: either an increase or a decrease in the response to taste stimulation. For 13 cells that were excited by LH stimulation, the average taste response doubled during LH stimulation (Fig. 8A; see also individual cells in Fig. 7). The responses to the best and second-best stimuli were tested on 12 cells, and the percent increase was actually larger for the second-best stimulus (Fig. 8B), indicating that LH activity has the potential of altering the relative responses to different stimuli in NST neurons. Indeed, the stimulus producing the largest response in 5 of these 12 cells changed following LH stimulation.

Although we did not test all 49 LH-responsive neurons with this protocol, the fact that modulation of taste responses mimicked the influence of single-pulse stimulation on ongoing activity in all 14 cells indicates that the descending input from the LH functions to modulate taste information processing in the NST. The “spontaneous” response to the 5-s prestimulus water rinse applied before each taste solution was not altered by LH stimulation (Figs. 7 and 8A) except in the case of inhibition, where the LH stimulus intensity was 100 μA (see METHODS). For the excitatory responses, stimulus intensity during the taste stimulus trials was below that which would induce an orthodromic action potential in the NST (see Figs. 7 and 8A). These data show that neuronal activity in the LH can serve to increase the excitability of NST neurons to gustatory stimuli, biasing them to be more responsive to gustatory stimulation. The increased signal-to-noise ratio produced by LH stimulation would have the effect of making the entire gustatory system capable of finer taste discriminations because neural discriminability is dependent on the relationship between signal and noise (Pfaff 1975). Such a mechanism would predict that discriminations between palatable and aversive stimuli would be enhanced when neurons in the LH are active as during feeding.

There has been only limited work on the role of LH in guiding responses to taste stimuli. Global stimulation of the LH leads to increases in food intake (Shiraiishi 1991; Sweet et al. 1999) and increased consumption of taste solutions, even aversive ones (Vasudev et al. 1985). Damage to this area of the hypothalamus results in disruptions in feeding behavior (Grossman and Grossman 1982) and has been shown to alter taste preference for saccharin (Touzani and Velley 1990). However, because LH stimulation and disruption affect the motivation to feed, the specific effects of LH manipulations on behavioral responses to taste stimuli are difficult to interpret in a straightforward manner.

Changes in such variables as blood glucose and insulin and gastric distension associated with satiety appear to specifically decrease the response to sweet stimuli (Giza and Scott 1983, 1987; Glenn and Erickson 1976). However, the present data suggest that global activation of the LH has a more general excitatory effect on taste responses in the NST. More specific effects might result from particular neurotransmitter actions within the LH as has been shown for the effects of carbachol and angiotensin II on taste preferences toward saccharin and NaCl (Vasudev et al. 1985). Further experiments should be able to demonstrate exactly how the LH modulates the neural code for taste by examining such effects on a broader array of gustatory stimuli, both physiologically and behaviorally. Recent data also show that descending input from the central nucleus of the amygdala, which is another major gustatory relay in the ventral forebrain, modulates taste responses of neurons in the NST of hamsters (Li et al. 2000) and the PbN of rats (Lundy and Norgren 2001). Taken together, these data show that the responses of taste neurons in the brain stem are clearly subject to descending forebrain modulation, most likely reflecting the animal’s physiological state.

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