Taste Responses of Neurons in the Hamster Solitary Nucleus Are Modulated by the Central Nucleus of the Amygdala

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Received 2 April 2002; accepted in final form 27 August 2002

Li, Cheng-Shu, Young K. Cho, and David V. Smith. Taste responses of neurons in the hamster solitary nucleus are modulated by the central nucleus of the amygdala. J Neurophysiol 88: 2979–2992, 2002; 10.1152/jn.00239.2002. Previous studies have shown a modulatory influence of forebrain gustatory areas, such as the gustatory cortex and lateral hypothalamus, on the activity of taste-responsive cells in the nucleus of the solitary tract (NST). The central nucleus of the amygdala (CeA), which receives gustatory afferent information, also exerts descending control over taste neurons in the parabrachial nuclei (PbN) of the pons. The present studies were designed to investigate the role of descending amygdaloïd projections to the NST in the modulation of gustatory activity. Extracellular action potentials were recorded from 109 taste-responsive cells in the NST of urethane-anesthetized hamsters and analyzed for a change in excitability following electrical and chemical stimulation of the CeA. Electrical stimulation of the CeA orthodromically modulated 36 of 109 (33.0%) taste-responsive NST cells. An excitatory response was observed in 33 (30.28%) cells. An initial decrease in excitability to electrical stimulation of the CeA, suggestive of postsynaptic inhibition, was observed in three (2.75%) NST taste cells. NST cells modulated by the CeA were significantly less responsive to taste stimuli than cells that were not. Many of these cells were under the modulatory influence of the contralateral CeA (28/36 = 77.8%) as well as the ipsilateral (22/36 = 61.1%); 14 (38.9%) were excited bilaterally. Latencies for excitation were longer after ipsilateral than after contralateral CeA stimulation. Microinjection of DL-homocysteic acid (DLH) into the CeA mimicked the effect of electrical stimulation on each of the nine cells tested: DLH excited eight and inhibited one of these electrically activated NST cells. Application of subthreshold electrical stimulation to the CeA during taste trials increased the taste responses of every CeA-responsive NST cell (n = 7) tested with this protocol. These effects would enhance taste discriminability by increasing the signal-to-noise ratio of taste-evoked activity.

INTRODUCTION

The rostral subdivisions of the nucleus of the solitary tract (NST) receive topographically organized input from gustatory and somatosensory afferent fibers of the facial, glossopharyngeal, and vagal nerves that terminate in a rostral-caudal sequence (Allen 1923; Aström 1953; Beckstead and Norgren 1979; Contreras et al. 1982; Hamilton and Norgren 1984). The chorda tympani (CT) branch of the facial nerve innervates taste receptor cells in the fungiform papillae on the anterior portion of the tongue (Miller and Smith 1984). Axons of the CT terminate onto second-order neurons in the rostral portion of the NST (Contreras et al. 1982; Hamilton and Norgren 1984). In hamsters, 80% of NST cells that respond to taste stimulation of the anterior tongue send their axons to the parabrachial nuclei (PbN) of the pons (Cho et al. 2002a). From the PbN, taste information is carried to the thalamus and insular cortex and to several areas of the ventral forebrain, including the lateral hypothalamus (LH) and the central nucleus of the amygdala (CeA) (Halsell 1992; Lasiter et al. 1982; Norgren 1976).

There are centrifugal inputs to the NST from several of these forebrain gustatory projection areas, including the gustatory neocortex, the LH, and the CeA (Halsell 1998; Shipley 1982; van der Kooy et al. 1984; Whitehead et al. 2000). After microinjection of biotinylated dextran into the CeA, labeled fibers were differentially distributed throughout the rostrocaudal extent of the rat NST (Halsell 1998). Within the rostral NST, the medial, rostral central and ventral subdivisions had the highest density of terminal endings. After rostral NST injection of cholera toxin B, the CeA contained numerous retrogradely labeled cells, although there was no anterograde labeling of axons in the hamster forebrain (Whitehead et al. 2000). Stimulation of gustatory areas of the insular cortex in rat (Di Lorenzo and Monroe 1995) and hamster (Smith and Li 2000) and the LH in hamster (Cho et al. 2002b) modulates taste activity in the NST. Although the projection from the CeA to the NST has been shown anatomically in several rodent species, there is no direct evidence of a modulatory influence of CeA on gustatory cells in the NST. Taste responses of the rat PbN, which also receives centrifugal input from the CeA (Halsell 1992), are modulated by CeA stimulation (Lundy and Norgren 2001), although such effects could possibly reflect an influence of the CeA mediated through the NST.

Both the CeA and the basolateral amygdaloid nuclei (BLA) are heavily involved in associative processes underlying appetitive and aversive emotional behavior (Everitt et al. 2000). The CeA of the rat contains neurons that respond differentially to hedonically positive and negative gustatory stimuli (Nishijo et al. 1998), and both the BLA and the CeA appear to be involved in acquisition and/or retention of a conditioned taste aversion (CTA) (Lamprech et al. 1997; Yamamoto et al. 1994). Acquisition of a CTA by rats has been shown to alter...
gestatory processing within the NST (Chang and Scott 1984) and behavioral taste reactivity to the conditioned stimulus (Grill 1985).

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

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 hamsters, Mesocricetus auratus (135–180 g, n = 30) were deeply anesthetized with urethane (1.7 g/kg ip), and additional anesthetic was given as needed during the course of each experiment. The animal was tracheotomized and mounted in a stereotaxic instrument with the incisor bar at the same level as the interaural line. The tissue overlying the parietal bone was removed and a hole was drilled on each side of the skull. A concentric bipolar stimulating electrode, constructed from 26-gauge stainless steel tubing and 140-μm-thick stainless steel wire, was lowered into the CeA on each side of the brain (coordinates: 0.57 mm posterior to bregma, 3.9 mm lateral to the midline, and 5.4 mm ventral to the brain surface) and secured with dental cement. The electrodes, except for the tip area, were insulated with Epoxylite 6001 (Epoxylite, Irvine, CA). After implanting the stimulating electrodes into the CeA, the animal was mounted in a nontraumatic head holder (Erickson 1966) with the snout angled downward 27° from horizontal to straighten the brain stem and minimize brain movement associated with breathing (Van Buskirk and Smith 1981). A sagittal skin incision was made through the midline overlying the posterior skull, and a portion of the occipital bone just dorsal to the foramen magnum was removed to reveal the cerebellum. The dura covering the cerebellum was removed and the posterior portion of the cerebellum was aspirated to expose the floor of the IVth ventricle for 3–4 mm anterior to the obex. Body temperature was monitored and maintained at 37 ± 1°C (mean ± SD) with an electric heating pad.

Single-unit recording and electrical stimulation

Single-barrel glass micropipettes (tip diameter = 2 μm, resistance = 7–10 MΩ) filled with a 2% (wt/vol) solution of Chicago Sky Blue dye in 0.5 M sodium acetate were used for the extracellular recording of action potentials from the rostral NST. The mean coordinates for the NST recordings were 2.05 ± 0.14 (SD) mm anterior to the obex, 1.30 ± 0.13 mm lateral to the midline, and between 0.51 and 1.20 mm ventral to the surface of the brain stem. Extracellular potentials were amplified with a band-pass of 16–3,000 Hz (NeuroLog, Digitimer, Hertfordshire, UK), discriminated with a dual time-amplitude window discriminator (Bak DD1S-1, Bak Electronics, Germantown, MD), displayed on oscilloscopes, and monitored with an audio monitor. A Dell Pentium computer configured with a CED 1401-plus interface board and Spike2 software (Cambridge Electronic Design, Cambridge, UK) controlled taste stimulus delivery and online data acquisition and analysis. The taste responsiveness of the NST cells was initially identified by a change in neural activity associated with the application of anodal current pulses (50 μA, 0.5 s, 1/3 Hz) to the anterior tongue (Smith and Bealer 1975) and confirmed by responses to chemical stimulation of the tongue. The chemical stimuli presented to the anterior tongue were: 0.032 M sucrose, sodium chloride (NaCl), and quinine hydrochloride (QHC), and 0.0032 M citric acid. These concentrations evoke approximately equal multiunit taste responses in the hamster NST (Duncan and Smith 1992). The tastants were delivered by a gravity flow system composed of a two-way solenoid-operated valve connected via tubing to a distilled water rinse reservoir and a stimulus funnel. The taste trial, during which the computer acquired data, was a continuous flow initiated by the delivery of 5 s of distilled water, followed by 10 s of stimulus, followed by 5 s of distilled water rinse. The flow rate was 2 ml/s. After each taste trial, the tongue was rinsed with distilled water (>30 ml) and individual stimulations were separated by ≥2 min to avoid adaptation effects (Smith and Bealer 1976).

After each NST cell was characterized for its taste responsiveness, rectangular pulses (0.5 ms, ±0.1 mA, 1/3 Hz) were delivered to the CeA through each bipolar electrode from an isolated stimulator (Grass S88, Grass Instruments, Quincy, MA) to examine the effect of electrical stimulation of the forebrain on ongoing spontaneous activity of the NST cell. Peristimulus time histograms (PSTHs) were created from data acquired on each NST cell in response to 50–200 CeA stimulus pulses from each of the two CeA electrodes.

To observe the influence of electrical stimulation of the CeA on the responses of NST cells to taste stimuli, the responses of a subset of the NST cells to taste stimulation were recorded before and during trains of constant square pulses (100 Hz, 0.2 ms) to the CeA on each side. Typically, all four tastants were presented alone, and then the two most effective stimuli for each cell were repeated during stimulation of the CeA. The electrical stimulation started at the beginning of each taste trial (i.e., 5 s prior to taste stimulus delivery) and lasted for 15 s. To prevent CeA-evoked spikes from contributing to the taste responses, the intensity of the CeA stimulation was adjusted to 0.9 times the minimum intensity that would orthodromically activate the NST cell using single pulses at 1/3 Hz. That is, train stimulation of the CeA did not produce orthodromic action potentials in any NST cell (see Results). Stimulus artifacts during the pulse train were eliminated from the recorded data using the dual time-amplitude window discriminator.

Pharmacology and microinjections

To examine the effect of chemical stimulation of the CeA on the spontaneous activity of taste-responsive NST cells, ~50 nl of 10 mM dl-homocysteic acid (DLH; Aldrich Chemical, Milwaukee, WI) in buffered physiological saline (pH = 7.4) was pressure injected into the CeA. DLH is an excitatory amino acid analogue that presumably excites neuronal somata but not fibers of passage (Goodchild et al. 1982; Yang and Coote 1998). Microinjections were made using a double-barrel glass pipette glued to the stimulating electrode implanted in the CeA (pipette tip = 35 μm diameter, 0.2 mm above the inner wire of the stimulating electrode). Pressure pulses (30 psi, 10 ms) from a Picospritzer II (General Valve, Fairfield, NJ) were used to trigger the microinjections. Injection volume was estimated by observing the meniscus in the injection pipette (Smith and Li 2000). Injections of physiological saline served as a control for possible pressure effects of microinjection.

Histology

At the end of each experiment, the last recording site of the day was marked by passing a 10-μA cathodal current through the recording electrode for 10 min (5 s on-off) to deposit a spot of Chicago Blue dye. The stimulation sites in the CeA were also marked by passing 10-μA anodal current through the inner wire of the stimulating electrode for 20–30 s to deposit a spot of iron. The hamster was then given an overdose of urethane and perfused through the heart with 4%
formalin containing 3% potassium ferrocyanide and ferricyanide. Brains were removed, fixed, frozen sectioned (40 μm) in the coronal plane, and stained with Neutral Red. The recording and stimulating sites were located microscopically and plotted on standard atlas sections (Morin and Wood 2001).

Data analysis

The responses of each cell to taste stimulation of the tongue were accumulated over three consecutive time periods: 5 s of prestimulus water rinse, 10 s of stimulus flow, and 5 s of poststimulus water rinse. The net response was calculated as the mean number of action potentials (imp/s) during the first 5 s of chemical stimulation minus the number during the 5-s prestimulus water rinse (Vogt and Smith 1993). Responses are reported as means ± SE For orthodromic responses of NST cells to electrical stimulation of the CeA, individual PSTHs were analyzed to determine excitatory or inhibitory epochs. A baseline period was defined as the 200 ms preceding stimulation; the mean ± SD of the number of spikes/1-ms bin in this baseline period were determined. An excitatory effect of CeA stimulation was defined as an epoch of at least five consecutive bins with a mean value ≥2 SD above the baseline mean. Using a 5-ms window allowed us to clearly identify the very short-duration responses (see following text) as well as those of longer duration; a mean response ≥2 SD above background occurs with a probability <0.05. Inhibitory responses were defined as ≥20 consecutive bins with a mean <50% of baseline firing rate. Because of the slow rates of spontaneous firing of many NST cells and their asynchronous discharge patterns, a criterion for inhibition based on variance is not practical; using 20 bins defines the inhibitory epoch as a relatively sustained decrease in firing rate. For statistical comparison of the effects of DLH on the responses of NST cells, the mean firing rate (imp/s) in each cell over a 1-min period before DLH administration was compared with the mean firing rate over a 1-min period following DLH injection.

Differences in mean firing rates between CeA-responsive and nonresponsive neurons and among taste stimuli were compared using ANOVA. The effect of DLH (or saline) on spontaneous activity, the effect of electrical stimulation on the mean firing rate to taste stimuli, and the difference of excitatory latency of ipsi- and contralateral CeA stimulation were compared using f-tests. The numbers of CeA-responsive neurons following ipsilateral or contralateral CeA stimulation were compared with the χ² test.

RESULTS

Histology

The recording and stimulating sites were examined histologically and representative examples are shown in Fig. 1. Although only the ipsilateral side of the brain is depicted here, a clear iron deposit from the tip of the stimulating electrode is located at the center of the CeA, within the capsular portion (CeC) of the nucleus (Fig. 1A). 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 where the caudal border of the dorsal cochlear nucleus (DC) is first apparent on the dorsolateral margin of the medulla, which is the area of the NST receiving its predominant gustatory input from the VIIth nerve (Whitehead 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).

Data were collected from 30 hamsters, and the locations of the stimulating electrodes in these animals were reconstructed on standard atlas sections of the hamster brain (Morin and Wood 2001), shown in Fig. 2. The areas encompassing the effective stimulating sites in the CeA are shown schematically in a midlevel section in Fig. 2A (same level as 2, E and F) and individual electrode placements are depicted in Fig. 2, C–H, arranged from rostral (C and D) to caudal (G and H). Filled circles show the placements that produced excitatory responses in gustatory cells of the NST, half-filled circles indicate the three sites that induced inhibitory responses, and open circles show electrode placements that did not alter NST activity. In two hamsters, in which the stimulating electrodes were outside the CeA, there was no effect of forebrain stimulation; these animals were not included in the analysis. The tips of the stimulating electrodes in the 30 hamsters included in the study were confined to the CeA, concentrated in the more dorsal portion (Fig. 2). The stimulating sites were distributed from the level of the first appearance of the anterodorsal nucleus of the
Stereotaxically activated action potentials in cells of the NST. Two
electrical stimuli were applied to the CeA orthodromically modu-
larized action potentials described above in Fig. 3: short-duration excitation (PSTH with <10-ms response; Fig.
4B) and longer-duration excitation (>10 ms in duration; Fig.
4A). The cells showing short-duration excitation often re-
ponded with a single action potential; the distribution of the
evoked spikes varied by <10 ms from sweep to sweep, as in
Fig. 3, D–F, and Fig. 4B (mean duration = 5.64 ± 0.82 ms,
n = 14). Across the 33 cells that were excited by CeA stim-
ulation, there were 47 excitatory responses: 14 short-duration
and 33 longer-duration responses. Five of 21 (23.8%) cells
showed short-duration excitation following ipsilateral CeA
stimulation and 9 of 26 (34.6%) showed this response pattern
following stimulation of the contralateral CeA.

Neurons showing longer duration excitatory responses (Fig.
4, A and E) displayed a more prolonged change in excitability
with durations ranging from 30 to 130 ms (mean = 26.21 ±
1.45 ms, n = 33). Sixteen of 21 (76.2%) and 17 of 26 (65.4%)
cells showed this response pattern after ipsilateral and con-
teralateral CeA stimulation, respectively. Neurons showing this
longer-duration response typically responded to electrical stim-
ulation of the CeA with more than one action potential per
sweep (as in Fig. 3, A–C).

These basic excitatory responses were sometimes accompa-
nied by a later inhibitory component, as illustrated in Fig. 4, C
and D. This cell showed a brief excitatory response followed
by a silent interval of 180 and 220 ms in response to ipsilateral
and contralateral CeA stimulation, respectively. In this case,
the ipsilateral excitation (Fig. 4C) was of the longer-duration
type and the contralateral (Fig. 4D) of short duration. In total,
there were nine instances of this type of postexcitatory depres-
sion: two after short-duration excitation and seven after longer-
duration excitatory responses. The presence of this prominent
postexcitatory inhibition suggests multiple synaptic in-
fluences from the CeA. Of these cells showing postexcitatory depres-
sion, four cells showed a more complicated response pattern
(Fig. 4F). These cells initially showed an excitatory response,
followed by a period of inhibition (as described in the preced-
ten text) and then by a very prolonged excitation with a
duration of 600–960 ms (as in Fig. 4F).

Three NST taste cells were simply inhibited by electrical
stimulation of the CeA (as in Fig. 4G); one cell was inhibited
by ipsilateral and the other two by contralateral CeA stimu-
lation. No cell was inhibited bilaterally. The duration of the silent
period in spontaneous firing produced by electrical stimulation
of the CeA ranged from 65 to 120 ms. In these three cells, no
enhancement of excitability was ever observed.

The orthodromic response latencies were determined for
each excitatory response to CeA stimulation. The latencies of the 14 NST cells that responded to CeA stimulation bilaterally are shown in Fig. 5 A. Each of these 14 cells displayed a longer latency after ipsilateral than after contralateral CeA stimulation ($t = 4.886$, df = 13, $P = 0.001$). The response latencies of these 14 cells varied from 8 to 44 ms (mean = 29.43 ± 2.88 ms) for ipsilateral stimulation and 4–32 ms (mean = 17.14 ± 2.46 ms) for contralateral stimulation (Fig. 5B).

The overall latencies for orthodromic excitation in all 33 NST cells after CeA stimulation ranged from 4 to 44 ms, with a mean latency of 22.8 ± 1.6 ms. The mean latency was significantly longer in response to ipsilateral CeA stimulation than with contralateral stimulation ($t = 4.618$, df = 45, $P < 0.001$), as depicted in Fig. 5C for all 33 neurons. Latencies ranged from 5 to 44 ms ($n = 21$ cells, mean = 29.67 ± 2.27 ms) for ipsilateral stimulation and 4–32 ms ($n = 26$ cells, mean = 17.19 ± 1.59 ms) for contralateral stimulation. In general, short-duration responses occurred with shorter latency than the longer-duration responses ($r = +0.63$, $P < 0.01$).

### Taste response characteristics of NST cells

The overall spontaneous firing rate of 109 taste-responsive NST cells varied between 0 and 15.9 imp/s and the mean firing rate was $2.12 ± 0.27$ imp/s. There was no significant difference in spontaneous firing rate between cells that received modulatory input from the CeA (range = 0–11.1 imp/s, mean = 1.64 ± 0.32 imp/s) and the cells that were not modulated by the CeA (range = 0–15.9 imp/s, mean = 2.35 ± 0.38 imp/s; $t = 1.221$, df = 107, $P = 0.225$). Each of the 109 NST cells was

<table>
<thead>
<tr>
<th>Best Stimulus</th>
<th>CeA-Responsive Cells</th>
<th>Nonresponsive Cells</th>
<th>CeA Response Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>3</td>
<td>1 (1)*</td>
<td>17</td>
</tr>
<tr>
<td>NaCl</td>
<td>2 (1)</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>Citric acid</td>
<td>2</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>QHCl</td>
<td>0</td>
<td>3 (1)</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>7 (1)</td>
<td>12 (2)</td>
<td>73</td>
</tr>
</tbody>
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* Inhibited cells are shown in parentheses, except for last column, which has percentages in parentheses.
tested for its responsiveness to the four basic taste stimuli and categorized as sucrose-, NaCl-, citric acid-, or QHCl-best on the basis of its best stimulus. Of the 109 cells, 23 were sucrose-best, 36 were NaCl-best, 31 were citric acid-best, and 19 were QHCl-best cells. These best-stimulus categories are indicated in Fig. 6, where the responses of the cells in each best-stimulus group are arranged along the abscissa in order of their response to the best stimulus for that group. The cells in Fig. 6 are further divided into CeA-responsive (Fig. 6A) and nonresponsive groups (B). Among 36 NST cells that were modulated by electrical stimulation of the CeA, 6 were sucrose-best, 10 were NaCl-best, 10 were citric acid-best, and 10 were QHCl-best (Fig. 6A). Ten of 19 QHCl-best NST cells (52.6%) were CeA-responsive, whereas the proportions of CeA-responsive neurons in each of the other three best-stimulus groups were <33% (see Table 1). A comparison of the numbers of cells in each best-stimulus group between the CeA-responsive and nonresponsive categories, however, demonstrated that these proportions were not significantly different ($\chi^2 = 2.852, df = 3, P = 0.415$).

The mean responses to each of the four taste stimuli and the mean spontaneous activity of the CeA-responsive and nonresponsive neurons are shown in Fig. 7. The mean net taste response of NST cells that were modulated by CeA stimulation was 2.80 ± 0.34 imp/s, whereas the mean response of those cells that did not respond to CeA stimulation was 4.59 ± 0.41 imp/s; this difference was statistically significant [$F(1,428) = 8.178, P < 0.005$]. The mean responses to each of the four taste stimuli did not differ significantly from one another [$F(3,428) = 1.063, P = 0.364$] nor was there any interaction between the response to taste stimuli and responsiveness to the CeA [$F(3,428) = 0.279, P = 0.841$].
Effects of electrical stimulation of the CeA on taste-evoked responses

In a second set of experiments, we examined the influence of electrical stimulation of the CeA on the response of NST cells to taste stimulation of the anterior tongue. This experiment was conducted on a subset (n = 7) of the 36 NST cells that were modulated by single-pulse (i.e., 1/3 Hz) stimulation of the CeA. After confirming a cell’s response to single-pulse stimulation, a train of rectangular pulses (100 Hz, 0.2-ms duration) was delivered to the CeA during taste stimulation trials. To avoid any contribution of orthodromically activated spikes, the intensity of the current delivered to the CeA was adjusted to 0.9 times the minimum current intensity necessary to elicit a response in the NST cell at 1/3 Hz. These electrical stimulus trains did not produce any response in the NST cells as evidenced by the failure to see any difference in firing rate during the prestimulus water rinse in control versus train stimulation conditions (see Fig. 8F).

Responses of these seven NST cells to taste stimuli presented to the anterior tongue before and during train stimulation of the CeA were compared. Two of the tested cells were NaCl-best, two were citric acid-best, one was sucrose-best, and two were QHCl-best cells. Among these seven cells, three were excited by ipsilateral and four by contralateral CeA stimulation. The effect of electrical stimulation on one of these cells is shown in Fig. 8, A and B. This QHCl-best cell was briefly excited by single-pulse electrical stimulation of the CeA (Fig. 8A). In Fig. 8B, the same cell’s activity (imp/s) during each taste stimulus is shown in 1-s bins for the 5-s prestimulus period (□), the 10-s stimulus period (◆), and the first 5 s of the poststimulus rinse (○). This cell was more responsive to QHCl than to sucrose, NaCl or citric acid. When the cell was tested with QHCl (Fig. 8B; QHCL + ES) and with citric acid (citric acid + ES) during train stimulation, its response to taste stimulation was increased by 43 and 256%, respectively. There was no increase in the response during the prestimulus rinse (□ prior to taste response) during which time the train stimulation was also present. In Fig. 8, C-E, are taste responses of three additional cells, each tested during train stimulation for response to the two most effective taste stimuli and each showing enhanced responses to these stimuli during CeA stimulation.

The mean responses of these seven NST cells to taste stimulation before and during train stimulation of the CeA are shown in Fig. 8F. The mean net response of these cells to 12 taste trials without electrical stimulation of the CeA was 8.96 ± 1.26 imp/s. Application of electrical stimulation to the CeA while repeating the same taste trials doubled the net response to the taste stimuli (17.74 ± 2.10 imp/s; t = 6.315, df = 11, P < 0.001), whereas the baseline activity (i.e., during the prestimulus rinse) of the cells was not significantly different before (1.86 ± 0.62 imp/s) and during (2.36 ± 0.58 imp/s) CeA stimulation. The enhancement of taste responses by train stimulation of the CeA occurred in all cell types: NaCl-best, sucrose-best, citric-acid-best, and QHCl-best cells. In every instance, train stimulation of the CeA enhanced taste responses of NST neurons that were excited by single-pulse stimulation.

Effects of chemical stimulation of the CeA on the activity of NST cells

In a third set of experiments, we tested the influence of microinjection of DLH on the spontaneous activity of another subset of taste-responsive cells in the NST. Because DLH is thought to act on receptors located on dendritic and somatic membranes and not on fibers of passage (Goodchild et al. J Neurophysiol • VOL 88 • DECEMBER 2002 • www.jn.org
1982; Yang and Coote 1998), this experiment was designed to demonstrate that the results of electrical stimulation of the CeA are attributable to cells in and around this nucleus. For nine of the NST cells that were orthodromically activated by single-pulse stimulation of the CeA, DLH (50 nl, 10 mM pipette concentration) and physiological saline were microinjected through a double-barrel glass injection pipette attached to the CeA stimulating electrode. Among eight of these cells, four were excited following ipsilateral DLH injection and another four after contralateral application. The effect of DLH microinjection on spontaneous activity of one such NST cell is shown in Fig. 9. This cell was briefly excited by single-pulse electrical stimulation of the contralateral CeA (Fig. 9A). Similarly, its ongoing spontaneous activity was increased over a period of about 2 min by application of DLH into the contralateral CeA (Fig. 9B); there was no effect of saline injection into the CeA.

Microinjection of DLH into the CeA significantly increased the spontaneous activity of these eight NST cells ($t = 5.666$, df = 7, $P < 0.001$), as seen in Fig. 9C. The mean response of the cells before DLH injection was 1.1 ± 0.06 imp/s, which was increased to 5.4 ± 1.0 imp/s by DLH. Saline injection was without effect (1.1 ± 0.5 vs. 1.2 ± 0.6 imp/s). There was one NST cell inhibited by both electrical and DLH stimulation of the contralateral CeA. DLH eliminated the firing activity of this cell over a 2-min period (Fig. 4H). In every instance, DLH stimulation of the CeA mimicked the effect of electrical stimulation: either excitatory or inhibitory.

**FIG. 6.** Taste responses (imp/s) of 36 CeA-responsive (A) and 73 nonresponsive (B) NST neurons. CeA-responsive neurons are arranged along the abscissa according to their best stimulus, with neurons 1–6 being sucrose-best (○), 7–16 NaCl-best (■), 17–26 citric acid-best (□), and 27–36 QHCl-best (●). In the same manner, nonresponsive neurons are arranged so that 37–53 are sucrose-best (○), 54–79 NaCl-best (■), 80–100 citric acid-best (□), and 101–109 QHCl-best (●). Cells are arranged within each best-stimulus group according to the magnitude of the response to their best stimulus. 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.
responsive to all stimuli than those that were unaffected by CeA stimulation.

Despite the significantly less firing of the 36 CeA-responsive neurons and the 73 nonresponsive NST cells. Cells that were modulated by the CeA were significantly less than those that were unaffected by CeA stimulation.

DISCUSSION

The present investigation has demonstrated for the first time that some taste-responsive NST neurons are subject to a modulatory influence from the CeA and that this descending projection affects the responsiveness of these neurons to taste stimulation. Several neuroanatomical studies have shown that the NST receives descending projections from the CeA in both rats and hamsters (Halsell 1998; Krettek and Price 1978; van der Kooy et al. 1984). Electrical stimulation of the NST both antidromically and orthodromically activates neurons in the CeA (Rogers and Fryman 1988). Earlier studies have shown that electrical stimulation of the gustatory cortex (Smith and Li 2000) and the LH (Bereiter et al. 1980; Matsuo et al. 1984, Murzi et al. 1986) also modulates the activity of NST cells, including their response to gustatory stimuli (Cho et al. 2002b). There is also evidence that the CeA (Lundy and Norgren 2001) and the gustatory cortex (Di Lorenzo and Monroe 1992) modulate the activity of taste cells in the Pnn. Generally, descending influences from the forebrain are excitatory, although there is some evidence for inhibitory effects as well (Di Lorenzo 1988; Di Lorenzo and Monroe 1995; Hayama et al. 1985; Lundy and Norgren 2001; Mark et al. 1988; Smith and Li 2000). Similar results were observed in the present study: 33 of 36 NST cells that were modulated by CeA stimulation were excited and only 3 were inhibited.

Connections between the CeA and the NST

Although neuroanatomical studies have shown that direct descending projections from the CeA to the NST are predominantly ipsilateral in rats (Halsell 1998; van der Kooy et al. 1984), the present data in hamsters show that there is an equally strong influence on NST neurons from the contralateral CeA. In all, 28 NST cells were modulated by contralateral CeA stimulation and 22 responded to stimulation of the ipsilateral CeA (see Table 1). More interestingly, the mean latency of orthodromic excitation was shorter after contralateral than following ipsilateral CeA stimulation (Fig. 5C), even although the distance from the ipsilateral CeA to the NST is undoubtedly shorter than from the contralateral. These differences in latency are most convincing for the 14 bilaterally modulated NST cells (Fig. 5, A and B), all of which showed shorter latency with contralateral stimulation. Such a result suggests that descending inputs from the contralateral CeA may be more direct than ipsilateral ones, although their latencies suggest that these pathways are predominantly multisynaptic.

Some axons from the CeA may synapse directly on taste-responsive neurons of the NST, as suggested by the shorter latencies observed for orthodromic activation of some cells (4–5 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 CeA through the Pnn (Moga et al. 1990), 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 in the hamster NST that exhibit orthodromic impulses following ipsilateral Pnn stimulation (Cho et al. 2002a). Descending connections may also flow from the CeA through the medullary reticular formation into the NST (Travers 1988). Such indirect connections would be reflected in the longer latencies (22–44 ms) observed in many cells.

Although the activation of taste-responsive NST neurons by electrodes implanted in the CeA could possibly modulate responses of NST cells by activating axons passing the vicinity of the CeA, the effects of DLH suggest that this is not the case. In the present study, the enhancement or suppression of NST responses produced by electrical stimulation of the CeA was reproduced in every cell tested by microinjection of the glutamate receptor agonist, DLH, into the CeA. DLH injection enhanced the spontaneous activity of seven NST cells that were excited by electrical stimulation of the CeA (Fig. 9) and depressed spontaneous firing of one cell that was inhibited by electrical stimulation of the CeA (Fig. 4H). Immunohistochemical studies have shown many glutamate immunoreactive neurons in the amygdaloid complex, including the CeA (McDonald et al. 1989) and the presence of glutamate receptors within the CeA (Rogers et al. 1991). The DLH results indicate that the effects observed in the present study with electrical stimulation were likely due to stimulation of cells within the amygdala and not fibers of passage.

Recent evidence has shown that in rats a major descending projection from the CeA to the NST is GABAergic (Saha et al. 2000). This projection terminates largely within the ventral portions of the entire rostrocaudal extent of the NST. These same authors also demonstrated that this descending pathway was not glutamatergic. Although the majority of the effects of CeA stimulation shown in the present experiments were excitatory, some of the longer latency excitatory responses could have resulted from inhibition of the tonic GABAergic network within the NST (Liu et al. 1993; Wang and Bradley 1993). It is also possible that the nature of this descending pathway differs between rats and hamsters.

FIG. 7. Mean net responses (±SE) to each of the 4 taste stimuli and mean spontaneous rate of the 36 CeA-responsive neurons and the 73 nonresponsive NST cells. Cells that were modulated by the CeA were significantly less responsive to all stimuli than those that were unaffected by CeA stimulation.
Taste response modulation by the CeA

Electrical stimulation of the CeA orthodromically activated ~1/3 of the cells in the NST that responded to taste stimulation of the anterior tongue. When a subset of these cells was tested with gustatory stimuli during stimulation of the CeA, their taste responses were significantly increased. Similar to what we observed following stimulation of the LH (Cho et al. 2002b), there did not appear to be any selectivity in enhancement of taste responsiveness. Rather, responses to all stimuli tested

FIG. 8. Taste response profiles of several NST neurons to taste stimulation before and during subthreshold CeA stimulation. A: this PSTH depicts the response of neuron CeA-NST-15 to single-pulse electrical stimulation of the CeA, showing a brief excitatory response. B: the control response to each of the 4 basic stimuli (sucrose, NaCl, citric acid, and QHCl) are shown first for the same NST neuron as in A. The responses (imp/s) during the 10-s stimulus; *, 5-s prestimulus and poststimulus water rinse periods; each histogram reflects 20 s of activity. After the 4 taste stimuli are the responses to the 2 most effective stimuli in the presence of electrical stimulus (ES) trains delivered to the CeA. The responses to both QHCl and citric acid were enhanced by CeA stimulation. The numbers above the stimulus ES histograms indicate the percent increase in net firing rate over the first 5 s of the taste response. C–E: control responses and responses to the 2 most effective taste stimuli during ES for 3 additional NST neurons. For each of these cells, CeA stimulation enhanced the response to taste stimulation (note percent increases) but had no effect on the firing rate during the water pre rinse. F: mean ± SE firing rates to all taste stimuli tested before (taste) and during (taste + ES) electrical stimulation of the CeA. Activity during the 5-s pre rinse period (□) was unaffected by ES, whereas the response to taste stimuli (■) was significantly (*) increased.

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after stimulation of the CeA were increased. Also, CeA stimulation modulated the response of all cell types (sucrose-best, QHCl-best, etc.). Because the taste response of every CeA-responsive cell tested with taste stimulation was modulated by the CeA, it is highly likely that all of the cells responsive to single-pulse electrical stimulation (Table 1) would show a similar enhancement or suppression of taste activity. These results suggest that descending input from the CeA modulates the processing of taste information in 1/3 of the taste-responsive cells of the NST.

The gustatory responses of the NST cells that were activated by stimulation of the CeA were significantly lower than those that were not activated by CeA stimulation (see Fig. 7). Although the significance of this difference needs to be explored further, it is interesting to note that cells of the NST that do not project axons to the PbN are also significantly less responsive to taste stimuli than those projecting information to the forebrain (Cho et al. 2002a). We did not determine in the present experiment whether each NST cell was antidromically activated from the PbN, but these findings suggest the possibility that the CeA could differentially influence NST cells involved in local brain stem connections more than those responsible for sending gustatory information to the forebrain. In rats, descending projections from the CeA terminate largely within the ventral subdivision of the NST (Halsell 1998), which contains fewer neurons projecting to the PbN than to the caudal brain stem.

These data show that neuronal activity in the CeA can serve to increase the excitability of NST neurons to gustatory stimuli, biasing them to be more responsive to gustatory stimulation while the background firing rate is unaffected. Signal detection theory would predict that the increased signal-to-noise ratio produced by CeA stimulation would have the effect of making the gustatory system capable of finer taste discriminations because neural discriminability is dependent on the relationship between signal and noise (Pfaff 1975; see also Green and Swets 1966). Such a mechanism would predict that discriminations between palatable and aversive stimuli would be enhanced when neurons in the CeA are active. Such enhancements could be particularly important in the control of brain stem motor neurons important for reflexive oromotor responses to gustatory stimulation, which are modified by conditioned taste aversion (CTA) learning (Grill 1985).

A recent study in rats demonstrated that electrical stimulation of the CeA alters the taste-evoked activity of PbN neurons (Lundy and Norgren 2001). Although the effects of CeA on PbN cells were mostly suppressive, the overall effect was to enhance the salience of the response to NaCl, suggesting that descending control from the CeA could play a role in the mechanisms of sodium appetite. In contrast, the most common effect of CeA stimulation on cells of the hamster NST in the present experiment was excitation. These differences in the influence of the CeA on pontine and medullary cells could
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reflected species differences or suggest differential effects on PbN versus NST processing.

Neurons in the CeA project to a vast array of sites in the diencephalon and brain stem (Price and Amaral 1981), with heavy projections to both the PbN and the NST (Whitehead et al. 2000). Several subnuclei of the amygdala are important for CTA learning, including the acquisition of taste information, hedonic evaluation of that information and integration, retention, and expression of a CTA (Yamamoto et al. 1994, 1997). Both the CeA and the BLA are heavily involved in associative processes underlying appetitive and aversive emotional behavior (Everitt et al. 2000). The CeA contains neurons that respond differentially to hedonically positive and negative stimuli (Nishijo et al. 1998), and both the BLA and the CeA appear to be involved in acquisition and/or retention of a CTA (Lampecht et al. 1997; Yamamoto et al. 1994). Therefore it is possible that the altered gustatory activity in the NST that results from CTA (Chang and Scott 1984) could be mediated through descending influences such as those shown in the present experiment. In addition to its effects on ingestion, CTA alters dramatically the reflexive responses to gustatory stimuli (Grill 1985), which undoubtedly depend on connections between the NST and brain stem oromotor nuclei (Travers and Norgren 1983).

Further experiments should be able to demonstrate exactly how the CeA modulates the neural code for taste by examining such effects on a broader array of gustatory stimuli, both physiologically and behaviorally. There is now evidence that descending influences from the gustatory cortex (Smith and Li 2000), the lateral hypothalamus (Cho et al. 2002b), and the amygdala (Lundy and Norgren 2001; present data) all modulate gustatory activity within the NST and/or PbN. 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 physiologic state or previous experience.

Thanks to Drs. Matthew Ennis, John D. Boughter Jr., and Christian H. Lemon for valuable comments on the manuscript. This work was supported in part by National Institute on Deafness and Other Communication Disorders Grant DC-00666 to D. V. Smith.


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