Activity in the Hypothalamus, Amygdala, and Cortex Generates Bilateral and Convergent Modulation of Pontine Gustatory Neurons

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Submitted 27 August 2003; accepted in final form 13 November 2003

Lundy, Robert F., Jr. and Ralph Norgren. Activity in the hypothalamus, amygdala, and cortex generates bilateral and convergent modulation of pontine gustatory neurons. J. Neurophysiol. 91: 1143–1157, 2004. First published November 19, 2003; 10.1152/jn.00840.2003. Evidence suggests that centrifugal modulation of brain stem gustatory cells might play a role in the elaboration of complex taste-guided behaviors like conditioned taste aversion and sodium appetite. We previously showed that activity in one forebrain area, the central nucleus of the amygdala (CeA), increased the chemical selectivity of taste cells in the parabrachial nucleus (PBN). The present study investigates how activity in 2 other similarly interconnected forebrain sites, the lateral hypothalamus (LH) and gustatory cortex (GC), might influence PBN gustatory processing in rats. The potential convergence of descending inputs from these sites, as well as the CeA, was also evaluated. After anesthesia (35 mg/kg Nembutal ip), 70 PBN gustatory neurons were tested before, during, and after electrical stimulation of these forebrain sites, while responding to 0.3 M sucrose, 0.1 M NaCl, 0.01 M citric acid, and 0.003 M HCl. Although each forebrain site modulated taste-evoked responses, more PBN neurons were influenced by stimulation of the GC (67%) and CeA (73%) than of the LH (48%). Activation of cortex (71%) and amygdala (85%) most often produced inhibition, whereas inhibition and excitation occurred equally often during hypothalamic stimulation. Of the neurons tested for convergence (n = 60), 88% were influenced by ≥1 of the 3 sites. Twenty were modulated by stimulation at all 3 sites and another 17 by 2 of the 3 sites. The net effect of centrifugal modulation was to sharpen the across-stimulus response profiles of PBN cells, particular with regard to the NaCl- and citric acid–best cells.

INTRODUCTION

Reciprocal connections between neocortex and subcortical relay nuclei are a common feature of all sensory systems. This anatomical arrangement provides a substrate for higher-order activity to modulate earlier stages of sensory processing. For the gustatory system, at least 3 forebrain sites, the lateral hypothalamus (LH), central nucleus of the amygdala (CeA), and gustatory cortex (GC), receive taste information (Nishijo et al. 2000; Norgren 1976; Yasoshima et al. 1995) and send projections back to brain stem taste relays (Hopkins and Holstege 1978; Van der Kooy et al. 1984; Veening et al. 1984). This feedback from more rostral structures might be functionally important because decerebrate animals that lack connections between the forebrain and brain stem fail to express learned and physiologically dependent taste-guided behaviors like conditioned taste aversion and sodium appetite (Grill 1985; Grill et al. 1986). In normal animals, the induction of both behaviors selectively alters gustatory-evoked responses in the brain stem (Chang and Scott 1984; Nakamura and Norgren 1995; Shimura et al. 1997a,b; Tamura and Norgren 1997; Yasoshima et al. 1995).

Prior studies have shown that descending input from the LH, CeA, and GC influences taste responsive neurons in the medulla and pons. In the medullary nucleus of the solitary tract (NST), electrical stimulation of the LH and CeA is known to modulate both background discharge and taste-evoked responses (Bereiter et al. 1980; Cho et al. 2002a; Li et al. 2002; Matsuo et al. 1984; Murzi et al. 1986). Cortex stimulation also modulates the spontaneous activity of taste responsive cells, but the effects on rapid stimulus responsiveness have not been tested (Smith and Li 2000). Nevertheless, temporary elimination of GC input does influence tastant-elicted responses in the NST (DiLorenzo and Monroe 1995). The influence of forebrain inputs on taste processing in the pontine parabrachial nucleus, one synapse further along in the central gustatory system, is less well characterized.

In the parabrachial nucleus (PBN), only the effects of electrical activation of the CeA have been tested on taste processing (Lundy and Norgren 2001). Amygdala stimulation produced both inhibitory and excitatory modulation of taste-elicited responses. The net effect of this influence was to increase the chemical selectivity of pontine taste neurons. Cortex and hypothalamus stimulation, on the other hand, have been tested only on the spontaneous activity of taste-responsive cells (DiLorenzo and Monroe 1992; Murzi et al. 1986). Only cortex stimulation altered PBN discharge rate, but the lack of effect in the LH study should be tempered by the small sample of neurons tested (n = 12). Nevertheless, one earlier study showed that reversible lesions of the GC reduced the number of taste stimuli to which a given neuron responded (DiLorenzo 1990).

The present study investigates the influence of electrical activation of the LH and GC on PBN taste processing. In addition, we tested for convergence of centrifugal input from the LH and GC, as well as the CeA onto the same taste-responsive cells. Recent evidence in hamsters indicates that descending projections from the LH and CeA can converge on the same gustatory neurons in the NST (Cho et al. 2003).

A portion of this work was presented as a poster at the 2001 meeting of the Society for Neuroscience.

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METHODS

Subjects

Neurophysiological recordings were made in 17 male Sprague-Dawley rats weighing 365–470 g [Cr:CD (SD) BR; Charles River Breeding Laboratories]. The animals were maintained in a temperature-controlled colony room on a 12-h light/dark cycle and allowed free access to normal rat chow (Teklad 8804) and distilled water.

Forebrain-stimulating electrodes

The rats were anesthetized with a 50 mg/kg injection [intraperitoneally (ip)] of pentobarbital sodium (Nembutal). Additional doses of Nembutal (0.1 ml) were administered as necessary to continue a deep level of anesthesia. Rectal temperature was monitored throughout a recording session and maintained at 37 ± 0.2°. Animals were secured in a stereotaxic instrument; after which the skull was exposed with a free access to normal rat chow (Teklad 8604) and distilled water.

Forebrain-stimulating electrodes

...recording session, these rods were slid forward about 1 cm and the rigidly to the stereotaxic ear bars (Norgren et al. 1989). During leads and the conical ends of 4 stainless-steel rods that were attached the wires. A cap of dental acrylic was anchored to the skull using in a stereotaxic instrument; after which the skull was exposed with a free access to normal rat chow (Teklad 8604) and distilled water.

The stimulating electrodes were lowered through the cortical tissue using a custom-made jig that spaced the electrodes 0.5 mm apart and oriented them in the anterior–posterior plane. Relative to β, the stereotaxic coordinates for LH placement were P −2.0, L 1.7, and V 8.6; for the CeA, P −1.8, L 4.0, and V 8.5; and for the GC, A +1.0, L 4.0, and V 7.7. Electrodes implanted in the GC were oriented 10° off the vertical with the tips pointed laterally. The anterior and lateral coordinates above correspond to the insertion point for GC with the final resting place of the electrodes being approximately 5.6 L and 7.0 V (Paxinos and Watson 1986). A stainless-steel wire twisted around a posterior skull screw served as an indifferent electrode, whereas a different wire twisted around a rostral screw served as a ground lead. A “gold pin” connector (A-M Systems) was crimped around each of the wires. A cap of dental acrylic was anchored to the skull using stainless-steel screws. The cap then was fashioned around the wire leads and the conical ends of 4 stainless-steel rods that were attached rigidly to the stereotaxic ear bars (Norgren et al. 1989). During recording sessions, these rods were slid forward about 1 cm and the tips resected in the head cap, thus recording. These rods were slid forward about 1 cm and the rigidly to the stereotaxic ear bars (Norgren et al. 1989). During...
means ± SE. Data analyses were done using SPSS and values of $P < 0.05$ were considered significant.

**Histological processing and analyses**

At the end of the final recording session, the animals were killed with a lethal dose of Nembutal (100 mg/kg, ip) and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde. The brain was removed, cut coronally (50 μm) using a freezing microtome, and stained with cresyl violet. The stimulating electrode tracks were localized using a light-field microscope.

**RESULTS**

**Histology**

The representative photomicrographs in Fig. 1, A, B, and C show a stimulating electrode track in the gustatory cortex, central nucleus of the amygdala, and lateral hypothalamus, respectively. The arrow in each picture indicates the terminal end of 1 of the 3 electrodes placed in each of the sites. The neurophysiological data in the present study were obtained from animals in which the stimulating electrodes were histologically confirmed to terminate in the gustatory cortex, central nucleus of the amygdala, and lateral hypothalamus.

**Neuronal categorization**

Seventy parabrachial gustatory neurons (PBN) were tested, 60 of them before, during, and after electrical stimulation at each of the 3 forebrain sites and while responding to 4 sapid stimuli. On the basis of the stimulus that evoked the greatest neural discharge, 6 ipsilateral and 5 contralateral PBN cells were categorized as sucrose-best, 34 ipsilateral and 13 contralateral as NaCl-best, and 9 ipsilateral and 3 contralateral as citric acid–best. The mean corrected response rates to each gustatory stimulus plotted as a function of neuron type are shown in Fig. 2. Separate repeated-measures ANOVAs revealed a main effect for stimulus in sucrose-best \( [F_{(3,30)} = 20.1, P < 0.01] \), NaCl-best \( [F_{(3,135)} = 111.9, P < 0.01] \), and citric acid–best neurons \( [F_{(3,33)} = 35.2, P < 0.01] \). The order of stimulus effectiveness was sucrose = NaCl > citric acid > QHCl for sucrose-best cells, NaCl > CA > sucrose = QHCl for NaCl-best cells, and CA = NaCl > sucrose = QHCl for citric acid–best cells \( (P < 0.01 \text{ or } P > 0.1) \).

**Forebrain stimulation**

Figure 3 shows the response of a NaCl-best neuron to NaCl, CA, sucrose, and QHCl with and without concurrent gustatory cortex stimulation. The horizontal black dots in each trace correspond to 10 s of water flow and the solid black bar to sapid stimulus application. The label above each raw trace indicates the sapid stimulus. The pair of traces labeled with the stimulus and cortex indicates applications during gustatory

![Fig. 1. Photomicrographs of a coronal section through the gustatory cortex (A), central nucleus of the amygdala (B), and lateral hypothalamus (C). For reference, dotted lines were drawn around the approximate boundary of specific nuclei in each forebrain region. Also, the approximate stereotaxic level of each section is given relative to bregma (Paxinos and Watson 1986). Abbreviations: CL, claustrum; MA, medial nucleus of the amygdala; CeA, central nucleus of the amygdala; LH, lateral hypothalamus; VMH, ventral medial hypothalamus; OT, optic tract.](http://jn.physiology.org/)

![Fig. 2. Mean corrected response rates to each sapid stimulus in parabrachial nucleus (PBN) neurons categorized based on the stimulus that evoked the greatest discharge. Left: sucrose-best neurons \( (n = 11) \). Middle: NaCl-best neurons \( (n = 47) \). Right: citric acid–best neurons \( (n = 12) \).](http://jn.physiology.org/)
The response profiles of NaCl-best neurons inhibited by forebrain stimulation are shown in Fig. 4. The taste stimuli and the discharge rate of each cell during the "ON SECONDS" (e.g., 1, 3, 5, 7, and 9) of the control (open bars) and test applications (filled bars) are arranged from top to bottom and the different forebrain sites from left to right. The individual neurons are arranged by their response rate to NaCl in descending order. Taste-elicited responses were suppressed by concurrent electrical stimulation at all 3 forebrain sites [CeA: \( F_{(1,26)} = 94.4, P < 0.01 \); GC: \( F_{(1,19)} = 92.3, P < 0.01 \); LH: \( F_{(1,11)} = 24.9, P < 0.01 \)]. During the “OFF SECONDS” when no brain stimulation occurred (e.g., 2, 4, 6, 8, and 10) the control and test series responses were equivalent for each stimulation site (not shown, \( P > 0.6 \)). Using our definition of a significant response (i.e., \( \pm 2.0 \) SD) from the mean response to water, stimulation of the amygdala, cortex, and hypothalamus reduced the effectiveness of sucrose, acid, and quinine by 59% (Table 2). The forebrain stimulation had a much smaller effect on responses to NaCl in neurons that did not respond best to this stimulus (19%).

The difference between control and test responses during ipsilateral and contralateral stimulation of each forebrain site is shown in Fig. 5, A, B, and C. The graphs on the left show the differences during concurrent brain stimulation and those on the right when no brain stimulation occurred. During the “ON SECONDS,” all sites were effective for almost all rapid stimuli. For cortex and amygdala, the pattern of inhibition was virtually identical with the contralateral side dominant and the effects on responses to NaCl most pronounced (\( P < 0.01 \)). Stimulation of the LH produced smaller effects with neither side dominant (\( P < 0.01 \)). Significant carryover of inhibition was not evident during the “OFF SECONDS” (\( P \geq 0.09 \)). A small rebound in spontaneous activity and the response to NaCl was evident during the “OFF SECONDS” in ipsilateral neurons responsive to CeA stimulation (\( P \leq 0.04 \)).

### Excitation

A smaller number of the NaCl-best cells (\( n = 29, 22\% \) of the sample) were facilitated by forebrain stimulation. Although not shown, a significant interaction between rapid stimulus and application series was evident for CeA [\( F_{(3,18)} = 22.4, P < 0.01 \), GC [\( F_{(3,27)} = 33.0, P < 0.01 \), and LH [\( F_{(3,33)} = 19.1, P < 0.01 \)]. The specificity of this augmentation to the most effective stimulus (i.e., NaCl) is shown in Fig. 6A (\( P < 0.01 \)). Unlike centrifugally induced inhibition, however, the excitatory influence persisted during the “OFF SECONDS” when no brain stimulation occurred (Fig. 6B, \( P < 0.01 \)). In fact, the discharge rates to sucrose and citric acid also were elevated during the times between pulse trains (\( P \leq 0.04 \)), even though the rates were either inhibited or unaffected during concurrent stimulation. All but one of these NaCl-best cells were recorded ipsilateral to the stimulation site.

The spontaneous activity of 2 neurons in this sample was increased during CeA stimulation and of another during LH stimulation. The variable latencies of the driven activity (5–15 ms) and their inability to follow low-frequency stimulation (10 pulses/s) suggest that the effects were not attributed to antidromic activation of PBN neurons projecting to the lateral hypothalamus.

### Citric acid–best neurons

As with NaCl-best neurons, taste responses in acid-best neurons were more often affected by amygdala (67%) and cortex (67%) stimulation than by the hypothalamus (45%). Inhibitory and excitatory effects also occurred with similar frequency during LH stimulation (60% inhibition), whereas CeA and GC activation produced only inhibition (Table 1).

Figure 7A shows the mean discharge during the “ON SECONDS” of the control (open bars) and test applications (filled bars) for each of the forebrain sites. Compared with control responses, stimulation of the central nucleus [\( F_{(1,7)} = 20.4, P < 0.01 \)] and cortex [\( F_{(1,7)} = 20.8, P < 0.01 \)], but not the hypothalamus [\( F_{(1,2)} = 3.9, P = 0.18 \)] suppressed responsive-

### Table 1. Neurons influenced by forebrain stimulation

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>CeA</th>
<th>GC</th>
<th>LH</th>
<th>CeA</th>
<th>GC</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Best Total</td>
<td>6/9</td>
<td>4/11</td>
<td>3/10</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>3</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Contralateral</td>
<td>3</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N-Best Total</td>
<td>27/45</td>
<td>20/44</td>
<td>12/45</td>
<td>7</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>19</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Contralateral</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>A-Best Total</td>
<td>8/12</td>
<td>8/12</td>
<td>3/11</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Contralateral</td>
<td>3</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
</tbody>
</table>

Shown is the number of neurons that were inhibited and excited by electrical stimulation of each forebrain site, listed as a function of neuron type and laterality of the recording site relative to the stimulating site. In the Inhibited column, the denominator in the cells labeled Total corresponds to the number of each neuron type tested during stimulation of a particular forebrain site.

cortex activation; the vertical deflections in the top trace mark the onset of an individual pulse in the five 1-s trains delivered to the cortex. This figure shows that electrical stimulation of the gustatory cortex produced a significant reduction in the taste-evoked discharge rates.

**NaCl-best neurons**

The number of NaCl-best neurons tested and influenced by electrical stimulation of the central nucleus of the amygdala (CeA), gustatory cortex (GC), and lateral hypothalamus (LH) is summarized in Table 1. Taste responses were more often affected by activation of the CeA (75%) and GC (68%) than the LH (53%). In addition, amygdala (79%) and cortex (67%) activation most often produced inhibition, whereas inhibition and excitation occurred equally often during LH stimulation (50% each).

**Inhibition**

The response profiles of NaCl-best neurons inhibited by forebrain stimulation are shown in Fig. 4. The taste stimuli and the discharge rate of each cell during the “ON SECONDS” (e.g., 1, 3, 5, 7, and 9) of the control (open bars) and test applications (filled bars) are arranged from top to bottom and the different forebrain sites from left to right. The individual neurons are arranged by their response rate to NaCl in descending order. Taste-elicited responses were suppressed by concurrent electrical stimulation at all 3 forebrain sites [CeA: \( F_{(1,26)} = 94.4, P < 0.01 \); GC: \( F_{(1,19)} = 92.3, P < 0.01 \); LH: \( F_{(1,11)} = 24.9, P < 0.01 \)]. During the “OFF SECONDS” when no brain stimulation occurred (e.g., 2, 4, 6, 8, and 10) the control and test series responses were equivalent for each stimulation site (not shown, \( P > 0.6 \)). Using our definition of a significant response (i.e., \( \pm 2.0 \) SD) from the mean response to water, stimulation of the amygdala, cortex, and hypothalamus reduced the effectiveness of sucrose, acid, and quinine by 59% (Table 2). The forebrain stimulation had a much smaller effect on responses to NaCl in neurons that did not respond best to this stimulus (19%).

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The spontaneous activity of 2 neurons in this sample was increased during CeA stimulation and of another during LH stimulation. The variable latencies of the driven activity (5–15 ms) and their inability to follow low-frequency stimulation (10 pulses/s) suggest that the effects were not attributed to antidromic activation of PBN neurons projecting to the lateral hypothalamus.

### Citric acid–best neurons

As with NaCl-best neurons, taste responses in acid-best neurons were more often affected by amygdala (67%) and cortex (67%) stimulation than by the hypothalamus (45%). Inhibitory and excitatory effects also occurred with similar frequency during LH stimulation (60% inhibition), whereas CeA and GC activation produced only inhibition (Table 1).

Figure 7A shows the mean discharge during the “ON SECONDS” of the control (open bars) and test applications (filled bars) for each of the forebrain sites. Compared with control responses, stimulation of the central nucleus [\( F_{(1,7)} = 20.4, P < 0.01 \)] and cortex [\( F_{(1,7)} = 20.8, P < 0.01 \)], but not the hypothalamus [\( F_{(1,2)} = 3.9, P = 0.18 \)] suppressed responsive-

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**Fig. 3.** Responses of NaCl-best neuron to NaCl, CA, sucrose, and QHCl with and without concurrent gustatory cortex stimulation. See text for details.
Neuron firing in response to electrical stimulation of the CeA and GC were turned off (i.e., “off seconds”), taste-elicted activity returned to control levels [not shown; $F_{1(17)} = 1.2, P \geq 0.29$]. The overall effect of forebrain stimulation was to reduce the effectiveness of sucrose and quinine by 63% (Table 2). The forebrain stimulation had a much smaller effect on the ability of NaCl and citric acid to elicit a response (10% each).

During amygdala stimulation, the magnitude of inhibition of ipsilateral (n = 5) and contralateral (n = 3) taste responses was comparable [$F_{1(30)} = 1.3, P = 0.26$]. Similar analyses were not performed on cortex and hypothalamus stimulation because stimulating these 2 sites inhibited an even smaller number of contralateral cells (Table 1). Thus subsequent analyses will not distinguish between ipsilateral and contralateral effects.

Figure 7B shows that the magnitude of inhibition during GC stimulation [$F_{4(35)} = 2.9, P = 0.03$] and CeA [$F_{4(35)} = 2.7, P = 0.04$] stimulation was greater for the most effective taste stimuli (i.e., citric acid and NaCl) than sucrose and QHCl ($P \leq 0.04$). LH-induced suppression was significant only for spontaneous activity and the responses to the most weakly effective stimuli, sucrose and QHCl. Post hoc analyses on the main effect for forebrain stimulation site [$F_{2(20)} = 5.0, P < 0.01$] showed that the amygdala and cortex produced greater suppression of neural activity than the hypothalamus ($P \leq 0.04$), but were equivalent to one another ($P = 0.13$).

Sucrose-best neurons

The number of sucrose-best neurons tested and influenced by electrical stimulation of the amygdala, cortex, and hypothalamus is shown in Table 1. Taste responses in these PBN neurons were more often affected by activation of the CeA (67%) and GC (64%) than the LH (30%). Only stimulation of cortex, however, produced both inhibitory (57%) and excitatory (43%) effects.

Inhibition

Although the corrected responses are not shown, taste-evoked activity was suppressed during stimulation of the CeA [$F_{4(15)} = 30.7, P < 0.01$] and GC [$F_{4(3)} = 96.3, P < 0.01$], but not the LH [$F_{1(2)} = 2.7, P = 0.23$]. Similar to the other neuron types, the inhibitory influence was not evident during the time between pulse trains when no brain stimulation occurred ($F = 0.18, P = 0.68$). Moreover, forebrain stimulation reduced the effectiveness of NaCl, citric acid, and quinine by 50% (Table 2). The forebrain stimulation had a much smaller effect on a sucrose-best neuron’s response to its best stimulus, sucrose (8%). In neurons that did not respond best to this stimulus, however, the effectiveness of sucrose was reduced by 71%

Figure 8A shows that, unlike forebrain inhibition of NaCl- and acid-best neurons, electrical stimulation of the CeA and GC inhibited taste responses in sucrose-best neurons ($P \leq 0.02$) without a significant effect on spontaneous discharge ($P \geq 0.07$). Post hoc analyses on the main effect for rapid stimulus during activation of cortex [$F_{4(15)} = 22.3, P < 0.01$] and amygdala [$F_{2(25)} = 10.5, P < 0.01$] indicated that the response to sucrose was inhibited significantly more than the response to citric acid and QHCl ($P < 0.01$), but not NaCl ($P \geq 0.07$). Although 3 neurons meet the criteria for inhibition during LH activation, the differences between control and test responses were not significantly different from 0 ($P \geq 0.07$). The magnitude of inhibition differed between forebrain stimulation sites [$F_{2(50)} = 33.0, P < 0.01$] where the order of impact was GC > CeA > LH ($P < 0.01$).

Excitation

Figure 8B shows that electrical stimulation of the GC also facilitated neural activity [$F_{1(2)} = 45.2, P = 0.02$]. Spontaneous activity and responses to each sapid stimulus were equally increased [$F_{1(10)} = 0.5, P = 0.7$]. Thus unlike in NaCl-best neurons, GC-induced excitation of sucrose-best neurons was not specific to its best stimulus. Moreover, the excitatory influence rapidly returned to control levels during the times between electrical pulse trains (Fig. 8D; $P < 0.01$). The variable latencies of the driven activity (6–18 ms) and their inability to follow low-frequency stimulation (10 pulses/s) suggest orthodromic effects. In rodents, there is no evidence that PBN gustatory neurons project to cortex directly (DiLorenzo and Monroe 1992).

Convergence of centrifugal input

Sixty PBN neurons were tested before, during, and after electrical stimulation at each of the forebrain sites: 9 neurons...
were sucrose-best; 40 NaCl-best; and 11 citric acid–best. Of these neurons, 88% were influenced by stimulation of ≥1 site. Moreover, the majority of forebrain-responsive neurons (70%) were influenced by multiple sources of descending input. Figure 9A shows the occurrence of different combinations of effective stimulation sites and the direction of change in neural discharge (−, inhibition; +, excitation; =, no change) as a function of neuron type. The bars to the left of the x-axis break show the neurons influenced by 2 stimulation sites (n = 17) and the bars to the right by all 3 sites (n = 20). Although most neurons were influenced in the same direction by convergent input (i.e., inhibition or excitation), the direction of change was not always the same for a given pontine taste cell. Shown in Fig. 9, B, C, and D are the difference scores for some of the various combinations of effective stimulation sites.

**Breadth of responsiveness**

The entropy measure (H value) ranges from 0 to 1. A value of 0 corresponds to a neuron that was activated by only one stimulus; a value of 1, to a neuron activated equally by all stimuli. Table 3 lists the H values during the “ON SECONDS” of the control and test applications as a function of neuron type and response category (e.g., inhibited or facilitated). Ipsilateral and contralateral values are combined because significant differences in entropy were not evident (P ≥ 0.06). Of the 9 possible combinations of forebrain stimulation and neural response, only 5 contained facilitated cells and the H values changed significantly in only two. The H value increased during GC stimulation of sucrose-best cells, which meant the neurons became less specific. Central nucleus stimulation decreased the H value for NaCl-best neurons, which meant they became more selective in their response to sapid chemicals. All 9 possible combinations contained neurons that were inhibited by forebrain stimulation and in 8 of these, the H values decreased significantly.

Another way to measure change in responsiveness in taste neurons is the percentage of overall evoked activity elicited by each of the sapid stimuli. Figure 10 shows the difference between the percentage of overall activity elicited by each of the stimuli during the control applications and those during concurrent stimulation of the amygdala, cortex, and hypothalamus. Positive percentages indicate that forebrain stimulation increased the relative contribution of a given stimulus to overall evoked activity, whereas negative ones reflect the opposite. Again, ipsilateral and contralateral values are combined because significant differences were not evident (P ≥ 0.06). Regardless of the forebrain stimulation site or the direction of the effect (e.g., inhibition or excitation), the pattern of change was similar across best-stimulus categories. During forebrain stimulation
stimulation, the best-stimulus carried more of the total activity of that set of neurons with the exception of cortex-induced facilitation of sucrose-best cells. This sharpening effect was most evident for the NaCl-best cells, in which 14 of the 24 possible changes were significant (Fig. 10, A and B) and least so for the sucrose-best group, in which only 2 of 24 possible changes were significant.

**DISCUSSION**

This study demonstrated that gustatory processing in the pontine parabrachial nucleus (PBN) was modulated bilaterally during electrical stimulation of separate forebrain sites, the central nucleus of the amygdala (CeA), lateral hypothalamus (LH), and gustatory cortex (GC). Prior studies demonstrated that stimulation in the LH and CeA can alter responses of nucleus of the solitary tract neurons (NST) to sapid stimulation (Cho et al. 2002a; Li et al. 2002), whereas the effects of only CeA stimulation were tested one synapse further centrally in the PBN (Lundy and Norgren 2001). Recent evidence also indicates that descending input from the LH and CeA converge to modulate some of the same NST taste cells (Cho et al. 2003). Stimulation at each of these forebrain sites produced a common effect, primarily excitatory. The present results also indicate that descending projections from multiple forebrain sites reach many of the same PBN neurons. Convergent inputs in the pons, however, can differentially influence taste-evoked responses.

**Modulation of PBN gustatory processing**

In our previous investigation, electrical stimulation of the CeA inhibited the spontaneous and taste-evoked activity of NaCl (18/32) and citric acid–best cells (13/14) in the PBN (Lundy and Norgren 2001). In other NaCl-best neurons (5/32), however, the response to NaCl was specifically augmented; the baseline discharge and responses to other classes of sapid stimuli were unaffected. The net effect of inhibition and excitation was to sharpen the across-stimulus response profile and, consequently, the distinction between classes of taste stimuli. The present data substantiate this characterization.

Electrical stimulation of the CeA, LH, and GC produced qualitatively similar effects on the responsiveness of PBN neurons to sapid stimuli. Gustatory neurons were more often affected by stimulation of the amygdala (48/66) and cortex (45/67) than the hypothalamus (32/65). Of these forebrain-responsive cells, inhibition of taste-evoked activity was the most common effect of CeA (41/48) and GC (32/45) stimula-
tion, whereas during LH stimulation inhibition (18/32) and excitation (14/32) were almost equally likely. Activity in the amygdala and cortex produced stronger inhibition of responses in NaCl- and acid-best cells than in the hypothalamus, but did not differ from one another. In sucrose-best cells, the degree of inhibition was GC > CeA > LH. When forebrain stimulation facilitated responses in NaCl-best neurons, the absolute change in response was comparable across stimulating sites. These findings indicate that, in terms of magnitude and number of neurons influenced, the transfer of taste information through the PBN is more effectively suppressed by activity in the amygdala and cortex than by the hypothalamus. It is possible, however, that increasing the sample size of sucrose-, acid-, and quinine-best neurons might alter our conclusions.

Despite these differences, however, the net effect of forebrain influence appears similar between sites; it sharpens the across-stimulus response profiles. Inhibition accomplishes this shift in chemical sensitivity by suppressing the overall response level of PBN neurons to sapid stimuli. This reduced the number of tastants to which a given unit responded, but the effect was often differential so that the response to the best-stimulus ended up even larger with respect to the other stimuli. The excitatory mechanism(s), on the other hand, sharpened the response profile of NaCl-best cells directly through stimulus-specific enhancement of responsiveness to NaCl. In fact, the background discharge and the responses to the other sapid stimuli were often suppressed, particularly during CeA and LH stimulation.

Evidence from our prior study suggested that a certain amount of afferent activity, normally achieved with above threshold concentrations of NaCl, might be required to engage this excitatory descending influence, in that NaCl-evoked responses were not enhanced when the solution contained the peripheral Na+ transduction antagonist amiloride (Lundy and Norgren 2001). Because the response to NaCl was not enhanced in PBN neurons that are not amiloride-sensitive (i.e., sucrose-best, acid-best, and NaCl-generalist neurons), the above results also suggest that amiloride-sensitive cells carry a specific message for Na+ salt (Lundy and Contreras 1999; Scott and Giza 1990).

**Bilateral modulation of taste processing**

The CeA and LH, but not the GC, receive primarily ipsilateral afferent gustatory information (Halsell 1992; Hopkins and Holstege 1978; Norgren 1976; Saper and Loewy 1980; Veenig et al. 1984). All 3 forebrain areas modulate the same system bilaterally (Cho et al. 2002a; DiLorenzo and Monroe 1995; Li et al. 2002). In fact, contralateral stimulation of the CeA and GC, but not the LH, more effectively suppressed responses in NaCl-best PBN cells than did ipsilateral activation. On the other hand, enhancement of PBN taste responses was almost exclusively ipsilateral; one contralateral PBN neuron was augmented by LH activation. Although descending projections from the central nucleus and cortex to the brain stem are bilateral, ipsilateral projections are more numerous.
and thus the mere density of direct connections cannot account for the difference in inhibitory magnitude (Hopkins and Holstege 1978; van der Kooy et al. 1984; Veening et al. 1984; Whitehead et al. 2000). Whether this discrepancy between anatomical and electrophysiological data reflects a multisynaptic pathway from the ventral forebrain to PBN remains an open question (Cho et al. 2003). Although it is unlikely that the PBN taste neurons were antidromically invaded, it is possible that other cells were antidromically activated by the forebrain stimulation and that their collateral axons, in turn, produced the effects on the gustatory neurons. One of our working hypotheses admits this possibility and it is under investigation. Regardless of the specific circuitry, the net effect of this forebrain influence on the response output of ipsilateral and contralateral PBN taste neurons was similar (see above).

**Convergence of forebrain inputs**

A recent study in hamster investigated the effects of CeA and LH stimulation on the same taste responsive neurons in the NST (Cho et al. 2003). In 46% (52/113) of the forebrain-responsive neurons (113/215), medullary neural activity was influenced by stimulation of both sites. The present data are in agreement with these findings, but show that a much greater proportion of PBN neurons received convergent inputs. Fifty-three of the 60 neurons tested were influenced by electrical stimulation of the CeA, LH, and/or GC; 70% of them responded to 2 or more sites. Similar to dually responsive NST cells, stimulation at each site produced a common effect on PBN taste cells, either inhibitory or excitatory. The neurons...
responsive to stimulation at all 3 sites, however, were often differentially influenced.

Intrinsic neurons versus fibers of passage

The limitation of using electrical stimulation is that it cannot distinguish between activation of intrinsic neurons and fibers of passage. This interpretational caveat applies to electrical stimulation of the LH and CeA. The fact that some PBN neurons were influenced by LH or CeA stimulation only or in the opposite direction of the effects produced by the other stimulation sites suggests activation of intrinsic neurons. This rationale is reinforced by the presence of monosynaptic projections from both the LH and CeA to the PBN and electrophysiolog-

**TABLE 3. Entropy for neurons inhibited and excited by forebrain stimulation**

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>CeA</th>
<th>GC</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibited</td>
<td>Facilitated</td>
<td>Inhibited</td>
</tr>
<tr>
<td>S-Best</td>
<td>$n = 6$</td>
<td>$n = 4$</td>
<td>$n = 3$</td>
</tr>
<tr>
<td>Control</td>
<td>$0.78 \pm 0.03$</td>
<td>$0.80 \pm 0.01$</td>
<td>$0.80 \pm 0.07$</td>
</tr>
<tr>
<td>Test</td>
<td>$0.72 \pm 0.01$</td>
<td>$0.68 \pm 0.07$</td>
<td>$0.98 \pm 0.01$</td>
</tr>
<tr>
<td>N-Best</td>
<td>$n = 27$</td>
<td>$n = 7$</td>
<td>$n = 10$</td>
</tr>
<tr>
<td>Control</td>
<td>$0.68 \pm 0.03$</td>
<td>$0.73 \pm 0.06$</td>
<td>$0.74 \pm 0.03$</td>
</tr>
<tr>
<td>Test</td>
<td>$0.42 \pm 0.05$</td>
<td>$0.62 \pm 0.08$</td>
<td>$0.50 \pm 0.07$</td>
</tr>
<tr>
<td>A-Best</td>
<td>$n = 8$</td>
<td>$n = 8$</td>
<td>$n = 3$</td>
</tr>
<tr>
<td>Control</td>
<td>$0.85 \pm 0.01$</td>
<td>$0.86 \pm 0.01$</td>
<td>$0.84 \pm 0.01$</td>
</tr>
<tr>
<td>Test</td>
<td>$0.67 \pm 0.06$</td>
<td>$0.65 \pm 0.05$</td>
<td>$0.71 \pm 0.03$</td>
</tr>
</tbody>
</table>

Shown are the entropy values for the different neuron types during the “On Seconds” of the control and test series applications, listed as a function of forebrain site and response category (e.g. inhibited or facilitated). The values are given as the means ± SE and those in bold signify a significant difference between control and test applications ($P < 0.05$).
Electrophysiological experiments find that from about 35 to 80% of NST taste neurons are anterogradely invaded by stimulation in the PBN (Cho et al. 2002a; Li et al. 2002). Moreover, stimulating the CeA, LH, and GC influences taste responses in both the NST and PBN cells. These findings suggest that the effects of forebrain activity on PBN taste responsiveness might be mediated through the NST. Although the present results cannot disprove this hypothesis directly, several lines of indirect evidence, in addition to the direct anatomical connections between the PBN and the ventral forebrain, suggest that centrifugal modulation of gustatory processing in the pons is not simply a reflection of altered medullary output.

For example, gustatory neurons in the NST were more often responsive to stimulation in the LH (49–70%, Cho et al. 2002a, 2003; Matsuo et al. 1984) than in the CeA (33%, Cho et al. 2003; Li et al. 2002) and GC (34%, Smith and Li 2000). In contrast, the present data indicate that the CeA (73%) and GC (67%) influenced more PBN neurons than the LH (48%). It should be noted that a previous study reported the baseline discharge of only 31% of PBN neurons tested was influenced by electrical stimulation of the GC, primarily excited (DiLorenzo and Monroe 1992). The stimulating procedures, however, appeared to predispose the identification of excitatory effects and thus likely underestimated the inhibitory component of descending GC projections.

In fact, additional data from these prior studies indicate that the proportion of forebrain excitatory and inhibitory effects shifts dramatically when the recording changes from the NST to the PBN. In the NST, the most common effect of CeA (91–93%) and LH (66–90%) stimulation was excitatory (but see Bereiter et al. 1980), whereas GC activation induced a more even distribution of excitation (47%) and inhibition (53%). For pontine neurons, however, CeA (85%) and GC (71%) activation most often produced inhibition, whereas inhibition and excitation occurred equally often during LH stimulation.

Additional evidence for a direct influence on PBN taste cells comes from the effects of temporary removal of cortical activity. When GC activity was suppressed using procaine HCl, specific changes in the across-unit patterns of response to sucrose and Na-saccharin were observed in the PBN (DiLorenzo 1990). Under identical experimental conditions, a similar effect on NST taste cell responsiveness was observed, but predominately in nonrelay units (DiLorenzo and Monroe 1995). These results suggest an independent effect of cortical input on the NST and PBN. It is important to note, however, that the bulk of the data showing centrifugal control of medullary taste neurons was collected in hamster. Thus variation in the absolute number of, and primary effect on, forebrain-responsive taste cells in the NST and PBN might reflect a true species differences. Although not a direct test, a better understanding of how ventral forebrain inputs modify taste processing at different levels of the central gustatory system will come from studies that repeat the present experimental conditions, but record from medullary taste neurons, as well as from those in the thalamic gustatory area.

### Implications for ingestive behaviors

Lesion-behavioral studies indicate some overlap in the participation of the LH, CeA, and GC in taste-guided behaviors. For example, each forebrain site has been implicated in the acquisition or expression of a conditioned taste aversion (CTA; Bermudez-Rattoni and McGaugh 1991; Cubero et al. 1999; Roth et al. 1973; Ruch et al. 1997, 1999; but see Dunn and Everitt 1988; Yamamoto et al. 1995). A common finding in electrophysiological studies of brain stem and GC taste cells is an enhanced response to the conditioned taste stimulus (CS) after acquisition (Chang and Scott 1984; Shimura et al. 1997a; Yasoshima and Yamamoto 1998). Whether the formation of a CTA increases the sensitivity of thalamic taste neurons to the CS is unknown; however, it is apparently without effect on the percentage of CS responsive neurons (Aleksanyan et al. 1976). If these lower-level modifications of sensitivity to the CS are not retained in the thalamus, it follows that the changes observed in GC might be mediated through PBN projections to the ventral forebrain. Prior evidence in conscious rats indicates that CTA induction increased the number of amygdala neurons responsive to CS reexposure (Yasoshima et al. 1995). The opposite effect on the incidence of CS-induced responses was observed in the LH (Aleksanyan et al. 1976). Nevertheless, the present results demonstrate that activation of ventral forebrain regions can increase the responsiveness of brain stem neurons to sapid stimulation, specifically or generally.

Prior lesion-behavioral studies suggest that CTA acquisition requires the cooperation of ipsilateral cortical and subcortical connections (Bielavska and Roldan 1996; Gallo and Bures 1991). Acquisition of a CTA proceeds normally when the amygdala and cortex are intact in the same hemisphere, but fails when the lesions are made in opposite hemispheres. If contralateral cortical and subcortical connections are not important for CTA learning, how can the present contralateral forebrain influences on PBN taste processing function in the context of our learning-related interpretation? Because this prior research indicates that only one intact hemisphere is required for CTA acquisition, the present forebrain modulation of contralateral PBN taste neurons is complementary. The logic is as follows. When lesions are placed in the amygdala and cortex in opposite hemispheres, modulation of PBN taste processing would remain intact through descending connections from the contralateral amygdala and cortex. Nevertheless, this altered information is unable to access relevant forebrain sites in both hemispheres because of the bilateral asymmetric lesions and failure of CTA acquisition. Conversely, when forebrain lesions are in the same hemisphere, the altered taste information can reach relevant forebrain sites in the opposite intact hemisphere and a CTA is acquired. The same interpretation holds when unilateral PBN lesions are combined with symmetric and asymmetric unilateral lesions of the amygdala and/or cortex (Bielavska and Roldan 1996; Gallo and Bures 1991).

Sodium appetite is another complex taste-guided behavior that requires some interaction between the brain stem, particu-
ularly the PBN, and the ventral forebrain. In the context of the present experiment, bilateral lesions of the LH and CeA, but not the GC, disrupt the expression of this taste-specific behavior (Galaverna et al. 1991; Ruger and Schulkin 1980; Wirsig and Grill 1982; Wolf et al. 1970; Zardetto-Smith et al. 1994). Electrophysiological studies have shown that sodium appetite induced under a variety of experimental conditions decreased the sensitivity of NST and PBN taste cells to sodium salt, particularly at the higher concentrations that control animals avoid ingesting (Jacobs et al. 1988; McCaughey and Scott 2000; Nakamura and Norgren 1995; Shimura et al. 1997b; but see Tamura and Norgren 1997). The present data also demonstrate that stimulation of the CeA and LH modulated PBN responsiveness to NaCl in a manner consistent with the changes produced by the motivational state itself. The effects of amygdala stimulation are of particular interest because cells within this nucleus respond to changes in sodium balance (Johnson et al. 1999) and the effects on NaCl responsiveness in the present study were far more robust than those produced by hypotalamic stimulation.

Taken together, lesion-behavior studies suggest that learned avoidance and physiologically dependent acceptance of sapid stimuli are correlated with, respectively, hypersensitive and hyposensitive responses of brain stem neurons to specific chemicals. Interestingly, acquisition of a conditioned taste preference for a normally avoided chemical also alters NST responses, but in a manner similar to sodium appetite, reduced responsiveness (Giza et al. 1997). Consistent with the overlapping contribution of the LH, CeA, and GC in the control of ingestive behavior, particularly learned and physiological state–dependent behaviors, the present findings reflect neurophysiological mechanisms whereby ongoing activity in higher-order nuclei might produce independent or coordinated modulation of earlier stages of gustatory processing.

Perspective

In the present experiments, we confirmed and extended prior data that demonstrate forebrain influence on brain stem gustatory neural activity. If we assume that pentobarbital anesthesia reduces the overall activity of the forebrain, then in our experiments, the electrical stimulation artificially restores activity that has been pharmacologically suppressed. Using this logic, we would expect that, in the awake behaving animal, the forebrain influences the same gustatory activity continuously and presumably dynamically. Thus gustatory neural activity recorded in the awake, behaving state might be expected to differ from responses in anesthetized animals in a manner similar to what we observed between our “ON” and “OFF” periods. On at least one dimension, this is exactly what occurred. Overall, the major effect of forebrain stimulation was to sharpen the responses of PBN taste neurons to sapid chemicals (i.e., to make the best stimulus better). When PBN taste neurons are tested in awake, behaving rats, their response profiles are sharper than similar activity recorded in anesthetized preparations (Nishijo and Norgren 1997). Given the vast differences in circumstances—a few pulses of electricity for a few seconds versus dynamic activity of the entire forebrain—the similarity in overall effect may be just a coincidence. On the other hand, it may reflect the value of simplifying the activity in an awake, behaving brain to uncover specific synaptic mechanisms.

GRANTS

This research was supported by National Institute on Deafness and Other Communication Disorders Grants DC-05156, DC-00240, and DC-05435.

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J Neurophysiol • VOL 91 • MARCH 2004 • www.jn.org