Pontine Gustatory Activity Is Altered by Electrical Stimulation in the Central Nucleus of the Amygdala

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Lundy, Robert F., Jr. and Ralph Norgren. Pontine gustatory activity is altered by electrical stimulation in the central nucleus of the amygdala. J Neurophysiol 85: 770–783, 2001. Visceral signals and experience modulate the responses of brain stem neurons to gustatory stimuli. Both behavioral and anatomical evidence suggests that this modulation may involve descending input from the forebrain. The present study investigates the centrifugal control of gustatory neural activity in the parabrachial nucleus (PBN). Extracellular responses were recorded from 51 single PBN neurons during application of sucrose, NaCl, NaCl mixed with amiloride, citric acid, and QHCl with or without concurrent electrical stimulation in the ipsilateral central nucleus of the amygdala (CeA). Based on the rapid stimulus that evoked the greatest discharge, 3 neurons were classified as sucrose-best, 32 as NaCl-best, and 16 as citric acid-best. In most of the neurons sampled, response rates to an effective stimulus were either inhibited or unchanged during electrical stimulation of the CeA. Stimulation in the CeA was without effect in two sucrose-best neurons, nine NaCl-best neurons, and one citric acid-best neuron. Suppression was evident in 1 sucrose-best neuron, 18 NaCl-best neurons, and 15 citric acid-best neurons. In NaCl-best neurons inhibited by CeA stimulation, the magnitude of the effect was similar for spontaneous activity and responses to the five taste stimuli. Nonetheless, the inhibitory modulation of gustatory sensitivity increased the relative effectiveness of NaCl resulting in narrower chemical selectivity. For citric acid–best neurons, the magnitude of inhibition produced by CeA activation increased with an increase in stimulus effectiveness. The responses to citric acid were inhibited significantly more than the responses to all other stimuli with the exception of NaCl mixed with amiloride. The overall effect was to change those CA-best neurons to responses to all other stimuli with the exception of NaCl mixed with amiloride. The overall effect was to change these CA-best neurons to responses to all other stimuli with the exception of NaCl mixed with amiloride. The overall effect was to change these CA-best neurons to responses to all other stimuli with the exception of NaCl mixed with amiloride.

INTRODUCTION

Visceral signals and experience modulate the acceptance of gustatory stimuli and, consequently, the consumption of foodstuff. For instance, the association of taste with gastrointestinal malaise (conditioned taste aversion) can prevent the subsequent ingestion of the tastant (Garcia et al. 1955; Grill 1985; Nachman and Ashe 1973), while dietary sodium deficiency can produce an avid consumption of previously avoided concentrations of NaCl (Berridge et al. 1984; Contreras and Hatton 1975; Nachman 1962; Richter 1936). The pontine parabrachial nucleus (PBN) appears to be a critical neural substrate for these taste-guided behaviors because bilateral lesions of this area severely disrupt their expression (Grigson et al. 1993; Scalera et al. 1995; Spector et al. 1992). In normal animals, both conditioned taste aversion and sodium appetite selectively alter gustatory evoked responses in the brain stem (Chang and Scott 1984; Nakamura and Norgren 1995; Shimura et al. 1997; Tamura and Norgren 1997; Yasoshima et al. 1995). One interpretation of this modulation in response to visceral signals and experience is that centrifugal activity normally regulates gustatory processing in the brain stem. This is supported by the fact that chronically decerebrate rats fail to acquire a learned taste aversion or to express a salt appetite (Grill et al. 1986).

The PBN distributes gustatory information to the hypothalamus, amygdala, bed nucleus of the stria terminalis, and, via the thalamus, to insular cortex and, in turn, receives projections from these brain structures (Halsell 1992; Hopkins and Holstege 1978; Norgren 1976; Roberts 1980; Saper and Loewy 1980; Veening et al. 1984). These forebrain regions participate in autonomic and endocrine functions, and each has been implicated in the expression of complex taste-guided behaviors (Bermudez-Rattoni and McGaugh 1991; Johnson et al. 1999; Loewy and Spyer 1990; Roth et al. 1973; Ruger and Schulkin 1980; Zardetto-Smith et al. 1994). The influence of descending input from these rostral structures on the neurophysiology of brain stem gustatory neurons has received little attention. Prior studies have shown that electrical stimulation in the lateral hypothalamus (Bereiter et al. 1980; Matsuo and Kusano 1984; Matsuo et al. 1984; Murzi et al. 1986) modulated gustatory-responsive neurons in the rostral nucleus of the solitary tract, but not in the PBN (Murzi et al. 1986). Activity in gustatory cortex, on the other hand, altered the ongoing spontaneous discharge of both nucleus of the solitary tract (NST) and PBN gustatory neurons (DiLorenzo and Monroe 1992; Smith and Li 2000). None of these studies tested for alterations in gustatory evoked activity.

The choice to stimulate in the central nucleus of the amygdala (CeA) was based on the fact that it is a major recipient of PBN gustatory afferent axons, as well as the major source of...
amygdaloid efferents to the brain stem (Halsell 1992; Norgren 1976; Veening et al. 1984). In addition, neurophysiological studies examining cardiovascular and respiratory sensitive neurons in the amygdala and PBN showed that neurons recorded in one area were influenced by electrical stimulation in the other (Cechetto and Calaresu 1983; Jhamandas et al. 1996). The effect of such forebrain stimulation on the responses of gustatory neurons in the PBN has not been examined.

The present study characterizes the influence of the CeA on gustatory processing at the level of the PBN. We recorded responses of single PBN neurons to gustatory stimuli applied before, during, and after concurrent electrical stimulation in the CeA. Amygdala stimulation was found to modulate both ongoing spontaneous discharge and taste-evoked responses in PBN gustatory neurons. These alterations in gustatory sensitivity mimic those produced by the induction of a sodium appetite.

A portion of this work was presented as a poster at the 2000 meeting of the Association for Chemoreception Sciences.

METHODS

Subjects

Neurophysiological recordings were made in 25 male Sprague-Dawley rats weighing 360–500 g [Crl: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 8604) and distilled water.

Amygdala stimulating electrodes

The rats were anesthetized with a 50-mg/kg injection (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 ± 2°C. The trachea was cannulated with polyethylene 210 tubing, and a small suture was attached to the ventral surface of the tongue. Animals were then secured in a stereotactic instrument using nonpuncture ear bars (45° taper) and a bite bar. A midline incision was made and the skull leveled between β and λ. Three 140-μm-diam stainless steel electrodes, insulated except at the cross section of the tip, were lowered into the central nucleus of the amygdala (CeA) using the following stereotactic coordinates relative to B: P = −1.8 mm, L 4.0 mm, and V 8.5 mm. A small hole was drilled through the skull at the P-L coordinates, and the dura was excised. 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 AP plane. The electrodes were anchored with dental cement via a stainless steel screw in the skull just posterior to the electrodes. Electrical stimulation in the amygdala was achieved using a Grass S 48 train generator attached to a constant current source (PSIU6), which, in turn, was connected to a switch box that could distribute the current to any possible pair of the 3 electrodes. Stimulation parameters were 1-s trains of 10 pulses delivered at 0.5 Hz for 10 s (total of 5 trains). The duration and amplitude of an individual pulse were 0.2 ms and 0.4 mA, respectively.

Localization of PBN gustatory neurons

After drilling a small hole through the interparietal bone, glass-insulated tungsten electrodes (resistance 2–8 MΩ) were lowered into the PBN using a Fredrick Haer micropositioner. To avoid the transverse sinus, the electrode was oriented 20° off the vertical with the tip pointed rostrally. A drop in background activity marked the boundary between the cerebellum and the pons, and, at that point, any neuron encountered was tested for its sensitivity to 0.1 M NaCl applied to the anterior two-thirds of the tongue. If a response was not obtained with NaCl, then 0.3 M sucrose, 0.01 M citric acid, and 0.003 M quinine hydrochloride were tested. Neural activity was amplified (>1,000), fed through an artifact suppressor, monitored with an oscilloscope and audiometer, and stored on a audio-cassette recorder for off-line analysis. The artifact suppressor was used to adjust the size of the electrical stimulation artifact to be easily distinguished from the neural signal during off-line analysis. Cambridge Electronic Design’s Spike2 hardware and software were used to convert the recorded data to digital format.

The neural signal and the artifact signal were individually classified and separated into two waveform channels using Spike2 hardware and software. The channel with the artifact signal was used to mark the onset and offset of the electrical pulses. This procedure removes the background noise from the recording (see Fig. 5) and functions as a window discriminator in conjunction with waveform matching. The analog signal is digitized at 20,000 Hz and templates formed during an initial sampling period (60 s). Subsequently, the matching algorithm is engaged only if the digitized voltage levels reach a prespecified value. Spikes are then included in a template only if more than a user-defined percentage of the points in a spike fall within the template. The present study characterized a spike with 75 points. It then required that >60% of the points match, and that the difference in amplitude between template and spike be <20% for any potential to be counted. In two instances, Spike2 was used to separate the activity of two different action potentials recorded from the same site.

Stimulus delivery and protocols

The tongue was gently extended out from the oral cavity using the ventral tongue suture. A computer-controlled delivery system was used for taste stimulus and water presentation to the anterior tongue. For 10 s before and for longer after stimulus offset, a separate outflow nozzle delivered deionized-distilled water to the same locus on the tongue. During the rinse period, the stimulus delivery line and nozzle were flushed with water. Gustatory neurons were tested for responsiveness to 0.3 M sucrose, 0.1 M NaCl, 0.1 M NaCl mixed with 10 μM amiloride, 0.01 M citric acid (CA), and 0.003 M quinine hydrochloride (QHCl) using a water, stimulus, water procedure. Stimulus order varied from one recording session to another. Briefly, water was applied to the tongue for 10 s followed by tastant application for 10 s. The water flow that followed tastant application was for 90 s, and the total time between different stimulus applications was 2 min. The stimulation protocol was as follows: control series 1–test series–control series 2. Tastant application during a control series was without CeA stimulation. Prior to the test series, baseline activity in the absence of fluid flowing over the tongue was recorded during CeA stimulation. After another 2–3 min, the test series began and the tasters were applied during concurrent CeA stimulation. In most cases a neuron was held long enough to complete the second control series. This was done to assess the stability of the recorded neuron and to determine whether CeA stimulation produced any prolonged effects on gustatory responsiveness.

Data analysis

Corrected neural responses to a taste stimulus were calculated by subtracting the 10-s discharge rate to each stimulus from its preceding 10-s discharge rate to water. The 10-s response measure was used to categorize individual neurons based on the stimulus that evoked the greatest discharge. When a neuron was tested with both control series 1 and 2, these response rates were averaged. During the test series, seconds 1, 3, 5, 7, and 9 of tastant application correspond to the time periods when pulse trains were delivered to the CeA, while seconds 2, 4, 6, 8, and 10 correspond to the time periods between pulse trains.
Neurons in the present study were classified as inhibited or augmented if these response measures differed from their corresponding control rates by $\pm 25\%$, respectively. A difference score was calculated by subtracting the test series response rates from the control series response rates during seconds 1, 3, 5, 7, and 9 and 2, 4, 6, 8, and 10. The breadth of responsiveness was calculated according to the formula $H = -K \sum p_i \log p_i$, where $K$ is a scaling constant (1.66 for 4 stimuli; sucrose, NaCl, CA, QHCl) and $p_i$ is the proportion of the response to each of the stimuli against the total response to all the stimuli (Smith and Travers 1979).

One- and two-way ANOVAs were performed to detect significant differences between response rates. In some instances, post hoc contrast analyses (least significant difference) were used to determine the source of statistically significant differences. One-sample $t$-tests were performed to determine whether the difference scores were significantly different from zero.

**FIG. 1.** Top left: photomicrograph of a coronal section through the parabrachial nucleus (PBN) where taste responsive neurons were isolated (cresyl violet stain). The black arrow points to a marking lesion made following the recording of a gustatory neuron; 3 electrode tracks are also visible. The approximate area in which taste-responsive neurons were isolated at various PBN levels is illustrated by a filled oval in the 3 line drawings. LC, locus coeruleus; LBP, lateral parabrachial nucleus; LSO, lateral superior olive; Me5, mesencephalic trigeminal nucleus; MPB, medial parabrachial nucleus; Mo5, trigeminal motor nucleus; Pr5DM, dorsomedial aspect of the principal sensory trigeminal nucleus; Pr5VL, ventrolateral aspect of the principal sensory trigeminal nucleus (redrawn from Paxinos and Watson 1986).

**FIG. 2.** A photomicrograph of a coronal section through the amygdala (cresyl violet stain). The track left by a stimulating electrode ends in the central nucleus of the amygdala (dashed line). Three additional amygdaloid nuclei are shown (dotted lines). MA, medial nucleus; BLA, basolateral nucleus; LA, lateral nucleus.
used to determine whether the absolute difference scores differed from 0. A change in stimulus-evoked activity was considered significant when it differed above or below the response rate to water flow by $\geq 2.5$ standard deviations. The results will be shown as the mean ± SE. Data analyses were done using SPSS, and $P$ values $<0.05$ were considered significant.

**Histological processing and analyses**

At the end of an experiment, a small electrolytic lesion (5 μA for 30 s) was made at the site of the last recorded gustatory neuron. The rats were given a lethal dose of Nembutal (100 mg/kg ip) and perfused intracardially with 0.9% saline followed by 10% Formalin. The brain was removed, cut coronally in 50-μm sections using a freezing microtome, and stained with the cresyl Lecht violet. The lesion mark and stimulating electrode tracks were detected using a light-field microscope and the loci noted.

**RESULTS**

**Histology**

Based on stereotaxic coordinates of the penetrations and the marking lesions, gustatory neurons were isolated in the caudo-medial quadrant of the PBN. Figure 1 shows a photomicrograph of a coronal section through the PBN and line drawings that correspond to three different levels of the PBN. The black arrow indicates the marking lesion made following the recording of a gustatory neuron; three electrode tracks are visible. The approximate area in which taste-responsive neurons were isolated at various PBN levels is illustrated by a filled oval in the line drawings (Paxinos and Watson 1986). The present taste-responsive zone matched closely the area reported in previous studies (Nishijo and Norgren 1990; Norgren and Pfaffmann 1975; Scott and Perrotto 1980). The photomicrograph in Fig. 2 shows the track of a stimulating electrode placed in the CeA. Neurophysiological data in the present study were obtained from animals in which the stimulating electrodes were histologically confirmed to terminate in the CeA.

**Neuronal categorization**

Fifty-one gustatory neurons were isolated in the PBN and tested with the five taste stimuli with and without concurrent
electrical stimulation in the CeA. The response profiles of these neurons are shown in Fig. 3. The taste stimuli tested and the activity of each neuron during the 10-s prestimulus water flow are arranged from top to bottom and the different neuron groups from left to right. Within each group, neurons are arranged by their response rate to the best stimulus in descending order. Neurons 1–3 were deemed sucrose-best, neurons 4–35 NaCl-best, and neurons 36–51 CA-best. Although neurons 31–35 were NaCl-best, they differed from the other NaCl-best neurons insofar as their response to NaCl was insensitive to amiloride. They were termed NaCl generalists (Lundy and Contreras 1999).

The mean corrected response rates to each stimulus as a function of neuron type is shown in Fig. 4. Separate one-way ANOVA tests revealed a significant main effect for stimulus in NaCl-best \( (F_{4,135} = 42.3, P < 0.01) \) and CA-best neurons \( (F_{4,76} = 19.8, P < 0.01) \). Post hoc analyses indicated that the order of stimulus effectiveness for NaCl-best neurons was NaCl > NaCl + amiloride = CA = sucrose > QHCl. In the case of CA-best neurons, the order was CA > NaCl = NaCl + amiloride > sucrose = QHCl. Figure 4 also shows clearly that the epithelial Na\(^+\) channel antagonist amiloride was only effective in suppressing response rates to NaCl in NaCl-best neurons. In subsequent analyses, the 2 NaCl-best groups were combined because no clear differences were observed in terms of the effects of CeA stimulation on neural responsiveness.

Amygdala stimulation

Figure 5 shows the response of a CA-best neuron to sucrose, NaCl, CA, and QHCl with and without concurrent CeA stimulation. The labels to the left of each raw record indicates the stimulus; the labels with the acronym CeA indicate stimulus applications during amygdala activation. With regard to the bottom record, the effect of CeA stimulation was assessed on the baseline activity when no fluid flowed over the tongue (Baseline/CeA). The first and third long black bar in each taste stimulus record corresponds to 10 s of water flow, and the second (middle) black bar is stimulus application. The short black bars in the records with the CeA acronym indicate the five 1-s pulse trains delivered to the CeA. The first three records show the responses to sucrose with and without concurrent CeA stimulation. To appreciate the typical signal-to-noise ratio obtained in the present study, the first two records are shown with the background noise included. Raw record 3 differs from record 2 only in that Spike2 was used to remove the background noise and extract the CeA stimulation artifacts (e.g., shorter vertical lines above pulse train marks in record 2), the spike data were unmodified. This figure shows that electrical pulses delivered to the CeA produced a significant reduction in the baseline and tant-evoked discharge rates.

NaCl-best neurons

Of the 32 NaCl-best neurons, electrical pulse trains delivered to the CeA inhibited gustatory responses in 18, augmented responses in 5, and were ineffective in 9. Only the results for neurons inhibited (amiloride sensitive and insensitive) and augmented (amiloride sensitive) will be shown. For the neurons that were inhibited, Fig. 6A shows the responses to the five stimuli applied before and after the applications during amygdala stimulation. The discharge rates were nearly identical, indicating that the recording was stable and CeA activation was without a prolonged influence on gustatory responsiveness \( (F_{1,160} = 0.0, P = 0.99) \). During delivery of the pulse trains to the CeA, the response rate to each stimulus was suppressed (Fig. 6B, \( F_{1,176} = 10.9, P < 0.01 \)). In contrast, the response rates to the taste stimuli between the pulse trains (seconds 2, 4, 6, 8, and 10) were comparable to the response rates during the same time periods in the control series (Fig. 6C, \( F_{1,176} = 0.2, P = 0.63 \)). This indicates that the effect of CeA activation rapidly reversed. The difference in response rates to the taste stimuli and the effect of the pulse trains on baseline activity during the same time periods are shown in Fig. 6D. One-
sample t-tests revealed that the baseline activity and the discharge rate to each stimulus differed significantly from 0 only during the pulse trains ($P$ values $\leq 0.01$). Although, the degree of inhibition was comparable for spontaneous activity and taste-evoked responses ($F_{5,105} = 1.7$, $P = 0.12$), CeA stimulation reduced the number of neurons that responded significantly to sucrose from 10 to 5, to NaCl/Amloride from 16 to 14, to CA from 13 to 8, and to QHCl from 11 to 2.

In another subset of NaCl-best neurons, CeA activation increased the responsiveness to NaCl. The stimulus-evoked responses in this group were stable between the two control series applications (Fig. 7A, $F_{1,40} = 0.02$, $P = 0.87$). Although the response rate of all five neurons to NaCl increased (range, 26–95%) during the pulse trains, the effect was not statistically significant (Fig. 7B, $F_{1,50} = 1.1$, $P = 0.3$). Analysis of the difference scores in Fig. 7D, however, showed that the increase in response rate to NaCl differed significantly from 0 during both concurrent CeA stimulation ($t$-test, $P < 0.01$) and between pulse trains ($t$-test, $P = 0.01$). A two-way ANOVA on the difference scores revealed a significant interaction between stimulus and measurement period ($F_{5,60} = 6.1$, $P < 0.01$). Post hoc analyses indicated that the increase in NaCl responsiveness between pulse trains was less than that during the pulse trains ($P < 0.01$). Interestingly, this amygdala-induced facilitation of NaCl-evoked responses was disrupted by amiloride and occurred in the absence of an effect on baseline activity. The number of neurons responsive to sucrose was reduced from three to two, to CA from four to two, and to QHCl from one to zero.

**CA-best neurons**

With the exception of one unaffected neuron, the influence of amygdala stimulation on CA-best neurons was always inhibitory. Before and after CeA stimulation, the evoked responses were stable ($F_{1,160} = 0.1$, $P = 0.72$; Fig. 8A). Electrical pulses delivered to the CeA dramatically suppressed the discharge rate of PBN cells to each taste stimulus (Fig. 8B, $F_{1,142} = 22.3$, $P < 0.01$). In fact, a post hoc analysis on the stimulus main effect during CeA stimulation ($F_{4,70} = 14.8$, $P < 0.01$) indicated that the response rates to NaCl and CA
were no longer significantly different ($P = 0.26$). During the time periods between pulse trains, the response rates tended to recover to control levels (Fig. 8C, $F_{1,142} = 2.7$, $P = 0.09$). One-sample $t$-tests indicated that the baseline activity and discharge rates to each tastant were significantly different from 0 during the pulse trains (Fig. 8D, $P$ values $<0.01$). The degree of inhibition varied with stimulus effectiveness ($F_{5,85} = 4.6$, $P < 0.01$); responses to CA were inhibited significantly more
than the responses to all other stimuli ($P$ values $\leq 0.03$) with the exception of NaCl mixed with amiloride ($P = 0.08$). Responses to NaCl, NaCl/Amiloride, and CA, but not to sucrose and QHCl, were inhibited to a greater degree than was the baseline activity. Between pulse trains, the inhibitory effect did not fully reverse when NaCl or CA was the stimulus ($t$-test, $P$ values $\leq 0.02$). The residual inhibition of CA responses was significantly different from sucrose, NaCl/Amiloride, QHCl,
and baseline activity (P values ≤0.01). As with NaCl-best neurons, the percentage of CA-best neurons that responded to stimuli other than their best stimulus was reduced during CeA stimulation. The number of neurons responding to sucrose decreased from 10 to 3, to NaCl and NaCl/Amloride from 15 to 14, and to QHCl from 10 to 5.
FIG. 9. The entropy measure for the NaCl-best and the CA-best neurons that were inhibited and the NaCl-best neurons that were facilitated by CeA stimulation (A). This metric of stimulus responsiveness was calculated using the neural responses to 0.3 M sucrose, 0.1 M NaCl, 0.01 M CA, and 0.003 M QHCl. A value of 0 indicates that neurons were responsive to only one of the four stimuli, while a value of 1 indicates that they were equally responsive to the 4 stimuli. In this and subsequent plots, the open bars represent values without concurrent CeA stimulation, and the dark bars represent values during concurrent CeA stimulation. B: the percentage overall evoked activity elicited by each stimulus in the NaCl-best neurons under inhibitory control by CeA stimulation. C: the NaCl-best neurons under excitatory control. D: the CA-best neurons under inhibitory control. *, test series significantly different from control series; †, significant difference between neuron types.
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. Amygdala stimulation reduced the entropy measure of each response category ($F_{1,70} = 9.1, P < 0.01$, Fig. 9A). Post hoc analysis of the main effect for neuron type ($F_{2,70} = 10.7, P < 0.01$) revealed that the H value was significantly lower for the NaCl-best cells compared with the CA-best cells. Another way to measure change in responsiveness in taste neurons is the percentage of overall evoked activity elicited by each of the sapid stimuli (Fig. 9, B–D). For the inhibited NaCl-best neurons, CeA stimulation increased the percentage of the response produced by NaCl (60–78%) and correspondingly decreased it for the other stimuli (Fig. 9B; sucrose, 11 to −1%; CA, 20 to 6%; QHCl, 9 to −3%). Proportional responses did not differ significantly in the NaCl-best neurons augmented (Fig. 9C, $F_{1,40} = 0.0, P = 0.98$), or in the CA-best neurons inhibited by CeA stimulation (Fig. 9D, $F_{1,96} = 0, P = 0.96$).

Discussion

We have shown that electrical stimulation in the CeA differentially controls gustatory neurons in the PBN. The present findings are the first to demonstrate that a subcortical forebrain area can modulate taste-evoked responses in brain stem gustatory neurons. Prior studies have demonstrated that the lateral hypothalamus (LH) (Bereiter et al. 1980; Matsuo and Kusano 1984; Matsuo et al. 1984; Murzi et al. 1986) and the gustatory cortex (GC) (Smith and Li 2000) modulate taste-responsive neurons in the nucleus of the solitary tract (NST). Stimulation of the GC (Di Lorenzo and Monroe 1992), but not the LH (Murzi et al. 1986), also has been shown to influence gustatory cells in the PBN. Descending activity was reported to be inhibitory, excitatory, or without effect on ongoing spontaneous discharge. Although modulation of taste-evoked responses was not assessed, these studies indicate that the stimulation sites in the LH and the GC, which receive afferent gustatory information, generate descending activity in the same system. Our data are consistent with these findings and extend them by showing how a different forebrain region modulates gustatory evoked responses in the PBN. Similar to the LH and the GC, the CeA receives taste information from the PBN, and the PBN, in turn, receives a reciprocal connection from the CeA (Halsell 1992; Norgren 1976).

Coding

Given what is known about the central anatomy of the gustatory system, both the site and parameters of electrical stimulation were largely arbitrary. Nevertheless, we observed considerable effects, and the changes were coherent. Amygdala stimulation inhibited the spontaneous activity and the response to each taste stimulus in 18 of the 32 NaCl-best neurons and 14 of the 15 CA-best neurons. In NaCl-best neurons, the degree of inhibition on spontaneous activity and responses to the five sapid stimuli was similar, but in CA-best neurons, inhibition was greatest for the most effective stimuli (e.g., CA and NaCl). When CeA stimulation facilitated responses in NaCl-best cells (5 of 32), the effect was specific to that sapid stimulus; spontaneous activity and the responses to sucrose, CA, QHCl, and NaCl mixed with amiloride were unaffected. The remaining NaCl-best neurons (9 of 32) were not influenced by electrically stimulating the CeA. The overall effect of CeA stimulation was to sharpen the gustatory response profiles in PBN neurons.

Specifically, the breadth of responses in NaCl-best and CA-best neurons was reduced during concurrent CeA stimulation, indicating that these neurons increased their chemical selectivity. This shift in chemical sensitivity was most pronounced in the NaCl-best neurons that were inhibited by forebrain stimulation. In this subset of neurons, sucrose, CA, and QHCl became relatively less effective during CeA stimulation, and the percentage of the total response profile produced by NaCl increased from 60 to 78%. At least as far as the present NaCl-best cells are concerned, the entropy measure during CeA stimulation (0.45 ± 0.05, mean ± SE) was near the value calculated for NaCl-specific PBN cells in awake behaving rats (0.39 ± 0.05) (Nishijo and Norgren 1990). In an anesthetized preparation, activity in the CeA modulated NaCl-best neurons in such a way that NaCl became a more salient stimulus. In contrast, the inhibitory effect of CeA stimulation in CA-best neurons disrupted the differential responsiveness to CA and NaCl that is typical of this category. The small number of sucrose-best neurons (n = 3) precludes any conclusion concerning the influence of CeA activation.

Possible neural connections

The differential effect of CeA stimulation on NaCl-best PBN neurons provides a hint of how forebrain activity might modulate the central gustatory system. The gustatory-evoked responses of different NaCl-best neurons isolated in the same preparation were inhibited, facilitated, or unaffected during electrical stimulation of the CeA. Modulation of spontaneous activity might underlie the inhibitory effect on gustatory responses because the degree of inhibition was similar in the presence and absence of a sapid stimulus on the tongue. In contrast, with regard to NaCl, the excitatory effect was dependent on taste-evoked activity. This differential effect of CeA stimulation on NaCl-best cells is further distinguished by the suppressive effect of amiloride on NaCl responses. The inhibited gustatory neurons included both amiloride-sensitive and amiloride-insensitive cells, while the facilitated gustatory neurons included only amiloride-sensitive cells. The excitatory effect, but not the inhibitory effect, of CeA stimulation on NaCl-evoked activity was disrupted by amiloride suppression of NaCl responses. One interpretation is that a certain amount of afferent activity might be required to engage the excitatory descending influence and that this is normally achieved only with above threshold concentrations of NaCl.

The only monosynaptic projections from the amygdala to the brain stem originate in the CeA (Halsell 1992; Veening et al. 1984). Nevertheless, electrical stimulation cannot differentiate between activation of intrinsic neurons and fibers of passage. Using small infusions of an excitotoxin that does not activate fibers of passage could test this possibility. If one assumes that fibers of passage were involved, then the activation of the CeA could have either influenced PBN neurons through direct syn-
aptic contact or through an indirect projection via some other forebrain region. As already mentioned, the PBN distributes axons at least to the hypothalamus, amygdala, and bed nucleus of the stria terminalis, and, in turn, receives projections from each of these brain structures. Moreover, these more rostral regions are connected with each other (see Norgren 1985 for review; Halsell 1992; van der Kooy et al. 1984; Yamamoto et al. 1984). The dissociation between these two alternative pathways might be tested by comparing gustatory-evoked responses in the PBN during CeA activation with and without concurrent inactivation in each of these other rostral structures. A final possibility is that the effects we observed in the PBN were first established in the NST and then transmitted rostrally to the PBN. In a prior study, stimulation in the LH modulated NST gustatory neurons, but not those in the PBN (Murzi et al. 1986). Any inferences drawn from these earlier data must be tempered by the small sample of PBN neurons tested (n = 12), and the obvious fact that centrifugal influences on brain stem gustatory neurons may differ between the LH and the CeA. A more direct test would be to repeat the present study, but record from gustatory neurons in the NST.

Other visceral sensory modalities also are subject to centrifugal modulation. Many cardiovascular-sensitive cells in both the NST and PBN respond to electrical stimulation in the hypothalamus and the amygdala (Cechetto and Calaresu 1983; Cox et al. 1986; Mifflin et al. 1988; Silva-Carvalho et al. 1995). In the case of hypothalamic stimulation, afferent activity evoked in the NST by inflating a balloon in the descending aorta or the carotid sinus was either inhibited, facilitated, or unaffected (Silva-Carvalho et al. 1995). Interestingly, the efficacy of afferent input was facilitated in two NST neurons without a synaptic response (e.g., excitatory postsynaptic potential) to the hypothalamic stimulation itself. The same types of influence, exerted by corticofugal input, have been reported in thalamic neurons that respond to auditory and somatosensory stimulation (Ghosh et al. 1994; He 1997; Villa et al. 1991). In the present study, electrical stimulation of the CeA also had variable effects on somatosensory cells in the dorsal pons (n = 15, data not shown). The spontaneous activity was unaffected (n = 11), inhibited (n = 2), or facilitated (n = 2).

Implications for sodium appetite

The central nucleus of the amygdala (CeA) participates in the maintenance of body fluid balance. For instance, c-fos expression in the central and medial divisions of the amygdala is increased by peritoneal dialysis-induced sodium depletion (Johnson et al. 1999). This immunocytochemical index of neural activation indicates that cells in these two amygdala subnuclei responded to the change in sodium balance. In addition, bi-lateral lesions of the CeA disrupt the sodium appetite that develops following furosemide (subcutaneous), DOCA (subcutaneous), or renin (intracerebroventricular) injection (Galaverna et al. 1991; Zardetto-Smith et al. 1994). The role of the gustatory system is to carry the neural information that signals the presence of the Na⁺ ion. Specifically, the amiloride-sensitive NaCl-best neurons are indispensable for the discrimination between sodium and nonsodium salts (Spector et al. 1996) and for the expression of sodium appetite (Bernstein and Hennessy 1987; Breslin et al. 1993; McCutcheon 1991).

One function of centrifugal projections to sensory nuclei might be to extract information from the incoming barrage based on current demands. A few electrophysiological studies have demonstrated that the induction of a sodium appetite alters the sensitivity of the gustatory system to sodium (Contreras and Frank 1979; Nakamura and Norgren 1995; Tamura and Norgren 1997). Whether sodium sensitivity is increased or decreased, however, seems to depend on the method of inducing the sodium need state. For instance, dietary sodium deprivation reduced the responsiveness to NaCl in both chorda tympani (Contreras and Frank 1979) and NST (Nakamura and Norgren 1995) NaCl-best neurons. In contrast, a sodium appetite induced with the diuretic furosemide increased specifically the response of NaCl-best NST neurons to NaCl (Tamura and Norgren 1997). Our findings complement these prior neurophysiological studies insofar as the activation of the CeA, a region known to be responsive to changes in sodium balance, also alters gustatory sensory processing of sodium. Activation of the CeA inhibited the responsiveness of some NaCl-best neurons to NaCl and other sapid stimuli (e.g., dietary sodium deprivation), while in other NaCl-best neurons the response to NaCl was increased selectively (e.g., furosemide sodium depletion).

Although the effect of this motivational state on gustatory sensory processing in the PBN has not been examined, the anatomical connections of the CeA are well suited to modulate gustatory information processing in the PBN based on physiological demand. Despite a lack of knowledge about how rostral brain structures might interpret this altered gustatory information, the present results provide clues to a neurophysiological relationship between the CeA and the PBN in the control of NaCl intake. This centrifugal modulation might serve to increase the relative detectability and motivational properties of the Na⁺ ion. Indeed, electrical stimulation in the amygdala of awake-behaving rats has been shown to alter saline preference (Gentil et al. 1971). The direction of the change in preference, however, seems to depend on the particular amygdaloid subnuclei that are stimulated.

Summary

The present study demonstrated that a subcortical forebrain area modulates pontine gustatory neurons. In 38 of 51 PBN gustatory cells, descending activity generated in the CeA influenced taste-evoke responses. Specifically, taste responses were inhibited in 33 cells and facilitated in another 5 cells. This centrifugal modulation increased the chemical selectivity of pontine taste cells, particular with regard to NaCl-best cells. The NaCl message transmitted through this subset of neurons was accentuated, which tends to mimic the changes in NST taste cell responsiveness produced by a motivational state, sodium appetite. Thus the gustatory neural code is not rigid, but subject to modulation by visceral signals and experience that likely involves centrifugal input from the forebrain. In this context, we have identified one forebrain area, the central nucleus of the amygdala, that participates in endocrine functions, receives afferent gustatory input, and generates descending activity that alters gustatory sensory processing in the dorsal pons.
REFERENCES


