Integration of gastric distension and gustatory responses in the parabrachial nucleus

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Baird, John-Paul, Susan P. Travers, and Joseph B. Travers. Integration of gastric distension and gustatory responses in parabrachial nucleus. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R1581–R1593, 2001.—Palatable gustatory stimuli promote feeding, whereas gastric distension generally inhibits this behavior. We explored a neural basis for integration of these opposing sensory signals by evaluating the effect of gastric distension on gustatory responses in the parabrachial nucleus (PBN) of anesthetized rats. Sixteen percent of 92 taste cells were coactivated; they responded to independent taste or gastric distension stimulus application. Modulation of taste responses by distension was more prevalent; taste responses declined 37% in response to distension in 25% of the cells and increased by 46% in 10% of cells. Across the whole population, however, the suppressive effect of distension on taste responses was small (6%). The incidence of modulation did not vary as a simple hedonic function of gustatory sensitivity, i.e., similar proportions of sucrose-, citric-acid-, and QHCl-best, but not NaCl-best, neurons were modulated by gastric distension. Coactivated, modulated, and nonmodulated gustatory-responsive cells were intermingled in the gustatory zone of the caudal PBN. The suppression of PBN taste responses by visceral stimulation may reflect a mechanism for satiation and further implicates the PBN in the control of ingestive function.

nucleus of the solitary tract; electrophysiology; satiation; satiety; rat; stomach

THE INTEGRATION OF TASTE and postingestive feedback signals is fundamental to feeding behavior. Indeed, the progress of meal taking has been modeled as a function of the changing balance of taste (excitatory) and postingestive (inhibitory) feedback signals derived throughout the meal (11–14, 57). For example, when gut accumulation of food is prevented during feeding through redirection of ingesta via an open gastric fistula (“sham feeding”), oral food intake is grossly elevated because of a lack of feedback inhibition (reviewed in Ref. 55). Conversely, taste reactivity is significantly diminished when rats are given a gastric load before taste sampling (8, also see Ref. 32). Yet, only a handful of studies have begun to uncover the neurophysiological concomitants of taste-gut integration (e.g., Refs. 24, 33, 40, 51).

Although areas throughout the brain have been implicated in taste-visceral processing, interpretation of

integration involving sensory, mnemonic, conditioned, and motivational factors that occurs in forebrain regions (see Ref. 51 for a review) may be clarified by understanding the initial integration of afferent stimuli (27). A brain stem locus for basic taste-visceral integration is implicated because chronic decerebrate rats, in which all neural connections between the forebrain and the brain stem are severed, can nonetheless modify intake of a sapid stimulus after a gastric preload (30, 54).

In the brain stem, both vagal visceral and taste afferent fibers synapse within the nucleus of the solitary tract (NST; for review, see Refs. 47 and 52), but they have a spatially distinct representation. Although previous investigators (20–24, 26) have reported that taste responses in the NST are modulated by longer-term postingestive treatments, such as sustained gastric distension or venous glucose infusions, it is not clear whether these effects result from primary afferent integration per se or from more indirect, perhaps hormonal, influences. In fact, Hermann et al. (37) failed to find coactivation of NST neurons during paired electrical gustatory and vagal stimulation.

There is, however, considerable neuroanatomical (35, 40, 46) and lesion/behavioral (e.g., Refs. 50 and 56) evidence to suggest that afferent signals from the oral cavity and the gastrointestinal tract are integrated in the parabrachial nucleus of the pons (PBN), which receives overlapping projections from the rostral (taste) and caudal (visceral) regions of the NST. The most direct confirmation of this integration is from electrophysiological studies. Hermann and Rogers (36) found single PBN units responsive to oral NaCl and to electrical vagal or caudal NST stimulation. Although provocative, the findings are limited because the visceral stimulus was not specific, and, moreover, only one tastant was applied to the anterior tongue, excluding the majority of taste receptors and three common taste qualities (59). However, recent work by Hajnal and colleagues (33) provides further insight into PBN integrative processes. They showed in awake rats that PBN neural responses to intraoral sucrose and NaCl were significantly diminished ~10 min after a duodenal intralipid infusion.

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In the present study, we used acute extracellular techniques to record responses of single PBN gustatory neurons to separate and conjoint applications of oral taste stimuli and gastric distension. Gastric distension represents a different visceral stimulus than that tested by Hajnal and colleagues (33). Unlike caloric stimuli, distension is rapidly reversible, thereby allowing repeated measurements in the same cell. Because the integration tests using distension are brief (<1 min), indirect mediation via hormonal contingencies is less likely than with caloric stimuli. In addition, the rapid reversibility of distension permits the following two forms of integration to be assessed: coactivation, as shown by Hermann and Rogers (36; a separate response to visceral and taste stimulation), and modulation (a change in the taste response during visceral stimulation despite no overt response to visceral stimulation), as shown by Hajnal et al. (33).

METHODS

Subjects

Fifty-four male Sprague-Dawley rats (Harlan) weighing 348.5 ± 5.3 (SE) g (range = 290–440) at the time of surgery were tested. Rats were maintained in individual plastic tubs on a 12:12-h light-dark schedule. Food (Purina Rat Chow no. 5001) and water were available ad libitum. Rats were injected for surgery at the same time each day, ~3 h after lights on. All procedures were approved by the Institutional Animal Care and Use Committee.

Surgery

Rats were anesthetized with Nembutal (50 mg/kg ip), with supplementary doses to maintain a nonreflexive level of anesthesia. On occasion, rats were pretreated with ethyl carbamate (1.0 g/kg) to prolong the viability of the experiment. All surgeries were performed under aseptic conditions. Core temperature was regulated by a rectal thermometer and adjustments to a heating pad.

The visceral surgery was based on protocols established in many previous gastric recording studies (e.g., Refs. 2, 4, 16, 17, 26, 40, 58). A laparotomy was made, and the antrum was exposed. Gastric contents were removed via a small incision, and a gastric balloon was inserted gently toward the corpus region. The balloon was made from a latex glove finger and was fastened via Teflon tape to one end of a Tygon tube (1/32 in. ID, 3/32 in. OD). The wound was ligated around the balloon's shaft. The stomach was then viewed via a surgical microscope over a range of balloon inflation volumes to ensure proper function and to evaluate possible tissue damage. If small tears (typically ~1 mm, perpendicular to the longitudinal axis) were observed, inflation was immediately halted, and larger inflation values were not tested. The laparotomy was then closed, a tracheotomy was performed, and an oral drain tube was inserted along with retractable mouth sutures to expose the oral cavity (34). The rat was then placed in a stereotaxic frame, and the skull was exposed and leveled. The left hemisphere was trephined 1.7 mm lateral and 0.4 mm anterior to lambda to expose ~15 mm² (5 mm × 3 mm) of brain surface, allowing full access to the PBN. The left side was tested because the left NST receives dominant innervation by the hepatic vagal branch, which carries gastric-antral fibers within it (1, 6).

Stimulation and Recording

Taste stimuli were delivered to the whole mouth from two pipettes fed by a pressurized solenoid-controlled manifold fluid delivery system. One pipette was directed at the nasoincisor ducts, and the stream of the other was directed over the whole tongue surface. Previous studies from this laboratory have shown that this method is adequate to evoke responses from the anterior tongue, nasoincisor ducts, soft palate, and foliate papillae and occasionally suffices to evoke responses from the circumvallate papilla (34). Fluid delivery was controlled by a computer program to achieve precise coordination of gustatory and visceral stimuli. An infusion/withdrawal pump (model 210P; KD Scientific) controlled by a computer program was used to deliver gastric stimuli. The gastric balloon was connected to a 140-ml syringe. Fluid stimuli initially included dH₂O, 0.3 M NaCl, and 0.3 M sucrose (appetitive stimuli) and 0.01 M HCl and 0.003 M QHCl (aversive stimuli). An undiluted "taste mixture" containing each of these tastants served as a "probe" taste stimulus for searching. Because initial experiments yielded few HCl-best neurons, HCl was replaced with 0.05 M citric acid after one-third of the rats had been tested. In addition, the concentration of NaCl was reduced to 0.1 M, and the tastant, 0.1 M NH₄Cl, was also added to the test battery to better discriminate broadly tuned electrolyte-responsive cells from neurons more narrowly tuned to sodium salts (45). Analyses based on taste quality were restricted to cells tested using the final stimulus array. Gastric volumes tested spanned the "normal range" of distension (see Ref. 4), and noxious volumes were avoided. For the current study, 6, 12, and 18 ml were selected as "anchor" volumes for initial testing. In about one-third of rats, 18 ml was not tested because it produced tissue damage.

Recording. Glass- and parylene-coated tungsten microelectrodes [impedance (Z) = 0.5–2.5 MΩ; conical tips ~7 μm × ~15 μm (base × height)] were used to record extracellular activity of single neurons in the PBN. Neural activity was amplified, monitored on a storage oscilloscope, and stored on magnetic tape. Stimulus markers were recorded on separate channels. Data were simultaneously collected using the MII (Modular Instruments Systems) or Spike2 (Cambridge Electronic Design) systems.

Search procedure. The electrode was inclined 20° posteriorly to prevent transverse sinus rupture and was advanced automatically using a piezoelectric microdrive. Once the brain stem was encountered, neural responses were evaluated in 25-μm increments. The taste mixture, followed by rinsing, was used as a search stimulus. The gastric probe stimulus was 6 ml air distension. Jaw stretch was tested by depressing the lower incisors. If a jaw stretch response was observed (indicating the mesencephalic trigeminal nucleus), the electrode was withdrawn and a new track was started. Oral tactile responses were evaluated using a glass probe to stroke the anterior tongue, foliate region, and occasionally the soft palate. Tracks were made at 100- to 200-μm points along a grid over the exposed region of the brain surface.

Stimulation protocol. GUSTATORY STIMULATION. If a single neuron responsive to the taste mixture was detected, the single most effective taste stimulus was evaluated. Taste stimulation (10 s) was preceded by a 30-s unstimulated period, the middle 10 s of which was the "spontaneous" period for data analysis. After taste offset (10 s), a dH₂O rinse was applied for 20 s (34), followed by a 50-s rest interval. Taste stimuli were presented in the order sucrose, NaCl, HCl (or citric acid), QHCl, NH₄Cl, and dH₂O. Taste stimuli and dH₂O were individually tested (2 min each), up to three times

each. If the “best” taste response was not clear, replications of suspected best stimuli were conducted.

DISTENSION STIMULATION. Cells were then tested for a response to gastric distension at 6 ml. The balloon was inflated for 10 s, held fully inflated for 10 s, and then deflated for 10 s. Gastric stimulation was preceded by 30 s of baseline recording and was followed by a 90-s pause. If no response was noted at 6 ml, then 12 ml was also tested. If there was a distension response, replications of the three gastric anchor volumes were conducted at least two times each as follows: 6, 12, and 18 ml (if possible). If this protocol was completed, 3-, 9-, and 15-ml (if possible) volumes were then tested.

MODULATION TESTING. Modulation was then evaluated by testing the best gustatory stimulus before, during, and after applying the best gastric stimulus (6 min/test). Modulation tests consisted of three taste tests [a “before” (taste only), “during” (taste + gastric), and “after” (taste only) distension taste test]. The before and after taste tests were identical in structure to those described for a single taste test. For the during condition, taste and gastric stimuli were presented simultaneously; thus, after the spontaneous period, the stomach was inflated for 10 s. Once full inflation was achieved, the taste stimulus was applied for 10 s. For the next 10 s, the stomach was deflated. For the next 20 s, a water rinse was applied, followed by an interstimulus interval of 120 s.

Cells were usually tested for modulation using 6 ml and 12 ml one time each. If modulation was suspected, replications were conducted, and an 18-ml modulation test was added (if this volume was testable). In a subset of cells ($n = 16$), we also performed “mock” modulation tests that were identical to modulation tests, except that the gastric stimulus was not applied. After modulation tests, if the cell remained isolated, additional replications of the taste-gastric modulation sequence were conducted at the same or different volumes.

Histological reconstruction. A lesion (anodal current: 3 $\mu\text{A} \times 3$ s) was usually made at the recording site or subjacent to it. Concluding testing, the rat was given a lethal dose of anesthetic and was perfused with isotonic saline followed by 10% buffered formalin. The brain was blocked in the recording plane, removed, and sectioned. Alternate sections were stained with Weil and cresyl violet to distinguish myelinated fibers from somatic components.

Neurophysiological data analysis. Electrophysiological data were analyzed off-line. Response measures used were the net spike counts during the stimulation period. Net spike counts were derived by subtracting the spike count during the spontaneous period before a given stimulation from the spike count during the stimulation period. All periods were matched in duration (10 s). For gastric stimulation, measurements were derived for three 10-s periods, during “inflation,” “hold,” and “deflation.” Responses were considered significant if they satisfied the appropriate criteria described below.

GUSTATORY AND GASTRIC RESPONSES. To be regarded as a significant taste response, the net response elicited by gustatory stimuli had to exceed 2 SD of the mean spontaneous rate for all spontaneous periods evaluated for that cell. In addition, the taste response had to exceed any response to dH_2O by at least 100% (i.e., two times the water response). Gustatory-responsive neurons were considered to be coactivated by gastric stimuli if the net response for any one of the three 10-s distension periods (inflation, hold, or deflation) exceeded 2 SD of the mean spontaneous rate for that cell, or, for inhibitory responses, the net spike total fell below 50% of the spontaneous rate.

MODULATED GUSTATORY RESPONSES. Although studies have used various criteria to evaluate changes in neural taste/visceral responses after treatment (modulation; see Refs. 2,

15, 22, 33, 61), we approached the issue empirically by evaluating mock modulation tests in a subset of cells ($n = 16$). For these 16 neurons, the mean of the absolute percent change from the “mock before” to the “mock during” stimulation was 10.5% and from the “mock before” to the “mock after” period was 12.5%. Thus the criterion for a taste “modulation” in actual distension tests was defined as a taste response change of 25% from baseline in either the during or after distension taste test, which is two times the average variation seen across repeated “taste-only” tests, i.e., mock trials. This criterion is thus analogous to our criteria for significant taste and gastric responses.

To simplify the modulation analysis, we selected one modulation test for each cell, the modulation test that used the most effective taste stimulus for that cell at the largest volume tested. Thus 71/92 cells had a modulation test using the most effective taste stimulus. For 4 of these cells this volume was 6 ml; for 2 cells, 9 ml; for 51 cells, 12 ml; for 1 cell, 15 ml; and for 13 cells, 18 ml. If a cell had multiple modulation tests at the same tastant and distension volume ($n = 35$), we used the average values across tests. Cells that were not used for analysis either had no modulation test ($n = 13$) or were tested for modulation using a tastant that was not the optimal stimulus for the cell ($n = 8$; also see Table 1).

Statistical Analysis

Electrophysiology. For hierarchical cluster analysis, the Pearson product-moment correlation coefficient and average-linkage methods were used to calculate taste profile similarities in cells tested using the final taste stimulus array ($n = 55$). Pearson correlations were also used to compare responses in coactivated neurons (net response to taste vs. net response to distension). Modulation effects across the population were evaluated using ANOVA. χ^2 analysis was used to determine if the chemosensitivity of neurons was systematically related to whether or not they showed modulation.

Histology. Recording sites were plotted on one of four representative serial sections of PBN, each section ~ 200 μm apart. If a lesion was made at the recording site before the electrode was moved for further sampling, then the cell could be localized within morphologically distinct PBN subnuclei (19, 34). However, if the lesion was not made at the recording site or was made at the site upon removal of the electrode, the precise location in the dorsoventral axis could not be specified because of factors such as tissue hysteresis and shrinkage. In the present study, we restricted our histological analysis to gustatory neurons marked with lesions at the recording site. In addition to analyzing the anatomical distribution of gustatory neurons, we also compared their locations with the distribution of a population of gastric-responsive neurons recorded in an earlier study (4). Because fewer neurons in the gastric study were marked with lesions at the recording site,

Table 1. Summary of neurons recorded

	Count
Overall	
Cells recorded	92
Cells tested for coactivation	92
Cells tested for modulation	71
Coactivated cells tested for modulation	12
Noncoactivated cells tested for modulation	59
Gustatory coding	
Cells tested using final taste battery	55
Cells tested for coactivation	55
Cells tested for modulation	38

we included cells with lesions made on the same track that were no more than 250 μm distant from the site to have an adequate sample for comparison. χ^2 analyses were used to evaluate whether neurons were differentially distributed according to their response characteristics.

RESULTS

Out of 92 taste cells only a small proportion showed a reliable response to gastric distension. For the most part, the effects of distension on these “coactivated” cells was inhibitory, although a few excitatory responses were observed. A greater proportion of cells, however, showed an appreciable modulation of taste responsivity by distension in the absence of a clear distension response per se. Table 1 shows which groups of cells were tested for coactivation and/or modulation.

Coactivated Taste Cells

Sixteen percent of the taste cells (15/92) exhibited an independent response to distension and were thus considered coactivated (e.g., Fig. 1). Gastric responses were generally inhibitory, and the taste responses were generally excitatory (Fig. 2, *inset*). Across coactivated cells, the mean response evoked by distension was significantly smaller than that evoked by taste; however, the response magnitude for distension responses was generally consistent with other reports in the PBN (e.g., Refs. 4 and 58). In coactivated neurons, distension evoked a 1.8 ± 0.4 net spikes/s suppression, whereas taste effected a 10.5 ± 3.7 spikes/s increase [$t(14) = 2.30$, $P < 0.04$]. Interestingly, the magnitude of the best taste response in coactivated cells was small compared with that for distension-insensitive taste cells; all but three coactivated cells had taste response rates below the median (Fig. 2).

Modulated Taste Responses

For 71/92 taste cells, we were able to test the modulatory effects of gastric distension on the response to the “best” tastant (Table 1). Across the population, the inhibitory effect of distension was small (6%) but statistically significant [$F(2,140) = 3.24$, $P < 0.04$]. In contrast, the spontaneous rate preceding each of the test periods (before, during, and after stomach inflation) did not vary significantly [$F(2,140) = 2.19$, not significant (NS)], suggesting that the decline in responsiveness was not the result of a general decrement in firing rate during the recording session. Similarly, it is important to point out that the decline in responsiveness related to gastric distension was not simply the result of an effect of repeated taste stimulation. Sixteen cells were evaluated with mock distension trials, and there was no significant difference across mock before, during, and after taste trials; the mean response during mock distension declined by just 2% [$F(2,32) = 0.37$, NS]. By comparison, for the same 16 neurons, the mean gustatory response declined by $\sim 14\%$ during real distension [$F(2,30) = 4.00$, $P < 0.03$]; again, there was no effect on spontaneous activity across the three trials [$F(2,30) = 1.54$, NS].

Fig. 1. Example of a coactivated cell. *A*: cell shows an excitatory response to 0.3 M sucrose application. *B*: same cell as in *A* shows an inhibitory response during 12 ml gastric distension.

Although distension effects on taste responsivity across the population were small, some individual cells showed robust effects. When the modulation criterion (see METHODS) was applied to responses obtained from each cell during its best taste response, a suppressive effect of gastric distension was noted in 18/71 (25%) taste-responsive cells, and a facilitatory effect was noted in 7/71 cells (10%). Examples of two neurons showing robust and replicable suppression and enhancement during gastric distension are shown in Fig. 3. *Neuron 9906-1* was a sucrose-best neuron that responded to 0.3 M sucrose with a mean net increase of 118 spikes/10 s before gastric stimulation. When the same taste stimulus was applied simultaneously with gastric distension (18 ml), the response declined, on average, to 71 net spikes/10 s and then returned to near baseline levels (122 net spikes/10 s) in the trials after distension. *Neuron 9859-3* was a slowly firing neuron that increased its average firing rate by 10 spikes/10 s in response to its most effective stimulus,

Fig. 2. Rank-ordered distribution of each cell's best net taste response (net spikes/10 s). "Coactivated" cells (filled bars) responded significantly to gastric distension ($n = 15$). *Inset*: Scatter plot of the magnitude of the best taste response and the gastric distension response (net spikes/10 s) in coactivated cells.

0.01 M HCl. When the stomach was inflated (12 ml), the mean response tripled to 32 net spikes/10 s and again returned to baseline in the trials after distension.

Across all neurons meeting inhibitory modulation criteria ($n = 18$), the mean taste response during distension declined by 37% relative to the average baseline response (Fig. 4A, *inset*). The suppressive effect was most apparent during the distension period and gradually returned to baseline as the stomach was deflated. For neurons meeting excitatory modulation criteria ($n = 7$), the mean taste response during distension increased by 46% (Fig. 4B, *inset*). As with the cells showing suppression, the effects of distension on taste were most apparent during the 10-s fully distended period and returned to baseline during deflation.

Thirteen cells meeting modulation criteria were similar to the examples depicted in Fig. 3 in that modulation occurred only during distension. However, in six cells, modulation occurred both during and after distension, and in six cells modulation was seen only after distension. The prolonged influence of distension on taste responses in this subset of cells is apparent in the mean after responses shown in Fig. 4, *insets*.

The subpopulation of coactivated cells was also evaluated separately for modulation effects (see Table 1). As might be expected in neurons independently responsive to distension, a larger proportion (7/12; 58%) showed modulation compared with those that were not coactivated (18/59; 31%). A two-way χ^2 test of this hypothesis was marginally significant [$\chi^2(1) = 3.84$, $P < 0.066$]. However, statistical analysis showed no main "before-during-after" effect across the 12 coactivated cells tested for modulation [$F(2,22) = 1.69$, NS], perhaps because of offsetting excitatory ($n = 2$) and inhibitory ($n = 5$) responses. Nevertheless, there was a positive correlation ($r = 0.78$, $P < 0.003$; Fig. 5) between the magnitude and direction of the gastric re-

sponse and the magnitude and direction of change in the taste response during the modulation test. Therefore, individual coactivated cells appeared to summate (linearly integrate) their intrinsic taste and gastric response propensities (for both polarity and magnitude) when exposed to conjoint stimulation.

Variations by Taste Quality

We restricted our analysis of the relationship between gustatory and gastric responsiveness to cells tested with the final stimulus battery ($n = 55$; see Table 1). A somewhat detailed description of the chemosensitivity of these neurons is appropriate, since previous investigators have shown that state manipulations modulate taste responses differentially according both to the optimal chemosensitivity of a neuron and its breadth of tuning (33, 43). The dendrogram in Fig. 6 shows the results of a cluster analysis, suggesting that the neurons are divisible into four groups, based on responsiveness to 0.3 M sucrose, 0.1 M NaCl, 0.05 M citric acid, 0.1 M NH_4Cl , and 0.003 M QHCl. The groups defined were similar to those reported previously for rodent brain stem taste neurons (15, 33, 34, 44, 45) and, for the most part, were well defined by their best stimulus. Mean profiles are shown in Fig. 7. The largest group of cells (Fig. 7C, $n = 31$, E, or "electrolyte" neurons), was, on average, most responsive to citric acid. However, mean responses of this group to NaCl, and particularly NH_4Cl , were robust (39 and 71%, respectively), which indicates a general electrolyte sensitivity (Fig. 6). A second group (Fig. 7B, $n = 14$, N neurons) was more narrowly tuned. Both on average and for every individual cell, N neurons responded best to NaCl (Fig. 6). The second-best stimulus was NH_4Cl , which evoked a mean response only 38% as great as NaCl. A third group (Fig. 7A, S neurons, $n = 8$) was characterized by an enhanced sucrose responsiveness. Sucrose nominally elicited the largest

Fig. 3. Individual examples of modulation. A: consecutive modulation tests for a sucrose-best cell that was suppressed during 18 ml gastric distension and returned to baseline after distension. B: consecutive modulation tests for an HCl-best neuron that was excited during 12 ml gastric distension. Filled bars, taste response before distension; open bars, taste response during distension; hatched bars, taste response after distension. Taste stimulation in the during conditions corresponded to the 10-s period of full distension (see Fig. 4).

mean response in S neurons, but the response to the second-best stimulus, NH_4Cl , was 82% as great. Indeed, only 5/8 S neurons actually responded best to sucrose (Fig. 6). The final two neurons formed a small "group" most responsive to QHCl (Fig. 7D). However, the mean response to QHCl for this group was small relative to the optimal responses for the other groups of cells, and the response to NH_4Cl was relatively large.

Similar to what was observed across the entire population, 14% of the cells tested with the final stimulus battery were coactivated. Coactivated neurons appeared evenly distributed across clusters. Two of 14 N cluster cells (14%), 4/31 E cluster cells (13%), 1/8 S cluster (13%) cells, and 1/2 quinine cluster cells were coactivated.

In contrast, there was evidence for a relationship between gustatory chemosensitivity and modulation. Thirty-eight of the 55 cells tested with the final stimulus battery (7 S, 11 N, 18 E, and 2 Q) were available for the modulation analysis, i.e., those tested for modulation of their cluster-best tastant by gastric stimulation with the highest volume tested for an individual cell. For ANOVA tests, the QHCl cells were removed because of the small sample size, leaving three factors (taste clusters) in the quality dimension. Across this sample, two-way ANOVA revealed no systematic effect of distension according to cluster type [cluster: $F(2,33) = 0.47$, NS; interaction: $F(4,66) = 0.53$, NS]. However, as for the entire population of cells, there was a main inhibitory effect of distension [$F(2,66) = 3.32$, $P < 0.04$]. For individual responses, 2/7 S, 6/18 E, 2/2 Q, but only 1/11 N cells showed a modulation that satisfied the criterion (see Table 2). χ^2 tests indicated that the N neurons were less likely to be modulated than the other types of cells [$\chi^2(1) = 7.37$, $P < 0.007$]. With respect to response direction, two of the six modulated acid-cluster cells showed excitatory modulation; all other cells showed inhibitory modulation.

Because Hajnal et al. (33) showed that the breadth of gustatory tuning was related to the magnitude of modulation, we correlated entropy to the magnitude of modulation (percentage of baseline), but no significant relationship ($r = -0.19$, NS) was observed. We also compared the breadth of tuning of cells that were or were not modulated, but the difference was not significant [$F(1,50) = 2.78$, $P < 0.10$].

Topography-Integration Relationships

The majority of histologically identified units (29/31) was located in the caudal half of the PBN (Fig. 8A, levels a and b). Most cells were recorded within the brachium itself, both ventral and lateral to the ventral lateral subnucleus, where a subset of cells ($n = 5$) was also localized. Several cells were located in the central medial subnucleus, ventral to the brachium. Taste cells that exhibited gastric responsivity (coactivation and/or modulation) were not uniquely distributed in the PBN compared with taste cells with no evidence of gastric input [$\chi^2(2) = 1.83$, NS]. However, compared with cells that responded only to gastric distension (replotted from Ref. 4; Fig. 8B), a greater proportion of gastric cells was located in the rostral levels of PBN than were taste cells [$\chi^2(3) = 17.92$, $P < 0.0001$]. Despite this statistical segregation, there was considerable overlap between gastric-only and taste-responsive cells in the caudal taste areas. Moreover, taste-responsive cells that were also gastric responsive were comingled with taste-specific neurons in the caudal PBN (Fig. 8A).

DISCUSSION

Approximately one-third of the gustatory cells tested were either coactivated or modulated by gastric distension. These data provide evidence of taste-visceral integration in the PBN using naturalistic stimuli and complement previous studies that implicate this nu-

Fig. 4. Modulation effect for cells satisfying the 25% criterion. *A*: mean time course response of 18 inhibitory cells to taste stimulation before and during gastric distension. *Inset*: mean + SE net response of 18 cells to taste stimulation before, during (middle 10 s of distension), and after gastric distension. The decline in gustatory response during distension in these 18 cells averaged 37% suppression from baseline. *B*: mean time course response of 7 excitatory cells to taste stimulation before and during gastric distension. *Inset*: mean + SE response of cells during taste stimulation before, during (middle 10 s of distension), and after gastric distension. The excitatory taste response during distension averaged 46% above baseline.

cleus as an important locus for the coordination of the afferent taste and visceral signals that structure feeding (e.g., Refs. 29, 33, 36, 40, 41, 50, 52, 56).

Inhibitory Effects of Distension

Overall, gastric distension had an inhibitory effect on taste-responsive cells. However, rather few taste

cells (16%) responded outright to gastric stimulation. This is notably smaller than the proportion of gustatory neurons coactivated by electrical stimulation of the cervical vagus and tongue (54%; see Ref. 36). The larger proportion of gustatory cells coactivated by vagal stimulation is not surprising, because this stimulus would be likely to evoke a simultaneous barrage of

Fig. 5. Response of coactivated cells to conjoint taste and gastric stimulation. There was a significant correlation between the net magnitude of the gastric distension response (net spikes/10 s) and the change in the taste response (net spikes/10 s) during distension vs. baseline tests ($r = 0.78$, $P < 0.003$). In all but three cases, the direction of change in the taste response during conjoint stimulation was in the same direction as the gastric response.

activity from many types of visceral afferent fibers in addition to gastric mechanoreceptors. As a group, taste responses of coactivated gustatory neurons in the present study were weak compared with the overall population (Fig. 2). Their distension responses were even smaller, although generally consistent with the magnitude of distension responses reported in the PBN (4, 58). However, their inhibitory nature distinguishes them from PBN gastric-responsive cells with no taste sensitivity, which are usually excitatory (4, 58). The inhibitory character of the present distension responses is emphasized by the overall suppressive effect seen during conjoint stimulation (modulation). Indeed, responses to distension were more commonly in the form of modulation, which was observed about two times as often as coactivation (35% modulated vs. 16% coactivated).

Although pervasive, the magnitude of inhibition and its association with specific gustatory responses differed from previous studies. Intestinal intralipid infusions depressed overall average gustatory responsiveness in the PBN by 16% compared with 6% in the present study (33). Similarly, intralipid infusion and other treatments intended to mimic or produce satiety, including intravenous glucose, glucagon, or insulin injections, all suppressed central gustatory responses to carbohydrates by ~30–50% (20, 21, 23, 24, 26, 33), compared with only 10% for the sucrose group in the present study. In addition, previous investigations reported that responses to palatable tastants (especially carbohydrates but sometimes NaCl) showed more prominent inhibitory modulation after peripheral treatment compared with responses to unpalatable stimuli, like acids or quinine (20, 21, 23, 24, 26, 33). Although the suppression we observed was significantly underrepresented in NaCl-best neurons, it was

otherwise apparent in all best-stimulus classes, i.e., those representing both appetitive and aversive taste stimuli.

In evaluating why our inhibitory effect was weaker, it is important to point out that a subset of the cells (25%) in the present investigation did show stronger (>25%) inhibitory modulation. In addition, a few taste responses were actually facilitated (>25%) by distension. An enhancement of taste responses by satiating stimuli has not been reported previously (20–24, 26, 33, and cf. Ref. 36), and their countervailing influences may partially account for the modest average decline. However, other variables are probably more important.

Assuming that suppression of PBN gustatory responses is a mechanism for producing satiety, we may speculate that the weaker effects of gastric distension on PBN gustatory responsiveness parallel a weaker potency of gastric distension to suppress feeding compared with caloric stimuli. In rats with closed pyloric cuffs, distension produced by intragastric saline loads of 5–10 ml (100–200% of baseline intake) suppressed intake by 30–70% of baseline (49). These volumes fall within the range of those tested here, where a mean suppressive effect on PBN taste responses of

Fig. 6. Cluster tree of 55 taste-responsive cells. Cells are labeled according to significant responses in order of magnitude. The first letter indicates the tastant that evoked the best response. Capital letters indicate that the response was at least 50% as great as that elicited by the best stimulus. A dash preceding a letter indicates that there was an inhibitory response to this tastant. A, NH_4Cl ; C, citric acid; N, NaCl; Q, QHCl; S, sucrose. Cells generally most responsive to A and C fell under one cluster, termed "E", which reflected a common electrolyte sensitivity cluster (see text for details).

Fig. 7. Mean response profiles for each of the 4 taste clusters. A: sucrose cluster. B: NaCl cluster. C: citric acid cluster. D: QHCl cluster. Spont, spontaneous.

6% was observed. By contrast, the same intralipid infusions that decreased PBN sucrose responses by 30% almost completely abated sucrose ingestion for many minutes (see Fig. 2 in Ref. 18). It is difficult, however, to separate the individual contributions of distension and calories across gastrointestinal compartments, independent of other hormonal, metabolic, and mechanical consequences of a postingestive stimulation.

Table 2. *Modulation by cluster quality*

Cluster Type	Modulated	Not Modulated	Total
NaCl	1(9)	10(91)	11
Sucrose	2(28)	5(72)	7
Citric acid	6(33)	12(66)	18
QHCl	2(100)	0(0)	2
Total	11	27	38

No. and proportion of cells in each taste cluster that did or did not show a significant change (>25% baseline) in taste response during gastric distension. Percentages are in parentheses.

It is equally difficult to compare the behavioral efficacy of different treatments against what occurs during normal digestion. When compartments of peripheral feedback are isolated and stimulated out of the context of normal digestion [e.g., the liver (3) or stomach distension (see above)], the inhibitory influences on intake are smaller compared with the effects of oral, gastric, or even intestinal nutrient preloads (e.g., Refs. 39 and 55). Thus stimulation of any single visceral compartment is likely to only partially reveal the capability of such stimuli to influence satiation mechanisms. Perhaps gastric distension initiates a process of gustatory response suppression that is accentuated over time if the gastric load is caloric and reaches the small intestine (see Ref. 55).

Another important variable to consider is the duration of the visceral stimulus. The duration of our visceral treatment (30 s) was much shorter than previous studies, and the metabolic load treatments used in the past likely had long-term influences (at least) tens of

Fig. 8. Schematic representation of the locations of neurons recorded in the parabrachial nucleus (PBN). Serial sections are arranged caudal (*a*) to rostral (*d*), ~200 μ m apart. *A*: distribution of taste neurons with no gastric sensitivity ($\circ$) and taste neurons that were either independently responsive to distension (coactivated; $\blacklozenge$) or showed taste modulation during distension but no intrinsic distension response per se ($\blacksquare$; see text for details). Only lesions made immediately after cell testing, without moving the electrode, were plotted. *B*: comparison of loci of taste cells (all types; $\triangle$) and gastric distension-responsive cells that had no taste sensitivity (*; replotted from Ref. 4). Gastric cells with lesions made at the recording site are plotted as symbols without accompanying tracks. Gastric cells with lesions made within 250 μ m of the recording site are denoted by symbols nominally placed at the top of the track. Tracks with two symbols indicate that two cells were found on the track. bc, Brachium conjunctivum; cl, central lateral subnucleus; dl, dorsal lateral subnucleus; el, external lateral subnucleus; em, external medial subnucleus; il, internal lateral subnucleus; LC, locus ceruleus; Me5, mesencephalic trigeminal nucleus; sl, superior lateral subnucleus; Mo5, motor trigeminal nucleus; Pr5, primary sensory trigeminal nucleus; vsc, ventral spinocerebellar tract; cm, central medial.

minutes long. Indeed, although an effect of intralipid stimulation on taste responses could be discerned ~2 min after infusion, it was very small compared with the maximum suppression observed ~10 min postinfusion (33). Although the relatively short latency argues for a preabsorptive action of intralipid infusion (33), the minutes-long delay before maximum efficacy suggests that intestinal modulation of taste responses may be more complex than a simple integration of intestinal inhibitory and gustatory excitatory signals. Further evidence for a more complicated mechanism by which visceral feedback modifies taste responses is evident in the classic study of Glenn and Erickson (26), which, like ours, used noncaloric gastric distension. These investigators found that NST multiunit sucrose responses were suppressed ~50%

during gastric distension. However, they used a prolonged distension period (~15 min). Inspection of their Fig. 1 and our own review of their raw data (obtained from Ref. 25) suggests that an early effect of distension (i.e., within 60 s of inflation) was not evident. Rather, the response suppression was, as they stated, "...slowly developing (several minutes)..." (26), reaching a nadir by about the 6th min. These findings suggest that more potent modulations of taste responses by postingestive signals may require some time-dependent process, such as hormone secretion, metabolic cascades, or prolonged neuromodulator release in the central nervous system (e.g., Refs. 3, 48, and 53).

In addition to their smaller magnitude, the modulatory effects in the present study were less specific than

in previous studies. Previous studies of the modulation of brain stem taste responses by satiety-mimicking manipulations have reported preferential effects on carbohydrate (glucose or sucrose)-elicited responses (20, 21, 23, 24, 26, 33). We only partially corroborated this finding. Sucrose responses were modulated more frequently than NaCl responses. However, they were no more susceptible to the effects of gastric distension than were citric acid responses. The reason for the lack of a more specific effect on sucrose responses is not obvious, but it may relate to properties of the sucrose-sensitive (S cluster) neurons we recorded. Specifically, in the only other single-unit study of brain stem visceral-gustatory modulation, sucrose responses were suppressed only in cells that responded best to this stimulus (33). Moreover, the largest modulations occurred in neurons specifically responsive to this stimulus, i.e., they were narrowly tuned cells with no response to any other class of tastant. By comparison, neurons in the sucrose-sensitive cluster of the present study were broadly responsive and only nominally sucrose best (Fig. 7A).

The lack of effect of gastric distension on the NaCl responses we observed is not incompatible with previous findings. Multiunit studies using intravenous glucose, insulin, and glucagon likewise noted that NST NaCl responses were unaffected by these treatments (20, 21, 23, 24). In addition, PBN NaCl responses were suppressed by intestinal lipid but less so than sugar responses. In fact, modulation of salt responses only occurred in broadly tuned NaCl-best cells. NaCl-specific cells were not affected. Although all of the NaCl-best cells in the present study displayed some response to other stimuli, as a class, they were the most narrowly tuned group.

The modulation of PBN acid responses by gastric distension in the present study starkly contrasts past studies where neither intravenous satiety factors nor gastric distension appeared to modulate similar taste responses in the NST (20, 21, 23, 24, 26). Intestinal intralipid also did not affect citric acid responses in the PBN, although a very small sample of acid-best cells ($n = 4/59$) was recorded in that study (33). Because all other instances of intestinal intralipid modulation for a given taste quality were confined to the subgroup of cells optimally responsive to that tastant, it is possible that the small sample of acid-best neurons obscured such an effect. It may be noteworthy that the only excitatory modulations we observed were from the acid cluster. Conceivably, if a larger proportion of acid cells showed excitatory responses than other cluster groups, it could account for the lack of an inhibitory effect in previous multiunit studies.

Mechanisms of Integration

The brief latency with which gastric effects occurred in PBN taste neurons suggests that they were mediated via gastric afferent pathways rather than indirect hormonal means. Previous studies suggest a simple anatomical substrate for taste-visceral integration in

PBN with pontine neurons innervated by separate populations of NST cells responsive to gustatory and gastric stimulation, respectively. This hypothesis received early support when Hermann and colleagues (37) demonstrated a lack of oral-vagal convergence in the NST, whereas PBN neurons were coactivated by both signals, with short latencies consistent with a direct medullary projection (36). Although electron microscopic data are necessary to confirm monosynaptic convergence, light microscopic analysis demonstrated intermingling of NST gastric and taste projections in the PBN waist region (40). On the other hand, there is no paucity of anatomical evidence for more complex taste-visceral circuits, including local PBN connections and descending forebrain projections (51).

The present study was not designed to define cellular mechanisms, but the nature of PBN taste-gastric integration provides clues to their general nature. Integration in taste neurons was most often evident only because the gustatory response was modified (modulated) by gastric stimulation; gastric stimulation itself did not elicit a response. Hajnal et al. (33) made a similar observation. Duodenal nutrient infusions had no effect on spontaneous rates of PBN taste cells, even though they profoundly inhibited gustatory responses. These observations imply that gastrointestinal modification of taste responses may result from presynaptic inhibition or from a chemical messenger acting as a neuromodulator (38). The PBN is replete with transmitters and receptors with neuromodulatory actions, including peptidergic and monoaminergic fibers (e.g., Refs. 7, 35, and 42), and a host of metabotropic receptors (e.g., Refs. 9, 10, and 31). In fact, several populations of peptidergic NST-PBN projection neurons originate in the caudal, visceral NST (35). However, in addition to neurons whose taste response was only modified by gastric stimulation, we observed another, smaller population of cells coactivated by gastric stimulation. For these cells, there was a significant correlation between the magnitude of the gastric response and the degree to which gastric stimulation modified the taste response. The linear integration in these neurons is more suggestive of simple spatial summation mediated by classic neurotransmitters and ionotropic receptors (38).

Topography of Taste and Distension Responses

It is significant that both modulated and coactivated taste cells extended to the most caudal reaches of the PBN (Fig. 8A), areas not traditionally associated with visceral sensitivity (e.g., Ref. 52). Coactivated cells intermingled with a substantial population of neurons purely responsive to taste stimulation and a smaller number responsive only to gastric stimulation. These observations emphasize that there is not a strict morphological division of the PBN into gustatory and visceral zones. However, this is not to suggest a lack of topographic organization. Indeed, there was a differential distribution of gastric- and taste-responsive cells, a distribution consistent with previous anatomical stud-

ies and classical descriptions of taste vs. visceral regions in the PBN (35, 40, 46). Taste-responsive neurons were distributed in the caudal waist region, spanning the brachium conjunctivum between the ventral lateral and central medial subnuclei. By comparison, gastric-responsive cells were preferentially distributed further anteriorly and laterally, with many located in or near the external lateral subnucleus. One caveat should be noted. In the present study, we recorded no gustatory neurons in the external subnuclei, an area where a small population of taste neurons was previously identified (34). The reason for this discrepancy is unclear. However, the "external" taste neurons observed previously were all responsive to posterior mouth stimulation. Although the whole mouth was stimulated in the present study, receptive fields were not determined, and we may not have recorded a representative sample of such neurons (e.g., Ref. 34). Thus the distribution of taste- and gastric-responsive neurons may be even more overlapping than that observed here.

Perspectives

An inhibitory influence of gastric distension on excitatory taste responses is consistent with a role for distension as an inhibitory feedback signal that enhances satiation and diminishes ingestive taste reactivity (8, 13, 32). More generally, the inhibitory distension effect can be taken on face value to support the model of feeding behavior proposed by Davis and Levine (13). In simple terms, the model states that feeding behavior varies as a function of the integration of excitatory afferent signals derived from palatable gustatory stimuli and inhibitory afferent signals derived from postingestive accumulation of ingesta. It is noteworthy, however, that inhibitory modulation was not confined to palatable stimuli in the present study. It is not immediately obvious how inhibition of aversive taste responses should be interpreted in behavioral terms. On the one hand, it could be argued that there is no clear role for gastric modulation of aversive gustatory signals, based on the assumption that aversive tastants are not ordinarily ingested. However, natural foods are complex mixtures that contain both aversive and palatable components. For example, carbohydrates are often present in combination with acids in fruit. Thus a general suppression of palatable and aversive gustatory input by satiating factors could be adaptive in certain contexts (also see Ref. 28). Indeed, one early study showed that intraperitoneal cholecystokinin injections suppressed intake of hypertonic saline and quinine in addition to glucose (5), but, for the most part, relatively little is known about how satiating treatments affect the hedonic evaluation and consumption of tastants other than carbohydrates (28).

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