Responsiveness of Neurons in the Hamster Parabrachial Nuclei to Taste Mixtures

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ABSTRACT   Responses from hamster parabrachial nuclei neurons to stimulation of the anterior tongue with sucrose, NaCl, HCl, quinine hydrochloride, and the six two-component mixtures of these stimuli were recorded. A cell's response to a mixture approached its response to the mixture's more effective component in the majority of cases, but was sometimes greater or smaller than this response. The best predictor of a neuron's response to a mixture, then, was its response to the mixture's more effective component. The single-component stimulus producing the maximum response was determined for each neuron and the response to this stimulus was compared with the responses evoked by the six mixtures. For 30% of the cells, a mixture elicited a response reliably, but only 1.1–2.1 times greater than the response to the best single-component stimulus. Thus, there were no neurons specialized to respond to these mixtures. The across-neuron patterns elicited by mixtures and the responses of best-stimulus classes to mixtures were studied for comparison with psychophysical data on taste mixtures. Mixtures were usually correlated with single-component stimuli in the mixture, but not with stimuli not in the mixture. In fact, five of the six mixtures fell directly between their components in a multidimensional scaling plot. In addition, a mixture was most effective in stimulating only those classes of neurons maximally stimulated by the mixture's components. These results correlate with psychophysical data suggesting that mixtures of taste stimuli evoke the same taste qualities as evoked by the mixture's components.

INTRODUCTION

In order to understand a sensory system, it is important to study it using stimuli that influence it under natural conditions. For the gustatory system, it is relevant to understand the effects of mixtures, since foods are often composed of two or more chemicals that individually elicit different taste qualities. The psychophysical effects of mixtures of pure taste stimuli of different quality have been fairly well studied. These experiments indicate that human subjects usually have no difficulty in analyzing the components present in these mixtures (Indow, 1969; Lawless, 1979; Pangborn, 1961, 1962; Pangborn and Trabue, 1967) and that

no "new" qualities emerge as a result of mixing individual stimuli (Bartoshuk, 1975). Erickson (1982) has shown that subjects do make mistakes when they are asked to identify the stimuli contained in certain mixtures, but the incidence of these errors is rather low. There have been similar findings in animal psychophysical studies. When a rat is conditioned to avoid a single-component stimulus, this aversion generalizes to that stimulus to a degree that is dependent upon its concentration in a mixture (Theodore, 1977). Conversely, hamsters generalize a mixture aversion to the components of the mixture when they are presented alone (Nowlis and Frank, 1977). Thus, mixtures of stimuli taste similar to the components in them to both rats and hamsters.

Although adding one chemical to another does not usually change the basic taste quality evoked by that stimulus, its intensity is often changed by the addition of other stimuli. When two stimuli having different tastes are mixed, the most common effect is a suppression of the intensities of the individual qualities (Pfaffmann et al., 1971; Moskowitz, 1972; Bartoshuk, 1975; Lawless, 1979; Pangborn, 1961). Enhancement of the intensity of one quality following the addition of a second, different-tasting stimulus has also been occasionally reported (Pangborn, 1962; Pangborn and Trabue, 1967), although this has usually occurred only when the added stimulus was weak.

The neurophysiological processing of mixtures has recently been studied in the peripheral nervous system. Hyman and Frank (1980a, b) investigated the integrated responses of the hamster chorda tympani (CT) nerve and the activity in single hamster CT fibers to two-component mixtures of sucrose, NaCl, HCl, and a number of other stimuli. It was found that the whole-nerve responses to mixtures were not readily predictable from the responses to their components, which suggested that the effects of mixtures on individual fibers might be complex. Indeed, these investigators found that the response of a unit to a mixture was dependent on the best-stimulus class of the cell. Other investigators have also found differential effects of mixtures on individual CT neurons in both the cat (Kruger and Boudreau, 1972) and rat (Miller, 1971; Sato et al., 1970; Wang, 1973). There have been no reports of the effects of taste mixtures on single neurons in the central nervous system.

The present study investigated the responses of single neurons in the third-order taste relay, the parabrachial nuclei (PBN), of the hamster to midrange concentrations of the four basic taste stimuli—sucrose, NaCl, HCl, and quinine hydrochloride (QHCl)—and to the six two-component, undiluted mixtures of these stimuli. The responses of each neuron were examined to determine whether they responded any differently to mixtures of two stimuli than to the stimuli presented alone. Since mixing two chemicals often causes changes in their perceived intensities, changes in the neural response to one chemical after the addition of a second chemical would not be surprising. Responses were also examined to determine if any cells were particularly sensitive to any of the mixtures tested, since investigations of other sensory systems have often revealed a special sensitivity to complex configurations of stimuli (Hubel and Wiesel, 1962, 1965; Kuffler, 1953; Suga et al., 1979). Psychophysical data on taste mixtures were then compared with the mixture responses of PBN neurons using

correlational (Erickson, 1963) and multidimensional scaling techniques (Smith et al., 1983b) and by using responses of best-stimulus categories (Frank, 1973). The neural data agreed well with the reported psychophysical effects of taste mixtures.

METHODS

Preparation and Recording

Subjects were 27 male hamsters (*Mesocricetus auratus*) ranging in weight from 98 to 149 g. After anesthetization with urethane and implantation of a tracheal cannula, the animal was placed in a nontraumatic head holder (Erickson, 1966) mounted in a stereotaxic frame. The head was angled 27° off the horizontal, nose downward, so that the transverse sinus was avoided and brainstem movement was minimized. A small hole (~2 mm in diameter) was drilled in the rostral portion of the right occipital plate. The anterior portion of the tongue was drawn into a glass tongue chamber for subsequent stimulation. The tongue chamber was fitted with a rubber membrane so that leakage was prevented and saliva was excluded. The obex was exposed by removing the overlying dura and sometimes moving the cerebellum and/or removing some bone at the caudal end of the occipital plate. A microelectrode (glass-insulated tungsten, 1–3 μm in diameter, 7–12 μm exposed tip) was positioned over the obex with a three-way micromanipulator. An indifferent electrode (alligator clip) was placed on the wound margin and the animal was grounded via the headholder. Neural activity was passed through a unity-gain high-impedance probe prior to amplification by a Grass P511 preamplifier (Grass Instrument Co., Quincy, MA). Action potentials were monitored on an oscilloscope and audio monitor and checked for spike amplitude and waveform consistency on a storage oscilloscope.

The coordinates for obex were determined and used as a reference point for the placement of the electrode. The search for taste-responsive activity in the PBN typically began at 4.1 mm anterior and 1.5 mm lateral to obex, although in some of the later preparations the search began somewhat anterior and medial to this (4.5 mm anterior, 1.3 mm lateral). The electrode was driven in the dorsal-ventral plane using a Narashige (Tokyo, Japan) hydraulic microdrive. Neural activity was tested for its responsiveness to gustatory stimulation every 50–100 μm beginning at 3.0 mm below the surface of the cerebellum. If taste-responsive neural activity was not encountered by a depth of 4.5 mm ventral to the surface, the electrode was withdrawn and positioned at a nearby site usually ~0.1 mm distant in either the anterior-posterior or medial-lateral plane. If taste-responsive activity was encountered, its depth was noted and the electrode was driven through the taste-responsive region very slowly, testing for the presence of taste-driven activity every 50 μm until action potentials from a single cell were isolated. All responses from single units were stored on magnetic tape for off-line analysis.

Solutions and Stimulation

The solutions used to test single neurons were: 0.03 M NaCl, 0.1 M sucrose, 0.003 M HCl, 0.001 M QHCl, and the six possible two-component, undiluted mixtures of these stimuli. These mixtures were prepared so that the molar concentrations of each of the components in the mixtures were the same as their concentrations in the single-component stimuli. These 10 solutions were prepared from reagent grade HCl and NaCl, commercial grade sucrose, and "Baker Grade" QHCl (J. T. Baker Chemical Co., Phillipsburg, NJ) dissolved in distilled H_2O (conductivity $\leq 4.0 \times 10^{-7}$ S/cm). These concentrations of the single-component stimuli produce approximately one-half the maximum integrated whole CT nerve response (Frank, 1973), but because the CT is differentially sensitive to some

of the chemicals tested, midrange concentrations are not equally effective concentrations of the four stimuli for the whole nerve. Rather, 0.03 M NaCl is slightly more effective than 0.1 M sucrose and 0.03 M HCl for the whole CT nerve and 0.001 M QHCl is much less effective than the other three stimuli (Frank, 1973). These concentrations of the four basic stimuli, however, did elicit roughly equal responses for PBN neurons in each of their respective best-stimulus categories and they have previously been used to characterize the sensitivity of single gustatory fibers in the hamster CT (Frank, 1973) and neurons in the hamster nucleus tractus solitarius (NTS) (Travers and Smith, 1979) and PBN (Van Buskirk and Smith, 1981). Hyman and Frank (1980b) used these concentrations of sucrose and HCl but a lower concentration of NaCl (0.01 M) to test mixture effects on single fibers in the hamster CT nerve.

Psychophysical studies have documented that stimulus concentration can be a variable in determining the effects of a particular taste mixture. The intensity of a taste stimulus is most often suppressed by the addition of a second stimulus evoking a different taste quality, but the magnitude of suppression can be affected by the concentrations of the mixture stimuli, and enhancement and suppression have occasionally been reported to occur for the same mixture at different concentrations (Pangborn, 1961, 1962; Pangborn and Trabue, 1967). Because each mixture was tested at a single concentration in the present study, the results obtained cannot be presumed to match the results that would be obtained for other mixtures of the same compounds at different concentrations, particularly with regard to the precise magnitudes of the mixture effects.

Stimulation with the 10 stimuli proceeded in a random order except that the four single-component stimuli were presented first. Each stimulation trial began with a 5-s distilled H_2O rinse, followed by presentation of the stimulus for 10 s, and concluded with a distilled H_2O rinse of at least 30 s. All stimuli were presented at room temperature (24°C) and flowed at a rate of 8 ml/s through a system of funnels and stopcocks. The interval between stimulations was at least 2 min to prevent the prolonged effects of adaptation (Smith and Bealer, 1976; Smith et al., 1978). When possible, the series of stimuli was presented three times and a different order was used for each of the series. Cells were also tested for their sensitivity to temperature changes when time allowed. The tongue was preadapted to room temperature (24°C) prior to the flow of warm (35–45°C, $\bar{X} = 39$°C) or cool (13–20°C, $\bar{X} = 16$°C) distilled H_2O.

Data Analysis

Action potentials were identified as arising from one cell on the basis of consistent spike amplitude and waveform. As in other investigations of central gustatory neurons (Travers and Smith, 1979), there is the possibility that some of this recorded unit activity may have come from presynaptic axons rather than postsynaptic cell bodies, but the relative size of these targets makes this unlikely. In addition, action potentials arising from axons have been reported to be of short duration (<0.5 ms; Cooper et al., 1969) and can be recorded for only short distances of microelectrode travel (Wolstencroft, 1964). No cells with these characteristics were included in these analyses. 34 taste-responsive neurons were isolated for a period of time sufficient to test each of the stimuli at least once. 23 of the cells were held long enough for at least two stimulus trials. The thermal sensitivity of 13 of the neurons was also tested.

Action potentials were passed through a window discriminator (model 121; W-P Instruments, Inc., New Haven, CT) and the pulsed output of the discriminator was counted in 500-ms bins with a PDP 11/03 computer (Digital Equipment Corp., Marlboro,

MA) equipped with a pulse-counting program. The numbers of action potentials occurring in successive 500-ms bins were accumulated for a 30-s period comprised of 5 s of the prestimulus period without H_2O flowing, 5 s of the prestimulus period with H_2O flowing, 10 s with the stimulus flowing, and 10 s of the rinse. Previous calibration had established that the stimulus contacted the tongue 1,185 ms (±164 ms, SD) after the verbal signal (Van Buskirk and Smith, 1981). Therefore, the stimulus usually contacted the tongue during the third 500-ms bin after this signal. The response measure used in the present analyses was the number of action potentials occurring in the 5-s period beginning with the third bin after the verbal signal (i.e., at about the time of stimulus contact). Spontaneous activity was routinely subtracted from this 5-s measure (Travers and Smith, 1979; Van Buskirk and Smith, 1981). The measure of spontaneous rate used was the mean number of action potentials occurring during two 5-s periods: the flow of distilled H_2O immediately prior to the presentation of the stimulus and the same period prior to the onset of the next stimulus. The standard deviation across all 5-s periods of spontaneous activity occurring during the flow of distilled H_2O before each stimulus trial was calculated and used in establishing a response criterion. The criterion for a gustatory response was applied only to the responses of the 23 cells with repeated stimulus trials. If a stimulus resulted in a change in spike frequency that exceeded 1 SD of the spontaneous rate on at least two stimulus trials, this change was considered to be a response. The criterion for a thermal response was the same as for a gustatory response for 11 out of 13 cells with repeated trials of the thermal stimuli. For two cells with only one test of the thermal stimuli, a response criterion of an increment or decrement that exceeded 2 SD was used. Increments of this type are referred to as excitatory responses and decrements are referred to as inhibitory responses with no reference to underlying synaptic events.

Some analyses required deciding whether there was a reliable difference between the responses evoked by two stimuli in the same neuron. Only cells for which each stimulus was tested at least twice were used in these analyses. Since two or three repetitions of each stimulus trial did not provide an adequate n for a statistical test, we adopted a criterion of response difference that used the standard deviations of both responses. Two responses were considered to be reliably different from one another if the difference between the two mean responses exceeded the sum of their standard deviations.

In another analysis, all 34 neurons were assigned to best-stimulus classes according to which of the four basic taste stimuli elicited the greatest response (Frank, 1973). Analyses were then performed on the mean responses of each of these four classes. For sucrose-best and NaCl-best neurons, there were a sufficient number of cells to use a statistical test (Sandler's A; Runyon and Haber, 1971) but there were not enough HCl- ($n = 4$) or QHCl- ($n = 4$) best cells for this test. Therefore, the mean responses of these classes were considered to be different if a difference occurred in the majority (three out of four) of cells in the class.

Histological Verification of Recorded Units

After recording from a cell, a lesion (10 μA for 10–15 s) was typically made to mark the recording site. The microlesion was made at the site of recording if the cell was the last to be recorded in the preparation or 1.0 mm dorsal and/or ventral to the site if further recording was to be done. At the end of the recording session, the animal was given a lethal dose of urethane and then perfused with 10% formalin. Serial sections of the pons were prepared using the Kluver method. Electrode tracks and lesions were reconstructed with the aid of a microprojector and plotted on standard diagrams of the pons.

RESULTS

Histological Verification

Fig. 1 shows the locations of the recording sites of 25 of the 34 taste neurons recorded in the present study that were marked by lesions. The position of the microelectrode (as measured from obex and the brain surface) and the location of electrode tracks suggested that the other nine neurons were also in the medial portion of the PBN.
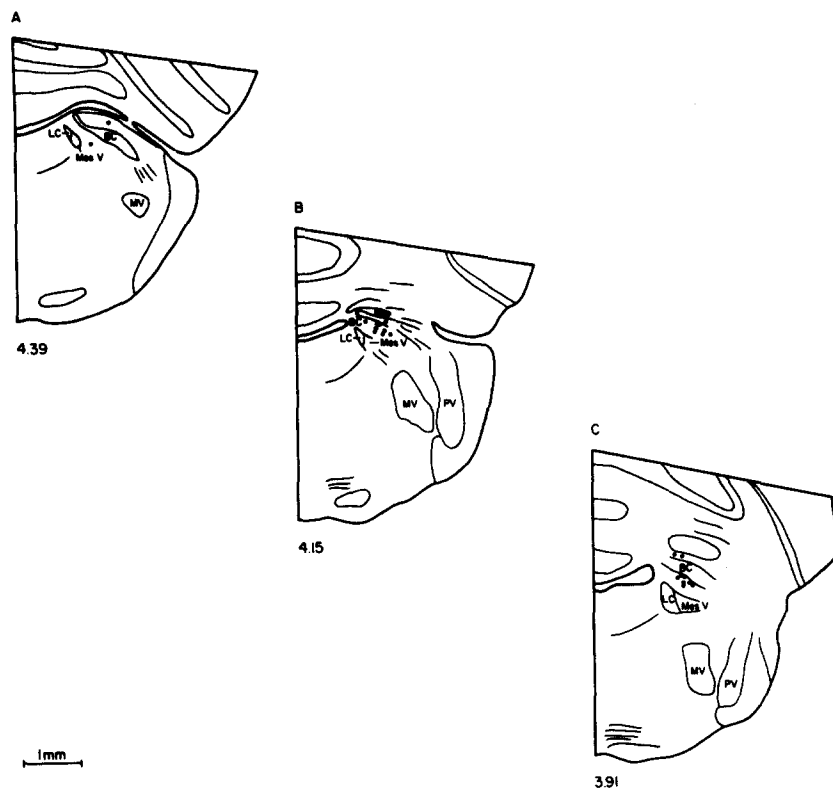

FIGURE 1. Traced sections from the PBN indicating the recording sites of 25 taste-responsive neurons. Sections A-C are arranged from anterior to posterior and the distance (in millimeters) anterior to the obex is given below each section. Positions of the recorded neurons appear as filled circles. Abbreviations: LC, locus coeruleus; BC, brachium conjunctivum; MV, trigeminal motor nucleus; PV, principal trigeminal nucleus; Mes V, mesencephalic trigeminal nucleus.

A small proportion of the neurons ($n = 2$, 8%) were recorded from the area of the PBN depicted in Fig. 1A, the most anterior section shown. The majority ($n = 14$, 56%) of the cells were recorded from the area of the PBN shown in the middle section (B), but a sizeable number of cells ($n = 9$, 36%) were also recorded from the posterior portion of the PBN depicted in section C. Twice as many

neurons were recorded ventral ($n = 16$, 64%) as were recorded dorsal ($n = 8$, 32%) to the brachium conjunctivum (BC) and only one cell was recorded within the borders of this structure.

Mixtures vs. Their Single Components (n = 23)

In the following analysis, the response of an individual neuron to a mixture was compared with that cell's response to the more effective component contained in that mixture. Since responses within single neurons were being compared, only neurons in which stimuli were tested at least twice were considered ($n = 23$). Altogether, 137 mixture responses were examined. Table I categorizes

TABLE I

Proportion of Mixture Responses Greater Than, Less Than, or Equal to the Response to the More Effective Component of the Mixture

Types of component responses*					
1st Component	2nd Component	Greater than[‡] (MEC, LEC, Δ)[§]	Less than (MEC, LEC, Δ)	Equal to (MEC, LEC, Δ)	Total
+	+	14 (9.5) (71, 34, 25)	7 (9.5) (132, 38, −46)	41 (43.0) (98, 40, N)	62
+	0	3 (7.4) (22, N, 29)	12 (7.4) (69, N, −28)	33 (33.3) (62, N, N)	48
0	0	3 (2.3) (N, N, 6)	0 (2.3)	12 (10.4) (N, N, N)	15
+	−	1 (1.7) (35, −24, 30)	2 (1.7) (122, −19, −34)	8 (7.6) (67, −19, N)	11
−	−	0 (0.15)	0 (0.15)	1 (0.71) (−32, −14, N)	1
All response pairs		21	21	95	137

* +, excitatory response; −, inhibitory response; 0, no reliable response.

‡ The number of cases observed appears first and the number of cases expected appears second, in parentheses.

§ MEC = $\bar{X}$ response to the more effective component; LEC = $\bar{X}$ response to the less effective component; Δ = $\bar{X}$ difference between response to mixture and response to MEC; N = no reliable response or no reliable difference.

responses to mixtures according to the types of responses evoked by their components. In 95 out of 137 cases (69%), there was no reliable difference between a mixture response and the response of a cell to the more effective component of that mixture. However, the remaining mixture responses (42 out of 137 = 31%) were reliably different from the responses evoked by their more effective component. 21 (15%) mixture responses were reliably greater than the responses evoked by their more effective component (Table I). In these cases, the mean difference between the response to the mixture and the response to its more effective component was 23 impulses/5 s; that is, the mixtures were 1.8 times as effective as the more effective component. 21 additional mixture responses (15%) were smaller than the responses to the more effective component. The mean of the differences between these two responses was 35 impulses/

5 s; i.e., the mixtures were only 0.48 times as effective as the more effective components.

A chi-square test was performed to determine whether the category of a mixture response (i.e., greater than, less than, or equal to its more effective component) was associated with the types of responses evoked by the mixture's components. These variables were found to be associated ($x^2 = 9.73$, $P < 0.05$, degrees of freedom $= 4$).[1] This relationship was examined more closely by comparing the observed frequencies of the various combinations of mixture response category with component response type to the frequencies expected if these two variables were unrelated. The observed and expected frequencies appear in Table I. Deviations from expected frequency were greatest for three particular combinations of mixture response category with component response type. Mixture responses greater than the response to their more effective component occurred more frequently than expected when both component responses were excitatory, but occurred less frequently than expected when one component response was excitatory and the other component stimulus elicited no response. In addition, mixture responses that were smaller than the response evoked by their more effective component occurred more frequently than expected when one component stimulus elicited an excitatory response and the other elicited no response. However, the relationship between component response type and category of mixture response was fairly weak, as suggested by the fact that all the deviations were small (<5). A measure of the degree of association between these two variables is provided by the contingency coefficient (C; Siegel, 1957). For a contingency table with these dimensions, a C of 0.00 would respresent no association, whereas the maximum degree of association between the two variables would be $+0.82$. The calculated value, $+0.26$, for this particular table is much smaller than this maximum value, which implies that the category of a mixture response is not tightly coupled to the type of response evoked by the mixture's components. Consequently, the category of a mixture response cannot always be predicted by assuming that component responses add in an algebraic fashion. For example, a neuron that increased its firing rate to two different stimuli did not necessarily respond better to the mixture of those stimuli than it did to the more effective component of that mixture presented alone. Further, a neuron that gave an excitatory response to one stimulus and did not respond to the other stimulus did not always respond to the mixture of these stimuli as if only the excitatory stimulus was present. In fact, the response was sometimes reduced. Figs. 2 and 3 show examples of some of these responses.

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[1] The chi-square test was performed after collapsing the three-component response types that occurred least frequently (00, +−, −−) into one category to increase the expected frequencies to an appropriate number (Siegel, 1957). Observed and expected frequencies are provided (in Table I) for the uncollapsed table to provide more detailed information. Note that the largest deviations from expected values are in the uncollapsed portion of the table; the expected and observed frequencies in these cells are the same in a collapsed or uncollapsed table. It is appropriate to draw inferences about the overall significance of the relationship between mixture category and component response type for the collapsed table only, i.e., about three groups of component response types (++, +0, and all others).

Fig. 2 depicts the responses of two neurons to mixtures whose components were both excitatory. The more common situation is shown in Fig. 2*A*. This cell (50-2) responded to both sucrose $\bar{X}$ = 73 ± 12.5 impulses/5 s, n = 3) and NaCl ($\bar{X}$ = 136 ± 28.8 impulses/5 s, n = 3), but the response to the mixture of these two stimuli ($\bar{X}$ = 134 ± 28.6 impulses/5 s, n = 3) was not reliably different from the response evoked by NaCl, the more effective component of the mixture. The responses of neuron 46-2 demonstrate a less common occurrence (Fig. 2*B*). Both sucrose ($\bar{X}$ = 48 ± 2.0 impulses/5 s, n = 3) and QHCl ($\bar{X}$ = 34 ± 9.8
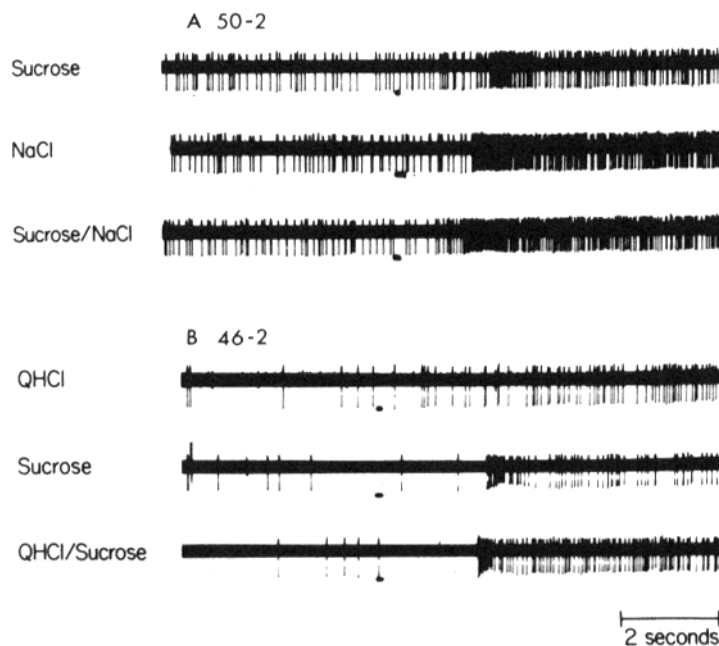

FIGURE 2. Oscillographic records for the responses of two PBN gustatory neurons. The marks beneath each neural record indicate the opening of the stopcock that allowed stimulus flow. The mean time of stimulus arrival at the tongue was 1,185 ms after this event. Action potentials are displayed with negative deflections downward. (*A*) Responses of neuron 50-2 to sucrose, NaCl, and the mixture of these stimuli. (*B*) Responses of neuron 46-2 to QHCl sucrose, and the mixture of these stimuli.

impulses/5 s, n = 3) elicited excitatory responses when presented alone, and the response to the mixture (X = 73 ± 4.8 impulses/5 s, n = 3) was greater than that evoked by sucrose, the mixture's more effective component.

Fig. 3 shows responses to two mixtures both containing one component stimulus that was excitatory and another that elicited no response. For this combination of component responses, the response to the mixture was usually no different from the response to the excitatory component. About 25% of the time, however, the response to the mixture was smaller than the response to the excitatory component alone. For example, neuron 55-3 (Fig. 3*A*) responded

quite vigorously to NaCl ($\bar{X}$ = 118 ± 19.2 impulses/5 s, n = 3), but did not respond to HCl. The response of this cell to the mixture of these stimuli ($\bar{X}$ = 55 ± 12.3 impulses/5 s, n = 3), however, was considerably smaller than the response to NaCl presented alone. An unusual response to a mixture with these types of component responses is shown in Fig. 3B. In neuron 14-1, NaCl elicited a small but reliable excitatory response ($\bar{X}$ = 5 ± 2.0 impulses/5 s, n = 2), but HCl was ineffective. The response to the mixture of these two stimuli ($\bar{X}$ = 24 ± 1.4 impulses/5 s, n = 2) was much greater than the response elicited by NaCl alone.
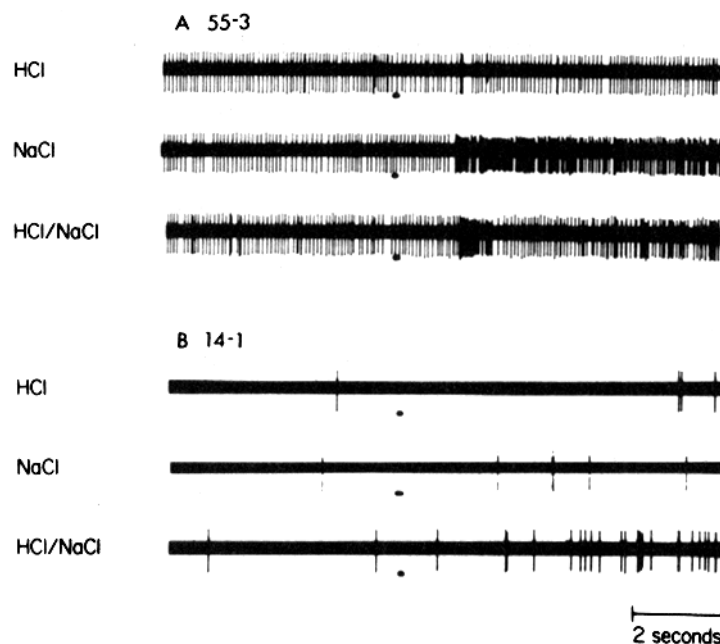

FIGURE 3. Oscillographic records for the responses of two PBN gustatory neurons displayed as in Fig. 2. (A) Responses of neuron 55-3 to HCl, NaCl, and the mixture of these two stimuli. (B) Responses of neuron 14-1 to HCl, NaCl, and the mixture of these two stimuli.

Mixture-Best Neurons (n = 23)

The responses of the 23 neurons with replications were also analyzed to determine whether any of the cells were particularly sensitive to any of the six mixtures tested in this investigation. The responses of a cell to the four basic stimuli were first examined to determine which of these stimuli elicited the greatest response. The response to this "best" stimulus for a neuron was then compared with that neuron's responses to the mixtures. A mixture reliably elicited a response greater than the response elicited by the best single-component stimulus in 7 of the 23 neurons. These cells are referred to as "mixture-best" neurons, and the mixture eliciting the most vigorous response is termed the "best mixture" for that cell. On average, the best mixtures for these cells elicited a response that was 1.3

times as great as that evoked by the cells' best single-component stimuli (range = 1.1–2.1 times). The best mixtures for each cell contained that cell's best single-component stimulus. Five of the seven mixtures (71%) were composed of the best stimulus and the second-best stimulus. Three of the seven mixture-best neurons responded better to more than one mixture than to their best single-component stimulus. For two of the cells, there were two mixtures that elicited greater responses than the best single-component stimuli, and in one neuron, there were three mixtures that evoked greater responses that the best single-component stimulus. All of the mixtures that elicited responses greater than the best single-component stimuli included that stimulus as a component. Fig. 2B shows the responses of a mixture-best neuron (46-2, described previously) to the best single-component stimulus (sucrose), the second-best single-component stimulus (QHCl), and the mixture of these two stimuli, which elicited a response that was 1.5 times greater than the response to sucrose alone.

Best-Stimulus Classification (n = 34)

All 34 neurons were categorized into one of four classes according to which of the four basic taste stimuli elicited the greatest response (Frank, 1973). The best-stimulus category of a neuron was quite stable across stimulus replication. Each of the four basic taste stimuli was presented at least twice to 29 neurons and the best-stimulus classification of only one neuron changed with repeated tests. Mean responses to the 10 stimuli were calculated for each of the four best-stimulus classes and are shown in Fig. 4. Even though some individual neurons consistently responded better to mixtures than to single-component stimuli, as discussed in the previous section, their effects were not apparent in the mean responses for a class of neurons when they were included. Mixtures of the four classical taste stimuli did not elicit mean responses that were appreciably greater than the response evoked by the best stimulus for a class of cells. None of the six mixtures evoked responses that were greater than the response to the best stimulus for the majority of either the HCl- or QHCl-best cells. The mixture of sucrose and NaCl did evoke a significantly (although only slightly) greater response in both sucrose- and NaCl-best neurons than did the best stimulus for each of these classes. This mixture evoked a response that was 1.1 times as great as both sucrose in sucrose-best neurons (Sandler's A test, $A = 0.243$, $P < 0.05$) and NaCl in NaCl-best neurons ($A = 0.273$, $P < 0.05$). Sucrose plus NaCl, which was more effective for sucrose- and NaCl-best neurons than the best stimulus alone, contained the best stimulus as a component of the mixture. However, some mixtures that contained the best stimulus for a class of cells actually evoked less vigorous responses than the best stimulus alone. In NaCl-best neurons, a mixture of NaCl plus HCl elicited a response that was 0.79 times as effective as NaCl alone ($A = 0.261$, $P < 0.05$). For three of the four QHCl-best cells, a mixture of NaCl and QHCl elicited a smaller response than that evoked by QHCl alone. This mixture was only 0.75 times as effective as QHCl in QHCl-best neurons.

Mixtures not containing the best stimulus for a class of cells (i.e., sideband mixtures) always evoked mean responses that were smaller than the response elicited by the best stimulus. For both sucrose- and NaCl-best neurons, the mean

responses evoked by sideband mixtures were significantly smaller than the mean responses evoked by the best stimuli ($P < 0.01$ for all comparisons). In QHCl-best cells, each of the sideband mixtures evoked smaller responses than QHCl in at least three of the four neurons. The mean responses to all of the sideband mixtures were smaller than the mean response to HCl in HCl-best cells. However, sucrose plus NaCl and NaCl plus QHCl did not elicit smaller responses than HCl in the majority of HCl-best neurons.
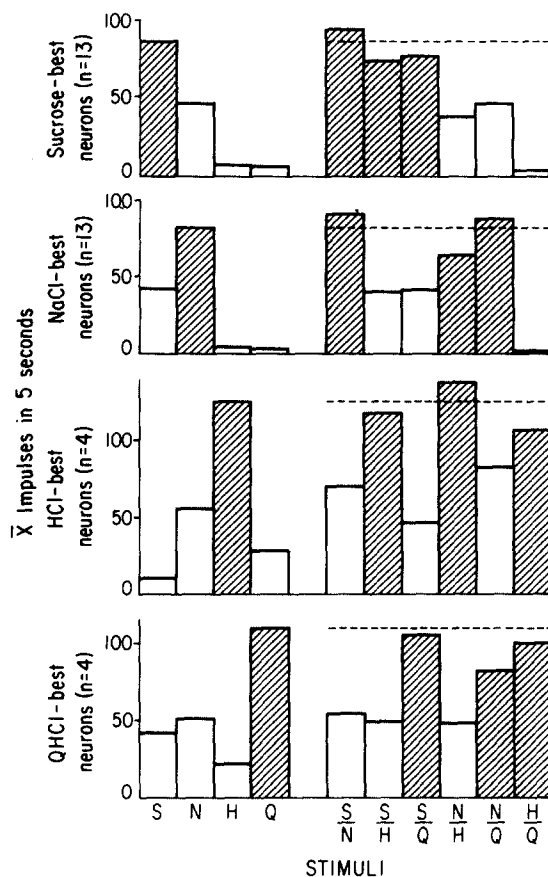

FIGURE 4. Mean firing rates to all 10 stimuli for each of the four best-stimulus classes. The shaded bars indicate the best stimulus and the mixtures that contain the best stimulus for each class of cells. The dotted lines denote the mean firing rate to the best stimulus. Abbreviations: S, sucrose; N, NaCl; H, HCl; Q, QHCl.

Across-Neuron Correlations (n = 34)

Across-neuron correlations have been used to quantify the similarity between the patterns of activity evoked by two stimuli across a population of cells (Erickson, 1963, 1968). If two stimuli evoke similar patterns, the across-neuron correlation between them will be high, but the across-neuron correlation will be

low if the stimuli generate dissimilar patterns. The across-neuron correlation for each of the pairs of these 10 stimuli was calculated and appears in Table II. The correlations can be divided into two groups: those corresponding to stimulus pairs that have a component in common (e.g., sucrose plus NaCl; NaCl plus HCl) or do not have a component in common (e.g., HCl; sucrose plus NaCl). Stimulus pairs having a common component tended to correlate significantly and more highly compared with those pairs not having a component in common. The mean correlation for all the stimulus pairs having a common component was +0.63, whereas the mean correlation for the pairs not having a common component was +0.21. These mean correlations are significantly different ($t = 6.716$, $P < 0.001$).

The relationships between the across-neuron correlations generated by the mixtures and those generated by the single-component stimuli were examined in more detail. In general, the across-neuron pattern for a mixture correlated significantly with each of the across-neuron patterns generated by the individual

TABLE II

Across-Neuron Correlations (Pearson r*) among the 10 Stimuli

	S‡	N	H	Q	S + N	S + H	S + Q	N + H	N + Q
H + Q	−0.05	+0.07	+0.77	+0.79	+0.03	+0.49	+0.39	+0.55	+0.34
N + Q	+0.48	+0.91	+0.23	+0.30	+0.75	+0.58	+0.59	+0.70	
N + H	+0.16	+0.59	+0.73	+0.11	+0.50	+0.72	+0.30		
S + Q	+0.85	+0.56	+0.11	+0.52	+0.77	+0.75			
S + H	+0.63	+0.54	+0.59	+0.21	+0.75				
S + N	+0.87	+0.85	+0.02	−0.02					
Q	+0.10	+0.06	+0.30						
H	−0.17	+0.04							
N	+0.61								

* For $n = 30$, a correlation of ±0.35 is significant ($P < 0.05$).

‡ Abbreviations for stimuli: S, sucrose; N, NaCl; H, HCl; Q, QHCl.

components of the mixture, but not with the patterns evoked by the stimuli not in the mixture. For example, the across-neuron correlation between sucrose plus NaCl and sucrose alone was +0.87, and between this mixture and NaCl it was +0.85, but the across-neuron correlations between the sucrose plus NaCl mixture and HCl and QHCl, respectively, were +0.02 and −0.02. Fig. 5 shows the across-neuron patterns for the sucrose plus NaCl mixture and for sucrose and NaCl alone. In this figure, the cells are ordered along the abscissa according to their responsiveness to sucrose. The mixture pattern bears some similarity to each of the patterns evoked by the components alone.

There were a few mixtures that were correlated with single-component stimuli not present, as well as present, in the mixture. In all of these cases, the mixture in question contained either sucrose or NaCl (plus another stimulus), but was correlated with both sucrose and NaCl. For example, the mixture sucrose plus QHCl is significantly correlated not only with sucrose (+0.85) and QHCl (+0.52), but also with NaCl (+0.56). These exceptions probably reflect the significant correlation between sucrose and NaCl.
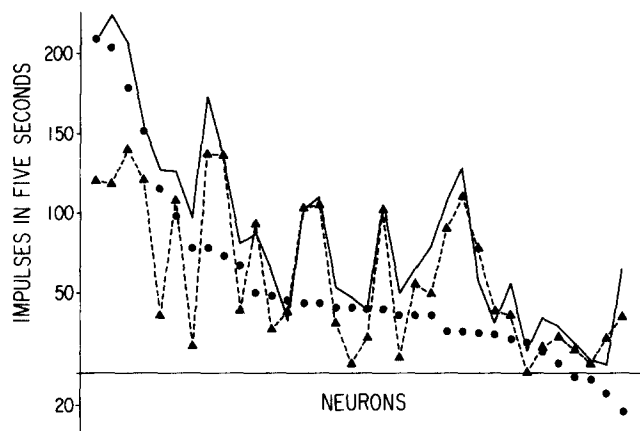
FIGURE 5.   Across-neuron patterns for sucrose, NaCl, and for the mixture of these stimuli. The neurons in each pattern are arranged according to their responsiveness to sucrose. The filled circles with no connecting line indicate the sucrose pattern, the filled triangles and dotted line designate the pattern for NaCl, and the solid line shows the pattern produced by the sucrose plus NaCl mixture.

The across-neuron patterns generated by the mixtures were well predicted from responses to the more effective components of the mixtures. For example, the across-neuron pattern for the sucrose plus NaCl mixture (Fig. 5) correlated very highly (+0.97) with a pattern composed of each cell's response to this mixture's more effective component stimulus, either sucrose or NaCl. That is, the observed mixture pattern was very similar to a hypothetical pattern composed of both sucrose and NaCl responses; the response included for a given cell depended on which stimulus was the more effective. The mixture patterns for the other five mixtures also correlated highly with hypothetical patterns derived in this fashion, and these correlations appear in Table III. The mean correlation between the mixture patterns and these hypothetical mixture patterns was +0.93.

In order to summarize graphically the relationships among the across-neuron

TABLE III

Across-Neuron Correlations (Pearson r*) Between Observed and Hypothetical Mixture Patterns

Mixture	Correlation
S + N	+0.97
S + H	+0.93
S + Q	+0.94
N + H	+0.87
N + Q	+0.91
H + Q	+0.98
$\bar{X}$	+0.93

* For $n = 30$, a correlation of ±0.35 is significant ($P < 0.05$).

patterns generated by all 10 stimuli, a multidimensional scaling (MDS) analysis (KYST, Bell Telephone Laboratories, Murray Hill, NJ) of the data was performed. The use of three dimensions optimally reduced the stress in the MDS solution. The three-dimensional solution for the stimuli is shown in Fig. 6. The placement of five of the six mixtures is in an intermediate position between the two single-component stimuli making up the mixtures. Certain mixtures do lie
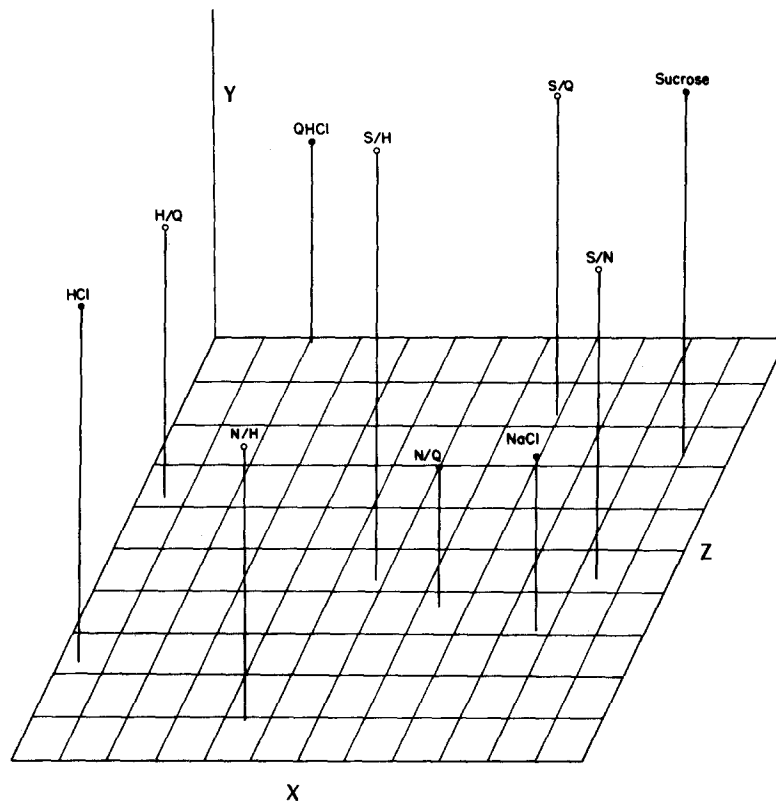

FIGURE 6.   Three-dimensional representation of the relationships among the 10 stimuli generated by the multidimensional scaling analysis. Filled circles designate the positions of the single-component stimuli and the open circles indicate the positions of the mixtures. Abbreviations are the same as in Fig. 4.

somewhat closer to one of their component stimuli than to the other (sucrose plus QHCl is closer to sucrose and NaCl plus HCl is closer to NaCl), and this is reflected in unequal aross-neuron correlations between these mixtures and their two components. However, all five of these mixtures are correlated significantly with both of their single components. A single mixture, NaCl plus QHCl, is correlated significantly with only one of its components (NaCl). This mixture lies much closer to NaCl than to QHCl on the MDS plot, a placement that is consistent with the across-neuron correlations among these stimuli (+0.91 be-

TABLE IV

Mean Responses* to Gustatory and Thermal Stimuli

	Stimuli[‡]					
	S	N	H	Q	Cooling	Warming
(A) Overall	64.7	71.2	19.2	41.9	37.6	50.2
(B) Classes						
(1) Sucrose-best	111.3	49.5	12.8	19.0	8.5	102.3
(2) NaCl-best	36.8	88.2	3.3	11.3	45.2	0.5

* Impulses/5 s.

[‡] Abbreviations for stimuli: S, sucrose; N, NaCl; H, HCl; Q, QHCl.

tween NaCl and the mixture, and +0.30 between QHCl and the mixture). There is a greater inequality between these two correlations than between comparable correlations associated with any other mixture. The relationship between NaCl and QHCl is considered further in the Discussion.

Thermal Responsiveness

13 of the taste-responsive cells were also tested for their responsiveness to warming and cooling. 12 of the cells that were tested responded to at least one of these thermal changes. Five cells were excited by both warming and cooling,
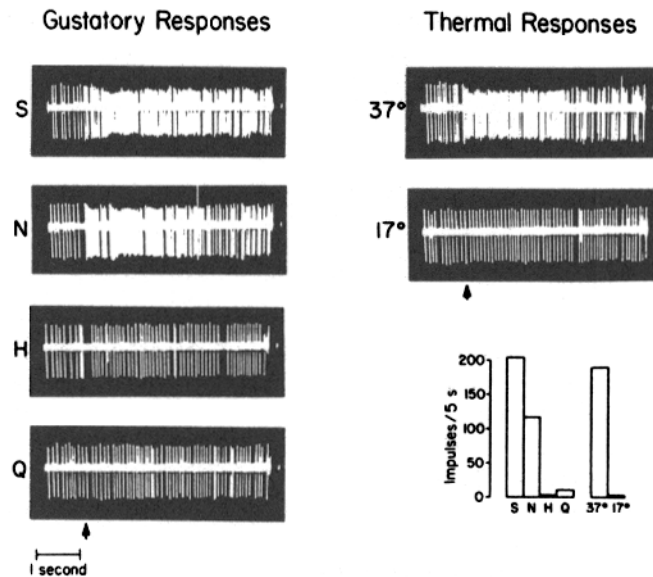

FIGURE 7. Oscillographic records for the responses of neuron 55-3 to the four classical gustatory stimuli; sucrose (S), NaCl (N), HCl (H), and QHCl (Q) and to warming (37°C) and cooling (17°C). The arrows indicate the approximate onsets of the responses. The histograms in the lower right-hand corner show the mean responses of this neuron to the six stimuli for three tests of the taste and two tests of the thermal stimuli.

three cells were excited by warming only, and two cells were excited only by cooling. The other two cells were inhibited by warming and excited by cooling. The mean firing rates to the four basic taste stimuli and the two thermal stimuli appear in Table IV. Both of the thermal stimuli elicited mean responses that were quite vigorous. In four cells, the response to warm distilled water was not reliably different from the response to the best gustatory stimulus. The neural records for one such cell appear in Fig. 7. Sucrose, the best gustatory stimulus for this cell evoked 204 ± 19.8 impulses/5 s ($n = 3$), whereas distilled water at $37^{\circ}C$ following adaptation at $24^{\circ}C$ evoked 191 ± 12.4 impulses/5 s ($n = 2$), a response that was not reliably different than that elicited by sucrose. In another case, a cell responded to cool distilled water, but gave no response to any of the four basic gustatory stimuli at the reference concentrations, although the cell did respond to 0.1 M NaCl and to certain mixtures of the gustatory stimuli. The across-neuron correlations (Pearson r) between the gustatory and thermal stimuli and between spontaneous rate and the thermal stimuli are shown in Table V. Responsiveness to warming was highly correlated with responsiveness to sucrose. The responsiveness of these cells to cooling was positively correlated with their responsiveness to HCl and with their spontaneous rate.

The relationship between gustatory and thermal sensitivities of these cells is also apparent from the mean firing rates within best-stimulus classes, although there were only two classes for which there were a sufficient number of cells for this comparison, the sucrose- and NaCl-best neurons. The mean firing rates for the four gustatory and two thermal stimuli for sucrose-best ($n = 4$) and NaCl-best ($n = 6$) cells are shown in Table IV. Sucrose-best cells responded vigorously to warming and very little to cooling, whereas NaCl-best cells responded well to cooling and not at all to warming.

DISCUSSION

Anatomical Distribution

Gustatory neurons in the present study were recorded from the portion of the PBN that receives input from the taste-responsive region of the NTS (Norgren and Leonard, 1971, 1973; Norgren, 1978; Travers, 1979). This region of the PBN forms part of the taste pathway from hindbrain to forebrain (Norgren and Leonard, 1971, 1973; Norgren, 1976) and overlaps that portion of the PBN from which taste responses have been recorded in other investigations of this nucleus in the hamster (Van Buskirk and Smith, 1981), rat (Norgren and Pfaffmann, 1975; Perrotto and Scott, 1976), and rabbit (Di Lorenzo and Schwartzbaum, 1982).

The anatomical distribution of gusatory-responsive neurons in the present study differed slightly from the distribution of hamster PBN taste neurons described by Van Buskirk and Smith (1981). These investigators encountered 44% of their cells in the anterior two-fifths of the taste-responsive PBN but only 8% of the neurons in the present sample were located in the anterior one-third

of this region. Further, 57% of the cells recorded by Van Buskirk and Smith (1981) were dorsal to the middle of the BC compared with 32% in the present sample. Differences in the proportion of HCl-best cells in these samples might be related to their anatomical distributions. Although 48% of the cells recorded by Van Buskirk and Smith (1981) were classified as HCl-best, only 12% of the cells in the present study could be so classified. 65% of the HCl-best cells encountered by Van Buskirk and Smith (1981) were in the anterior two-fifths of the taste-responsive area, and 73% of these cells were dorsal to the middle of the BC. Since fewer of the neurons in the present study were recorded from the anterior and dorsal regions of the PBN, this sampling difference probably accounts for the smaller percentage of HCl-best cells encountered.

TABLE V

Across-Neuron Correlations (Pearson r^*) Between Responses to Gustatory and Thermal Stimuli

Stimulus pair	Correlation
Warming × sucrose	+0.86
Warming × NaCl	+0.20
Warming × HCl	+0.01
Warming × QHCl	+0.49
Warming × spontaneous	+0.20
Cooling × sucrose	−0.07
Cooling × NaCl	+0.45
Cooling × HCl	+0.62
Cooling × QHCl	+0.47
Cooling × spontaneous	+0.63

For $n = 13$, a correlation of ± 0.51 is significant ($P < 0.05$).

Mixtures vs. Single Components

EQUAL MIXTURE RESPONSES   One principal finding of the present investigation was that well over half of the responses (95 out of 137) to mixtures were not reliably different from the response to the more effective component of the mixture. Thus, the best predictor of the magnitude of a cell's response to a two-component mixture was its response to the mixture's more effective component. It is not surprising, then, that the across-neuron patterns evoked by the mixtures are well predicted by hypothetical patterns derived from the responses to the more effective components of the mixtures (Table III).

Mixture responses approached the response to the more effective component of the mixture in the majority of cases, regardless of the types of responses elicited by a mixture's components (Table I). Of the 62 cases in which both component stimuli elicited excitatory responses, 41 mixture responses were no different from the response to the more effective component of the mixture. That is, there was little summation of excitatory responses. This lack of summation is not simply due to a lack of effectiveness of the less effective components of the mixtures, since the mean response to the less effective components was

robust—40 impulses/5 s (Table I), with 66% of these responses exceeding 20 impulses/5 s. Further, because stimulus concentrations were midrange, the lack of summation is not likely to be attributable to the mixture's more effective component driving the cell at its maximum frequency. In fact, in 54% of the cases showing lack of summation of excitatory responses, another stimulus (not contained in the mixture in question) evoked a greater response than the mixture's more effective component.

In 11 cases, one mixture component evoked an excitatory response and the other elicited an inhibitory one, but well over half (8 out of 11) of these mixture responses were not reliably different from the response to the excitatory stimulus. That is, stimuli that inhibited the spontaneous firing in a cell did not usually reduce the excitatory response elicited by another stimulus.

GREATER MIXTURE RESPONSES Mixtures reliably elicited a greater response than the mixture's more effective component in 15% of the cases. This effect occurred for four response type combinations (Table I), but occurred more often than expected from chance when both component stimuli elicited excitatory responses and less often when one component stimulus evoked an excitatory response and the other elicited no response.

In human psychophysical experiments, the intensity of a taste stimulus has sometimes been reported to be enhanced after the addition of a second, usually weak, stimulus evoking a different quality (Pangborn, 1962; Pangborn and Trabue, 1967). The greater mixture responses in the present study are not exactly analogous to psychophysical mixture enhancement since mixtures in the current study contained stimuli of midrange concentration.

SMALLER MIXTURE RESPONSES The remaining 15% of the mixture responses were smaller than the responses to the more effective components of those mixtures. Smaller mixture responses occurred for three component response type combinations, including several cases in which both component stimuli evoked excitatory responses. When one component elicited an excitatory response and the other evoked an inhibitory one, mixture responses were smaller than the responses to their more effective component no more frequently than expected by chance. A mixture elicited a smaller response than its more effective component more often than expected when one component stimulus elicited an excitatory response and the other elicited no response. Suppression was evident not only in the responses of certain individual neurons, but also in the mean responses of NaCl- and QHCl-best neurons. The suppression of the neural response to one stimulus following the addition of a second stimulus may be an analogue of psychophysical mixture suppression. Mixture suppression is a reduction in perceived intensity that occurs often when a stimulus is contained in a mixture with a qualitatively different taste stimulus (Pfaffmann et al., 1971; McBurney, 1978).

Mixture Suppression and Ionic Activity Changes

Since cases of mixture suppression have been reported at the level of the peripheral nerve (Hyman and Frank, 1980b; Kruger and Boudreau, 1972; Miller,

1971; Sato et al., 1970; Wang, 1973), one mechanism for suppression must be at or before the level of the first-order neuron. The ionic activity of an electrolyte is a measure of its effective concentration in solution. The presence of more than one electrolyte in a solution reduces the ionic activities of the individual electrolytes. Table VI shows the ionic activities of the electrolytes used in the present study for each chemical alone and in the mixtures. Although reductions in activity for chemicals in the mixtures are small, it is conceivable that some of the response suppression seen was due to ionic activity changes. We will consider this possibility for the reduction of the response of QHCl-best neurons to QHCl following the addition of NaCl.

The magnitude of the effect of NaCl upon QHCl's ionic activity was greater than the effects seen for the other electrolyte mixtures. The ionic activity of 0.001 M QHCl alone was 0.000964, and in the mixture it was 0.000814, a decrease in concentration of 0.07 log steps. The response-concentration function for QHCl in the CT nerve (from Frank, 1973) would predict, in this concentration range, that a decrease of 0.07 log steps would result in a 4% decrement in

TABLE VI

Ionic Activities of Electrolytes and Electrolyte Mixtures

Second component	Activity of first component		
	0.03 M NaCl	0.003 M HCl	0.001 M QHCl
None	0.0245	0.00281	0.000964
NaCl	—	0.00242	0.000814
HCl	0.0242	—	0.000929
QHCl	0.0244	0.00279	—

the neural response. However, the response of QHCl-best neurons to a mixture of QHCl plus NaCl was 20% smaller than their response to QHCl, a greater reduction than predicted by the ionic activity change alone. Although a response-concentration function for the whole CT is considered an average function for anterior tongue sensitivity, there will be variability in individual elements and, further, the concentration functions may be different for the PBN neurons of interest here. Because the concentration functions of the present QHCl-best neurons were not investigated, it is impossible to know for certain whether reduction in ionic activity is an important mechanism for suppression of the QHCl plus NaCl response, but it seems likely that it is only a partial explanation. Similar arguments hold for the explanation of the suppression of the response of NaCl-best fibers to NaCl after the addition of HCl and for cases of response suppression seen with other electrolyte mixtures in certain neurons. However, the effective concentration of sucrose is not changed by the addition of electrolytes, nor does added sucrose change the ionic activity of NaCl, HCl, or QHCl. Mechanisms other than ionic activity changes must therefore be entirely responsible for the suppression observed in nine cases for mixtures containing sucrose.

Comparison with Data from the Hamster Periphery

Hyman and Frank (1980a) recently investigated responses elicited by two-component taste mixtures in hamster CT fibers. These investigators assigned CT fibers to one of three best-stimulus categories—sucrose-, NaCl-, or HCl-best—and analyzed responses of each group separately. The present discussion will focus on responses of these best-stimulus classes in the CT and PBN to stimulation with the mixtures used in both studies—sucrose plus NaCl, sucrose plus HCl, and NaCl plus HCl.

SUCROSE-BEST NEURONS In the CT, the response of sucrose-best neurons to sucrose was found to be reduced by the addition of NaCl, HCl, and a number of other electrolytes. There was less suppression of the response of sucrose-best pontine neurons to sucrose by NaCl and HCl. The response of sucrose-best CT fibers to sucrose was reduced by 12% (not significant) after the addition of NaCl, but the response of sucrose-best pontine neurons to this mixture was actually 10% greater ($P < 0.05$) than the response to sucrose alone. This difference may be due to the greater responsiveness of sucrose-best pontine neurons to NaCl relative to CT fibers.[2] NaCl was an ineffective stimulus for this group of fibers in the periphery, but it was about half as effective as sucrose for sucrose-best fibers in the PBN. Presumably, addition of component responses to sucrose and NaCl accounts for the greater response of pontine neurons to sucrose plus NaCl. However, the pontine response to sucrose plus NaCl was smaller (28%, $P < 0.02$) than the algebraic sum of the responses to sucrose and NaCl presented alone. Suppression of the sucrose response by NaCl at the periphery (as seen by Hyman and Frank, 1980b) could be partially responsible for this incomplete summation.

Adding HCl to sucrose suppressed the response of sucrose-best neurons to sucrose in both the periphery and PBN, but the amount of suppression for CT fibers (46%, $P < 0.05$) was greater than for PBN neurons (16%, not significant). The attenuation of suppression in the pons does not appear to be due to differential responsiveness of these two groups of neurons to HCl, since sucrose-best neurons at both levels responded similarly (i.e., poorly) to this chemical. It is possible that there is a nonlinear transformation of information from sucrose-best CT fibers to such neurons in the PBN. This idea could be tested if concentration-response functions for sucrose-best neurons were compared for these levels.

NACL- AND HCL-BEST NEURONS Hyman and Frank (1980b) reported that NaCl- and HCl-best CT fibers processed electrolyte mixtures differently. For NaCl-best fibers, adding a second electrolyte was nearly equivalent to increasing the NaCl concentration by an analogous amount, whereas in HCl-best neurons the responses to mixtures of electrolytes approached the sum of the component responses. The fact that the concentration-response function for NaCl is com-

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[2] The greater relative response to NaCl in these neurons could be due to the higher concentration of NaCl used in the present study compared with Hyman and Frank (1980b), although other data indicate that sucrose-best pontine neurons in the hamster respond better to NaCl than do comparable CT units even when the same concentrations of salt are tested at both levels (Van Buskirk and Smith, 1981).

pressed in CT fibers means that component responses to electrolytes sum more completely in HCl- than in NaCl-best fibers. Since concentration functions were not tested in the present study, we will compare the processing of electrolyte mixtures at the two levels by comparing the ratios of responses to mixtures relative to the sum of the responses to the mixture's components, i.e., by comparing the amount of summation. (These ratios have been estimated for the CT from Figs. 7 and 8 of Hyman and Frank, 1980b). For NaCl-best neurons, the response to the mixture, NaCl plus HCl is smaller than the sum of the responses to NaCl and HCl presented alone for both the CT (~36%, not significant) and PBN (26%, $P < 0.05$). Although component responses add similarly at the two levels, responses to the mixtures relative to the responses to the more effective components of the mixtures are different at the two levels, probably because of differences in responses to NaCl and HCl presented alone. For CT NaCl-best fibers, NaCl and HCl are nearly equally effective and the response to the mixture of the two stimuli is greater (~20%, not significant) than the response to the more effective component of the mixture. The NaCl-best pontine neurons, however, responded poorly to HCl, and the response to the mixture was only 75% as great ($P < 0.05$) as the response to NaCl alone. This suggests that one mechanism for the incomplete summation of NaCl and HCl responses in the CT could be the reduced effectiveness of NaCl in the presence of HCl evident in the responses of these PBN neurons since they were not sensitive to HCl.

In contrast to NaCl-best neurons, HCl-best neurons sum responses to NaCl and HCl differently in the PBN than in the CT. In the CT, the response to NaCl plus HCl approaches the sum of the component responses, but the pontine response to this mixture was smaller (28%, occurring in three of the four HCl-best neurons) than the sum of the component responses. Thus, mixtures of NaCl and HCl add similarly for NaCl- and HCl-best pontine neurons but differently for these groups of fibers in the CT. This conclusion must be tentative, since only four HCl-best pontine neurons were studied. It should also be kept in mind that a higher concentration of NaCl was used in the PBN study.

Taste Mixtures and Gustatory Neuron Types

There has been a great deal of interest in the grouping of mammalian gustatory neurons into types (Boudreau and Alev, 1973; Frank, 1974; Kruger and Boudreau, 1972; Nowlis and Frank, 1977; Ogawa et al., 1968; Pfaffmann et al., 1976; Smith et al., 1983a), although there has not been universal agreement that this is appropriate (Erickson et al., 1980; Woolston and Erickson, 1979). Most researchers studying the hamster taste system (Frank, 1973; Smith et al., 1983a; Travers and Smith, 1979; Van Buskirk and Smith, 1981), though not all (Gill et al., 1982), have agreed there are a limited number of gustatory neuron classes in this species. This idea of a finite number of neuron types, each tuned to stimuli evoking an individual taste quality, is supported by the lack of taste neurons with a special sensitivity to mixtures of stimuli evoking different taste qualities. Hyman and Frank (1980b) do not address this question directly, but an inspection of their published data suggests that best-stimulus classes are not well tuned to such

mixtures. 30% of the PBN neurons investigated in the present study did respond more vigorously to at least one of the mixtures tested than to any single-component stimulus. However, on average, "mixture-best" neurons were not very specifically tuned to their best stimiuli, i.e., mixtures. Sucrose-best cells responded 1.9 times, NaCl-best 2.0 times, HCl-best 2.3 times, and QHCl-best 2.2 times as well to their best stimulus as to their second-best stimulus, whereas mixture-best neurons responded only 1.3 times as well to their best mixtures as to their best single-component stimuli. If mixture-best neurons formed distinct classes based on their sensitivites to particular combinations of stimuli, one would expect mixtures to be more specific in activating these cells. Instead, a mixture-best neuron's response to its best mixture (which always contains that cell's best single-component stimulus) is more clearly related to its sensitivity to the four basic stimuli than to any special tuning to the mixture per se.

Responses to taste mixtures have not been extensively studied in other species but those single neuron studies that include mixtures (goat geniculate ganglion: Boudreau et al., 1982; cat geniculate ganglion: Kruger and Boudreau, 1972; rat CT: Miller, 1971; rat CT: Wang, 1973) do not demonstrate specific, mixture-senstive neurons in these species either. Neurons in other sensory systems do show specific sensitivities for combinations of stimuli (e.g., Suga et al., 1979) and for other types of complex stimuli (Kuffler, 1953; Hubel and Wiesel, 1962, 1965), but are often located at more rostral levels of the nervous system and/or have precise stimulus requirements related to the organism's behavioral repertoire.

Psychophysical and Behavioral Correlations with Neural Responses

Although the labeled-line and across-neuron pattern theories are often viewed as opposite approaches, recent discussions (Smith et al., 1983b; Van Buskirk and Smith, 1981) of taste quality coding have pointed out similarities between them. The essence of the labeled-line theory is that separate classes of neurons code for different taste qualities (Nowlis and Frank, 1977; Pfaffmann et al., 1976). Neurons are separated into classes according to which of the four basic taste stimuli evokes the best response in a given cell and each class is presumed to signal the quality evoked by this best stimulus. The across-neuron pattern theory (Pfaffmann, 1941, 1955; Erickson, 1963, 1968, 1974), on the other hand, posits that a particular taste quality is coded by the pattern of activity across the entire population of neurons. The similarity between the patterns that two stimuli evoke is quantified with the use of the across-neuron correlation coefficient. Smith and his colleagues (Smith et al., 1983b; Van Buskirk and Smith, 1981) examined the relationship between the two theories and showed that the neurons which contribute most to the across-neuron pattern for a particular quality are largely the same as those classified "best" for that quality. In fact, the inclusion of this group of cells is usually necessary to define the similarity (expressed by across-neuron correlations) among chemicals of the same quality. Thus, it is not surprising that the effects of single-component taste stimuli upon neurons correlate well with behavioral effects of such stimuli when analytical methods associated with either quality coding theory are used (Smith et al., 1979; Nowlis

and Frank, 1977; Scott, 1973; Erickson, 1963). Further, the present study has shown that results of analyses associated with both the labeled-line (best-stimulus class responses) and across-neuron pattern theories (across-neuron correlations) are congruent with psychophysical and behavioral data on gustatory mixtures which indicate that these stimuli evoke the same taste qualities elicited by the individual components in the mixture (Bartoshuk, 1975; Nowlis and Frank, 1977; Theodore, 1977). Accordingly, a particular taste mixture was usually most effective in activating only those classes of neurons that responded "best" to that mixture's components. For example, both sucrose- and NaCl-best neurons were stimulated more vigorously by a sucrose plus NaCl mixture than were HCl- or QHCl-best cells. In fact, neither HCl- nor QHCl-best cells responded much better to the sucrose plus NaCl mixture than they did to sucrose or NaCl presented alone. The cross-correlational analysis also showed that mixtures activated the same set of neurons as their individual components. In general, the pattern of activity evoked across these PBN neurons by a mixture was correlated with both the patterns evoked by that mixture's components, but not with the patterns of activity evoked by other single-component stimuli. In fact, the MDS analysis demonstrated that five of the six mixtures evoked patterns of activity in the hamster PBN intermediate to the patterns evoked by their components (Fig. 6).

One of the six mixtures, however, elicited a pattern that was not intermediate to the patterns evoked by its components. The QHCl plus NaCl mixture evoked a pattern very similar to the pattern elicited by NaCl ($r = +0.91$) but not to that elicited by QHCl ($r = +0.30$). In Fig. 6, this mixture lies in close proximity to NaCl but not to QHCl. This anomaly in the across-neuron patterns has a psychophysical correlate in humans and a behavioral correlate in hamsters. For humans, the bitterness of QHCl is suppressed more than the saltiness of NaCl in a QHCl plus NaCl mixture (Bartoshuk, 1979). Although hamsters usually generalize an aversion conditioned to a two-component mixture to both stimuli tested individually, an aversion to a mixture of 0.1 M NaCl and 0.001 M QHCl generalizes strongly to NaCl but not to QHCl (generalization to quinine is observed only after tripling the QHCl concentration) (Nowlis and Frank, 1977). This behavioral-neural correlation can also be detected when responses of best-stimulus categories of neurons are analyzed. The mean response of NaCl-best neurons to a mixture of NaCl plus QHCl was no different from the response to NaCl alone, but in QHCl-best neurons the mean response to QHCl plus NaCl was only 75% of the response to QHCl presented alone.

PBN Mixture Responses: Conclusions

The response of a neuron to a mixture was best predicted simply from the neuron's response to that mixture's more effective component. That is, mixture responses usually approached, rather than exceeded or fell short of, the response produced by the more effective component of a mixture. This was true regardless of the types of responses produced by the mixture's components.

There was no evidence that there are classes of PBN neurons especially

sensitive to mixtures. About one-third of the PBN neurons recorded did respond somewhat better to a mixture than to the single-component stimuli, but, on average, these neurons were not as specifically tuned to their best mixtures as the classic best-stimulus groups were to their best single-component stimuli.

Data from both human and animal psychophysical studies indicate that taste mixtures evoke the same taste qualities as are evoked by the components in the mixture presented alone. The neural data from the PBN are congruent with these findings since the same neurons are activated by a mixture as are activated by that mixture's components presented individually. These results are obvious when the responses of best-stimulus classes are studied and when correlational methods are used.

Thermal Responsiveness

The majority of hamster PBN neurons tested with thermal stimuli responded to warming and/or cooling. The thermal responses are due in part to information traveling to the central nervous system via the chorda tympani nerve since hamster CT fibers are responsive to both thermal and taste stimuli (Ogawa et al., 1968). However, some of the thermal sensitivity may be due to input from the trigeminal system since the lingual nerve of the hamster projects to the NTS (Whitehead and Frank, 1983), which in turn projects to the PBN (Travers, 1979). Because the temperature changes used to investigate the thermal sensitivity of hamster CT neurons were somewhat greater than those employed in the present study, it is not possible to directly compare thermal responses at these two synpatic levels. However, it is evident that at least as great a proportion of PBN neurons as CT fibers responded to cooling (CT, 57%; PBN, 59%) and warming (CT, 71%; PBN, 77%). In addition, the responsiveness of cool-sensitive neurons to cooling (CT, $\bar{X}$ = 44 impulses/5 s; PBN, $\bar{X}$ = 51 impulses/5 s) and warm-sensitive neurons to warming (CT $\bar{X}$ = 67 impulses/5 s; PBN, $\bar{X}$ = 65 impulses/5 s) is comparable at both levels. However, two inhibitory responses to warming were observed in PBN neurons, whereas none were reported for CT fibers. Gustatory neurons responsive to thermal stimulation have also been reported in the rat (Norgren and Pfaffmann, 1975; Ogawa et al., 1968) and cat (Nagaki et al., 1964; Sato, 1963).

The mean response evoked by warming in the hamster PBN neurons studied here was greater than that elicied by cooling, perhaps because the temperature change for warming (15°C warmer) was greater than that for cooling (8°C cooler). At any rate, responses elicited by both warming and cooling were vigorous; every thermally sensitive neuron responded to either warming or cooling at least as vigorously as it did to its second-best gustatory stimulus. In fact, some of these neurons responded as well to warming as they did to their best gustatory stimulus. The mean response of the four sucrose-best cells to warming was nearly as great (102 impulses/5 s) as these cells' response to sucrose (113 impulses/5 s), and the across-neuron pattern for sucrose across all 13 neurons was highly correlated with the pattern for warming (r = +0.86). However, there is no evidence that warm water tastes sweet. It seems possible

that the nervous system can assess the taste quality message of PBN neurons such as these only in relation to activity occurring in neurons in other portions of the nervous system, such as cells in the trigeminal system that respond to thermal changes of the anterior tongue. This is an extension of the hypothesis that the quality message of taste neurons is given in the activity across taste neurons
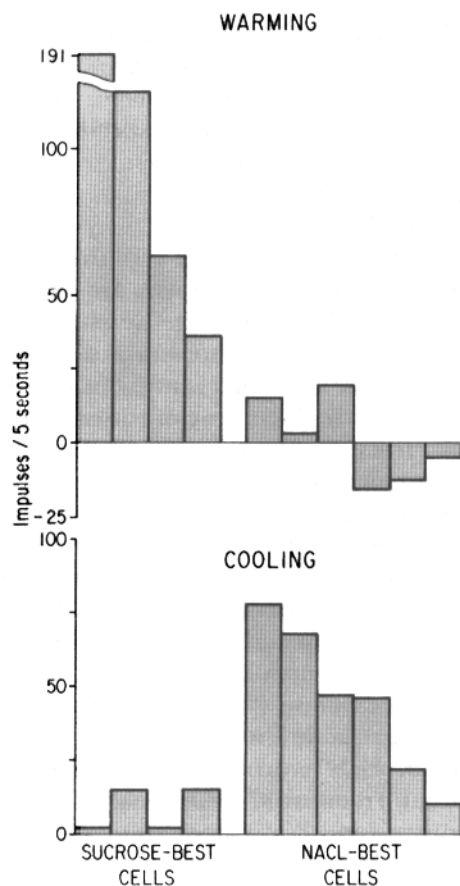

FIGURE 8.   Responses of the four sucrose-best and six NaCl-best cells to warming (upper histograms) and cooling (lower histograms). The sucrose-best cells are ordered according to their responsiveness to warming and the NaCl-best cells are arranged according to their responsiveness to cooling.

(Erickson, 1963, 1968, 1974) or across taste neuron types (Smith et al., 1983b). Alternatively, it is possible that certain PBN cells highly sensitive to both gustatory and thermal stimulation do not code for taste quality at all but for some more general dimension of these stimuli.

The thermal sensitivity of PBN neurons was related to their differential gustatory sensitivity. The relationships between gustatory and thermal responses seen in the across-neuron correlations (Table V) were the same as those in the

hamster CT (Ogawa et al., 1968), except that the across-neuron correlation between QHCl and warming was negative in CT fibers (−0.40) but was positive, although not significant, in PBN neurons (+0.49). The difference in the thermal responsiveness of PBN sucrose- and NaCl-best cells was striking. Table IV shows that the mean response of sucrose-best neurons to warming was quite vigorous but that the cells responded little to cooling. Conversely, NaCl-best neurons responded vigorously to cooling but not to warming. Each sucrose-best and NaCl-best cell tested displayed the same kind of differential thermal sensitivity shown in the mean responses (Fig. 8). The differential thermal sensitivity of sucrose- and NaCl-best cells is an additional characteristic that distinguishes these two groups of neurons, which have previously been characterized on the basis of their differential chemical sensitivity (Smith et al., 1981; Travers and Smith, 1979; Van Buskirk and Smith, 1981).

The temperature of a solution has been reported to affect the perceived intensity of a compound (Bartoshuk et al., 1982; Moskowitz, 1973) as well as its threshold (McBurney et al., 1973), and some investigators have reported that temperature has varying effects for different qualities (Pfaffmann et al., 1971; Moskowitz, 1973). For example, a recent study (Bartoshuk and Hooper, 1981) showed the sweetness of sucrose to be more dramatically affected by temperature than the intensity of the other basic taste qualities. Bartoshuk et al. (1982) have found that the sweetness of low concentrations of sucrose is enhanced by warming, a finding consistent with the correlation between sucrose and warming in the present study. The relationship between sweetness and warming is complex, however. There is no increase in sweetness at higher concentrations of sucrose, and the sweetness of saccharin (Bartoshuk and Hooper, 1981) is not affected by temperature. Previous studies of the relationship between temperature and taste intensity have often yielded conflicting results (Pfaffmann et al., 1971; Sato, 1967), which is not surprising in light of such complexities. An understanding of a possible relationship between the effects of temperature on taste-sensitive neurons and the effects of solution temperature on the perception of taste quality requires further study of both these phenomena in the same species.

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