Sensitivities of Single Nerve Fibers in the Hamster Chorda Tympani to Mixtures of Taste Stimuli

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ABSTRACT Responses of three groups of neural fibers from the chorda tympani of the hamster to binary mixtures of taste stimuli applied to the tongue were analyzed. The groups displayed different sensitivities to six chemicals at concentrations that had approximately equal effects on the whole nerve. Sucrose-best fibers responded strongly only to sucrose and D-phenylalanine. NaCl-best and HCl-best fibers responded to four electrolytes: equally to CaCl₂ and nearly equally to HCl, but the former responded more to NaCl, and the latter responded more to NH₄Cl. The groups of fibers dealt differently with binary mixtures. Sucrose-best fibers responded to a mixture of sucrose and D-phenylalanine as if one of the chemicals had been appropriately increased in concentration, but they responded to a mixture of either one and an electrolyte as if the concentration of sucrose or D-phenylalanine had been reduced. NaCl-best fibers responded to a mixture as if it were a "mixture" of two appropriate concentrations of one chemical, or somewhat less. But, responses of HCl-best fibers to mixtures were greater than that, approaching a sum of responses to components. These results explain effects on the whole nerve, suggest that the sensitivity of a mammalian taste receptor to one chemical can be affected by a second, which may or may not be a stimulus for that receptor, and suggest that some effects of taste mixtures in humans may be the result of peripheral processes.

INTRODUCTION

Neurophysiological study of the sensory effects of taste stimuli in mixtures is important in view of the prevalence of multicomponent stimuli in natural feeding situations. In the first systematic study of mixtures at any neural level in mammals, the responses of the whole chorda tympani of the hamster to binary stimuli were found not to be predictable from the responses to individual components (Hyman and Frank, 1980). Yet, the overlap in sensitivities to pairs of stimuli in individual afferent nerve fibers was the most useful predictor: the greater the overlap, the more closely a mixture of different chemicals has an effect indistinguishable from a "mixture" of two concentrations of one chemical. However, even this was not a satisfactory predictor of
most effects of mixtures, and it was concluded that it was necessary to present
the mixture itself to know its effect.

The chorda tympani, which innervates taste receptors on the anterior
tongue in mammals, is composed of hundreds of nerve fibers with diverse
sensitivities, which are summed in the recorded response. It is thought that
taste quality is represented in relative amounts of activity in differentially
sensitive neurons. If this is true, an understanding of effects on the intensity of
a given taste quality requires knowledge of sensitivities of individual neurons
to mixtures. The discovery that many effects of mixtures on the responses of
the whole nerve could not be predicted from the sensitivities of single fibers to
individual chemicals argues for a determination of their sensitivities to mix-
tures of chemicals; the effects of mixtures on single fibers must also not be
predictable from sensitivities to individual components if the whole nerve
response reflects the sum of single fiber responses. Knowledge of responses of
single fibers to mixtures should explain, then, the responses of the whole nerve.
More importantly, sensitivities of single taste fibers to mixtures are also
necessary to understand the variables determining such mixture effects. Neu-
rions innervate receptors that differ in their sensitivities to single-component
stimuli; these receptors may also differ in the way they process two stimuli
encountered simultaneously.

Chorda tympani nerve fibers of various mammals are differentially sensitive
to chemicals that evoke different taste qualities in the human (for examples,
see Pfaffmann [1955], Fishman [1957], and Erickson [1963]). Most fibers
display one of a limited number of general sensitivity patterns (for examples,
see Boudreau and Alev [1973], Frank [1973], Sato et al. [1975], and Pfaffmann
et al. [1976]) that can be related to one of the four taste qualities described by
humans, i.e., sweet, salty, sour, or bitter (Nowlis and Frank, 1977). In
particular, the single fibers of the hamster chorda tympani can be divided
into three groups (Frank, 1973). Fibers within a group have similar sensitivity
profiles across a number of chemicals (Frank, 1975) and have been named
sucrose-best, NaCl-best, and HCl-best because they respond more to a mod-
erate intensity of the chemical indicated in their name than to others of four
test stimuli. The test stimuli are single concentrations of sucrose, NaCl, HCl,
and quinine hydrochloride and are prototypes of the human’s taste qualities.

The response profiles of all nerve fibers within a group are certainly not
identical. The variability within a category leads to some question as to the
validity of the categorization (Erickson, 1977). However, the variability can,
at least in part, be attributed to intraneuron changes, changes in sensitivities
of fibers or taste cells that occur over time after the fibers are cut from their
cell bodies (Berland et al., 1977), or to changes that occur with repeated
stimulation of the taste receptors (Kruger and Boudreau, 1972). Both of these
effects probably played a role in the variability reported for eight rat chorda
tympani fibers (Ogawa et al., 1973); responses to six repetitions of a set of
stimuli showed that some neurons displayed increased responsiveness, that
others decreased, and that others both increased and decreased, depending
upon the stimulus considered. With such changes occurring in sensitivities of
a single fiber, diversity in the responsiveness of different fibers need not be attributed to systematic variation in sensitivity among neurons.

Although it is possible that gustatory neurons cannot be divided into such categories and that their inputs are not separately processed in the nervous system, it is likely that separate stimulation of the sucrose-best, the NaCl-best, or the HCl-best fibers will evoke very different taste qualities. In this regard, division of single nerve fibers into a few groups according to their relative sensitivity to four chemicals has been useful for the prediction of: (a) the responsiveness of afferent nerve fibers to stimuli other than the four prototypic stimuli in the hamster (Frank, 1974), rat (Frank, 1975), squirrel monkey (Pfaffmann, 1974; Pfaffmann et al., 1976) and macaque (Sato et al., 1975); (b) the squirrel monkey's preference for sugars (Pfaffmann, 1974; Pfaffmann et al., 1976; and (c) the behavioral classification of taste stimuli by hamsters and rats (Nowlis and Frank, 1977). Whether these three groups of fibers process mixtures in distinct ways is of considerable interest in view of the fact that sensations of different taste qualities are affected differentially in mixtures.

The literature of human taste psychophysics abounds with studies of mixtures of stimuli, but there are fewer studies on mixtures of stimuli that individually elicit the same taste quality than studies on mixtures of stimuli that individually elicit different qualities. Cameron (1943, 1944, 1945, and 1947) concluded in a classic set of studies of the relative sweetness of various sweet-tasting substances and their mixtures that the sweetness of a mixture could be predicted from the sum of the concentrations of a reference chemical, when the concentrations of the reference chemical correspond in taste intensity to the individual components of the mixture. In terms of more modern psychophysics (Stevens, 1957), the sum of subjectively equivalent concentrations is transformed into a sensation whose magnitude is given by the psychophysical function for taste intensity of the reference. One restriction on this formulation is that the psychophysical functions for the component stimuli and the reference must be similar for the prediction to be accurate. Moskowitz's determinations of sweetness (1973 and 1974 a) or sourness (1974 b) of mixtures of similar-tasting chemicals agree with Cameron's predictions; however, because the exponents of the power functions relating sensation to stimulus intensity are close to +1.00 for these data, it is also accurate to state that the taste intensity of these mixtures of similar-tasting substances is equal to the sum of the intensities of the components presented individually. Bartoshuk and Cleveland (1977) clarified the situation by manipulating exponents of psychophysical functions with changes in method of stimulus presentation. The magnitude of a sensation evoked by a mixture of similar-tasting substances could be predicted by the psychophysical functions for the component chemicals, as Cameron would have predicted.

Some generalizations formulated on the basis of the many studies of mixtures of chemicals that individually elicit different taste qualities and that may have relevance to neural recordings from the hamster chorda tympani follow. The effects of mixtures of salts, sugars, and acids on evoked sweetness, saltiness, and sourness will be considered since it is responses of sucrose-best,
NaCl-best, and HCl-best units of the hamster chorda tympani to mixtures of these kinds of stimuli which will be reported here.

The sweetness of sucrose solutions is increased by the addition of subthreshold concentrations of NaCl (Fabian and Blum, 1943; Beebe-Center et al., 1959; Kamen et al., 1961; Pangborn, 1962; Indow, 1969), perhaps due to the reported sweetness of weak salt solutions (Richter and MacLean, 1939; Kahn, 1951; Bartoshuk et al., 1964; O'Mahoney et al., 1976; Cardello and Murphy, 1977). This may also be true of other sugars (Fabian and Blum, 1943). In contrast, moderate or intense concentrations of NaCl suppress the sweetness of sucrose (Beebe-Center et al., 1959; Kamen et al., 1961; Pangborn, 1962; Indow, 1969); glucose and fructose solutions are affected similarly (Moskowitz, 1972). Citric acid can reduce the sweetness of solutions of sucrose (Pangborn, 1960 and 1961), glucose, or fructose (Stone et al., 1969; Moskowitz, 1972). The suppression of sweetness by threshold and moderate concentrations of citric acid is much more marked than the effect of threshold or moderate concentrations of NaCl on the concentrations of sucrose (Pangborn, 1960, 1961, and 1962). Indeed, concentrations of citric acid sufficient to reduce the pH of sugar solutions from 5.8 to 2.7 cause a 50% reduction in sweetness (Stone et al., 1969). Corresponding concentrations of HCl have not been tested. However, weak solutions of HCl (Fabian and Blum, 1943) or the addition of citric acid sufficient to reduce the pH of sugar solutions to 4.0 (Stone et al., 1969) do not influence sweetness.

Sucrose suppresses the sourness of citric acid (Fabian and Blum, 1943; Pangborn, 1960; Kamen et al., 1961) and tartaric acid solutions (Fabian and Blum, 1943; Indow, 1969). Small amounts of NaCl, when added to citric acid solutions, reduce their sourness (Fabian and Blum, 1943; Pangborn, 1960; Kamen et al., 1961; Pangborn and Trabue, 1967), but there are conflicting reports as to whether the sourness of citric acid solutions is increased (Kamen et al., 1961) or decreased (Pangborn and Trabue, 1967) by the addition of larger quantities of NaCl.

Although Kamen et al., (1961) report that the addition of sucrose produces no effect on the saltiness of NaCl solutions, the more common finding is that saltiness is suppressed by a small amount of sucrose (Fabian and Blum, 1943; Beebe-Center et al., 1959; Pangborn, 1960 and 1962; Indow, 1969). Again, this may also be true of other sugars (Fabian and Blum, 1943). The saltiness of NaCl solutions is not affected by the addition of near-threshold concentrations of HCl (Fabian and Blum, 1943), but it is increased by the addition of citric acid at near-threshold concentrations (Fabian and Blum, 1943; Pangborn and Trabue, 1967) and decreased by the addition of citric acid at higher concentrations (Pangborn and Trabue, 1967).

In summary, the psychophysical reports state that (a) the sweetness of sugars is suppressed more by the addition of moderate amounts of citric acid than moderate amounts of NaCl and is enhanced by small amounts of NaCl, (b) that the addition of sucrose or small amounts of NaCl suppress the sourness of acids, and (c) that the saltiness of NaCl is typically suppressed by the addition of small amounts of sugars and large amounts of citric acid but is enhanced by small amounts of acid.
Unlike the psychophysics of taste stimuli in mixtures, the neurophysiology of taste stimuli in mixtures has not been studied systematically. However, in studies with major aims other than a description of effects of mixtures, both suppression and enhancement have been documented. Responses of some chorda tympani fibers of the rat to 0.1 M NaCl may be halved when 0.1 M potassium benzoate is added to the solution; whereas, responses of other fibers may not be affected at all by such an addition (Miller, 1971). Comparably, responses of acid-sensitive units (type I) in the geniculate ganglion (where cell bodies of chorda tympani fibers are located) of the cat to NaH₂PO₄ are halved when an equal concentration of NaCl is added to the solution. On the other hand, in units of the same ganglion that are sensitive to NaCl (type II), responses to mixtures of equimolar NaCl and NaH₂PO₄ are greater than the sum of the responses to the components presented individually (Kruger and Boudreau, 1972). This enhancement contrasts with the suppression of responses of type I units to this mixture. The responses of sucrose-sensitive chorda tympani fibers of rats to mixtures of 0.016 M monosodium glutamate and 0.00073 M inosine 5'-monophosphate (disodium salt) or other 5'-ribonucleotides (e.g., guanosine 5'-monophosphate) were also enhanced; responses to the same mixtures were not enhanced in fibers which were not sensitive to sucrose (Sato et al., 1970). These examples illustrate that the neurophysiological effects of taste stimuli in mixtures can differ for units which differ in their sensitivities to single-component stimuli. In fact, suppression and enhancement can be present to similar degrees in different groups of units (Kruger and Boudreau, 1972). A summation of these opposite effects would make them observable in recordings from the whole nerve.

In the present experiments, single nerve fibers of the hamster chorda tympani were divided into three groups according to their predominant sensitivities to single-component stimuli; the responses of the groups were analyzed separately. The effects of taste stimuli in mixtures proved to be considerably different for the three groups of nerve fibers.

METHODS

Recording Technique, Stimulation Scheme, and Response Measurement

Surgical and stimulating techniques were identical to those reported for whole nerve recordings (Hyman and Frank, 1980), with the following exceptions. For single-nerve-fiber recording, after the chorda tympani was dissected free and cut, the sheath surrounding the nerve was removed, and the nerve trunk was teased apart into small bundles of fibers. Each nerve bundle was in turn lifted onto the recording electrode. Action potentials were recorded differentially with respect to an indifferent electrode positioned on muscle close to the chorda tympani. Solutions of 0.01 M NaCl, 0.003 M HCl, and 0.1 M sucrose were flowed over the tongue, and their effect on the neural activity of each nerve bundle was observed. If the response of a single nerve fiber could be identified reliably, as judged by the amplitude and wave form of action potentials displayed on an oscilloscope and audiometer, stimulation proceeded according to a predesigned schedule.

In the following schedule of stimulation, the letters A, B, C, D, E and F refer to 0.01 M NaCl, 0.05 M NH₄Cl, 0.1 M sucrose, 0.01 M CaCl₂, 0.07 M D-phenylalanine, and
0.003 M HCl, respectively. A mixture of any two stimuli is designated by the appropriate two letters; e.g., AB refers to the mixture of 0.01 M NaCl and 0.05 M NH₄Cl. The concentration of a component in the mixture equals its concentration when presented alone. For any stimulus X, +X is one-half log step higher, −X is one-half log step lower, and --X is one log step lower in concentration than that in the mixtures; e.g., +A designates 0.03 M NaCl, −A designates 0.003 M NaCl, and --E designates 0.007 M D-phenylalanine.

In the first stage of stimulation, responses to the single-component stimuli were obtained (i.e., responses to A, B, C, D, E, and F, in random order). In the second stage of stimulation, stimulus-response functions (i.e., the variation of response with stimulus concentration) and responses to mixtures were obtained in the following order: (1) the A series: −A, +A, then A, AB, AC, AD, AE, and AF in random order; (2) the B series: −B, +B, then B, BC, BD, BE, and BF in random order; (3) the C series: −C, +C, then C, CD, CE, and CF in random order; (4) the D series: −D, +D, then D, DE, and DF in random order; (5) the E series: --E, −E, then EF and E in random order; and (6) the F series: −F, F, and +F. If a nerve fiber was lost before presentation of all six series, stage two of the experiment on the next fiber was begun with the series that was not completed in the previous experiment, and that series was rotated to (1). For example, if a nerve fiber were lost after completion of the A (NaCl), B (NH₄Cl), C (sucrose), and D (CaCl₂) series, the E (D-phenylalanine) series would be the first series to be presented to the next fiber; under these circumstances, the D-phenylalanine series would consist of eight stimuli: --E, −E, then E, EA, EB, EC, ED, and EF in random order.

There was an interval of at least 1 min between successive stimulations, and the tongue was rinsed with distilled water for at least 15 s before each stimulus presentation. The chemicals and their concentrations in mixtures were the same as those reported for whole-nerve experiments (Hyman and Frank, 1980). Only single nerve fibers for which responses to all of the single component stimuli were obtained are reported here. 44 single nerve fibers were functionally isolated from 20 hamster nerves. Responses of these fibers were recorded on analogue tape for future “off-line” processing.

Tape recordings were played back and oscilloscope displays were photographed. The number of action potentials occurring in the first 5 s of stimulation was determined from the photographs. Fig. 1 is a series of photographs of one fiber’s responses to nine stimuli. Spontaneous response rate, measured as the mean response rate to distilled water in the 2-s periods preceding each stimulation, varied from 0.0 to 4.4 action potentials per second for different fibers and was not subtracted from responses to stimuli. The 5-s response measure was chosen because it had been used successfully in previous analyses of afferent taste nerve responses (Frank, 1973, 1974, and 1975; Pfaffmann, 1974; Pfaffmann et al., 1976; Sato et al., 1975).

Classification of Nerve Fibers

The single nerve fibers were divided into three groups on the basis of their responses to the single-component stimuli presented at the beginning of each experiment. The criteria are similar but not identical to those used by Frank (1973), who compared taste-fiber responses to four gustatory stimuli, each at one test concentration: 0.1 M sucrose, 0.003 M HCl, 0.03 M NaCl, and 0.001 M quinine hydrochloride. The term “best stimulus” refers to the test stimulus that elicits the largest number of action potentials in the first 5 s of stimulation. In the present study, six stimuli were used, only two of them the same as those used by Frank (i.e., the HCl and sucrose stimuli). The NaCl test stimulus used here is of lower intensity (i.e., 0.01 M) than that used by
Frank, and a quinine hydrochloride test stimulus was not used here because it had not been found useful in the classification of hamster chorda tympani nerve fibers (i.e., it is neither a very effective nor a selective stimulus).

17 single nerve fibers were classified as sucrose-best (S-best). In 15 of these fibers, the responses to 0.1 M sucrose and 0.07 M D-phenylalanine were at least twice the magnitude of the responses to 0.003 M HCl, 0.01 M NaCl, 0.05 M NH₄Cl, and 0.01 M CaCl₂; in two less sensitive fibers, the responses to the former stimuli were greater but not twice as great as responses to the other stimuli.

Twelve single nerve fibers were classified as acid-best, specifically HCl-best (H-best). In all of these fibers, the responses to 0.003 M HCl and 0.05 M NH₄Cl were at least five times the size of the responses to 0.1 M sucrose and 0.07 M D-phenylalanine. In 10 of these fibers, the responses to 0.003 M HCl and 0.05 M NH₄Cl were greater than twice the magnitude of the response to 0.01 M NaCl; in the other two fibers, the
responses to HCl and NH₄Cl were much greater than, but not quite twice as great as, the response to NaCl. All fibers classified as HCl-best responded more, or nearly as much, to 0.05 M NH₄Cl as to 0.003 M HCl, and less to 0.01 M CaCl₂.

15 single nerve fibers were classified as salt-best, specifically NaCl-best (N-best). In all of these fibers, the response to 0.01 M NaCl was greater than twice the magnitude of the responses to 0.1 M sucrose and 0.07 M D-phenylalanine. In six of these fibers, the response to 0.01 M NaCl was greater than twice the magnitude of the responses to 0.003 M HCl, 0.05 M NH₄Cl, and 0.01 M CaCl₂; in four other fibers the response to NaCl was greater than, but not twice as great as, the responses to HCl, NH₄Cl, and CaCl₂. In yet five more fibers, the response to NaCl was slightly less than the response to HCl; these fibers were relatively nonspecific electrolyte-sensitive fibers, and were classified as NaCl-best rather than HCl-best because they (a) responded more to 0.01 M NaCl than any of the fibers classified as HCl-best and (b) because they responded more to 0.01 M CaCl₂ than to 0.05 M NH₄Cl, which was characteristic of NaCl-best and contrary to the response characteristics of all HCl-best fibers.

Although the criteria for classification differ slightly from those of Frank (1973), it was expected that the two methods would result in nearly identical grouping of the nerve fibers. With regard to sucrose-best fibers, 0.001 M quinine-HCl produced no effect in the earlier study; therefore, its omission here is not critical. The use of the stronger NaCl stimulus would not have affected classification, as is evidenced by the nearly equal responses to 0.03 M NaCl and 0.01 M NaCl in the 11 sucrose-best fibers that were so stimulated in the second stages of the experiments. With regard to HCl-best fibers, 0.001 M quinine-HCl was only weakly effective in the earlier study so that, again, its omission here is not critical; and in all but one of the nine HCl-best fibers stimulated with 0.03 M NaCl, that stimulus was less effective than the initial 0.003 M HCl application. With regard to the NaCl-best fibers, 0.001 M quinine-HCl was only weakly effective in the earlier study so that, again, its omission here is not critical; and in the five NaCl-best fibers that were stimulated with 0.03 M NaCl, it was more effective than the 0.01 M NaCl and 0.003 M HCl tested initially. Moreover, these five fibers include two fibers in which 0.01 M NaCl was less effective than 0.003 M HCl in the initial tests. Therefore, the five fibers that responded more to 0.003 M HCl than to 0.01 M NaCl would likely have been classified as NaCl-best on the basis of the Frank (1973) method as well.

Data from each group of nerve fibers were analyzed separately. Responses to the six single-component stimuli to variation in their concentrations and to each of the 15 two-component mixtures were considered separately for sucrose-best, NaCl-best, and HCl-best fibers. 13 of the 17 sucrose-best fibers, 10 of the 12 HCl-best fibers, and 9 of the 15 NaCl-best fibers contributed to at least one of the six sets of data on response vs. stimulus concentration of each fiber group. These stimulus-response functions were necessary to determine whether the response to a two-component mixture could be accounted for as a response to the sum of two concentrations, equal in intensity to the two stimuli in the mixture, of one chemical. 12 of the sucrose-best, nine of the HCl-best, and seven of the NaCl-best fibers contributed to the data from two-component mixtures. Responses to at least two complete mixture series were recorded from each of these fibers. Note that a smaller percentage of NaCl-best fibers survived long enough to be tested with mixtures. Only responses to mixture components of the fibers which contributed to the mixture data were used in the analysis of mixtures.

RESULTS

Effects of Single-component Stimuli on Single Nerve Fibers

Responses of single nerve fibers to the single-component stimuli presented at the beginning of each recording session are plotted in Fig. 2; response profiles
Figure 2. Response profiles of HCl(H)-best, NaCl(N)-best, and sucrose(S)-
best hamster chorda tympani nerve fibers, as well as the mean profile for each
group, to 0.07 M D-phenylalanine, 0.1 M sucrose, 0.01 M NaCl, 0.01 M CaCl_2,
0.003 M HCl, and 0.05 M NH_4Cl. Profiles for 12 fibers in each group are
illustrated; 12 HCl-best, 15 NaCl-best and 17 sucrose-best fibers were sampled.
Profiles in the latter two groups which showed the largest responses to their
"best stimulus" (eight NaCl-best, six sucrose-best) and alternates of the less
responsive are illustrated. All fibers' responses were included in the calculation
of means. The response measure was the number of action potentials in the first
5 s of a response.

for the sucrose-best, HCl-best, and NaCl-best groups of fibers are plotted on
separate sets of axes. In this and in similar figures in this paper, the discrete
points along the abscissa indicate the chemicals presented, each at one test
concentration. The order along the abscissa is arbitrary and does not imply a 
continuous scale or function; each line merely connects the points that 
represent the responses of a particular fiber to the indicated stimuli.

Responses to each stimulus vary in magnitude across fibers. At the test 
centrations, NaCl, HCl, and sucrose are approximately equally good 
stimuli: 17 of the sampled single fibers respond best to sucrose; 15 fibers 
respond best to NaCl, and 12 fibers respond best to HCl. The number of 
single fibers responding with over 20 action potentials in the 5-s stimulation 
period is 22 for NaCl, 22 for HCl, and 17 for sucrose; over 100 action potentials 
in 5 s were elicited by NaCl in nine fibers, by HCl in eight fibers, and by 
sucrose in eight fibers; and within each group there are fibers whose best 
response was nearly 200 action potentials in 5 s.

All of the sucrose-best fibers are quite specific in their sensitivities, although 
the magnitude of the response to sucrose varies considerably across these 
fibers, ranging from 50 to 223 action potentials in 5 s. Responses to the 
electrolytes are small, if they occur; only two such responses exceed 30 action 
potentials in 5 s. In general, the fibers respond as much to D-phenylalanine as 
to sucrose.

All of the HCl-best fibers respond more to NH₄Cl than to CaCl₂ and NaCl. 
The order of responsiveness is NH₄Cl > CaCl₂ > NaCl for every HCl-best 
fiber except one, whose response to NaCl slightly exceeds its response to CaCl₂. 
The response to HCl ranges from 50 to 218 action potentials/5 s in these 
fibers. The response to NH₄Cl approaches that to HCl and even exceeds it in 
several fibers. The response to NaCl is generally small. Responses to sucrose 
and D-phenylalanine, if they occur, are small; none exceeds 30 action poten-
tials in 5 s.

The NaCl-best fibers whose best-response is among the lowest of the group 
respond little, if at all, to the other stimuli. However, with only one exception, 
fibers responding with at least 30 action potentials to either NH₄Cl or CaCl₂ 
respond more to CaCl₂ than to NH₄Cl. The response to NaCl ranges from 65 
to 205 action potentials/5 s in the NaCl-best fibers. At these test concentra-
tions, some NaCl-best fibers respond as much to HCl as to NaCl, or even 
more. These fibers are considered NaCl-best because of their large response to 
NaCl, their projected (or known) response to a stronger concentration of NaCl, 
and their greater sensitivity to CaCl₂ than NH₄Cl (see Methods). In the more 
sensitive NaCl-best fibers, responses to the other electrolytes are also high. 
Responses to sucrose and D-phenylalanine are very small; four exceed 30 
action potentials in 5 s.

Mean responses to the single-component stimuli for the sucrose-best, HCl-
best, and NaCl-best fibers are plotted on a fourth set of axes in Fig. 2. The 
mean best-responses (specifically, the mean responses to the three stimuli 
being used as markers to classify fibers) are nearly equal for the three groups. 
However, response profiles for the three groups of fibers are strikingly different. 
Fig. 3 presents mean relative response profiles (in which mean responses to the 
other stimuli are expressed as percentages of the mean best-response) for the 
more and less sensitive halves of each group, divided on the basis of the size 
of the response to the best-stimulus. In general, the same pattern of respon-
siveness is exhibited by the halves of each group, but there are a few trends that are noteworthy. Responses to other stimuli tend to be slightly larger percentages of the best response in less sensitive units; this is true for the means of all five other stimuli in sucrose-best units. On the other hand, mean responses to the other three electrolytes are larger percentages of the response to NaCl in more sensitive NaCl-best units. These trends can also be seen in the response profiles of individual nerve fibers. The increase in relative effect of less effective stimuli is expected if "noise" remained constant and "signal" decreased in the less responsive units; however, the relatively greater effect of CaCl₂ and HCl in the more responsive NaCl-best units could not be the result of such a reduction in "signal".

The responses of single nerve fibers were recorded as the concentration of each single-component stimulus was varied over a limited range. The resulting stimulus-response functions for the mean responses calculated across the fibers of each group which had been so stimulated are presented in Fig. 4. Power functions were fitted to the data for each chemical with considerable effect for a given group of fibers. The exponents of the power functions (slopes of the straight lines fit to log stimulus concentration v. log mean response) are presented in Table I, with the correlation coefficients attesting to goodness of fit; all of the exponents are positive and less than +1.00.

Again, it is clear, as it was from the data on single concentrations of each stimulus, that the different groups of fibers exhibit different sensitivities, in
this case to three concentrations spanning a log unit of molarity of a chemical's dynamic range of effect. Sucrose and D-phenylalanine are very effective stimuli for the sucrose-best fibers; the electrolytes hardly stimulate them at all. Of the electrolytes, NH₄Cl is the most selective in its effect on the other

![Graph](image-url)

**Figure 4.** Mean responses of sucrose(S)-best, NaCl(N)-best, and HCl(H)-best nerve fibers of the hamster chorda tympani as a function of log concentration (millimoles per liter) of six chemicals. Lines connect points which represent the mean effect of each stimulus on one group of fibers. The response measure was the number of action potentials in the first 5 s of a response. Responses to electrolytes in N-best and H-best fibers, and responses to sucrose or D-phe in S-best fibers, are significantly greater (Wilcoxon test, \( P \leq 0.05 \)) at the highest concentration than at the lowest concentration and are monotonically related to concentration; these properties do not hold for responses to sucrose or D-phe in N-best or H-best fibers, or for responses to electrolytes in S-best fibers. Responses of N-best and H-best units to NH₄Cl and NaCl differ significantly (Wilcoxon test, \( P \leq 0.05 \)) in size.

Two groups of fibers in that the HCl-best are much more responsive to it than the NaCl-best fibers. NaCl is also quite selective in effect, affecting the NaCl-best considerably more than the HCl-best fibers. HCl alone could not be used to separate the two fiber groups unless it were presented at a concentration of 10 mM, at which it tends to stimulate HCl-best fibers more than NaCl-best fibers. Finally, CaCl₂ is the least discriminating of the salts, affecting both NaCl-best and HCl-best fibers nearly equally at the three concentrations tested.
Exponents of the power functions fit to the responses to HCl, CaCl₂, and NH₄Cl are similar for the NaCl-best and HCl-best groups of fibers, varying from 0.32 to 0.42, and these values are similar to the exponent of the power function fit to the responses of sucrose-best fibers to sucrose (i.e., 0.31). The exponent for the responses to NaCl is higher than the exponents for the other electrolytes in both the NaCl-best and HCl-best groups of fibers, and the exponent for D-phenylalanine is higher than that for sucrose in the sucrose-best group of fibers. However, the range of concentrations tested is closer to threshold for NaCl and D-phenylalanine than for the other chemicals. Many NaCl-best and HCl-best fibers do not respond to 3 mM NaCl, and many sucrose-best fibers do not respond to 7 mM D-phenylalanine. Therefore, differences in exponents presented here may be more characteristic of different portions of the stimulus-response function being represented than of the chemical itself.

### Effects of Two-component Mixtures on Single Nerve Fibers

Mean responses of sucrose-best, NaCl-best, and HCl-best fibers to the single-component stimuli and their mixtures are represented in Fig. 5. The responses were obtained during the second part of each recording session from twelve sucrose-best, seven NaCl-best, and nine HCl-best fibers.

Only sucrose-best fibers responded well to sucrose, D-phenylalanine, and their mixture, and these fibers responded relatively little to the electrolytes presented individually or mixed with other electrolytes. Mixtures of sucrose or D-phenylalanine with each electrolyte did stimulate sucrose-best fibers, but generally less so than either sucrose or D-phenylalanine alone, the one excep-
tion being the mixture of D-phenylalanine and CaCl₂. Therefore, one of these electrolytes, although itself rather ineffective as a stimulus for sucrose-best fibers, reduces the effectiveness of sucrose and D-phenylalanine as stimuli for these fibers when presented in a mixture.

**Figure 5.** Mean responses of sucrose(S)-best, NaCl(N)-best, and HCl(H)-best nerve fibers of the hamster chorda tympani to 0.1 M sucrose, 0.07 M D-phenylalanine, 0.05 M NH₄Cl, 0.003 M HCl, 0.01 M CaCl₂, and 0.01 M NaCl presented individually and in binary mixtures. Each triplet of bars represents (from left to right) the mean responses of sucrose-best, NaCl-best, and HCl-best fibers, respectively, to the stimulus indicated directly below and shows the average pattern of activity across the three unit groups for each stimulus. The dark vertical line atop each bar indicates plus and minus one standard error of the mean and shows how much that average might vary in random samples of the same number. The response measured was the number of action potentials in the first 5 s of a response.

The addition of sucrose or D-phenylalanine, comparably weak stimuli for NaCl-best and HCl-best fibers, does not have a dramatic effect on the response of these fibers to electrolytes. For example, the mean response of NaCl-best fibers to NaCl or HCl is but slightly changed in the presence of the two effective stimuli than D-phenylalanine alone. HCl reduces the stimulatory
nonelectrolytes. However, there is a dramatic reduction in the response of 
HCl-best fibers to HCl when mixed with D-phenylalanine; but this effect is 
probably the result of the chemical interaction in the mixture.

The four electrolytes and the six two-component mixtures of electrolytes all 
strongly stimulate NaCl-best and HCl-best fibers. It is not as easy to interpret 
the responses to these mixtures as it is to interpret responses to mixtures of 
stimuli which differ so much in effect. However, a hint of the different ways 
in which the two groups of electrolyte-sensitive nerve fibers treat mixtures is 
given by two examples in Fig. 5. Consider the stimuli NH₄Cl, NaCl, and their 
mixture. NH₄Cl and NaCl are the most selective of the electrolytes tested: 
NH₄Cl stimulates HCl-best fibers more than twice as much as NaCl-best 
fibers; and NaCl stimulates NaCl-best fibers more than twice as much as HCl-
best fibers. When these two salts are mixed, their effect on NaCl-best fibers is 
less than the effect of NaCl alone, but their effect on HCl-best fibers is more 
than the effect of NH₄Cl alone. Next, consider responses to NH₄Cl, HCl, and 
their mixture: the response of NaCl-best fibers to the mixture of NH₄Cl and 
HCl is less than their response to HCl alone; but the response of HCl-best 
fibers to this mixture is much larger than their response to HCl alone. These 
examples are a preview of more detailed analyses presented below, which 
show that responses of NaCl-best fibers to two-component mixtures approach 
a response predicted for a single-component “mixture” of one chemical, 
whereas responses of HCl-best fibers to mixtures approach the sum of the 
responses to the components presented individually.

Sucrose-best fibers: the effects of sucrose and D-phenylalanine are 
reduced by electrolytes Mean responses of sucrose-best fibers to the 
single-component stimuli and their mixtures are plotted in Fig. 6. 12 of the 15 
mixtures elicit mean responses smaller than the response to the more effective 
component of the mixture presented alone. One of the other three mixtures is 
sucrose mixed with D-phenylalanine; the response to this mixture is nearly 
equal to the response predicted from the stimulus-response function for sucrose 
for the sum of two concentrations of sucrose (100 mM and 110 mM) equal in 
intensity to the sucrose and D-phenylalanine stimuli. The sucrose-equivalence 
prediction (Sucr') is plotted to the right of the bar representing the actual 
response of the mixture.

All of the mixtures of sucrose and an electrolyte are less effective than 
sucrose alone. Electrolytes have little positive effect by themselves, and the 
effect of their presence in a mixture can be considered negative (i.e., a 
reduction in the effect of the stimulating chemical). The reduction in the 
response to sucrose varies from 46% with HCl to 12% with NaCl. The great 
majority of nerve fibers (see Fig. 1 for a sample) showed this reduction; 
specifically, 10 of the 12 sucrose-best fibers so stimulated showed a reduced 
response to sucrose with HCl (or NaCl) added. The effect of the electrolytes 
in reducing the response to sucrose is ordered HCl > NH₄Cl > CaCl₂ > NaCl, 
with HCl and NH₄Cl more severely affecting the response to sucrose than 
CaCl₂ or NaCl.

Three of the four mixtures of D-phenylalanine with electrolytes are less
effective stimuli than D-phenylalanine alone. HCl reduces the stimulatory effect of both sucrose and D-phenylalanine to about half. NH₄Cl reduces the mean response to D-phenylalanine only slightly in comparison with its effect on the response to sucrose. CaCl₂ does not reduce the effect of D-phenylalanine; indeed, the response to this mixture is slightly greater than predicted for sucrose-equivalence (Sucr').

The electrolyte mixtures are without major effect on sucrose-best fibers, as are the component electrolytes when presented alone.

In summary, the sucrose-best fibers exhibit three characteristics in response to mixtures of taste stimuli: (a) in the presence of a second chemical that is itself a very effective stimulus, they respond as if confronted with a higher concentration of the first such stimulus; (b) the effectiveness of a stimulus is generally reduced by the presence of an electrolyte that itself is not a very effective stimulus; and (c) the effect of an electrolyte on responses to two different very effective stimuli can be similar but is not necessarily so. Statistical support for these descriptions of sucrose-best fibers' responses to mixtures is found in Table II.

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**Figure 6.** Mean responses of sucrose(S)-best nerve fibers of the hamster chorda tympani to 0.1 M sucrose, 0.07 M D-phenylalanine, 0.01 M CaCl₂, 0.01 M NaCl, 0.05 M NH₄Cl, and 0.003 M HCl; the binary mixtures of these six stimuli; and the response predicted for a “mixture” of two appropriate concentrations of sucrose (Sucr'), if the response to a mixture was greater than the response to the more effective component. Responses are represented by crosshatched bars and predictions by gray bars; the former are numbers of action potentials in the first 5 s of a response, and the latter were obtained from a power function fit to the data presented in Fig. 4.
NaCl-best fibers: addition of a second electrolyte has an effect which nearly equals the effect of increasing the NaCl concentration. Mean responses of NaCl-best fibers to the single-component stimuli and their mixtures are represented in Fig. 7. 12 of the 15 mixtures elicit a response either smaller than the response to the more effective component presented alone or smaller than the response predicted from the NaCl stimulus-response function for the sum of two concentrations of NaCl equal in intensity to the components in the mixture.

NH₄Cl mixed with each of the other electrolytes elicits a mean response slightly smaller than that elicited by the other electrolyte presented alone; in contrast, the mean responses to NH₄Cl mixed with either sucrose or D-phenylalanine (i.e., two relatively ineffective stimuli for these fibers) are approximately equal to the sum of the responses to the components. NH₄Cl is the least effective of the electrolytes in stimulating NaCl-best fibers, and only for mixtures containing NH₄Cl were small reductions in the effect of the electrolytes or increases in the effect of nonelectrolyte stimuli observed.

The other three electrolyte mixtures (NaCl and HCl, CaCl₂ and HCl, and NaCl and CaCl₂) elicit mean responses somewhat greater than that elicited by the more effective component presented alone but a little less than the response predicted from the stimulus-response function for the sum of the two concentrations of NaCl equivalent in intensity to the components of the mixture.

Mean responses to mixtures of HCl or NaCl with sucrose or D-phenylalanine, or of CaCl₂ with D-phenylalanine were slightly smaller than the response to the electrolyte component presented alone. The addition of D-phenylalanine to HCl produced a larger reduction in the response of NaCl-best fibers to an...
electrolyte, but this effect most probably results from the chemical interaction in the mixture. The nonelectrolyte mixture of sucrose and D-phenylalanine had a slightly greater mean effect on NaCl-best fibers than did either of the components presented alone.

In summary, the response of NaCl-best fibers to mixtures is generally characterized by being somewhat smaller than or indistinguishable from the response to a single-component “mixture” of NaCl; none of the mixtures had an effect very different from the effect an appropriate increase in NaCl would have produced.

HCl-best FIBERS: THE EFFECTS OF ELECTROLYTES IN MIXTURES ARE NEARLY EQUAL TO THEIR INDIVIDUAL EFFECTS Mean responses of HCl-best fibers to the single-component stimuli and their mixtures are represented in Fig. 8. 13 of the 15 mixtures elicit responses that exceed the response predicted from the stimulus-response function for a concentration of HCl equal to the sum of two concentrations equivalent in intensity to the components of the mixture. None of the mixtures of electrolytes elicit a mean response from these fibers less
than that elicited by the more effective component. Indeed, the effect of each is greater than that of a single-component "mixture" of HCl and approaches to various degrees the sum of the effects of the components presented individually. For example, the mean response to the mixture of HCl and NH₄Cl is 92% of the sum of the responses to the components and 39% greater than the response predicted for a single-component "mixture" of equivalent concentra-

![Figure 8](image-url)

**Figure 8.** Mean responses of HCl(H)-best nerve fibers of the hamster chorda tympani to 0.003 M HCl, 0.05 M NH₄Cl, 0.01 M CaCl₂, 0.01 M NaCl, 0.1 M sucrose, and 0.07 M D-phenylalanine; the binary mixtures of these six stimuli; the response predicted for a "mixture" of two appropriate concentrations of HCl (HCl'), if the response to a mixture was greater than the response to the more effective component; and the sum of the responses to the components (Sum), if the response to the mixture was greater than that sum. Crosshatched bars represent obtained responses, dotted bars, the summed component responses, and gray bars, the predicted responses; the former two are numbers of action potentials in the first 5 s of a response; the latter were obtained from a power function fit to the data presented in Fig. 4.

The mean response to the mixture of NH₄Cl and CaCl₂ is nearly equal to the response predicted for the sum of equivalent concentrations of HCl (i.e., 1.5 mM and 0.8 mM HCl). The mixture of NH₄Cl and CaCl₂ is the only electrolyte mixture whose effect closely approaches the response predicted for a single-component "mixture" of HCl. The mean effect of the HCl and NaCl mixture approaches the sum of the responses to the components more closely than the response to a
"mixture" of two HCl concentrations; but the effects of the HCl and CaCl₂, the NaCl and NH₄Cl, and the NaCl and CaCl₂ mixtures fall about halfway between these two limits.

The mixture of sucrose and D-phenylalanine is as weak a stimulus for HCl-best fibers as either of its components. Mean responses to seven of the eight mixtures of sucrose and D-phenylalanine with an electrolyte exceed the response predicted for a single-component "mixture" of HCl and are nearly equal to the sum of the responses to the components, but these limits are similar due to small responses to nonelectrolytes. The one exception is the mixture of HCl and D-phenylalanine, which is about 75% as effective as HCl presented alone, which can be reasonably explained by the chemical interaction of the components in the mixture.

In summary, responses of HCl-best fibers to mixtures are generally characterized by their surpassing the responses to a single-component "mixture" of HCl and being nearly equal to the sum of responses to the components presented individually. In particular, mixtures of HCl and NaCl with each other or of either with NH₄Cl or CaCl₂ have effects which considerably exceed the effect of a concentration of HCl equal to the sum of two concentrations equivalent in intensity to the components in the mixture. On the other hand, one mixture of electrolytes (i.e., NH₄Cl and CaCl₂) elicits a response equal to that of a single-component "mixture" of HCl. As seen in Table II, the mixture of NH₄Cl and CaCl₂ is the only two-component mixture of electrolytes tested that elicits a response in HCl-best fibers which is statistically significantly smaller than the sum of the component responses.

DISCUSSION

Single-component Stimuli

Classification of fibers Clearly, responsiveness differs for the three groups of nerve fibers, but response profiles also vary within each group of fibers. Some fibers are less responsive than others to the stimulus that elicits the largest response; sizes of responses to stimuli other than the most effective vary from fiber to fiber. This variation is comparable to that for fibers previously sampled from peripheral taste nerves (Fishman, 1957; Ogawa et al., 1968; Frank, 1973). Several recent observations argue for attributing such within-group variation to changes in responsiveness of individual fibers rather than to systematic variations in sensitivity from fiber to fiber (see Introduction).

Diversity in response profile is greatest for the NaCl-best group of fibers. In fact, responses to the less effective electrolytes tend to be larger percentages of the best response in the more sensitive of these fibers (Fig. 3). This result is opposite to that for the sucrose-best or HCl-best fibers and contradicts the notion of a proportional loss in sensitivity and specificity with deterioration of a preparation. However, Kruger and Boudreau (1972) found that after repeated stimulation some cat geniculate ganglion units (type I) decreased in sensitivity to H⁺ and NaCl, whereas other units (type II) increased in sensitivity to both NaCl and H⁺. If a change in sensitivity occurred in NaCl-
best fibers of the hamster chorda tympani similar to that which occurred in
the type II units, the result could be as reported here, i.e., the most sensitive
NaCl-best fibers would be the least selective.

Moreover, the diversity of the NaCl-best group of fibers is exaggerated by
the use of a weak NaCl stimulus. The NaCl stimulus used here, although only
one-half log step lower in concentration than that used by Frank (1973), elicits
a more specific response than the stronger concentration of NaCl. Sucrose-
best fibers respond hardly, if at all, to 0.01 M NaCl and HCl-best fibers
respond only weakly to it; in contrast, 0.03 M NaCl affects sucrose-best fibers
somewhat and is half as effective as HCl for the HCl-best fibers (Frank, 1973).
The effect on response profiles is to accentuate the specificity of HCl-best
fibers while broadening the profiles of NaCl-best fibers. The effect on the
correlations (see below) between response to pairs of stimuli is to sharpen the
apparent distinctiveness of the three sensitivities. A weaker NaCl stimulus also
affects the proportion of fibers sampled that are classified as being most
sensitive to NaCl because it is apparently very close to threshold. Only a third
of the fibers sampled here responded best to NaCl, in comparison with more
than half in a previous sample (Frank, 1973).

CORRELATIONS BETWEEN RESPONSES TO PAIRS OF STIMULI

The differential
sensitivity of hamster chorda tympani nerve fibers, evident in the response
profiles of individual fibers (fig. 2), is reflected in the correlation coefficients
between responses to pairs of stimuli calculated across all fibers. Correlation
coefficients were instituted as measures of the similarity of effects of two
stimuli by Erickson (1963). The correlations indicated by the coefficients in
Table III are consistent with the existence of fibers with three distinct sets of
sensitivities in the hamster chorda tympani. One group of fibers is sensitive to
sucrose and D-phenylalanine: responses to these stimuli are highly correlated
(i.e., \( r = +0.95 \)), indicating covariation in absolute sensitivities; strong re-
sponses to these stimuli and electrolytes do not occur in the same fibers, which
is reflected by coefficients of ca. \(-0.5\). At least two different sets of fibers must
be sensitive to the electrolytes because the correlations between responses to
some electrolyte pairs are quite low. The correlations between responses to
NaCl and the other electrolytes are much lower (i.e., \(+0.24\) to \(+0.55\)) than those
for HCl, NH4Cl, and CaCl2 (i.e., \(+0.81\) to \(+0.92\)). The sensitivities to HCl,
NH4Cl, and CaCl2 are distributed similarly among hamster chorda tympani
nerve fibers; in contrast, many fibers that respond to NaCl do not respond to
other electrolytes, other fibers respond to both NaCl and other electrolytes,
and many fibers that respond the most to the other electrolytes respond only
weakly to NaCl. However, correlations between responses to NaCl and CaCl2
or to NaCl and NH4Cl, much lower than those for HCl and these two salts,
do not indicate that all NaCl-best fibers are more specific in their sensitivities
than HCl-best fibers. Rather, the NaCl stimulus is more specific in its effects
than the other electrolytes, affecting predominantly NaCl-best and not HCl-
best fibers.

The correlation coefficients between responses to pairs of stimuli are com-
parable to those reported earlier for hamster chorda tympani nerve fibers for
sucrose, NaCl, and HCl by Ogawa et al. (1968) and Frank (1973). In the
earlier reports, the same response measure was used, but some of the stimuli were tested at different concentrations. Yet, coefficients between responses to sucrose and electrolytes were negative, and coefficients between responses to electrolytes were positive, as they are here. The exact value of the correlation coefficient depends on the relative sensitivities of the fibers in the population to the test stimuli, and because changes in concentration affect the ratios of sensitivities of the various fibers, they consequently affect the coefficients generated.

**RESPONSES PREDICTED TO SINGLE-COMPONENT “MIXTURES”** The power functions, describing response as a function of stimulus concentration (see Results), serve perfectly well for predicting the responses to single-component “mixtures” providing the responses to the mixture components are within the range of responses used to develop the fitted function. Responses to the components of the electrolyte mixtures are within this range with one exception. The mean response of HCl-best fibers to NaCl (i.e., 30 action potentials/5 s) is below the range elicited in these fibers by HCl (i.e., 61-149 action potentials/5 s). If the exponent of the power function for HCl is higher at concentrations lower than those tested, this small response might require a higher equivalent concentration of HCl than that estimated by extending the power function to the lower concentration. It is possible that the exponent in the range of concentrations of HCl which encircles the equivalent concentration of NaCl is 0.82 (as it is in these fibers for the NaCl stimulus-response function) instead of 0.39 (i.e., the exponent of the function fit to the range of HCl concentrations tested). Yet, even if such an extreme change in slope did occur, only relatively small changes in the predicted responses would result. In this case, the predicted response of HCl-best fibers to the mixture of NaCl and CaCl₂ would be most severely affected and would be increased by only 10%.

To predict the responses to single-component “mixtures,” the power function fit to the stimulus-responses function of NaCl was used for NaCl-best fibers, HCl for HCl-best fibers, and sucrose for sucrose-best fibers. These functions were selected because: (a) the stimuli are the most effective for the respective fiber groups; (b) the range of responses elicited is appropriate (see above); (c) the goodness of fit of the functions are among the best.
Two-component Mixtures

Suppression of responses of sucrose-best fibers by electrolytes. The suppressive effects of electrolytes on the responses of sucrose-best fibers to sucrose are not correlated with concentration, activity, ionic strength, or the magnitude of the response to the electrolyte in these fibers. But the order of effectiveness of the electrolytes in reducing the response of sucrose-best fibers to sucrose in a mixture is the same as the order of their effectiveness in stimulating HCl-best fibers. Possible inhibitory interconnections between sucrose-best and HCl-best fibers and/or the cells they innervate could explain this. However, sucrose and D-phenylalanine are equally good stimuli for sucrose-best fibers; the response they elicit should then be suppressed to the same degree by each electrolyte. The suppression of the response to D-phenylalanine by HCl is as dramatic as that for sucrose. But NH₄Cl, which strongly suppresses the response to sucrose, does not suppress the response to D-phenylalanine very much. Similarly, the effects of CaCl₂ on the responses to sucrose and D-phenylalanine do not appear to be equivalent. The sensitivity of HCl-best fibers to these electrolyte stimuli does not predict their effect on a response to D-phenylalanine. Thus, their suppressive effects in mixtures cannot be due to inhibition of sucrose-best fibers by HCl-best fibers or the taste cells they innervate. The possibility of interaction between the taste cells innervated by HCl-best fibers and the cells sensitive to sucrose cannot be excluded, although these sucrose-sensitive cells could not be the cells which are sensitive to D-phenylalanine.

The electrolytes could interact chemically with, but not stimulate, receptors on the taste cells innervated by sucrose-best fibers. CaCl₂ and NH₄Cl might each affect the receptor(s) so as to reduce the effectiveness of the receptor's interaction with sucrose more than that with D-phenylalanine. In contrast, HCl could interfere with the receptor(s) interaction with sucrose and D-phenylalanine to the same degree. However, it is impossible to conclude from these data whether the receptors for sucrose and D-phenylalanine are the same. It is unlikely that there are distinct sucrose-sensitive and D-phenylalanine-sensitive taste cells because the response to the mixture of these stimuli very closely approximates the response to the sum of two equivalent concentrations of sucrose. If there were two types of taste cells innervated by each sucrose-best fiber, it would be necessary to postulate an inhibitory interaction for the response to the mixture to be so much less than the sum of the responses to the components presented individually. A more parsimonious explanation for the data is two receptors on the same taste cell or one receptor for both sucrose and D-phenylalanine, whose interaction with the two stimuli can be affected differentially.

The acid-base interaction of D-phenylalanine and HCl when mixed is too small to account for the suppression of the response to D-phenylalanine. The HCl in the mixture cannot reduce below 0.067 M the concentration of D-phenylalanine available for interaction with the taste receptors. The value predicted from the stimulus-response function for the response of sucrose-best fibers to 0.067 M D-phenylalanine is 97% of the response to D-phenylalanine.
at the 0.07 M test concentration; in contrast, the mixture of HCl and D-phenylalanine elicits a mean response that is 51\% of the response to 0.07 M D-phenylalanine. However, because of the acid-base interaction, the pH of the D-phenylalanine and HCl mixture is higher than the pH of the sucrose and HCl mixture; since the suppressive effect of HCl on responses to sucrose and D-phenylalanine is the same, it cannot be attributed to the pH of the solution. An effect of hydrogen ions on the receptors of the taste cells innervated by the sucrose-best fibers might explain the suppression if the receptors are very strong bases capable of attracting hydrogen ions equally well from the two mixture solutions.

**Differential effects on NaCl-best and HCl-best fibers** When an electrolyte is mixed with another in solution, its effective concentration is reduced by the presence of the other component. Activity is a measure of effective concentration and depends upon ionic strength and charge as well as concentration. The slight suppression observed in responses of NaCl-best fibers to electrolytes in mixtures could be partly a result of reductions in activity. The order of mean percent suppression (measured relative to the predicted response to a single-component “mixture” of two concentrations of NaCl equivalent in intensity to the components of the mixture) is CaCl₂ and NH₄Cl (31\%) > NaCl and NH₄Cl (24\%) > HCl and NH₄Cl (20\%) > NaCl and HCl (17\%) > CaCl₂ and HCl (9\%) > NaCl and CaCl₂ (1%). The greatest percent suppression occurs with the CaCl₂-NH₄Cl pair and corresponds to the greatest percent reduction in activity (i.e., 19\%) of all the electrolytes in mixtures. Thus, 0.05 M NH₄Cl, a relatively ineffective stimulus for NaCl-best fibers, may effect a slight suppression by reducing the activity of the other component (e.g., NaCl, CaCl₂, or HCl) in a mixture. However, the small effects of the other electrolytes in mixtures on these fibers are not attributable to changes in activity; nor do they parallel changes in the ionic strength of the solution or the concentration of chloride ions present. The pH change when HCl is added may alter the effectiveness of stimuli but it cannot explain all effects.

The order of the small suppressive effects of the electrolytes in mixtures on NaCl-best fibers does not follow the order of their effectiveness as stimuli for either sucrose-best or HCl-best fibers. Therefore, interfiber interactions need not be considered. Indeed, the characteristic effect of electrolytes in mixtures on the responses of NaCl-best fibers may be due to several variables operating simultaneously, such as chemical effects of the electrolytes on each other (e.g., activity), on receptor processes, or, possibly, on the electrogenic abilities of the taste cell.

In contrast to their effects on NaCl-best fibers, the electrolytes do not...
suppress the responses of HCl-best fibers. Mean responses of HCl-best fibers to mixtures of electrolytes are either equal to or greater than the response predicted in terms of equivalent concentrations of HCl. The order of measured increases (i.e., the percentage greater than the response predicted in terms of HCl equivalence) is HCl and NH₄Cl (39%) > HCl and CaCl₂ (23%) > HCl and NaCl (21%) > NaCl and CaCl₂ (20%) > NaCl and NH₄Cl (17%) > NH₄Cl and CaCl₂ (1%). This order does not follow reductions in activity upon mixing or chloride ion concentration. Beidler (1975) has noted that an acid may have increased effectiveness when the ionic strength of the solution is increased. The responses of HCl-best fibers follow this rule for HCl mixed with each of the salts; specifically, the addition of 0.05 M NH₄Cl to HCl increases the ionic strength and the response more than the addition of 0.02 M CaCl₂ or 0.01 M NaCl.

The degree of augmentation of the responses to mixtures above the values predicted for equivalent concentrations of HCl does not parallel the effects of the electrolytes as stimuli for sucrose-best or NaCl-best fibers or the suppressive effects of electrolytes on either of these groups of fibers. Therefore, the observed effects on HCl-best fibers could not be mediated via interneural interactions.

Typically, the response of HCl-best fibers to mixtures of electrolytes exceeds the response predicted for a single-component “mixture.” The one exception is the response to NH₄Cl mixed with CaCl₂, which equals that predicted value. The notion of distinct and independent receptors for HCl, NH₄Cl, and NaCl (with NH₄Cl and CaCl₂ stimulating the same receptor), although attractive, cannot explain all of the results. The NaCl and NH₄Cl, HCl and CaCl₂, and NaCl and CaCl₂ mixtures do not show simple addition of their individual effects as this notion would predict; indeed, the response to each of these mixtures is between the value expected for independent receptors (i.e., the sum of the responses to the components) and the response predicted for a common receptor for the chemicals in the mixture (i.e., the response to a single-component “mixture” of two concentrations of one chemical). Another possibility is an enhancement of the effect of one electrolyte by the presence of another in the mixture produced by processes similar to those suggested to yield suppression of responses of NaCl-best fibers.

Fig. 9 graphically portrays differences in the mean effects of electrolytes in mixtures on the HCl-best and NaCl-best fibers of the hamster chorda tympani, showing HCl-best fibers diverging from predictions for “mixtures” of one chemical (i.e., HCl equivalence) in the positive direction (toward the sum of the responses to the components) but responses of the NaCl-best fibers diverging from the predicted value (i.e., NaCl equivalence) in the negative direction (with the components of the mixtures apparently reducing each other’s stimulatory effects).

**Explanation of the Whole-Nerve Responses to Mixtures**

**Mixtures containing sucrose and/or D-phenylalanine** Sucrose and D-phenylalanine strongly stimulate the same group of fibers; the electrolytes stimulate those fibers minimally but strongly stimulate different fibers that, in turn, are stimulated minimally by sucrose and D-phenylalanine. On the basis
of this small overlap in sensitivities to single-component stimuli, one would expect mixtures of sucrose or D-phenylalanine with an electrolyte to elicit whole-nerve responses that approach the sum of responses to the components. In fact, they are much smaller, with responses to mixtures of sucrose and the three salts falling further below this sum than responses of D-phenylalanine and the salts (Hyman and Frank, 1980). The greater suppression of responses to sucrose than responses to D-phenylalanine in sucrose-best fibers by the salts is the explanation.

Although HCl equally reduces the effectiveness of sucrose and D-phenylalanine as stimuli for sucrose-best fibers, sucrose and D-phenylalanine do not equally reduce the effectiveness of HCl as a stimulus for NaCl-best or HCl-best fibers. D-Phenylalanine especially reduces the responses of HCl-best fibers to HCl, whereas sucrose produces little change. This reduction in response magnitude is predictable from the acid-base interaction of D-phenylalanine and HCl. In the whole nerve, the reduction in response of sucrose-best fibers to D-phenylalanine by HCl adds to the reduction in effectiveness of HCl as a stimulus for HCl-best and NaCl-best fibers by D-phenylalanine. The result is a summed response to the mixture of HCl and D-phenylalanine that is less than the summed response to the mixture of HCl and sucrose.

The addition of 0.07 M D-phenylalanine to 0.003 M HCl considerably reduces the concentration of hydrogen ion available to stimulate the taste receptors, increasing the pH to 3.6. This concentration of hydrogen ion, if provided by a strong acid such as HCl, would hardly stimulate the NaCl-best and HCl-best fibers (cf. Hyman and Frank, 1980). However, their responses

![Figure 9](image-url)
are reduced by 15–20%, not 50–60%, as predicted on the basis of pH. Therefore, the free hydrogen ion concentration, although important, is not the sole determinant of response size in these fibers. Equal sourness measurements (Taylor et al., 1930) and whole-nerve recordings from the rat (Beidler, 1967) are in complete agreement with the finding that solutions of equal pH do not elicit responses of equal magnitude (Beidler, 1975).

Knowledge of the whole-nerve response to the stimuli presented individually and the overlap in the fiber groups stimulated by the mixture components permitted successful prediction of the whole-nerve response to just one mixture tested here: sucrose and D-phenylalanine. There is complete overlap of the sets of responding nerve fibers and the response of the whole nerve to their mixture was indistinguishable from the response predicted for a “mixture” of two concentrations of one stimulus (Hyman and Frank, 1980).

Mixtures of Electrolytes

Whole-nerve responses to mixtures of electrolytes (Hyman and Frank, 1980) can be explained by the suppressive effects of the electrolytes on the responses of NaCl-best fibers summed with their augmentative effects on the responses of HCl-best fibers; these opposite effects neutralize and yield a response indistinguishable from that predicted for a single-component “mixture.” Neutralization is not complete when the opposite effects are of different magnitudes. For example, the mean response of NaCl-best fibers is suppressed the most by the CaCl2 and NH4Cl mixture, but the effects of these chemicals presented together are not augmented in terms of the responses of HCl-best fibers. Without an augmented effect to compensate for any suppressive effect on the activity of NaCl-best fibers, the whole-nerve response shows considerable suppression; on the other hand, the mixture of NaCl and CaCl2 elicits a mean response in HCl-best fibers greater than that predicted by HCl equivalence, but produces no suppression in NaCl-best fibers; the augmented effects of the stimuli are not compensated for by opposing suppressive effects, allowing the balance to tilt in the additive direction, in the response summed across all fibers.

Comparison of Neural Recordings and Psychophysical Measurements

Although the neural records are from the chorda tympani of hamsters, comparison with taste intensity assessments by humans is not inappropriate. Recordings of summed responses of the human chorda tympani indicate an agreement between neurophysiological and psychophysical measures of thresholds for some stimuli and the relative effectiveness of various sugars (Diamant et al., 1963). Also, the taste of many single-component stimuli seems to be similar for the two species. Magnitude estimates by humans of the sweetness, saltiness, sourness, or bitterness of a large number of stimuli (McBurney and Shick, 1971) correspond well to the extent to which aversions learned to these chemicals are generalized to sucrose, NaCl, HCl, or quinine-HCl by hamsters (Nowlis and Frank, 1977); these four chemicals taste sweet, salty, sour, and bitter, respectively, to humans. Therefore, humans and hamsters similarly characterize the tastes of some stimuli. However, measurements of an animal’s generalizations of gustatory stimuli and psychophysical measurements in humans could be influenced by more than the activity of
the chorda tympani. There are other gustatory inputs (e.g., those transmitted via the glossopharyngeal nerve), nongustatory inputs (transmitted via the trigeminal nerve), and complex processes mediated centrally in the nervous system. Therefore, agreement between recordings from the chorda tympani and psychophysical assessments of taste intensity would indicate that this one gustatory input is not altered by other sensory inputs and that peripheral processes are preserved in the course of transmission to central structures.

Suppression of the sweetness of sucrose in humans by moderate intensities of NaCl or citric acid (see Introduction) corresponds to a suppression of the response to sucrose by salts and HCl in sucrose-best fibers of the hamster chorda tympani. For both perceived intensity and chorda tympani activity, the suppressive effect of acid on the response to sucrose is much more dramatic than the effect of NaCl. Sucrose-best fibers are the only ones that respond vigorously to the two chemicals which taste sweet to humans (i.e., sucrose and L-phenylalanine). These data suggest that sucrose-best fibers mediate the sensation of sweetness in hamsters. Moreover, making the analogy across species, the suppression of sweetness by acid or salt in humans may simply be a reduction in the peripheral effect of sucrose on the taste receptors when acid or NaCl is present.

The perceived intensity of saltiness evoked by NaCl in humans is reduced by moderate concentrations of sucrose or acid (see Introduction). Sucrose and L-phenylalanine slightly reduce the mean response of NaCl-best fibers of the hamster chorda tympani nerve to NaCl; however, they do not reduce the response of HCl-best fibers to NaCl. These data suggest that NaCl-best fibers mediate the sensation of saltiness in hamsters, and by correspondence, that this peripheral process is the mechanism of the suppression observed in humans. However, there is no reduction in the response of NaCl-best fibers to NaCl with the addition of HCl; in fact, HCl is a very effective stimulus for these fibers. But the psychophysical results in humans refer to citric acid suppressing saltiness, and the effects of this weaker acid on NaCl-best fibers may be different from the effects of HCl.

The suppression of sourness of an acid mixed with sucrose or NaCl (see Introduction) does not have a parallel in the responses of the hamster chorda tympani. The response of HCl-best fibers is increased very slightly when sucrose is added to HCl, as it is when NaCl is added. The neural activity evoked in NaCl-best fibers by HCl is slightly less with sucrose added, but NaCl increases the activity. However, many of the studies of the intensity of sourness in humans used citric acid, not HCl, and sensations evoked by citric acid and HCl may be affected differently. For example, the intensity of the sensation evoked by citric acid is enhanced after a human taste papilla is pretreated with sucrose (Kuznicki and McCutcheon, 1979), but the perceived intensity of HCl is not.

Conclusions
The effects of taste stimuli in mixtures on responses of the hamster chorda tympani are complex and require, at this stage, a stimulation of the taste receptors with the mixtures themselves for their appreciation. The effects are
unpredictable because one chemical can affect the response of an afferent fiber to other stimuli; this cannot be observed by extracellular recording of responses to individual stimuli. However, fibers of one class (sucrose-best, NaCl-best, or HCl-best) tend to react in the same way to mixtures of chemicals of a given variety; fibers in different classes can react in an opposite way. These observed effects of mixtures on responses of afferent nerve fibers indicate that receptors innervated by distinct fiber populations process taste stimuli in mixtures differently, demonstrate that the sum of the several treatments of mixtures can account for the whole-nerve response to them, suggest that a quantitative study of effects of mixtures could lead to an understanding of the physical and chemical variables that affect different mechanisms of taste receptivity, and indicate that some effects of mixing on intensities of elicited taste qualities documented by studies of human taste psychophysics may be attributable to peripheral effects at the receptors.

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