

Effects of Binary Taste Stimuli on the Neural Activity of the Hamster Chorda Tympani

ANGELA M. HYMAN and MARION E. FRANK

From The Rockefeller University, New York, New York 10021

ABSTRACT Binary mixtures of taste stimuli were applied to the tongue of the hamster and the reaction of the whole chorda tympani was recorded. Some of the chemicals that were paired in mixtures (HCl, NH₄Cl, NaCl, CaCl₂, sucrose, and D-phenylalanine) have similar tastes to human and/or hamster, and/or common stimulatory effects on individual fibers of the hamster chorda tympani; other pairs of these chemicals have dissimilar tastes and/or distinct neural stimulatory effects. The molarity of each chemical with approximately the same effect on the activity of the nerve as 0.01 M NaCl was selected, and an established relation between stimulus concentration and response allowed estimation of the effect of a "mixture" of two concentrations of one chemical. Each mixture elicited a response that was smaller than the sum of the responses to its components. However, responses to some mixtures approached this sum, and responses to other mixtures closely approached the response to a "mixture" of two concentrations of one chemical. Responses of the former variety were generated by mixtures of an electrolyte and a nonelectrolyte and the latter by mixtures of two electrolytes or two nonelectrolytes. But, beyond the distinction between electrolytes and nonelectrolytes, the whole-nerve response to a mixture could not be predicted from the known neural or psychophysical effects of its components.

INTRODUCTION

Mammalian gustatory systems are simultaneously stimulated by several chemicals in natural feeding. However, neurophysiological and psychophysical investigations have principally considered the effects of individual chemicals. Knowledge of the gustatory effects of pure chemicals is a necessary beginning in an understanding of mechanisms of taste. There have been descriptions of the effects of single-chemical stimuli on multi-unit and single-unit neural activity at several levels of the nervous system in a number of species of mammals, as well as descriptions of the sensations they evoke in animals and humans. From studies of human taste, psychophysicists have begun an attempt to predict the sensations evoked by mixtures of chemicals whose individual effects are known. The results indicate the existence of complexities. Specifically, enhancement or suppression of taste sensations often result upon mixing of stimuli. Each of these psychophysical complexities could be the result of multifarious influences of the central nervous system or the result of peripheral

processes transmitted centrally by sensory neurons. The present study is an initial attempt to assess the role of peripheral sensory processes on the effects of combinations of stimuli with known individual effects.

In mammals, the chorda tympani provides sensory innervation to taste receptor cells grouped in taste buds in fungiform papillae on the anterior two-thirds of the tongue. The neurons whose peripheral processes travel in this nerve have been studied electrophysiologically more extensively than any other part of the gustatory system. Numerous investigators have recorded the summed neural activity of the hundreds of fibers in this nerve. These whole-nerve recordings in mammals have been used to obtain information about a variety of aspects of the peripheral gustatory apparatus: temporal coupling of the response with differing parameters of stimulation of the taste receptors (Smith and Bealer, 1975; Marowitz and Halpern, 1977); the overall effect of different chemicals on taste receptors of various species (Beidler et al., 1955; Zotterman, 1956; Hagstrom and Pfaffmann, 1959); mechanisms of taste reception (Beidler, 1954; Jakinovich, 1976; Mooser, 1976); effects of dietary manipulations (Nachman and Pfaffmann, 1963) or crossed neural innervation (Oakley, 1967) on receptor sensitivity; and, as is reported here, the manner in which gustatory receptors and their peripheral neural associates deal with complex stimuli (Beidler, 1961; Tateda and Hidaka, 1966; Sato et al., 1971). The whole-nerve response is a conglomerate of the responses of neurons with widely varying sensitivities (Pfaffmann, 1955; Ogawa et al., 1968; Frank, 1973) and must be recognized as such in the interpretation of recordings. In fact, it can most simply be related to the overall activity of all receptors and thus the overall intensity of evoked taste sensation, that is, the sum of all taste qualities evoked by any stimulus.

Neurophysiological evidence for the effects of taste stimuli in mixtures in mammals is meager; it typically has been obtained for particular purposes and has been in the form of peripheral whole-nerve recordings. Three types of effects have been observed in whole-nerve recordings from the rat chorda tympani: (a) a response to a mixture of two stimuli can equal the response to a "mixture" of two concentrations of one of the components (e.g., 0.05 M LiCl and 0.05 M NaCl produce equal responses when presented individually and elicit a response when mixed that equals the response to 0.1 M NaCl or 0.1 M LiCl [Beidler, 1953]); (b) a response to a mixture of two stimuli can be smaller than the response to one of the stimuli presented alone (e.g., 0.1 M potassium benzoate, which elicits a small response, and 0.1 M NaCl, which elicits a large response, together elicit a response less than two-thirds the size of the response to 0.1 M NaCl alone [Miller, 1971]); and (c) a response to a mixture of two stimuli can be greater than the sum of the responses to the components presented individually (e.g., 0.0001 M inosine 5'-monophosphate (disodium salt) and 0.3 M monosodium glutamate together elicit a response twice as large as the sum of the responses to the components presented individually [Sato et al., 1971]). The first type of response to a mixture has been taken to indicate that the two chemicals are interchangeable in affecting the receptors so that *summation* of stimulus intensities occurs when the gustatory system is confronted with the mixture. The second type of response to a

mixture has been taken to indicate inhibitory effects or *suppression* of the effective stimulus intensities in the mixture. The third type of response to a mixture has been taken to indicate potentiation or *enhancement* of the stimulatory effectiveness of the chemicals in the mixture. Finally, although no such finding has been reported with the whole-nerve recordings, a response to a mixture that equals the sum of the responses to the components could be taken as indicating noninteraction or *independence* of the effects of the stimuli in the mixture. Independence indicates that the magnitudes of sensations or responses elicited by stimuli are summed when the gustatory system is confronted with a mixture. Independence and summation cannot be differentiated if the response to a chemical increases by the same factor as its concentration; in this case, the response to the sum of two concentrations of one stimulus will equal the sum of the responses to the two stimuli presented individually.

The choice of the hamster chorda tympani for an investigation into the peripheral effects of taste stimuli in mixtures was based on several factors. Some recordings have been made from presumed mammalian taste receptor cells, those structures derived from epithelial tissue and likely to be responsible for the receipt and transduction of chemical stimuli in the sense of taste (Kimura and Beidler, 1961; Ozeki and Sato, 1972; Sato and Ozeki, 1972). However, this receptor cell electrophysiology is not sufficiently developed and, to date, recording the electrophysiological responses of afferent nerve fibers or of the cell bodies of these fibers in the geniculate ganglion of larger mammals is the most reliable and efficient method for measuring the output of the taste receptor systems. Moreover, it is the output of the afferent neurons that is transmitted centrally and processed into sensations.

Extensive recordings from the whole nerve (e.g., Beidler et al., 1955; Noma et al., 1974) and single nerve fibers (e.g., Fishman, 1957; Ogawa et al., 1968; Frank, 1973) have characterized the sensitivities of the hamster chorda tympani to a large number of pure chemicals in aqueous solution. Furthermore, extensive behavioral testing of reactions to tastant solutions, both preference (Carpenter, 1956; Faull and Halpern, 1971) and generalizations of conditioned aversions (Nowlis and Frank, 1977), have characterized the taste sensations of the hamster better than any species other than man. With such knowledge of peripheral neural and behavioral discrimination of individual tastants, pairs of chemicals can be chosen that have either similar or very different neural and behavioral effects. It can then be determined if this knowledge of individual effects of taste stimuli is a predictor of their effects in mixtures. Neural responses elicited by binary mixtures of these different types of tastants should be informative about the ways in which the mammalian peripheral gustatory apparatus deals with complex stimuli.

METHODS

Preparation

All subjects were adult male golden hamsters (125–155 g) deeply anesthetized by intraperitoneal injection of sodium pentobarbital (initial dose of 7.5 mg/100 g and

25% of that when necessary). The animal's body temperature was monitored via a rectal probe and maintained at 36–37°C. The trachea was cannulated. The animal's head was clamped into an appropriate position, and the head clamp served to electrically ground the animal. The right chorda tympani was dissected free of surrounding tissue from its exit from the tympanic bulla to its juncture with the lingual nerve. In no case was the nerve sheath removed. The chorda tympani was cut, and its peripheral end was raised onto a Nichrome (Driver-Harris Co., Harrison, N. J.) wire recording electrode. Electrophysiological responses of the whole nerve were

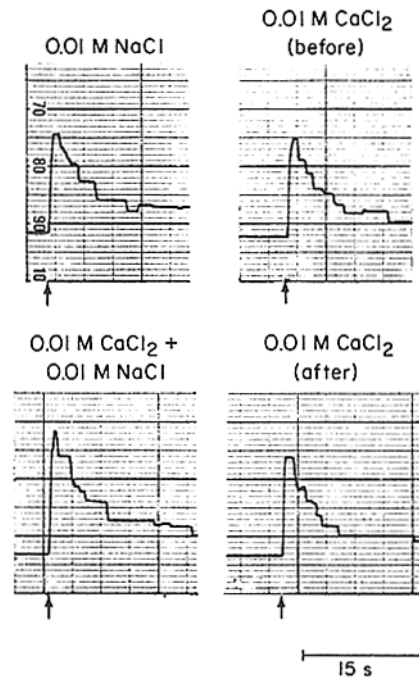


FIGURE 1. Photographs of chart recorder displays of one hamster chorda tympani's summed responses to the indicated stimuli. 0.01 M CaCl_2 was presented before and after the mixtures of its series (see text). Arrows indicate the opening of a stopcock controlling the flow of stimuli to the tongue.

recorded differentially with respect to a Nichrome wire indifferent electrode placed on muscle close to the chorda tympani. Responses were amplified by a Grass model P-4 (Grass Instrument Co., Quincy, Mass.), summed and displayed on a chart recorder whose pen deflected at time constants 0.3 s rise and 1.3 s fall. Nerve activity was also monitored with an oscilloscope and audiometer. The measure of the response (in units of the chart recorder paper) was the maximum deflection in the first 5 s of stimulation minus the average level of deflection to distilled water in the 5 s preceding s. Fig. 1 shows typical records from one preparation.

The animal's tongue was fitted into a water-tight chamber that allowed solutions to flow over the anterior portion from a funnel located above. 50 ml of each test solution was delivered across the tongue in 15 s. Each stimulus was followed by two rinses of distilled water, 50 ml each, delivered at the same rate.

Stimuli

All stimuli (analytic reagents) were prepared with distilled water and presented at room temperature, 23–24°C. All solutions containing sucrose or D-phenylalanine were prepared from 1 to 3 d before use and stored in capped glass bottles at 5°C until 1 h before use when they were brought to room temperature. This procedure minimized the growth of microorganisms in the solutions and the acid-catalyzed hydrolysis of sucrose. Acid and salt solutions were prepared in liter quantities once every two weeks and stored at room temperature.

To select the stimuli to be studied in mixtures and to determine their stimulus-response functions (i.e., the variation of response with stimulus concentration), solutions were prepared at approximately one-half log step differences in concentration by serial dilution. Table I lists the chemicals and the ranges of concentrations tested. Solutions were delivered in series of ascending concentration, with 0.03 M NaCl presented before and after each concentration series. The means of each pair of responses to 0.03 M NaCl were arbitrarily assigned the value of 100 U, and the

TABLE I
DATA USED IN THE CHOICE OF STIMULI AND INTERPRETATION OF
MIXTURE RESULTS

Chemical	Range of concentrations <i>mol/liter</i>	Number of nerves	Concentration in mixtures <i>M</i>	Power function fitted to mean responses		
				Concentrations <i>mol/liter</i>	Exponent	Correlation coefficient
NaCl	0.0003 to 0.3	11	0.01	0.003 to 0.03	0.39	0.982
KCl	0.001 to 0.3	5	—	—	—	—
CaCl ₂	0.0003 to 0.3	8	0.01	0.003 to 0.03	0.25	0.988
NH ₄ Cl	0.0003 to 0.3	6	0.05	0.01 to 0.10	0.35	0.999
MgCl ₂	0.0003 to 0.3	5	—	—	—	—
HCl	0.0001 to 0.01	9	0.003	0.001 to 0.01	0.25	0.989
Citric acid	0.0001 to 0.01	4	—	—	—	—
Sucrose	0.01 to 1.0	9	0.1	0.03 to 0.3	0.30	0.993
D-Phenylalanine	0.003 to 0.1	7	0.07	0.01 to 0.1	0.45	0.995

responses to the members of the concentration series they surrounded were adjusted proportionately. For the NaCl concentration series, the 0.03-M member was used for adjustments. These adjustments were made to equalize the contribution of each nerve to a mean.

Means of adjusted responses to each stimulus were calculated when a concentration series was repeated for a given preparation. The order of presentation of the concentration series varied across preparations. Adjusted response values were then averaged across nerves (numbers noted in Table I), and the means are plotted in Fig. 2.

The concentrations of the stimuli selected for study in mixtures are indicated in Table I. All of the chemicals listed in this table could not be included in the mixture study because the severed nerve retains a stable response for a limited time. NaCl, HCl, and sucrose were selected for study; these chemicals represent the taste qualities salty, sour, and sweet, respectively, and fiber populations of the hamster chorda tympani respond well to them (Frank, 1973). D-Phenylalanine was included because of recent behavioral evidence that hamsters generalize its taste to that of sucrose; specifically, a conditioned aversion established to D-phenylalanine is generalized to sucrose but not to other chemicals tested here (Nowlis and Frank, 1977). Also, D-phenylalanine tastes sweet to humans. NH₄Cl was selected because it is both a salt

and a weak acid to which the hamster chorda tympani responds well. CaCl_2 was arbitrarily chosen as a representative of divalent salts. The concentrations selected are of moderate strength and elicit responses approximately equal in magnitude. Responses to these concentrations fall within a range that follow a straight line when plotted on log-log coordinates.

Power functions were fitted to the mean responses to each chemical for the range

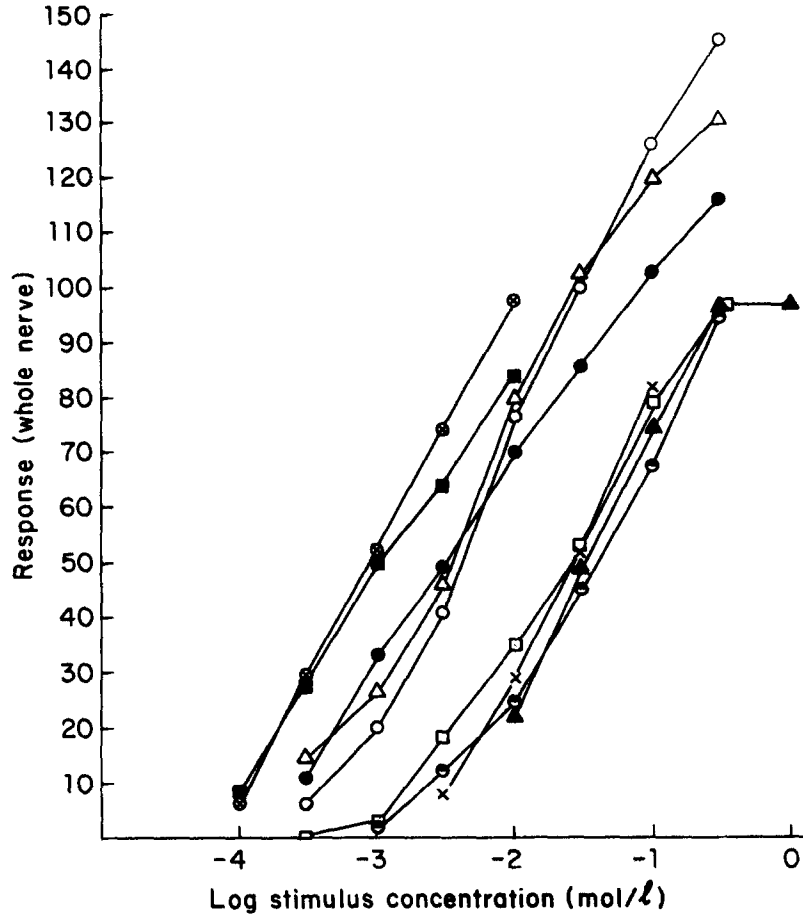


FIGURE 2. Whole hamster chorda tympani response vs. log stimulus concentration for nine chemicals. Means of responses, adjusted so that the response to 0.03 M NaCl equals 100 U, are plotted for each concentration series. (○) NaCl. (●) CaCl_2 . (□) NH_4Cl . (■) Citric acid. (△) MgCl_2 . (▲) Sucrose. (×) D-Phenylalanine. (⊗) HCl. (⊙) KCl.

of concentrations listed in Table I. This procedure enabled the prediction of the response to a single-component stimulus at a concentration equal to the sum of two concentrations equivalent in intensities to two different stimuli in a mixture. Exponents of the power functions and correlation coefficients attesting to goodness of fit are presented in Table I. The exponents are not significantly different ($F = 0.95$, $df = 5/38$, $P \gg 0.01$). It is important to realize that these exponents are the same for the absolute and adjusted responses (i.e., absolute responses multiplied by a constant).

Binary Mixtures

Each selected chemical was mixed with every other one such that the concentrations of the components in the resulting 15 two-component mixtures equaled the values noted in Table I. For example, 1 liter of the mixture of sucrose and NaCl contains 0.1 mol of sucrose and 0.01 mol of NaCl. Of all the stimuli studied here in mixtures, only two are known to interact chemically: the pK_a of D-phenylalanine is 2.58, and a rapid acid-base reaction ensues upon mixing with HCl, resulting in an increase in pH from 2.6 (i.e., the pH of 0.003 M HCl) to 3.6. At room temperature, there is a slow hydrolysis of the glycosidic bond of sucrose in the presence of HCl, but under the conditions of fresh preparation and cold storage <5% hydrolysis (as measured polarimetrically) would have occurred.

Mixtures were presented in series, the members of each series having a common component. There were six series of five mixtures each. The component common to all members of a particular series (e.g., sucrose for the sucrose mixture series) was presented before and after the mixtures of that series. The order of presentation of the various mixture series and the order within each series was varied. All single-component stimuli were usually presented before every second mixture series. When a series or a single-component stimulus was repeated for a particular preparation, mean responses were used for the calculation of the means across nerves. Eleven nerves were sampled in total, nine or ten for each series. The response of a nerve to a particular mixture was included in the analysis only if the responses to the components presented before and after the mixture series differed by <20% and if responses to all other members of the mixture series and the components of the particular mixture had also been obtained. Thus, responses to a mixture and its components were obtained in close temporal proximity and during a period in the life of a preparation when it was quite stable.

For each nerve, means of the responses to each mixture, presented as a member of two mixture series, and the means of the responses to each component of a mixture were calculated. These means were then averaged across nerves. This procedure produced three mean values for each mixture: the response to the mixture of stimulus *a* and stimulus *b* (R_{ab}); the response to component *a* presented alone (R_a); and the response to component *b* presented alone (R_b).

The sum of the responses to the components of a mixture presented individually ($R_a + R_b$) was calculated to compare the effect of the mixture with the sum of the effects of the components. If the components have completely independent effects, the response to their mixture will equal the sum of the responses to the components. The response to a "mixture" composed of two concentrations of one chemical will be less than the sum of the responses to the two concentrations presented individually if the exponent of the power function describing the stimulus-response relation is <1.00; this situation prevailed for all of the chemicals tested. Therefore, a response to a binary mixture that is either more or less than the sum of the responses to the components indicates a lack of independence, specifically, enhancement in the former case and, in the latter case, either inhibition or a lack of discrimination between the components. If the components of a mixture are perceived as the same stimulus by the gustatory system, the response to the mixture will equal the response to either component at a concentration equal to the sum of two concentrations equivalent in effect to the components in the mixture. Fig. 3 shows the average stimulus-response function for six chemicals tested in mixtures and illustrates the derivation of a measure (R'), the response when two concentrations of one chemical (those two equivalent in effect to the two separate components) are added.

The goodness of fit of power functions of response vs. concentration was reasonable

(c.f., Table I), and the exponents of these functions did not differ significantly; therefore, the mean responses to the lowest, middle (one-half log step stronger), and highest (one log step stronger) concentrations of each chemical were averaged across chemicals. The mean relative response to an increase in stimulus concentration is plotted in Fig. 3. The straight line is the power function fit to the points; it has a slope of 0.325 on log-log coordinates, and the correlation coefficient attesting to goodness of fit is 0.986.

To determine the effect of mixing two concentrations of one stimulus, the responses

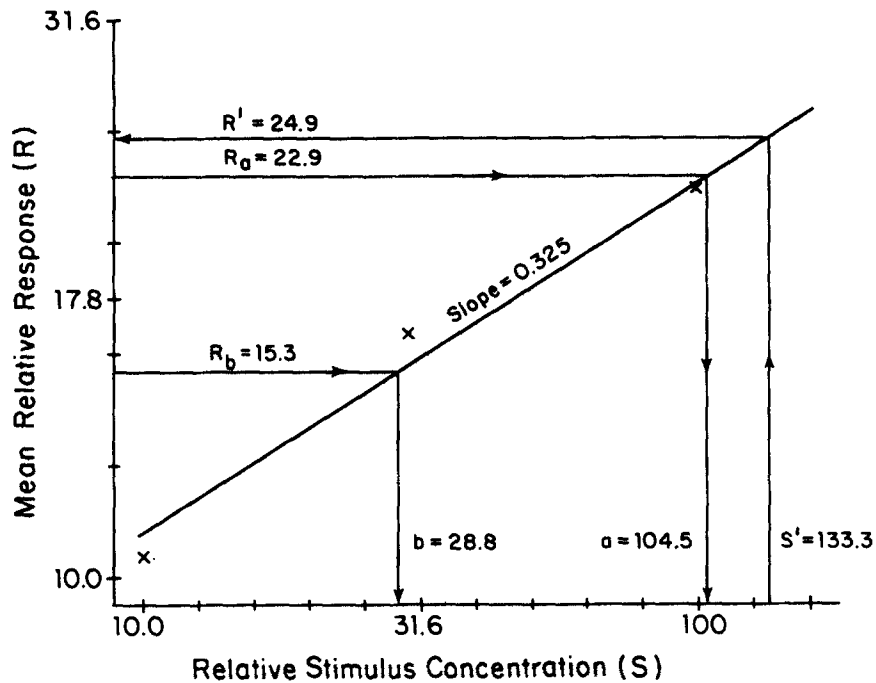


FIGURE 3. Mean response as a function of relative stimulus concentration plotted on log-log coordinates. The three Xs indicate means of the mean adjusted responses to six chemicals at about the concentration used in mixtures, about one-half log step lower and one-half log step higher; a straight line of slope 0.325 fits the points best. Relative concentrations of any single chemical (a , b) that would elicit the responses (R_a , R_b) evoked by individual application of mixture components to the tongue can be determined from the graph, as can the response (R') elicited by the sum of these concentrations (S').

elicited by the components of a mixture are needed. The concentrations of one stimulus that will elicit these responses are determined, and the response elicited by their sum is the predicted (R') response. In the example illustrated in Fig. 3, the mean effect of stimulus a alone (R_a) is 22.9 and of stimulus b alone (R_b) is 15.3; the relative concentrations of a stimulus that would elicit these responses are 104.5 (a) and 28.8 (b), the sum of which is 133.3 (S'). S' elicits a mean response of 24.9 (R'). This predicted response permits assessment of the degree to which a mixture of two chemicals is discriminated by the receptors. If the response to a mixture is greater than or less than the response function prediction (R'), the addition of a second chemical is, in the former case, adding to, and in the later case, subtracting from the

expected effect of a "mixture" of two concentrations of a single chemical. If the response to a mixture equals R' , the summed effects on all receptors does not differentiate between the presence of a second chemical and an appropriately high concentration of a single chemical in the stimulus solution.

RESULTS

Numerical values necessary for the analysis of sensitivities of the whole hamster chorda tympani to binary mixtures of taste stimuli are presented in Table II.

TABLE II
MEAN WHOLE NERVE RESPONSE TO A MIXTURE (R_{ab}),* THE SINGLE COMPONENTS THAT COMPRISE IT (R_a , R_b), AND VALUES PREDICTED FOR A "MIXTURE" OF TWO CONCENTRATIONS OF ONE CHEMICAL (R')

Stimulus <i>b</i>						Stimulus <i>a</i>
CaCl ₂	NH ₄ Cl	HCl	Sucrose	D-Phenylalanine		
23.8‡	23.7‡	23.8	26.9	27.9	R_{ab}	NaCl
18.2	18.6	18.6	18.6	17.7	R_a	
18.9	19.0	22.3	15.0	12.6	R_b	
23.2	23.5	25.8	21.2	19.5	R'	
0.64	0.63	0.58	0.80	0.92	$R_{ab}:(R_a + R_b)$	
1.03	1.01	0.92	1.27	1.43	$R_{ab}:R'$	
	22.1	24.3‡	24.7	29.7	R_{ab}	CaCl ₂
	20.0	19.6	18.2	19.8	R_a	
	20.9	21.3	13.9	13.5	R_b	
	25.6	25.6	20.5	21.6	R'	
	0.54	0.59	0.77	0.89	$R_{ab}:(R_a + R_b)$	
	0.86	0.95	1.20	1.38	$R_{ab}:R'$	
		25.5‡	27.9	28.6	R_{ab}	NH ₄ Cl
		20.3	20.7	20.0	R_a	
		22.0	16.4	12.7	R_b	
		26.7	23.6	21.5	R'	
		0.60	0.75	0.87	$R_{ab}:(R_a + R_b)$	
		0.96	1.18	1.33	$R_{ab}:R'$	
			29.3	23.6‡	R_{ab}	HCl
			22.9	22.7	R_a	
			15.3	12.3	R_b	
			24.9	23.8	R'	
			0.77	0.67	$R_{ab}:(R_a + R_b)$	
			1.18	0.99	$R_{ab}:R'$	
				19.4‡	R_{ab}	Sucrose
				15.9	R_a	
				13.2	R_b	
				18.4	R'	
				0.67	$R_{ab}:(R_a + R_b)$	
				1.05	$R_{ab}:R'$	

* The mean response to each mixture (R_{ab}) was smaller (t , $P \leq 0.05$) than the sum of the mean responses to its components ($R_a + R_b$); each mean response to a mixture not marked ‡ differed (t , $P \leq 0.05$) from the response predicted for a mixture of two appropriate concentrations of one component (R'). Both ratios, $R_{ab}:(R_a + R_b)$ and $R_{ab}:R'$, were smaller (t , $P \leq 0.05$) for mixtures of sucrose with a salt than for D-phenylalanine with a salt. The average standard error of the ratios across all mixtures was 4%, with a standard deviation of 1%.

Listed are: (a) the mean response to each two-component mixture (R_{ab}); (b) the mean response to each component presented individually (R_a or R_b); and (c) the predicted response to a single-component "mixture," which is the sum of two concentrations of one chemical equivalent in intensities to the components of the mixture (R'). Also listed are two ratios: $R_{ab}:(R_a + R_b)$, the ratio of the response to a mixture and the sum of the responses to its components, and $R_{ab}:R'$, the ratio of the response to a mixture and the predicted response to a single-component "mixture" of two concentrations of one chemical equivalent in intensity to the components of the mixture. We named the first ratio the *independent component index*; it compares the effects of stimuli in mixtures to their effects when presented individually. The independent component index equals 1.00 when the effects of stimuli in a mixture equal their effects when presented individually, specifically when $R_{ab} = R_a + R_b$. We named the second ratio the *mixture discrimination index*; it equals 1.00 when the whole nerve does not discriminate between two chemicals in a mixture so that a two-component mixture elicits a response identical to that of a single-component "mixture" of two concentrations of one chemical (i.e., $R_{ab} = R'$).

The Independent Component Index

The mean response to each binary mixture is significantly less than the sum of the responses to the components. Therefore, it appears that none of the stimuli are completely independent in effect. But, the degree of independence varies considerably across stimuli: the response to the mixture of NaCl and D-phenylalanine approaches a value expected for stimuli with completely independent effects; and the response to the mixture of CaCl₂ and NH₄Cl is about half of that value.

The degree of interaction between the effects of the stimuli in each pair is indicated by the length of the bars plotted in Fig. 4; the bars represent deviation from a value of 1.00 for the independent component index. Pairs of electrolyte stimuli show the least independence of effect on the whole nerve, with responses to mixtures ranging from one-half to two-thirds of the sum of the responses to the components. Similarly, mixtures of sucrose or HCl and D-phenylalanine elicit responses which are only two-thirds of the sums of the effects of the components presented individually. However, responses to mixtures of either sucrose or D-phenylalanine and an electrolyte (with the one exception noted above) approach the sum of the responses to the components, assuming at least three-fourths of that value. Moreover, responses to mixtures of electrolytes and D-phenylalanine indicate significantly greater independence of the effects of components (about nine-tenths) than do responses to mixtures of electrolytes and sucrose (about three-fourths).

Separating the stimuli into two sets aids in describing their effects on the whole nerve: one set consists of NaCl, CaCl₂, NH₄Cl, and HCl; the other set comprises D-phenylalanine and sucrose. Binary mixtures of stimuli belonging to the same set elicit responses much smaller than the sum of responses to components. Brackets *A* in Figs. 4 and 5 surround bars representing indices for these stimulus pairs. Brackets *B* and *C* surround bars representing the

indices for between-set pairs of stimuli. Responses to mixtures of stimuli from different sets elicit responses approaching the sum of responses to components in all cases (B) but one (C); the one mixture for which a significant chemical interaction occurs (D-phenylalanine and HCl) does not obey this rule.

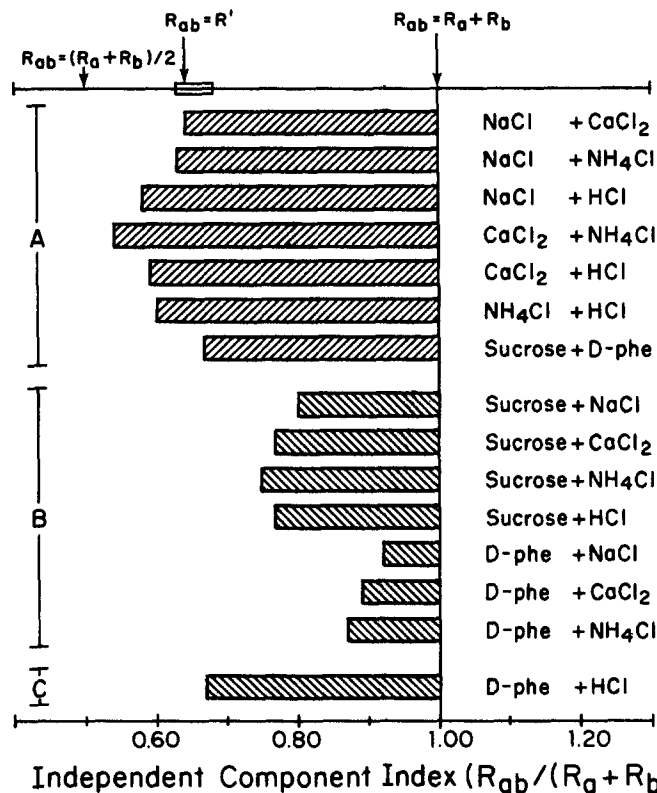


FIGURE 4. Deviations of responses to the fifteen indicated binary mixtures from an independent component index of 1.00. This index equals 1.00 when the response to the mixture (R_{ab}) equals the sum of the responses to the components ($R_a + R_b$); it equals ~ 0.50 when the response to the mixture equals the response to either component ($[R_a + R_b]/2$) since responses to the component stimuli were approximately equal; it equals 0.64 (mean, range indicated by horizontal extent of small rectangle) when the response to the mixture equals the response to a "mixture" of two appropriate concentrations of one chemical (R'). Arrows indicate these three values.

Mixture Discrimination Index

The mean responses to 6 of the 15 two-component mixtures are not significantly different from the predicted response to a single-component "mixture" of two concentrations of one chemical equivalent in effect to the components of the mixture; the response to two of the mixtures is slightly (but significantly) less than that value; but the response to the other seven mixtures is significantly

greater. Therefore, the whole nerve could discriminate the presence of two components in a mixture in seven cases on the basis of an increase in effect and in two cases on the basis of a decrease in effect, but could not discriminate between a two-component mixture and a single-component "mixture" in six of the fifteen cases.

The degree to which the presence of two stimuli in a mixture could be discriminated by the whole nerve is represented by a horizontal bar for each stimulus pair in Fig. 5. An absence of discrimination is indicated by a value

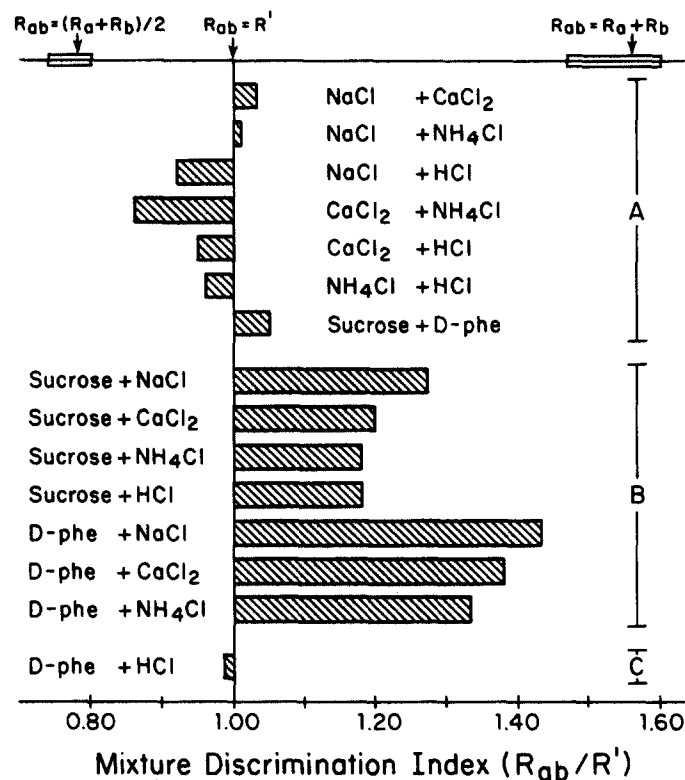


FIGURE 5. Deviations of responses to the fifteen indicated binary mixtures from a mixture discrimination index of 1.00. This index equals 1.00 when the response to the mixture (R_{ab}) equals the response to a "mixture" of two appropriate concentrations of one chemical (R'); it equals 0.78 (mean, range indicated by horizontal extent of small rectangle) when the response to the mixture equals the response to either component ($(R_a + R_b)/2$) since responses to the component stimuli were approximately equal; it equals 1.56 (mean, range indicated similarly) when the response to the mixture equals the sum of the responses to the components ($R_a + R_b$). Arrows indicate these three values.

of 1.00 for the mixture discrimination index; deviations from that value are plotted. Bars extending to the left of the vertical line (drawn at an index value of 1.00) indicate that the response to the mixture is less than predicted for a single-component "mixture" of two concentrations of one chemical. Two such

suppressions are notable: the CaCl_2 and NH_4Cl mixture and the NaCl and HCl mixture are somewhat less effective than predicted. However, responses to other mixtures of within-set stimuli (*A*) very closely approach the predicted response (*R'*). It is noteworthy that the response to the mixture of HCl and *D*-phenylalanine (a between-set pair of stimuli) also closely approaches the predicted response *R'*; but the chemical interaction between the components in this mixture may be the cause of this relation.

Deviations of the mixture discrimination index represented by bars extending to the right of the vertical line in Fig. 5 indicate that the effect of the second chemical in the mixture is greater than the effect of an increase in concentration of one component. With the exception noted above, all mixtures of between-set stimuli elicit such an additional effect (*B*). However, the effects of sucrose when mixed with electrolytes less closely approximate the effects of the components presented individually than do the effects of *D*-phenylalanine mixed with electrolytes. Consequently, the effects of sucrose mixed with the electrolytes are closer to the effects of a single-component "mixture" of two concentrations of one stimulus than are the effects of *D*-phenylalanine mixed with the electrolytes. The sucrose mixtures elicit whole-nerve responses that are about one-fourth greater than the value predicted for a single-component "mixture," whereas the *D*-phenylalanine mixtures elicit responses more than one-third greater than that value. Yet, mixtures of *D*-phenylalanine with NaCl , CaCl_2 , and NH_4Cl are progressively less discriminable by the whole nerve, as are the mixtures of sucrose with these three electrolytes.

DISCUSSION

A Distinction Between Mixtures of Electrolytes and Nonelectrolytes

These studies of summed activity of the chorda tympani of the hamster indicate that not one of the pairs of stimuli tested are completely independent in their effects if independence is indicated by a response to a mixture equal to the sum of the responses to the individual components. The lack of independence, however, was never manifested in an enhancement; all mixtures had an effect significantly smaller than that predicted for independence.¹

Some pairs of chemicals more closely approached the indication of independence of effect than other pairs. The effects of mixtures of *D*-phenylalanine and each of the three salts (NaCl , CaCl_2 , and NH_4Cl) most closely approached the sum of effects of the components. Mixtures of sucrose and each of the four electrolytes (NaCl , CaCl_2 , NH_4Cl , and HCl) evoked responses which fell shorter of an indication of independence. Yet, with the exception of the *D*-phenylalanine and HCl mixture, for which a chemical interaction between

¹ The recorded whole nerve response was amplified by an ac-coupled instrument. If the response was not reduced by a constant factor because of cancellations of positive by negative going portions of recorded nerve impulses, and if the factor increased with increasing activity, then the sum of two smaller responses (recorded to the components) would be greater than the larger response (recorded to the mixture), even if the actual activity emanating from the nerve were exactly the same in the two cases. Such a possibility could explain why all of the mixtures had a lesser effect than that predicted for independence. But it would not explain the large variation in the amount that responses to different mixtures were less than predicted.

components is known to occur, each D-phenylalanine and electrolyte mixture more closely approached the prediction for independent stimuli than it approached the prediction for interchangeable stimuli. Also, each sucrose and electrolyte mixture approached the prediction for independence as closely as it approached the prediction for interchangeability of stimuli. An independence of stimulus effects should easily have been distinguished from an interchangeability, that is, a summation of stimulus intensities, because the determined exponent of the power function describing response as a function of stimulus concentration (over the range used) was considerably different from 1.00, being <0.50 for all chemicals tested.

All six mixtures of two electrolytes, as well as the mixture of D-phenylalanine and sucrose evoked responses that approached the prediction for interchangeable stimuli. Five of these mixtures had effects that were not significantly different from the prediction for interchangeability of components (NaCl and CaCl_2 , NaCl and NH_4Cl , HCl and CaCl_2 , HCl and NH_4Cl , and D-phenylalanine and sucrose). The effects of these pairs of different chemicals in a mixture are the same as a single component mixture of one chemical or one chemical presented at an appropriate concentration. Two pairs of chemicals (CaCl_2 and NH_4Cl , and HCl and NaCl) evoked whole-nerve responses significantly smaller than the response predicted for an equivalent increase in concentration of one chemical. These responses hint at the presence of inhibitory effects, a suppression of effective stimulus intensities, in some mixtures.

The whole-nerve response can clearly differentiate between two sets of stimuli: electrolytes and nonelectrolytes. Pairs of stimuli within a set, when applied to the tongue in a mixture, evoked a response resembling in size that evoked by an appropriate increase in concentration of one chemical. However, when members of a pair of stimuli are from different sets, unless there is a chemical interaction between components, the mixture evoked a response more closely resembling in size the sum of the responses to the components. Finally, an acid-base reaction, which occurs when HCl and D-phenylalanine are mixed, resulted in changes in the proportions of the different chemical species present in the mixture as compared with those present in the individual components; this is a reasonable explanation for the smaller response to this one between-set mixture.

Single-fiber Sensitivities to Components and Prediction of Responses to Mixtures

Division of the six test stimuli into a set of electrolytes and a set of nonelectrolytes, with between-set mixtures showing greater independence of effects than within-set mixtures, could have been predicted from the known sensitivities of single nerve fibers of the hamster chorda tympani (Frank, 1973; Frank, 1974). Most fibers that are sensitive to sucrose are not very sensitive to HCl, NaCl, or NH_4Cl . On the other hand, most electrolyte-sensitive fibers display an acute sensitivity to two or three of the electrolytes; very few of these units respond well to sucrose. Thus, there is little overlap of sensitivities to one of the nonelectrolytes with any of the three electrolytes tested, but there is

considerable overlap of sensitivities to the electrolytes. Therefore, the extent of overlap of single-fiber sensitivities to components appears to be a determinant of the independence of action of stimuli in mixtures, as measured by the whole-nerve response.

However, the extent to which the sensitivity to sucrose is segregated into one set of fibers is not mirrored by a near independence of effect of this chemical when it is mixed with electrolytes. For example, sensitivities to HCl and sucrose are likely to occur in completely different sets of fibers; but the response to the mixture of these two chemicals is only about one-fifth greater than a response to an appropriate increase in concentration of one chemical and only about three-fourths of the sum of component responses. This is also true of the mixture of NaCl and sucrose or NH_4Cl and sucrose: little overlap of effects on single fibers but lack of independence of effects in a mixture.

Furthermore, mixtures of electrolytes elicit whole-nerve responses that approach the expectation for a complete lack of independence of effects, a result that a complete overlap of sensitivities would engender. This is known not to be the case. NaCl, for example, selectively stimulates fibers that are not very sensitive to NH_4Cl ; whereas NH_4Cl maximally stimulates fibers that are not very sensitive to NaCl. The response to a mixture of NaCl and NH_4Cl does not reflect this relative selectivity, being nearly equal to a response to an appropriate increase in concentration of a single chemical.

Finally, some pairs of electrolytes elicit whole-nerve responses that indicate that they may suppress the effects of one another. For example, the response to a mixture of NH_4Cl and CaCl_2 is somewhat less than the response predicted for an appropriate increase in concentration of one chemical. Such inhibitory interactions could not, of course, be predicted from single component effects on neurons; the mixture itself must be applied to the receptors for their discovery.

Taste Quality of Components and Predictions of Responses to Mixtures

D-Phenylalanine was chosen for study because its taste is sweet, as is the taste of sucrose, to humans. Also, hamsters generalize a learned aversion to this amino acid to sucrose but not to NaCl, HCl, or quinine hydrochloride (G. H. Nowlis, M. Frank, and C. Pfaffmann, unpublished data), none of which is predominantly sweet. Sucrose and D-phenylalanine evoke a very similar taste quality, and, when they are mixed, elicit a response of the hamster chorda tympani nearly equivalent to that elicited by an increase in concentration of a single chemical. In contrast, NaCl and D-phenylalanine evoke very different taste qualities and, when mixed, elicit a chorda tympani response close to the sum of the responses to the components. These two examples illustrate that chemicals having similar tastes can approach interchangeability of effect in a mixture and those with different tastes can be independent in a mixture. Although none of the other pairs of stimuli tested here has highly similar tastes, other pairs are as dissimilar as D-phenylalanine and NaCl: NaCl and HCl is an example of such an electrolyte pair. Unlike D-phenylalanine and NaCl, these two chemicals of very different taste quality, when mixed, do not

display an independence of effect; in fact, the mixture of the two dissimilar tasting electrolytes evokes a neural response even smaller than that predicted for interchangeable stimuli. Therefore, as noted above in reference to prediction of responses to mixtures on the basis of single-fiber sensitivities to components, the evoked taste qualities of individual chemicals can be helpful for such predictions in some instances but are relatively useless in others.

Functions Describing Responses to Components and Prediction of Responses to Mixtures

It has been suggested that the mathematical functions that describe magnitude estimates of taste intensity to increasing concentrations of component chemicals may be more useful in the prediction of magnitude estimates of taste intensities of mixtures than the taste quality of the components (Bartoshuk, 1977). Taste intensities of mixtures of chemicals with similar tastes (Bartoshuk and Cleveland, 1977) and with dissimilar tastes (Bartoshuk, 1975) have been effectively predicted using the rule that intensities of several chemicals add in mixtures in the same way as intensities of one chemical add with increases in concentration. The evoked activity recorded from the whole nerve may be comparable to the total intensity of a stimulus and, responses predicted for a summation of stimulus intensities via a power function can be compared with responses obtained from mixtures.

For the two similar tasting stimuli used here, sucrose and D-phenylalanine, the chorda tympani response to the mixture does closely approximate the prediction from the power function. Some dissimilar tasting chemicals (if there is no known chemical interaction between components) elicit neural responses that are close to the response predicted by the power function, and some do not. Those stimulus pairs that approximate the prediction for stimulus interchangeability (the electrolyte mixtures) do. Mixtures of sucrose or D-phenylalanine and an electrolyte do not, however; their responses exceed the predicted response. Therefore, as noted above in reference to prediction of responses to mixtures on the basis of either overlap of single-fiber sensitivities to components or evoked taste qualities of components, the function that describes the dependence of response on the stimulus concentration of a component is a predictor in some cases, but not in others.

In conclusion, the utilization of known effects of a mixture's components is not always reliable in predicting the effect of the mixture. Perhaps the overlap in single-fiber sensitivities to the single-component stimuli is the most useful predictor of the response to a mixture, allowing a division of stimuli into two sets: electrolytes and nonelectrolytes. However, even this predictor cannot handle most of the finer differences in responses to different mixtures. Knowledge of the effects of many mixtures appears to require presentation of the mixture itself.

We appreciate the helpful advice of T. Hettinger and the support and suggestions of C. Pfaffmann.

A report of preliminary analyses of the data presented in this manuscript was given at the Seventh Annual Meeting of the Society of Neuroscience (1977. *Soc. Neurosci. Symp.* 3:80). The

work was supported by the Alcohol, Drug Abuse and Mental Health Administration (Service Award 07524 from the National Institute of General Medical Sciences), by the National Science Foundation (grant BNS78-16533), and by a Biomedical Research Support Grant (PHS 5 SO7 RR-07065).

Received for publication 28 November 1979.

REFERENCES

- BARTOSHUK, L. M. 1975. Taste mixtures: is mixture suppression related to compression? *Physiol. Behav.* **14**:643-649.
- BARTOSHUK, L. M. 1977. Psychophysical studies of taste mixtures. *Olfaction Taste Proc. Int. Symp.* **6**:377-384.
- BARTOSHUK, L. M., and C. T. CLEVELAND. 1977. Mixtures of substances with similar tastes: a test of a psychophysical model of taste mixture interactions. *Sensory Processes.* **1**:177-186.
- BEIDLER, L. M. 1953. Properties of chemoreceptors of tongue of rat. *J. Neurophysiol. (Bethesda).* **16**:595-607.
- BEIDLER, L. M. 1954. A theory of taste stimulation. *J. Gen. Physiol.* **38**:133-139.
- BEIDLER, L. M. 1961. Taste receptor stimulation. In *Progress in Biophysics and Biophysical Chemistry*, Vol. 12. J. A. V. Butler, H. E. Huxley, and R. E. Zirkle, editors. Pergamon Press, Inc., Elmsford, N. Y. 107-151.
- BEIDLER, L. M., I. Y. FISHMAN, and C. W. HARDIMAN. 1955. Species differences in taste responses. *Am. J. Physiol.* **181**:235-239.
- CARPENTER, J. A. 1956. Species differences in taste preferences. *J. Comp. Physiol. Psychol.* **49**:139-144.
- FAULL, J. R., and B. P. HALPERN. 1971. Reduction of sucrose preference in the hamster by gymnemic acid. *Physiol. Behav.* **7**:903-907.
- FISHMAN, I. Y. 1957. Single fiber gustatory impulses in rat and hamster. *J. Cell. Comp. Physiol.* **49**:319-334.
- FRANK, M. 1973. An analysis of hamster afferent taste nerve response functions. *J. Gen. Physiol.* **61**:588-618.
- FRANK, M. 1974. The classification of mammalian afferent taste nerve fibers. *Chemical Senses and Flavor.* **1**:53-60.
- HAGSTROM, E. C., and C. PFAFFMANN. 1959. The relative taste effectiveness of different sugars for the rat. *J. Comp. Physiol. Psychol.* **52**:259-262.
- JAKINOVICH, W., JR. 1976. Stimulation of the gerbil's gustatory receptors by disaccharides. *Brain Res.* **110**:481-490.
- KIMURA, K., and L. M. BEIDLER. 1961. Microelectrode study of taste receptors of rat and hamster. *J. Cell. Comp. Physiol.* **58**:131-139.
- MAROWITZ, L. A., and B. P. HALPERN. 1977. Gustatory neural response of the chorda tympani to lick-duration stimuli. *Chemical Senses and Flavor.* **2**:457-485.
- MILLER, I. J., JR. 1971. Peripheral interactions among single papilla inputs to gustatory nerve fibers. *J. Gen. Physiol.* **57**:1-25.
- MOOSER, G. 1976. N-substituted maleimide inactivation of the response to taste cell stimulation. *J. Neurobiol.* **7**:457-468.
- NACHMAN, M., and C. PFAFFMANN. 1963. Gustatory nerve discharges in normal and sodium deficient rats. *J. Comp. Physiol. Psychol.* **56**:1007-1011.
- NOMA, A., M. SATO, and Y. TSUZUKI. 1974. Taste effectiveness of anomers of sugars and glycosides as revealed from hamster taste responses. *Comp. Biochem. Physiol.* **48A**:249-262.

- NOWLIS, G. H., and M. FRANK. 1977. Qualities in hamster taste: behavioral and neural evidence. *Olfaction Taste Proc. Int. Symp.* **6**:241-248.
- OAKLEY, B. 1967. Altered temperature and taste responses from cross-regenerated sensory nerves in the rat's tongue. *J. Physiol. (Lond.)* **188**:353-371.
- OGAWA, H., M. SATO, and S. YAMASHITA. 1968. Multiple sensitivity of chorda tympani fibers of the rat and hamster to gustatory and thermal stimuli. *J. Physiol. (Lond.)* **199**:223-240.
- OZEKI, M., and M. SATO. 1972. Responses of gustatory cells in the tongue of rat to stimuli representing four taste qualities. *Comp. Biochem. Physiol.* **41**:391-407.
- PFÄFFMANN, C. 1955. Gustatory nerve impulses in rat, cat and rabbit. *J. Neurophysiol. (Bethesda)* **18**:429-440.
- SATO, M., H. OGAWA, and S. YAMASHITA. 1971. Comparison of potentiating effect on gustatory response by disodium 2-methyl mercapto-5'-inosinate with that by 5'-IMP. *Jpn. J. Physiol.* **21**:669-679.
- SATO, M., and M. OZEKI. 1972. Transduction of stimuli into electrical events at the gustatory cell membrane in the rat fungiform papillae. *Olfaction Taste Proc. Int. Symp.* **4**:252-258.
- SMITH, D. V., and S. L. BEALER. 1975. Sensitivity of the rat gustatory system to the rate of stimulus onset. *Physiol. Behav.* **15**:303-314.
- TATEDA, H., and I. HIDAOKA. 1966. Taste responses to sweet substances in rat. *Mem. Fac. Sci. Kyushu Univ. Ser. E Biol.* **4**:137-149.
- ZOTTERMAN, Y. 1956. Species differences in the water taste. *Acta Physiol. Scand.* **37**:60-70.