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# Effects of *Gymnema Sylvestre* on Complex Tastes Elicited by Amino Acids and Sucrose<sup>1</sup>

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MEISELMAN, H. L. AND B. P. HALPERN. *Effects of Gymnema sylvestre on complex tastes elicited by amino acids and sucrose.* PHYSIOL. BEHAV. 5 (12) 1379-1384, 1970.—The effects of an aqueous decoction of the taste modifier, *Gymnema sylvestre*, were studied on two amino acids, glycine and dl-alanine, and on sucrose. Each subject (human) estimated the magnitude of each taste quality response category (sour, salty, bitter and sweet) on each presentation of a solution. G extracts produced the expected depression of sweetness for all three chemicals, and also produced depressions and enhancements of some of the other taste quality categories for these stimuli. These results are discussed in terms of: (1) possible reciprocal characteristics of taste quality categories; (2) taste mixtures; and (3) simple and complex taste sources.

Human taste    Taste modifiers    Amino acids    Gustatory qualities    Psychophysics    *Gymnema sylvestre*

THE EFFECTS of active components from the leaves of the plant *Gymnema sylvestre* (G) on human judgments of tastes of different compounds have been studied with raw leaves and with several different preparations from the leaves. Suppression of sweetness has been reported with every sapid compound except chloroform: raw leaves with sugar [12]; aqueous extract with sucrose [13, 17]; aqueous extract or potassium gymnemate with sucrose or sodium saccharin [27-29]; gymnemate salts with sucrose [1, 17]; potassium gymnemate with sucrose, cyclamate, D-tryptophan, D-leucine, beryllium chloride, lead acetate but not chloroform [14]. In addition, suppression of bitterness has been reported using the raw leaves with quinine sulfate (QSO<sub>4</sub>) [13], aqueous extract or sodium gymnemate with QSO<sub>4</sub> [14], and an alcoholic extract with QSO<sub>4</sub> [14]. Although no significant depression of sourness has been noted, depression of saltiness has been reported with both an aqueous extract and sodium gymnemate used on NaCl [21], and with gymnemic acid (HG) in alcohol and NaCl [13].

Bartoshuk *et al.* [1] have suggested that previously reported bitter suppression might have been cross-adaptation between bitter tasting raw leaves or extracts of G and the bitter test compounds such as quinine. They demonstrated that rinsing the mouth for 40 sec with distilled water after HG treatment eliminated the taste of HG and thereby eliminated the effect on bitter tasting quinine hydrochloride (QHCl). They further suggested that previously reported depression of saltiness

might have been due to slight cross-adaptation with sodium salts present in the G extract preparation.

The studies reported above have generally used single compound (i.e. not mixtures) stimulus sources thought to elicit a simple taste, with which to test the gustatory effects of G extracts. For example, bitter suppression has been tested by observing whether a G extract could lower the estimated intensity or elevate the threshold of a classical bitter stimulus such as quinine. Such use of presumed simple stimuli may have avoided the problems of using the functional taste mixtures present in complex stimuli at a time when the effect of G extracts on simple tasting stimuli was as yet unclear. Conversely, this choice of stimulus sources, and their assumed simple effects, may have hampered a full exploration of the actions of G extracts.

With these presumed simple stimuli, subjects have been asked for only unidimensional judgments, e.g. the bitterness of quinine. Bartoshuk *et al.* [1] did permit subjects to use all desired taste quality categories for each stimulus, although subjects were not required to report on each taste category with every stimulus. In general, prior experiments indicate that active G extracts suppress human responses to sweet tasting compounds only, at least when a multidimensional answer is not encouraged and when presumably simple tasting compounds are used.

The present experiment was undertaken to assess the effects of an aqueous extract of G on potentially more representative

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(complex) stimulus sources, and to more fully explore the interactions of this G extract with taste quality categories. Two compounds which are recognized to elicit several taste quality response categories which vary with concentration (complex stimuli: glycine and dl-alanine [26, 27]) and one compound which is assumed to elicit a single taste quality response category (simple stimulus: sucrose) and which has been previously reported to be strongly suppressed by G extracts were used. Further, the present experiment made use of a profile technique which asked the subjects (Ss) for ratings of stimulus intensity for each of four taste quality categories in each trial.

## METHOD

*Subjects*

Subjects were six nonsmoking male undergraduate students at Cornell University enrolled in Introductory Psychology. Their ages ranged from 17 to 20. These six were chosen from a larger group on the basis of their performance in a gustatory screening task previously described by Meiselman and Dzendolet [16]. None of the Ss had previous experience with gustatory research or psychophysical research. Subjects were paid for their participation in the experiment.

*Stimuli*

All solutions were made with distilled water (conductivity  $< 6 \times 10^{-6}$  mhos/cm, refractive index = 1.3330), sucrose of reagent grade, and glycine and dl-alanine meeting NRS specifications. A series of five concentrations of each compound was chosen on the basis of a pilot study which used a separate group of subjects.

From this pilot study, corresponding concentrations (1-5) of the three chemicals which gave approximately the same average magnitude estimations of total taste intensity were determined. The concentrations of the three compounds used in the present experiment are given in Table 1. Solutions were refrigerated until placed in a water bath for presentation to the Ss at 32°C. All solutions were used within 24 hr of preparation.

Aqueous decoctions of leaves of G or a control plant were prepared in the following manner: 5.0 g of either G leaves or orange pekoe tea leaves were added to 75 ml distilled water in a 250 ml beaker, which was placed in a water bath at 95°C for one hour. The mixture was occasionally stirred while in the water bath. The leaves and distilled water were then suction-filtered through smooth surface, high retention, medium speed filter paper (Will No. 13061) for 15 min. The filtrate was refrigerated at 4°C.

The orange pekoe leaves were a suitable control source because their aqueous decoction (AP) receives a taste profile similar to that which the aqueous decoction of G (AG) receives [17].

*Procedure*

The middle concentration of the sucrose series, 0.125 M sucrose, was used as the standard for magnitude estimation throughout the experiment and was assigned a value of 10 by the experimenter. During each session S was blindfolded and seated in the experimental room in front of a funnel used for expectoration. A session was initiated by the presentation of 10 ml of the standard, which was held in the mouth for 5 sec. The S was told to assign the resulting stimulus strength a value of 10. The standard was then expectorated into the

TABLE I

PERCENTAGE OF THE TOTAL MAGNITUDE ESTIMATE ASSIGNED TO EACH OF THE FOUR TASTE QUALITY CATEGORIES (CONCENTRATION IN MOLES PER LITER). FOR EACH TASTE QUALITY CATEGORY AND CONCENTRATION THE VALUE OBTAINED WITH GYMNEMA TEA IS SHOWN ABOVE THE VALUE OBTAINED WITH ORANGE PEKOE CONTROL TEA (AG/AP)

	Sucrose				
	0.05	0.08	0.125	0.205	0.3
Sour	26 22	16 20	9 18	7 7	7 6
Salty	10 0	6 1	6 1	3 0*	3 0*
Bitter	56 46	44 8	24 3	10 0*	7 0*
Sweet	8 32	34 71	61 78	80 93	83 93
	Glycine				
	0.2	0.32	0.5	0.8	1.25
Sour	31 22	46 33	63 42	55 47	59 34
Salty	4 5	9 9	1 7	21 9	17 17
Bitter	56 53	35 38	27 23	12 10	8 11
Sweet	9 20	10 20	9 28	12 34	16 38
	dl-Alanine				
	0.1	0.175	0.315	0.555	1.0
Sour	31 22	35 22	42 26	47 36	40 30
Salty	7 18	3 8	11 17	15 17	15 17
Bitter	55 39	48 23	29 6	21 2	21 3
Sweet	7 21	14 47	18 51	17 45	24 50

\* < 1.0.

collecting funnel. The standard was presented and identified twice more in succession with 90 sec rest intervals separating the presentations. After another 90 sec pause, 10 ml of either AG or AP were presented and held in the mouth for 60 sec. During this period, S was asked to move his tongue. This was done to improve penetration of the solution into the tongue folds. After a 5 min rest, 5 ml of the first test stimulus was presented for 5 sec, and S was asked to rate the sensory magnitude of the sample. Additional samples were presented 90 sec apart. Each rating was done by first assigning a magnitude estimate to the total taste intensity of the stimulus. Subject was then asked to break down this total into the portions attributable to each of 4 quality categories: sour, salty, bitter and sweet. In this way a quadrifid taste profile of each stimulus was obtained. All conditions of the experiment were presented in random order with the exception that all Ss

received the same single test compound (sucrose, glycine, or dl-alanine) on the same day, although not necessarily with the same pretreatment or in the same order of concentrations. Each concentration was presented 4 times per session. No rinses were used. The liquids were presented in plastic beakers which were discarded after one presentation.

The 6 min period from initiation of the 60 sec presentation of AG to presentation of the first test stimulus permitted the effect of AG to reach a submaximal but only slowly changing level. For HG, a 27 per cent decrease from maximum reduction of sweetness estimates for sucrose occurs in the first 6 min, and a further 40 per cent reduction in the next 48 min [17]. This latter, slow decrease in effectiveness of G extracts in reducing sweetness estimates was not evident during the 30 min test session of the present experiment. An analysis of variance of the four successive estimates of the same concentration during a single AG session revealed no significant differences ( $F = 1.47, df = 3/15, p > 0.05$ ).

RESULTS

Table 1 presents the per cent contribution of each taste category to the total magnitude estimation. Generally, the percentage of the total attributed to sweetness increased with increasing concentration for both control tea and G conditions. This effect was most marked for sucrose: percentage sweetness increased from 32 to 93 per cent under the control tea condition and from 8 to 83 per cent under the G condition. The per cent sweetness of the amino acids rarely exceeded 50 per cent. For all stimuli, the percentage sweetness under the G condition was always less than the percentage sweetness under the corresponding control condition.

Percentage of total response attributed to sourness decreased with increasing concentration of sucrose, but increased with increasing concentration of glycine and dl-alanine. The per cent sourness of sucrose rarely exceeded 25 per cent but reached 59 per cent for glycine. With G, the percentage sourness contribution decreased for the amino acids, but showed no systematic change for sucrose.

For all solutions and pretreatments, the percentage bitter contribution decreased with increasing concentration. The lowest concentration of all stimuli was regarded as approximately 50 per cent bitter for both G and control tea. For all conditions, the saltiness contribution showed little systematic change and was relatively low ( $\leq 21$  per cent), especially for sucrose ( $\leq 10$  per cent).

Geometric mean magnitude estimation under both pretreatments show regular increases or decreases with concentration changes (Fig. 1), although some reversals of trends occur at either the highest or lowest concentrations. The geometric mean is the appropriate measure of central tendency for magnitude estimation data [23]. Least-squares estimation of the slope of the best-fitting straight line of the functions relating log magnitude estimation to log stimulus concentration was done. This yields the exponent (n) of the best fitting power function ( $R = KS^n$ ) (Table 2). AG did not have a unidirectional effect common to the exponents of these power functions.

The effects of AG on the geometric mean magnitude estimations depended upon compound and taste quality category (Fig. 1). Multiple *t*-tests were performed on the log magnitude estimations, and the *t*-test significance level was corrected for error rate per experiment by dividing the level by the number of comparisons. One-tailed tests were used for total intensity and sweetness: two-tailed, for all other categories.

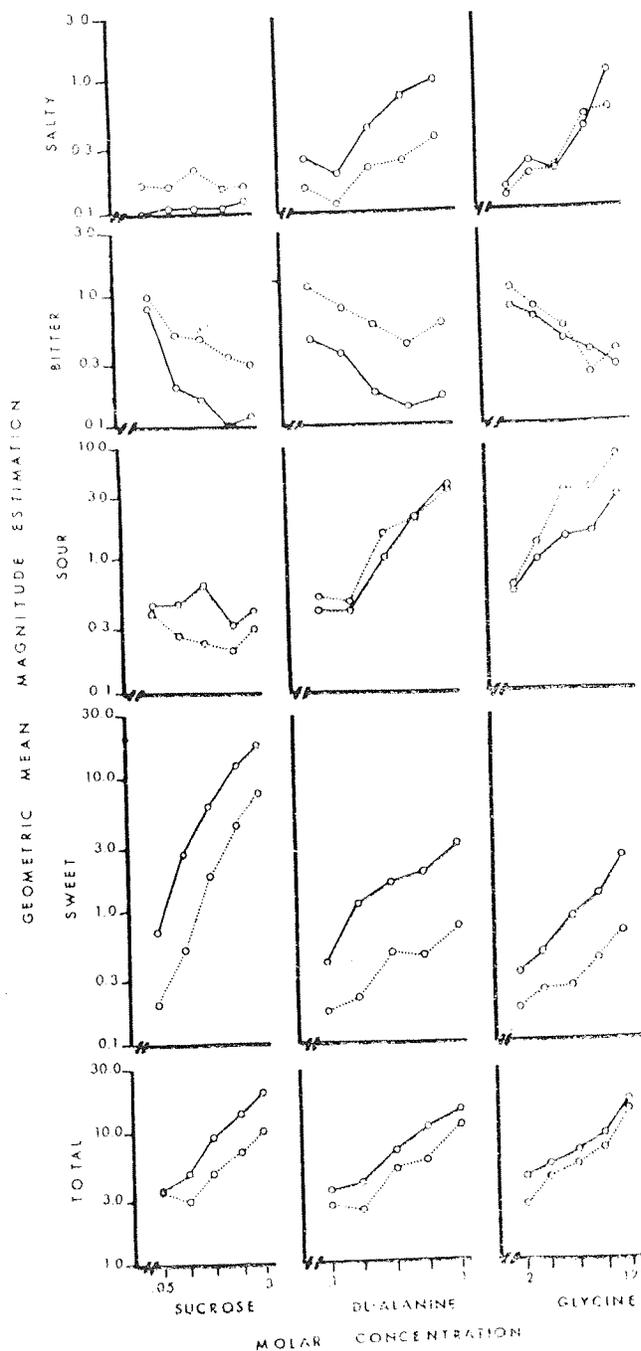


FIG. 1. Geometric mean magnitude estimation as a function of molar concentration, plotted on log-log coordinates. Each point represents four repeated measurements on each of six Ss, or 24 measurements. The control decoction of orange pekoe leaves (AP) is shown in solid lines, and G decoction (AG) in hatched lines. The molar concentrations of each test compound were as follows: 0.05, 0.08, 0.125, 0.205 and 0.30 M sucrose; 0.2, 0.32, 0.5, 0.8 and 1.25 M glycine; and 0.1, 0.175, 0.315, 0.555 and 1.0 dl-alanine.

Magnitude estimations for the AG condition were significantly ( $p < 0.01$ ) lower than for the control condition for both total intensity and sweet estimations of all three compounds, and for the sourness of sucrose and the saltiness of

TABLE 2

LEAST-SQUARES ESTIMATES OF THE SLOPES OF THE BEST-FITTING STRAIGHT LINES FOR THE FUNCTIONS IN FIG. 1. CALCULATED VALUES OF  $r^2$  ARE GIVEN IN PARENTHESES

	Total	Sour	Salty	Bitter	Sweet
Sucrose					
Gymnema	0.7 (0.88)	-0.2 (0.28)*	—	-0.6 (0.90)	2.0 (0.98)
Control	1.0 (0.99)	-0.1 (0.15)*	0.1 (0.77)	-1.0 (0.78)	1.8 (0.96)
DL-alanine					
Gymnema	0.6 (0.91)	0.9 (0.90)	0.4 (0.78)	-0.4 (0.67)	0.6 (0.92)
Control	0.7 (0.97)	1.1 (0.92)	0.7 (0.87)	-0.5 (0.81)	0.8 (0.91)
Glycine					
Gymnema	0.8 (0.95)	1.2 (0.94)	0.9 (0.94)	0.8 (0.80)	0.7 (0.93)
Control	0.7 (0.94)	0.8 (0.95)	1.0 (0.86)	-0.6 (0.99)	1.1 (0.99)

\*Clearly, these data aren't fit by power functions.

dl-alanine. In contrast, magnitude estimations for the AG condition were significantly higher ( $p < 0.01$ ) than for control condition for the sourness of glycine, the bitterness of sucrose and dl-alanine, and the saltiness of sucrose.

#### DISCUSSION

##### *Taste Category Specificity of G Extracts*

In view of the apparent psychophysical [1] and electrophysiological [2] evidence in favor of a specific action of G extracts on sweetness judgments or response, the finding of other effects of the G deserves special attention. Three possible explanations can be considered. The first is that the effect of active G extracts is not restricted to the sweet taste category. Past studies with G extracts have used different preparations, which vary in the degree to which the different gymnemic acids (usually labeled  $A_1$ ,  $A_2$ , etc.) are present [25]. It is possible that these different acids differ in their activity, both quantitatively and qualitatively. Also more components may exist, or some components already uncovered might be further separable.

A second possibility is that the removal of sweetness by G extracts produces the analog of modifying a taste mixture. For example, adding a moderate amount of sucrose to solutions which are described as salty, bitter, or sour generally depresses the estimated magnitude of the latter taste quality categories [19], an effect which is probably central in origin [9]. If adding a source of sweetness estimates depresses other tastes, then decreasing sweetness might produce apparent enhancement through release of suppression. This could account for four of the present six statistically significant effects of AG, other than its effects on total taste intensity and sweet intensity: Increased saltiness of sucrose, bitterness of sucrose and dl-alanine, and increased sourness of glycine. This explanation would not account for the reduction of dl-alanine saltiness or the reduction of sucrose sourness, both quantitatively small, though statistically significant effects, nor would it account for the lack of enhancement for the saltiness of glycine and the sourness of dl-alanine.

The third possibility is that procedural differences between the present study and the previous report of sweet specificity

of G extracts [1] might account for some of the results. Bartoshuk *et al.* presented HG before each stimulus, while the present study used only one 60 sec AG presentation which ended five minutes before each 30 min session. While a sweet reducing effect of G extracts certainly lasts this long [14, 17], the qualitatively effects of AG might vary over time. Therefore, effects of AG on complex stimuli should perhaps be investigated using the peak effect of G at about one min after application [17]. However, repeated presentations of G extracts may also introduce problems, because of prolonged yet slowly developing effect on sweetness estimates for sucrose ([14, p. 467; [17]), and the noncompetitive inhibition of sweetness estimates for amino acids [7]. These factors may suggest possible penetration into the receptor cells, or a gradual allosteric interaction [6].

##### *Taste Psychophysics*

The present results confirm a changing proportion of quality categories with changing concentration of the amino acids (Table 1). While the specific percentages for glycine are not identical with those previously reported [26], the trends are in the same directions: with increasing concentration, there is increasing percentage sweetness and sourness, decreasing bitterness, and negligible saltiness. A similar pattern held for dl-alanine although saltiness was at a higher but stable percentage. Sucrose, usually considered a pure or simple sweet stimulus, showed the same degree of bitterness as the two amino acids, with relatively small saltiness and sourness. Halpern [8] had also noted increasing bitterness reports with decreasing sucrose concentration. Perhaps other pure stimuli should be reinvestigated. This bitterness of sucrose at low concentration is possibly responsible for the lower exponents reported for the power functions relating sucrose concentration and total taste intensity rather than just sweetness intensity. The former are usually less than 1.0, whereas the latter are usually greater than 1.0 (Meiselman, unpublished manuscript). The addition of bitterness at lower intensities raises the lower end of the function producing a flatter function with lower slope, i.e., bitterness is decreasing while sweetness is increasing as concentration increases, thus producing a slower rise for total intensity than for sweetness.

Several prior experiments have attempted to determine the sweetness of the amino acids relative to sucrose [5, 10, 11, 20, 22, 31]. Alanine has been reported to be more sweet than sucrose or glycine. Although the relationship between glycine and sucrose is not clear, glycine is probably more sweet. The magnitude estimation quadrifid category profile data (Fig. 1) permit determination of equal taste contours. A line parallel to the abscissa intersects points of the functions at levels of equal taste intensity for each taste quality category. Dropping a vertical from these points to the abscissa allows one to read off the concentration corresponding to an equal taste intensity of that taste category. Thus, for any level of sweetness the concentrations of the compounds are ordered as follows: sucrose < dl-alanine < glycine. That is, to obtain a constant level of sweetness one would have to choose a higher concentration of dl-alanine than sucrose and an even higher concentration of glycine. The results can also be viewed in terms of what levels of sweetness are produced by the same concentration of the stimuli. It is not always clear from earlier experiments using amino acids whether subjects were judging the sweetness component of the overall taste, or whether they were judging the overall taste at a concentration which has a large percentage contribution of sweetness. Although this problem is eliminated in the present data, no attempt was made to qualitatively compare the sweetness.

The exponents generated by fitting power functions to the log-log converted data (Table 2) indicate that for these multi-dimensional taste stimuli, such functions can describe the estimations of more than just the predominant taste. Thus, power functions adequately approximate the relationship between the physical intensity of all three compounds and the total taste intensity, as well as the sweet intensity and bitter intensity. This relationship also holds for the sourness and saltiness of the amino acids.

Stone and Oliver [27] recently reported power functions with exponents near 1.0 obtained through magnitude estimations for dl-alanine, glycine and sucrose. However, it should be noted that not all dimensions of a multidimensional taste stimulus are related to concentration by a power function. If one assumes that a linear addition of simple taste intensities produces compound tastes, and if one assumes that total taste intensity is related to stimulus concentration by a power function [18, 25], then all dimensions of a stimulus should not be power functions since power functions do not sum to produce a power function.

The values of the exponents from the control conditions provide interesting comparisons with exponents for power functions previously reported for these compounds and these quality categories (Table 3). All exponents that are reported were obtained by using the sip technique. For several conditions, the exponent is similar for the same quality category previously determined for an assumed simple tasting compound and now determined for the same qualitative dimension for a known complex stimulus source. For example, previously reported exponents for the sourness of two commonly used acids, hydrochloric and citric, are close to the exponent of sourness of the amino acids. This suggests the possibility that the exponents considered to be characteristic of taste functions might be related to the taste response category (quality) rather than the compound. In other words, if variability among exponents due to many factors of procedure could be removed, then a unique exponent might be representative of each response category, that is, each qualitative dimension. To be most effective, qualitative descriptions must be used that fully encompass the stimulus dimension.

TABLE 3

POWER FUNCTION EXPONENTS FOR SEVERAL TASTE QUALITY CATEGORIES AND STIMULI

Quality Category	Present Experiment	Other Experiments Range	Reference	Reference Compound
Sucrose (sweet)	1.8	1.6-1.4	Moskowitz 1970	sucrose
			Moskowitz 1970	sucrose
Sucrose (total)	1.0	1.4-0.6	Moskowitz 1970	sucrose
			Gregson and Russell 1965	sucrose
dl-Alanine (sour)	1.1	0.9-	Meiselman 1968	HCl
Glycine (sour)	0.8	0.7	Moskowitz 1970	citric acid
dl-Alanine (salty)	0.7	0.9-	Bruvold and Gaffey 1965	NaCl
Glycine (salty)	1.0	1.6	Ekman 1961	NaCl
Sucrose (bitter)	-1.0	0.54-	Meiselman 1970	QSO <sub>4</sub>
dl-Alanine (bitter)	-0.5	1.0	Stevens 1969	QSO <sub>4</sub>
dl-Alanine (sweet)	0.8	1.0	Stone and Oliver 1969	dl-Alanine
Glycine (sweet)	1.1	1.0	Stone and Oliver 1969	glycine

G Extracts and Taste Psychophysics

AG treatment produced a decrease of percentage sweetness for all stimuli used. In other conditions where AG had a large and stable effect across concentrations on one quality category, e.g. the bitterness of dl-alanine, there was a concomitant change in the percentage contribution of each taste category with AG, relative to control (Table 1). In other words, when a taste quality category decreased or increased in estimated magnitude with AG treatments, the relative contribution of that quality category also decreased or increased. Moreover, both the magnitude estimates and percentage contributions of sweet and bitter tend to show reduction of one quality category along with growth of another quality category. This reciprocity is clearer with another plant product with taste modifying properties, miracle fruit [1, 15]. With miracle fruit, sweetness increase is accompanied by sourness reduction. With AG, the reciprocal relationship is less consistent, and incomplete.

The power functions indicate the types of transformation which AG performs on the taste functions. For both total and sweet categories for all three compounds, the AG function and control function are near parallel. This also applies to the bitterness and saltiness of dl-alanine. The constant separation on log-log coordinates indicates that the AG treatment is removing (or adding) a constant percentage of the stimulus intensity measured under control tea conditions. Thus, rather than adding or subtracting a fixed amount to

the control functions (which would produce diverging or converging functions on log-log coordinates), AG produces a fixed magnification or contraction of the control functions.

Since the effect of G varies with the concentration of the stimulus, some concentration-dependent interaction be-

tween the two such as competition must be posited. This supports previous suggestions [3, 7, 28] that G extracts have an inhibitory action on membrane receptor sites involved in eliciting sweetness, and perhaps other quality categories also. The detailed nature of the inhibition is still unclear.

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