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Taste Confusions following Gymnemic Acid Rinse

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Abstract

The effect of a gymnemic acid (GA) rinse, which simulated a sweet-taste deficit, was measured on human taste perception and identification. Taste ratings showed that GA reduced the intensities of sucrose and aspartame to 14% of pre-rinse levels; over the recovery interval of 30 min, these values increased linearly to 63% of the pre-rinse levels. Repeated presentations of a set of 10 stimuli (five primarily or partly sweet—sucrose, aspartame, and NaCl–sucrose, acid–sucrose and quinine–sucrose mixtures; and five nonsweet—NaCl, KCl, Na glutamate (MSG), quinine·HCl and citric acid) for identification following water and GA rinses produced 'taste confusion matrices' (TCMs). Correct identification of the sweet-tasting stimuli was reduced by 23% in presentations closely following the GA rinse, an effect that dissipated with time. Most misidentifications involved sucrose and mixtures containing sucrose. In a second TCM experiment, GA was presented frequently within each session to maintain the sweet taste deficit, which revealed itself as specific confusions. Rinsing with GA impaired discriminability of sweet–nonsweet pairs of stimuli but enhanced discriminability of the aspartame–(NaCl–sucrose) pair. GA had no effect on discriminability of nonsweet stimulus pairs. The results suggest that specific error patterns in the TCM could be used to identify quality-specific taste disorders.

Introduction

Our interest in exploring the use of stimulus identification reflects our goal of constructing objective, performance-based measures of suprathreshold taste function. This interest arises from our experience with patients presenting to the Taste and Smell Clinic of the Connecticut Chemosensory Clinical Research Center. Measures of identification have two virtues: they reveal perceptual performance at levels of stimulation that characterize much daily experience and they are objective, in the sense that responses can be classified as correct or incorrect. In a recent paper (Hettinger *et al.*, 1999), we characterized a stimulus-identification task which has potential for evaluating suprathreshold taste function. The resulting 'taste-confusion matrix' is composed of identification response frequencies to a set of 10 stimuli presented 10 times each; responses were chosen from among a closed set of 10 stimulus names. The response matrix reveals not only the number of correct identifications, but also the pattern of incorrect choices for each stimulus in the set.

Although taste-quality identification is routinely assessed in chemosensory clinical centers (Frank *et al.*, 1995; Gent *et al.*, 1997), patients are typically asked to identify perceptual qualities, such as sweet, sour, salty or bitter, rather than stimuli, such as sugar, acid, salt or quinine. The data are reported simply in terms of frequency of quality identification (Cowart, 1989; Deems *et al.*, 1991). In contrast, clinical tests of smell emphasize stimulus identification (Doty *et al.*, 1984; Cain and Gent, 1986; Wright, 1987). Patterns of errors in stimulus-identification tasks may provide important diagnostic information regarding smell function (Wright *et al.*, 1991). Such an approach has not been attempted before for taste function.

The array of 10 taste stimuli that we use includes both single substances and mixtures. We ask the subjects to identify each substance from a set of response labels containing the names of all of the stimuli. Groups of subjects with normal taste function performed consistently at this task (Hettinger *et al.*, 1999). If a stimulus-identification test designed along these lines is to be useful in clinical settings, it needs to identify deficits and/or distortions in taste function. To this end, the present study seeks to show that the 'taste-confusion matrix' (TCM) is sensitive to a simulated deficit in sweet taste perception engendered by application of gymnemic acid (GA). Work on the olfactory system shows that a smell deficit results in poor stimulus identification (Cowart *et al.*, 1997).

Gymnemic acid, a mixture of triterpene saponins, was discovered in 1847 to temporarily reduce or abolish the sweet taste of sugar in humans (Hellekant and van der Wel, 1989). Because GA has a specific (Bartoshuk *et al.*, 1969; Oakley, 1985) and profound (Frank *et al.*, 1992) effect on the

sweetness of sugars and other sweet substances, the application of GA serves as a useful model of dissociated taste loss (Tomita and Horikawa, 1986). Thus, in the present study we examine how the application of GA to the tongue modifies the pattern of taste identification for a TCM. In particular, we hypothesized that pre-rinsing with GA would make stimuli that are primarily sweet tasting more difficult to identify. Furthermore, because binary mixtures are normally confused with the mixture components (Hettinger *et al.*, 1999), we hypothesized that after GA treatment, mixtures containing one sweet-tasting and one nonsweet-tasting substance (e.g. sucrose plus NaCl) would be more difficult to distinguish from the nonsweet component (i.e. NaCl alone).

We report here how the application to the tongue of GA modifies the TCM. From the matrix of correct and incorrect responses for each subject (the TCM) we calculate two quantitative measures of performance, namely, percent correct and transmitted information, T (Hettinger *et al.*, 1999).

Experiment 1. Taste perception after GA rinse

Experiment 1 measured how the perceived intensity of sucrose, aspartame and NaCl taste varied as a function of time following application of GA.

Subjects

Six subjects (three women, three men), aged 20–55 (mean = 42, SD = 9 years), participated in one session. Subjects for this and subsequent experiments were recruited from the students and staff of the University of Connecticut Health Center. This study was approved by the Institutional Review Board of the University of Connecticut Health Center. All subjects gave informed consent for participation.

Stimuli and treatment rinse

The stimuli used were 0.3 M sucrose (reagent grade; Baker, Phillipsburg, NJ), 3.0 mM aspartame (for laboratory use; Searle, Arlington Heights, IL), 0.1 M NaCl (Baker, reagent grade) and water (reverse osmosis deionized). Sucrose and aspartame are primarily sweet and NaCl is primarily salty (DuBois *et al.*, 1991; Smith and van der Klaauw, 1995; Hettinger *et al.*, 1996). The GA treatment rinse was prepared by a modification of a previously published procedure (Warren and Pfaffmann, 1959). Dried leaves of *Gymnema sylvestre* (50 g) were mixed with 1000 ml of deionized water and heated at 80°C for 4 h. After pressing out the liquid, remaining solids were removed by centrifugation. The cooled extract (~800 ml) was acidified with 50 ml of 1 M HCl and the tan-brown precipitate collected by centrifugation. The dried product (~1 g) was dissolved in 200 ml of 0.1 M $NaHCO_3$ to give a concentration of 0.5% w/v. Solutions were refrigerated, then used within a few days or frozen. After correcting for losses, we estimate this crude GA constituted 2.5% of the weight of the original dry leaves. The product was mostly GA because our yield was comparable to yields of 1.30–1.56% obtained with more complex procedures that likely resulted in additional losses but gave essentially pure products (Kurihara, 1969; Riskey *et al.*, 1982). Thus, ~2.5% of dry leaves of *Gymnema sylvestre* is GA, a useful figure when comparing potencies of extracts with purified GA.

Psychophysical method

Subjects rated the intensity of the stimuli on a 10-point scale, ranging from 0 (tasteless) to 9 (extremely strong), using a 'sip-and-spit' procedure with water rinses between trials. Stimuli were presented for rating before subjects were asked to rinse with GA (two 1 min rinses with 5 ml of GA, followed by a water rinse) and again immediately after and 5, 10, 20 and 30 min after the GA rinse.

Data analysis

The effect of GA on perceived taste intensity over time was examined using a two-way, repeated measures ANOVA. The two factors of interest were compound (NaCl, sucrose and aspartame) and time (six intensity rating points from before to 30 min after the GA rinse). Final post-treatment ratings were compared with pre-treatment ratings using *t*-tests. To establish any difference in the effect of GA on the two sweeteners, sucrose and aspartame, the five post-GA ratings were compared with the pre-GA ratings by a two-way, repeated measures ANOVA. The ratings for water had no variance for many of the times it was rated and were therefore excluded from the analysis.

Results and discussion

Rinsing with GA had an immediate effect on the intensity of sucrose and aspartame, but no effect on water or NaCl

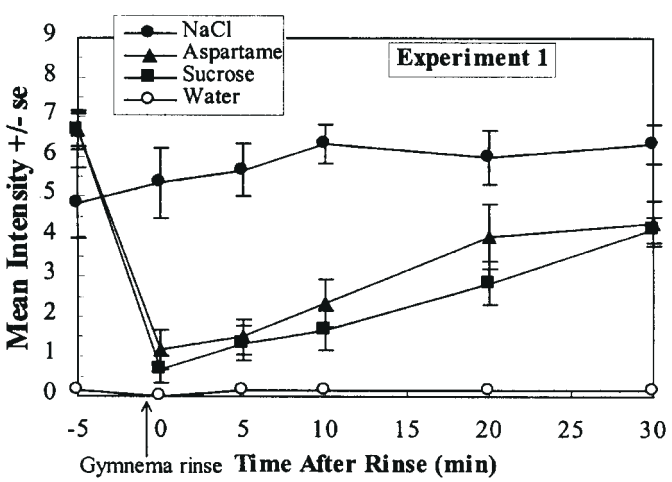

Figure 1 Mean intensity ratings (n = 6) for sucrose, aspartame, NaCl and water before and at various times following a GA rinse (experiment 1).

(Figure 1). The repeated measures tests of within-subjects effects were significant for compound [$F(2,10) = 21.96$, $P < 0.0001$], for time [$F(5,25) = 24.01$, $P < 0.0001$] and for their interaction [$F(10,50) = 10.86$, $P < 0.0001$]. The intensity of sucrose and aspartame was significantly reduced immediately following GA: the mean intensity rating for sucrose fell from 6.7 ± 0.4 to 0.7 ± 0.3 ($t = 10.39$, $P < 0.0001$); and that of aspartame fell from 6.7 ± 0.5 to 1.2 ± 0.5 ($t = 5.74$, $P < 0.002$). Recovery of intensity showed significant linear trends for both compounds [sucrose: $F(1,5) = 52.23$, $P < 0.001$; aspartame: $F(1,5) = 14.55$, $P < 0.012$], but neither returned to its pre-treatment level by 30 min after treatment. At time 30 min the rating of sucrose was 4.2 ± 0.3 ($t = 5.84$, $P < 0.002$) and the rating of aspartame was 4.3 ± 0.6 ($t = 4.18$, $P < 0.009$). The proportional effect of GA on pre-GA taste intensity ratings for the two sweeteners did not differ; neither the main effect of stimulus nor the stimulus by time interaction was significant.

Our results are consistent with previous work attributing to GA a selective and profound effect on sweet but not nonsweet stimuli, yet a general and equal effect on most if not all sweet stimuli irrespective of chemical structure (Riskey *et al.*, 1982; Frank *et al.*, 1992). The time course of recovery that we observed also compares well with earlier data obtained with a 0.05% GA solution (Riskey *et al.*, 1982); the intensity of sucrose (average for 0.08, 0.16, 0.32 and 0.64 M) rose to ~60% of the pre-GA value in 40 min.

Experiment 2. Effect of GA rinse on identification

Using a within-subjects experimental design, experiment 2 compared effects of GA on identification of sweet and nonsweet stimuli at 0–10 min versus 20–30 min following GA application.

Subjects

Ten subjects participated (three women and seven men), aged 17–38 (mean = 23, SD = 6 years).

Stimuli and treatment rinse

The 10 stimuli (and their names) were identical to those used in our other study (Hettinger *et al.*, 1999), and included five solutions that were primarily or partly sweet: 0.3 M sucrose ('sugar'), 3 mM aspartame ('artificial sweetener'), and NaCl–sucrose ('salt–sugar'), citric acid–sucrose ('acid–sugar') and quinine–sucrose ('quinine–sugar') mixtures; and five that were nonsweet: 0.1 M NaCl ('salt'), 0.1 M KCl ('salt substitute'), 0.1 M Na glutamate ('MSG'), 0.1 mM quinine·HCl ('quinine') and 3 mM citric acid ('acid'). The concentrations used to generate components of mixtures were the same as those used in single stimuli. Sucrose, NaCl and KCl were all reagent grade compounds from Baker; citric acid (reagent grade), quinine·HCl and MSG were all from Sigma (St Louis, MO) labeled for laboratory use.

The GA rinse was prepared in the manner described for experiment 1, resulting in a 0.5% GA solution.

Psychophysical method

As in our other study (Hettinger *et al.*, 1999), 100 trials were presented in 10 replicates of 10 stimuli; within each replicate, all 10 stimuli were presented in random order. Using the 'sip-and-spit' method, with water rinses between trials, subjects were asked to taste 5 ml of solution, then name the solution using only names on the list provided. Subjects received no feedback as to the correct label for each of the stimuli. One hour or less was needed to complete each of two sessions; each replicate took 5–6 min to run. In addition, subjects received a 2 min treatment rinse before replicates 1 and 6 (trials 1 and 51). The rinse was water in session 1 and 0.5% GA in session 2.

Data analysis

A matrix containing frequencies of each response for all stimuli was generated for each subject—for examples see Table 1 and our other study (Hettinger *et al.*, 1999). From each subject's TCM we derived the percent correct identification for each stimulus. Using percent correct for the sweet stimuli from session 2, in which GA was applied, we used a *t*-test to examine the effects of time after GA rinse. Because the effect of GA fades with time (experiment 1), performance should be reduced more for replicates closest to GA rinse (replicates 1, 2, 6 and 7) than for replicates farthest from the rinse (replicates 4, 5, 9, 10). The effect of the GA rinse was examined separately for the sweet and nonsweet stimuli with a two-way analysis of variance (ANOVA) with two main effects: session (1: water rinse, 2: GA rinse) and stimulus. Separate analyses were necessary because GA selectively affects sweet intensity (Riskey *et al.*, 1982), and identification performance with the nonsweet stimuli improves with practice (Hettinger *et al.*, 1999). Given the result of the *t*-test described above, we also repeated the two-way ANOVA using data for sweet stimuli from replicates 1, 2, 6 and 7.

Results and discussion

In session 2, the sweet stimuli were identified less accurately in the four replicates immediately following the GA rinse (36 ± 5% correct) compared with the four replicates further from the GA rinse (59 ± 6% correct) ($t = 3.36$, $P < 0.008$). Patterns of errors for 'near' replicates (1, 2, 6 and 7) were compared with patterns of errors for 'far' replicates (4, 5, 9 and 10). On average, the three mixtures were more frequently misidentified as a nonsweet mixture component in the 'near' (34.2%) than 'far' replicates (12.5%) and sucrose was more frequently misidentified as a mixture or nonsweet stimulus in the 'near' (20.0%) than 'far' replicates (7.5%).

Table 1 Identification response matrix for one of the subjects in the GA treatment group

Stimulus	Response label										
	'Salt'	'Salt-sub'	'MSG'	'Qui.'	'Acid'	'Sugar'	'Art swtnr'	'Salt–sugar'	'Acid–sugar'	'Quin–sugar'	Row sum
NaCl	**8**	0	0	0	0	0	0	2	0	0	10
KCl	2	**7**	0	1	0	0	0	0	0	0	10
MSG	0	6	**0**	0	0	0	1	2	1	0	10
Quinine·HCl	0	0	2	**8**	0	0	0	0	0	0	10
Citric acid	0	0	0	0	**8**	0	0	0	1	1	10
Sucrose	0	1	1	1	1	**0**	***4***	0	1	1	10
Aspartame	0	0	0	1	2	***1***	**4**	0	2	0	10
NaCl–sucrose	***6***	0	0	0	0	0	0	**4**	0	0	10
Acid–sucrose	0	0	0	0	***7***	0	0	0	**3**	0	10
Quinine–sucrose	0	0	2	***4***	0	0	1	1	0	**2**	10
Column sum	16	14	5	15	18	1	10	9	8	4	100

Each cell represents the number of times a particular stimulus (far left column) was called by a particular name ('response label'). 'Correct' responses (bold) and typical errors in sweet-stimulus identification made by subjects in this group (bold italic) are indicated.

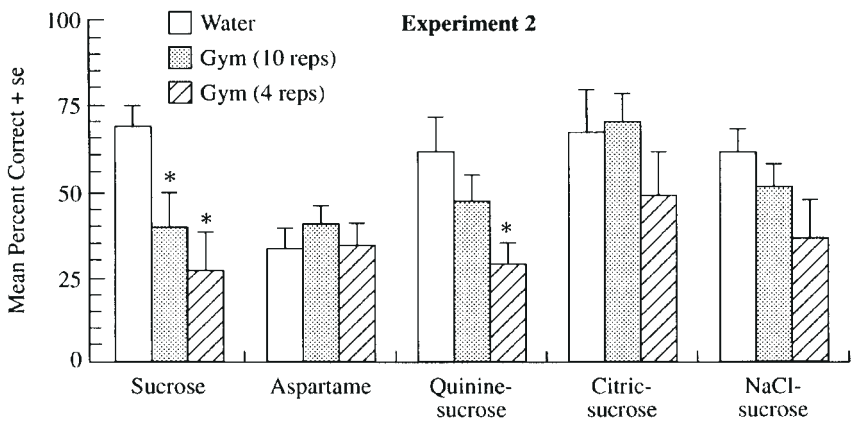

Figure 2 Mean percent correct for sweet stimuli (sucrose, aspartame, NaCl–sucrose, acid–sucrose, quinine–sucrose) in experiment 2 ($n = 10$) following water rinses in session 1 (open bars), or GA rinses in session 2 represented by all replicates (stippled bars) or the four replicates closest to the GA rinse (striped bars). Significant differences in rinse condition are indicated (t-test, $P < 0.01$, one-tailed).

The ANOVA comparing percent correct for all 10 replicates of the sweet stimuli in water and GA sessions did not reveal a significant effect of rinse ($P < 0.062$), even though average percent correct fell from 59 ± 4% in session 1 (water) to 50 ± 3% in session 2 (GA). However, rinse condition had a significant effect on performance in the four 'near' replicates: 1, 2, 6 and 7 [$F(1,9) = 17.39$, $P < 0.002$], with percent correct falling from 59% in session 1 (water) to 36% in session 2 (GA) (Figure 2). Collapsed across sessions, the accuracy with which the five different sweet stimuli were correctly identified in all 10 replicates differed [$F(4,36) = 3.22$, $P < 0.023$], as expected, given that normal individuals identify aspartame less accurately than the other sweet stimuli (Hettinger *et al.*, 1999). More to the point, there was a significant interaction of rinse condition and sweet stimulus both for all 10 replicates [$F(4,36) = 2.793$, $P < 0.041$] and for the four 'near' replicates: 1, 2, 6 and 7 [$F(4,36) = 2.787$, $P < 0.041$]. Post-hoc contrasts ($\alpha = 0.01$) demonstrated significant decreases in percent correct in session 2 (GA) compared with session 1 (water). For sucrose, percent correct fell from 69.6 ± 6% to 40 ± 10% in all 10 replicates, and to 28 ± 11% in replicates 1, 2, 6 and 7; for the quinine–sucrose mixture it fell from 62 ± 10% to 30 ± 6% in replicates 1, 2, 6 and 7.

The ANOVA for the nonsweet compounds showed no significant effect of rinse or rinse by stimulus interaction. There was a significant effect of stimulus [$F(4,36) = 8.78$, $P < 0.0001$], confirming the differences in correct identification for the stimuli shown in our other report (Hettinger *et al.*, 1999).

In summary, the profound decrease in the intensity of sweet stimuli immediately following application of a 0.5% solution of GA (experiment 1) results in decreased accuracy in identifying sweet stimuli. Accuracy of identification improves as the effect of GA wears off with time. In fact, for the replicates farthest from the GA rinse (4, 5, 9 and 10), identification performance in session 2 (GA) was the same as in session 1 (water) at 59%.

In experiment 1 we showed that the perceived taste intensity of either sucrose or aspartame, which have a sweet taste quality, was reduced to ~ 15% of pre-rinse intensity by a GA rinse but recovered linearly with time. Intensity recovered another 15% of its pre-rinse value every 10 min. Given that each session took about 60 min to run, in the 20 min spent running replicates 1–3 or 6–8, intensity would recover to ~50% of its value before GA. Apparently, subjects could identify stimuli at half strength as accurately as stimuli at full strength. In our other report, we addressed effects of stimulus intensity on identification (Hettinger *et al.*, 1999). Those data and the current finding support the idea that identification improves as intensity increases until a peak is reached, beyond which further increase in stimulus intensity may result in decreased accuracy.

Experiment 3. Effect of multiple GA rinses on identification

In order to maintain its maximal effect on sweet stimulus intensity, subjects in experiment 3 rinsed with GA frequently during the course of a session. A between-subjects experimental design was used to assess the consequent decrease in sweetness on the identification of sweet and nonsweet stimuli. The measures of stimulus identification used in the analyses were percent correct and measures derived from information theory that quantify consistency and discriminability. We discuss the results of experiment 3 in the context of the oft-cited confusions in taste labels made by normal people (Kuznicki *et al.*, 1983; Cowart *et al.*, 1997; Ossebaard *et al.*, 1997), and in the face of evidence that practice and training improve accuracy of taste-stimulus identification (Hettinger *et al.*, 1999).

Subjects

Twenty-four subjects participated in experiment 3. Twelve served in a control (water rinse) group (six women and six men), aged 22–52 (mean = 38, SD = 12 years), and 12 served in the GA-rinse group (eight women and four men), aged 20–64 (mean = 36, SD = 13 years).

Stimuli and treatment rinse

Stimuli and their names were the same as those used in experiment 2. The GA rinse was prepared in the manner described in experiment 1 and 2, resulting in a 0.5% GA solution.

Psychophysical method

Subjects were asked to choose correct stimulus names for the 10 test stimuli, again presented 10 times each in a single session without feedback, as in experiment 2. Each subject received a 2 min rinse every two replicates (i.e. prior to replicates 1, 3, 5, 7 and 9), instead of every five replicates as in experiment 2. Twelve subjects (control group) rinsed with water and another 12 (GA group) rinsed with 0.5% GA.

Data analysis

The resulting TCMs were used to generate measures of performance based on information theory. Two measures of T, bits of information transferred, were calculated as described elsewhere (Hettinger *et al.*, 1999). The first was an overall measure of consistency of performance using the full 10 × 10 response matrix: T_{10}. The second was T_2, a measure of pairwise stimulus discriminability using the forty-five 2 × 10 response matrices. T_2 quantifies the difference in the response patterns for each pair of stimuli. In this analysis, $T = H_x + H_y - H_{xy}$ bits of information, where $H_x = \Sigma[p_x \times \log_2(1/p_x)]$, $H_y = \Sigma[p_y \times \log_2(1/p_y)]$ and $H_{xy} = \Sigma[p_{xy} \times \log_2(1/p_{xy})]$. H_x is the information contained in the 10 stimuli, H_y is the information in the 10 response labels and H_{xy} is the information in the 100 stimulus–response combinations. The probabilities of stimulus presentations (p_x) were set at 0.1, i.e. 10 out of 100; probabilities of response–label use (p_y) and of each stimulus–response combination (p_{xy}) were determined by a subject's performance on the identification task (see Table 1 for an example). Theoretically, T_{10} can range from 0 to 3.32 bits; likewise, T_2 can range from 0 to 1.00 bit. T is maximal when the 10 response labels are used with equal frequency and each response label is used for one stimulus only (e.g. for T_{10}, $H_x = 3.32$, $H_y = 3.32$ and $H_{xy} = 3.32$). T is minimal when each response label is used for every stimulus (e.g. for T_{10}, $H_x = 3.32$, $H_y = 3.32$ and $H_{xy} = 6.64$) and all stimulus–response combination frequencies are 1. Close to maximum T (perfect performance) has been achieved by some subjects, but minimum T is very unlikely with merely 10 replicates of random assignment. For further discussion of these topics, see our other study (Hettinger *et al.*, 1999).

Subjects' taste confusion matrices were also used to generate the measures of performance used in experiment 2: percent correct and error patterns for each sweet stimulus.

We tested with a one-tailed, independent t-test whether the overall consistency of response, as measured by T_{10}, was greater for the water-rinse than the GA-rinse group. We examined with three separate two-way ANOVAs the effect of GA on T_2 (Hettinger *et al.*, 1999). The three ANOVAs were for (1) the 25 stimulus pairs composed of one primarily or partly sweet and one nonsweet stimulus; (2) the 10 stimulus pairs composed of two sweet stimuli; and (3) the 10 stimulus pairs composed of two nonsweet stimuli. The main variables were rinse group and stimulus pair.

Analysis of percent correct was similar to that described for experiment 2. Separate, repeated measures ANOVAs were performed for the sweet and nonsweet stimuli. The between-subjects factor was rinse (water or GA); the within-subjects repeated measure was stimulus. Percent correct was calculated in two ways: (1) for 10 'correct labels' as in experiment 2 and (2) with 'correct' defined in terms of five response categories. Using response categories based on errors made by normal subjects (Hettinger *et al.*, 1999) minimized between-group differences due to unfamiliar stimuli or stimulus names. The five categories were created as follows: (1) a response of 'salt', 'salt substitute' or 'MSG' was treated as 'correct' for salts: NaCl, KCl, and MSG; (2) either 'sugar' or 'artificial sweetener' was treated as correct for sucrose and aspartame; (3) either 'quinine' or 'acid' was treated as correct for quinine·HCl and citric acid; (4) either 'acid–sugar' or quinine–sugar' was treated as correct for the acid–sucrose and quinine–sucrose mixture; and (5) 'salt–sugar' remained the only correct label for the NaCl–sucrose mixture.

Percent correct calculated on the basis of the five response categories was used for additional analyses that examined the effect of GA rinse on the pattern of response errors for the three sucrose mixtures. A two-way ANOVA with multiple comparisons was run with rinse condition (either GA or water) as the between-subjects factor and response label (limited to three categories: the mixture label and the labels for each of the components) as the within-subjects factor.

Results and discussion

The use of frequent rinses maintained the efficacy of GA throughout the test session. Average percent-correct identification of the five sweet stimuli was 36 ± 5% both for replicates 1, 3, 5, 7 and 9, which immediately followed a GA rinse, and for replicates 2, 4, 6, 8 and 10, which immediately preceded the next GA rinse. Thus, we succeeded in maintaining a maximal effect of GA on stimulus identification.

Overall consistency of performance as measured by T_{10}, which has a maximum value 3.32 bits, was lower for the GA-rinse group (mean = 1.84 ± 0.07 bits) than for the water-rinse group (mean = 2.08 ± 0.10 bits) ($t = 2.04$, $P < 0.026$). The percent correct for all 10 stimuli (sweet and nonsweet stimuli combined) was 45 ± 3% for both the GA-rinse and water-rinse groups. Thus consistency for the entire 10 × 10 matrix was more sensitive to the effect of GA than percent correct, a result that is explained by a consistent but incorrect use of labels by some subjects (Hettinger *et al.*, 1999). T_{10} and average percent correct (10 response labels) were correlated for all 24 subjects: $r = 0.77$ (significantly greater than 0, $P < 0.0001$). A similar significant correlation between T_{10} and percent correct was also observed for 42 subjects (Hettinger *et al.*, 1999). As was pointed out in that study, a high level of percent correct necessarily yields high values of T_{10}; however, low values of percent correct may not yield low values of T_{10} if subjects use 'incorrect' labels consistently.

Repeated measures ANOVAs of the pairwise stimulus discriminability, as measured by T_2, showed the ability of the GA-rinse group to discriminate the 25 pairs representing one nonsweet and one sweet stimulus to be inferior to the ability of the water-rinse group. The average value of T_2 of 0.79 ± 0.02 bit for the GA-rinse group was lower [$F(1,22) = 25.36$, $P < 0.0001$] than the average T_2 of 0.93 ± 0.02 bit for the water-rinse group. The value for the water-rinse group is close to the theoretical maximum 1.0 bit and is identical to the performance for sweet–nonsweet pairs observed previously (Hettinger *et al.*, 1999). The reduced intensity of the sweeteners presumably made it more difficult to distinguish them consistently from nonsweet stimuli.

The significant interaction term for rinse and stimulus pair [$F(24,528) = 5.06$, $P < 0.0001$] suggests a variable effect of GA on T_2, the nature of which we addressed with additional analyses. Table 2 shows results of analyses for the 25 nonsweet–sweet pairs grouped as follows: the 10 pairs involving a nonsweet stimulus and sucrose or aspartame and the three sets of five pairs involving a nonsweet stimulus and a sucrose mixture. For each of these sets of stimulus pairs, the water-rinse group performed better than the GA-rinse group. Figure 3 shows the significant post-hoc comparisons ($\alpha = 0.01$), all of which involve discriminations of the sweet mixtures with a nonsweet stimulus. An analysis of the exact errors that the subjects in the GA group made follows after our presentation of percent-correct measures below.

Rinse condition had no overall effect on the 10 pairs representing the discrimination of two sweet stimuli. However, there was a significant interaction of rinse and stimulus pair [$F(9,198) = 3.09$, $P < 0.002$]. The GA-rinse group was significantly better at discriminating aspartame from the salt–sucrose mixture ($T_2 = 0.97 \pm 0.03$ bit) than the water-rinse group ($T_2 = 0.65 \pm 0.09$ bit) (post-hoc comparisons, $\alpha = 0.01$). Table 1 is the matrix of an individual in the GA group who discriminated this pair of stimuli perfectly ($T_2 = 1.0$); there was no overlap in responses. However, subjects who rinsed with water identified the NaCl–sucrose mixture as 'sugar' or 'artificial sweetener' 21% of the time (Hettinger *et al.*, 1999). Such mistakes would be expected to be less frequent, given the reduction in sweetness of sucrose in this mixture, and would improve discriminability.

Finally, rinse had no effect and there was no rinse by pair interaction for the 10 pairs representing the discrimination of two nonsweet stimuli.

The results of the repeated measures ANOVAs of the percent-correct responses calculated using the original 10 response labels are shown in Table 3. Rinse condition (group) failed to reveal a significant effect on correct identification of the sweet solutions; although averages were 50 ± 5% correct for the water-rinse group and 36 ± 5% for the GA-rinse group. However, the rinse condition by compound inter-

Table 2 Analysis of nonsweet versus sweet stimulus discriminability in experiment 3

Sweets (no. of pairs)	T_2 (± SE)		ANOVA			
	Rinse group		Effect: rinse		Rinse × pair	
	Water	GA	$F(1,22)$	$P <$	F	$P <$
Sucrose or aspartame (10)			5.59	0.0270	1.37	ns
Mean	0.98 ± 0.01	0.92 ± 0.03				
Min	0.96 ± 0.03	0.82 ± 0.07				
Max	1.00 + 0.00	0.99 + 0.01				
Acid–sucrose (5)			19.36	0.0001	10.71	0.0001
Mean	0.90 ± 0.03	0.73 ± 0.03				
Min	0.82 ± 0.06	0.28 ± 0.05				
Max	0.95 ± 0.02	0.88 ± 0.04				
Quinine–sucrose (5)			7.04	0.0150	2.8	0.0310
Mean	0.85 ± 0.04	0.72 ± 0.03				
Min	0.73 ± 0.09	0.39 ± 0.08				
Max	0.96 ± 0.02	0.85 ± 0.04				
NaCl–sucrose (5)			23.57	0.0001	8.06	0.0001
Mean	0.92 ± 0.03	0.68 ± 0.04				
Min	0.82 ± 0.05	0.35 ± 0.06				
Max	0.99 ± 0.01	0.95 ± 0.03				
All sweet (25)	0.93 ± 0.03	0.79 ± 0.02	25.36	0.0001	5.06	0.0001

Mean values of T_2 are shown for each rinse group for pairs representing the ability to discriminate between a nonsweet stimulus and the sweet stimuli. Results of the repeated measures ANOVA are shown for the effect of rinse (GA or water) and the rinse by stimulus pair interaction.

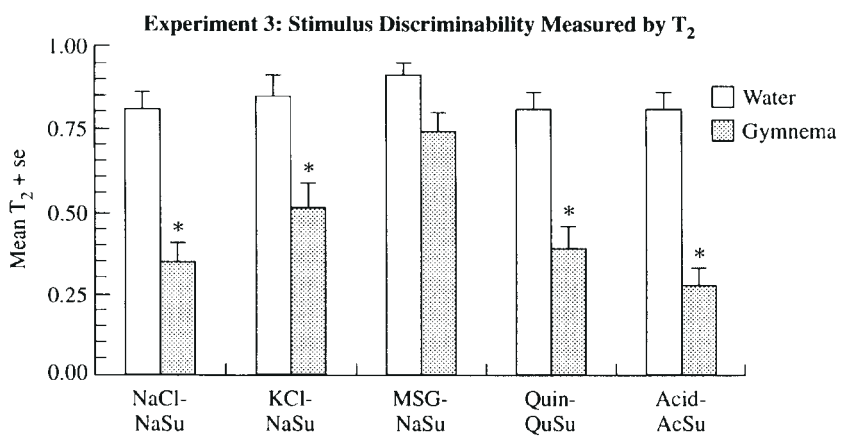

Figure 3 Discriminability, as measured by T_2, of the sweet mixtures versus nonsweet stimuli in experiment 3. Significant differences between subjects in the water ($n = 12$, open bars) and GA ($n = 12$, stippled bars) rinse conditions are indicated (t-test, $P < 0.01$, one-tailed)

action was significant and, as in experiment 2, the greatest effect was for identification of sucrose. The water-rinse group identified sucrose correctly 67 ± 8% of the time but the GA group identified it correctly 32 ± 8% of the time.

The results for the sweet stimuli were more straightforward when percent correct was calculated from the five response–label combinations (Table 3). The average 55 ± 6% correct performance for sweet stimuli by the GA group was significantly lower than the average 76 ± 3% correct performance by the water control group. There was no rinse by stimulus interaction.

Although there was no between-subjects group effect of rinse condition for the nonsweet stimuli for either the 10- or the 5-label analysis, there was a significant group by compound interaction with the 10-label analysis (Table 3). This result may be explained by a fortuitous assignment of subjects: the water-rinse group correctly identified quinine-HCl and citric acid less frequently than the GA-rinse group

Table 3 Repeated measures analysis of percent correct data from experiment 3

	df	10 labels		5 labels	
		F	*P* <	*F*	*P* <
Sweet					
Group	1,22	3.92	0.061	9.55	0.005*
Compound	4,88	3.76	0.007*	15.76	0.0001*
Grp × cmp	4,88	2.53	0.046*	0.87	0.483
Nonsweet					
Group	1,22	0.70	0.413	1.20	0.285
Compound	4,88	5.81	0.0001*	3.00	0.023*
Grp × cmp	4,88	2.62	0.041*	0.04	0.997

Analyses were performed on percent correct calculated using all 10 responses and on five response–label combinations (see text). The between group factor is rinse condition (water, $n = 12$, or GA, $n = 12$).

(44 ± 11 versus 71 ± 10% and 44 ± 11 versus 64 ± 9%, respectively) . This disparity in percent-correct performance, attributable to the taste-quality label confusions made by normal people (Kuznicki *et al.*, 1983; Cowart *et al.*, 1997; Ossebaard *et al.*, 1997), necessarily disappeared when the response labels for quinine and acid were combined. Similarly, the significant group by compound interaction for the 10-label analysis for the sweet stimuli is partly attributable to the same fortuitous group difference, specifically with regard to correct identification of the quinine–sucrose and acid–sucrose mixtures. In this case, when labels were combined, the effect of GA on group performance was revealed.

Figure 4 shows the effect of GA rinse on the pattern of five response–label combinations used for each sweet stimulus. For the analysis of the sucrose mixtures, we were interested in the number of times out of the 10 stimulus presentations that each subject used the 'correct' mixture label compared with one of the two mixture-component labels. Label had a significant effect [$F(6,17) = 10.90$, $P < 0.0001$], which indicated that subjects applied different labels to the different mixtures. More relevant to the effects of GA, the label by rinse-group interaction was also significant [$F(6,17) = 5.42$, $P < 0.003$]. Contrasts for interactions of labels and rinse group were significant for all three mixtures: NaCl–sucrose [$F(1,22) = 11.42$, $P < 0.003$]; acid–sucrose [$F(1,22) = 11.04$, $P < 0.003$]; and quinine–sucrose [$F(1,22) = 6.29$, $P < 0.02$] (Figure 4a–c). In each case, the label for the nonsweet component was used more frequently and the mixture label less frequently by the GA group compared with the control group. The patterns for sucrose and aspartame are also shown (Figure 4d,e). Sucrose was correctly identified significantly less often by the GA group than by the water group ($t = 3.09$, $P < 0.005$) and the results for aspartame showed a similar trend ($t = 1.56$, $P < 0.07$).

This analysis points to the similarity in results from experiments 2 and 3. Given the GA rinse, subjects more frequently

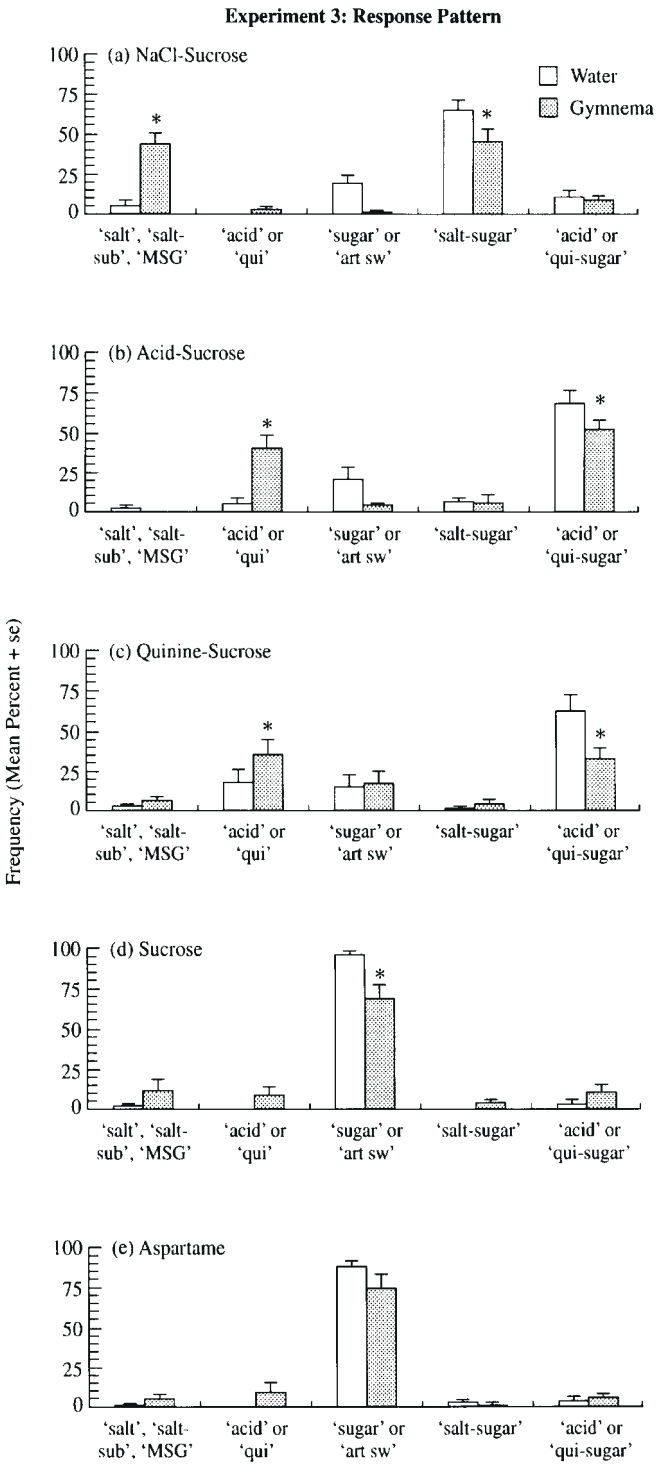

Figure 4 Frequency (mean percent) of five-label categories used for the five sweet stimuli, NaCl–sucrose **(a)**, acid–sucrose **(b)**, quinine–sucrose **(c)**, sucrose **(d)** and aspartame **(e)** presented in experiment 3.

mistook the nonsweet component of the sucrose-containing mixture for the mixture (experiment 3, Figure 4), which resulted in a reduced percent correct (experiment 2, Figure 2). The subjects misidentified sucrose and aspartame more

frequently in the GA-rinse group, but in these cases responses were distributed across many incorrect label categories (Figure 4d,e). Finally, stimulus-pair discriminability, as measured by T_2, was poorer for mixture versus nonsweet component pairs in the GA group (Figure 3). This effect can be seen in the individual matrix for a member of the GA group presented in Table 1. For this subject, T_2 calculated for the 'citric acid' versus 'acid–sucrose' rows reflects 80% response overlap. In the average matrix for 42 people who were not treated with GA, 12% of the responses in these rows overlapped (Hettinger *et al.*, 1999).

Measures of T_2 for the NaCl–sucrose versus nonsweets showed that subjects selected the label for KCl as well as NaCl instead of the mixture label. This is understandable because use of response labels 'salt' and 'salt substitute' for KCl and NaCl overlapped 39% in subjects not treated with GA (Hettinger *et al.*, 1999). Finally, comparison of Figure 4a with 4e further demonstrates why T_2 for the NaCl–sucrose mixture versus aspartame pair was larger for the GA- than the water-rinse group (also see above): mistaking the NaCl–sucrose mixture for aspartame was less likely when the perceptual intensity of sucrose was weak.

General discussion and conclusions

In this general discussion we address the utility of an objective, performance-based, stimulus-identification task, which results in a response–frequency matrix (TCM), in the diagnosis of a specific taste deficit. We comment on prevalence of dissociated taste losses in patients, the relationship between taste-stimulus identification performance and subjective intensity ratings, and specific misidentification patterns for binary stimuli made by individuals experiencing specific taste deficits. We also address the value of performance measures based on 'correct identification' versus measures based on information theory (consistency of identification or discrimination).

Dissociated loss of the sweet taste in humans

Rinsing with GA produced a deficit specific for sweet compounds in normal subjects. This simulates the nearly complete loss of sweet taste, but not other taste qualities, reported for a Japanese population (Tomita and Horikawa, 1986). However, such specific sweet-taste losses are rare among the patients of our Taste and Smell Clinic at the University of Connecticut Health Center. During the 24 month period of 09/96 to 09/98, we identified 88 patients (38 male, mean age 53.2 ± 13.4 years, and 50 female, mean age 53.5 ± 16.4 years) with hypogeusia/ageusia (Gent *et al.*, 1997) for at least one of four prototypic taste stimuli: sucrose, NaCl, citric acid and quinine·HCl. Of these patients, 45 were hypogeusic/ageusic for sucrose, rating sucrose intensity (totaled for five concentrations: 0.01. 0.03, 0.10, 0.30 and 1.0 M), on average, half as strong as did a control population. However, only two of these patients experienced a 'dissociated' loss for sweet sucrose, rating nonsweet NaCl, citric acid and quinine·HCl in the normal range of values. Thus, in our patients, a sweet taste loss is rarely 'dissociated;' rather, it is usually accompanied by losses in other taste modalities.

Correct identification is related to perceived intensity in a complex way

Correct identification of sweet-tasting stimuli was reduced by GA but the effect disappeared once stimulus intensity had recovered to 50% of pre-GA intensity. This information is useful for the design of a TCM that would identify hypogeusic patients. Data from a psychophysical function for sucrose (magnitude estimation of intensity versus concentration) produced by normal controls (n = 159) reveal that the intensity of 0.1 M sucrose is rated half as strong (14.20 ± 0.60) as 0.3 M sucrose (28.16 ± 0.84) (Bartoshuk and Marks, 1986). Our results on the recovery of identification performance with time following GA rinses suggest that 0.1 M sucrose would be as readily identified as 0.3 M sucrose. However, weaker sensations, such as those produced by lower concentrations in normals, would yield more misidentifications. Given that, on average, our hypogeusic patients perceive sucrose as half as intense as normals do, they would generally not make more errors than normals if 0.3 M sucrose were used in a TCM. However, they would be expected to make more errors than normals if 0.1 M sucrose were in the TCM. This idea could be tested in the TCM on normal subjects by using 0.04 M sucrose, which normals perceived as half as intense as 0.1 M sucrose.

When the sucrose concentration increased from 0.3 to 0.9 M, more rather than fewer misidentifications of sweet stimuli resulted (Hettinger *et al.*, 1999). In particular, sucrose-containing mixtures were mistaken for sucrose more frequently. Although 0.9 M sucrose results in a 30% increase over 0.3 M in perceived intensity in normals, discriminability as measured by T_2 for sweet–sweet pairs was reduced. Thus, discriminability of sweet–sweet pairs worsens when sucrose intensity is increased above midrange, whereas discriminability of sweet–nonsweet pairs worsens when sucrose intensity is decreased below midrange. Thus, discriminability and stimulus intensity are related in a complex way and, compared with midrange intensities (e.g. 0.1–0.3 M sucrose), distinct patterns of errors are seen for weaker and stronger intensities, as may be experienced by patients.

Identification of sucrose-containing mixtures was most sensitive to sweet deficit

Subjects treated with GA had particular difficulty correctly identifying the sucrose mixtures as mixtures, labeling them instead as the nonsweet components. It is interesting to compare this result with our observations of 42 subjects who performed the same identification task after water rinses (Hettinger *et al.*, 1999). These subjects mistook components for the mixture 23% of the time, on average,

but only 8% of the choices were for the nonsweet component; 15% were for the sweet component. In the current studies, the weaker 'sweetness' of the sucrose component after GA resulted in an increase in mistakes of identifying the mixture as its nonsweet component. Among the subjects from our other study (Hettinger *et al.*, 1999) who had water rinses, the 0.3 M sucrose was hardly ever mistaken for a nonsweet stimulus. These TCM results are compatible with the perspective that taste is an analytic system and that each of the mixture components is separately identifiable within the mixture (Bartoshuk and Gent, 1985; Schiffman and Erickson, 1993). Those arguing that taste is synthetic point out that the subject is not presented with a response label appropriate for the mixture in most protocols. In the identification protocol, however, both mixture and component labels are presented.

Subjects who rinsed with water (Hettinger *et al.*, 1999) hardly ever mistook 0.3 M sucrose or 3 mM aspartame for a nonsweet stimulus. Thus, if GA were to reduce sweetness intensity to zero, there would be no appropriate response such as 'no taste' for sucrose and aspartame. For these 'pure sweet' stimuli, erroneous choices were thus distributed across the incorrect categories following the GA rinse.

Data from a study on the effect of stimulus intensity on TCM (Hettinger *et al.*, 1999) suggest that the off-taste of aspartame (DuBois *et al.*, 1991) resulted in mistaking 3 mM aspartame (the same concentration used in the present study) for a sucrose mixture 7% of the time and mistaking the more intensely sweet 20 mM aspartame for a sucrose mixture 30% of the time. Rinsing with GA, which reduced the intensity of the 3mM aspartame in this study, decreased the frequency of mistaking aspartame for the NaCl–sucrose mixture, which increased discriminability (T_2). In conclusion, although responses to sucrose mixtures are sensitive to a taste deficit produced by GA, some response errors become more likely, such as identifying a salt–sugar mixture as a salt, but others become less likely, such as identifying the mixture as a 'pure' sweet stimulus.

The TCM measures based on information transmitted (T) fared well compared with simple percent-correct measures but both benefited by considering normal error patterns. Measures from information theory were used to quantify consistency in identification and pairwise discrimination of stimuli. Consistency in general and discrimination of sweet from nonsweet stimuli in particular were significantly impaired in subjects treated with GA. T measures consistency of identification, not correct identification. For example, if a subject consistently identified quinine as 'acid' and acid as 'quinine', information would be transmitted perfectly about the tastes of the stimuli by the gustatory system. T has an advantage over correct identification when a subject is unfamiliar with names of stimuli, especially if the 'wrong' name has been learned for a substance. This advantage was observed in the current study, with T_{10} showing a significant effect of GA on the entire 10 × 10 TCM. T_{10} 'ignored' differences in use of incorrect names for nonsweets by the two groups. The discriminability of stimulus pairs, as measured by T_2, was also useful, given expected effects of GA on particular stimulus pairs. T_2 has the advantage of quantifying overlapping responses for pairs of stimuli; again consistency, not correct identification, is key. However, overall T_2 may show no effect; discriminability would be expected to improve for some pairs but decline for other pairs, given a particular taste weakness. The 'percent correct' measure can better detect deficits if response categories based on common errors in normal populations are devised. Then naming errors that people frequently make becomes irrelevant to the result. Response categorization with an error analysis demonstrated the effect of GA very well. A deficit for the taste of one component of a binary, heterogeneous mixture (McBride, 1989) decreased the frequency of mistaking the mixture for that component but increased the frequency of mistaking it for the other component. We conclude that a dissociated sweet deficit would be identified with greatest sensitivity by examining key changes in error patterns (more mistakes toward nonsweet and less toward sweet stimuli) and key changes in pairwise discriminability (decreases for sweet versus nonsweet, increases for sweet versus partially sweet). The key changes would be a 'signature' for this particular dissociated taste disorder, which would differ from the 'signatures' for other dissociated deficits affecting other single taste modalities. However, given that 74% of our patients who have taste loss perceive stimuli of several taste qualities weakly, most patients would display multiple shifts in error patterns and discriminability.

Summary and conclusions

Treatment of the tongue with GA produced a sweet-taste intensity deficit in subjects who were then asked to identify 10 chemicals, each presented 10 times. Consistent sweet-stimulus identification, as measured by information transmitted (T), was specifically impaired, suggesting that 'objective' tests could serve to characterize taste deficits in patients.

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