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The influence of sodium salts on binary mixtures of bitter-tasting compounds

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Running head; Bitterness suppression of binary bitter mixtures

Abstract

In order to study potential mixture interactions among bitter compounds, selected sodium salts were added to five compounds presented either alone or as binary bitter-compound mixtures. Each compound was tested at a concentration that elicited 'weak' perceived bitterness. The bitter compounds were mixed at these concentrations to form a subset of possible binary mixtures. For comparison, the concentration of each solitary compound was doubled to measure bitterness inhibition at the higher intensity level elicited by the mixtures. The following sodium salts were tested for bitterness inhibition: 100mM sodium chloride (salty), 100mM sodium gluconate (salty), 100 & 20mM monosodium glutamate (umami), and 50mM adenosine monophosphate disodium salt (umami). Sucrose (sweet) was also employed as a bitterness suppressor. The sodium salts differentially suppressed the bitterness of compounds and their binary combinations. Although most bitter compounds were suppressed, the bitterness of tetralone was not suppressed, nor was the bitterness of the binary mixtures that contained it. In general, the percent suppression of binary mixtures of compounds was predicted by the average percent suppression of its two components. Within the constraints of the present study, the bitterness of mixtures was suppressed by sodium salts and sucrose independently, with few bitter interactions. This is consistent with observations that the bitter taste system integrates the bitterness of multi-compound solutions linearly.

Key words:

Bitter taste, bitterness inhibition/supression, sodium salts, taste psychophysics, binary mixtures

Introduction

Everyday life exposes us to complex mixtures of bitter tasting compounds. For example, many foods contain multiple compounds that can elicit bitterness (e.g., catechin, theophylline, theobromine, and caffeine in black tea). Similarly, Over-The-Counter pharmaceuticals are often co-delivered within a formulation (e.g., dextromethorphan, acetaminophen, and pseudoephedrine in cough syrups). Despite the potential for interactions via the cellular complexity of the bitter taste system (multiple G-protein-coupled receptors and post-receptor transduction mechanisms (Kinnamon and Margolskee, 1996; Wong *et al.*, 1996; Rossler *et al.*, 1998; Huang *et al.*, 1999; Adler *et al.*, 2000; Chandrashekar *et al.*, 2000)), bitterness perception often appears additive when compounds are mixed in binary combination (Keast *et al.*, 2003). For example, adding a weakly bitter alkaloid (e.g., quinine-HCl) to a weakly bitter amino acid (e.g., L-tryptophan) results in a final bitterness that is equal to the addition of the weakly bitter alkaloid to itself, or the weakly bitter amino acid to itself (see Figure 1, Equation 4). Therefore, the processes of increasing concentration and mixing together different compounds are related to each other in that they produce similar levels of perceived bitter taste intensity.

We investigated the influence of bitterness suppression on binary mixtures of bitter compounds as yet another test of binary bitter mixture interactions. If the suppression of the individual compounds predicts the suppression of bitter mixtures, then there is little evidence of interactions. When bitter compounds are mixed together, the combination solution appears more bitter than either compound would alone. This

creates the opportunity for the mixture to appear more difficult to suppress than its components. Because bitterness is more difficult to suppress as perceived intensity increases (Breslin and Beauchamp, 1995), we employed the additional comparison condition of adding salts to individual bitter compounds at double their respective concentrations in the binary mixtures.

There are few known bitterness inhibitors, but sodium (Na^+) salts have been shown to suppress the bitterness of certain compounds in human psychophysical studies (Bartoshuk and Seibyl, 1982; Breslin and Beauchamp, 1995; Keast and Breslin, 2002b; a). This suppression is mainly an oral peripheral effect of ions (at the cellular/epithelial level) rather than a cognitive effect (central process) of the perceived taste. To demonstrate the peripheral effect, Kroeze & Bartoshuk (1985) applied a bitter stimulus to one side of the tongue and a Na^+ salt to the other side of the tongue (split-tongue methodology). The stimuli were applied independently and simultaneously. The intensity of bitterness was reduced more when the stimuli were applied to the tongue in mixture together, compared to independent simultaneous application of the two stimuli on different sides of the tongue. This conclusion is possible because the two lateral halves of the tongue are neurologically independent until the ascending neurons interact in the brain (Tucker and Smith, 1969). This peripheral interaction between sapid compounds could occur with a number of molecules in the taste receptor cells (Keast *et al.*, 2001). Several studies have investigated the effect of these bitterness inhibitors on a variety of individual bitter compounds, but there are few, if any, reports of bitterness inhibition of binary mixtures of bitter compounds.

Sodium gluconate was used in this study because of the reduced salty taste (compared to NaCl) associated with its large anion (Ossebaard and Smith, 1995); low perceived saltiness allows us to distinguish between the peripheral inhibition of bitterness by Na^+ ions and the central cognitive inhibition of bitterness by perceived saltiness (Breslin and Beauchamp, 1995). Kroeze & Bartoshuk (1985) demonstrated cognitive taste suppression using the same split-tongue methodology described above. They reported mutual suppression of individual suprathreshold taste qualities, such as sweet and bitter, regardless of whether the compounds were applied independently to either side of the tongue or together as a mixture. This demonstrated that suppression could have a central cognitive, rather than just a peripheral oral, effect.

Umami-tasting Na^+ salts were also included in the present study because a comparison of the bitterness inhibition of a several Na^+ salts revealed that monosodium glutamate (MSG) and adenosine monophosphate (Na_2AMP) were the most effective at inhibiting bitterness (Ming *et al.*, 1999; Keast and Breslin, 2002b). It was not known if the added bitterness inhibition efficacy was due to the cognitive influence of the umami taste quality or an oral peripheral effect of the salts. To help further understand the central or peripheral influence on bitterness inhibition, we used the rare phenomenon of within quality taste synergy. Mixtures of certain 5'-ribonucleotides (NaIMP or NaGMP) with MSG enhance umami taste beyond additivity (Yamaguchi, 1967; Rifkin and Bartoshuk, 1980). Therefore, the central effect of umami taste can be compared with the peripheral effects of Na^+ & glutamate on bitterness by using iso-intense umami solutions containing different molarities of MSG (100mM MSG or 20mM MSG & 2.4mM IMP).

Materials and Methods

Subjects

Subjects (n=12, 32±5 years old, 6 female) between the ages of 21 and 51 were paid to participate after providing informed consent on an Institutional Review Board approved form. Nine were employees of the Monell Chemical Senses Center. The participants were asked to refrain from eating, drinking or chewing gum for one hour prior to testing.

Subject Training

Participants were initially trained in the use of the general Labeled Magnitude Scale (gLMS) following the published standard procedures (Green *et al.*, 1993; Green *et al.*, 1996) except the top of the scale was described as the strongest imaginable sensation of any kind (Bartoshuk, 2000). The gLMS is a psychophysical tool that requires participants to rate perceived intensity along a vertical axis lined with adjectives: barely detectable = 1, weak = 5, moderate = 16, strong = 33, very strong = 51, strongest imaginable = 96; the adjectives are placed semi-logarithmically, based upon experimentally determined intervals to yield data equivalent to magnitude estimation (Green *et al.*, 1993; Green *et al.*, 1996). The scale only shows adjectives, not numbers, to the participants, but the experimenter receives numerical data from the computer program.

Participants were trained to identify each of the five taste qualities by presenting them with exemplars. Salty taste was identified as the predominant taste quality from 150mM NaCl, bitterness as the predominant quality from 0.50mM quinine-HCl, sweetness as the predominant quality from 300mM sucrose, sourness as the predominant

quality from 3mM citric acid, and umami the predominant quality from a mixture of 100mM MSG and 50mM IMP. To help subjects understand a stimulus could elicit multiple taste quality, 300mM urea (bitter and slightly sour) and 50mM NH_4Cl (salty, bitter, and slightly sour) were employed as training stimuli.

Stimuli

Ranitidine (RAN) was purchased from ICN Pharmaceuticals (Aurora, OH), quinine-HCl (QHCl) was purchased from Fluka Chemika (Buchs, Switzerland), tetralone (TET) (family of iso-α-acids, the primary bittering compounds in beer) was purchased from Kalsec (Kalamazoo, MI), L-tryptophan (TRP), urea and denatonium benzoate (DB) were purchased from Sigma Chemical (St. Louis, MO). Sucrose was purchased from USB (Cleveland, OH). All salts were purchased at highest purity available: sodium chloride (NaCl) from Fisher (Fair Lane, NJ), sodium gluconate (NaGlc) and inosine 5'-monophosphate monosodium salt (IMP) from Sigma Chemicals (St. Louis, MO), adenosine 5'-monophosphate disodium salt (Na_2AMP) from ICN Pharmaceuticals (Aurora, OH), and monosodium glutamate (MSG) from USB (Cleveland, OH).

All solutions were prepared with deionized (*di*) Millipore® filtered water and were stored in amber glass bottles at 4°-8°C and were brought to room temperature prior to testing with the aid of a water bath. Millipore® filtered *di* water was used as the blank stimulus and the rinsing agent in all experiments.

Intensity matching of 100mM MSG and a mixture of MSG and IMP

The intensity matching procedure involved adjusting the concentrations of MSG:IMP solution until the intensity was rated iso-intense to 100mM MSG on the gLMS. The matching methodology follows: Subjects were instructed to wear nose clips

to eliminate olfactory cues when sampling, and to rate the perceived total intensity of the solution presented while it remained in the subjects mouth. Taste intensity was recorded on a computerized gLMS and transferred in real time to the technician making solutions. The first session was to determine the intensity of 100mM MSG. Four concentrations of MSG (2, 20, 100, 300mM) were assessed to minimize the risk that ratings would be confined to a small region of the scale. Subjects rated the intensity of 100mM MSG a minimum of five times. Subsequent sessions assessed the intensity of a variety of MSG:IMP solutions (10mM MSG:3mM IMP, 20mM MSG:3mM IMP, 30mM MSG:3mM IMP, 10mM MSG:2.4mM IMP, 20mM MSG:2.4mM IMP, 30mM MSG:2.4mM IMP, 10mM MSG:1.8mM IMP, 20mM MSG:1.8mM IMP, 30mM MSG:1.8mM IMP). There was an interstimulus interval of approximately 60sec, during which time the subject was required to rinse with *di* water at least 4 times. When randomly retested with 100mM MSG, we required subjects to provide ratings that were within 25% of their previous ratings as a screen for consistency of ratings. On average the mixture of 20mM MSG + 2.4mM IMP matched the umami intensity of 100mM MSG.

Method for setting the concentration of single bitter compounds

The procedure involved presenting subjects with varying concentrations of bitter stimuli and assessing the average concentration required to elicit "weak" bitterness on the gLMS. The protocol follows: Subjects were instructed to wear nose clips and to rate the perceived bitterness intensity of the solution while it remained in his or her mouth. Subjects rated the intensity of predetermined concentrations of bitter solutions (initial range of concentration is in parentheses): DB ($5x10^{-9}$M-5 $x10^{-7}$M), RAN (5 $x10^{-5}$M-5

x10^{-3}M), TET (1 x10^{-7}M-9 x10^{-4}M), TRP (2 x10^{-3}M-8 x10^{-2}M), and QHCl (5 x10^{-6}M-5 x10^{-4}M). Taste intensity was recorded on a computerized gLMS. There was an interstimulus interval of approximately 60sec, during which time the subject was required to rinse with *di* water at least 4 times. A group average concentration eliciting 'weak' bitterness was determined for each compound. The subjects were retested to verify the chosen concentration of bitter compounds was, on average, perceived as weakly bitter. If the perceived bitterness rating (gLMS 6±25%) did not match "weak" on subsequent evaluations, the concentration was adjusted up or down depending on whether more or less bitterness intensity was required. This procedure continued until a weak bitter concentration was found.

Consistency of ratings during the matching phase and throughout the study was good. We did not use 2-AFC methods to confirm the matched compounds, as we did not require this degree of precision and were confident with the gLMS system. Weak intensity concentrations were: DB (4.92 x10^{-8}M), RAN (9.59 x10^{-4}M), TET (8 x10^{-5}M), TRP (2.58 x10^{-2}M), and QHCl (5.5 x10^{-5}M).

Doubled-concentration and binary-mixture bitter compounds

The concentration required to elicit "weak" intensity bitterness of single-concentration (SC) bitter compounds was doubled (DC) as an intensity-matched comparison solution for the binary mixtures. To construct the binary-bitter mixture solutions each component was dissolved into solution at its respective single concentration. The perceived bitterness of the binary-bitter mixtures was expected to be equal to the perceived bitterness of the DC bitter compounds based on previous work (Keast *et al.*, 2003).

Design

The sixteen bitter compounds/mixtures used in this experiment were; single compounds: DB ($4.92x10^{-8}$M), RAN ($9.59x10^{-4}$M), TET ($8x10^{-5}$M), TRP ($2.58x10^{-2}$M), and QHCl ($5.5x10^{-5}$M); doubled concentration compounds; DB+DB ($9.84x10^{-8}$M), RAN+RAN ($1.92\ x10^{-3}$M), TET+TET ($1.6x10^{-4}$M), TRP+TRP ($5.16x10^{-2}$M), and QHCl+QHCl ($1.15x10^{-4}$M); binary mixture compounds; DB($4.92x10^{-8}$M)+RAN($9.59\ x10^{-4}$M), TET($8x10^{-5}$M)+RAN($9.59\ x10^{-4}$M), TRP($2.58x10^{-2}$M)+TET($8x10^{-5}$M), TET($8x10^{-5}$M)+DB($4.92x10^{-8}$M), TRP($2.58x10^{-2}$M)+QHCl($5.5x10^{-5}$M), and RAN($9.59x10^{-4}$M)+QHCl($5.5x10^{-5}$M). Not all possible binary combinations were included in this study in order to keep the total number of bitter-tasting sessions manageable for subjects. The six binary combinations were selected to incorporate each bitter stimulus in at least 2 binary mixtures. The eight Na^+ salts (Na^+) were (primary taste quality in parentheses); 100mM NaCl (salty), 100mM NaGlc (salty), 100mM MSG (umami), 50mM Na_2AMP (umami), 20mM MSG + 2.4mM NaIMP (umami), 20mM MSG (umami), and 2.4mM NaIMP (umami). Bitter intensity of the salts was negligible. Sucrose (200mM) (sweet) was included in the experiment as a control to assess the influence of a non-salty compound on bitterness and as a general cognitive suppressor of bitterness (mixture suppression).

Table 1 lists list all bitter compounds and binary bitter combinations in Column 1 and all Na^+ salts in Column 2. In each session all bitter compounds or binary mixtures from Column 1 was presented with one Na^+ salt (or water) from Column 2 resulting in 17 samples per session and nine sessions to complete the matrix (Table 1). The matrix was performed in triplicate for a total of 27 separate sessions.

Stimulus delivery

An aliquot of 10 ml of each solution (n=20) was presented in 30 ml polyethylene medicine cups (Dynarex, NY) on a numbered tray. Randomized solutions (10ml) were presented in 30ml plastic medicine cups and on numerically labeled trays. Subjects rinsed with *di* water at least four times over a 2 min period prior to testing. Each subject tasted, and then rated each solution for sweetness, sourness, saltiness, bitterness and savoriness, prior to expectorating. All subjects rinsed with *di* water 4 times during the interstimulus interval of 90 sec. The gLMS was used as the rating method. Each sample was tasted only once per session and there were three sessions in total as a test of reliability of rating. Subjects wore nose-clips to eliminate olfactory input.

Statistical analysis

Numerical results are expressed as geometric means ± geometric error. All 0 ratings were substituted with the value 0.24 prior to converting ratings to log values. Statistical variation was determined by 1 or 2 way analysis of variance (ANOVA) using Statistica 6 software package. P values <0.05 were considered statistically significant. All post-hoc pairwise comparisons were conducted with the Tukey HSD test.

Results

INFLUENCE OF SODIUM SALTS ON SINGLE AND DOUBLED CONCENTRATION BITTER COMPOUNDS

A three-way ANOVA (2 concentrations v 5 bitter compounds v 7 Na+ salts) revealed there was a significant main effect of concentration [$F(1,11) = 106$, $p<0.0001$], which shows that increasing the concentration of the bitter compound significantly increased bitterness. There was a main effect of bitter compound [$F(4,44) = 19$, $p<0.0001$], indicating that the bitterness of the compounds differed overall when pooled across added Na^+ salts. There was a significant main effect of Na^+ salts [$F(6,66) = 12$, $p<0.0001$], demonstrating that the Na^+ salts differentially affected bitterness. There was a significant interaction between the two concentration levels and the bitter compounds [$F(4,44) = 4.9$, $p<0.001$] indicating that there was a difference in the bitterness of the compounds at different concentrations. There was a significant interaction between the bitter compounds and the Na^+ salts [$F(24,264) = 5.9$, $p<0.001$] because that the Na^+ salts differentially affected the bitterness of compounds.

Pairwise comparisons revealed there was no difference in the bitterness of the SC or DC bitter compounds without Na^+ (Figure 2). But, the bitterness of the different SC and DC compounds was differentially suppressed. The Na^+ salts did not inhibit the bitterness of TET. When Na^+ salts were added, the bitterness of RAN and QHCl was significantly less than the bitterness of TET. The Na^+ salts (pooled across salts) suppressed the bitterness of the compound RAN significantly more than they did the

other four bitter compounds ($p<0.05$). All Na^+ salts significantly inhibited bitterness ($p<0.05$) (Figure 3). The Na^+ salts were less effective at suppressing the bitterness of TRP at the doubled concentration than at the single concentration ($p<0.05$).

When sucrose and a ribonucleotide are included in the analysis, results from a three-way ANOVA (2 concentrations v 5 bitter compounds v 9 taste modifiers (including Na^+ salts, 2.4mM IMP and sucrose)) revealed sucrose significantly suppressed the bitterness of the compounds. There were few differences in bitterness inhibition efficacy between sucrose and the Na^+ salts (Figure 3), except sucrose significantly suppressed the bitterness of TET more than the Na^+ salts did ($p<0.05$).

INFLUENCE OF SODIUM SALTS ON BINARY-MIXTURE BITTER-COMPOUNDS

A one-way ANOVA of DC bitter compounds and binary-mixture bitter compounds (both without Na^+ salts) revealed no significant differences in the bitterness among the compounds [$F(1,11) = 0.8$, $p=0.6$], which supports previous observations that perceived bitterness of binary mixtures is an additive function of the bitterness of its components (Keast *et al.*, 2003).

Results from a 6 x 7 (binary-mixture bitter compound v Na^+ salts) two-way ANOVA for binary bitter-compounds were similar to the individual compound results (see above). There was a significant main effect of binary-mixtures [$F(5,55) = 4.6$, $p<0.05$], a significant main effect of Na^+ salts [$F(6,66) = 5.6$, $p<0.0001$], and a significant interaction between the binary-mixtures of bitter compounds and Na^+ salts [$F(30,330) = 3.1$, $p<0.0001$].

Tukey HSD revealed there was no difference in the bitterness of the binary-mixture bitter-compounds without Na^+ (Figure 4). However, the Na^+ salts differentially suppressed the bitterness of the binary-mixture compounds. The Na^+ salts did not inhibit the bitterness of any binary mixture containing TET (TET-RAN, TRP-TET, TET-DB), while the Na^+ salts suppressed the bitterness of the remaining three binary-mixtures ($p<0.05$) (DB-RAN, TRP-QHCl, RAN-QHCl). All Na^+ salts significantly inhibited bitterness ($p<0.05$) (Figure 5).

When sucrose and a ribonucleotide are included in the analysis, results from a two-way ANOVA (5 bitter compounds v 9 taste modifiers (including Na^+ salts, 2.4mM IMP and sucrose)) revealed sucrose significantly suppressed the bitterness of the binary-mixtures of compounds and there was a significant difference in the bitterness suppression efficacy between sucrose and the Na^+ salts [$F(40,440) = 2.8$, $p<0.0001$]. An ANOVA revealed that sucrose significantly suppressed the bitterness of binary mixtures containing TET more than the Na^+ salts ($p<0.05$).

BITTERNESS SUPPRESSION BY UMAMI TASTING SALTS

Results from a two-way ANOVA (16 bitter compounds v 4 umami salts) revealed no significant difference in bitterness inhibition efficacy among 100mM MSG, 20mM MSG and 20mM MSG+2.4mM IMP [$F(3,33)=1.5$, $p=0.2$]. Interestingly, the influence of Na^+ at 100mM was not more effective at suppressing bitterness than 20mM Na^+. In addition, the higher perceived umami quality of 100mM MSG and 20mM MSG + 2.4mM IMP had no additional bitterness suppression efficacy over 20mM MSG (Figures 3&5).

CAN BITTERNESS SUPPRESSION OF A MIXTURE BE PREDICTED FROM BITTERNESS SUPPRESSION OF ITS COMPONENTS?

Figure 1 graphically outlines the comparisons made in this study. Intensity ratings for the binary mixtures with Na+ salts were calculated from the empirical data from the bitterness suppression of single and double concentration components. Results from a two-way ANOVA (6 binary mixtures v 3 prediction) revealed that there was no main effect of prediction [$F(2,10)=0.07$, $p=0.93$] indicating that, overall, the bitterness ratings predicted by the single concentrations and the predictions based upon the doubled concentrations did not differ from the actual bitterness ratings. However, there was an interaction between the binary bitter mixtures and the predictions [$F(10,50)=10.6$, $p=0.001$] indicating specific differences between the predictions and actual bitterness of the binary mixtures with salt (Figure 6).

Post hoc Tukey HSD showed that the doubled concentration prediction was significantly different from the actual bitterness of TRP-TET binary mixture ($p<0.05$). Figure 6 illustrates, however, that this is a small magnitude effect.

Discussion

Sodium salts differentially suppressed the bitterness of both the solitary and binary mixtures of compounds used in this study. At two extremes, the bitterness of RAN was suppressed more than any other compound, while the bitterness of TET was not suppressed by the Na^+ salts and at times was even enhanced. The suppression of the binary bitter mixtures by Na^+ salts was generally predicted by the degree of suppression of the individual components of the mixture at either intensity. Thus, the bitter taste system appears to ingrate bitterness of a binary mixture of compounds that is inhibited by Na^+ as if the components of the mixture were independent and non-interacting.

Predicting the suppression of bitter taste of binary mixtures

Figure 6 shows that the bitterness of the binary mixtures with Na^+ salts does not generally differ from the additive combination of bitterness from the individual components with Na^+ salts. For example, Na^+ salts did not suppress the bitterness of TET and the bitterness of mixtures containing TET was not significantly suppressed. Similarly, the bitterness of the components RAN and QHCl was suppressed by Na^+, and so too was the mixture RAN-QHCl. Keast *et al.*, (2003) reported that, in general, bitter taste is additive when two compounds are mixed together, and this study finds that bitterness suppression of binary mixtures is also generally additive. Regardless of the complexity of the bitter taste system at both the receptor cell and cognitive levels, the system appears to keep an additive tally of bitter system activation, whether the mixtures of compounds are inhibited or not.

The sole deviation from bitterness additivity was the TRP-TET mixture that was suppressed more than was predicted (based upon the suppression of the double concentration components). The reason for this small interaction is unknown.

Differential inhibition of bitterness by the sodium salts

The differential bitterness suppression by the salts suggests that the effect of Na^+ occurs peripherally (at a receptor/transduction mechanism) rather than cognitively as a function of the salty or the umami tastes, since all bitter solutions were equally intense prior to the addition of Na^+ salts. In contrast, sucrose suppressed the bitterness of all compounds equally, as expected, since the suppressive effect of sucrose on bitterness is primarily cognitive (Kroeze and Bartoshuk, 1985a). Keast *et al.*, (2001) suggested four possible modes of action of Na^+ at an oral peripheral level. In a review of G-protein coupled receptors (GPCR) Christopoulos and Kenakin, (2002) report the mode of action of Na^+ on receptors may be an allosteric site in the 2^{nd} transmembrane region, specifically an aspartic acid that is highly conserved across GPCRs. Based upon this analysis, a change in activation or conformational state of a GPCR by Na^+ could cause an altered signal from the taste cell that would result in bitterness inhibition.

Surprisingly, the Na^+ salts failed to inhibit the bitterness of TET, though they were effective at significantly suppressing the bitterness of the other compounds used in this experiment. This suggests that the bitterness of TET is unique because it is the only bitter-tasting compound we tested that Na^+ failed to suppress. If TET bitterness is initiated by a GPCR, it may not contain the highly conserved aspartic acid in TM II on which Na^+ may act. Not only do Na^+ salts fail to suppress the bitterness of TET, but one

Na^+ salt, NaGlc, significantly enhanced the bitterness of TET. This supports a recent finding by Mennella *et al.*, (2003) who reported that the preference for TET was decreased when it was mixed with NaGlc, suggesting that Na^+ may increase the bitterness of TET. We suggest that Na^+ ions may generally enhance the bitterness of TET regardless of the associated anion. Evidence of this, however, would be difficult to see in psychophysical experiments, since the enhancing effects of non-gluconate Na^+ salts may be negated by the cognitive bitter taste suppression of their stronger salty taste (Keast and Breslin, 2003). Interestingly, when TET was a component of a binary mixture, 100mM Na^+ salts were overall less effective at suppressing the mixtures than the 20mM Na^+ salts. This is consistent with the interpretation that higher concentrations of Na^+ enhance the bitterness of solutions containing TET more than do lower concentrations.

Bitterness suppression efficacy of umami-tasting salts

Keast and Breslin, (2002b) reported that the umami tasting salts MSG and Na_2AMP inhibited the bitterness of certain pharmaceuticals more than non-umami-tasting Na^+ salts. This difference among the salts was not clear in the present study, and may be due to the lower concentration of salts used (100mM versus 300mM) or the inclusion of different bitter stimuli. The minimum practical concentration of MSG for bitterness inhibition remains to be determined. The umami taste intensity was not important to the suppression of bitterness, since 20mM MSG, 100mM MSG and the MSG + IMP synergy solution all showed comparable levels of bitterness suppression (Figures 3&5). This may mean that glutamate's bitterness inhibiting effects are peripheral, rather than based on perceived umami intensity.

Conclusion

The bitter taste system appears to be sensitive to the total level of activation by combinations of bitter compounds. Components of a bitter mixture appear independent -- both in terms of additive taste intensity (Keast *et al.*, 2003) and in terms of suppression of bitter mixtures. These observations are consistent with the idea that the bitter system tracks absolute net activation by all bitter tasting compounds and that suppression of bitterness mixtures will be related to the overall levels of activation within the bitter taste receptor cells and the sensitivity of the respective receptors to the inhibitors.

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Table I Matrix design of the study

Column 1	Column 2
H_20	H_20
Denatonium Benzoate (DB)	100mM NaCl
Ranitidine (RAN)	100mM NaGlc
Tetralone (TET)	100mM MSG
L-tryptophan (TRP)	50mM Na_2AMP
Quinine-HCl (QHCl)	MSG:IMP 20mM:2.4mM
DB-DB	20mM MSG
RAN-RAN	2.4mM IMP
TET-TET	200mM Sucrose
TRP-TRP	
QHCl-QHCl	
DB-RAN	
TET-RAN	
TRP-TET	
TET-DB	
TRP-QHCl	
RAN-QHCl	

Figures

Figure 1 **Schematic design of this study**

Each equation is a hypothetical example of what happens to bitterness intensity of compounds A and B when they are mixed together and/or a sodium salt is added. Equation 1 shows that a mixture of A+B has a bitter intensity of gLMS 12 (general Labeled Magnitude Scale). When a sodium salt is added to the mixture, the bitterness intensity is reduced to gLMS 6. Equations 2 & 3 show the bitterness of the individual components of the mixture, A & B (both gLMS 8), and the bitterness of each component after a sodium salt has been added (A gLMS 6, B gLMS 1). Equation 4 (box) illustrates that doubling the concentration of bitter compounds and mixing together their components are related to each other in that they produce similar levels of perceived bitter taste intensity, if the components are equally intense initially (Keast *et al.*, 2003). Equation 5 & 6 use the model in Equation 4 to assess the bitterness suppression of the mixture components, A & B, at double their concentration; therefore each component has the same intensity as the mix A+B, gLMS 12. Addition of sodium salt suppresses bitterness of 2A to gLMS 9, and 2B to gLMS 4. We investigated whether the observed bitterness suppression of the mixture A+B (Equation 1) can be predicted from bitterness suppression of its components single concentration (A&B, Equations 2&3) or double concentration (2A&2B, Equations 5&6). The predictions based upon summing Equation 2+3 and averaging Equation 5+6 are found to the right. Trapezoids represent the medicine cup from which the solutions were sampled, and the numbers inside represent the bitterness ratings.

Figure 2 **The average effect of sodium salts on the bitterness intensity of single- and double-concentration bitter-compounds**

Each bar represents the bitterness of one compound when mixed with seven sodium salts (NaCl, NaGlc, MSG[100], Na_2AMP, MSG:IMP, MSG[20], IMP – see Figure 3 for abbreviations). The solid or dashed line above each bar indicates the bitterness of the single or doubled concentration compound without the sodium salts. (There was no significant difference in bitterness intensity of the single concentration compounds without sodium salts, nor was there a difference for the double concentration compounds without salts). The Y-axis represents average bitterness rating on the gLMS (geometric mean ± geometric error) for each bitter compound. The right hand vertical axis lists the verbal descriptors from the gLMS. Abbreviations and concentrations (single concentration first) for the bitter compounds are: DB=denatonium benzoate ($9.6X10^{-8}M$ & $9.84X10^{-8}M$), RAN=ranitidine ($9.59X10^{-4}M$ & $1.92X10^{-3}M$), TET=tetralone ($5X10^{-2}M$ & $1.6X10^{-4}M$), TRP=L-tryptophan ($2.58X10^{-2}M$ & $5.16X10^{-2}M$), and QHCl=quinine-HCl ($5.5X10^{-5}M$ & $1.1X10^{-4}M$). a,b,c symbolize a statistically significant ($p<0.05$) difference in bitterness intensity among single concentration compounds with added sodium salts. z,x,y symbolize a statistically significant ($p<0.05$) difference in bitterness intensity among double concentration compounds with added sodium salts. * indicates a significant difference in bitterness between the compound with and without sodium salts (bar height versus horizontal lines over bars).

Figure 3 **Specific effects of sodium salts on the average bitterness of single-and double-concentration bitter-compounds**.

Each bar represents the effect of the named sodium salt (x-axis) on the pooled bitterness of five single- and doubled-concentration bitter compounds. The x-axis lists the sodium salts. The Y-axis represents average bitterness rating on the gLMS (geometric mean ± geometric error) for each sodium salt averaged across all five bitter compounds (denatonium benzoate, ranitidine, tetralone, L-tryptophan, and quinine-HCl). The right hand vertical axis lists the verbal descriptors from the gLMS. The first two bars are the average bitterness without any added sodium salt. The last two bars show the impact of sucrose on the bitterness of the SC and DC bitter compounds. Abbreviations of sodium salts are: NaCl= 100mM sodium chloride, NaGlc= 100mM sodium gluconate, MSG[100]= 100mM sodium glutamate, AMP= 50mM di-sodium adenosine monophosphate, MSG:IMP= 20mM sodium glutamate and 2.4mM di-sodium inosine monophosphate, MSG[20]= 20mM MSG. Different letters a,b indicate a difference in the bitterness intensity of pooled bitterness of doubled concentration compounds ($p<0.05$). Different letters z,y indicate a difference in the bitterness intensity of pooled bitterness of single concentration compounds ($p<0.05$).

Figure 4 **Bitterness intensity of binary-mixtures of bitter-compounds**

Each bar represents the bitterness (geometric mean + geometric error) of one binary bitter-mixture when mixed with seven sodium salts (NaCl, NaGlc, MSG[100], AMP, MSG:IMP, MSG[20], IMP – see Figure 3 for abbreviations). The dashed line above each bar indicates the bitterness of the compound without the sodium salts (There were no significant differences in the bitterness intensity of the binary mixtures). The right hand vertical axis lists the verbal descriptors from the gLMS. Abbreviations and concentrations for the binary mixtures are: DBRAN=denatonium benzoate ($9.6X10^{-8}$M) and Ranitidine ($9.59X10^{-4}$M), TETRAN= tetralone ($8X10^{-5}$M) & ranitidine ($9.59X10^{-4}$M), TRPTET= L-tryptophan ($2.58X10^{-2}$M) & tetralone ($8X10^{-5}$M), TETDB= tetralone ($8X10^{-5}$M) & denatonium benzoate ($9.6X10^{-8}$M), TRPQHCl= L-tryptophan ($2.58X10^{-2}$M) & quinine-HCl ($5.5X10^{-5}$M), and RANQHCl= ranitidine ($9.59X10^{-4}$M) & quinine-HCl ($5.5X10^{-5}$M). Different letters symbolize a statistically significant ($p<0.05$) difference in bitterness intensity.

Figure 5 **Specific effects of sodium salts on the average bitterness of binary-mixtures of bitter-compounds**.

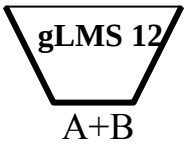
Each bar represents the effect of the named sodium salt (x-axis) on the average bitterness of six binary bitter mixtures. The x-axis lists the sodium salts. The Y-axis represents average bitterness rating on the gLMS (geometric mean ± geometric error) for each sodium salt pooled across all six binary-mixtures of bitter compounds: DBRAN, TETRAN, TRPTET, TETDB, TRPQHCl, and RANQHCl. Abbreviations and

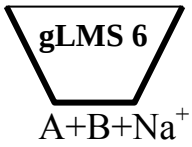
concentrations of the binary-mixtures of bitter compounds are the same as Figure 4. The first bar is the average bitterness without any added sodium salt. The last bar shows the impact of sucrose on the bitterness of the mixtures. Abbreviations of sodium salts are listed in Figure 3.

Figure 6 **Predicting the bitterness of binary mixtures after addition of sodium salts by calculating bitterness suppression of the mixture components**

See Figure 4 for a description of the axes, abbreviations, and concentrations. The solid black line adjoining each bar is the predicted bitterness calculated from bitterness suppression of the mixture components at double concentration. The dotted line adjoining each bar is the predicted bitterness calculated from bitterness suppression of the mixture components at single concentration. See Figure 1 for the prediction equations. An * beside the line indicates a significant difference between the predicted bitterness and the actual bitterness.

Suppression of the mixture A+B

1. 

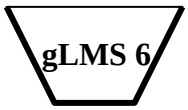
A+B + **Na^+** = 

A+B+Na^+

Observed
gLMS 6

Suppression of individual components at single concentration of the mixture A+B

2. 

A + **Na^+** = 

A+Na^+

3. 

B + **Na^+** = 

B+Na^+

Predicted
(Eq. 2 + Eq. 3)=
(6 + 1) =
gLMS 7

Equality of added bitter compounds

4. (

A + 

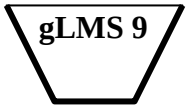
B) = 

A+B = 

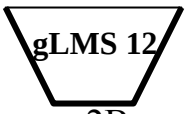
2A or 2B

Suppression of individual components at double concentration of the mixture A+B

5. 

2A + **Na^+** = 

2A+Na^+

6. 

2B + **Na^+** = 

2B+Na^+

Predicted
(Eq. 5 + Eq. 6)/2=
(9 + 4)/2 =
gLMS 6.5

Equation 1. ≈ (Eq. 2 + Eq. 3) OR (Eq. 5 + Eq. 6)/2

Figure 2

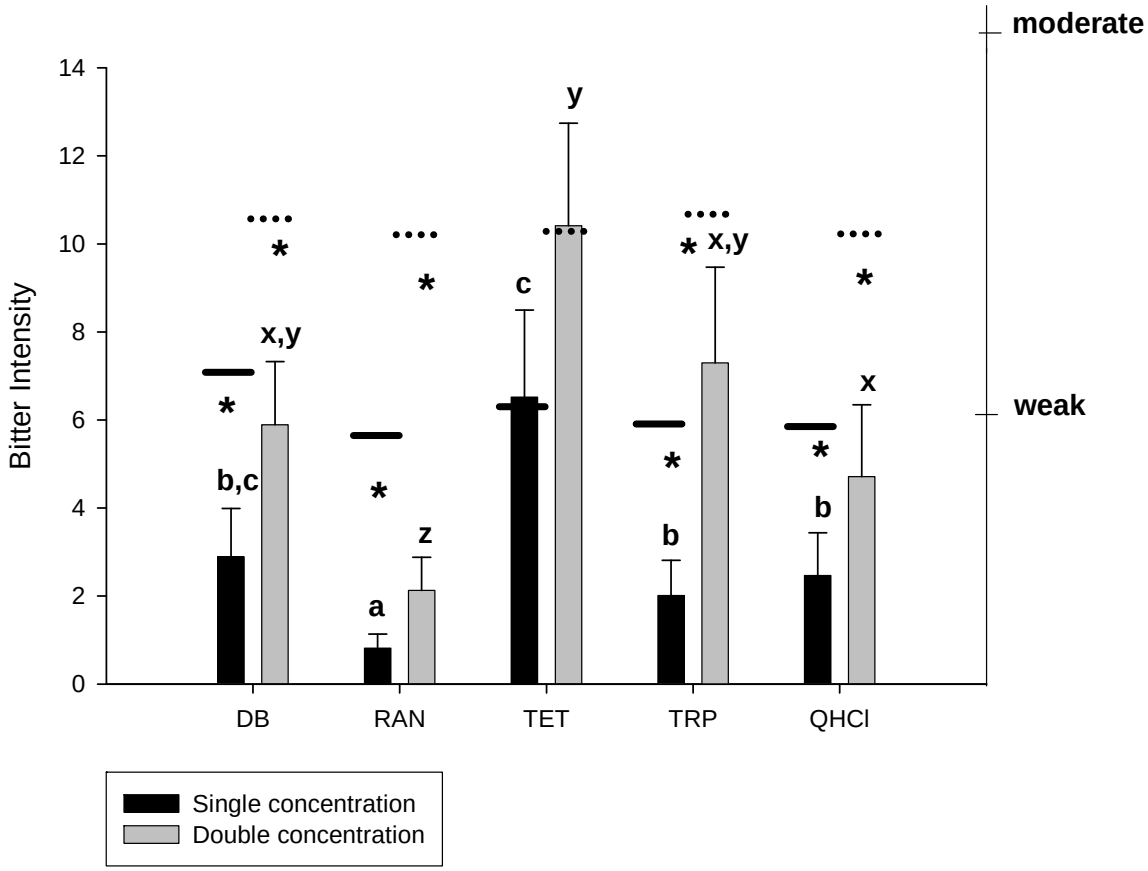

moderate
weak
Bitter Intensity
14
12
10
8
6
4
2
0
DB
RAN
TET
TRP
QHCl
b,c
x,y
a
z
c
y
b
x,y
b
x
Single concentration
Double concentration

Figure 3

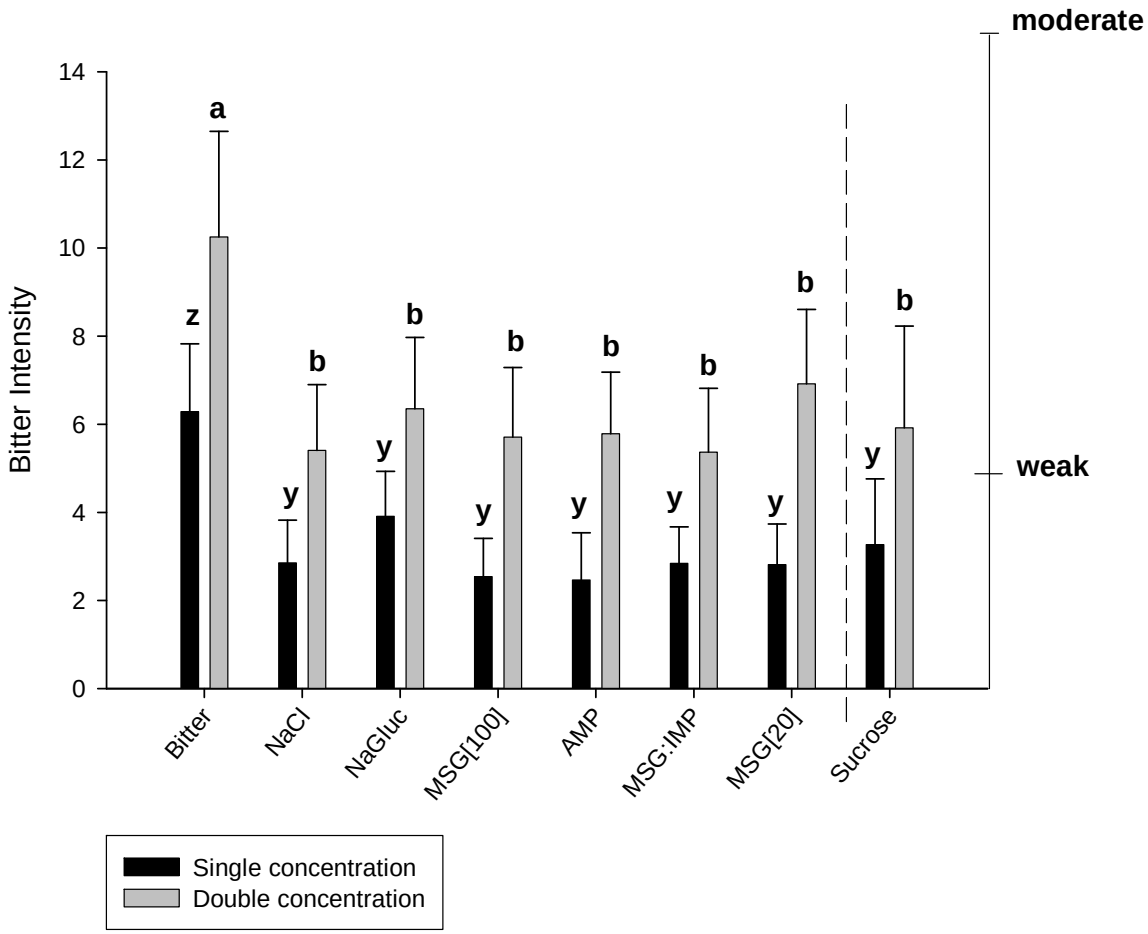

moderate
weak
Bitter Intensity
14
12
10
8
6
4
2
0
a
z
b
y
b
y
b
y
b
y
b
y
b
y
b
y
Bitter
NaCl
NaGluc
MSG[100]
AMP
MSG:IMP
MSG[20]
Sucrose
Single concentration
Double concentration

Figure 4

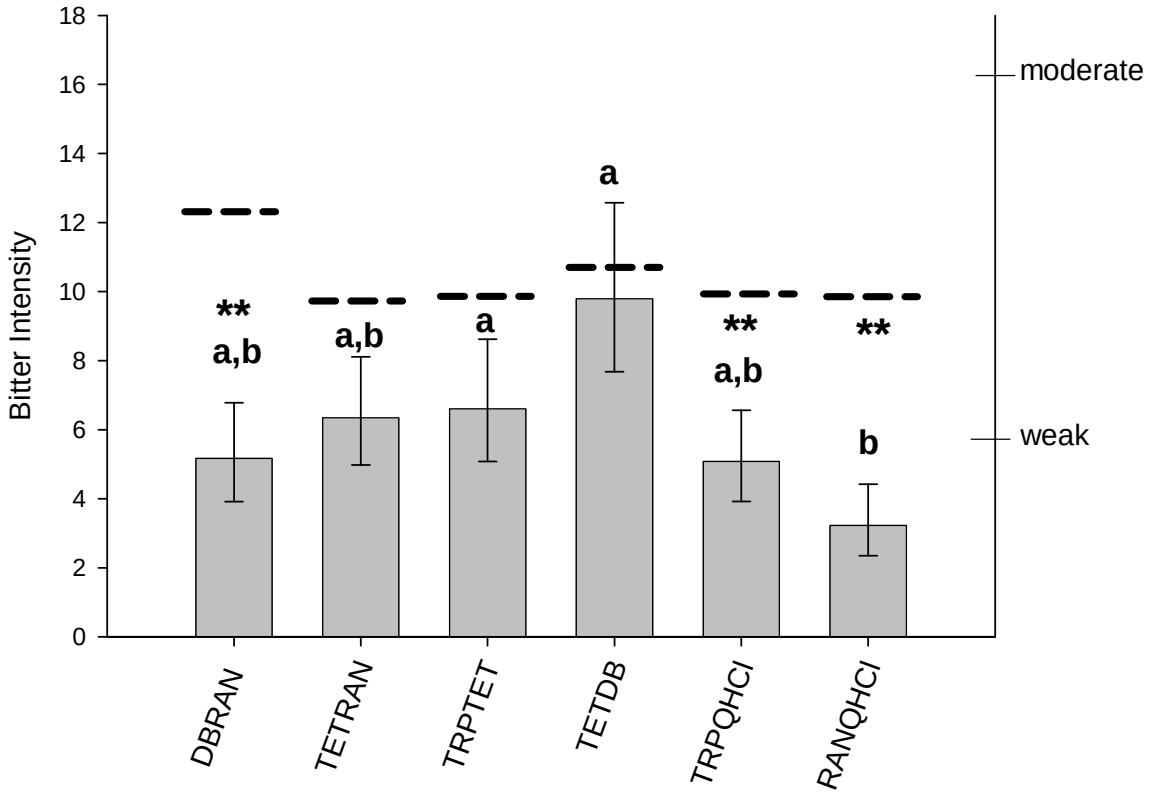

Bitter Intensity
18
16
14
12
10
8
6
4
2
0
moderate
weak
**
a,b
a,b
a
a
**
a,b
**
b
DBRAN
TETRAN
TRPTET
TETDB
TRPQHCl
RANQHCl

Figure 5

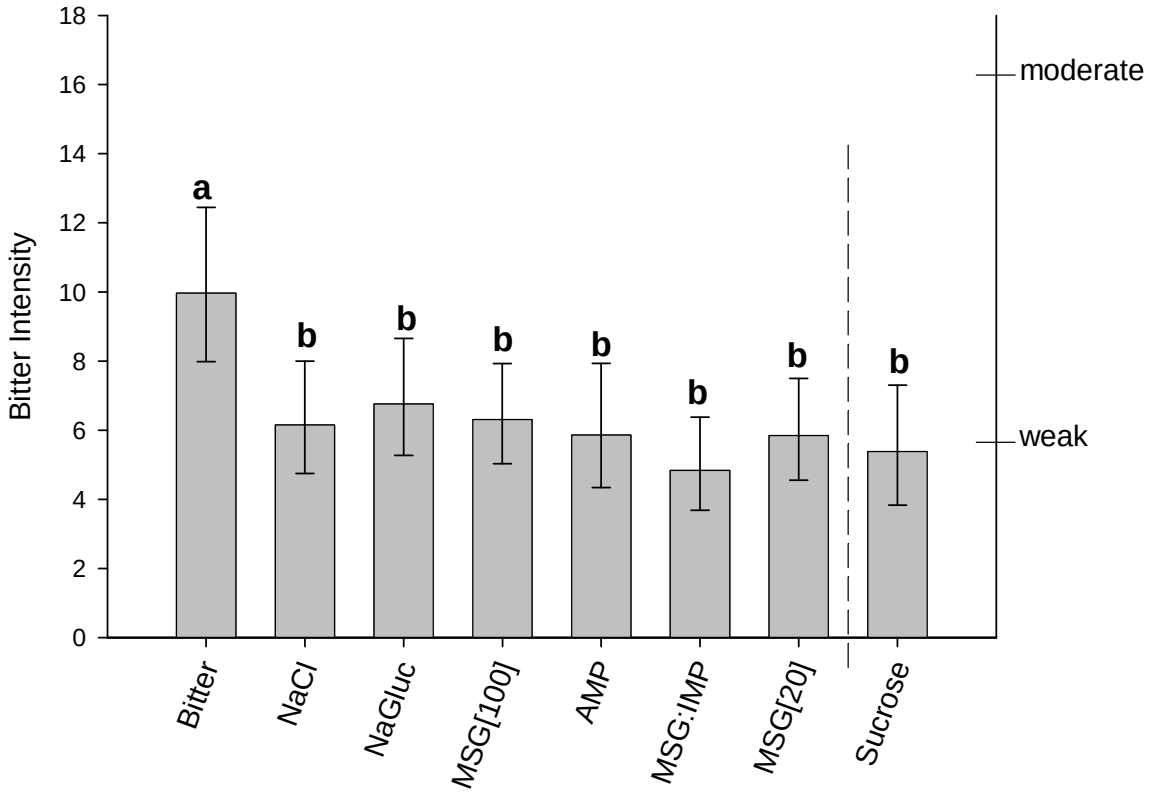
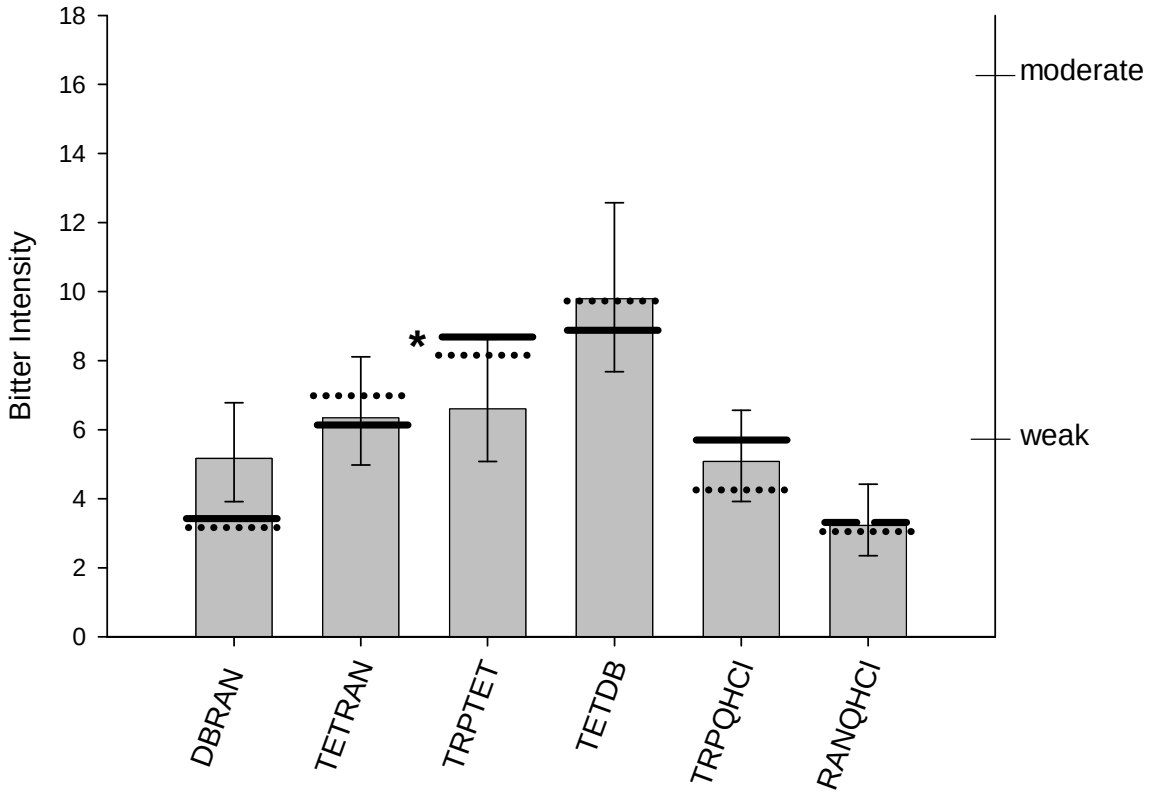

Bitter Intensity
18
16
14
12
10
8
6
4
2
0
a
b
b
b
b
b
b
b
moderate
weak
Bitter
NaCl
NaGluc
MSG[100]
AMP
MSG:IMP
MSG[20]
Sucrose

Figure 6


Bitter Intensity
18
16
14
12
10
8
6
4
2
0
*
moderate
weak
DBRAN
TETRAN
TRPTET
TETDB
TRPQHCl
RANQHCl