# Dissociating Pleasantness and Intensity with Quinine Sulfate/Sucrose Mixtures in Taste

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## Abstract

Independent experimental manipulation of subjective intensity and hedonic tone is required if one wants to study their separate effects on brain activity and behavior. This is problematic because hedonic tone and subjective intensity are related, leading to a pleasantness change each time the stimulus intensity is altered. In the present study, a solution to this problem was explored by combining a pleasant-tasting substance (sucrose) and a bad-tasting substance (quinine sulfate) into a number of different isointense mixtures. Here we show that subjective intensity as well as pleasantness can be accurately predicted, particularly in midrange, only if one corrects for mixture suppression.

Key words: binary mixtures, human, psychohedonic, taste hedonics

### Introduction

By changing its subjective intensity, the pleasantness of a stimulus can be manipulated. Wundt (1896) was the first to describe this relationship as an inverted U–shaped function, since then known as the Wundt curve: as subjective intensity increases, pleasantness increases as well up to a certain maximum, after which pleasantness will decrease with increasing intensity. The Wundt curve has been established for many taste substances (Ekman and Åkesson, 1965; Moskowitz, 1971; Moskowitz et al., 1974, 1975; de Graaf et al., 1996).

Coombs and Avrunin (1977) interpreted the often asymmetrical shape of the Wundt curve as evidence for two different underlying processes, a slow positive and a fast negative one, which combine into one single-peaked psychohedonic function. Cacioppo and Berntson (1999) similarly proposed two partially segregated systems in the brain underlying approach-avoidance behavior: an appetitive (positive) system and an aversive (negative) system. Results of a functional magnetic resonance imaging study by Rolls et al. (2003) confirmed this in the olfactory modality: bad- and good-smelling odors indeed activated separate locations in the orbitofrontal cortex, suggesting their independent processing. Clearly distinct gustofacial reactions to sucrose and quinine (Steiner, 1973; Rosenstein and Oster, 1988; Steiner *et al.*, 2001) also point in the direction of independent processing of pleasant and unpleasant tastes.

Pleasantness may also be manipulated by directly addressing the underlying positive and negative processes. This may be accomplished by mixing two stimuli of opposite hedonic value in different proportions. Djordjevic et al. (2004) used this approach with isointense odors. In the present study, sucrose and quinine sulfate were used. Rather than merely matching two or three different stimuli for subjective intensity and then using their different hedonic tones as an independent variable (Zald et al., 1998; Anderson et al., 2003; Small *et al.*, 2003; Winston *et al.*, 2005), we prepared a whole series of subjectively isointense mixtures of different quinine sulfate and sucrose intensities. This makes our approach less vulnerable to the confounding effect of quality seen in previous approaches where different hedonic value always fully coincided with different quality.

The aim of the present study is to explore the feasibility of dissociating subjective intensity and hedonic value by proportionally mixing an unpleasant and a pleasant taste substance. By also measuring the separate subjective intensities of the unmixed components, we enable correction for mutual mixture suppression (Keast and Breslin, 2003), thereby possibly improving the appropriateness of the mixtures as stimuli in taste pleasantness studies.

The present study proceeds in two steps. First, sucrose and quinine sulfate psychophysical functions will be established,

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which will then be used in the experiment to compose the required binary mixtures.

#### Materials and methods

#### Subjects

Nineteen subjects (5 males and 14 females), ranging in age from 18 to 27 years (mean 21.05 years), were recruited by advertisements in university buildings. They were informed about the general procedure but not the purpose of the experiment. All subjects gave their written consent, and all procedures adhere to the ethical policy of Utrecht University. Before participating, subjects filled out a questionnaire in order to check their general health and to avoid subjects with chemosensory deficits. No subjects were excluded on the basis of this screening procedure, although one subject dropped out due to family circumstances after the matching procedure (step 1). All subjects abstained from drinking, eating, and smoking 1 h prior to testing. They received a monetary incentive for their participation.

#### Stimuli

We used seven different concentrations of sucrose (1.63  $\times$  $10^{-2}$ ,  $1.80 \times 10^{-1}$ ,  $3.44 \times 10^{-1}$ ,  $5.08 \times 10^{-1}$ ,  $6.72 \times 10^{-1}$ ,  $8.36 \times 10^{-1}$ , and  $1.00 \times 10^{0}$  M; CSM [Diemen, the Netherlands], commercial grade) and quinine sulfate  $(7.660 \times 10^{-6},$  $1.532 \times 10^{-5}$ ,  $2.298 \times 10^{-5}$ ,  $3.064 \times 10^{-5}$ ,  $3.830 \times 10^{-5}$ ,  $4.596 \times$  $10^{-5}$ , and  $5.362 \times 10^{-5}$ ; Fluka [Buchs, Switzerland], pharmaceutical grade) dissolved in demineralized water (produced by a Millipore Milli-U10 water purification system, resistivity  $> 10$  M $\Omega$ ). Both sucrose solutions and quinine sulfate solutions started at near-detection threshold concentrations (Stahl, 1973) and increased up to a concentration that was rated as either pleasant (sucrose) or unpleasant (quinine sulfate) by 95–100% of the participants in a study by Engel (1928). Solutions were presented in amounts of 5 ml in 25 ml polystyrene medicinal cups at room temperature  $(\sim 22^{\circ}C)$ . The solutions were prepared at least 24 h before the experiment and were used for no longer than 3 days.

The psychophysical functions for sucrose and quinine sulfate resulting from step 1 were used to compose the subjectively equi-intense 20/80%, 40/60%, 50/50%, 60/40%, and 80/ 20% mixtures of quinine sulfate and sucrose. Another five binary mixtures inducing a larger intensity range were added in order to prevent effects of a small range of stimuli (R. Teghtsoonian and M. Teghtsoonian, 1978). These ''context'' mixtures consisted of the same concentrations and compounds as the other binary mixtures except that we presented them in 20/20%, 40/40%, 50/50%, 60/60%, and 80/80% combinations, thus varying in subjective intensity.

#### Procedure and design

Before the first part of the experiment, each subject was given written and verbal instructions and three practicing trials.

The subject was seated in front of a computer monitor and used a mouse for controlling an arrow to rate each stimulus on a visual analogue line scale on the screen. The left and right end points of the scale were labeled with the Dutch equivalent of ''very weak'' and ''very strong,'' respectively. After being cued by a beep from the PC, the subject sipped the entire content of the cup, kept it in his/her mouth for 3 s, and then spat it into a disposal container. The subject then immediately rated the stimulus and rinsed his/her mouth thoroughly with demineralized water. After 55 s, the next stimulus was cued. The session was divided into two blocks per subject, one block for the quinine sulfate solutions and the other for the sucrose solutions. Between blocks, there was a short break. This part of the experiment lasted approximately 50 min. Blocks were counterbalanced over subjects, and the stimuli were randomized within each block. Each of the seven solutions of both substances was rated three times, thus resulting in a total of 42 stimuli.

The second part of the experiment contained three experimental sessions. The first two sessions of 60–65 min were at approximately the same time on 2 consecutive days. During these two sessions, pleasantness, intensity, bitterness, and sweetness of the mixtures had to be rated. Each session consisted of two blocks with a short pause in between. Before a subject began each block of ratings, he was familiarized with the dependent variable by means of written instructions and three practicing trials. We counterbalanced the order of the sessions, but since there were only 18 subjects during the experimental session, counterbalancing was not perfect. Each binary mixture was presented three times within a block. The context mixtures were all presented once. This added up to a total of 20 stimuli per block of which the order was randomized. For each variable, we used a separate scale. The scale for intensity was the same as in the matching experiment. The pleasantness, sweetness, and bitterness scales were also visual analogue line scales, with on the left end the Dutch equivalents of "very unpleasant," "not sweet at all," and ''not bitter at all,'' respectively, and on the right end the Dutch equivalents of "very pleasant," "very sweet," and "very bitter," respectively.

We collected ratings to two additional dependent variables in a third session: the pleasantness of unmixed quinine sulfate and sucrose solutions at the same concentrations as those used in the binary mixtures. In this session, all five concentrations of both solutions were presented three times in a randomized order, making a total of 30 trials.

#### Results

#### Psychophysical functions

The mean intensity ratings of the seven sucrose and quinine sulfate solutions, obtained in the first step of the investigation, are displayed in Figure 1. After log transformation, the data were entered into a regression analysis. A linear



Figure 1 Mean subjective intensity ratings and standard error of mean (averaged over subjects and replications) of the quinine sulfate (Q, left panel) solutions and sucrose (S, right panel) and the fitted linear regression lines in the matching procedure.

regression line was fitted to each of the variables with statistical computing software package ''R'' (Team, 2005) with an  $R^2$  of 0.97 and 0.99 for quinine sulfate and sucrose, respectively.

The entire subjective intensity range of modeled functions was used to match the intensity for the five binary solutions. The range was based on the log subjective intensity range, the upper value being 125 for both solutions and the lowest value being the intercept of the modeled function. The log subjective intensity ratings and concentrations were then all transformed back. Combining these values in a complementary way (e.g., adding up to 100%) resulted in the five binary mixtures. The total predicted intensities, obtained by adding the subjective intensities of the individual components, in the binary mixtures were almost, but not perfectly, constant (Figure 2).

#### Individual data of mixtures

We observed inverted U–shaped pleasantness functions in 16 out of the 18 subjects. Two subjects showed a monotonic function, suggesting a high optimal point. The individual subjective intensity data points showed no systematic increase or decrease. In binary mixture 2, we observed complete masking of sweetness (a rating of <5 on a scale of 0–150) in four subjects, and in binary mixture 8, we observed complete masking of bitterness in eight subjects.

#### Mixture intensity

Figure 3 shows the mean observed subjective intensity for the five binary mixtures. We carried out a  $5 \times 3$  (mixture  $\times$  replication) within-subject multivariate analysis of variance (SPSS 10.1) with subjective intensity, pleasantness, sweetness, and bitterness as dependent variables. Subjective intensity of the mixtures appeared to deviate from constancy  $(F(4,68) = 20.84, P = 0.000)$ , as can be seen in the differences between predicted and observed subjective intensity functions.



Figure 2 Means and standard error of mean of subjective intensity of the mixtures averaged over subjects and replications (closed squares), predicted intensity from the unmixed components (open squares), and predicted intensity from the unmixed components corrected for the amount of mixture suppression (open stars).

It is known that individual taste qualities may be suppressed in binary mixtures. For quinine sulfate, bitterness suppression increases with increasing amounts of sucrose. For sucrose, both enhancement (in mixtures with small to medium amounts of quinine sulfate) and suppression (in mixtures with the highest quinine sulfate concentration) can be observed. The predicted subjective intensity curve of the mixture was adjusted for this mixture suppression. This adjustment considerably improved the prediction of subjective intensity (Figure 2).

#### Pleasantness integration

Figure 3A shows psychohedonic curves of the two unmixed components quinine sulfate and sucrose. This figure also shows the mean pleasantness rating of the mixtures averaged over subjects and replications. The observed pleasantness ratings varied over mixtures  $(F(1.93, 32.9) = 16.46, P = 0.000)$ .

Similarly as with the intensity ratings, we predicted the total pleasantness of the binary mixtures from the pleasantness



Figure 3 (A) Means and standard error of mean (SEM) of the pleasantness of the unmixed components quinine sulfate (closed triangles) and sucrose (closed circles) and of the predicted pleasantness (obtained by averaging the pleasantness of the unmixed components, open diamonds) and the observed pleasantness ratings of the mixtures (closed diamonds). (B) Means and SEM of the observed pleasantness ratings of the mixtures (closed diamonds) and the predicted pleasantness from the unmixed components corrected for the amount of mixture suppression (open stars).

of the unmixed components by arithmetically averaging these two. The resulting curve is shown in Figure 3A. As can be seen, this gives a poor description of the observed pleasantness of the mixtures. Also here, adjusting predicted pleasantness for mixture suppression provided a much better description of the observed total pleasantness of the mixtures, especially in the midrange mixtures 4, 5, and 6 (Figure 3B).

#### **Discussion**

The aim of the present study was to explore if subjective intensity and hedonic value can be dissociated experimentally. As expected, we observed mixture suppression. When we corrected for this mixture suppression, it resulted in a better prediction of subjective intensity as well as the pleasantness of the mixture. This means that isointense stimuli in mixtures cannot be achieved without correction for asymmetric effects of either mixture suppression or enhancement. In the past, such corrections have also given an increased fit to observed data (Moskowitz and Klarman, 1975; Lawless, 1977; Frank and Archambo, 1986). The benefit of correcting for mixture suppression is smallest for extreme mixtures.

This may be explained by looking at the psychohedonic curves of the unmixed components. In both mixtures 2 and 8, the pleasantness of the sucrose component in the mixture starts to decrease, which is predicted by the inverted U– shaped psychohedonic function. The psychohedonic curve for unmixed sucrose (Figure 3A) confirms this. Independent positive and negative processes underlying the pleasantness of taste could also explain the lower than expected pleasantness at the extreme ends of the binary mixtures since it has been claimed that negative aspects increase faster than positive aspects (Coombs and Avrunin, 1977; Cacioppo and Berntson, 1999; Rozin and Royzman, 2001). This also agrees with the observation of Lawless (1977) that unpleasant components contribute more than pleasant components to the overall hedonic tone of a taste mixture.

Somatosensory effects due to increased viscosity with increasing sucrose levels may also have contributed to this asymmetric mixture suppression of intensity. It is often observed that increased viscosity leads to decreased sweetness (Calvino et al., 1993; Theunissen and Kroeze, 1995), which may explain the decrease of pleasantness observed in mixture 8, which is the mixture containing most sucrose.

In conclusion, we varied the pleasantness of taste with binary mixtures of isointense quinine sulfate and sucrose. Even though we were able to systematically manipulate pleasantness in the midrange of the binary mixtures, we were not able to do this completely independently of intensity. It is possible to manipulate pleasantness and intensity systematically and independently when two requirements are met. First and foremost, a correction for mixture suppression is needed in order to obtain subjectively isointense stimuli. Second, due to the different characteristics of the positive and negative processes underlying pleasantness, it is important to avoid the extreme ends of the psychohedonic curve.

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