Impact of Novel Olfactory Stimuli at Supra and Subthreshold Concentrations on the Perceived Sweetness of Sucrose after Associative Learning

D. Labbe and N. Martin

Department of Food Consumer Interaction, Nestlé Research Center, Vers-chez-les-Blanc, PO Box 44, CH-1000 Lausanne 26, Switzerland

Correspondence to be sent to: David Labbe, Department of Food Consumer Interaction, Nestlé Research Center, PO Box 44, CH-1000 Lausanne 26, Switzerland. e-mail: david.labbe@rdls.nestle.com

Abstract

The impact of coexposure to a novel olfactory stimulation in combination with sweet taste on the construction of perceptual interaction was studied. The first objective was to explore whether a new flavoring perceived retronasally at a subthreshold concentration could enhance the perceived sweetness after a coexposure with sucrose using an approach encouraging associative learning. After validating the associative learning by showing an increase of the perceived sweetness by the flavoring at a suprathreshold concentration, we showed that the flavoring stimulation did not impact the perceived sweetness when presented at a subthreshold concentration. The second objective was to validate the absence of associative learning when subjects were exposed to the sucrose flavored solution in a context of coexposure akin to sensory profiling training. As expected, we confirmed that coexposure following sensory profiling training did not promote associative learning, probably because this approach encouraged subjects to consider the olfactory and sweet taste combination as a set of distinct qualities. The potential role of neural integration processes in these results was discussed.

Key words: associative learning, neural processes, olfaction, subthreshold, taste

Introduction

Flavor is a perception resulting from a complex combination of the olfactory, gustatory, and trigeminal sensations perceived during tasting and modulated by multisensory interactions (Prescott 1999; Auvray and Spence 2007; Small 2008). Interactions between the olfactory and sweet taste perception are among the most commonly reported. First, the impact of strawberry flavoring on the perceived sweetness was highlighted in a sucrose solution (Frank et al. 1989); then, other flavorings were also found to enhance the perceived sweetness of a sucrose solution, for example, pineapple and raspberry (Prescott 1999) and maracuja and caramel (Stevenson et al. 1999). Such odors are generally perceived in the sweet foods, and these are therefore congruent with the sweet taste stimulus. Congruency between sensory qualities is an important factor for perceptual interaction between senses. Congruency is the extent to which 2 stimuli are appropriate for combination in a food product (Schifferstein 2006). Several studies showed that, contrary to congruent odors, odors that are incongruent with sweet taste do not increase sweetness (Stevenson et al. 1999; Djordjevic et al. 2004). After coexposure to the olfactory and taste stimuli in mixture, the odor acquires the taste property of the coexposed tastant (Stevenson and Case 2003; Prescott et al. 2004). Perceptual associations therefore result from associative learning between sensory modalities previously encountered during everyday food experience (Dalton et al. 2000; Small and Prescott 2005; Bult et al. 2007). Although a lot of evidence showed that associative learning occurs implicitly, that is, without awareness (De Houwer et al. 1997, 2001; Stevenson and Boakes 2004; Wong et al. 2004), this topic is still today being debated (Olson et al. 2009).

The attentional strategy during exposure is an additional important factor influencing the construction of perceptual associations. Prescott et al. (2004) compared the impact of 2 different coexposure conditions on the construction of perceptual associations between a novel olfactory stimulus and sucrose, both at suprathreshold level. Each task encouraged subjects to consider the olfactory and taste sensory dimensions either analytically (by giving people a training that helps them to separate the 2 sensory aspects) or synthetically (by letting people consider the percept as a whole). Comparing the post- and preexposure results, Prescott found that the
synthetical attentional strategy (SYN) group rated the flavored sucrose solution sweeter than the unflavored sucrose solution but the analytical attentional strategy (ANA) group did not. However, another study showed that the construction of perceptual associations may occur after coexposure in a context of sensory profiling training, a procedure that nevertheless encourages an ANA (Stevenson and Case 2003). In this latter study, subjects also rated their liking of the solutions during the coexposure period. According to Prescott et al. (2004), who discussed results from Stevenson and Case (2003), the rating of the liking may have encouraged subjects to consider the olfactory and taste sensory dimensions synthetically, and this may explain why perceptual associations were built.

A few studies focused on the olfactory and taste interactions with stimuli at a subthreshold concentration (Dalton et al. 2000; Pfeiffer et al. 2005). In these 2 later studies, the authors explored the impact of an in-mouth saccharine solution (sweet tastant) at a subthreshold concentration on the detection threshold of benzaldehyde (an almond-like odor) delivered orthonasally. The orthonasal olfactory detection threshold of benzaldehyde significantly decreases with the presence of the saccharine solution in the mouth. In addition, Labbe et al. (2006) showed that the sweetness rating of a sucrose solution is increased by a simultaneous retronasal olfactory stimulation by a subthreshold concentration of ethyl butyrate (a strawberry-like odor). More recently, Miyazawa et al. (2008) demonstrated that a subthreshold concentration of acetic acid increases the perceived retronasal olfactory intensity of 3 volatile coffee aroma compounds.

The first objective of this study was to construct perceptual associations between a novel olfactory stimulation and the sweet taste of sucrose, with stimuli at suprathreshold concentrations (Experiment 1), and then to investigate the impact of this olfactory and taste associative learning on the perceived sweetness of a sucrose solution flavored with the olfactory stimuli at a subthreshold concentration (Experiment 2).

The second objective of the first experiment was to validate the absence of associative learning between olfactory and taste perception when coexposure is carried out in a context of sensory profiling training without hedonic evaluation.

**Experiment 1: impact of 2 implicit associative learning procedures on the construction of perceptual interaction with stimuli at suprathreshold level**

**Materials and methods**

**Flavoring selection**

Two commercial flavorings, elderflower (product code CD95904) from Givaudan SA at 1200 ppm and cactus (product code 505898 A) from Firmenich at 350 ppm were selected among 12 flavorings during a preliminary study conducted with 11 subjects. The subjects were asked to taste and swallow the flavored solutions and to indicate how familiar the olfactory stimulations were to them on a 10-cm scale anchored at the extremities (left: not at all familiar; right: “extremely familiar”). Familiar was defined as “how much does this odor resemble odors you know.”

The flavorings were evaluated in Vittel water solution with and without 7% sucrose. Among the 12 flavorings, the elderflower and cactus flavorings were scored the lowest in familiarity (±standard error) in unsweetened water (3.8 ± 1.5 and 4.9 ± 1.5, respectively) and in a 7% sucrose solution (3.8 ± 1.6 and 3.7 ± 1.9, respectively).

An unflavored sucrose solution at 7% was also evaluated. All 1-L solutions were prepared each morning prior to the test and stored at room temperature (22 °C) until use.

**Subjects and procedure**

Twenty-four untrained women between 40 and 45 years old took part in the study. Subjects were previously selected for normal olfactory and taste acuity based on the procedure (NF ISO 8586-1 [1995]).

The preexposure (PRE) session and the postexposure (POST) session were conducted by all assessors and consisted in scoring: 1) the sweetness of the 2 flavored unsweetened solutions, the 2 flavored sucrose solutions and the unflavored sucrose solution (which was replicated), and 2) the familiarity (by smelling) and the retronasal olfactory intensity of the 2 flavored unsweetened solutions.

The group of 24 assessors was then split randomly into 2 groups of 12, each group being coexposed with 1 of the 2 flavorings. Within each group of 12 assessors: l) 6 assessors were coexposed following a coexposure condition encouraging an ANA, that is, sensory profiling training, and 2) 6 assessors were coexposed following a coexposure condition encouraging a SYN, that is, triangle test.

Three coexposure sessions lasting 1 h were carried out for the ANA and the SYN groups using 4 solutions obtained by successive dilution of the flavored sucrose solution evaluated in the PRE and POST sessions with a dilution step of 1.2. The aim was to limit boredom by presenting solutions with different olfactory and taste intensities.

Each of the 4 solutions of 50 mL was presented 3 times during each coexposure session. Subjects were not informed about the 3 replications of the 4 solutions. They were asked to taste the whole volume so that at the end of the coexposure phase, subjects from the ANA or SYN groups tasted 1800 mL of solution (50 mL × 4 solutions replicated 3 times × 3 sessions) and consequently had been exposed to the same number and volume of olfactory and taste stimuli. This factor was important to control because a difference in stimulus exposure between the SYN and ANA groups may impact the associative learning effect.
Each of the 5 sessions, that is, the PRE, the POST, and the 3 coexposure sessions, were conducted on 5 separate and consecutive days (see Table 1).

**Sensory profiling training coexposure.** The sensory training aimed at promoting ANA during the coexposure because assessors were encouraged by this procedure to consider the olfactory and taste sensory dimensions independently. The 6 assessors exposed to the cactus flavoring and the 6 assessors exposed to the elderflower flavoring conducted the coexposure sessions separately. The first session of the coexposure consisted in sniffing and tasting the 12 flavored sucrose solutions (4 triplicate solutions) and in describing the olfactory and taste characteristics of the solutions with their own vocabulary. During the second session, the attribute list was reduced by removing redundant and confusing attributes (NF ISO 11035 1995). The assessors tasted the 12 flavored sucrose solutions again and selected the attributes they considered relevant for each solution. At the end of this session, sweetness and 2 attributes related to olfactory perception were kept for the third session. Finally, the last training session consisted in a series of ranking tests where a total of 12 solutions were tasted. In fact, for each of the 3 attributes, the four 50-mL flavored sucrose solutions were ranked from the least to the most intense. At the end of the sensory profiling training coexposure, a total of 36 solutions of 50 mL had been tasted.

**Triangle test coexposure.** The aim of conducting triangle tests was that the subjects were encouraged to acquire a SYN so that they merged the olfactory and taste stimuli as an integrated flavor perception. In fact, subjects were asked to pick the odd sample based on the overall perception and not to focus independently on each sensory dimension. The triangle tests were carried out in separate booths, and the 6 subjects that were exposed to the cactus flavoring and the 6 exposed to the elderflower flavoring took part simultaneously in all 3 sessions. Four triangle tests were performed per session. Within each triangular test, 3 identical flavored sucrose solutions were presented. Each of the 4 triangle tests was conducted with 1 of the 4 flavored solutions as previously described. The same 4 triangle tests were repeated during the 3 sessions. Similarly to the subjects from the ANA group, the subjects from the SYN group tasted a total of 36 solutions of 50 mL.

**Tasting conditions**

Solutions were coded with 3-digit random numbers and 50-mL portions were served in 100-mL plastic cups. Assessors were asked to sip and swallow the solutions. Rinsing was done between products with water and unsalted crackers for the PRE and the POST sessions and between each triangle test for the SYN group coexposure sessions. For the PRE and the POST evaluations, the 6 samples (the 2 flavored unsweetened solutions, the 2 flavored sucrose solutions, and the 2 unflavored sucrose solutions) were presented according to a presentation design, based on Williams Latin squares, balancing position, and order effects. The design was identical for both the PRE and the POST evaluations. For each SYN group coexposure session, the 4 triangle tests were presented in the same order within each group of 6 assessors being exposed to the same flavoring. Data were collected on a computer screen with FIZZ software version 2.20E (Biosystemes) for the PRE and the POST evaluations and during the SYN group coexposure. The same 10-cm scales as those described for the flavoring selection were used for both the PRE and the POST evaluations. Tests were conducted in an air-conditioned room (22 °C), under white light in individual booths.

**Statistical analyses**

**Unsweetened flavored solutions.** The objective was to determine whether the flavoring and sucrose coexposure impacted on: 1) the flavoring familiarity when the solution was sniffed and 2) the retronasal olfactory intensity and the sweetness evoked by the flavoring when the solution was tasted.

The flavoring serving as the coexposed stimulus was named “TEST,” and the flavoring serving as the not coexposed stimulus was named “CONTROL.”

For each attribute, individual scores obtained in the PRE evaluation were subtracted from individual scores obtained in the POST evaluation (POST–PRE) within each experimental condition (TEST and CONTROL) and within each coexposure condition (ANA and SYN). A positive value means that coexposure induced an increase of the attribute intensity, a negative value indicates that coexposure induced a decrease of the attribute intensity and a value close to zero means that coexposure did not change perception.

An experimental conditions (TEST and CONTROL) × coexposure conditions (ANA and SYN) analysis of variance (ANOVA) with interaction was performed on the POST–PRE evaluation scores to explore the impact of the both factors on the familiarity, aroma intensity, and perceived sweetness.

**Sweetened flavored solutions.** The objective was to investigate if, after to the coexposure stage, the flavoring impacted on mean sweetness of the flavored sucrose solution. This was conducted in 3 steps. Step 1: the mean sweetness of the 2 unflavored sucrose solutions was calculated per assessor and for each session (PRE, POST). Each value was used as a sweetness baseline for each stage. Step 2: the ability of the olfactory stimuli to modulate the perceived sweetness

### Table 1  Steps and duration of the experiments 1 and 2

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Days 2–4</th>
<th>Day 5</th>
<th>Days 6–7</th>
<th>Days 8–9</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE session</td>
<td>Exposure</td>
<td>POST</td>
<td>Break</td>
<td>Olfactory threshold</td>
<td>3-AFC tests</td>
<td>Experiment 1</td>
</tr>
</tbody>
</table>

**Figure 1**
was measured for each period, each subject, and each flavoring by subtracting the sweetness of the unflavored sucrose solution calculated in step 1 from the sweetness of the flavored sucrose solution (called relative sweetness score).

Step 3: the individual relative sweetness score calculated in the PRE evaluation was subtracted from the individual relative sweetness obtained in the POST evaluation (POST – PRE).

An experimental conditions (TEST, CONTROL) × coexposure conditions (ANA, SYN) ANOVA with interaction was calculated to explore the impact of both factors on the perceived sweetness as defined in step 3.

ANOVA were calculated using NCSS software version 2007 (Number Cruncher Statistical Systems). Post hoc pair comparisons were conducted by a Student’s t-test. Confidence level was set to 95% for all analyses.

Results

Results of the PRE evaluation confirmed that the cactus and elderflower flavorings did not differ (Student’s t, 2-tailed, paired): 1) in familiarity ($P = 0.23$) with mean scores of 6.1 and 6.9, 2) in retronasal olfactory intensity ($P = 0.59$) with mean scores of 6.8 and 6.6, and 3) in sweetness ($P = 0.51$) with mean scores of 0.6 and 0.4, respectively.

Unsweetened flavored solutions

Change in familiarity, in retronasal olfactory intensity, and in sweetness between the POST evaluation and the PRE evaluation was not affected by the coexposure conditions according to ANOVA with ($F_{1,44} = 0.4$), ($F_{1,44} = 0.69$), and ($F_{1,44} = 0.10$) for each of the 3 attributes, respectively.

Change in sweetness ($F_{1,44} = 1.49$) and in retronasal olfactory intensity ($F_{1,44} = 2.69$) between the POST evaluation and the PRE evaluation was not affected by the experimental conditions (TEST, CONTROL). The change in familiarity of the unsweetened flavored solution depended marginally ($F_{1,44} = 3.27$, $P$ value = 0.07) on the experimental conditions (TEST, CONTROL). As expected, the increase in familiarity (see Figure 1) was higher for the flavoring when serving as the coexposed stimulus (TEST) than for the flavoring when serving as the not coexposed stimulus (CONTROL).

Sweetened flavored solutions

Change in sweetness between the PRE evaluation and the POST evaluation was not significantly impacted by the coexposure conditions (ANA vs. SYN) ($F_{1,44} = 0.74$) and the experimental conditions (TEST, CONTROL) ($F_{1,44} = 0.4$). But, the interaction between both factors was significant ($F_{1,44} = 5.92$, $P$ value < 0.05; see Figure 2). Pair comparisons by Student’s-t test revealed a significant difference in sweetness ($P$ value < 0.05) for the TEST flavoring between the 2 coexposure conditions (see Figure 2). In addition, change in sweetness for the sucrose solution flavored with the TEST flavoring was significantly higher than zero for SYN and significantly lower than zero for ANA.

Experiment 2: impact of olfactory stimuli at a subthreshold concentration on sweetness after implicit associative learning

The second experiment was conducted to investigate the impact of the 2 flavorings (TEST and CONTROL) on the perceived sweetness of a sucrose solution when presented at a subthreshold level. For each flavoring, 2 sucrose solutions, with and without flavoring, were compared by a 3-Alternative Force-Choice (3-AFC) discrimination test. We supposed that a sucrose solution containing the TEST flavoring at a subthreshold concentration, but not the CONTROL flavoring,
should be perceived differently from the unflavored sucrose solution due to sweetness change resulting from perceptual interaction.

The experiment started after a 2-day break (weekend) following the first experiment and consisted in 2 sessions for the determination of the individual detection threshold for each flavoring and 1 session for the evaluation of the impact on sweetness of both flavorings at a concentration leading to a subthreshold retronasal olfactory stimulation. These 3 sessions were conducted on 3 separate and consecutive days (see Table 1). The same 24 assessors as in the first experiment participated in the second experiment.

Materials and methods

Procedure and statistical analyses

Determination of the flavoring concentration leading to a subthreshold retronasal olfactory stimulation. First, we measured the individual retronasal olfactory detection threshold of each flavoring in Vittel mineral water using the forced-choice ascending concentration series method of limit (ASTM 1991) during 2 sessions (one for each flavoring). For each flavoring, each subject performed a series of fifteen 3-AFC tests with an ascending concentration of flavoring using a dilution factor of 2, as described in Labbe et al. (2006).

In accordance with the suppliers’ recommendations and after preliminary trials, the ranges of concentration were chosen from 6.7E-04 to 11 ppm for the cactus and from 4.8E-03 to 78 ppm for the elderflower flavoring. For each subject and flavoring, the concentration above which all 3-AFC tests were correctly performed was considered as the detection threshold. Finally, the subthreshold concentration was obtained by dividing the threshold value by 64 in order to stay clearly below the threshold. This value was chosen because in our previous study we highlighted an enhancing impact of ethyl butyrate volatile retronasal olfactory stimulation on sweetness when added at a concentration 64 times lower than the threshold (Labbe et al. 2006).

Criteria to conclude that the subthreshold flavoring concentration has an impact on the sucrose solution perception. For each flavoring, assessors carried out a 3-AFC test in a 7% sucrose solution, 1 of the 3 sucrose solutions being flavored with the subthreshold flavoring concentration. A minimum of five 3-AFC tests out of 6 had to be solved to consider the flavored and unflavored sucrose samples as significantly different according to the binominal law with a confidence level set at 95%.

Tasting conditions

Solutions were coded with 3-digit random numbers and 50-mL was served in 100-mL plastic cups. Assessors were asked to sip and swallow the solutions. Rinsing was done between each 3-AFC test with water and unsalted cracker. Data were collected on a computer screen with FIZZ software version 2.20E (Biosystemes). Tests were conducted in an air-conditioned room (22 °C), under white light in individual booths.

Results

The lowest and highest threshold concentrations within the 24 subjects were as follows: 1) 1.34E-03 and 1.10 ppm with a panel geometric mean of 4.2E-02 ppm for the cactus flavoring and 2) 7.6E-02 and 2.45 ppm with a panel geometric mean of 3.8E-01 ppm for the elderflower flavoring.

Only 1 subject out of 24 significantly distinguished the flavored from the unflavored sucrose solution for the TEST flavoring (see Figure 3). Neither differences in experimental conditions (TEST vs. CONTROL) nor differences in coexposure conditions (ANA vs. SYN) influenced the distinction between the flavored and the unflavored sucrose solution significantly.

Discussion

Impact of associative learning on the perception of sucrose with a subthreshold concentration of flavoring

As expected, the flavoring and tastant coexposure using an approach encouraging a SYN led to the construction of perceptual olfactory-taste associations as previously shown by Stevenson and Case (2003), Prescott et al. (2004), and Yeomans et al. (2006).

The impact of subthreshold olfactory stimuli on sweetness has already been demonstrated with common flavorings congruent with sweetness (Dalton et al. 2000; Pfeiffer et al. 2005; Labbe et al. 2006). In the present study, the expected enhancing effect of the flavoring coexposed with sucrose on sweetness was not obtained at subthreshold level whatever the attentional condition applied during coexposure. In fact, flavored and unflavored sucrose solutions were not discriminated by a 3-AFC procedure suggesting that the sweetness of the flavored sucrose solution was not increased by olfactory and taste perceptual interactions. In the context of our study, sweetness was not impacted by a subthreshold concentration of an olfactory stimulus perceptually associated with sucrose after a short coexposure, contrary to what is shown with common olfactory stimuli congruent with sweet taste such as almond (Dalton et al. 2000) and strawberry (Labbe et al. 2006).

Impact of coexposure conditions on associative learning

When exposure of the flavoring and the sweet tastant was combined with a sensory profiling training approach, the coexposed flavoring did not enhance the perceived sweetness. This result supports the assumption of Prescott et al. (2004) that this approach encourages the subjects to consider the perceptual dimensions analytically. This finding shows that a descriptive profiling training carried out with references
mixing olfactory and taste stimuli that will be later conjointly experienced in the evaluated products could reduce the impact of perceptual interaction on product description. The perceived sweetness of the flavored sucrose solution was weaker after coexposure than before coexposure as previously reported by Prescott et al. (2004). As assessors were trained not to overevaluate the sweetness of the flavored sucrose solution by dissociating the olfactory and taste perception, one may argue that the training instructions led them to underevaluate the sweetness in presence of the coexposed flavoring may have affected sensory magnitude judgment. This kind of bias related to the impact of training instructions on sensory magnitude judgment has been described by Poulton (1979) as a “transfer bias.” Whatever the exposure conditions, when the unsweetened solution was in mouth, the flavoring did not enhance the perceived sweetness. However, Prescott et al. (2004) showed that after coexposure, the smelled flavoring was perceptually associated with sweetness. In the present study, where we focused on retronasal olfactory perception, a minimum amount of the sweet tastant seemed to be required in the mouth to induce the perceptual olfactory and taste associations.

Role of neural integration processes in the construction of perceptual associations

Small et al. (2004) showed that neural processes underlie the olfactory and taste interactions. Using functional magnetic resonance imaging (fMRI), the authors highlighted a supra-additive effect of a congruent vanilla flavoring and sucrose mixture on the neuron activity from the insula/orbitofrontal cortex compared with the activation induced by each ingredient independently. The authors suggested that these observations may be explained by the presence of specific neurons of the insula/orbitofrontal cortex that may integrate both the olfactory and taste stimuli when congruent. Such bimodal neurons have been highlighted in the macaque orbitofrontal cortex (Rolls and Baylis 1994) and amygdala (Kadohisa et al. 2005). They may result from repeated and simultaneous exposure to a given olfactory and taste combination during lifetime. Other key structures may be involved in olfactory and taste integration processes such as the frontal operculum and anterior cingular cortex (Small and Prescott 2005).

Different studies also report perceptual interaction between the perception of sucrose and subthreshold concentration of olfactory stimuli experienced because childhood in sweet food such as almond (Dalton et al. 2000) and strawberry (Labbe et al. 2006). The role of coexposure duration seems to be important, and the absence of impact of the olfactory stimuli at a subthreshold concentration on the perceived sweetness in our study may be explained by the too short period used to experimentally build perceptual associations.

Perspectives

Additional psychophysics and neuroimaging research might extend our understanding about the plasticity of the flavor network during associative learning and on how unitary perception is generated over time. The impact of a new flavoring on the sucrose perception could be evaluated before and repeatedly after several coexposures by measuring: 1) the activity of the orbitofrontal cortex, amygdala, operculum, and cortex cingular anterior by fMRI and 2) the perceived sweetness by sensory evaluation.

Acknowledgements

We gratefully acknowledge Givaudan Schweiz AG, Duebendorf, Switzerland and Firmenich SA, Geneva, Switzerland who kindly provided the flavorings and Elizabeth Prior and Jeremy Pace for reviewing the English manuscript.

References


Accepted July 2, 2009