Chem. Senses 35: 767–776, 2010
doi:10.1093/chemse/bjq075
Advance Access publication August 11, 2010

Evidence that the Sweetness of Odors Depends on Experience in Rats

Shree Hari Gautam and Justus V. Verhagen

The John B. Pierce Laboratory, New Haven, CT 06519, USA and the Department of Neurobiology, Yale School of Medicine, New Haven, CT 06520, USA

Correspondence to be sent to: Justus V. Verhagen, The John B. Pierce Laboratory, 290 Congress Avenue, New Haven, CT 06519, USA. e-mail: jverhagen@jbpierce.org

Accepted July 3, 2010

Abstract

Humans describe their perception of certain odorants in terms of taste qualities (e.g., sweet). It has also been found that in humans, novel odorants can rapidly and irreversibly acquire a taste, even after just a single pairing with a taste. It remains unclear whether flavor objects in general, and odor–taste generalizations in particular, are experience-dependent. Interactions might result from a failure by humans to sufficiently analyze the olfactory and gustatory components of compound flavorants. Here, we tested odor–taste generalizations in rats with or without paired exposure to an odorant and a tastant. We evaluated the generalization of conditioned odor aversion to tastants by rats. Our findings suggest that rats behave toward putatively tasteless retronasal odorants as if they were sweet only after prior paired experience of the odorant with a sweet tastant. These data support the hypothesis that taste-like qualities of odors are learned and are not innate. Furthermore, the present results suggest that acquisition of a taste quality by an odor need not depend on higher cognitive abilities. This study helps to establish the rat as a model for the study of behavioral neuroscience of flavor.

Key words: COA, conditioned odor aversion, CTA, flavor, multisensory integration, paired experience, perceptual learning, taste acquisition

Introduction

The flavor of a food consists of its taste, retronasal odor, and oral somatosensation. The perception of flavor is hence a multisensory process involving the integration of all of these sensory modalities. Of these, taste–odor integration has been of particular interest in flavor research. It is notable that odorants often can be described in terms of basic tastes. For example, vanilla and amyl acetate have been reported as smelling ''sweet'' and hexanoic acid as ''sour'' (Burdach et al. 1984; Dravnieks 1985; Stevenson and Boakes 2004). Adding a quality-congruent odorant to a tastant increases the perceived taste intensity, consistent with the notion that the reported odor-evoked taste is a true gustatory experience (Prescott 1999; Stevenson and Boakes 2004; Small and Prescott 2005; Verhagen and Engelen 2006). These odor–taste interactions have been described as taste–odor synesthesia, brought about by the pairing of the 2 modalities via oral and retronasal stimulation when food is ingested (e.g., Verhagen and Engelen 2006; Stevenson and Tomiczek 2007). Indeed, it has been found in humans that novel odors can acquire a taste during a single pairing (Stevenson et al. 1998; Stevenson and Boakes 2004). One prediction based on this view is that without paired odor–taste experience, odors would not have a taste component.

Despite these findings, it has been suggested that the enhancement of tastes by odors in human subjects depends on factors like ''halo dumping'' (Frank et al. 1993; Clark and Lawless 1994), that is, the enhancement is not a true odor–taste interaction but a cognitive artifact. Others have reported that odor–taste enhancement may depend on whether the subjects treat the experience analytically or synthetically (Prescott et al. 2004). Accordingly, this interpretation would predict that tests that do not rely on psychophysical scaling and cognitive processes would show no evidence of odors enhancing tastes, and, by extension, would show no evidence of odors acquiring tastes.

In order to test these predictions, we used Wistar rats. We are establishing the rat as a neuro-behavioral model of multimodal flavor integration. This will allow us to perform neurophysiological experiments not otherwise feasible, to complement the neurobiological work performed on human subjects. Although it is unknown whether odors can acquire a taste in rodents, several experiments suggest they can, at

least in rats (Sakai and Imada 2003; Dwyer 2005; Harris and Thein 2005).

In the first experiment, we used conditioned taste aversion (CTA) and conditioned odor aversion (COA) generalization tests to first determine whether for flavor-naive rats, orally presented odorants have a taste component. In 2 other experiments, we provided odor–taste experiences in otherwise flavor-naive rats and tested whether these experiences led to the acquisition of a taste quality by an odor and/or the acquisition of odor by a taste. Despite some inconsistencies, as these results were based on a fairly small number of animals, the results supported the hypothesis that, in rats, an odor can acquire a specific taste quality only after paired taste–odor experience but that a taste cannot acquire an odor. It should be noted that it could not unambiguously be determined whether odors actually induced a taste in rats or whether some other process underlay these results. This result suggests that the acquisition of a taste by an odor may not require higher cognitive abilities. This new combination of methods allows a new way to study flavor-related multi-stimulus integration (MSI) in rats and opens the door for procedures that would not be possible in humans.

Materials and methods

Subjects

A total of 64 male Wistar rats weighing 180∼200 g were purchased from Charles River Laboratories Inc. in 4 batches of 16 each and housed individually with controlled humidity (40%) and temperature (22 °C). The vivarium was set with 12:12 h light:dark cycles, and all the experiments were carried out in the light phase. For each conditioned taste/odor generalization experiment, rats were randomly divided into 7 (Experiment 1) or 4 (Experiment 2 and 3) conditioned stimulus (CS) groups (including one water CS, control group) of 2 or 4 rats each as detailed in the Results section. Animals in each group were conditioned to avoid one of the CSs. Food and water were available ad libitum except during behavioral testing. Rats were on a 22-h water deprivation schedule throughout behavioral testing, starting one day prior to familiarization. All the animals were treated according to the guidelines established by the US National Institutes of Health (1986) and the American Psychological Association (1996), and the experimental protocols were approved by the Institutional Animal Care and Use Committee of the John B. Pierce Laboratory.

Chemicals and equipment

All the chemicals used for this study, including tastants and odorants, were obtained from Sigma-Aldrich. Almond paste (45% almond, 50% sugar) and green unripe banana used for the experience studies were purchased from local stores.

The stimuli were delivered using a custom-built 8-channel gustometer system. All procedures, namely, familiarization, conditioning, and brief access tests, were fully automated using Labview. Valves were individually calibrated to provide 5 ± 0.5 μL of fluid per lick. A lickometer (Med. Associates) was connected to the raised metal grid of the cage and the lick spout. Upon switching stimuli, the lick manifold was rinsed and emptied by vacuum such that the next lick refilled the manifold's dead space. Eight rats were tested daily per setup, using 2 identical setups.

Retronasal odor stimulation

As retronasal sweet odorant CSs we chose 0.01% amyl acetate (the smell of banana) and 0.01% benzaldehyde (the smell of almonds) dissolved in deionized water. The same odorants were used as test odorants during the brief access tests. Amyl acetate is known to be tasteless up to 0.1% to male Wistar rats (Slotnick et al. 1997). When rats licked odorized water from the spout, the chance of orthonasal release of the odorant from the lick spout was minimized by surrounding the lick spout with a stainless steel conical ring and applying a continuous vacuum at 5 L/min in the ∼2 mm concentric space between the ring and spout (Supplementary Figure 1).

Conditioned taste/odor generalization procedure

The taste/odor aversion generalization experiments employed in this work were performed according to the CTA generalization paradigm established by Yamamoto (Yamamoto et al. 1985) with some modifications to include 2 oral odorant solutions, 0.01% benzaldehyde and 0.01% amyl acetate, together with the 4 prototypical tastants: 500 mM sucrose, 100 mM NaCl, 30 mM HCl, and 0.5 mM quinine HCl, all dissolved in deionized water. The sequence of experimental events is summarized in Table 1 and explained next.

During a 3-day familiarization period, a rat was placed in a test chamber (a modified home cage with raised metal grid and raised lid) for 30 min and allowed to lick water from a spout (8-channel taste manifold). The spout presented 5 ± 0.5 μL of deionized water as the animal licked, up to 3 times per second (to sustain motivation for the entire duration of the test procedure) for 2 min, followed by a 30 s break. During this break, the manifold was rinsed for 5 s with 5 mL deionized water and vacuumed for 10 s (starting at the same time as the rinse) to empty the dead space. Rinsing was necessary to prevent cross-contamination of test stimuli during brief access tests performed at a later stage. During familiarization, our rats drank 11.83 ± 0.23 mL of water in the test cage. Subsequently, each rat drank an additional 9.17 ± 0.03 mL of water in its home cage starting 45 min after the

Table 1 Conditioned taste/odor aversion generalization protocol

Day	1 2 3	4	5	6 7 8
Procedure	Familiarization	Conditioning	Rest	Brief access test
Drink	Water	CS	Water	Test stimuli

session, when a water bottle was placed on the home cage for 35 min. For conditioning (CS-US pairing), on the fourth day, the water was replaced by a conditioning stimulus (CS), and the procedure was otherwise the same as familiarization. On average, our rats drank 11.78 ± 0.54 mL of the CS during conditioning. This was followed by immediate (<3 min) bilateral LiCl (0.15 M i.p. at 2% (v/w) of body weight) injection using a short-beveled needle. No additional water was provided in their home cage on the conditioning day. This was followed by a recovery/rest day during which the rats underwent the familiarization procedure. This was then followed by 3 consecutive days (6th, 7th, and 8th day) of brief access (2-min short-term taste preference) tests. On each day, the rats were presented with all 7 test stimuli 2 times for 2 min each in the order water, sucrose, NaCl, quinine HCl, HCl, benzaldehyde, and amyl acetate solutions. In this paradigm, optimized for such a large brief access test array, rats consumed nearly the same amount of fluid (~93%) during the second half as they did in the first half of the test session. During these tests, they drank on average 10.94 ± 0.24 mL, and 45 min after the test they drank an additional 11.04 ± 0.38 mL when a water bottle was presented on the home cage for 35 min. The test chamber, used throughout the experiment, was filled with fresh bedding, and the grid cleaned and dried prior to starting each rat's session. All stimuli were presented at room temperature (~22 °C).

We avoided cross-contamination of the various stimuli in the lick manifold. During CS-US pairing, only a single line of the 8-channel gustometer was employed, others being filled with water. These lines were thoroughly rinsed with 50 mL of water after each CS, with odorants being presented last. During brief access tests, the manifold was rinsed with 5 mL of water during 5 s after each test stimulus and cleared using a vacuum and subsequently refilled with the new stimulus upon the first lick. We thus ensured that both during CS-US pairing and during testing, stimuli could not contaminate each other and that hence any generalization was extrinsic to the stimuli.

Description of the 3 experiments

Experiment 1: conditioned taste and odor aversion generalization tests in flavor-naive rats

The aim of this experiment was to understand how similar an orally ingested (retronasal) odorant is to tastants and to other retronasal odorants in flavor-naive rats (i.e., without flavor-enriched diets and having had no prior exposure to the odorants and specific singular tastants being investigated) (see Tables 2 and 3). To this end, we were particularly interested in evaluating whether aversion to retronasal odorants (CSs) would generalize to sweet tastants and to each other (Figure 1). In this experiment, 2 rats were used for each of the 6 CS groups and 4 rats for the water CS control group. All the rats were flavor naive. The test array consisted of 7 stimuli, each of which was presented twice per testing session for 2 min.

Table 2 Time course of flavor experience manipulation in the 3 experiments

Experiment 1	None			COA/CTA (Table 1)
Experience Expt 1		7-day odor–taste	7-day delay	
Experience Expt 2	2-day odor–taste	14-day delay		

Table 3 Odor-taste experiences per CS group for both experiments

CS	Experience Expt 1	Experience Expt 2
Water	Almond paste and banana	Almond paste and amyl acetate–sucrose solution
Sucrose	Almond paste	Almond paste
Benzaldehyde	Almond paste	Almond paste
Amyl acetate	Banana	Amyl acetate–sucrose solution

Experiment 2: conditioned taste and odor aversion generalization tests in flavor-experienced rats (Experience experiment 1)

We hypothesized that the odorants would attain a sweet quality only after consumatory pairing with a sweet gustatory stimulus. To test this hypothesis, we performed 2 experience experiments. In both experiments, we used 16 rats, 4 rats per CS (water control, sucrose, benzaldehyde, and amyl acetate). For the first experience experiment, we allowed them to experience an odor–taste mixture for 1 week, added to the regular diet, followed by 1 week of rest before starting familiarization, conditioning, and testing (Table 2). The odor–taste mixture consisted of almond–sugar paste (almond paste), 5 g/day, for the sucrose CS and benzaldehyde CS rats. For amyl acetate CS rats, the odor–taste mixture consisted of fresh banana, 5 g/day. Control rats were given both almond paste and banana (Table 3). The bananas tasted both sweet and sour to humans. All additions were consistently eaten in full.

Experiment 3: conditioned taste and odor aversion generalization tests in flavor-experienced rats (Experience experiment 2)

In the second flavor experience experiment, we had 3 goals. First, we reasoned that if the odor–taste learning in rats is like that in humans, it should be establishable using a shorter experience period and should survive a longer delay to allow extinction. We therefore shortened the experience period from 7 days to 2 and increased the delay period from 7 to 14 before starting familiarization (Table 2).

Second, we tested whether odor to sucrose generalization would occur only after taste–odor pairing using a solid food (banana, almond paste, in experience experiment 1) or whether such generalization could also be established with

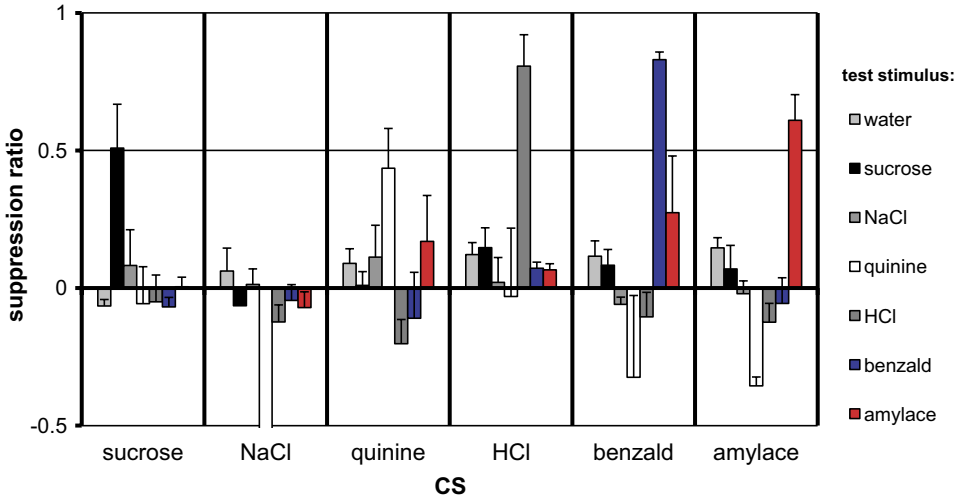

Figure 1 Conditioned taste and odor aversion generalization tests in flavor-naive rats (n = 16, male Wistar). The CSs included the 4 prototypical tastants and 2 water-dissolved oral odorants, 0.01% benzaldehyde (benzald) and 0.01% amyl acetate (amylace). Both of these odorants have been reported to be perceived as sweet by human subjects. None of the odorants generalized to sucrose, neither did sucrose CS generalize to the odorants. The odorants also did not clearly generalize to each other, suggesting that they do not share a common quality-like sweetness to rats. (average of brief access test day 1, 2, and 3; + standard error of the mean indicates variation across test days; *n* = 2 rats per CS; control = 4 water CS rats; 6 two-min trials/rat/test stimulus).

a watery solution. Furthermore, we sought tighter control of the chemicals presented for the odor–taste pairing. Therefore, we replaced banana with an amyl acetate–sucrose mixture (0.01% amyl acetate + 0.5 M sucrose, presented in a second bottle on the cage, ad lib for 48 h) in this experiment for amyl acetate CS rats (Table 3). The sucrose and benzaldehyde CS rats received the same almond paste (5 g/day) as in the first experience experiment (Table 3).

Third, we sought to reduce one source of variability in the first experience experiment. In that experiment, during the brief access tests, we allowed 2 min per stimulus before moving to the next test stimulus. There was, however, a variable delay between the start of each trial and the rat's first lick (especially when grooming). For data analysis purposes, we ignored the few trials in which rats did not engage at all. To reduce this source of variability, in the third experiment the rat's first lick started the 2 min trial time, with a maximum wait of 1 min.

Data analysis

For each rat, the number of licks (per 2 min) was recorded for each of the 7 test stimuli separately, and the number of licks for the same CS was averaged across the same CS rats. The rats presented water as a conditioning stimulus (water CS rats) served as the control/standard for the number of licks to each test stimulus. These behavioral data were then expressed in terms of the suppression ratio, defined as: 1 – (mean no. of licks *E*/mean no. of licks *C*), where E is the test stimulus of any CS group and C is the test stimulus of the water CS group (Yamamoto et al. 1985). These data were further analyzed by correlation analysis, analysis of variance (ANOVA) (group × test stimulus) and planned *t*-tests. Averages are reported ± standard error of the mean (standard deviation/$\sqrt{n}$). Alpha level was set at 0.05.

Results

Experiment 1: conditioned taste and odor aversion generalization tests in flavor-naive rats

Averaged across brief access test day 1, 2, and 3 to reduce day-to-day variation ($n = 2$ rats per CS group, $n = 4$ for controls), the mean suppression ratio for CS-specific stimuli was 0.53 ± 0.12 (range 0.01 ± 0.06, for NaCl, to 0.83 ± 0.03, for benzaldehyde; see Figure 1). This was significantly higher than suppression ratios to non-CS stimuli (–0.04 ± 0.04; $P < 0.003$, unpaired 1-way *t*-test). Tastant CSs generalized rather specifically to the same tastants except for NaCl, for which no aversion was obtained. The average-specific suppression ratio, excluding NaCl, was 0.64 ± 0.08. Odorant CS rats specifically avoided the respective oral odorants with suppression ratio of 0.83 ± 0.03 and 0.61 ± 0.10 for benzaldehyde and amyl acetate, respectively. We found no evidence that the 2 sweet odorant CSs generalized to sucrose (suppression ratio: 0.08 ± 0.06 for amyl acetate and 0.07 ± 0.09 for benzaldehyde) in flavor-naive rats. Symmetrically, sucrose CS also did not generalize to either of the 2 odorants (suppression ratio: –0.07 ± 0.03 for benzaldehyde and 0.00 ± 0.14 for amyl acetate). It was also noted that odorant CSs did not consistently generalize to each other. The suppression ratio of benzaldehyde CS to amyl acetate was a highly variable 0.27 ± 0.21 and of amyl acetate CS to benzaldehyde a low –0.06 ± 0.09, suggesting that they do not evoke a common quality, like sweetness, in flavor-naive rats.

Experiment 2: conditioned taste and odor aversion generalization tests in flavor-experienced rats (Experience experiment 1)

The results of the brief access test day 1 for the first experience experiment are shown in Figure 2 (n = 4 rats per CS group). Sucrose CS rats showed significant suppression of sucrose intake ($P < 0.004$) and did not generalize to the odorants (suppression ratio: benzaldehyde 0.02 ± 0.12, amyl acetate 0.09 ± 0.10). None of the benzaldehyde CS stimuli showed significantly larger suppression ratio than that of water. For the amyl acetate group, both amyl acetate (suppression ratio 0.43 ± 0.16, $P < 0.04$) and HCl (suppression ratio 0.56 ± 0.15, $P < 0.02$) were avoided more than water (Figure 2). The latter was expected as the unripe green bananas were both sweet and sour to humans in informal testing. Both odorant CS groups appeared to generalize to sucrose (suppression ratio of 0.28 ± 0.18 and 0.40 ± 28, respectively, for benzaldehyde and amyl acetate CSs), although this did not reach statistical significance (n.s. to water suppression). Nonetheless, benzaldehyde CS and amyl acetate CS suppression to sucrose were approximately 87% and 95% (average 92%) of the respective conditioned odorant suppression. Across odorant CS rats, the strength of the odor aversion correlated highly ($r^2 = 0.49$) with the strength of the sucrose aversion (strength of aversion estimated as stimulus intake divided by water intake, Figure 2 insert). For unclear reasons, amyl acetate CS rats appeared to avoid benzaldehyde (0.32 ± 0.22; n.s. from water).

The data were fairly noisy (n = 4 rats per CS group, see Materials and methods), in that one-factor ANOVA showed a significant effect of stimulus on suppression ratios for only the sucrose CS group ($F_{6,21} = 4.0$, $P < 0.008$). For the benzaldehyde CS group, this nearly reached significance ($F_{6,20} = 2.0$, $P < 0.12$) but did not for the amyl acetate group ($F_{6,21} = 1.4$, $P < 0.27$).

We continued the brief access tests the subsequent days to test how rapidly the taste, tentatively acquired by the odorants, would extinguish. Because rats repeatedly experienced the taste and odor elements of the previously paired odor–taste object separately during the brief access test, we expected a fairly rapid extinction of the taste acquired by the odorants. Complete extinction of the odorant-acquired taste was observed during the 3 days of brief access testing, being 13–15 days post odor–taste pairing (Figure 4, Table 2).

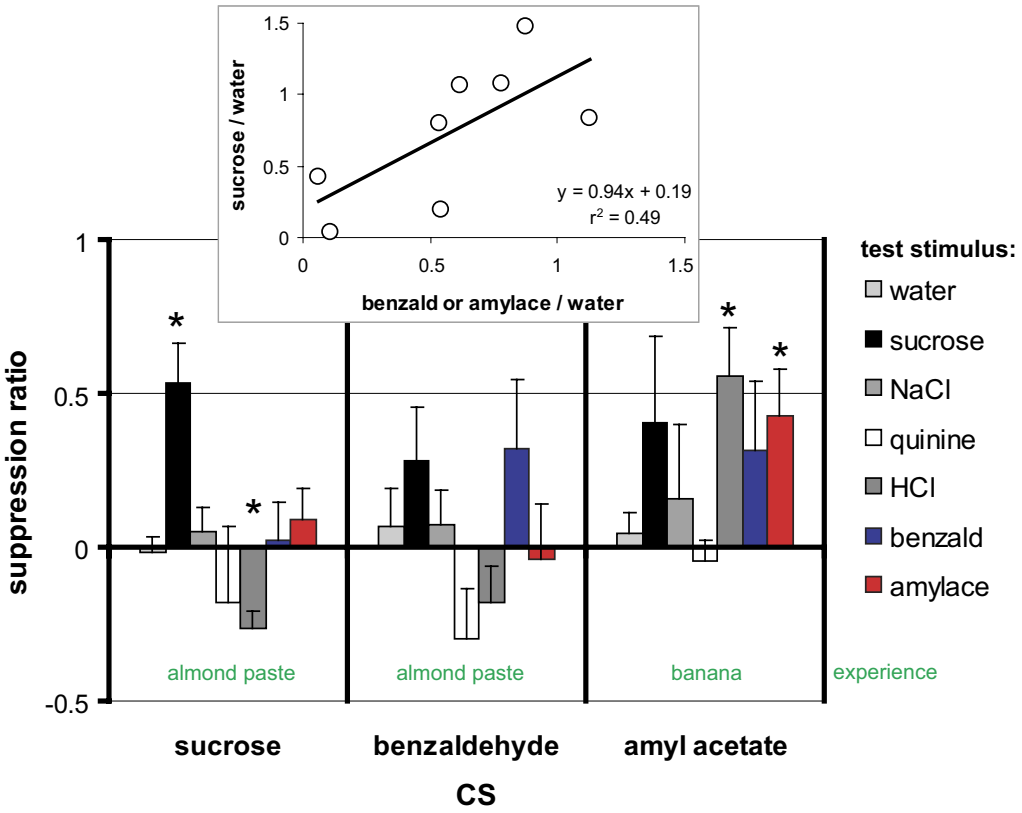

Figure 2 Experience Study 1. Conditioned taste and odor aversion generalization tests in flavor-experienced rats (n = 16, male Wistar; n = 4 rats per CS; control = 4 water CS rats; 2 two-min trials/rat/test stimulus; mean suppression ratio ± standard error of the mean). The sucrose and benzaldehyde CS rats both received almond-sugar paste (almond paste), and the amyl acetate CS rats received unripe green banana for 7 days followed by 7 days rest. Sucrose CS rats did not generalize to the odorants, but both odor CS rats did generalize to sucrose. Amyl acetate CS rats also generalized to HCl and benzaldehyde. Inset: correlation between the strengths of odor aversion and sucrose aversion in the odor CS rats. *$P < 0.05$ versus water suppression (one-sided unpaired t-test).

Experiment 3: conditioned taste and odor aversion generalization tests in flavor-experienced rats (Experience experiment 2)

The results of the first day of the brief access test are shown in Figure 3. Similar to the previous experiment, sucrose CS rats did not generalize to the odorants (suppression ratio: 0.06 ± 0.11 for benzaldehyde and 0.04 ± 0.02 for amyl acetate) and the odor CS rats generalized their suppression to sucrose (suppression ratio: 0.25 ± 0.10 and 0.34 ± 0.10 for benzaldehyde and amyl acetate CS rats, respectively). In this experiment, for benzaldehyde CS rats and amyl acetate CS rats, the suppression of sucrose intake was 32% and 115% (average 74%) of the respective conditioned odorant suppression. Across odor CS rats, the strength of the odor aversion correlated highly ($r^2 = 0.34$, $r = 0.59$, $n = 8$) with the strength of the sucrose aversion (corrected for water intake). This did not seem to be the case with the other tastants, HCl, NaCl, and QHCl ($r = 0.18$, $r = 0.13$, and $r = 0.33$, respectively, $n = 8$, data not shown), although these correlations did not reliably differ from the one with sucrose ($Z < 1.2$, Fisher's Z-test). A one-factor ANOVA showed a significant effect of stimulus on suppression ratios for both the sucrose CS group ($F_{6,21} = 7.5$, $P < 0.001$) and the benzaldehyde CS group ($F_{6,21} = 3.0$, $P < 0.01$). Only for the amyl acetate CS group did the suppression ratio not reliably vary across test stimuli, due to 2 of 4 rats not having developed an effective aversion (ANOVA, $F_{6,21} = 1.6$, $P < 0.2$). Nevertheless, for this CS group, there was a strong correlation between the water-normalized strength of amyl acetate CS aversion and the strength of sucrose aversion ($r^2 = 0.49$). The suppression ratio of sucrose was invariably higher than that of water (planned one-way unpaired t-test, $P < 0.05$) in all 3 groups (Figure 3; asterisks). Thus, in all 3 CS groups, rats reliably drank less sucrose than water, implying that the odorants by themselves were perceived as sweet by the rats during conditioning.

Nearly a complete extinction of the odorant-acquired taste in this study was observed as early as the second day of the brief access test (Figure 4), being 21 days after the 2 days of odor–taste pairing (Table 2).

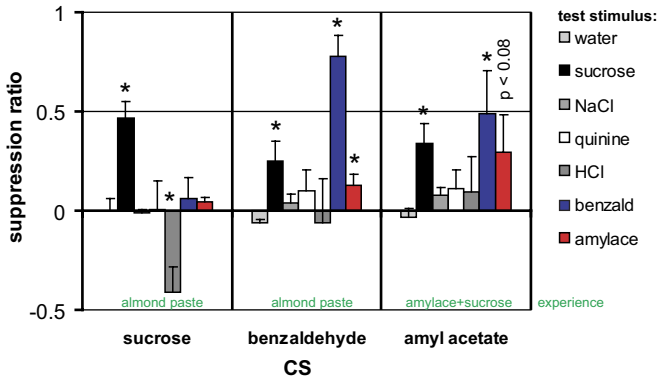

Figure 3 Experience Study 2. Conditioned taste and odor aversion generalization tests in flavor-experienced rats ($n = 16$, Wistar; $n = 4$ rats per CS; control = 4 water CS rats; 2 two-min trials/rat/test stimulus; mean suppression ratio ± standard error of the mean). In this study, the sucrose and benzaldehyde CS rats received the same almond-sugar paste (almond paste), but amyl acetate CS rats received a mixture of sucrose (0.5 M) and amyl acetate (0.01%) in water, and the duration of experience was reduced to 2 days followed by 14 days rest. Again sucrose did not generalize to the odorants but both odorants did generalize to sucrose. Note that amyl acetate no longer generalized to HCl. *$P < 0.05$ versus water suppression (one-sided unpaired t-test). One-factor (test stimulus) ANOVA: sucrose CS $P < 0.001$; benzaldehyde CS $P < 0.01$; and amyl acetate CS $P < 0.2$. Amyl acetate group n.s. because of 2 of 4 rats having no effective aversion.

Experiment 2 and 3: pooled statistical results

We also analyzed the combined data sets of Experiment 2 and 3 to investigate the statistics with larger numbers of animals (Supplementary Figure 2; $n = 8$ rats per CS group). Given the similarity in design between these 2 experience experiments, we felt this addition was justified. One-factor ANOVA showed a significant effect of stimulus on suppression ratios for the sucrose CS group ($F_{6,49} = 10.9$, $P < 0.001$) and for the benzaldehyde CS group ($F_{6,48} = 4.5$, $P < 0.001$). For the amyl acetate group, this nearly reached significance ($F_{6,49} = 2.2$, $P < 0.060$).

For the sucrose CS group only, only licks to sucrose ($P < 0.001$) were suppressed more than licks to water (unpaired one-sided t-test). Licks to HCl were increased over water ($P < 0.001$). Intake of benzaldehyde ($P < 0.28$) and amyl acetate ($P < 0.12$) were not suppressed. For the benzaldehyde CS group, only sucrose ($P < 0.021$) and benzaldehyde ($P < 0.003$) intake were more suppressed than water. For the amyl acetate CS group, sucrose ($P < 0.013$) and amyl acetate ($P < 0.007$) intake were suppressed more than water.

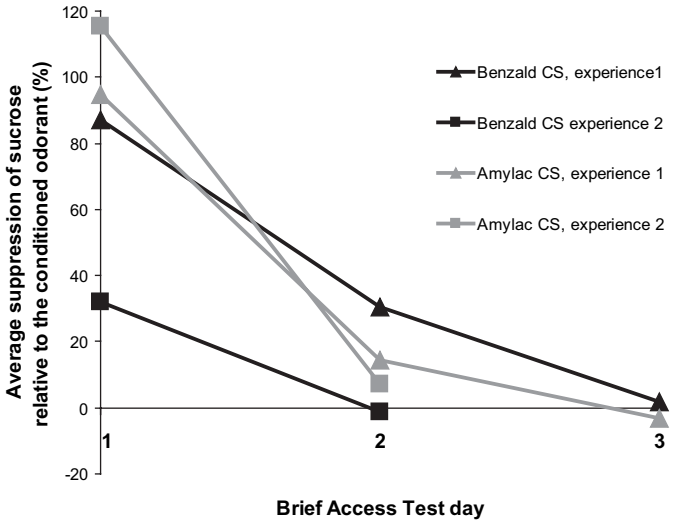

Figure 4 Rapid extinction of taste acquired by the odorant after interference. Average percentage of sucrose suppression (derived from the suppression ratio) relative to the odorant CS suppression, for both of the experience studies, is shown.

Additionally, HCl intake ($P < 0.024$) and benzaldehyde intake ($P < 0.012$) were suppressed (Supplementary Figure 2).

Discussion

Tasteless odorants are perceptually dissimilar to tastants in flavor-naive rats

In this study, we followed the classical CTA generalization test established by Yamamoto et al. (Yamamoto et al. 1984, 1985; Yamamoto et al. 1994), which has been useful in understanding similarities among tastants. We extended this approach to compare responses between the taste and odor modalities in rats. We asked rats that were naive to specific flavors how similar 2 orally ingested odorants (typically perceived by humans as sweet) were to the 4 basic tastes and to each other.

The 4 basic taste CS's generalized specifically to the same test tastants, as previously reported (Yamamoto et al. 1985) (Figure 1). The average CS-specific suppression ratio, excluding NaCl CS rats, was 0.64 ± 0.08, indicating that CTA/COA generalization was successfully established. Conversely, the average non-CS suppression ratio (conservatively excluding the unusually low values of the responses of NaCl CS rats to quinine of –1.03 ± 0.14) was –0.01 ± 0.02, significantly below the CS-specific suppression ratio ($P < 0.004$). These data confirm that our approach had sufficiently high consistency, sensitivity, and specificity despite rather small groups of animals. However, we do point out that no aversion was obtained to 0.1 M NaCl, which tends to be a problematic stimulus in CTA experiments. For example, in a study by Giza and Scott (1987), rats only failed to develop an aversion to NaCl. Rather, the NaCl CS rats drank more quinine than our control rats did (water CS group). We do not know the reason for this, but it was also evident in the study by Yamamoto et al., where suppression ratios of –0.2 to –0.3 occurred in 5 of the 12 CS groups (see Figure 8 in Yamamoto et al. 1985).

We were particularly interested in evaluating whether odorants (CSs) (which humans typically perceive as sweet) would generalize to the sweet tastant (sucrose), and to each other, in flavor-naive rats as these odorants could hypothetically be natively sweet to rats (as they also could be to humans). The 2 odorants neither generalized to sucrose nor to any other tastant nor to each other.

Amyl acetate has been demonstrated to be tasteless up to 0.1% to male Wistar rats (Slotnick et al. 1997), but no similar report existed for our other CS, benzaldehyde. A possible taste of benzaldehyde would somewhat limit our conclusions. Note that this only means that amyl acetate (≤0.1%) is not orally detectible in anosmic rats, which was useful to us to prevent false-positive findings, but importantly, it does not rule out the possibility that its odor could evoke a taste-percept (not tested by Slotnick and colleagues).

Our experimental set up was designed to ensure that rats received the odorants (as CS or test stimuli) via the oral–retronasal route but not the orthonasal route. First, we used a fully automated gustometer to present stimuli dissolved in water. Second, we employed a novel taste-manifold that sucks in the air around the lick spout (see arrows in Supplementary Figure 1). Hence, we presume that both during acquisition and expression of the association the retronasal route was mainly involved.

Paired odor–taste experience leads to specific generalization from odorant to tastant but not vice versa

The findings of the experiment with flavor-naive rats described above led us to hypothesize that odorants would attain a sweet perceptual quality only after consummatory pairing with a sweet gustatory stimulus, and hence would depend on the animal's flavor experience. This hypothesis is consistent with reports on flavor psychophysics (Stevenson et al. 1998; Stevenson and Boakes 2004; Stevenson and Tomiczek 2007) and neuroimaging data (Small et al. 2004).

The 2 flavor experience studies differed with regard to the duration/frequency of the paired taste–odor experience and regarding the delay between the paired experience and the CS-aversion-conditioning phase. The first experiment employed longer experience and shorter delay, whereas the second one was shorter experience and longer delay. Both experiments yielded similar results, and the pooled data analysis provided further robust results in support of our hypothesis. That is, the flavor-experienced rats in both experiments, after subsequent conditioning of only the odor (CS), avoided both the odor and the sucrose. This implies that the odor was perceived as sweet (i.e., sucrose-like) to them during conditioning. These findings tightly parallel taste acquisition of odors in humans (Stevenson et al. 1998; Small et al. 2004; Stevenson and Boakes 2004).

One important feature of the taste acquisition reported in humans is that it is highly resistant to extinction (Stevenson et al. 2000) and interference (Stevenson and Case 2003). Indeed, in our rodent paradigm, the acquired taste did not extinguish during the 2 weeks of delay between the paired odor–sucrose experience and the second short-term taste test. During this period, the rats were only exposed to regular food and water. The acquired sucrose-like taste was nevertheless extinguished within a matter of days upon COA learning (Figure 4). This suggests that resilience to spontaneous extinction is as robust as in humans but that resilience to interference may not be. Whether the COA tests may present stronger interference than interference tests in humans is unknown. It would also be of use to approach these questions using a different paradigm to allow more direct comparisons with humans. Indeed, the acquired preference for almond odor, after pairing with sucrose, has been shown to be highly resistant to extinction in rats (Boakes 2005; Albertella and Boakes 2006).

Sucrose CS rats did not conversely avoid the odorants they had prior paired flavor experience with. This too is analogous to the asymmetry observed in human odor–taste integration studies in that tastes do not acquire odors (Stevenson et al. 1998; Stevenson and Boakes 2004; Small and Prescott 2005; Stevenson and Tomiczek 2007). Such asymmetry would not have been expected if it were merely a case of associative learning, although asymmetry may be expected for adaptive ingestive reasons, that is, it would be maladaptive to avoid all sweet energy-rich foods but adaptive to avoid a specific food (-odorant). The asymmetry could hence be a product of the vastly larger odor-space (thousands of odor receptor proteins) than taste-space (tens of gustatory receptor proteins and 5 basic tastes) and hence that foods are identified more by their unique odor profile than their taste profile. We conclude that paired odor–taste experience leads to specific taste acquisition by odorants, but not vice versa, which may be further evidence that the data represent true taste quality acquisition by an odor rather than an odor–taste associative process.

It should be noted that amyl acetate CS rats which received fresh banana in the Experience Study 1 generalized to HCl and benzaldehyde in addition to sucrose. Replacing banana with amyl acetate–sucrose solution in the Experience Study 2 removed such generalization to HCl, suggesting that banana did have a sour taste component in it and apparently paired together with sucrose and amyl acetate. The unexpected generalization of avoidance to benzaldehyde by amyl acetate CS rats persisted in the Experience Study 2. Due to our manifold rinsing procedure, it is unlikely to have been caused by cross-contamination.

The current findings complement the human literature in that they suggest that no explicit psychophysical scaling is required for the phenomenon of taste acquisition by odors. This suggests that halo dumping, typically invoked in context of taste enhancement (Frank et al. 1993; Clark and Lawless 1994), is not a prerequisite for taste acquisition of odors in humans either, consistent with the hypothesis that this is a true learned synesthesia.

Compound conditioning and flavor MSI

These experiments complement experiments employing taste–odor compound conditioning, where individual stimuli (elements) are presented together (as well as separately) as the CS (Rescorla and Cunningham 1978). In our studies, we paired the stimuli only well before conditioning to serve as flavor experience but not during conditioning.

Compound conditioning can lead to overshadowing and potentiation, in that when tested one element produces a weaker, respectively, a stronger conditioned response than when this stimulus alone was the CS (Durlach and Rescorla 1980). When the compound CS consists of an odorant and tastant, the odorant is typically potentiated and the tastant overshadowed (Schnelker and Batsell 2006). This odorant potentiation is termed taste-mediated odor potentiation (or taste-potentiated odor aversion) (Rusiniak et al. 1979) and potentiation decreases as odorant concentration increases (Bouton et al. 1986; Slotnick et al. 1997).

In addition to providing paired odor–taste flavor experience, our experiments expand on this paradigm by also including test stimuli that were not CSs to investigate generalization of the taste and odor elements across an array of tastants and the CS odor. Slotnick et al. (1997) have shown that not only tastants can potentiate odorant intake suppression ($OT \rightarrow O > O \rightarrow O$; before the arrow indicates the stimulus during CS, after the arrow during testing, O, odorant, T, tastant) but also that odorants can potentiate tastant aversion ($OT \rightarrow T > T \rightarrow T$), potentiation again being negatively correlated with the concentration of the CS stimulus in both cases.

The findings of overshadowing and potentiation have typically been interpreted in a learning theoretical context. The illness would be associated with each element, and the elements would be mutually associated to each other (within-compound associations) (Durlach and Rescorla 1980). It has, however, been shown that within-compound associations are too weak to explain potentiation (Schnelker and Batsell 2006). Indeed, Batsell and colleagues have suggested that odor–taste potentiation depends on the additional formation of ''configural'' associations between the odor–taste compound as-a-whole and the illness (Trost and Batsell 2004) (see also Pearce and Bouton 2001). By extension, a flavor MSI interpretation could shed interesting new light on the compound conditioning findings.

Indeed, one ''rule'' in MSI is that it is most likely to occur when the concentrations of the elements are not too high (inverse effectiveness) (Meredith 2002). Thus, this predicts potentiation to be negatively correlated with stimulus concentration (or show an inverted-U shape). As described above, this has indeed been found to be the case.

Flavor MSI also predicts that such potentiation will occur most when stimuli are most congruent in both perceived flavor quality (e.g., sweet) and in time (simultaneously) (Small and Prescott 2005; Verhagen and Engelen 2006). Quality congruence may result from experiencing a combination of multimodal stimuli. The role of odor–taste quality congruence has not yet been addressed in the nonhuman literature because flavor experience has thus far not been manipulated. Thus, even though tastant and odorant pairs could have been congruent for human subjects (e.g., amyl acetate and saccharine; Slotnick et al. 1997), they would not be congruent for animals naive to these stimuli. It will be of interest to establish whether flavor experience modifies the odor–taste compound conditioning outcomes.

Tasteless odorants, odorless tastants?

As an important control, Slotnick et al. (1997) showed that the odorant (amyl acetate) could not be discriminated in anosmic bulbectomized rats even at the highest concentrations (0.1%) used in their aversion paradigms, strongly suggesting it was devoid of any orosensory component. In the current

experiments, we employed the same odorant at one-tenth this concentration (0.01%).

Vice versa, the sucrose (or other tastants) could have had an odor. This concern was addressed by Capaldi et al. (2004), showing that CTA learning may depend both on the taste and the odor of the conditioned taste stimulus. We attempted to avoid this pitfall at least orthonasally by using our vacuuming taste manifold. To be sure, the sucrose solution may have had a retronasal odor component, which we could not control. We believe that the putative retronasal smell of the sucrose solution played at best a minor role, however, because in both experience experiments, the sucrose CS group did not generalize to the retronasal benzaldehyde odorant with which it had been paired. Had sucrose had a substantial odor component, such odor–odor association would have been expected to be expressed. Instead, sucrose CS rats only generalized to sucrose itself. We conclude that despite evidence of an odor component, it played a relatively minor role in this study. In future studies, it would be of great interest to employ a wider variety of tastants for pairing.

The present results support the use of rats as a model for flavor perception. Because rodents allow investigations of flavor perception without the potential confounds of higher-order cognitive influences, such as language or the complications of scaling, they can complement studies in humans. In particular, they allow for deeper investigations into the neural processes underlying flavor perception and how they relate to cross-modal integration, experience, and learning.

Taken together, we conclude that flavor perception critically depends on, and evolves from, multimodal ingestive food experience.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/.

Funding

National Institutes of Health/National Institute on Deafness and Communication Disorders (R01DC009994).

Acknowledgements

We gratefully acknowledge Dr Patricia Di Lorenzo for her generous help with the gustometer design including the taste manifold. We would like to thank Dr Barry Green for helpful discussions. We thank Drs Barry Green, Larry Marks, and Thomas R. Scott for helpful comments on an earlier draft. We would like to acknowledge the John B. Pierce shop for making these experiments technically possible: John Buckley, Michael Fritz, Ronald Goodman, Tom D'Alessandro and Angelo DiRubba.

References

Albertella L, Boakes RA. 2006. Persistence of conditioned flavor preferences is not due to inadvertent food reinforcement. J Exp Psychol Anim Behav Process. 32:386–395.

Boakes RA. 2005. Persistence of acquired changes in the properties of odors and flavors for both humans and rats. Chem Senses. 30:i238–i239.

Bouton ME, Jones DL, McPhillps SA, Swarzentruber D. 1986. Potentiation and overshadowing in odor-aversion learning: role of method of odor presentation, the distal-proximal cue distinction and the conditionability of odor. Learn Motiv. 17:115–138.

Burdach KJ, Kroeze JH, Koster EP. 1984. Nasal, retronasal, and gustatory perception: an experimental comparison. Percept Psychophys. 36: 205–208.

Capaldi ED, Hunter MJ, Privitera GJ. 2004. Odor of taste stimuli in conditioned "taste" aversion. Behav Neurosci. 118:1400–1408.

Clark CC, Lawless HT. 1994. Limiting response alternatives in time-intensity scaling: an examination of the halo-dumping effect. Chem Senses. 19: 583–594.

Dravnieks A. 1985. Atlas of odor character profiles. Philadelphia (PA): ASTM.

Durlach PJ, Rescorla RA. 1980. Potentiation rather than overshadowing in flavor-aversion learning: an analysis in terms of within-compound associations. J Exp Psychol Anim Behav Process. 6:175–187.

Dwyer DM. 2005. Reinforcer devaluation in palatability-based learned flavor preferences. J Exp Psychol Anim Behav Process. 31: 487–492.

Frank RA, van der Klaauw NJ, Schifferstein HN. 1993. Both perceptual and conceptual factors influence taste–odor and taste–taste interactions. Percept Psychophys. 54:343–354.

Giza BK, Scott TR. 1987. Blood glucose level affects perceived sweetness intensity in rats. Physiol Behav. 41:459–464.

Harris J, Thein T. 2005. Interactions between conditioned and unconditioned flavor preferences. J Exp Psychol Anim Behav Process. 31:407–417.

Meredith MA. 2002. On the neural basis for multisensory convergence: a brief overview. Cogn Brain Res. 14:31–40.

Pearce JM, Bouton ME. 2001. Theories of associative learning in animals. Annu Rev Psychol. 52:111–139.

Prescott J. 1999. Flavour as a psychological construct: implications for perceiving and measuring the sensory qualities of foods. Food Qual Prefer. 10:349–356.

Prescott J, Johnstone V, Francis J. 2004. Odor–taste interactions: effects of attentional strategies during exposure. Chem Senses. 29:331–340.

Rescorla RA, Cunningham CL. 1978. Within-compound flavor associations. J Exp Psychol Anim Behav Process. 4:267–275.

Rusiniak KW, Hankins WG, Garcia J, Brett LP. 1979. Flavor-illness aversions: potentiation of odor by taste in rats. Behav Neural Biol. 25:1–17.

Sakai N, Imada S. 2003. Bilateral lesions of the insular cortex or of the prefrontal cortex block the association between taste and odor in the rat. Neurobiol Learn Mem. 80:24–31.

Schnelker J, Batsell WR Jr. 2006. Within-compound associations are not sufficient to produce taste-mediated odor potentiation. Behav Processes. 73:142–148.

Slotnick BM, Westbrook F, Darling FMC. 1997. What the rat's nose tells the rat's mouth: long delay aversion conditioning with aqueous odors and potentiation of taste by odors. Anim Learn Behav. 25: 357–369.

Small DM, Prescott J. 2005. Odor/taste integration and the perception of flavor. Exp Brain Res. 166:345–357.

Small DM, Voss J, Mak YE, Simmons KB, Parrish T, Gitelman D. 2004. Experience-dependent neural integration of taste and smell in the human brain. J Neurophysiol. 92:1892–1903.

Stevenson RJ, Boakes RA. 2004. Sweet and sour smells: the acquisition of taste-like qualities by odors. In: Calvert G, Spence C, Stein B, editors. Handbook of multisensory processing. Boston (MA): MIT Press.

Stevenson RJ, Boakes RA, Prescott J. 1998. Changes in odor sweetness resulting from implicit learning of a simultaneous odor-sweetness association: an example of learned synesthesia. Learn Motiv. 29:113–132.

Stevenson RJ, Boakes RA, Wilson JP. 2000. Resistance to extinction of conditioned odor perceptions: evaluative conditioning is not unique. J Exp Psychol Learn Mem Cogn. 26:423–440.

Stevenson RJ, Case TI. 2003. Preexposure to the stimulus elements, but not training to detect them, retards human odour-taste learning. Behav Processes. 61:13–25.

Stevenson RJ, Tomiczek C. 2007. Olfactory-induced synesthesias: a review and a model. Psychol Bull. 133:294–309.

Trost CA, Batsell WR Jr. 2004. Taste + odor interactions in compound aversion conditioning. Learn Behav. 32:440–453.

Verhagen JV, Engelen L. 2006. The neurocognitive bases of human multimodal food perception: sensory integration. Neurosci Biobehav Rev. 30:613–650.

Yamamoto T, Shimura T, Sako N, Yasoshima Y, Sakai N. 1994. Some critical factors involved in formation of conditioned taste aversion to sodium chloride in rats. Chem Senses. 19:209–217.

Yamamoto T, Yuyama N, Kato T, Kawamura Y. 1984. Gustatory responses of cortical neurons in rats. I. Response characteristics. J Neurophysiol. 51: 616–635.

Yamamoto T, Yuyama N, Kato T, Kawamura Y. 1985. Gustatory responses of cortical neurons in rats. III. Neural and behavioral measures compared. J Neurophysiol. 53:1370–1386.