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The effect of multisensory context and experience on flavor preference decisions in rats

Alex Hua, Timothy V. Dong, Joost X. Maier *

Department of Neurobiology & Anatomy, Wake Forest School of Medicine, Winston-Salem, NC, USA

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ABSTRACT

Flavor is perceived through multiple senses, including gustation and olfaction. Previous studies have shown that different sensory qualities that make up flavor are integrated to inform perceptual judgements. Psychophysical work in humans further suggests a prominent role for congruency (i.e., the learnt correspondence between taste and odor components of flavor through eating experience) in shaping multisensory interactions underlying perceptual judgments of flavor. However, eating experience cannot be controlled in humans, and depending on the type of judgement, these studies yielded mixed findings. Here, we used rats to test how experimentally-controlled experience with specific flavor mixtures ($Odor_A$+$Taste_A$ and $Odor_B$ +$Taste_B$) from weaning to adulthood affects subsequent flavor preference judgements in a series of two-bottle preference tests. In unisensory conditions, animals made odor or taste preference decisions (i.e., $Odor_A$ versus $Odor_B$ and $Taste_A$ versus $Taste_B$, respectively). In multisensory conditions, animals made identical decisions, but the addition of the other modality rendered one solution congruent; the other one incongruent (e.g., $Odor_A$+$Taste_A$ versus $Odor_B$+$Taste_A$). The results show that animals effectively learned congruency associations between the taste and smell components of experienced flavor mixtures. Comparing unisensory and multisensory conditions revealed no systematic effect of congruency on the magnitude of flavor preference, but preferences were less variable in multisensory compared to unisensory conditions. Results from a second group of naïve animals further demonstrate that increased reliability of preference judgements in multisensory conditions was independent of experience.

1. Introduction

Most foods that constitute a typical diet consist of both taste and smell characteristics. During consumption, taste compounds stimulate the lingual epithelium and give rise to the perception of gustatory qualities (sweet, bitter, sour, salty and umami). At the same time, volatile compounds released in the oral cavity travel up the nasopharynx to stimulate the olfactory epithelium retronasally and give rise to the perception of a near-endless variety of qualities [5] that allow us to uniquely identify foods. Both gustatory and retronasal olfactory modalities contribute substantially to our sense of flavor (often referred to as “taste” in everyday language) [27,28,31,38].

Previous work in humans demonstrated that both gustatory and retronasal olfactory signals contribute to flavor perception [36]. Moreover, central integration of signals from both modalities is thought to enhance perceptual judgements. For example, detection of taste-smell mixtures is faster than can be predicted on the basis of independent processing of the two modalities [35,43]. Some of these studies further suggest a role for experience in the ability to integrate gustatory and retronasal olfactory inputs [20,21,46]. In particular, enhanced detection [8,35] and pleasantness [1,14,33,34] of multisensory mixtures has been shown to critically depend on “congruency” of taste and smell components of a mixture. Congruency is typically defined as learned associations between taste and odor components of multisensory flavor mixtures through consumption [40,46–48]. However, work in humans precludes control over (or knowledge of) flavor experience, and congruency can at best be determined post-hoc, and is typically assumed based on arbitrary criteria [8,27,29,33–35,37]. Indeed, findings regarding the effect of congruency on flavor judgements have been inconsistent. Intensity judgements generally do not appear to be affected by congruency [15,17,22,27,37], and whereas some studies have shown that the addition of a congruent odor enhances the pleasantness/unpleasantness of palatable/unpalatable tastes [1,14,33,34], others found no effect of congruency on pleasantness ratings [37].

We previously used consumption tasks in rats to overcome some of the limitations of working with human subjects. Consumption is a

* Corresponding author.
E-mail address: jmaier@wakehealth.edu (J.X. Maier).

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behavioral read-out of hedonic flavor perception, and working with rats allows for experimentally manipulating flavor experience. Elliott & Maier [10] showed that lifelong experience with specific taste-smell mixtures had no apparent effect on consumption of taste-smell mixtures during subsequent testing sessions. That is, the amount consumed from a unisensory taste or odor solution was not differentially affected by adding a congruent versus an incongruent component of the other modality. However, since this study used a one-bottle design, a direct comparison between congruent and incongruent flavors was lacking. Moreover, a second study [23] found that gustatory and olfactory components of flavor stimuli were integrated to inform preference judgements in a manner that did not appear to depend on experience. Here, we exposed rats to specific taste-smell mixtures throughout development, thereby creating (in)congruent flavor combinations. Following training, we used a two-bottle design that allows us to directly contrast unisensory and multisensory preference decisions using congruent, incongruent and novel taste-smell mixtures. We assessed the effect of multisensory context on both the magnitude and the variability of flavor preference decisions. Based on work in humans, we hypothesized that multisensory congruency increases flavor liking. In the context of the present experiment, we thus predicted that adding a congruent compound of a different modality to a unisensory (taste or odor) flavor should increase the preference for that flavor. Based on our previous work in rats, we also considered the possibility that multisensory context affects flavor preferences in a manner that is independent of experience. Previous work suggests that access to gustatory and retronasal flavor qualities not only affects preference magnitude, but also increases the reliability (i.e., lowers the variability) of flavor preference decisions relative to unisensory stimuli [23]. In the context of the present experiment, we thus predicted that preferences in multisensory conditions were less variable than preferences in unisensory conditions.

2. Methods

2.1. Subjects

A total of 45 Long-Evans rats (20 males) were used for this study. Pregnant dams were obtained from www.criver.com and litters were kept together until weaning on postnatal day (PND) 21. All animals were kept on a 12 hour light cycle (6AM-6PM) and had *ad lib* access to food and fluids at all times during the experiment. All procedures took place in animals' housing facilities. Animals were treated in accordance with the *Guide for the Care and Use of Laboratory Animals*, and all procedures were approved by the Institutional Animal Care and Use Committee of Wake Forest University School of Medicine.

2.2. Stimuli

Stimuli consisted of aqueous solutions of taste and/or smell compounds (obtained from www.fischersci.com and www.sigmaaldrich.com; >98 % purity; dissolved in distilled water). Tastants: 10 mM saccharin (sweet), 58 mM sucrose (sweet), 50 mM sodium chloride (salty) and 100 mM monopotassium glutamate+0.17 mM inosine monophosphate (umami). Odorants (both at 0.025 % weight/volume): amyl acetate (CAS#: 628–63–7) and 2-hexanone (CAS#: 591–78–6). Since our lifelong exposure paradigm relies on animals' willingness to consume the presented flavors, only palatable tastes were used.

2.3. Procedures

Two groups of animals were used in this study. One group underwent training followed by testing sessions; the other group underwent only testing sessions. Table 1 summarizes the groups and associated experimental phases. For animals in group 1, training occurred over a period of four weeks, from postnatal day (P) 21–23 to P47–49; testing started two days after the last training day, from P49–51 to P60–62. Animals in group 2 remained naïve until testing, from P56–67.

Table 1
Overview of experimental timeline and procedures for group 1 and 2.

	Training	Testing Block 1	*Block 2*
Group 1 ($n = 33$)			
# Days	24	6	6
Age	~22–48	~50–55	~56–61
Group 2 ($n = 12$)			
# Days	–	6	6
Age	–	56–61	62–67

2.3.1. Training

Each week for four weeks, animals were exposed to taste-smell mixtures for 6 consecutive training days. Each animal was exposed to two unique bimodal mixtures consisting of one tastant and one odorant (T_A+O_A, T_B+O_B). On each training day, animals received 20 ml of a single mixture for 16 h (from 5 to 6PM to 9–10AM). Mixture identity alternated each training day (A-B-A-B-A-B), resulting in 3 exposures to each mixture per week (12 total over the course of training). During mixture exposure, animals were single-housed; in between exposures (from 9 to 10 AM to 5–6 PM), animals were pair-housed with a littermate and received *ad lib* water. Each block of six training days was followed by one day of *ad lib* access to plain water. For all animals, the taste component of one of the training mixtures was saccharin (per convention arbitrarily referred to as mixture A); depending on the animal, the other mixture (per convention referred to as mixture B) contained sodium chloride, sucrose or umami. Odor components used were identical for each animal (amyl acetate and 2-hexanone); the identity of the odor component paired with each taste was stable within each animal across training, but counterbalanced between animals. The training conditions are summarized in Table 2. To ensure equal exposure to mixtures A and B, the number of exposures to each mixture was identical for each animal, and we monitored consumption throughout the training period. Consumption did not differ between mixtures A and B (independent samples *t*-test comparing daily consumption of mixtures A and B: $t_{789} = 0.96$, $p = 0.34$). Training resulted in the formation of two congruent flavors (i.e., T_A+O_A and T_B+O_B) and two incongruent ones (i.e., T_A+O_B, T_B+O_A).

2.3.2. Testing

Testing occurred over a period of 12 consecutive days. Each day, rats drank freely from two bottles containing taste, odor, or taste+odor mixture solutions for 16 h (from 5 to 6PM to 9–10AM), effectively asking animals to make preference judgments in the following conditions: unisensory (O_A vs. O_B, T_A vs. T_B) or multisensory (O_A+T_A vs. O_B+T_A, O_A+T_B vs. O_B+T_B, T_A+O_A vs. T_B+O_A, T_A+O_B vs. T_B+O_B). Each bottle contained 200 ml of solution, ensuring animals had *ad lib* access to both solutions. Table 3 summarizes the testing conditions. In between tests, animals had *ad lib* access to water. Two blocks of testing were conducted. Within each block, each condition was presented once, in random order. To demonstrate that the testing procedure reliably measures preferences, we compared relative preferences in each condition between the two testing blocks. Preferences were significantly

Table 2
Overview of training conditions and number of animals assigned in group 1.

n	TasteA	OdorA	TasteB	OdorB
6	Saccharin	AA	NaCl	2H
7	Saccharin	2H	NaCl	AA
5	Saccharin	AA	Sucrose	2H
5	Saccharin	2H	Sucrose	AA
5	Saccharin	AA	Umami	2H
5	Saccharin	2H	Umami	AA

Table 3
Overview of testing conditions.

#	Modality	Bottle 1 Odor	Bottle 2 Odor	Bottle 1&2 Taste
1	Unisensory	OdorA	OdorB	No Taste
2	Multisensory	OdorA	OdorB	TasteA
3	Multisensory	OdorA	OdorB	TasteB
		Bottle 1 Taste	**Bottle 2 Taste**	**Bottle 1&2 Odor**
4	Unisensory	TasteA	TasteB	No Odor
5	Multisensory	TasteA	TasteB	OdorA
6	Multisensory	TasteA	TasteB	OdorB

correlated between blocks ($r = 0.50$, $p<0.001$), and preferences did not consistently differ between testing blocks (paired samples *t*-test comparing preferences in each animal and condition between the two blocks: $t_{240} = 0.88$; $p = 0.38$), indicating stable preference throughout the testing phase.

2.4. Data analysis

Consumption was measured by weighing bottles before and after training/testing sessions ($Consumption = Weight_{Before} - Weight_{After}$). Relative preference was calculated as $Consumption_{BottleA}/(Consumption_{BottleA}+Consumption_{BottleB})$, where a score >0.5 indicates a preference for bottle A; a score <0.5 indicates a preference for bottle B. For a total of 33 bottles (3.1 % of all bottles), consumption could not be determined due to spillage of displacement in the cage. Preferences in these instances were excluded from the analyses. Comparison of preference magnitude was based on the average preference over the two repetitions for each condition (i.e., blocks 1 and 2). Variance in preference was calculated across the two repetitions. Because preference and variance in preference were approximately normally distributed, we compared these two dependent variables between conditions defined by various independent variables using *t*-test and analysis of variance (ANOVA). Preferences were primarily compared between multisensory conditions and their corresponding unisensory conditions (factor Modality). We further broke down the data into cases where animals made odor decisions versus taste decisions (factor Decision), and into cases where different taste or odor compounds made up the congruent flavor (factors Taste and Odor Identity respectively). Finally, results were compared across groups that did or did not receive prior flavor experience (Factor Experience).

3. Results

We investigated the effect of multisensory context on the expression of flavor preferences in a two-bottle task. One group of rats ($n = 33$; 15 males) was first exposed to specific taste-smell mixtures in a controlled manner from weaning to adulthood during a training phase (see Methods), and then subjected to a two-bottle preference testing phase. A second group of animals ($n = 12$; 5 males) remained naïve until adulthood and were subjected to the same two-bottle preference tests without prior exposure. This design eliminates any possible confounding contribution of generalization between exposed and novel odor and taste compounds. The timeline of the experiments is illustrated in Table 1; Table 2 lists the two-bottle testing conditions.

3.1. Efficacy of congruency training on unisensory preferences

Extensive experience with a specific mixture of taste and odor components is thought to result in that mixture to be perceived as congruent. Thus, during training, each animal was exposed to two binary taste-smell mixtures. Although the use of animal models precludes direct assessment of congruency perception, our paradigm does allow us to test for the formation of associations between taste and odor components of mixtures. Based on previous work in rodents and humans showing that odors can acquire taste qualities when experienced in mixture [3,7,16,40–42,47], we predicted that if animals formed associations between the taste and odor components of the mixtures they were exposed to during training, relative preferences for tastes and their associated odors (conditions #1 and #4, respectively) should be correlated. For example, if an animal was exposed to saccharin+amyl acetate (A) and sodium chloride+2-hexanone (B), the preference for saccharin over sodium chloride should be similar to the preference for amyl acetate over 2-hexanone. Indeed, preferences in the two conditions were significantly correlated ($r = 0.45$, $p<0.01$; Fig. 1), demonstrating that animals learned associations between multisensory components of the training mixtures. We did not observe significant differences within unisensory preferences. That is, taste preferences relative to saccharin did not differ significantly between sucrose, NaCl and umami (one-way ANOVA on preference with factor Taste: $F_{2,32} = 2.94$, $p = 0.07$). As a consequence, odor preferences relative to the saccharin-associated odor did not differ between odors associated with sucrose, NaCl and umami ($F_{2,32} = 2.14$, $p = 0.14$). We speculate this is due to the fact that we used moderate concentrations of tastes that are equally palatable. We also note that whereas taste and odor preferences are correlated, taste and odor stimuli are detected and processed by (at least partly) separate neural systems. Indeed, previous work has shown that naïve animals have different preferences for the monomolecular odorants used here [3,25]. This offers a potential explanation for individual variability of differences in preference for tastes and their associated odors seen in Fig. 1. In the following analyses, conditions in which animals made taste-based versus odor-based decisions are treated as different (factor Decision, see below).

3.2. The effect of congruency on preference magnitude

Testing conditions were designed to directly test the effect of congruency on flavor preference decisions. In condition #1, animals made a unisensory preference decision between two odor stimuli they were previously exposed to. In the multisensory conditions #2 and 3, animals were confronted with the same odor choice, but in the presence of a taste stimulus that rendered one solution congruent; the other incongruent. Thus, in addition to a choice between two odors, animals made—at the same time—a choice between a congruent and incongruent flavor. Note that because the same taste stimulus is added to both bottles in the

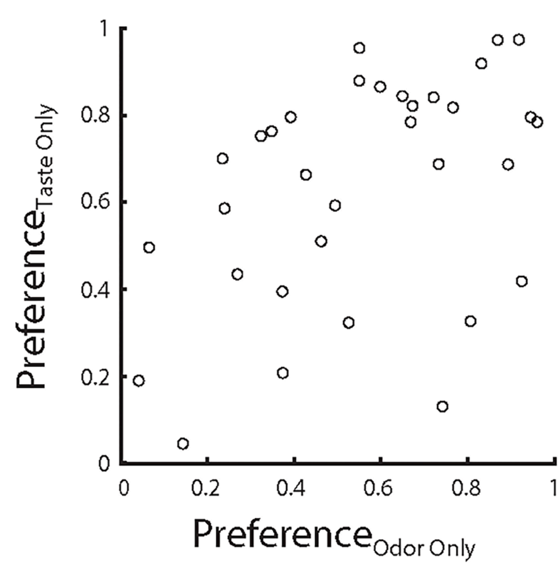

Fig. 1. Efficacy of congruency training. Each data point represents preference for taste stimuli (i.e., condition #4) plotted against preference for taste-associated odor stimuli (i.e., condition #1) for each animal ($n = 33$). Preferences in the two conditions were significantly correlated ($r = 0.45$, $p<0.01$).

multisensory conditions, taste information could not by itself affect preference. Therefore, any difference between unisensory (#1) and multisensory (#2–3) conditions would reflect an effect of taste congruency on odor preference. More specifically, based on our hypothesis that congruency increases liking, we predict that preferences for the congruent flavor in multisensory conditions should be greater than preferences for the odor component of the congruent flavor in the corresponding unisensory condition. Conditions #4–6 follow a similar design, but constitute taste-based choices (instead of odor-based choices). For all analyses and graphs, preferences in multisensory conditions were expressed as preference for the congruent flavor relative to the incongruent flavor; preferences in the corresponding unisensory conditions were expressed as preference for the odor/taste component of the congruent flavor, depending on whether animals made odor/taste discriminations, respectively. Fig. 2A shows that preferences in multisensory and corresponding unisensory conditions were highly correlated (r = 0.73, p<0.001), demonstrating that odor-based and taste-based choices were consistent in unisensory and multisensory conditions. Fig. 2B shows the difference in preference between multisensory and corresponding unisensory conditions for all animals, broken down by factors Modality (i.e., odor-based versus taste-based choice) and Taste (i.e., identity of the congruent taste). Unisensory and multisensory preferences did not differ when combining both taste and odor-based decisions involving all stimulus identities, although there was a (non-significant) trend toward lower preferences in multisensory versus unisensory contexts (t_{127} = 1.9, p = 0.06), indicating that incongruent flavors were slightly preferred over congruent ones. Post-hoc comparisons revealed that this was particularly apparent in cases where animals made odor-based choices with NaCl as the congruent taste (t_{12} = 4.04, p<0.01). This was likely due to a ceiling effect, since animals in this condition showed the highest preferences in the corresponding unisensory condition, and differences in preference between unisensory and multisensory conditions were overall negatively correlated with unisensory preference magnitude (r = -0.28, p<0.01). That is, when unisensory preferences were higher, corresponding multisensory preferences tended to be lower. Two-way ANOVA on the difference in preference between unisensory and multisensory conditions did not yield an effect of Decision ($F_{1,127}$ = 0.63, p = 0.43) or Taste Identity ($F_{3,127}$ = 2.74, p = 0.05; trending toward significance due to ceiling effect), nor an interaction effect ($F_{3,127}$ = 1.84, p = 0.14). We also directly compared preferences in cases where the congruent odor was amyl acetate versus 2-hexanone. No significant effects of Odor Identity (i.e., amyl acetate versus 2-hexanone in odor-based decisions or amyl acetate-associated tastes versus 2-hexanone-associated tastes in taste-based decisions) or Decision (i.e., odor-based versus taste-based decisions) were observed (main effect of Odor Identity: $F_{1,127}$ = 2.87, p = 0.09; main effect of Decision: $F_{1,127}$ = 1.51, p = 0.22; interaction: $F_{1,127}$ = 0.61, p = 0.43). Thus, flavor congruency did not affect the magnitude of odor or taste preferences in our two-bottle test.

3.3. The effect of congruency on variance in preference decisions

The results above suggest that congruency may not affect the magnitude of flavor preferences. However, it is possible that preference decisions are otherwise affected by multisensory context. Previous work suggests that multisensory flavor preferences are more reliable than preferences for their unisensory components. We tested the effect of multisensory context on the reliability of taste-based and odor-based choices in our paradigm by comparing preferences from the same animal and condition across the two testing blocks (see Table 1). Variance in multisensory judgements within the same condition has previously been used to assess judgment reliability [11,23]. Variance in preference across repetitions was significantly lower in the multisensory conditions as compared to the corresponding unisensory conditions (t_{102} = 2.39, p< 0.05; Fig. 3, left). Two-way ANOVA on the difference in variance between multisensory and unisensory conditions did not yield significant effects (Decision: $F_{1,102}$ = 0.03, p = 0.87; Taste Identity: $F_{3,102}$ = 0.28, p = 0.84; interaction: $F_{3,102}$ = 0.45, p = 0.72). Thus, multisensory context increased the reliability of preferences for flavor solutions.

3.4. The effect of multisensory context on preference variance in naïve animals

Preference is a relative measure, and it is therefore unclear whether the decrease in variance reported above resulted from more reliable judgements of congruent solutions, incongruent solutions or both. To test for the contribution of congruency to the reliability of flavor preferences, we performed an identical series of preference tests in a second group of naïve animals that were not experimentally exposed to taste-smell mixtures (see Table 2). Each animal in this group was tested on the same odor-based (amyl acetate versus 2-hexanone) and taste-based (saccharin versus sodium chloride) choices. Since we did not observe an effect of Taste in the data from group 1 presented above, only two taste compounds were used in group 2. Since these animals were not exposed to flavors, they had no opportunity to form taste-odor associations. As was the case for the first group of animals, multisensory preferences were correlated to their corresponding unisensory preferences (r = 0.50, p<0.001). Fig. 3 (right) shows variances in unisensory and multisensory preference across testing block 1 and 2 for this group of animals. Two-way ANOVA on variance with factors Modality

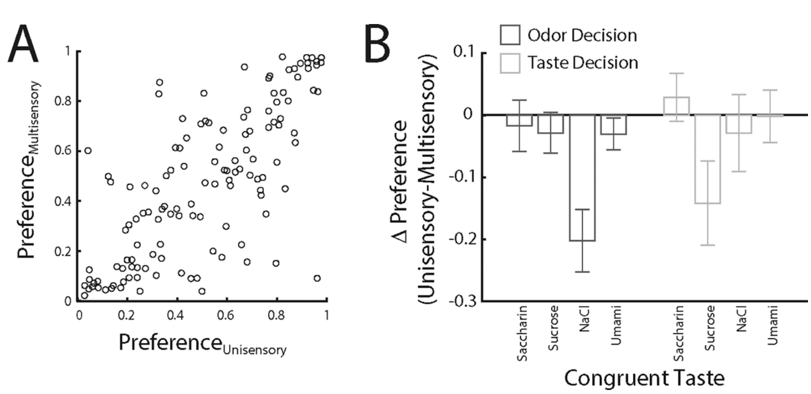

Fig. 2. The effect of congruency on magnitude of flavor preferences. **A.** Each datapoint represents preference in a multisensory condition (expressed as preference for the congruent relative to the incongruent flavor), plotted against preference in the corresponding unisensory condition (n = 132). No significant difference between the two conditions was observed. **B.** Bars represent differences in preference between multisensory and corresponding unisensory conditions, averaged (±SEM) across all animals and conditions. Preferences are shown separately for odor-based decisions (dark gray) and taste-based decisions (light gray), and for cases where saccharin (n = 33), sucrose (n = 10), sodium-chloride (n = 13) and umami (n = 10) was the congruent taste. Two-way ANOVA revealed no significant effects of Modality or Taste.

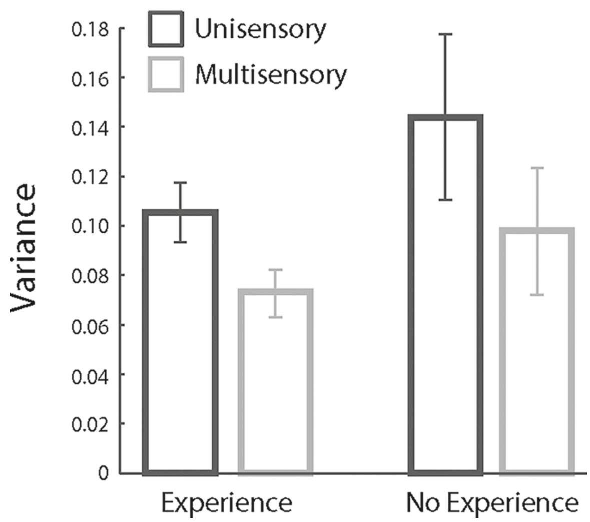

Fig. 3. Preference decisions are less variable in a multisensory context and independent of experience. Bars represent within-condition variance, averaged (±SEM) across conditions and animals (n = 132, 132, 48 and 48, respectively). Two-way ANOVA on variance with factors Condition (unisensory, multisensory) and Experience (experience, no experience) yielded a significant effect of Condition ($F_{1,321} = 4.30$, $p<0.05$), but no effect of experience ($F_{1,321} = 2.82$, $p = 0.09$).

(unisensory, multisensory) and Experience (experience, no experience) yielded a significant effect of Modality ($F_{1,321} = 4.30$, $p<0.05$), but no effect of experience ($F_{1,321} = 2.82$, $p = 0.09$) or an interaction effect ($F_{1,321} = 0.13$, $p = 0.71$). Thus, multisensory context increases the reliability of flavor preference decisions independent of experience with specific taste-smell mixtures.

4. Discussion

In the present study, we experimentally manipulated life-long experience with specific taste-smell mixtures to create congruent and incongruent multisensory flavor associations. Subsequent taste and smell preference testing in unisensory and multisensory context showed that congruency did not affect the magnitude of preferences. However, multisensory context did increase the reliability of preferences in a way that was independent of experience with specific taste-smell mixtures.

Our results show that taste-smell congruency does not increase (or decrease) taste or smell preferences as measured in a two-bottle choice test. Previous work in humans has provided evidence that perceptual judgements of multisensory flavor stimuli are affected by congruency [1,8,14,35]. For example, congruency enhances the detectability of taste-smell mixtures relative to incongruent mixtures or their unisensory components [35]. However, preferences observed in the present study are unlikely to have been affected by detectability since all stimuli used were well-above threshold. Future work using near-threshold concentrations of taste and odor stimuli during testing will investigate how congruency training affects flavor detection. Congruent tastes (as compared to incongruent ones) have also been shown to increase pleasantness ratings of odor stimuli [34], and congruent odors increase the saltiness/sweetness ratings of salty/sweet taste stimuli [29,33]. In our design, preferences for congruent flavors were measured relative to incongruent flavors, and congruency-enhanced pleasantness (or incongruency-suppressed pleasantness) would thus be expected to have increased preferences. These findings seem at odds with the findings from the present study, however, note that there is a significant portion of the literature on human flavor perception that does not support a role for congruency in perceptual flavor judgements [15,17,19,22,27,37]. Below, we will discuss several factors that could contribute to discrepancies within the human psychophysics literature, and between perceptual judgments in humans and rats. First, humans made pleasantness judgements following explicit experimental instruction. Such judgments are subject to interpretation, and only indirectly relate to preference and choice behavior. Second, congruency is poorly defined in the extant literature, and few studies explicitly measured perceived congruency of stimuli [1,33,40]. Therefore, choice of congruent stimulus pairings is relatively arbitrary. For example, sucrose-lemon [33,35], citric acid-grapefruit [46] and saccharin-almond [8]—pairings that may not necessarily be thought of as congruent—have all been used as congruent mixtures. Perhaps the most intuitive objective definition of flavor congruency includes taste-smell mixtures that have been experienced through eating [39,46,47]. However, life-long, subjective experience with an enormous variety of flavor stimuli precludes precise knowledge of which taste-smell combinations are congruent for a given individual. Our rat model allowed us to greatly simplify eating experience, and experimentally manipulate congruency of arbitrary taste and smell components in a highly controlled manner. Even though animal models preclude assessment of subjectively perceived congruency, our behavioral data demonstrate that animals effectively learned associations between taste and smell components experienced during training, consistent with the definition of congruency resulting from eating experience. Finally, it is possible that the effects of congruency observed in human subjects are species-specific. For example, it is possible that humans have evolved hard-wired connections between certain taste and smell components. It may also be the case that the subjective experience of congruency is unique to humans and affects pleasantness judgements.

The present results are consistent with previous work in rats showing that taste-smell interactions in the context of preference judgements are independent of prior experience with specific taste-smell mixtures. Using an identical training procedure to the one used here followed by a series of one-bottle consumption tests, Elliott and Maier [10] showed that the amount animals consumed from taste-smell mixture solutions constituted a weighted sum of the amount consumed from the unisensory component solutions. This multisensory operation did not depend on congruency. Consistent with this finding, Maier and Elliott [23] measured preferences for taste, smell and mixture solutions in a series of two-bottle tests without experimentally manipulating prior exposure to specific taste-smell mixtures, and showed that mixture preferences could be explained by a weighted sum of unisensory component preferences. This pattern of results is consistent with the framework of maximum-likelihood integration [12]. According to this framework, perceptual judgements of multisensory stimuli are the result of "weighting" the unisensory component judgements, as has been observed for judgements of multisensory stimuli in a variety of modalities and task contexts, including visual-haptic [11], auditory-haptic [4] and visual-vestibular [6]. This operation is highly adaptive in that the weight placed on a given unisensory component increases with its reliability, resulting in multisensory judgements that are more reliable (i.e., less variable) than either unisensory component judgement in isolation. Our finding that flavor preference judgements are more reliable in a multisensory as compared to a unisensory context is consistent with the idea that taste and smell components of mixtures are integrated to inform preference judgements that are more reliable than the unisensory component judgements. Our findings further suggest that multisensory integration of flavor components is independent of experience with specific taste-smell mixtures. This independence would allow for the ability to evaluate any multisensory flavor stimulus, regardless of whether it has been experienced before. Future work that independently quantifies variance in taste, smell and mixture conditions will establish whether multisensory preferences can be accurately predicted using the maximum likelihood integration framework.

In summary, the present work provides insight into the multisensory computations underlying flavor preference judgements: the ability to integrate taste and smell components of flavor solutions results in more reliable preference judgements, and this ability does not depend on prior experience with these taste and smell components (not together in mixture nor in isolation). Our rat model allowed us to experimentally manipulate flavor experience in a controlled manner, overcoming some major challenges presented by work on the topic in human subjects. Rats also present an experimentally-tractable model system in which to study the circuit-level mechanisms underlying multisensory integration of flavor inputs. Previous work has established multisensory convergence of taste and smell inputs to several brain regions, including classical “association” areas such as the orbitofrontal and anterior cingulate cortex [9,30,37], as well as primary olfactory (piriform) [2,24–26] and gustatory cortices [32,44,45]. Recent work has made important progress in enhancing our understanding of how circuits in these regions integrate their multisensory inputs [13,18], and future work combining behavioral and circuit-level approaches will investigate how the neural computations performed in these circuits produce the adaptive advantages associated with multisensory integration of flavor.

CRediT authorship contribution statement

Alex Hua: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Timothy V. Dong:** Writing – original draft, Visualization, Formal analysis. **Joost X. Maier:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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