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Effects of chemosensory experience and context on consummatory behaviors.

Saphira M. Chiu
University of Louisville

EFFECTS OF CHEMOSENSORY EXPERIENCE AND CONTEXT ON CONSUMMATORY BEHAVIORS

By

Saphira M. Chiu
B.A., Western Kentucky University, 2015
M.S., University of Louisville, 2021

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By the following Thesis Committee:

Thesis Director: Dr. Chad Samuelsen

Second Committee Member: Dr. Cynthia Corbitt

Third Committee Member: Dr. Robert Lundy

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ABSTRACT

EFFECTS OF CHEMOSENSORY EXPERIENCE AND CONTEXT ON CONSUMMATORY BEHAVIORS

Saphira M. Chiu

November 30, 2021

Eating food generates associations between odors and tastes (i.e., flavor) that guide future choices. Experience with an odor-taste mixture links an odor with a taste's quality and hedonic value, resulting in a preference for an odor paired with a palatable taste over an odor paired with an unpalatable taste. However, experience with a neutral stimulus (i.e., latent inhibition) or environment (i.e., context) can influence the formation of conditioned associations. Here, I used a two-bottle brief-access task to determine whether rats display an innate preference between unpaired odors (isoamyl acetate and benzaldehyde), how preexposure to the unpaired odors impacts mixture-dependent consummatory behaviors, and to understand how the context in which mixtures are sampled informs consummatory behaviors. I found that odors are equally palatable prior to being paired with a taste, that experience with unpaired odors did not impact mixture-dependent consummatory behaviors, and that context may influence the formation of odor-taste associations.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE PAGE

INTRODUCTION

Eating is a multisensory experience. Although all the senses contribute, those of taste and smell are required for the perception of flavor (Small, 2012). The co-activation of the olfactory and gustatory systems generates robust odor-taste associations that link an odor with a taste's quality and hedonic value (pleasantness or unpleasantness) (Fanselow and Birk, 1982; Holder, 1991; Stevenson et al., 1995; Prescott et al., 2004; Gautam and Verhagen, 2010; Green et al., 2012). These experiences with flavors guide future food choices; foods with pleasant flavors are consumed again, and those with unpleasant flavors are avoided. When food enters the mouth, nonvolatile chemicals dissolve in the saliva to activate taste receptor cells clustered in taste buds, while volatile chemicals travel retronasally to activate olfactory sensory neurons in the nasal epithelium. Although both represent chemosensory information, the gustatory and olfactory systems convey these signals through different pathways.

The gustatory system is tasked with representing five taste qualities: sweet, salty, sour, bitter, and umami (savory). Once these chemicals (i.e., tastants) bind to the taste receptors, taste signals are transmitted through three routes to the central nervous system, depending on the location of the taste cells. The chorda tympani branch of the facial nerve (CN VII) carries gustatory signals from the anterior two-thirds of the tongue, the glossopharyngeal nerve (CN IX) carries gustatory signals from the posterior one-third of the tongue, and the vagus nerve (CN X) carries gustatory signals from the oropharynx. These nerves terminate in the brain stem, specifically in the nucleus of the solitary tract (NST). In most mammals, the taste signals are relayed to the parabrachial nucleus (PBN) before continuing to the parvocellular portion of the ventroposteromedial nucleus of the

thalamus (VPMpc). In primates, the taste signals are sent directly to the ventroposteromedial nucleus of the thalamus bypassing the parabrachial nucleus (Beckstead et al., 1980). The taste information is then transmitted to the gustatory cortex and to the hypothalamus. The gustatory cortex communicates with a number of higher order-areas, including the amygdala, mediodorsal thalamus, and orbitofrontal cortex, important for processing the sensory and affective information associated with flavors (Carleton et al., 2010; Kandel et al., 2013).

Volatile chemicals (i.e., odorants) are detected by the olfactory system via two routes. Orthonasal, where odorants enter through the nostrils and retronasal, where odorants from the mouth reach the nasal cavity by passing through the oropharynx. Retronasal olfaction is an essential component of flavor perception (Lim and Johnson, 2011; Prescott, 2012). Odorants are detected by olfactory sensory neurons in the nasal epithelium. Their axons make up the olfactory nerve (CN I), which transmits olfactory signals to mitral and tufted neurons encapsulated in the olfactory bulb. Mitral and tufted neurons are the main output neurons of the olfactory bulb and send projections to a number of cortical areas that process olfactory information, the largest of which is the piriform cortex (Neville and Haberly, 2004; Wilson and Sullivan, 2011). The olfactory system is the only sensory system that does not communicate with the thalamus before reaching the cortex. Cortical neurons transmit olfactory signals to higher-order limbic, thalamic, and cortical regions involved in processing the sensory and affective properties of flavors (Zald and Pardo, 1997; Courtiol and Wilson, 2014; Maier et al., 2015). Interactions between the gustatory and olfactory systems are crucial for the perception of flavor (Schul et al., 1996), where signals from the two chemosensory systems, along with visual, auditory, and somatosensory signals, are integrated to generate the perception of flavor (Small, 2012).

While the perception of flavor relies on experience with foods, novel or unfamiliar foods are often avoided, a phenomenon known as neophobia (Barnett, 1958). Rodents are especially hesitant when sampling novel foods (Rzóska, 1953), preferring to consume familiar ones (Barnett, 1956). For example, when given a single bottle containing a novel odor dissolved in water, rats will initially avoid it but begin to sample from it over subsequent days (Miller et al., 1986; Lin et al., 2009; Fredericksen et al., 2019). However, when given the choice, rats prefer consuming water to a novel odor dissolved in water but change their preference after sampling the same odor mixed with sucrose. These results show that odor preferences are modulated by experience, but it is unclear whether there are innate preferences between novel odors. Experience with odors that have not been paired with a taste (i.e., unpaired odors) reduces neophobia. However, it may perturb the formation of odor-taste associations because pairing a familiar stimulus with a new stimulus often impedes the association, an effect called latent inhibition (Hall, 2009; Lubow, 2009). Latent inhibition modulates associative learning when a subject is given experience with a neutral stimulus before being paired with a positive or aversive consequence through classical conditioning. Having experience with a once neutral stimulus can weaken its ability to potentiate a conditioned behavior (Lubow, 2009). In the case of conditioned taste aversion (CTA), pairing a novel taste with an injection of lithium chloride induces gastrointestinal malaise and potentiates a robust aversion to the taste (Freeman and Riley, 2009). However, pairing lithium chloride with an experienced taste, one that has been sampled many times without malaise (preexposure), attenuates the aversion to the taste (Bills et al., 2005). Therefore, giving rats experience with odors before pairing them with a taste (preexposure) may perturb the formation of odor-taste associations, thus inhibiting experience-dependent consummatory behaviors.

Most studies of how odor-taste associations influence consummatory behaviors are conducted under laboratory conditions, where the stimuli and environment are under

strict experimental control. However, novel and familiar foods are often consumed during different times and in various places. Since the environment (i.e., context) can influence the acquisition and expression of experience-dependent behaviors (Bouton and Nelson, 1998), it is unknown to what extent variations in context during consumption influences future consummatory behaviors. In other words, how does experience with odor-taste mixtures in a familiar context, but outside the experimental context, influence mixture-dependent consummatory behaviors.

A gap in knowledge is whether rats have innate preferences between unpaired odors and what impact preexposure with unpaired odors has on mixture-dependent consummatory behaviors. Furthermore, how does the context in which odor-taste mixtures are sampled influence mixture-dependent consummatory behaviors? To answer these questions, I used a two-bottle brief-access task to measure consummatory behaviors before and after experience with chemosensory stimuli. This task uses a fixed number of trials, with a limited amount of time per trial, for a rat to drink from two simultaneously presented bottles. The number of times each bottle is sampled (i.e., licks) and the number of trials the rat chooses to engage are measures of consummatory behavior, while the difference in the number of licks between the two bottles is a measure of preference. I hypothesized that 1) prior to being paired with tastes, rats will consume the odors isoamyl acetate and benzaldehyde similarly. 2) Experience with the two unpaired odors will not perturb mixture-dependent consummatory behaviors; where after mixture experience rats will prefer to consume the odor previously paired with sucrose and avoid the odor previously paired with citric acid. Furthermore, I tested the hypothesis that 3) rats that receive mixture experience in the two-bottle brief-access apparatus will form a robust preference for the odor previously paired with sucrose, but those rats that receive mixture experience only in the home cage will not form an odor preference.

MATERIALS AND METHODS

Animals. All experimental procedures were performed in accordance with university, state, and federal regulations regarding research animals and were approved by the University of Louisville Institutional Animal Care and Use Committee. Twenty-three female Long Evens rats (250-300g; Charles Rivers) were single-housed and maintained on a 12/12-h light–dark cycle with ad libitum access to food and water prior to each experiment.

Chemosensory stimuli. Chemical stimuli were selected because of their prior use in chemosensory research involving rats (Gautam and Verhagen, 2012; Samuelsen and Fontanini, 2017; Bamji-Stocke et al., 2018; Fredericksen et al., 2019; McQueen et al., 2020). The odor stimuli, isoamyl acetate and benzaldehyde, were used at 0.01% concentration. We chose a concentration of sucrose (0.1M) that is consumed significantly more than water (Spector et al., 1993; Grobe and Spector, 2008; Treesukosol et al., 2014) and a concentration of citric acid (0.1M) that is consumed significantly less than water (Grobe and Spector, 2008; Treesukosol et al., 2014). Taste stimuli were obtained from VWR (Radnor, PA). Odor stimuli were obtained from Sigma–Aldrich (St. Louis, MO). All stimuli were mixed with distilled water.

Two-bottle brief-access task. All experiments employed a computer-controlled two-bottle brief-access apparatus directed by customized LabVIEW scripts (Fredericksen et al., 2019; McQueen et al., 2020). Briefly, the two-bottle brief-access apparatus consists of a test chamber, two motorized shutters to control access to two ports, and a motorized stage for the pseudorandom positioning of bottles containing chemosensory stimuli. Each two-bottle brief-access session began with a 2-minute period for the rats to acclimate to the test chamber. The two-bottle brief-access task started with the opening of the 2

shutters, allowing access at each port to a bottle with a stainless steel sipper tube. Rats had 15 s to contact either of the sipper tubes (contact window), if either sipper tube was contacted the shutters remained open for an additional 15 s (sampling window). The 15 s sampling window allowed rats to switch between ports within a trial. If no contact was made during the 15 s contact window, the shutters closed and a new trial began. Each trial was counted as one of the 20 trials whether the rat chose to engage or not. Once a trial was complete, the shutter doors closed, a 30 s intertrial interval began, and the computer moved the bottles for the next trial into position. Bottles were presented pseudorandomly and counterbalanced such that each chemosensory stimulus was presented 10 times at each port (20 trials total). Licks were recorded by a grounded contact circuit. Rats had to lick a minimum of 3 times at either port to qualify as an engaged trial. Data are presented as the mean licks per engaged trial, mean number of engaged trials, mean total licks, and preference ratio. The preference ratio is calculated as (S1 − S2) / (S1 + S2), where S1 is the total number of licks for bottles containing stimulus 1, and S2 is the total number of licks for bottles containing stimulus 2. A positive preference ratio indicates a preference for stimulus 1, and a negative preference ratio indicates a preference for stimulus 2.

Experiment 1. Five rats were placed on a water regulation schedule three days prior to the first rig-training session in the two-bottle brief-access apparatus, whereby access to distilled water was allowed for 4 h/day in their home cages. On the first day of rig-training, rats were habituated to the two-bottle brief-access apparatus, where they were allowed to drink from bottles of distilled water (10 ml each) through either of the two ports for 15 minutes. During the next three days of rig-training, rats were introduced to the two-bottle brief-access task, where they had 20 trials to drink distilled water from either port. After rig-training days, consummatory choice between novel odors dissolved in water (i.e., unpaired odors), 0.01% isoamyl acetate and 0.01% benzaldehyde were tested for the next

7 days. Rats had no experience with either odor (unpaired odor sessions). Following the seventh day of the unpaired odor sessions, the consummatory choice between two odor-taste mixtures (0.01% isoamyl acetate-0.1M sucrose and 0.01% benzaldehyde-0.1M citric acid) were tested for the next 4 days. Following the fourth day of experience with the two odor-taste mixtures, the consummatory choice between the two odors dissolved in water were retested for the next 7 days (paired odor sessions). After each experimental session, rats were given access to distilled water in their home cage for 4 hours. Data are presented as the mean (± standard error of the mean [SEM]) licks per engaged trial, mean (± SEM) total licks, mean (± SEM) number of engaged trials, and preference ratio. A positive preference ratio indicates a preference for isoamyl acetate or isoamyl acetate-sucrose, and a negative preference ratio indicates a preference for benzaldehyde or benzaldehyde-citric acid.

Experiment 2. Eighteen rats were separated into three groups, where the different groups received experience with odor-taste mixtures in different contexts (Table 1). Due to the number of subjects, Experiment 3 was run in two sections of 9 rats. Each section had 3 rats from each of the 3 groups. Rats were placed on a water regulation schedule and trained in the two-bottle brief-access apparatus for 3 days as in Experiment 1. After rig-training, the 3 groups of rats were given experience with odor-taste mixtures for 3 days. Sessions of odor-taste mixture experience were split into four 10-min blocks. Blocks 1 and 2 occurred in the two-bottle brief-access apparatus and blocks 3 and 4 occurred in the home cage. All rats were given access to a total of 10 ml of distilled water and 10ml of odor-taste mixtures (5 ml of palatable 0.01% isoamyl acetate-0.1M sucrose and 5 ml of unpalatable 0.01% benzaldehyde-0.1M citric acid). Rats in Group 1 (n = 6) were given access to the odor-taste mixtures in the two 10-min blocks inside the two-bottle brief-access apparatus, followed by distilled water in the home cage for both of the 10-min

blocks. Rats in Group 2 (n = 6) were given access to distilled water in both 10-min blocks inside the two-bottle brief-access apparatus and access to odor-taste mixtures in the home cage for both 10-min blocks. Rats in Group 3 (n = 6) were given access to odor-taste mixtures in the two-bottle brief-access apparatus for the first 10 minutes (block 1) then distilled water for the second 10 minutes (block 2). The rats were transferred to their home cage and given access to odor-taste mixtures for 10 minutes (block 3) followed by 10 minutes of distilled water (block 4). Each bottle was filled with 2.5 ml fluid. Bottle position was switched halfway through each 10-min block. New bottles were used for each 10-min block. This design ensured that all rats had access to the same amount of liquid and spent the same amount of time in each context. Following the last day of odor-taste mixture experience, we used the two-bottle brief-access task to measure the rats consummatory behaviors to distilled water. Next, the consummatory choice between the two odors dissolved in water were tested for 7 days. Data were collected and analyzed as above. A positive preference ratio indicates a preference for isoamyl acetate, and a negative preference ratio indicates a preference for benzaldehyde.

Water deprivation (3 Days)									
4 hours access to water in the home cage									
Rig habituation (1 Day)									
15 minute access to water in the two-bottle apparatus									
Rig training (3 Days)									
Two-bottle brief-access task: water vs. water									
Mixture experience (3 Days)									
Group 1: Rig only		**Group 2: Home cage only**		**Group 3: Rig & Home cage**	
Rig experience		**Rig experience**		**Rig experience**	
Block 1	**Block 2**	**Block 1**	**Block 2**	**Block 1**	**Block 2**
Isoamyl acetate-sucrose vs. Benzaldehyde-citric acid	Isoamyl acetate-sucrose vs. Benzaldehyde-citric acid	Water vs. Water	Water vs. Water	Isoamyl acetate-sucrose vs. Benzaldehyde-citric acid	Water vs. Water
Home cage experience		**Home cage experience**		**Home cage experience**	
Block 3	**Block 4**	**Block 3**	**Block 4**	**Block 3**	**Block 4**
Water vs. Water	Water vs. Water	Isoamyl acetate-sucrose vs. Benzaldehyde-citric acid	Isoamyl acetate-sucrose vs. Benzaldehyde-citric acid	Isoamyl acetate-sucrose vs. Benzaldehyde-citric acid	Water vs. Water
Consummatory behavior (1 Day)									
Two-bottle brief-access task: water vs. water									
Consummatory behavior (7 Days)									
Two-bottle brief-access task: isoamyl acetate vs. benzaldehyde									

Table 1: Schematic outline of Experiment 2. All rats were water deprived for 3 days, given one day of habituation in the two-bottle apparatus, and trained to drink water in the two-bottle brief-access task. Rats were divided into three groups and given experience with odor-taste mixtures either in the two-bottle apparatus (Rig only), in their home cage (Home cage only), or in both locations (Rig & Home cage). Next, consummatory behaviors were measured for all rats for the choice between water bottles (1 day) and the choice between odorized water (7 days).

Statistical analyses. All statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA). One-way repeated-measures analysis of variance (ANOVA) was used to test whether the number of licks between the two bottles differed across days. Differences in total number of licks across days were tested using a one-way repeated-measures ANOVA. Differences between preference ratios were tested using one-way repeated-measures ANOVA. Post hoc analyses were performed using Holm–Sidak tests to correct for familywise error. Fisher's exact test with the Dunn-Sidak correction for familywise error were used to compare the proportion of trials in which both bottles were contacted across days.

RESULTS

Experiment 1. A principal factor guiding consummatory choice is the association between odor and taste generated by sampling an odor-taste mixture; rats prefer to consume an odor previously paired with a palatable taste to an odor previously paired with an unpalatable taste (McQueen et al., 2020). Here, I used a two-bottle brief-access task to determine whether odors are preferred prior to being paired with tastes and investigate how the previous experience with unpaired odors influences mixture-dependent consummatory behaviors. Rats preference and consummatory behaviors were measured during the choice between bottles containing water (water sessions, days 1-3), bottles containing 0.01% isoamyl acetate and 0.01% benzaldehyde dissolved in water (unpaired-odor sessions, days 4-10), bottles containing odor-taste mixtures 0.01% isoamyl acetate-0.1M sucrose and 0.01% benzaldehyde-0.1M citric acid (mixture sessions, days 11-14), and retested with bottles containing 0.01% isoamyl acetate and 0.01% benzaldehyde dissolved in water (paired-odor sessions, days 15-21).

Figure 1 shows the rats consummatory behavior during each two-bottle brief-access session. There was a significant difference in the mean licks per engaged trial across the two-bottle sessions ($F(41,164) = 35.69$, $P < 0.001$). A pairwise *post hoc* analysis showed that rats sampled similarly from the two bottles during each of the water sessions (day 1: $t_{(164)} = 0.34$, $P > 0.9$; day 2: $t_{(164)} = 0.60$, $P > 0.9$; day 3: $t_{(164)} = 0.97$, $P > 0.9$) and during each of the unpaired-odor sessions (day 4: $t_{(164)} = 0.15$, $P > 0.9$; day 5: $t_{(164)} = 0.94$, $P > 0.9$; day 6: $t_{(164)} = 0.39$, $P > 0.9$; day 7: $t_{(164)} = 0.37$, $P > 0.9$; day 8: $t_{(164)} = 0.42$, $P > 0.9$; day 9: $t_{(164)} = 0.35$, $P > 0.9$; day 10: $t_{(164)} = 0.84$, $P > 0.9$). When given the choice between odor-taste mixtures, rats sampled significantly more from the bottle containing

isoamyl acetate-sucrose than benzaldehyde-citric acid (day 11: $t_{(164)}$ = 6.99, P < 0.001; day 12: $t_{(164)}$ = 12.11, P < 0.001; day 13: $t_{(164)}$ = 12.60, P < 0.001; day 14: $t_{(164)}$ = 11.38, P < 0.001). After experience with odor-taste mixtures, rats sampled significantly more isoamyl acetate than benzaldehyde (day 15: $t_{(164)}$ = 11.38, P < 0.001; day 16: $t_{(164)}$ = 11.73, P < 0.001; day 17: $t_{(164)}$ = 12.29, P < 0.001; day 18: $t_{(164)}$ = 9.92, P < 0.001; day 19: $t_{(164)}$ = 12.89, P < 0.001; day 20: $t_{(164)}$ = 11.59, P < 0.001; day 21: $t_{(164)}$ = 9.78, P < 0.001). The preference ratio indicates which of the odorized stimuli were sampled more during each two-bottle session. Here a positive preference ratio indicates a preference for stimuli containing isoamyl acetate, and a negative ratio indicates a preference for stimuli containing benzaldehyde (Fig. 1B). There was no significant difference in the preference ratios within each session, so the preference ratios were averaged for the water sessions (days 1-3), the unpaired-odor sessions (days 4-10), the mixture sessions (days 11-14), and the paired-odor sessions (days 15-21). There was a significant difference in the preference ratios (F(3, 17) = 220.3, P < 0.001) (Fig. 1B). Post hoc analyses revealed that

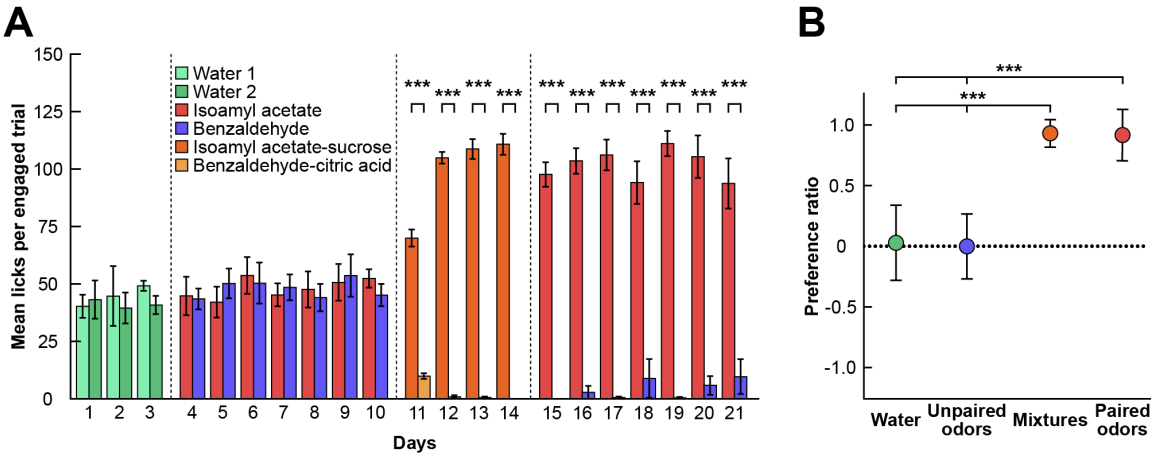

Figure 1: Rats choose to consume unpaired odors similarly but prefer the sucrose paired odor after mixture experience. (**A**) Mean number of licks per engaged trial (± SEM) during the four two-bottle brief-access task. Rats sampled similarly from the two bottles during the water sessions (days 1-3) and during the unpaired odor sessions (days 4-10). Rats sampled significantly more isoamyl acetate-sucrose than benzaldehyde-citric acid during the mixture sessions (days 11-14) and sampled significantly more isoamyl acetate than benzaldehyde during the paired odor sessions (days 15-21). (**B**) Preference ratios (± SEM) were averaged for each two-bottle brief-access task. The preference ratios for the mixture and paired odor sessions significantly differed from the water and unpaired odor sessions. Rats preferred to consume stimuli containing an odor (isoamyl acetate) when it had been paired with sucrose. The preference ratios did not significantly differ for mixture and unaired odor sessions. *** P < 0.001.

the preference ratio for the mixture sessions significantly differed from the water sessions ($t_{(17)}$ = 14.50, P < 0.001) and unpaired-odor sessions ($t_{(17)}$ = 18.26, P < 0.001), but not the paired-odor sessions ($t_{(17)}$ = 0.27, P > 0.8). The preference ratio for the paired-odor sessions also significantly differed from the water ($t_{(17)}$ = 15.81, P < 0.001) and unpaired-odor sessions ($t_{(17)}$ = 21.10, P < 0.001). There was no difference in the preference ratio between the water and unpaired-odor sessions ($t_{(17)}$ = 0.54, P > 0.8).

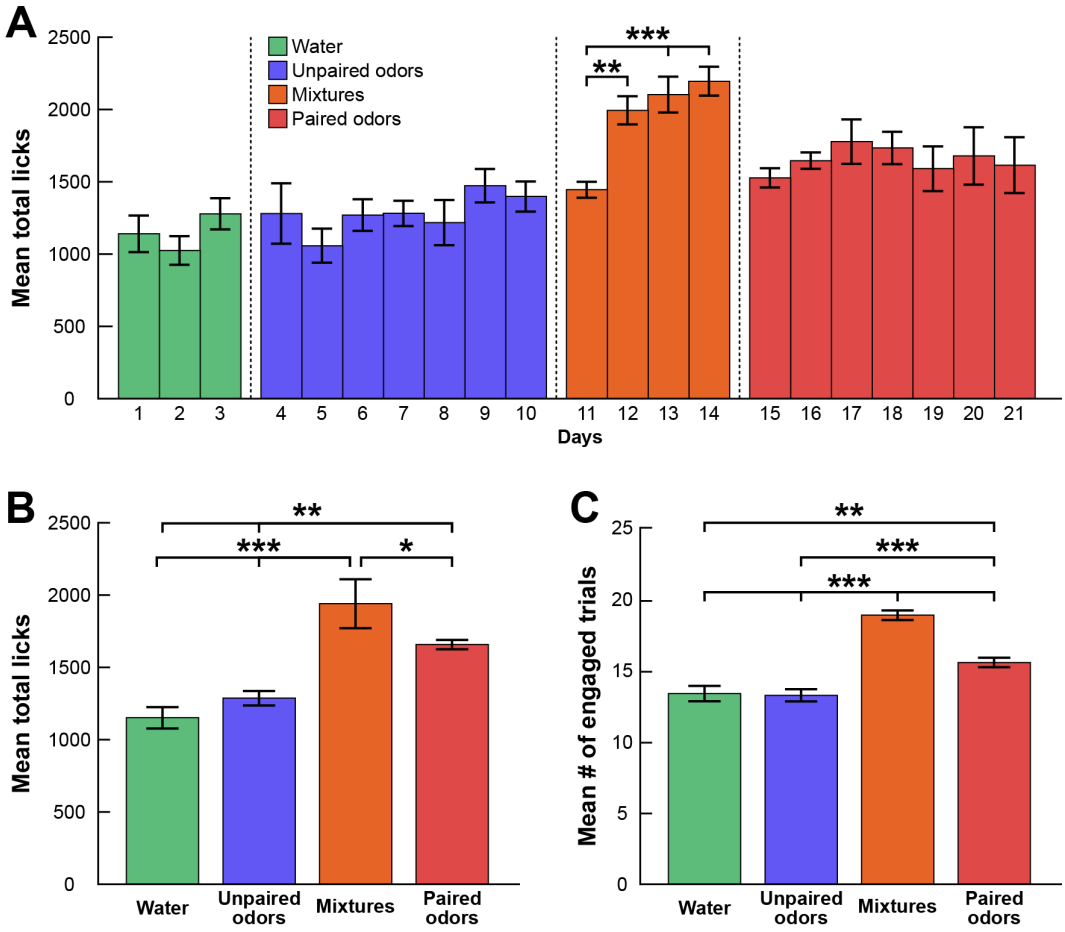

Figure 2: Mixture experience changes consummatory behaviors. (**A**) Mean total licks (± SEM) during each day of the 2-bottle brief-access task. Compared to the first mixture session (day 11), rats sampled significantly more from the bottles during subsequent mixture sessions. (**B**) Mean total licks (± SEM) for each 2-bottle brief-access session. Rats sampled the mixtures significantly more than all other sessions, but also sampled the paired odors significantly more than during either the water or unpaired odors sessions. There was no difference in the total number of licks between the water and unpaired odors sessions. (**C**) Rats engaged in significantly more trials when the bottles contained odor-taste mixtures than all other two-bottle brief-access sessions. Rats also engaged in significantly more trials during the paired odors sessions than either the water or unpaired odors sessions. There was no difference in the number of engaged trials between the water and unpaired odors sessions. ***P < 0.001; **P < 0.01; *P < 0.05.

As preference is only one measure of consummatory behavior, we examined the number of engaged trials and total number of licks (Fig. 2). There was a significant difference in the total number of licks across all sessions ($F(20, 80) = 9.92$, $P < 0.001$) (Fig. 2A). Next, we examined whether there was a difference in the total licks across days within each of the four sessions. There was a significant difference for the mixture sessions ($F(3, 12) = 16.74$, $P < 0.001$), but not for the water ($F(2, 8) = 2.99$, $P = 0.11$), unpaired-odor ($F(6, 24) = 1.61$, $P = 0.19$), or paired-odor sessions ($F(6, 24) = 0.75$, $P = 0.61$). A post hoc analysis revealed that the total number of licks on the first day of mixtures (day 11, 1459.8 ± 56.66) was significantly less than each of the following mixture days (day 12: 2014.8 ± 98.34, $t_{(12)} = 4.72$, $P < 0.01$; day 13: 2124.6 ± 124.96, $t_{(12)} = 5.66$, $P < 0.001$; day 14: 2218.0 ± 100.69, $t_{(12)} = 6.45$, $P < 0.001$). Next, we compared the average total number of licks during the water sessions, the unpaired-odor sessions, the mixture sessions, and the paired-odor sessions (Fig. 2B). There was a significant difference in the total number of licks across the four sessions ($F(3, 17) = 17.62$, $P < 0.001$). A post hoc analysis found that rats sampled significantly more during the mixture sessions compared to the water ($t_{(17)} = 5.89$, $P < 0.001$), unpaired-odor ($t_{(17)} = 5.94$, $P < 0.001$), and paired-odor sessions ($t_{(17)} = 2.57$, $P = 0.01$). Additionally, rats sampled significantly more during the paired-odor sessions compared to the water ($t_{(17)} = 4.18$, $P < 0.01$) and unpaired-odor sessions ($t_{(17)} = 3.96$, $P < 0.05$). There was no difference in the total number of licks between the water and unpaired-odor sessions ($t_{(17)} = 1.12$, $P > 0.27$).

The number of trials the rats chose to engage differed across experimental days (Fig. 2C). As there was no significant difference in the number of engaged trials within each session, the number of engaged trials was averaged for the water sessions, the unpaired-odor sessions, the mixture sessions, and the paired-odor sessions. There was a significant difference in the number of engaged trials across the four sessions ($F(3, 17) = 33.09$, $P < 0.001$). A post hoc analysis revealed that rats performed significantly more

trials during the mixture sessions compared to the water ($t_{(17)}$ = 7.51, P < 0.001), unpaired-odor ($t_{(17)}$ = 9.36, P < 0.001), and paired-odor sessions ($t_{(17)}$ = 5.53, P < 0.001). Additionally, rats performed significantly more trials during paired-odor sessions compared to the water ($t_{(17)}$ = 3.29, P < 0.01) and unpaired-odor sessions ($t_{(17)}$ = 4.49, P < 0.001). There was no difference in the number of engaged trials between the water and unpaired-odors sessions ($t_{(17)}$ = 0.19, P > 0.8).

On the first day of the mixture sessions, rats preferred isoamyl acetate-sucrose (Fig. 1) and engaged in a similar number of trials but sampled significantly less compared to the following three days (Fig. 2). These results indicate that consummatory behavior changed after the first mixture session. The two-bottle brief-access task allows rats to choose between 2 simultaneously presented bottles within a set amount of time. The 15 second presentation window affords rats time to switch between bottles. Therefore, we quantified the proportion of trials in which both bottles were sampled to determine whether the reduction in sampling was related to switching between bottles within the same trial (Fig. 3). Using Fisher's exact test with a Dunn-Sidak correction for multiple comparisons, we compared the proportion of trials in which both bottles were sampled within each of the

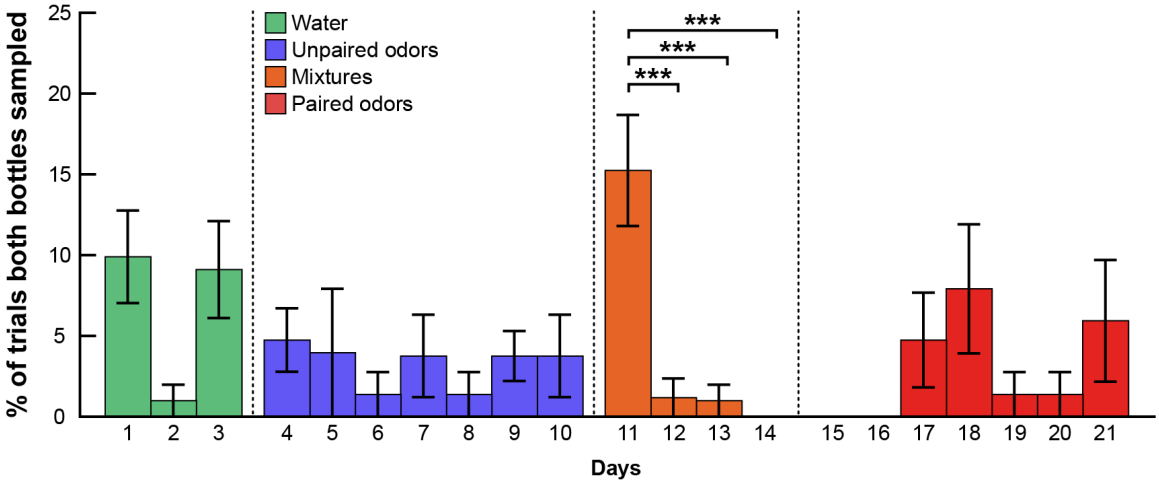

Figure 3: On the first day of odor-taste mixture experience (day 11), rats switched between bottles for a significantly greater proportion of trials compared to all other mixture tasks (days 12-14). There was no significant difference in switching during the other two-bottle brief-access sessions. ***P < 0.001.

four sessions. We found that the proportion of trials in which rats sampled from both bottles was significantly greater on the first mixture session (day 11: 15.4%, 14/91) compared to all other mixture days (day 12; 1.1%, 1/94, $P < 0.001$; day 13: 1.0%, 1/96, $P < 0.001$; day 14: 0%, 0/99, $P < 0.001$). There were no significant differences for the water, unpaired-odor, or paired-odor sessions. These results revealed that after rats had experience with odorized water, they spent a greater proportion of trials switching between bottles on the first day when given a choice between odor-taste mixtures.

Experiment 2. To determine how context influences consummatory behaviors, we provided three groups of rats with odor-taste mixture experience in different contexts, then measured preference and consummatory behaviors using the two-bottle brief-access task (Table 1). All rats were trained to sample water in the two-bottle brief-access task prior to mixture experience. After water training, rats in Group 1 (n = 6) received the odor-taste mixtures in the two-bottle brief-access apparatus, rats in Group 2 (n = 6) received the odor-taste mixtures in the home cage, and rats in Group 3 (n = 6) received the odor-taste mixtures in both the two-bottle brief-access apparatus and the home cage. During mixture

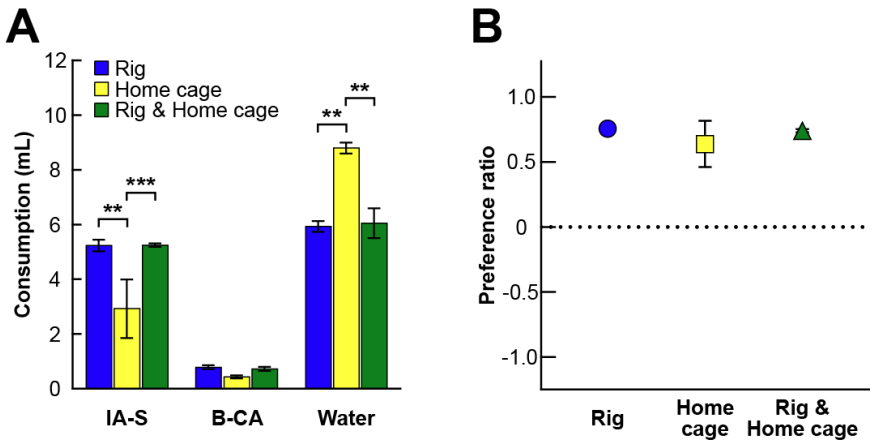

Figure 4: Consumption of mixtures and water in the different contexts (**A**) Mean volume consumed of 0.01% isoamyl acetate-0.1M sucrose (IA-S), 0.01% benzaldehyde-0.1M citric acid (B-CA), and water for the different contexts. Rats that received mixtures only in the home cage (yellow) consumed significantly less IA-S, but significantly more water, than rats with mixture experience only in the rig (blue) or those that had mixture experience in both locations. (**B**) The preference ratio (± SEM) for mixtures did not differ between groups. *** $P < 0.001$. ** $P < 0.01$.

experience, rats in Group 2 consumed significantly less isoamyl acetate-sucrose, but significantly more water, than rats in either Group 1 or Group 3 (Fig. 4A). However, there was no significant difference in the preference ratios between groups, indicating that all groups preferred to sample isoamyl acetate-sucrose to benzaldehyde-citric acid (Fig. 4B). After mixture experience, rats consummatory behavior was measured for the choice between water and then for the following seven days, the three groups of rats were given

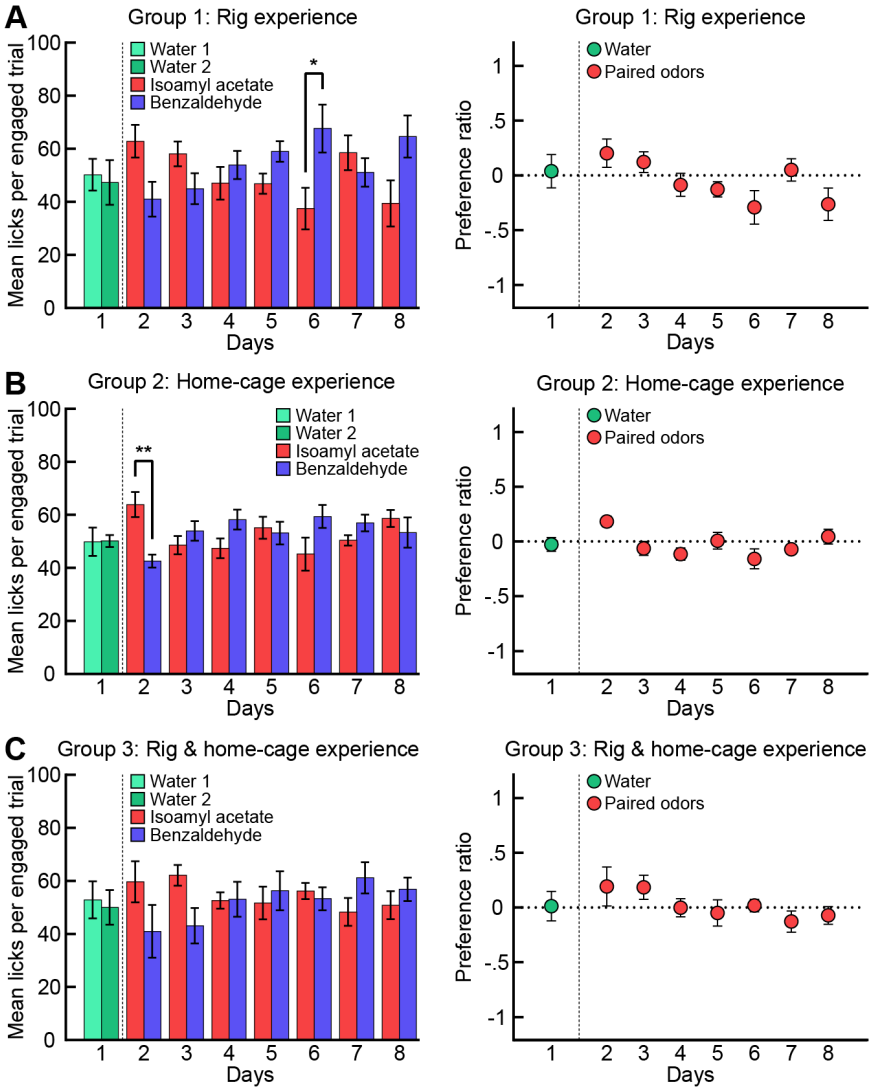

Figure 5: Consummatory behaviors were largely unaffected by mixture experience in the different contexts. (**A**) Rats that experienced mixtures only in the two-bottle brief-access apparatus sampled significantly more of the citric acid-paired odor (benzaldehyde) during the fifth paired odor session. (**B**) Rats that experienced mixtures only in the home cage sampled significantly more of the sucrose-paired odor (isoamyl acetate) during the first paired odor session. (**C**) Rats that experienced mixtures in both locations sampled odors similarly. The preference ratios between odors did not significantly differ from the preference ratio between water for any of the groups. $^{**}P < 0.01$; $^{*}P < 0.05$

the choice between bottles containing isoamyl acetate and benzaldehyde in the two-bottle brief-access apparatus. To obtain a baseline measure of consummatory behavior, rats were given the choice between bottles containing water during the two-bottle brief-access task on the first day after mixture experience. Over the next seven days, consummatory behavior was measured in the two-bottle brief-access task during the choice between bottles containing isoamyl acetate and benzaldehyde.

Figure 5 shows the consummatory behaviors of the three groups during each of the two-bottle brief-access sessions. The group of rats that experienced mixtures only in the two-bottle apparatus (Group 1) showed a significant difference in the number of licks per engaged trial ($F(15, 75) = 1.96$, $P = 0.030$). Group 2, those rats with mixture experience only in the home cage, also showed a significant difference in the number of licks per engaged trial ($F(15, 75) = 2.11$, $P = 0.018$). The group of rats that experienced mixtures in both locations did not differently consume the two odorized waters ($F(15, 75) = 0.90$, $P = 0.569$). Post hoc analyses showed that the only significant difference for the rats in Group 1 was sampling significantly more of the citric acid-paired odor (i.e., benzaldehyde) on the fifth paired-odor session ($t_{(75)} = 3.25$, $P = 0.014$) (Fig. 5A, left), while the only significant difference for the rats in Group 2 was sampling significantly more of the sucrose-paired odor (i.e., isoamyl acetate) during the first paired-odor session ($t_{(75)} = 3.85$, $P = 0.002$) (Fig. 5B, left). Groups 1 and 2 showed a significant difference in preference ratios (Group 1: $F(7, 35) = 2.68$, $P = 0.025$; Group 2: $F(7, 35) = 2.76$, $P = 0.021$), while Group 3 did not ($F(7, 35) = 1.03$, $P = 0.431$). Post hoc analyses revealed that neither group's odor preference ratio differed from the preference ratio for water, thus indicating that all three groups sampled from the two bottles containing odorized water similarly to the two bottles containing water (Fig. 5, right). Furthermore, we found no significant difference for any of the groups in the total number of licks (Fig. 6, left. Group 1: $F(7, 35) = 0.74$, $P = 0.64$; Group 2: $F(7, 35) = 1.17$, $P = 0.35$; Group 3: $F(7, 35) = 0.57$, $P = 0.78$)

or the number of engaged trials (Fig. 6, right. Group 1: $F(7, 35) = 1.12$, $P = 0.37$; Group 2: $F(7, 35) = 1.14$, $P = 0.36$; Group 3: $F(7, 35) = 1.58$, $P = 0.17$). Taken together, these results indicate that the mixture experience in all three contexts failed to generate odor-taste associations powerful enough to consistently influence consummatory behaviors.

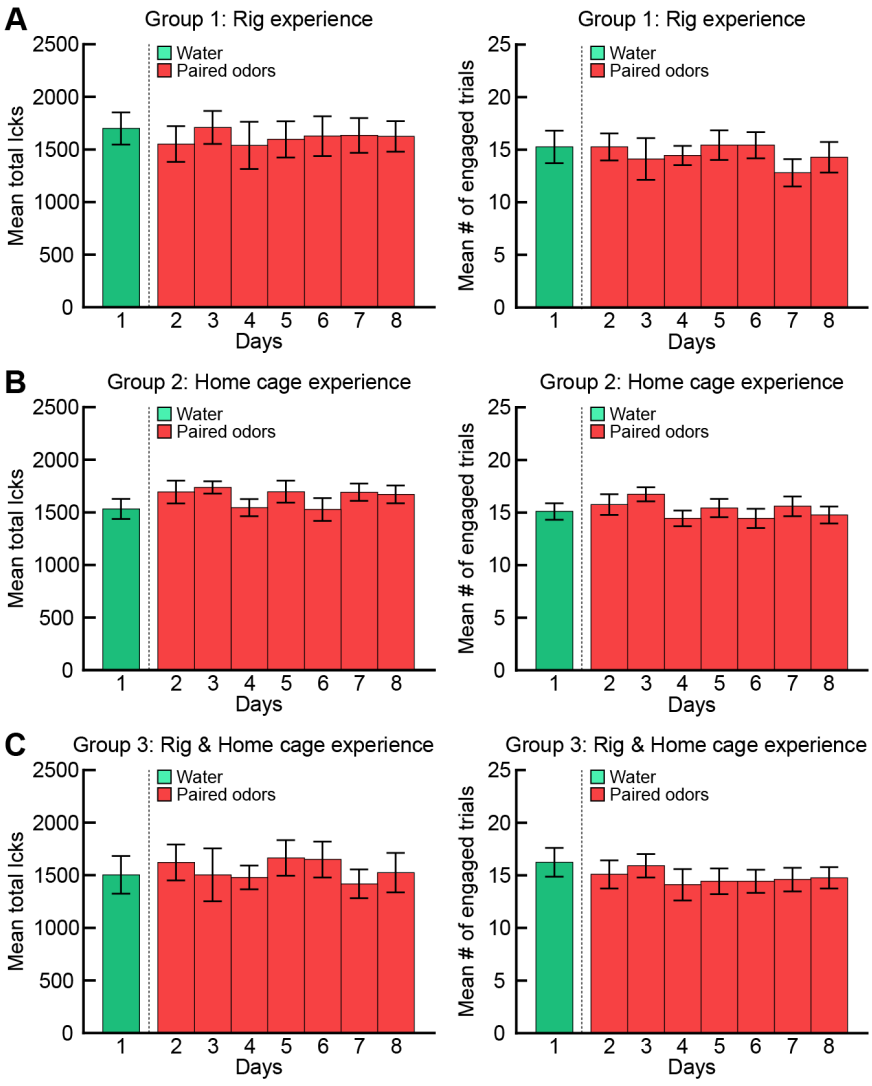

Figure 6: There was no significant difference in the total number licks (left) nor in the number of engaged trials (right) for (**A**) rats that experienced mixtures only in the two-bottle brief-access apparatus, (**B**) experienced mixtures only in the home cage, or (**C**) experienced mixtures in both locations.

DISCUSSION

In Experiment 1, I investigated whether rats show a preference for odors that have never been paired with a taste (i.e., unpaired odors) and how preexposure with unpaired odors influences mixture-dependent consummatory behaviors. My results demonstrate that unpaired odors dissolved in water are preferred equally and consumed similarly as water. Furthermore, even after experience with the unpaired odors, sampling mixtures generates odor-taste associations that guide consummatory choices; rats preferred to consume the odor that was paired with sucrose. These findings are consistent with studies in humans (Stevenson et al. 1995; White and Prescott 2007; Green et al. 2012) and rodents (Sakai and Yamamoto, 2001; Sakai and Imada, 2003; Fredericksen et. al, 2019; Elliot and Maier, 2020; Maier and Elliot, 2020; McQueen et. al 2020) demonstrating that experience with odor-taste mixtures influences consummatory behaviors.

As tastes have inherent hedonic value (Grill and Norgren, 1978a, 1978b), experience with an odor-taste mixture associates the odor with the identity and hedonic value of the taste (Fanselow and Birk, 1982; Holder, 1991; Gautam and Verhagen, 2010). Researchers often use the odors isoamyl acetate and benzaldehyde to pair with hedonically varied tastes when investigating experience-dependent consummatory behaviors (Sakai and Yamamoto, 2001; Gautam and Verhagen, 2010; Fredericksen et al., 2019; McQueen et al., 2020). Although previous experiments show that rats do not exhibit preferences between 2 orthonasally presented odors (Torquet et al., 2014), it was unknown whether rats prefer to sample either isoamyl acetate or benzaldehyde when dissolved in water. Our results show that rats choose to consume isoamyl acetate and benzaldehyde similarly. Also, there was no difference in consummatory behaviors

between the water sessions and the unpaired odor sessions. As discussed above, humans and rodents tend to avoid novel foods, a behavior known as neophobia (Barnett, 1958; Corey, 1978; Dematte et al., 2014). The fact that rats engaged in a similar number of trials and sampled similar amounts (Fig. 2B, 2C) during the water and unpaired-odor sessions indicates a similar level of motivation and suggests that neophobia was not a significant factor in the consummatory choice between unpaired odors. Since stimulus neophobia is less pronounced in familiar contexts (Mitchell, 1976), it is possible that experience gained during the three days of water training in the two-bottle brief-access task reduced the negative aspects of the novel unpaired odors.

Next, we asked if previous experience with the unpaired odors influenced consummatory choices that depend upon mixture experience? As expected, rats engaged in significantly more trials and sampled significantly more during the mixture sessions (when bottles contained mixtures of isoamyl acetate-sucrose and benzaldehyde-citric acid) than any other session. Interestingly, rats engaged in significantly more trials and sampled significantly more odorized water after mixture experience (paired-odor sessions) compared to either the water sessions or the unpaired odor sessions. These findings suggest that mixture experience increased motivation by altering the odor's incentive value (Berridge, 2004), where isoamyl acetate odorized water became more palatable and benzaldehyde odorized water became less palatable.

The first time rats were given odor-taste mixtures, they sampled significantly more of the mixture containing sucrose than the mixture containing citric acid, but they switched between bottles for a significant proportion of trials (Fig. 3). In addition, rats consumed significantly less from the two bottles compared to the subsequent mixture sessions but performed a similar number of trials (Fig. 2A). The switching behavior most likely explains the lower sampling during the first mixture session, suggesting that being exposed to odor-taste mixtures for the first time influenced consummatory behavior. Taken together, these

results show that sampling odor-taste mixtures informs consummatory choice even after previous experience with unpaired odors.

In experiment 2, I asked how the context in which the odor-taste mixtures were sampled influenced the acquisition and long-term expression of experience-dependent consummatory behaviors. Three groups of rats were given mixture experience in different contexts: only in the two-bottle brief-access apparatus (Group 1), only in the home cage (Group 2), or in both locations (Group 3). My results suggest that sampling mixtures in any of the three contexts did not establish odor-taste associations. There were only two sessions where rats showed significantly different consummatory behaviors. The group of rats that received mixture experience in the home cage consumed significantly more of the sucrose-paired odor during the first odor session (Fig. 5B, left) and the group of rats that received experience only in the two-bottle brief-access apparatus consumed significantly more of the citric acid-paired odor during the fifth odor session (Fig. 5A, left). However, the preference ratios across all groups and sessions never significantly differed from the preference ratio for water (Fig. 5, right). Furthermore, there were no differences in the number of engaged trials or total amount sampled (Fig. 6). These results indicate that the mixture experience was insufficient to impact their consummatory behavior, likely due to a failure to form odor-taste associations.

Although odor-taste associations can occur in as little as a single pairing (Stevenson et al., 1995; Blankenship et al., 2019) and, once acquired, are extremely resistant to extinction or interference (Sakai and Yamamoto, 2001; Harris et al., 2004; Albertella and Boakes, 2006; González et al., 2016), a variety of factors in our experimental design may have perturbed the establishment of odor-taste associations. First, the number of mixture experience sessions may not have been sufficient. Sakai and Yamamoto (2001) showed that the amount of mixture training directly influences consummatory choice for odorized water. They showed that three training days (consisting

of mixture experience in the test apparatus in the morning and mixture experience in the home cage at night) led to long-term preference for a sucrose-paired odor. However, just one training day failed to drive consistent odor preferences. Our lab has used multiple methods to establish odor-taste associations to investigate consummatory behavior, including multiple days (4 days) of 1-hour mixture experience in the home cage, multiple days (3 days) of overnight mixture experience in the home cage (McQueen et al., 2020), and multiple days (4 days) of mixture experience solely during the two-bottle brief-access task (Experiment 1). Although these studies demonstrate that multiple days of mixture experience can establish strong odor-taste associations, the different contexts may have exasperated neophobia. Rats are particularly hesitant to consume novel odorized stimuli (Miller et al., 1986; Lin et al., 2009; Fredericksen et al., 2019) and novel contexts can amplify stimulus neophobia (Mitchell, 1976). In Experiment 2, the mixtures and different contexts were introduced during the same session. The combination of a novel context and novel mixtures may have contributed to the failure to drive odor-taste associations. It is possible that an additional mixture experience session would have mitigated the effects of neophobia and enable the formation of odor-taste associations.

Another possible factor that may have disrupted the formation of odor-taste associations during Experiment 2 was the limited access to mixtures. In Experiment 1, rats consume ~20-25 ml of mixture (mostly isoamyl acetate-sucrose) during the odor-taste mixture sessions and formed robust odor-taste associations. In Experiment 2, I controlled the amount of time and the total volume available to consume (10 ml of each mixtue, see Methods) to ensure that the groups had similar mixture experiences in the different contexts. Although limiting the volume was meant to ensure consistency across groups, the limited amount of odor-taste mixtures may not have provided sufficient sensory information to drive the formation of odor-taste associations. Furthermore, due to their

water regulated state, the limited volume coupled with the rewarding aspects of quenching thirst may have shifted the hedonic value of the citric acid mixture making it less aversive.

Future experiments examining the role of context will use a modified approach of Experiment 1, where mixture experience clearly influenced consummatory behaviors. As in Experiment 1, we will train two groups of rats in the two-bottle brief-access task using water, but after each training session rats will be presented with two bottles containing water in the home cage. On the fourth day, Group 1 will receive odor-taste mixture during the two-bottle brief-access task and two bottles of water in the home cage. Group 2 will continue to receive water during the two-bottle brief-access task but receive bottles of odor-taste mixtures in the home cage. We will match the volume and time available to drink stimuli in the home cage to the maximum time available during the two-bottle brief-access task (15 min). After 3 days of mixture experience, both groups of rats will be given the choice between the paired odors dissolved in water during the two-bottle brief-access task for 14 days. This will allow us to measure how the different contexts influence consummatory behaviors, including consumption, preference, and extinction.

In summary, the results of my experiments show that prior to being paired with a taste, odors are equally palatable. Additionally, odor-taste mixture experience guides consummatory behaviors even after a week of experience with unpaired odors. Finally, establishing odor-taste associations to guide consummatory behaviors requires more than just sampling odor-taste mixtures.

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CURRICULUM VITAE

Saphira Chiu

511 South Floyd Street, MDR 433
Louisville, KY 40202-1825

saphira.chiu@louisville.edu

EDUCATION

2015	B.A. in Psychology, Western Kentucky University, Bowling Green, KY
2021	M.S. in Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY

EMPLOYMENT

2015-2019	Brand Activation Team NAF US Army FMWR Fort Knox Fort Knox, KY
2017-2018	Media Specialist Elizabethtown Community and Technical College Elizabethtown, KY
2018-2019	Research Laboratory Technician Department of Anatomical Sciences and Neurobiology University of Louisville Louisville, KY

PROFESSIONAL MEMBERSHIPS AND ACTIVITIES

2013-2015	Western Kentucky University Psychology Club, active member
2019-	Science Policy & Outreach Group, active member
2020-2021	University of Louisville Graduate Student Council, department representative
2021-	Association for Chemoreception Sciences, member
2021-	Kentucky Academy of Science, member and volunteer

HONORS AND AWARDS

2013	Elizabethtown Community and Technical College 4.0 Medallion
2015	Western Kentucky University Magna Cum Laude
2021	University of Louisville Graduate Student Council Travel Grants

EDUCATIONAL ACTIVITIES

2019-2021 Graduate Teaching Assistant
University of Louisville, Louisville, KY
Courses: Brain and Behavior, Statistics for Psychology, Neuroscience, Research Methods

2019- High School Science Fair Mentor
Central High School, Louisville, KY