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Sucrose-Based Flavor Preferences in Rats: Factors Affecting Detection of Extinction

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Rats that have consumed a novel target flavor added to a sucrose solution will develop a preference for that flavor. Such preferences appear to persist over the course of many presentations of the flavor alone when animals are not food-deprived. However, previous research indicates that an extinction effect (a reduction in preference) can be obtained when training or testing is carried out in animals that are hungry. In a series of experiments that produced flavor preferences in hungry rats by adding the flavor to a sucrose solution, three (Experiments 1, 2A, 2B) established that the concentration of sucrose and the nature of the flavor influenced the results but failed to detect extinction. Two-bottle choice tests showed some loss of preference but this occurred both in subjects given the extinction treatment (flavor-only presentations) and in control subjects given just water. A loss of preference in rats given an extinction treatment as opposed to controls given only water was, however, found in Experiments 3 and 4. These experiments differed from Experiments 1 and 2 in that the extinction stage involved the presentation of two bottles containing the flavor, thus matching the two-bottle procedure used in the test phase. These results confirm that experiencing a flavor alone can result in extinction of a conditioned flavor preference in hungry rats but indicate that the effect is highly context-specific, requiring the conditions of the test to match those of the extinction procedure.

Keywords: flavor preference learning, extinction, sucrose concentration, spaced training, rats

Rats given access to a novel flavor such as vanilla mixed with a sucrose solution will develop a preference for vanilla that becomes evident when they are given a choice between vanilla and water. This effect has been interpreted as an instance of classical conditioning, with the vanilla flavor serving as the conditioned stimulus (CS) and some aspect of the sucrose as the unconditioned stimulus (US). Given the complex nature of sucrose, a number of associative interpretations are possible: Links have been suggested between the CS and the sweet taste of sucrose, between the CS and the hedonic response to sucrose, and between the CS and the nutritional properties of sucrose (e.g., Harris et al., 2000; Harris et al., 2004).

A challenge to any simple associative account comes from the fact that flavor preferences can be remarkably persistent. Presentation of the target flavor alone, in the absence of sucrose, constitutes an extinction procedure and loss of a conditioned preference would be expected if the preference depends on an orthodox association. However, it has been demonstrated repeatedly that the preference can be sustained despite extended extinction training (e.g., Albertella & Boakes, 2006; Dwyer et al., 2009; González et al., 2016, Experiments 1B and 2; Harris et al., 2004, Experiments 1A and 1B; but see Delamater, 2007). A feature of these experiments is that the subjects have not been food-deprived during training, leading to the suggestion that the critical property of sucrose in these circumstances will not be its nutritional properties but rather its sweet taste. This has prompted the proposal that the source of the preference in these conditions is not a simple CS-US link, but some other form of learning that produces a change in the perceptual properties of the flavor, a change that is not susceptible to extinction (for different versions of this general proposal see, e.g., Boakes, 2005; Campbell et al., 1988; Myers & Sclafani, 2006; Pearce, 2002).

These results come from experiments in which the rats were not food-deprived during training. There is evidence to suggest, however, that manipulation of the animals' motivational state can promote learning about the relation between a flavor and nutritional consequences, a form of learning that follows standard associative principles. Using sucrose to produce flavor-preference learning, Capaldi et al. (1994) found stronger preferences in rats that were trained when access to food was highly restricted than in rats on a low level of restriction; on the other hand, degree of food restriction did not have this effect when saccharin was used. This suggests that a high level of deprivation during training can enhance

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the acquisition of preferences based on nutrients, but not those based on taste. This analysis raises the possibility that the resistance of sucrose-based flavor preferences to extinction might be restricted to procedures in which subjects are not food-deprived, in which case learning (and performance) would be supported by the sweetness and/or hedonic properties of sucrose. By contrast, making the rats hungry would promote the formation and use of an orthodox association between flavor and nutritional consequences and such an association would be subject to normal extinction effects. Just this result, an effect of the extinction treatment in hungry rats but not in rats that are sated, has been reported by González et al. (2016, Experiments 1A and 1B) and by Harris et al. (2004, Experiments 2A and 2B).

In their Experiments 2A and 2B, Harris et al. (2004) first trained rats on an almond-flavored sucrose solution and then gave repeated tests of preference for almond in the absence of sucrose (i.e., extinction procedure) relative to water. Across 14 two-bottle test sessions, rats that were sated during both training and testing showed no evidence of extinction. However, preferences did decline when rats were hungry, either during training or testing. They interpreted their results as showing that preferences based on flavor-nutrient associations do extinguish, whereas those based on the sensory properties of sucrose do not. It may be noted, however, that at least part of the decrease in preference ratios was due to the general increase in intakes of both the almond-flavored water and water only, such that the difference in these intakes did not change.

The first three of the present experiments (Experiments 1, 2A, and 2B) were closely based on the procedure used by Harris et al. (2004). The original intention of this study was to investigate further the effect of motivational state on the extinction of sucrose-based flavor preferences; in particular, the aim was to test whether, after being trained, extinguished, and tested while food-deprived, an extinguished flavor preference could be restored by satiating the animals. As detailed below, however, it turned out to be difficult to obtain an extinction-produced decrease in flavor preference even in animals that were hungry throughout the procedure. Accordingly, our focus shifted to an analysis of the conditions necessary for obtaining an extinction effect in flavor-preference procedures. Our findings indicate, to anticipate, the importance of context during the flavor presentations in an extinction stage and that a one-bottle extinction procedure is less effective than repeated two-bottle tests.

Experiment 1

As noted above, the aim of Experiment 1 was to examine whether, after extinction of a flavor preference in hungry animals, the preference would recover when animals were sated and retested. In a 2 × 2 between-subjects design, one factor was pairing, whether CS-US presentations during initial training were either simultaneous (Sim: vanilla added to sucrose) or sequential (Seq: vanilla followed by sucrose 60 min later); the Seq condition served as a control and was not expected to produce any flavor learning. The other factor was extinction, whether after training rats were given repeated exposure to vanilla in water (Extn) or water only (NoExtn). Thus, the four groups were: Sim-Extn, Sim-NoExtn, Seq-Extn, and Seq-NoExtn. As shown in Table 1, the experiment comprised four stages, each concluding with a preference test. Rats were food- and water-restricted throughout Stages 1 and 2. Test 1 was conducted immediately after initial training and Test 2 after half of the animals from each training condition had been given the extinction treatment. To assess the effect of satiation on flavor preferences, all rats were sated before Test 3, and then food-restriction was reinstated for Test 4. In Test 1, strong preferences for vanilla were expected only in rats that had

Table 1
Experimental Designs: Experiments 1, 2A, and 2B

Experiment 1 Groups	Training *hungry*	Test 1 *hungry*	Extn *hungry*	Test 2 *hungry*	Test 3 *sated*	Test 4 *hungry*
Sim-Extn	Van+10%Suc	Van+base vs. base	12x Van	Van+base vs. base	Van+base vs. base	Van+base vs. base
Sim-NoExtn			12x Water			
Seq-Extn	Van+base →		12x Van			
Seq-NoExtn	*60 min* → 10%Suc		12x Water			
Experiment 2A Groups	**Training *hungry***	**Test 1 *hungry***	**Extn *hungry***	**Test 2 *hungry***	**Test 3 *hungry***	
Hi-Extn	Van+10%Suc	Van vs. Water	8x Van	Van vs. Water	Van+base vs. base	
Hi-NoExtn			8x Water			
Lo-Extn	Van+4%Suc		8x Van			
Lo-NoExtn			8x Water			
Experiment 2B Groups	**Training *hungry***	**Test 1 *hungry***	**Extn *hungry***	**Test 2 *hungry***	**Extn *hungry***	**Test 3 *hungry***
Van-Extn	Van+10%Suc	Van vs. Water	8x Van	Van vs. Water	8x Van	Van vs. Water
Van-NoExtn			8x Water		8x Water	
Alm-Extn	Alm+10%Suc	Alm vs. Water	8x Alm	Alm vs. Water	8x Alm	Alm vs. Water
Alm-NoExtn			8x Water		8x Water	

Note. Motivational state indicated by *hungry* or *sated*. Van = 1% artificial vanilla flavoring; Suc = sucrose; Alm = 1% almond essence; base = 2% sucrose solution.

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received the Sim condition. Test 2 was expected to reveal lower vanilla preferences in the Sim-Extn group compared with the Sim-NoExtn group, and we anticipated these preferences might be restored by the shift to satiation in Test 3.

Method

Subjects

Thirty-two male Sprague-Dawley rats were purchased from ARC Perth. They were 6 weeks old on arrival, with no prior experimental history, and an average weight of 250 g (range 222–269 g) at the start of the experiment. In this and the following experiments the food was standard rodent chow (Specialty Feeds, 14.2 kJ/g, Glen Forrest, WA). Rats were housed four to a cage under a reverse light cycle (lights on 9 p.m. to 9 a.m.). Cage floors were covered in wood shavings. Six days after arrival animals were weighed and progressive restriction of food and water commenced, with 6-hr, then 4-hr, then 2-hr access per day. Two-hour daily access to chow and water was provided in home cages immediately after the experimental procedure, until after Test 2. Access to both water and food was restricted because in past experiments in our laboratory it was generally found that rats drank very little of unsweetened solutions if unrestricted access to water accompanied food restriction. Water pretraining started 10 days after arrival and four cages (16 rats) were allocated to each of the Sim and Seq conditions, matching for body weight.

Apparatus and Solutions

Twelve acrylic cages measuring 23 cm × 35 cm × 19 cm, with steel wire lids, served as the drinking chambers; floors were covered with commercial cat litter. Fluids were presented in plastic bottles with stainless steel ball-bearing spouts, inserted between wires of the cage lids. To record fluid intake, bottles were weighed to the nearest 0.1 g before and after each session. The target flavor was a solution of 1% imitation vanilla (Queen brand). A 10% sucrose (commercial white sugar) solution was used during the training stage and a 2% sucrose solution was used as a base solution in test sessions, so as to increase intakes. All solutions were mixed in tap water.

Procedure

All sessions in the chambers lasted 15 min, unless otherwise noted. There were six daily sessions a week (Monday to Saturday), starting at 9 a.m. Rats were run in three squads, 12 rats in the first two and eight rats in the final squad, counterbalanced for conditions. All rats received three initial sessions of water pretraining in the drinking chambers to habituate them to drinking in this context. Over six training sessions, both Sim groups received 15-min access to the vanilla-flavored sucrose solution on three sessions that were interleaved with three 15-min sessions of water. The Seq groups received three sessions of, first, 5-min access to vanilla in base (2% sucrose), followed 60 min later by 10-min access to 10% sucrose, also interleaved with three sessions of water. Rats in the Seq groups were returned to their home cages during the 60-min interval. The spaced training sequence was: sucrose-water-water-sucrose-sucrose-water.

To familiarize rats with the testing procedure, the final two training sessions presented two bottles containing the same solution. In this and the following experiments, left and right bottle intakes from these two-bottle training days were measured to check whether any rat displayed a persistent side preference of >80% drinking to one side. In the rare cases where such a side preference was found, further two-bottle training designed to ensure substantial drinking from each bottle was conducted. Testing took place over two sessions in which rats were given a two-bottle choice between vanilla in the 2% base versus the base only, with vanilla on the left in the first session and on the right in the second.

Following Test 1, rats from each condition were allocated to the Extn and NoExtn conditions, matching for flavor preferences in this test. In each of the 12 sessions of the extinction stage, rats in the Sim-Extn and Seq-Extn groups were given vanilla in water, while those in the Sim-NoExtn and Seq-NoExtn groups were given only water. The procedure for Test 2 was identical to that for Test 1. Afterward, all rats were given 5 days of unrestricted access to chow and water in preparation for Test 3. Following this test, food restriction was reintroduced for all rats by returning for 5 days to the schedule of 2-hr daily access to chow and water. Finally, Test 4 was given. Procedures for Tests 3 and 4 were identical to those used in the first two tests.

Data Analysis

Test intakes (summed over the two sessions comprising each test in Experiments 1, 2, and 4) were first analyzed to check that groups did not differ in terms of total intakes. These analyses are reported only when such a difference was found. As long as differences in groups' total test intakes were not large, percentage preferences were calculated on intakes (summed over the two test sessions for Experiments 1, 2, and 4) as [total intake of flavor solution/total intake of both solutions] × 100. Percentage preferences were analyzed using 2 × 2 ANOVAs; for statistical comparison the Extn factor was included in the analyses of all tests including those prior to the extinction phase. Where appropriate, mixed ANOVAs were used to compare preference ratios between tests, with test as the within-subjects factor. ANOVAs, trend analyses and planned contrasts were applied to training and extinction intakes; extinction sessions were averaged over every 2 days into blocks, analyses were only applied to the Extn groups' flavor intakes (NoExtn group water intakes are shown in the figures for reference). Results were considered significant when $p < .05$. In the event of potentially interesting null effects, Bayesian analyses were applied to compare the relative likelihood of the data under the null hypothesis. The reported Bayes factors were estimated using JASP Version 0.11.1 (JASP Team, 2019) and indicate the probability of the alternative hypothesis relative to the null model.

Results

Intakes of solutions generally increased across the three conditioning sessions. Average intakes of the vanilla-flavored sucrose solution in Sim groups were 7.2 ml, 13.2 ml, and 14.0 ml. In the Seq groups, intakes of the vanilla-flavored base solution were 3.0 ml, 5.9 ml, and 5.1 ml and intakes of 10% sucrose were 8.7 ml, 12.2 ml, and 12.9 ml. A 3 × 2 (training solution × Extn) ANOVA with planned contrasts was conducted on total mean intakes summed across all three sessions. This confirmed lower intake of the solution containing vanilla in the Seq groups combined (M = 14.0 ml) compared with the Sim groups (M = 34.5 ml), $F(1, 42)$ =

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125.79, $p < .001$, $\eta_p^2 = .75$, and thus less exposure to the flavor. There was no significant Sim versus Seq group difference in the total consumption of solutions containing sucrose ($M = 34.5$ ml and 33.9 ml, respectively) nor was there an effect of extinction condition or interaction (Fs < 1).

Mean preference ratios of the four groups in Test 1 after the allocation to Extn/NoExtn conditions (but prior to the extinction stage) can be seen on the right of Figure 1A. These preference ratios revealed a clear main effect of pairing, $F(1, 28) = 131.25$, $p < .001$, $\eta_p^2 = .82$; averaged across the Extn condition, flavor preferences were much higher in the Sim groups ($M = 79.6\%$) than the Seq groups ($M = 35.4\%$). A difference between the four groups in total test intakes was found only in Test 1. The 2 × 2 analysis of Test 1 intakes (left panel of Figure 1A) produced main effects of pairing, $F(1, 28) = 4.53$, $p = .04$, $\eta_p^2 = .14$, and Extn, $F(1, 28) = 4.92$, $p = .04$, $\eta_p^2 = .15$, with no interaction, confirming that the Sim groups drank more than the Seq. These effects were driven by a higher total intake in the Sim-Extn group ($M = 18.4$ ml) compared with all other groups (Sim-NoExtn: 13.2 ml, Seq-Extn: 13.3 ml, and Seq-NoExtn: 11.8 ml). This higher intake was most likely a product of conditioning, which would be expected to stimulate vanilla intake in animals that received simultaneous training. That the same did not occur in the Sim-NoExtn group is likely due to imbalance in the matching procedure; in all the present experiments, group allocation was made after Test 1 based on preference ratios, not on total intakes.

Intakes during the 12 extinction sessions were averaged into six two-session blocks (see Figure 2). The Extn groups' vanilla intakes were analyzed with a 2 × (6) mixed ANOVA. Fluid intakes were low, as expected from the suppression of thirst in rats when hungry. There was a quadratic effect of block, $F(1, 14) = 13.73$, $p = .002$, $\eta_p^2 = .50$, with no difference between the Sim-Extn and Seq-Extn groups and no interactions (ps $> .10$), indicating the pattern of consumption did not differ between groups. For the Sim-Extn group, mean intake of vanilla on Day 1 of extinction was 1.7 ml and 1.2 ml on Day 12, suggesting that, following conditioning of the preference, the extinction treatment had little effect on rats' willingness to drink vanilla. It may be seen in Figure 2 that intakes on Sessions 7–8 were atypical; there was no obvious reason for this discrepancy but we should note that these sessions followed a Sunday in which no treatment was given and the rats remained in their home cages.

Test 2 was conducted postextinction. As seen in the right panel of Figure 1B, preferences in the two Sim groups were unexpectedly similar. The analysis confirmed that there was still a main effect of pairing, $F(1, 28) = 31.95$, $p < .001$, $\eta_p^2 = .53$, but failed to detect either a main effect of Extn or an interaction (Fs < 1). A Bayesian analysis indicated that the extinction data were 2.7 times more likely to occur under the null hypothesis, namely, that there was no main effect of Extn, compared with the alternative hypothesis ($BF_{10} = .37$, error % $< .001$). Preferences for both the Sim-Extn ($M = 69.6\%$) and Sim-NoExtn ($M = 69.1\%$) groups were equivalent and slightly lower than in Test 1. An analysis comparing Sim group preferences from Tests 1 to 2 found this reduction to be significant, $F(1, 14) = 6.85$, $p = .02$, $\eta_p^2 = .33$, and equivalent across Extn conditions (Fs < 1 for main effect and interaction).

Test 3 was conducted with rats sated. The analysis again yielded no effect of Extn or interaction (Fs < 1, see right panel of Figure 1C). For the Extn factor, the data were 2.5 times more likely to occur under the null hypothesis ($BF_{10} = .40$, error % $< .001$). The main effect of pairing was weaker than in Tests 1 and 2, but still significant, $F(1, 28) = 6.28$, $p = .02$, $\eta_p^2 = .18$. Test 3 preferences were somewhat lower, though not significantly so ($p = .06$), than those in Test 2 for both the Sim-Extn ($M = 58.3\%$) and Sim-NoExtn ($M = 60.6\%$) groups.

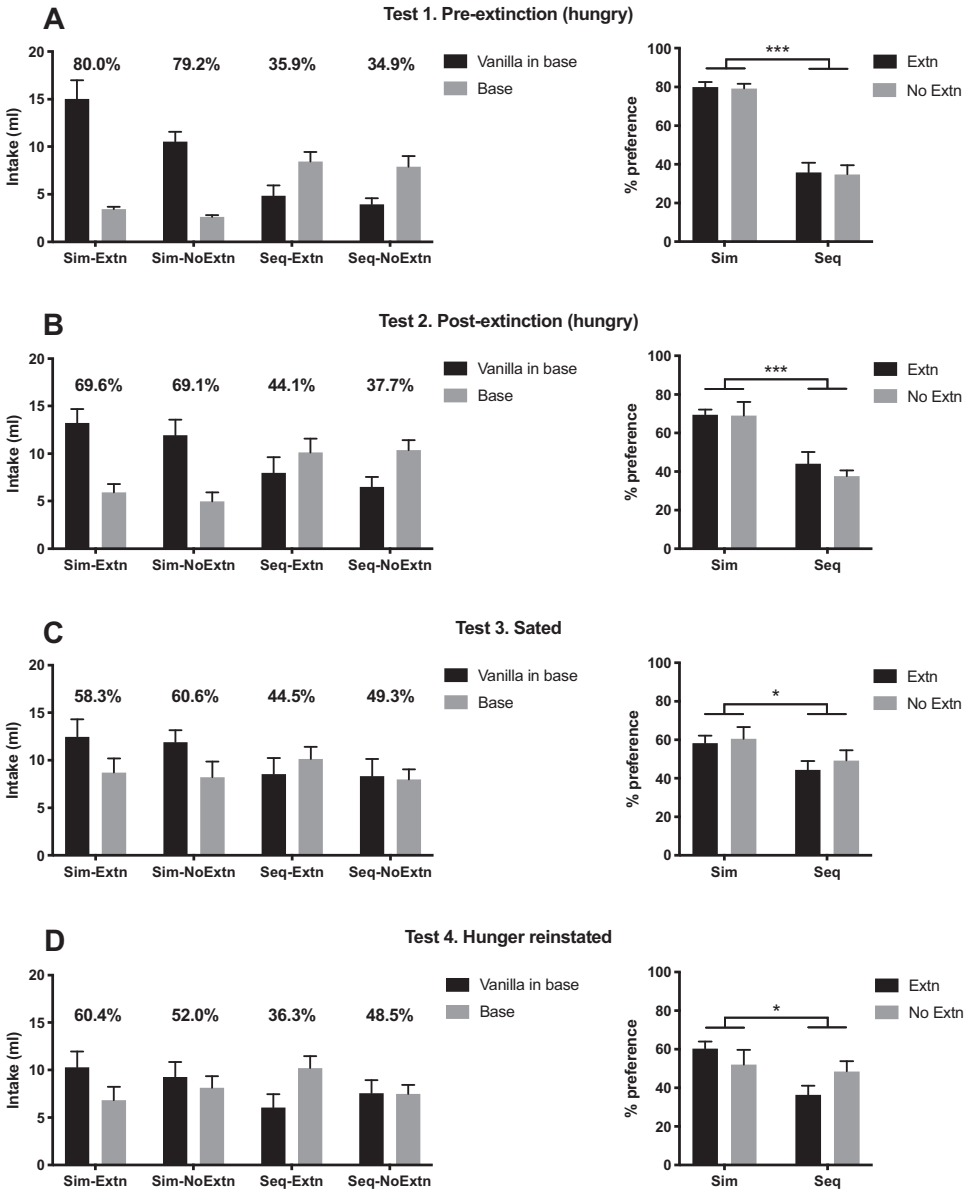
Test 4 was conducted when rats were again food-restricted (see Figure 1D). As previously, there was a significant main effect of pairing, $F(1, 28) = 6.22$, $p = .02$, $\eta_p^2 = .18$, no effect of Extn ($F < 1$), although the interaction almost reached significance ($p = .07$). The Bayesian analysis indicated that the data were 2.9 times in favor of the null hypothesis that there was no main effect of Extn ($BF_{10} = .35$, error % = .04). It was notable that the reinstatement of hunger did not greatly affect preferences in the Sim-Extn ($M = 60.4\%$) and Sim-NoExtn ($M = 52.0\%$) groups compared with the results from Test 3. The 2 × 2 analysis comparing preferences obtained in Tests 3 and 4 failed to detect any significant effects (ps $> .10$).

Discussion

Adding vanilla to a 10% sucrose solution produced a robust preference for this flavor, as expected. However, in contrast to previous findings of extinction in hungry animals (González et al., 2016; Harris et al., 2004), no evidence for subsequent extinction was found either in the preference tests or in terms of a reduced intake of vanilla across the extinction sessions. It may be noted that the acquired preference for vanilla, indicated by the differences between Sim and Seq groups, was maintained throughout the remainder of the experiment, even though there were only three initial training sessions in which the Sim rats drank the vanilla-flavored sucrose solution. It is also notable that the motivational shift from hunger (Test 2) to satiation (Test 3) did not produce the anticipated increase in preferences in the "extinguished" group. In fact, the change of motivational state between the final three tests made no discernible difference to preferences in either of the simultaneously conditioned groups. Preferences generally declined between tests, significantly from Test 1 to 2 ($p = .02$) and almost significantly from Test 2 to 3 ($p = .06$), but this occurred regardless of motivational state.

There are two potentially important differences between Experiment 1 and previous between-subjects experiments that have reported reduced flavor preferences postextinction treatment when rats were hungry during training or testing. First, the experiment reported by Harris et al. (2004) used a 4% sucrose solution during training. This suggests that a robust extinction effect might be detected with the use of 4% sucrose, instead of the 10% used here. Second, similar experiments have used almond essence as the target flavor, rather than vanilla (Garcia-Burgos & González, 2012; Harris et al., 2004; Higgins & Rescorla, 2004). The almond essence used in our lab, and presumably in the labs of other researchers, contains alcohol and we have previously found this flavoring to be slightly more aversive to rats than vanilla. Although vanilla flavoring does not appear to be naturally preferred by rats, as seen in initial test intakes of the unconditioned Seq groups in left panels of Figure 1A and 1B, a recent study reported the relative neutrality of vanilla flavoring compared with almond. In an examination of latent inhibition by Morillas et al. (2019), preexposure to an almond CS facilitated the conditioning of a flavor preference, while preexposure to vanilla produced the expected latent inhibition effect (i.e., weaker preferences in preexposed subjects).

Figure 1
Experiment 1



Note. Mean (+*SEM*) of total test intakes (summed over two test days of two-bottle choice tests) on the left and mean preference ratios (+*SEM*) on the right. Percentages noted in the left panels indicate preference ratios represented in the right panels. Preference ratio: Sim/Seq main effect * $p < .05$. *** $p < .001$. No effect of Extn or interaction in any test.

The authors proposed that the difference in hedonic value between the two flavors was responsible for the opposing effects. Although speculative, these observations suggest that the choice of a slightly aversive flavor could be critical to achieving extinction in a between-subjects design and that the failure of our attempt in Experiment 1 may be related to using a more neutral flavor. It is possible that presenting an inherently disliked flavor (such as those containing alcohol) in a compound with sucrose masks the initial aversiveness to produce a flavor preference. During subsequent extinction exposure in the absence of sucrose, the inherent dislike of the flavor might emerge and conditioned preferences decrease. With an alcohol-free flavor, such as imitation vanilla, this is less likely to occur. These two possibilities were examined next.

Another procedural difference in the preference tests administered in this first experiment was to offer the rats a choice between the flavor in 2% sucrose and 2% sucrose alone, whereas for the rats in Harris et al. (2004) the choice was between the flavor in water and water alone.

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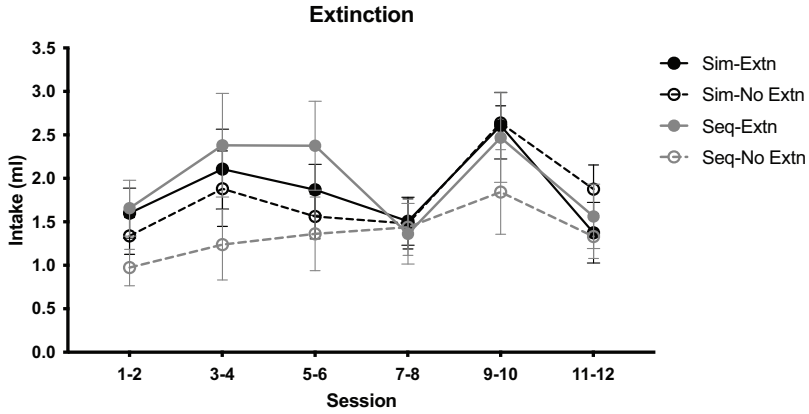
Figure 2
Experiment 1



Note. Mean (±*SEM*) intakes during the extinction phase averaged across two-session blocks. Solid lines: vanilla-only extinction exposure for Sim-Extn and Seq-Extn groups; the groups did not differ in acceptance of the flavor. Dashed lines: water-only intake for the NoExtn groups (data not analyzed).

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Experiments 2A and 2B

These experiments had two aims. First, to determine whether extinction of a flavor preference is more likely to occur when the preference is based on 4%, rather than 10%, sucrose (Experiment 2A). Second, to determine whether the use of a slightly aversive flavor is critical to achieving extinction in a between-subjects design; effects of extinction were compared between vanilla and almond as target flavors (Experiment 2B). In 2 × 2 between-subjects designs, one factor was Extn, while the other factor was sucrose concentration (Hi: 10% vs. Lo: 4%, Experiment 2A) or flavor (Van: imitation vanilla vs. Alm: almond essence, Experiment 2B). The experimental designs are shown in Table 1.

Because the use of a 2% sucrose base during testing in the previous experiment might have produced some weak conditioning effect, rats were presented with a choice between the flavor in water versus water alone in all tests other than Test 3 in Experiment 2A.

In Experiment 2A, the use of 10% sucrose was expected to generate stronger flavor preferences than when 4% was used. We also predicted that extinction of a 4%-trained preference would be evident after only a few extinction sessions and so reduced the number of these sessions from 12 to eight. In Experiment 2B, two courses of extinction were conducted, each comprising eight sessions of flavor-only exposure. We expected to see an effect of Extn for the animals trained with almond and, on the basis of results from Experiment 1, we did not expect extinction of a vanilla preference.

Method

Experiment 2A Subjects

Thirty-two, naïve male Sprague-Dawley rats were obtained from the same source as Experiment 1. They were aged 6 weeks on arrival, with an average weight of 295 g (range 264–328 g) at the start of the experiment. Rats were housed four to a cage under reverse light cycle conditions (lights on 10 p.m. to 10 a.m.) and cage floors were covered in corncob bedding. Seven days after arrival, animals were weighed and progressive food and water restriction commenced, as in Experiment 1, after which rats were maintained on 2-hr daily access to food and water. Water pretraining started 11 days after arrival, when four cages were allocated to each of the Hi/Lo conditions, matched for body weight.

Apparatus and Solutions

The acrylic cages used in Experiment 1 again served as the drinking chambers. However, for incidental reasons the floors were now covered with paper chip bedding. Bottles, vanilla, and sucrose (either 4% or 10%) solutions were as previously described.

Procedure

Sessions were 15 min long, starting at 1 p.m., with six daily sessions a week (Monday to Saturday). Rats were run in two squads, with 16 rats per squad, one cage from each group. Following three water pretraining sessions, six sessions of training comprised three flavored sucrose exposures interleaved with three sessions of water in the sequence previously described. On sucrose days, 16 rats in the Hi condition were given a vanilla-flavored 10% sucrose solution and 16 rats in the Lo condition were given a vanilla-flavored 4% sucrose solution. Two-bottle training was conducted as in Experiment 1. The tests that followed comprised two sessions (over 2 days) that presented a choice between the flavor in water and water only (one exception noted below), with the flavor on the left in the first session and on the right in the second. After Test 1, rats from each training condition were allocated to either the Extn or NoExtn conditions, matching for flavor preferences in Test 1, thus forming four groups (each $n = 8$).

Eight extinction sessions followed, in which the Extn groups received their training flavor in water and NoExtn groups received water only. As reported below, the postextinction test (Test 2) did not reveal an extinction effect and we considered this may have been due to very low fluid intakes. Consequently, in order to

increase intakes, testing was repeated (Test 3) using the 2% sucrose base (i.e., vanilla+base vs. base only).

Experiment 2B Subjects

Thirty-two, naïve male Sprague-Dawley rats, obtained from the same source, were aged 8 weeks on arrival and weighed an average of 351 g (range 323–384 g) when the experiment started. Housing conditions and the food/water restriction schedule was as previously described. Water pretraining started 11 days after arrival, when four cages were allocated to each of the Van/Alm conditions, matched for body weight.

Apparatus and Solutions

The cages were the same as those used in Experiment 2A. Bottles, vanilla, and 10% sucrose solutions were as previously described. The almond flavoring was a 1% solution of natural almond essence (Queen brand, 15% vol/vol alcohol content).

Procedure

Sessions proceeded as for Experiment 2A, except that on flavor+sucrose training days 16 rats were given vanilla-flavored 10% sucrose and 16 were given almond-flavored 10% sucrose. Testing and extinction sessions were as in Experiment 2A. At Test 2, although an extinction effect was not detected, an effect of flavor suggested that the eight extinction sessions had a more profound effect on the almond flavor than on vanilla. Therefore, it was decided to run a further eight sessions of extinction before giving a final test (Test 3).

Results

Experiment 2A

Intakes of the vanilla-flavored sucrose solutions in the three training sessions were 3.5 ml, 7.6 ml, and 10.6 ml for the Hi condition, and 3.5 ml, 6.5 ml, and 8.7 ml for the Lo. Intakes for the Hi group increased at a greater rate, confirmed by a linear trend over the three sessions, $F(1, 30) = 187.22$, $p < .001$, $\eta_p^2 = .86$, and an interaction between sucrose concentration and trend, $F(1, 30) = 4.30$, $p = .047$, $\eta_p^2 = .13$. A Concentration × Extn ANOVA of total intakes summed across all sessions produced a significant interaction, $F(1, 28) = 5.21$, $p = .03$, $\eta_p^2 = .16$. This was driven by lower total intakes, and thus less exposure to the conditioning compound, in the Lo-NoExtn group ($M = 15.9$ ml) compared with all other groups (Hi-Extn: 20.9, Hi-NoExtn: 22.6, and Lo-Extn: 21.4 ml). While more conditioning exposure in the group destined to be extinguished was a potential limitation for the interpretation of extinction results, the difference in total intake did not produce a discernible difference in the expression of preferences, as seen in Figure 3. Averaged across the Extn condition, flavor preferences in Test 1 were stronger in the Hi group ($M = 76.9\%$) than in the Lo ($M = 67.2\%$), $F(1, 28) = 20.79$, $p < .001$, $\eta_p^2 = .43$ (see right panel of Figure 3A).

Intakes during extinction sessions, averaged into four two-session blocks, are shown in Figure 4. As this figure suggests, in the two groups exposed to vanilla (Extn groups) there was no effect of group, block, or interaction (ps $> .10$). Thus, as in Experiment 1, the extinction treatment produced no detectable effect on the acceptance of vanilla in either group. In the Hi-Extn group, the mean intakes of vanilla were 2.3 ml on Day 1 of extinction and 2.4 ml on Day 8; in the Lo-Extn group the comparable intakes were 2.0 ml and 2.2 ml.

As seen in Figure 3B, Test 2 (postextinction) failed to yield a main effect of Extn or interaction (Fs < 1). The Bayesian analysis indicated that the data were 2.7 times more likely to occur under the null hypothesis of no main effect of Extn ($BF_{10} = .37$, error $\% < .001$). Averaged across the Extn factor, the difference in preferences between Hi ($M = 64.7\%$) and Lo ($M = 58.6\%$) groups was no longer significant, $p = .06$. Separate 2 × 2 mixed analyses for each sucrose concentration compared preferences between Tests 1 to 2. These found preferences were significantly lower for all four groups in Test 2: $F(1, 14) = 42.37$, $p < .001$, $\eta_p^2 = .75$, for the Hi conditions, and $F(1, 14) = 7.71$, $p = .02$, $\eta_p^2 = .36$, for the Lo. Neither analysis found any main effect of Extn or interaction (Fs < 1).

Test 3 provided a choice between vanilla-flavored base versus base only; it may be seen that the introduction of the base increased intakes, as intended (see left panel, Figure 3C). Analysis of these total intakes found an unexpected main effect of Extn, $F(1, 28) = 7.96$, $p = .01$, $\eta_p^2 = .22$, whereby the Extn groups ($M = 15.1$ ml) drank more than NoExtn groups ($M = 12.0$ ml). This effect was likely due to the exposure to vanilla in Extn groups during flavor-only extinction sessions and an increased acceptance of the flavor. Because the difference was not large, analysis of preferences was undertaken as previously. As shown in the right panel of Figure 3C, the difference in preferences between Hi ($M = 60.2\%$) and Lo ($M = 52.1\%$) groups returned to significance, $F(1, 28) = 8.09$, $p = .01$, $\eta_p^2 = .22$, but there was still no evidence of an Extn effect or interaction (Fs < 1). The Bayesian analysis found these data were almost three times in favor of the null hypothesis, that there was no main effect of Extn ($BF_{10} = .34$, error $\% = .04$). Separate 2 × 2 comparisons between Tests 2 and 3 for each concentration found preferences were significantly lower in Test 3 only for the Lo groups, $F(1, 14) = 5.05$, $p = .04$, $\eta_p^2 = .27$, the reduction in preferences was not significant in the Hi condition, $p > .10$. Again, neither analysis found a main effect of Extn or interaction (Fs < 1).

Experiment 2B

The groups drank similar amounts of the flavored sucrose solutions during conditioning: 4.5 ml, 11.4 ml, and 12.8 ml for rats given vanilla, and 3.2 ml, 10.0 ml, and 11.3 ml for those given almond. Total mean intakes of the four groups summed across all sessions were Van-Extn: 30.0, Van-NoExtn: 27.3, Alm-Extn: 23.3, and Alm-NoExtn: 25.9 ml. A Flavor × Extn ANOVA failed to detect any differences in amount of the conditioning compound consumed (largest $F = 2.99$). As shown in the right panel of Figure 5A, flavor preferences in Test 1 were high in both the Van ($M = 80.3\%$) and Alm ($M = 78.2\%$) conditions; the analysis did not yield any significant effects, Fs < 1.

Intakes during the extinction phases were averaged into two-session blocks (see Figure 6). For the two groups that received flavor exposure (Extn groups), a 2 × 4 mixed ANOVA on the first eight sessions found no main effect of flavor or interactions (largest $F = 3.30$), indicating similar intakes of vanilla and almond. An increase in consumption across the course of extinction was indicated by a linear effect of block, $F(1, 14) = 6.30$, $p = .03$, $\eta_p^2 = .31$. In the Van-Extn group, mean intakes of vanilla were 2.6 ml on

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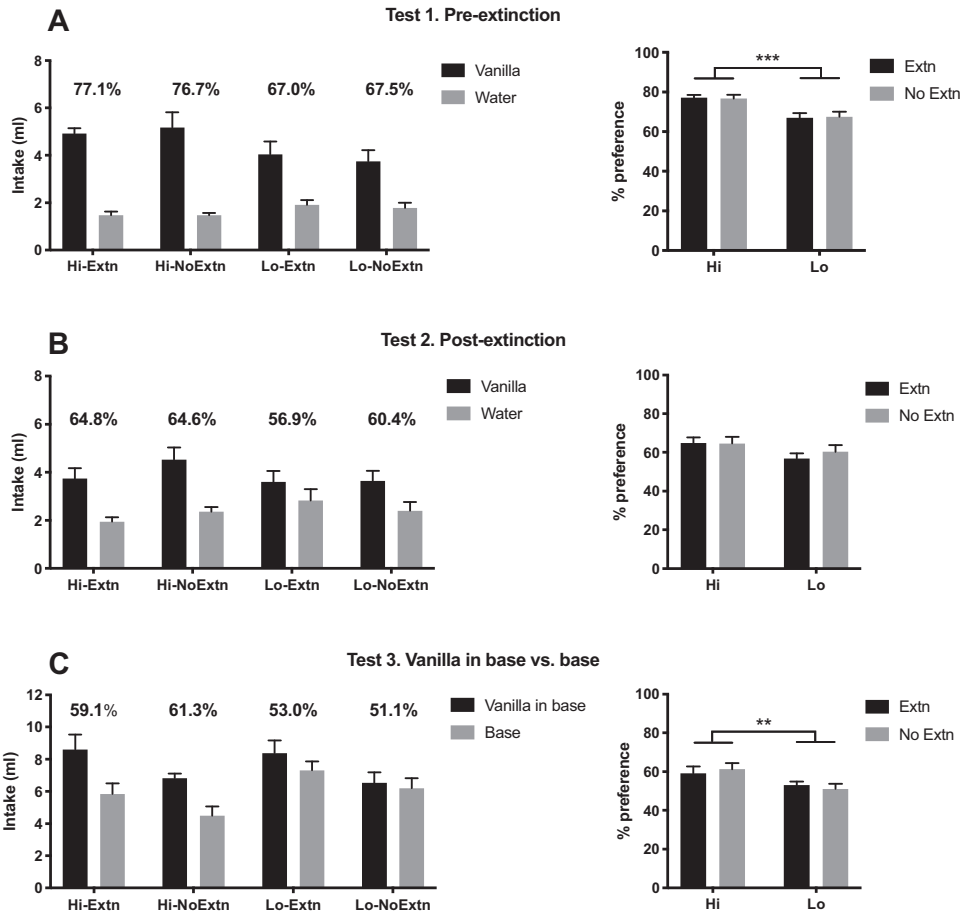
Figure 3
Experiment 2A



Note. Mean (+*SEM*) of total test intakes (summed over two test days of two-bottle choice tests) on the left and mean preference ratios (+*SEM*) on the right. Percentages noted in the left panels indicate preference ratios represented in the right panels. Tests 1 and 2, vanilla versus water; Test 3, vanilla in base versus base. Preference ratio: Hi/Lo main effect ** $p = .01$. *** $p < .001$. No effect of Extn or interaction in any test.

Day 1 of extinction and 3.7 ml on Day 8; for the Alm-Extn group, the comparable intakes were 3.1 ml and 3.3 ml.

As seen in the right panel of Figure 5B, postextinction testing (Test 2) produced lower preferences than in Test 1 for all groups. Analysis of preference ratios found an effect of flavor, $F(1, 28) = 7.41$, $p = .01$, $\eta_p^2 = .21$, but no effect of Extn or interaction ($ps > .10$). The absence of an Extn effect also received some support from the Bayesian analysis that found the data were 2.9 times more likely under the null ($BF_{10} = .34$, error % = .04). Simple effects analyses indicated that the flavor effect was driven by a significant difference in preferences between the Van-Extn ($M = 71.1\%$) and Alm-Extn ($M = 61.3\%$) groups, $F(1, 28) = 7.13$, $p = .01$, $\eta_p^2 = .20$. Despite no main effect of Extn or interaction, this simple effect suggested that the first eight extinction sessions had a greater effect on almond than on vanilla.

Separate 2 × 2 mixed analyses for each flavor compared preferences between Tests 1 to 2. Preferences were significantly lower in Test 2 both for the Van groups, $F(1, 14) = 31.54$, $p < .001$, $\eta_p^2 = .69$, and for the Alm groups, $F(1, 14) = 71.12$, $p < .001$, $\eta_p^2 = .84$. No main effects of Extn or interactions were detected in these analyses ($Fs < 1$).

Intakes across the second course of extinction are shown on the right of Figure 6. The analysis of the flavor-exposed groups failed to detect any main effects of flavor or block ($Fs < 1$). There was a significant cubic interaction, $F(1, 14) = 8.83$, $p = .01$, $\eta_p^2 = .39$, indicating differing patterns for each flavor in the rise and fall of intakes. In the Van-Extn group, mean intake of vanilla on Day 9 of extinction was 2.9 ml and on Day 16 was 2.6 ml; for the Alm-Extn group, the comparable intakes were 3.2 ml and 2.7 ml. As for all experiments reported here, the flavor-only exposures of extinction had no apparent effect on acceptance of either flavor.

After further extinction, the analysis of preference ratios in Test 3 still failed to find any main effects or interaction, $Fs < 1$ (see right panel, Figure 5C). For the main effect of Extn, the Test 3 data were 2.8 times more likely under the null hypothesis ($BF_{10} = .36$, error % < .001). Separate mixed ANOVAs for the two flavors compared preferences from Test 2 to Test 3. In the Van groups, preferences in Test 3 were lower than in Test 2, $F(1, 14) = 9.25$, $p = .01$, $\eta_p^2 = .40$. No such

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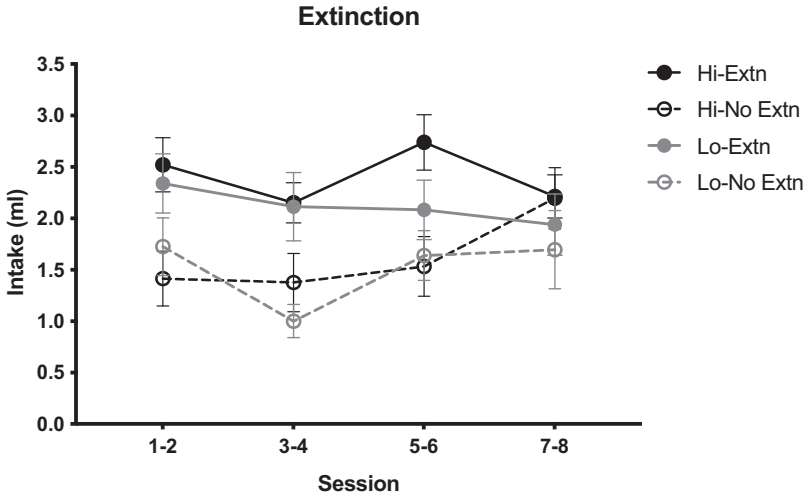
Figure 4
Experiment 2A



Note. Mean (± *SEM*) intakes during the extinction phase averaged across two-session blocks. Solid lines: vanilla-only extinction exposure for Hi-Extn and Lo-Extn groups; the groups did not differ in acceptance of the flavor. Dashed lines: water-only intake for the NoExtn groups (data not analyzed).

effect was found in the Alm groups, $p > .10$. Neither analysis found any main effects of Extn or interactions (Fs < 1).

Discussion

In Experiment 2A, adding vanilla to a 10% sucrose solution produced a greater vanilla preference than adding it to a 4% solution and this difference was generally maintained throughout testing. However, there were no differences between Extn/NoExtn groups after training with either of the sucrose concentrations. This outcome is particularly surprising given the between-subjects procedure and 4% sucrose concentration were the same as those used by Harris et al. (2004), in which high preferences appeared to extinguish by the second day of testing. Similarly, Experiment 2B failed to confirm the prediction that an effect of extinction would be observed when almond was used as the target flavor; differences between Extn and NoExtn groups were not seen with either flavor. We speculated that, prior to conditioning, 1% vanilla is more palatable than 1% almond. However, few differences between these flavors were detected during training and subsequent extinction stages. Where a difference was detected this was in the predicted direction: In Test 2 preferences for vanilla were higher than for almond.

For all the experiments reported here, regardless of the target flavor used, flavor preferences were high in the first test but declined, most often significantly, as tests were repeated. Notably, this decline occurred in the continued absence of any difference between Extn and NoExtn control groups. These reductions in preferences from test to test suggest that extinction occurred during the two-bottle tests, which are, in effect, extinction procedures themselves (i.e., nonreinforced presentations of the flavor). However, further to any extinction occurring during two-bottle tests, it was expected that Extn groups would show a greater loss of preference due to the additional flavor-only exposures during the extinction phases. The absence of any sign of this additional extinction in these experiments is at odds with the claim that learned flavor preferences extinguish when rats are food deprived. A possibly crucial procedural difference between the present experiments and that reported by Harris et al. (2004) that first led to the above claim is in the sequence of conditioning sessions in the initial training phase. This was investigated in the next experiment.

Experiment 3

The training method used in the experiments reported so far is one that has been standard in our laboratory. It comprises sessions in which rats are given the flavored sucrose solution intermixed with sessions in which rats are given only water; see also Higgins and Rescorla (2004). The intention behind the introduction of water sessions that generate a "spaced" training procedure is to reduce context conditioning and thus the possibility that contextual cues might compete with the flavor cue. In the absence of such interleaved water sessions—a "massed" procedure—it seems likely that rats develop strong associations between the context and the flavored sucrose solution and, if rats are hungry, contextual cues may come to signal the expectation of nutrition provided by the solution. Thus, when extinction conditions are introduced with the flavor presented alone for the first time, the flavor becomes paired with the absence of nutritional consequences in a context in which these are expected. Such conditions favor inhibitory learning (Garcia-Burgos & González, 2012) or the "missing calorie effect" (Boakes et al., 2010). When, as in the experiments reported so far, water sessions are interleaved during training, a hungry rat is likely to have a weaker context-generated expectancy of nutritional benefits and therefore little or no inhibitory learning should take place.

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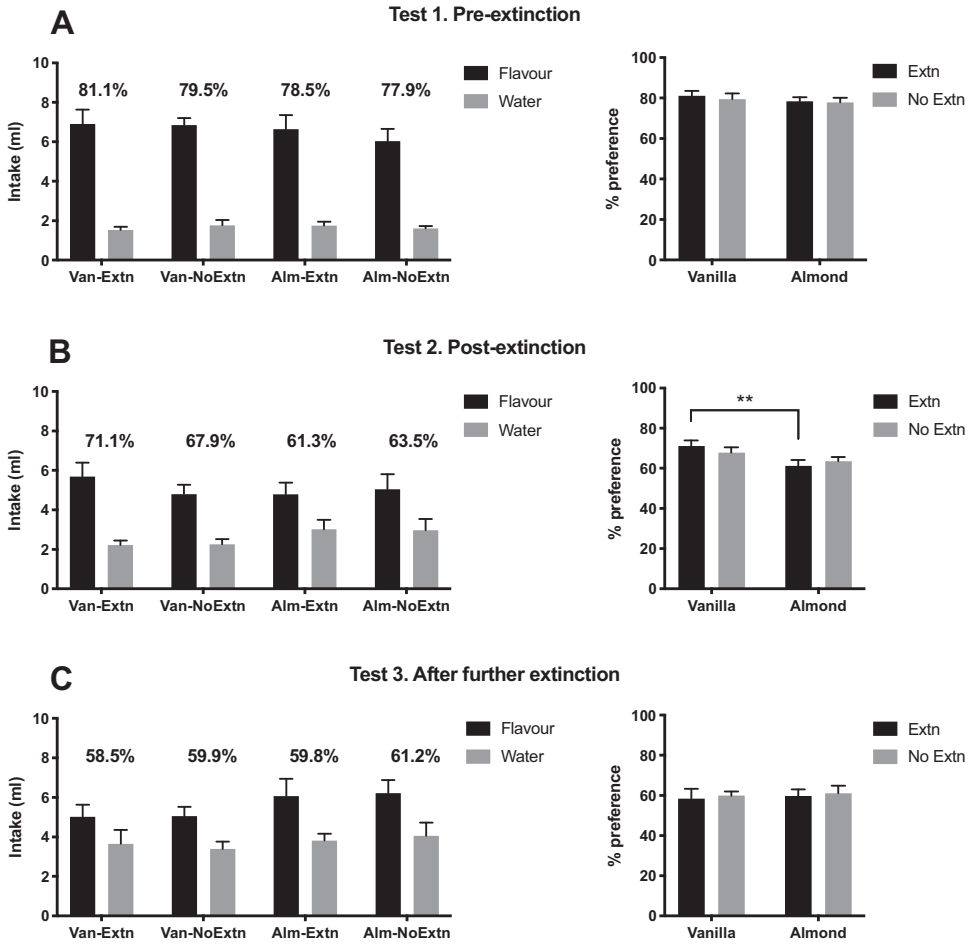
Figure 5
Experiment 2B



Note. Mean (+*SEM*) total test intakes (summed over two test days of two-bottle choice tests) on the left and mean preference ratios (+*SEM*) on the right. Percentages noted in the left panels indicate preference ratios represented in the right panels. Preference ratio: Van-Extn/Alm-Extn simple effect ** $p = .01$. No effect of Extn or interaction in any test.

In light of this consideration, Experiment 3 examined the effects of the extinction procedure used previously on rats given either spaced or massed training. As shown in Table 2, the experiment comprised four groups in a 2 × 2 design: Spaced-Extn, Spaced-NoExtn, Massed-Extn, Massed-NoExtn.

In our previous experiments, tests were conducted and averaged over two sessions. In order to minimize extinction occurring in all groups during two-bottle tests, in Experiment 3 the tests were conducted in single sessions. A further procedural change was to present fluids in two bottles that both contained either the flavored solution (Extn condition) or water (NoExtn condition) throughout the extinction stage; this was done to ensure minimal change in context between this stage and the subsequent test. To maximize the formation of context-sucrose associations during massed training (i.e., to minimize latent inhibition to the context), the initial water sessions for the massed group were conducted in a different context (steel cages rather than acrylic chambers). Finally, to enhance the intended effect of our spaced training sequence (i.e., to minimize the development of context-sucrose associations), three additional water sessions were interleaved into the spaced sequence, as shown in Table 2.

Method

Subjects

Thirty-two female Sprague-Dawley rats had previously served in an appetitive conditioning study in which visual and auditory cues were paired with the delivery of food pellets. The rats had no prior experience of either vanilla or sucrose. The use of these females as opposed to the experimentally naïve males used in the previous experiments in this series was due to the nonavailability of the latter at the time this and the next experiment were run.

Rats were housed under the same conditions as in Experiment 2 and aged 23 weeks on arrival in our colony room. After 5 days of acclimatizing and handling, progressive food and water restriction began as previously. The day before water pretraining commenced,

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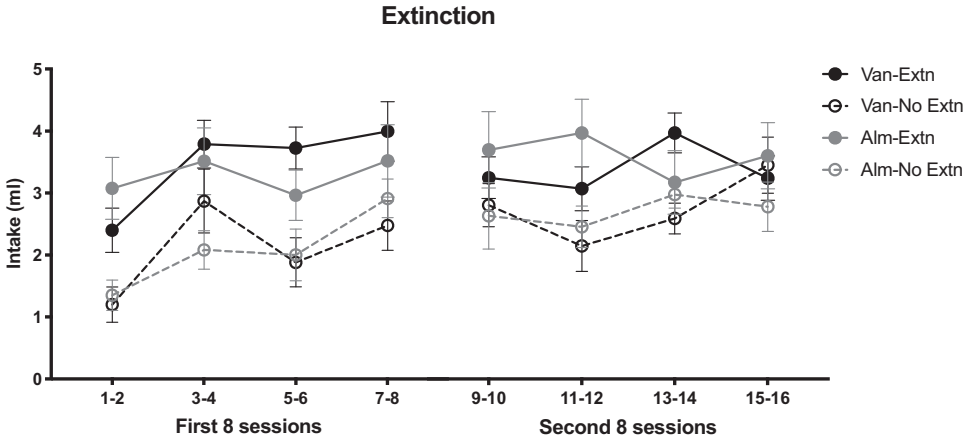
Figure 6
Experiment 2B



Note. Mean (±*SEM*) intakes during extinction phases averaged across two-session blocks: first course of extinction (after Test 1) on the left, second course of extinction (after Test 2) on the right. Solid lines: flavor-only extinction exposure for the Van-Extn and Alm-Extn groups; the groups did not differ in acceptance of the flavors. Dashed lines: water-only intake for the NoExtn groups (data not analyzed).

rats were weighed and allocated to the spaced or massed groups, matched for body weight. Average weight was 282 g (range 234–318 g) at the start of the experiment.

Apparatus and Solutions

The same acrylic cages, with paper chip bedding covering the floor, served as the drinking chambers. The first eight water presentations for the massed group were conducted in steel cages measuring 19.5 cm × 28 cm × 18 cm. Both drinking environments were located in the same procedure room. Bottles, 10% sucrose and vanilla solutions were as previously.

Procedure

There were six 15-min sessions each week (Monday to Saturday), starting at 12 p.m. Rats were run in two squads, with 16 rats per squad, one cage from each group. Following three water pretraining sessions—in acrylic chambers for the spaced group and steel cages for the massed—there were nine sessions of vanilla preference training. As shown in Table 2, the spaced group received one vanilla+sucrose session, followed by two water sessions, and this sequence was repeated three times. The training phase for the massed group comprised five water sessions in the steel cages, followed by one session of water in the acrylic chambers, then three sessions of vanilla+sucrose also in the acrylic chambers. From this point onward, all sessions for all groups were conducted in the acrylic chambers.

In preparation for the following two-bottle procedures (tests and extinction), the final two training sessions presented rats with two bottles, both containing either vanilla-flavored sucrose (massed training) or water (spaced training). To familiarize rats with the switch of bottles to be used in the tests (described below), after 5 min the bottles were withdrawn and quickly replaced in the same position. All sessions used two bottles from this point on.

Test 1 presented a single two-bottle choice between vanilla in water versus water only. To control for side preferences, for half of the rats in each group the test started with vanilla on the left and for the other half vanilla was on the right. As a further control measure, 5 min into the test the positions of the bottles were reversed for the remaining 10 min. The spaced and massed groups were then allocated to the Extn and NoExtn conditions, matching for flavor preferences in Test 1.

During the two-bottle extinction phase, the Spaced-Extn and Massed-Extn groups were given vanilla in water and the Spaced-NoExtn and Massed-NoExtn groups received water only. To match the bottle switch procedure used during tests, after 5 min the bottles were withdrawn and immediately replaced in the same position. Test 2 procedures were exactly as for Test 1. As an effect

Table 2
Experimental Design: Experiment 3

Groups	Training	Test 1	Extn	Tests 2 and 3
Spaced-Extn	Van+ W W Van+ W W Van+ W W	Van vs. Water	8x Van	Van vs.Water
Spaced-NoExtn			8x Water	
Massed-Extn	W W W W W W Van+ Van+ Van+		8x Van	
Massed-NoExtn			8x Water	

Note. Van = 1% artificial vanilla flavoring; Van+ = vanilla+% sucrose; W = water.

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of the extinction procedure was almost significant in Test 2, a final test (Test 3) was given the following day.

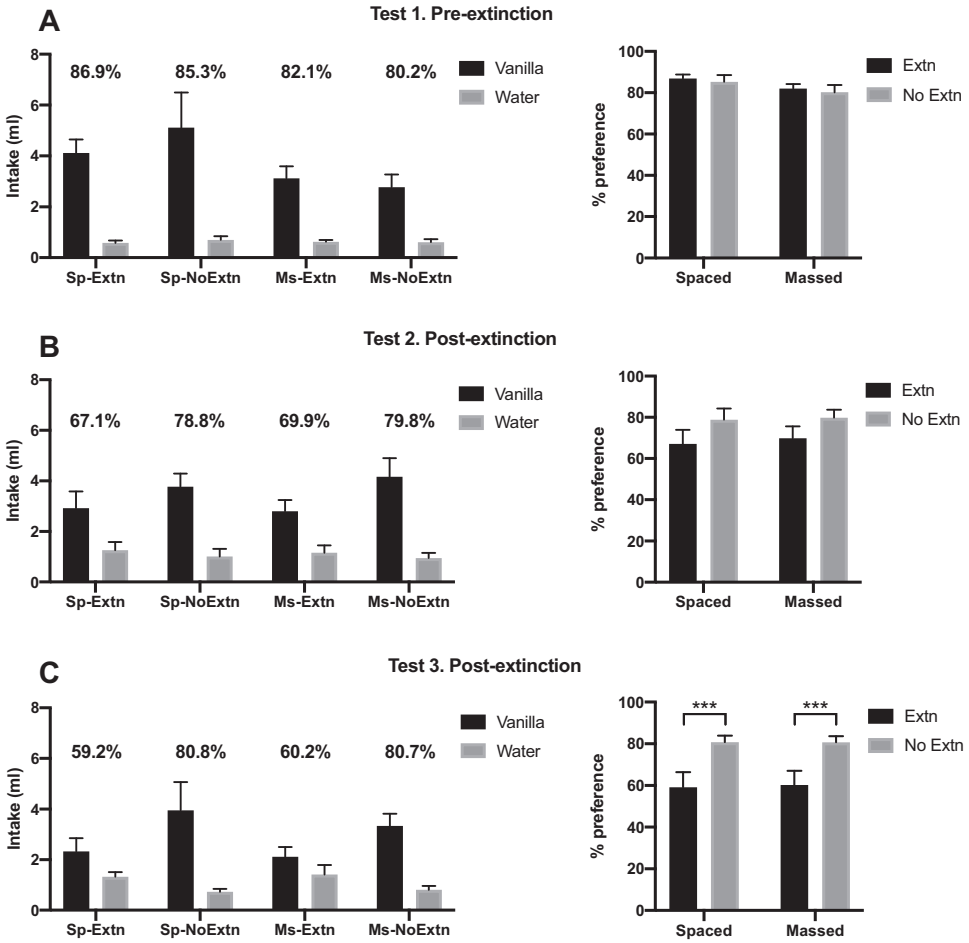
Results

During the training phase, intakes of the vanilla+sucrose solution increased for both the spaced (M = 4.0 ml, 7.5 ml, 8.2 ml) and massed (M = 4.6 ml, 12.1 ml, 11.1 ml) training groups. The massed group consumed more of the compound than the spaced group in the second two training sessions. When the extinction factor was included, total mean intakes across all conditioning sessions in ml were Spaced-Extn: 20.2, Spaced-NoExtn: 19.1, Massed-Extn: 29.4, and Massed-NoExtn: 26.4. A 2 × 2 (Training × Extn) ANOVA found no effects involving the Extn factor (Fs < 1), but confirmed higher intakes in the massed groups with an effect of training, $F(1, 28) = 15.72$, $p < .001$, $\eta_p^2 = .36$, an effect that is discussed later. Test 1 revealed vanilla preferences that were slightly higher after spaced (M = 86.1%) than after massed (M = 81.2%) training; however, a 2 × 2 ANOVA applied to these preferences did not detect any group differences, largest $F = 3.19$ (see right panel of Figure 7A).

Intakes during the eight sessions of the extinction phase were averaged over four two-session blocks (see Figure 8). A 2 × 4 (Training × Block) analysis of the Extn groups found no main effect of training nor interactions (largest F = 1.72). This confirmed what is suggested by the figure, namely, that intakes of vanilla in Extn groups were similar regardless of training sequence. There was also a linear trend across blocks, $F(1, 14) = 11.87$, $p = .004$, $\eta_p^2 = .46$.

Test 2 intakes and preferences are shown in Figure 7B; as seen in the right panel, postextinction preferences in the Extn groups had decreased compared with Test 1. The 2 × 2 analysis of preferences found nonsignificant effects of training and interaction (Fs < 1), while the main effect of Extn almost reached significance ($p = .06$). Mixed 2 × 2 analyses were conducted separately for the Extn and NoExtn conditions to compare preferences from Test 1 with Test 2. These found preferences were significantly lower for Extn groups in Test 2,

Figure 7
Experiment 3



Note. Mean (+*SEM*) test intakes (during a single two-bottle choice test) on the left and mean (+*SEM*) preference ratios on the right. Percentages noted in the left panels indicate preference ratios represented in the right panels. Preference ratio: main effect of Extn *** $p = .001$.

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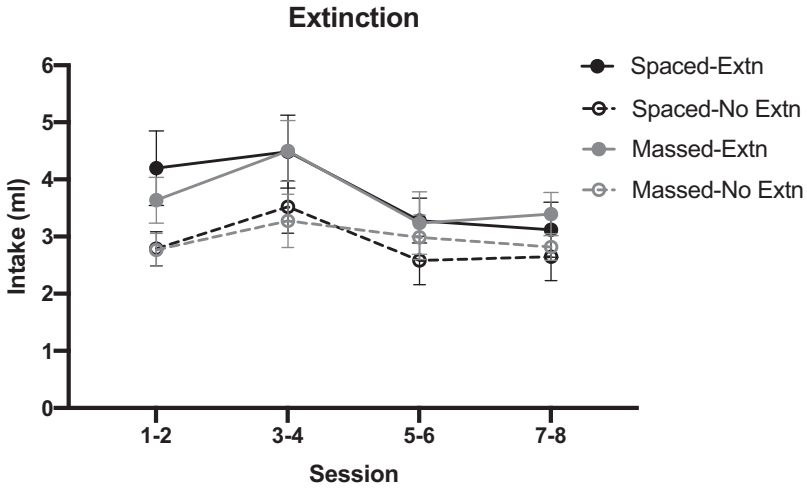
Figure 8
Experiment 3



Note. Mean (±*SEM*) intakes during extinction averaged across two-session blocks. Solid lines: vanilla-only extinction exposure for the Spaced-Extn and Massed-Extn groups; the groups did not differ in acceptance of the flavor. Dashed lines: water-only intake for the NoExtn groups (data not analyzed).

$F(1, 14) = 14.97$, $p = .002$, $\eta_p^2 = .52$, whereas there was no such difference between tests in the NoExtn groups, $p > .10$. At Test 3, a main effect of Extn was found at last (right panel, Figure 7C), confirmed by the 2 × 2 analysis, $F(1, 28) = 15.27$, $p = .001$, $\eta_p^2 = .35$, with no effect of training nor interaction (Fs < 1). The mixed analysis comparing Test 2 with Test 3 found an almost significant drop in preferences in Test 3 for the Extn conditions, $p = .065$, and no such reduction for the NoExtn groups, $p > .10$.

Discussion

The aim of Experiment 3 was to examine the possible contribution of context-sucrose associations by comparing spaced and massed training. We predicted that massed training would produce weaker conditioned preferences and a greater decrease in those preferences during the extinction stage. Neither prediction was confirmed. What we did see for the first time was evidence for extinction following both types of training. Experiment 3 differed in three potentially important ways from the earlier experiments. First, female rats were used instead of the males in the previous experiments. Second, preference testing was carried out in a single session instead of the two sessions used previously. Although it is unlikely that this generated the decrease in preferences shown by the Extn groups, it did produce an outcome not seen in Experiments 1 and 2: Whereas in the latter experiments repeated tests revealed decreases more or less equally in Extn and NoExtn conditions, in Experiment 3 the NoExtn groups showed no such decrease. As discussed more fully in the General Discussion, this supports the suggestion that the two-session test procedure promoted extinction.

The third difference was the use of a two-bottle extinction procedure, in which Extn groups were repeatedly given two bottles, each containing vanilla, and NoExtn controls were given two bottles, each containing water. This resulted in there being a smaller change of context from extinction session to test session than when the one-bottle extinction procedure was used. Thus, whether a rat faces two bottles or one bottle could be an important contextual cue.

The availability of multiple bottles containing taste solutions has been found to increase voluntary intakes of solutions such as sucrose, saccharin, and ethanol in rats and mice (Morales et al., 2020; Tordoff, 2002). Rodent drinking behavior appears to be stimulated by multiple sources of a tastant as the animals sample from all sources available. Such an effect appears to have occurred in the second and third training sessions of the present experiment in which the massed groups drank more vanilla-flavored sucrose in two-bottle presentations than the spaced groups drank in one-bottle presentations. Although spillage may have accounted for some of the group difference, it is unlikely to have accounted for all of it. It remains the case, however, that the different intakes of training solution did not affect preference ratios in any of the tests (i.e., there was no effect of training in any test, as seen in the right panels of Figure 7).

Extinction learning is context-specific (Bouton, 2004). Conducting an extinction procedure in one context and a test procedure in another can produce renewal of conditioned responding in either the original or a new context. Thus, in the present setting a context change from an extinction stage to a test could mean that what a rat may have learned during one-bottle, flavor-only exposures does not transfer to a two-bottle test. The possible importance of this factor was tested in the final experiment.

Experiment 4

Experiment 4 compared rats that were given two bottles in the extinction stage, as in Experiment 3, with rats that were given a single bottle during this stage, as in the earlier experiments. Table 3 shows the 2 × 2 design, in which one factor was Extn and the other factor, Bottles, was whether one or two bottles were used in

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Table 3
Experiment 4: Experimental Design

Groups	Training	Test 1	Extn	Test 2
1-Extn	Van+ W W Van+ Van+ W	Van vs.Water	1-bottle 8x Van	Van vs.Water
1-NoExtn			1-bottle 8x Water	
2-Extn			2-bottle 8x Van	
2-NoExtn			2-bottle 8x Water	

Note. Van = 1% artificial vanilla flavoring; Van+ = vanilla+10% sucrose; W = water.

the extinction phase. The four groups were labeled: 1-Extn, 1-NoExtn, 2-Extn, and 2-NoExtn.

Females were again the subjects. A comparison between the results from the present 1-Extn and 1-NoExtn groups and those from the males given similar conditions in Experiments 1 and 2 would indicate whether sex is an important factor in experiments of this kind.

Experiment 3 indicated that whether spaced or massed training was used had little consequence. Therefore, our standard six-session spaced training procedure was used in the present experiment, as this would facilitate comparison with the results from Experiments 1 and 2. For the same reason, we also reverted to using the two-session test procedure of the earlier experiments.

Method

Subjects

Thirty-two, female Sprague-Dawleys had previously served in the type of appetitive Pavlovian conditioning experiment as the rats in Experiment 3. Rats were housed under the same conditions and were 18 weeks old on arrival in our colony room. After 3 days acclimatizing, progressive food and water restriction began, as previously. The mean body weight was of 252 g (range 195–298 g) at the start of the experiment. One rat in the 1-NoExtn group drank nothing during most experimental sessions and was excluded from all analyses; its behavior suggested that it had somehow acquired an aversion to the spout in its drinking chamber.

Apparatus and Solutions

Sixteen of the acrylic chambers were used. The bottles and the 10% sucrose and vanilla solutions were as used previously.

Procedure

There were six 15-min sessions each week (Monday to Saturday), starting at 12 p.m. Rats were run in two squads, as previously. Following three water pretraining sessions, all groups received the same training: three sessions of vanilla-flavored sucrose solutions interleaved with three sessions of water. The sequence was: sucrose-water-water-sucrose-sucrose-water. In preparation for the following two-bottle procedures (tests and extinction sessions), the final two training sessions presented rats with two bottles, both first containing vanilla-flavored sucrose and then containing water in the final session. Left and right bottle intakes were measured to assess potential side preferences.

Test 1 comprised two sessions of a two-bottle choice between vanilla in water versus water only. For the first session, the bottle containing vanilla was on the left and water was on the right; these positions were reversed for the second test session. Rats were then divided into the four groups, matching for flavor preferences in Test 1 and ensuring one cage from each group was run in each squad.

During the extinction phase, the 1-Extn and 2-Extn groups were given vanilla in water, in one-bottle or two-bottle presentations, respectively. The 1-NoExtn and 2-NoExtn groups received water only, also in one- or two-bottle presentations. The experiment ended with Test 2, using an identical two-session procedure to that of Test 1.

Results

During the three sessions of training, mean intakes of the vanilla + sucrose solution across all groups were 3.8 ml, 6.1 ml, and 11.1 ml. Total mean intakes of the four groups across all three sessions in ml were 1-Extn: 22.4, 1-NoExtn: 23.0, 2-Extn: 19.3, and 2-NoExtn: 19.9. A 2 (Bottles) × 2 (Extn) ANOVA detected no group differences in the amount of vanilla+sucrose consumed ($p > .10$). As seen in Figure 9A, preferences in Test 1 were above 80% in all four groups, with an overall mean of $M = 82.5\%$. A 2 × 2 analysis confirmed no significant effects, Fs < 1.

Intakes during the extinction stage were averaged into two-session blocks, as shown in Figure 10. A 2 × 4 mixed ANOVA applied to vanilla intakes produced a main effect of Bottles, $F(1, 14) = 18.0$, $p = .001$, $\eta_p^2 = .56$, such that the group presented with two bottles of vanilla drank more. This was likely due to the stimulation of intake by the availability of two bottles, as described above in the Discussion section of Experiment 3. There was also a quadratic effect of block, $F(1, 14) = 7.07$, $p = .02$, $\eta_p^2 = .34$, with no interactions (ps $> .10$).

Test 2 preferences in all groups decreased from their values in Test 1 (see right panel of Figure 9B). The 2 × 2 analysis of preference ratios found no main effect of Bottles ($F < 1$), but there was a main effect of Extn, $F(1, 27) = 8.90$, $p = .006$, $\eta_p^2 = .25$, and an interaction, $F(1, 27) = 4.65$, $p = .04$, $\eta_p^2 = .15$. Simple effects confirmed the interaction was driven by a significant difference between the 2-Extn ($M = 56.7\%$) and 2-NoExtn ($M = 76.9\%$) groups, $F(1, 27) = 13.68$, $p = .001$, $\eta_p^2 = .34$. Mixed 2 × 2 analyses separately compared the change in preferences between tests for the Extn and NoExtn groups. These found a robust decrease in Test 2 for the Extn groups, $F(1, 14) = 45.99$, $p < .001$, $\eta_p^2 = .77$, and a similar, though weaker, effect for the NoExtn groups, $F(1, 13) = 15.96$, $p = .002$, $\eta_p^2 = .55$.

Discussion

The aim of Experiment 4 was to test the prediction that two-bottle presentations during the extinction stage would be more effective than one-bottle presentations in producing a detectable extinction

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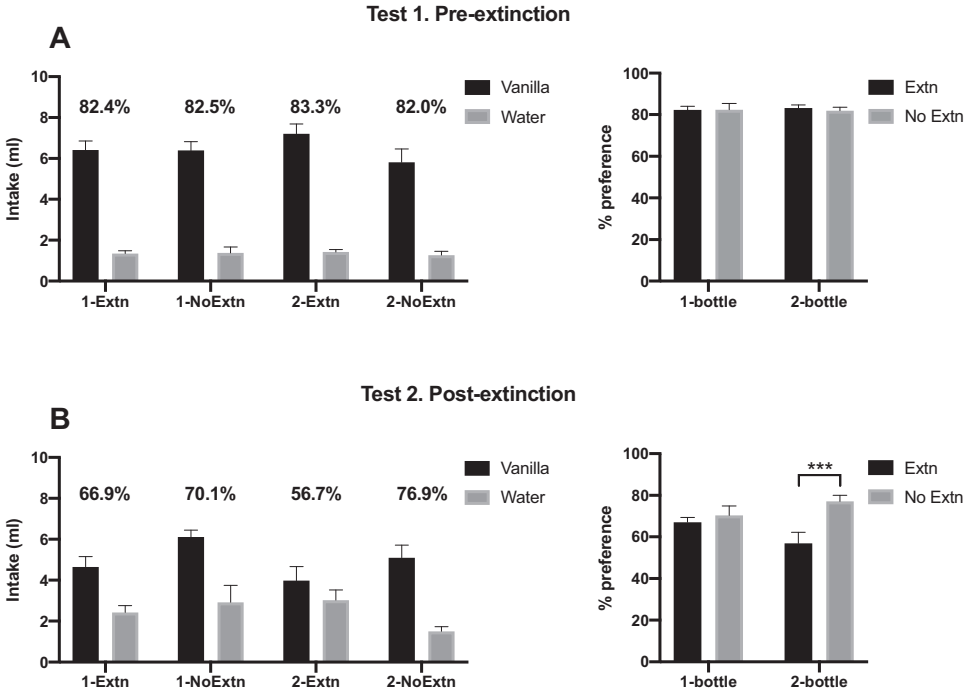
Figure 9
Experiment 4



Note. Mean (+*SEM*) total test intakes (summed over two test days of two-bottle choice tests) on the left and mean (+*SEM*) preference ratios on the right. Percentages noted in the left panels indicate preference ratios represented in the right panels. Preference ratio: 2-Extn/2-NoExtn simple effect *** $p = .001$.

effect. The results of Test 2 confirmed this prediction. The absence of an extinction effect in the one-bottle condition replicated the results obtained with male subjects in Experiments 1 and 2, indicating that the use of females in the final two experiments was not an important factor. Experiment 4 returned to the two-session test procedure used in Experiments 1 and 2, this generated the same test-to-test reduction in preferences, even in the NoExtn groups, that has been seen in all experiments other than Experiment 3, where the one-session test procedure was used. However, in this experiment we also found a clear indication that the intervening two-bottle presentations of vanilla in the extinction phase contributed an additional effect. The between-test comparison showed extinction occurred in both Extn and NoExtn groups, but the decline in preference was more pronounced for the Extn condition.

As suggested above, extinction may be easier to detect using the two-bottle extinction procedure because it involves less of a change in context when the test is introduced. However, the data on intakes during the extinction procedure suggest an alternative factor, in that, as shown in Figure 10, rats in the 2-Extn condition drank more of the vanilla solution than those in the 1-Extn condition. The relative importance of the two factors is discussed in the following section.

General Discussion

The starting point for the present series of experiments was the conclusion reached by Harris et al. (2004) that sucrose-based conditioned flavor preferences are subject to extinction as long as rats are food deprived. It therefore came as a surprise when Experiments 1, 2A, and 2B failed to detect any evidence for the extinction of a similarly conditioned, sucrose-based flavor preference. These initial experiments were sufficiently powered to detect other effects, such as the weaker preferences obtained when 4% sucrose was used instead of 10% sucrose (Experiment 2A) and the greater decline in conditioned preferences when almond was used as the target flavor instead of vanilla (Experiment 2B).

The failure to detect extinction in these early experiments suggested two possibilities: Either (a) the data reported by Harris et al. (2004) were misleading or (b) the procedures used in the present experiments were inappropriate for detecting extinction effects. One version of the first possibility was mentioned above: The decrease in preference for almond as tests were repeated was accompanied by an increase in intakes of both the almond-flavored water and water without a noticeable change in the difference between these intakes. Thus, even in the final test intakes of almond by the hungry group of rats exceeded intakes of water by a similar amount as in the early tests, even though the preference ratio calculation was lower.

The second of the two possibilities raised above—namely, that the procedures used in the initial experiments were inappropriate for detecting extinction—was confirmed by the results of Experiments 3 and 4. These showed that extinction could be detected when two bottles contained the target flavor during the extinction stage. The reason for the importance of this factor was not entirely

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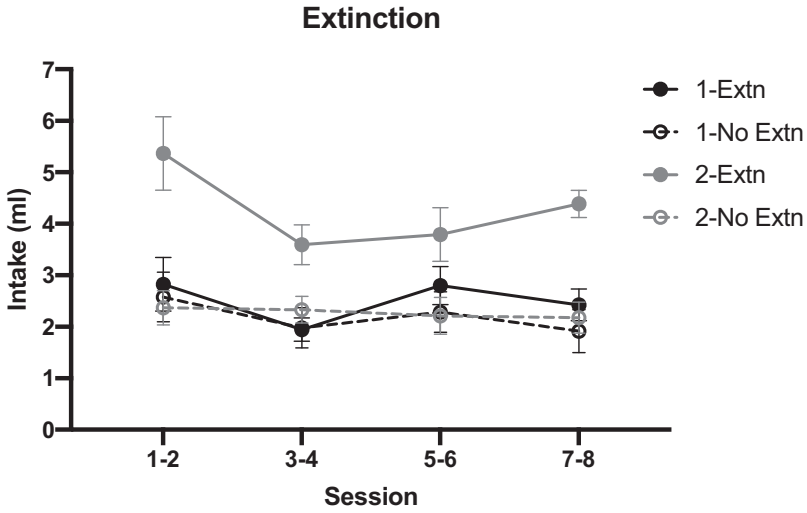
Figure 10
Experiment 4



Note. Mean (±*SEM*) intakes during extinction averaged across two-session blocks. Solid lines: vanilla-only extinction exposure for the 1-Extn and 2-Extn groups; the 2-Extn group drank more than the 1-Extn group, $p < .001$. Dashed lines: water-only intake for the NoExtn groups (data not analyzed).

clear. Our preferred explanation is in terms of context shift, as outlined previously. A less interesting alternative is that, when two bottles are used, rats drink more, as found in both previous studies (e.g., Morales et al., 2020; Tordoff, 2002) and in the present Experiment 4 (see Figure 10); greater intakes mean greater exposure to the to-be-extinguished flavor.

The intakes of the flavor solutions during initial tests and subsequent extinction sessions in the present experiments have some bearing on this issue and on the decline of preferences from test to test. In Experiments 2A and 2B, rats in the Extn conditions had consumed totals that ranged from 23 ml to 34 ml of the target flavor in water prior to the postextinction test (Test 2). Whereas total intakes of the flavor in water for rats in the NoExtn conditions comprised only what they had consumed during Test 1 and ranged from 4 ml to 6 ml. This Extn:NoExtn ratio of flavor-only intake prior to Test 2 was in the order of almost 6:1. Despite these large differences in intake, there were consistent failures to find any differences in preferences in the postextinction tests carried out in these early experiments. Therefore, we conclude that the differences in intake between the one-bottle and two-bottle groups during the extinction stage of Experiment 4 were unlikely to have made a major contribution to the differences in preferences found in subsequent choice tests and that the major contribution to the apparent lack of extinction following the one-bottle procedure is the change of context.

A question raised by the present results is whether, when testing for extinction of a conditioned flavor preference, one needs to include control groups that are not given access to the target flavor during the extinction stage, as in the present experiments, or—more simply—just give repeated preference tests, as in Harris et al. (2004). It may be noted that in Experiments 1, 2A, and 2B preferences decreased as the two-session tests were repeated to essentially the same extent in rats given the target flavor during the extinction stages as in rats given only water (see Figures 1, 3, and 5). However, when a single-session test procedure was used in Experiment 3, no such decline was found in the rats given water during the extinction stage (see Figure 7, NoExtn groups). When the two-session test procedure was reintroduced in the final experiment, the decline of preference was again seen in the one-bottle group given water during the extinction stage (see Figure 9, 1-NoExtn group). To return to the question of what extinction procedure is to be preferred, an answer requires further investigation that directly compares the two methods in order to determine whether they produce differences in the degree of extinction and in the extent to which extinction is context-specific.

As far as we are aware, no other report of flavor preference learning has considered the possibility that whether a chamber contains one or two spouts is an important contextual cue to a rat. Consequently, it is of interest to examine whether it may have influenced the results of previous studies. In particular, the results reported by Garcia-Burgos and González (2012) may need to be reevaluated in the light of the present study.

A series of five experiments reported by Garcia-Burgos and González (2012) led these authors to conclude that, following training with a flavored sucrose solution, the subsequent decrease in preference for the flavor produced by an extinction procedure was not "true" extinction but was an example of inhibitory learning. Their first three experiments used as an extinction procedure, the same repeated two-bottle preference testing that Harris et al. (2004) employed. In turn, these failed to obtain spontaneous recovery following a 2-week delay in testing (Experiment 1), a reinstatement effect after the rats were given postextinction exposure to sucrose and then tested again the following day (Experiment 2), or any impact on the flavor preference of postextinction LiCl-based devaluation of sucrose in an extinction group (Experiment 3).

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The Methods section does not specify for Experiment 2 whether the postextinction exposure to sucrose consisted of giving access to this solution in a single bottle but this seems likely. If so, then the failure to obtain reinstatement in the subsequent two-bottle preference test may have resulted from the shift in contexts. Similarly, if in Experiment 3 the sucrose devaluation procedure consisted of giving access to sucrose in a single bottle before the lithium chloride injection, then the failure of this intervention to affect preference in animals that had undergone two-bottle extinction could also be seen due to the change in context.

The final two experiments reported by Garcia-Burgos and González (2012) used a different extinction procedure from that of the first three experiments, in that, following the training stage, the target flavor was presented in water using a single bottle. In contrast to the results of the present experiments that used a one-bottle extinction procedure, in both of the Garcia-Burgos and González (2012) final experiments a decrease in flavor preferences was found in postextinction two-bottle preference tests. One aspect of their procedure may be important. During the posttraining transition from fluid deprivation alone to deprivation of both food and fluid (the animals were rendered hungry for the test phase) the rats were given three sessions of two-bottle exposure to water; this may have provided a first stage in learning that no sucrose was available in a two-bottle context.

Putting aside the issue of whether or not the new results reported here require reevaluation of those from previous studies, the present experiments reinforce two general conclusions. First, learned flavor preferences are unusually sensitive to changes in context, as previously indicated by studies such as Albertella et al. (2008). And second, small and seemingly unimportant changes in the procedure used in some experiments can have major consequences.

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