The Extinction Procedure Modifies a Conditioned Flavor Preference in Nonhungry Rats Only After Revaluation of the Unconditioned Stimulus

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In 3 experiments rats experienced 2 flavors, each paired with sucrose, in order to establish a conditioned preference to each. One (flavor Fe) was then presented alone (an extinction procedure) prior to a choice test between Fe and the flavor that did not undergo extinction (Fne). Hungry rats showed a preference for Fne over Fe (Experiment 1A); but rats that were not food-deprived showed no effect of extinction when given a choice between Fe and Fne immediately after extinction (Experiment 1B) or after an interval in which reexposure to sucrose was given (Experiment 2). The extinction procedure was not without effect, however, as Fe was preferred over Fne after sucrose had been devalued by pairing with lithium chloride, and Fne was preferred over Fe after a procedure likely to enhance the value of the sucrose (Experiment 3). The explanations considered propose that preference conditioning establishes a range of associations between the flavor and the various properties of sucrose (its nutritional value, its taste, the hedonic reaction it evokes). It is suggested that the form of learning that mediates revaluation effects is sensitive to extinction whereas that responsible for performance on a consumption test is not.

Keywords: rats, flavor preference, extinction, hunger, US devaluation

In flavor-preference conditioning subjects (rats in the experiments to be reported here) are allowed to consume a neutral or a nonpreferred flavor that is presented in compound with a substance such as sucrose that has positive hedonic and motivational properties. After this training, rats, given a choice between plain water and water containing the flavor, show an increased preference for the latter, an outcome that has been interpreted as an instance of conditioning with the flavor serving as the conditioned stimulus (CS) and sucrose as the unconditioned stimulus (US). Consumption of sucrose is, of course, a complex event with many aspects, which allows for a range of associative interpretations of the source of the preference. In particular, it has been suggested that an association might be formed between the flavor and the sweet taste of sucrose, between the flavor and the hedonic response evoked by the sucrose, and between the flavor and the nutritional/motivational properties of the sucrose; or all of these (e.g., Harris, Gorissen, Bailey, & Westbrook, 2000; Harris, Shand, Carroll, & Westbrook, 2004).

Such preferences have proved to be remarkably persistent. For most conditioning paradigms (see, e.g., Mackintosh, 1974) repeated presentation of the CS alone, in the absence of the US (the extinction procedure) leads to a loss of the conditioned response (CR). For rats that have acquired a preference for a given flavor as a result of experiencing a mixture of that flavor and a preferred substance such as sucrose, the extinction procedure consists of giving the experience of the flavored solution in the absence of sucrose. That consumption of the flavored solution does not reliably decline in these conditions is not, in itself, of theoretical significance. In this experimental procedure the rats are usually on a water deprivation schedule, and thus will continue to drink in spite of any loss of the initial preference. It is significant, however, that when rats are given a choice between flavored water and plain water (both of which will alleviate thirst) they continue to show a preference for the flavor over the course of repeated choice tests (e.g., Dwyer, Pincham, Thein, & Harris, 2009; Harris et al., 2004), or when the choice test is given after extensive exposure to the flavor alone (e.g., Albertella & Boakes, 2006).

This outcome, the failure to show normal extinction, is not obtained when the rats are trained or tested in a state of food deprivation (Harris et al., 2004, Experiment 2). This observation has been taken to support the suggestion that, when the rats are hungry, the preference is based on just one of the available associations—that between the flavor and its nutritional consequences (Fedorchak & Bolles, 1987). This association is assumed to obey the standard principles of Pavlovian conditioning, and is thus susceptible to the effects of extinction. When the animals are not hungry, however, the preference is assumed to depend on some other form of learning that follows different principles. The nature of this “other form” has been expressed in a variety of ways, but the central notion is that, when subjects are not hungry, the important feature of the sucrose is its sweet taste, rather than its
nutritional consequences (see, e.g., Boakes, 2005; Campbell, Capaldi, Sheffer, & Bradford, 1988; De Houwer, Thomas, & Baeyens, 2001; Harris et al., 2000; Myers & Sclafani, 2006). In terms of the possible associations listed in the introduction, one interpretation of this notion is that establishing a flavor-taste association changes the perceptual properties of the flavor (e.g., Campbell et al., 1988), and that such a change is not sensitive to extinction (e.g., Pearce, 2002). An alternative, suggested by Harris et al. (2004), is that the critical association is between the flavor and the hedonic response to sweetness, and that this form of learning resists extinction.

The need to take this radical theoretical step is challenged by results reported by Delamater (2007) showing that, with a modified experimental design, it is possible to demonstrate an effect of extinction even in rats that are not food-deprived. Delamater pointed out that the choice test used in earlier studies is unlikely to be a sensitive measure of the effects of an extinction procedure. When given a choice between a more-valued and a less-valued substance, subjects might show a substantial preference for the more valued, even when the difference between the two substances is small. Thus, although the extinction procedure might in some way reduce the acquired value of the CS, if this reduction is not complete, a clear preference could still be evident in a choice between the trained flavor and water. In order to deal with this issue, Delamater (2007) used a design in which the rats were trained with two flavors, each paired with sucrose. One of these flavors then underwent extinction, prior to a choice test between the two flavors. The choice was thus between a valued flavor and one that might, as a result of the extinction trials, be somewhat less valued. Using this procedure, Delamater was able to show an effect of extinction not only in rats that were hungry during training and testing, but also in rats that were not food-deprived. Delamater concluded that the use of this more sensitive test had revealed that conditioned flavor preferences based on the taste of the US are subject to the effects of extinction, just as are other CRs.

Before accepting Delamater’s (2007) conclusion, however, we should note (as he did himself) that his experimental procedure involved factors other than those designed to produce an increase in the sensitivity of the test measure. Specifically, Delamater adopted a technique, previously used by Harris et al. (2004), in which the choice test was given after a phase of training designed to reduce the value of the sucrose reinforcer. In this technique, rats were given access to sucrose, followed by an injection of lithium chloride (LiCl). This training produced a reduction in the readiness with which sucrose was consumed and, in the choice test, a shift in preference in favor of the flavor that had undergone extinction. This shift provides indirect evidence of an extinction effect, being consistent with the notion that extinction had reduced the effectiveness of an associative link between the flavor and the now-devalued sucrose. More direct evidence came from control subjects who were given unpaired presentation of sucrose and LiCl prior to the test; these showed a preference for the nonextinguished flavor.

Although this pattern of results is consistent with the proposal that an effect of extinction can be revealed by using an appropriately sensitive choice test, the use of the devaluation technique allows only an indirect demonstration of the effect. The result for the control subjects is more straightforward but it might, as Delamater (2007) acknowledged, be in some way a consequence of exposure to the US per se during the phase of unpaired presentations. There is evidence to show that posttraining exposure to sucrose will reduce the magnitude of a conditioned preference (e.g., Boakes, Albertella, & Harris, 2007, Experiment 3; Harris et al., 2004, Experiment 4; Kawai & Nakajima, 1997); and it will do so when it is combined with the extinction procedure (e.g., Boakes et al., 2007, Experiment 2). Delamater suggested that it would be useful to determine whether an extinction effect could be obtained, using his procedure, when no US presentations intervened between extinction and the test. Accordingly, in the first experiment to be reported here, we repeated the essential features of Delamater’s procedure—that is, we gave training to two flavors and a choice test between one that had undergone the extinction treatment and one that had not—but we did not give exposure to the sucrose US (or to LiCl) prior to the test. To anticipate, we found (for rats that were not food-deprived) no effect of extinction in these circumstances. In subsequent experiments we investigated what features of the postextinction (and pretest) treatment were responsible for the effects observed by Delamater. A summary of the experimental designs is presented in Table 1.

Experiments 1A and 1B

In both experiments, rats received a first phase of training in which they experienced two flavors, each presented mixed with a sucrose solution. As in many studies of flavor-preference conditioning, the flavorants used are likely to function primarily as odors; however, as they are consumed orally and may also have a taste component, we will continue to refer to them as flavors. All subjects then received a phase of training in which one of the flavors (to be referred to as Fe, for extinguished flavor) was presented alone, in the absence of sucrose. In the final test phase the subjects were given a choice between Fe and the flavor that did not undergo extinction (Fne). We also included tests in which the choice was between one of the flavors and water.

The experiments differed only in that in Experiment 1A the rats were hungry throughout, whereas in Experiment 1B they had free access to food. Experiment 1A should thus allow us to replicate the well-established effect of extinction in hungry animals. At issue was whether or not this procedure, which omits the phase of postextinction training, but otherwise follows that used by Delamater (2007) in his Experiments 1 and 3, would allow us to replicate (in our Experiment 1B) his finding of an effect of extinction in rats that were not food-deprived.

Method

Subjects and apparatus. The subjects in each experiment were 16 naïve male Wistar rats (from Janvier, France) with a mean body weight of 345 g (Experiment 1A) or 290 g (Experiment 1B) at the start of the experiment. They were housed in individual home cages and kept in a colony room at the Biomedical Research Center of the University of Granada that was lit from 8:00 a.m. to 8:00 p.m. each day. Experimental procedures took place with the rats in their home cages and during the light period of the cycle. Inverted 50-ml plastic tubes equipped with stainless steel ball-bearing-tipped spouts were used to present fluids in these cages. Consumption was estimated by weighing the tubes before and after fluid presentation to the nearest 0.1 g. The rats were maintained on a schedule of restricted access to water throughout the experiment; access to food was controlled, as detailed below. The flavors used
consisted of 0.6% (vol/vol) solution of almond essence (Shepcote Distributors Ltd, Yorkshire, U.K.), and a 0.07% (vol/vol) solution of vanilla concentrate essence (Manuel Riesgo, S.A., Spain). These were made up with tap water for the extinction and test phases, and with solution of 20% (wt/vol) of sucrose (AB Azucarera Iberia S.L., Madrid, Spain) during conditioning.

**Procedure.** The experimental procedures were approved by the University of Granada Ethics Committee. To initiate the deprivation schedule, the water bottles were removed 24 hr before the start of the experiment. In Experiment 1A, food was also removed at this stage; in Experiment 1B, food was removed from the cages during the experimental sessions (see below) but was otherwise always available. The rats were then given 3 days to accommodate to a deprivation schedule, in which access to water was allowed twice a day at approximately 10 a.m. and 1:30 p.m. In Experiment 1B each drinking session was of 30 min; in Experiment 1A the afternoon session was extended to 90 min and food was made available at this time (a schedule maintained for the rest of the experiment).

Conditioning occurred over the following four days with two trials per day, one in the morning (10 a.m.) and the other in the afternoon (1 p.m.). Each trial consisted of 5 min of access to 6 ml of a flavored sucrose solution. There were four trials with flavor Fe (the flavor to be extinguished) and four with Fne (nonextinguished). The identity of the flavors serving as Fe and Fne was distinguished. The identity of the flavors serving as Fe and Fne was counterbalanced, presented as a simultaneous compound with sucrose during conditioning. After conditioning, Fe was extinguished and rats were given a two-bottle choice test pitting Fe against Fne (Test 1). Tests on subsequent days pitted the flavors against water (Tests 2 and 3). In Experiment 2 half of animals received exposure to sucrose or water after extinction and before testing. In Experiment 3, half of rats receive pairing of sucrose and lithium chloride, whereas the rest received unpaired presentations of sucrose and lithium chloride.

**Results**

**Statistics.** In these experiments, and in those to be reported subsequently, scores were analyzed using mixed analyses of variance (ANOVAs) in order to determine the locus of significant main effects and interactions. Holm’s sequential Bonferroni post hoc test (the Holm-Bonferroni method, hereafter) was used to control for the family-wise error rate when making multiple comparisons. The Greenhouse–Geisser correction was applied in case of violation of the assumption of sphericity, but we report the nominal $F$’s degrees of freedom for simplicity. For comparisons involving just two means, we used Student’s $t$-tests (two-tailed). Calculated effect sizes were Cohen’s $d$ for $t$-tests (Cohen, 1988), and $η^2$ for ANOVAs. Jeffreys-Zellner-Siow (JZS) prior (Rouder, Speckman, Sun, Morey, & Iverson, 2009) was utilized to estimate Bayes factor using JASP software (JASP, 2014). Here we follow conventional interpretation of JZS-values proposed by Wagenmakers, Wetzels, Borsboom, and van der Maas (2011).

**Experiment 1A.** Apart from the very first trial (an Fe trial), on which consumption was slightly reduced, the rats drank almost all of the fluid presented during the conditioning sessions. Group means for the Fe trials were 3.9, 5.6, 5.7, and 5.7 g; and for the Fne trials: 5.3, 5.5, 5.5, and 5.9 g. A repeated measures ANOVA with flavor (Fe vs. Fne) and trial (1–4) as the variables revealed significant main effects of both flavor, $F(1, 15) = 20.86, p < .01, η^2 = 0.58$, and trial, $F(3, 45) = 21.13, p < .01, η^2 = 0.58$, as well as a significant interaction, $F(3, 45) = 20.40, p < .01, η^2 = 0.58$. Post hoc Holm-Bonferroni tests revealed that consumption on the
first Fe trial was significantly lower than the other three Fe trials (largest \( p < .02 \)); in addition, consumption on the first Fne trial was lower than the fourth Fne trial (\( p < .01 \)). Consumption on the first Fe trial was less than on the first Fne trial, \( p < .01 \), but there were no differences on subsequent trials (smallest \( p = .36 \)). This pattern of results presumably reflects a certain degree of neophobia occurring on the first encounter with both flavor-sucrose compounds, but especially in the case of the very first one (Fe).

Consumption of the Fe flavor fluctuated over the course of the nonreinforced trials, but there was no convincing evidence for the occurrence of extinction. Group means over the six trials were: 4.0, 2.8, 2.5, 2.9, 3.6, and 3.5 g. An ANOVA showed there to be a difference among the trials \( F(5, 75) = 3.71, p = .02, \eta^2_g = .20 \). The Holm-Bonferroni test revealed a difference between the first and the third trial. No other differences were significant (smallest \( p = .005 \); corrected \( p \) value = .003).

The results of the preference tests are shown in Figure 1. The left panel shows that, when given a choice between Fe and Fne, the rats consumed more of Fne than of Fe, \( t(15) = 7.37, p < .01, d = 1.84, B_{10} > 100 \), extreme evidence of the alternative according to Wagenmakers et al. (2011). Thus, as in previous experiments (e.g., Delamater, 2007; Harris et al., 2004) the extinction procedure is found to be effective in hungry animals. The remaining tests confirmed this result. The central and right panels of Figure 1 show the results for the tests in which the choice lay between a flavor and water. The extinguished flavor, Fe, was consumed no more readily than water, \( t < 1, B_{10} = 0.15, \) but Fne was preferred over water, \( t(15) = 7.18, p < .01, d = 1.79, B_{10} > 100 \). Expressed as preference ratios (flavor consumption/total consumption) the score for the test with Fe was .46, and that for the test with Fne was .79. These differed significantly, \( t(15) = 6.66, p < .01, d = 1.66, B_{10} > 100 \). In terms of the Bayesian analysis, therefore, we found extreme evidence in favor of the alternative hypothesis: consumption and preference scores for Fe differed from those for Fne.

**Experiment 1B.** As in Experiment 1A, the rats consumed almost all of the fluids offered during the conditioning phase, except on the very first trial. Group means for the Fe trials were 5.0, 5.5, 5.6, and 5.6 g; and for the Fne trials: 5.5, 5.5, 5.7, and 5.6 g. A repeated measures ANOVA with flavor (Fe vs. Fne) and trial (1–4) as the variables revealed main effects of both flavor, \( F(1, 15) = 8.71, p = .01, \eta^2_g = 0.37, \) and trial, \( F(3, 45) = 8.74, p < .01, \eta^2_g = 0.37, \) as well as a significant interaction, \( F(3, 45) = 5.88, p < .01, \eta^2_g = 0.28 \). Post hoc Holm-Bonferroni tests revealed that consumption on the first trial (Fe) was significantly lower than on the others (largest \( p < .001 \)), which did not differ among themselves (smallest \( p = .04 \); corrected \( p \) value = .008).

Group means for consumption of the Fe flavor over the six extinction trials were: 11.6, 11.6, 11.3, 11.2, 10.4, and 10.2. An ANOVA showed there to be a difference among the trials \( F(5, 75) = 3.05, p = .01, \eta^2_g = .17 \); but subsequent post hoc testing failed to reveal a difference at the corrected \( p \) value (for the comparison between the second and last trials, \( p = .005; \) corrected \( p \) value = .003).

The results of the choice tests are shown in Figure 2. The left panel shows the group mean amounts consumed on the Fe versus Fne test. Although consumption of Fe was somewhat less than that of Fne the difference was small and not statistically significant, \( t(15) = 1.29, p = .22, d = 0.32, B_{10} = 0.30, \) providing evidence in favor of the null hypothesis \( (B_{10} < 1/3) \). Thus, in common with the results of previous studies using nondeprived subjects (e.g., Albertella & Boakes, 2006; Harris et al., 2004), and in contrast to the analysis offered by Delamater (2007), there was no evidence that the extinction treatment had shifted preference in favor of the nonextinguished flavor. The results of the subsequent flavor versus water tests (presented in the central and right sections of Figure 2) show that both Fe and Fne were consumed significantly less than water: for the comparison of Fe with water, \( t(15) = 3.69, p < .01, d = 0.92, B_{10} = 17.26; \) for Fne, \( t(15) = 2.24, p = .04, d = 0.56, B_{10} = 1.21 \). The preference ratios, .30 for Fe and .38 for Fne, did not differ significantly, \( t < 1, B_{10} = 0.20 \). That these ratios were less than .5 does not mean that conditioning was ineffective. The particular flavors used in these experiments appear to be disliked by rats. Our pilot work has confirmed that pairing with sucrose can shift the preference in favor of the flavor, but, evidently, it will generate greater consumption of the flavor than of water only when the rats are hungry (as in Experiment 1A). The possible implications of this will be discussed later.

**Discussion**

Experiment 1A has successfully replicated the results of previous, similar, experiments (e.g., Harris et al., 2004, Experiments 2A and 2B; Delamater, 2007, Experiment 3), showing that, for hungry rats, a preference established by pairing a flavor with sucrose can be extinguished by presentations of the flavor alone. We take this result to support the view (e.g., Harris et al., 2000, 2004) that, in these animals, the preference is controlled by an association between the flavor and its outcome (i.e., a flavor-nutrient association), and that this association is subject to the effects of extinction as are other instances of Pavlovian conditioning. The results of Experiment 1B are also in accord with those of previous studies (e.g., Albertella & Boakes, 2006; Dwyer et al., 2009; Harris et al., 2004) in showing the extinction procedure to be ineffective in rats that are not food-deprived. Critically, however, they failed to replicate the extinction effect to be expected on the basis of the results of Delamater, and this in spite of the fact that we made use of a test procedure that involved a choice between extinguished and nonextinguished flavors. As we have acknowledged, this procedure is likely to be especially sensitive to the effects of extinction. Our failure to find an effect with this test challenges the idea that the absence of extinction in previous studies was simply a consequence of their use of a different, less sensitive test. It also implies that some other factor is responsible for the effects obtained by Delamater. We investigate this issue in the next experiment.

**Experiment 2**

The aims of this experiment were twofold. First, given the discrepancy between the results of Experiment 1B and those of Delamater (2007), we wanted to look again at the effects of

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1. Based on this rather large effect size (Cohen, 1988), 95% CI [1.01; 2.65], and following the recommendations for selecting the Cauchy prior width according to previous knowledge about effect sizes, we decided to set the value of the parameter \( r = \sqrt{2} \) in this and following Bayesian factor calculations, as suggested by Schönbrodt, Wagenmakers, Zehetleitner, and Perugini (2015).
extinction in nonhungry rats, in a study with modified parameters. In particular, the flavors used in Experiment 1 seemed to be disliked by the rats (even after conditioning, as indicated by the water vs. flavor preference tests of Experiment 1B). Although it is not obvious why this factor might be of importance, we thought it worthwhile to repeat the central features of Experiment 1B using flavors that we thought would be neutral prior to conditioning.

In addition, we wanted to investigate the role of the postconditioning treatment given by Delamater (2007) in generating his results. It will be recalled that Delamater’s procedure involved a phase of training interpolated between the extinction phase and the test trials; this, we must assume, is in some way responsible for making the effects of extinction evident. It is certainly possible that devaluing the sucrose reinforcer, by associating it with the effects of LiCl, allows a particularly sensitive test of the strength of the flavor-sucrose association, and the effect obtained by Delamater with this procedure has also been demonstrated in a study using a between-groups comparison (Harris et al., 2004, Experiment 3). This latter experiment, however, found no effect in the control groups given unpaired presentations of sucrose and LiCl, whereas Delamater did. A possible interpretation of this finding comes from the analysis of the effects of sucrose exposure offered by Boakes et al. (2007). This makes use of the concept of adaptation level, and holds that a phase of exposure to sucrose shifts the level, and thus reduces the perceived sweetness of flavors that have acquired this property by previous association with sucrose. Such a reduction, it can be argued, could move the values associated with the Fe and Fne cues into a range in which differences in behavior are more easily observed.

In order to test this hypothesis, we included two groups in the present experiment, one of which received exposure to sucrose prior to the test, whereas the other group did not. We expected that the latter group would replicate the results of Experiment 1B, with the choice between Fe and Fne being unaffected by extinction. Would exposure to sucrose in the other group shift the preference to Fne—that is, produce the extinction effect obtained by Delamater?

**Method**

The subjects were 16 naïve male Wistar rats (Janvier, France) with a mean body weight of 346 g at the start of the experiment. They were housed and maintained as in Experiment 1B (i.e., they were on a schedule of water deprivation but had free access to food). On the basis of pilot work, we chose concentrations of the flavors that were approximately neutral (with respect to water) and a concentration of sucrose that generated a clear preference with these flavors. We reduced the concentration of vanilla to 0.035%.

![Figure 1](image1.png)

**Figure 1.** Experiment 1A: group mean consumption on choice tests of water (Wt), an extinguished flavor (Fe), and a nonextinguished flavor (Fne). Vertical bars represent standard error of the mean (corrected for within-subject design according to O’Brien & Cousineau, 2014).

![Figure 2](image2.png)

**Figure 2.** Experiment 1B: group mean consumption on choice tests of water (Wt), an extinguished flavor (Fe), and a nonextinguished flavor (Fne). Vertical bars represent standard errors of the mean (corrected for within-subject design according to O’Brien & Cousineau, 2014).
did not differ among themselves (smallest \( F \)). The interaction was not significant, \( p > .01 \).

Overall means for consumption of the Fe flavor on the six trials of the extinction phase were 12.6, 11.5, 11.1, 11.4, 10.9, and 11.2 g. Thus there was some fluctuation over trials; an ANOVA showed a significant effect of trial, \( F(5, 75) = 2.53, p = .03, \eta^2_p = .14 \). Holm-Bonferroni tests showed that the first trial differed significantly from the fifth (\( p < .01 \)). No other differences were significant (smallest \( p = .006; \) corrected \( p \) value = .003).

Group mean consumption over the three trials of the reexposure phase was 9.9, 9.7, and 9.9 g, for the sucrose reexposed group, and 9.4, 9.0, and 9.6 g, for the no-reexposure group. Although fluid exposure was limited to 10 ml, rats given sucrose consumed more than rats exposed to water. The ANOVA, with group and trials as the variables, confirmed this impression, yielding a main effect of group, \( F(1, 14) = 10.56, p < .01, \eta^2_p = .43 \). There was no effect of trial and no interaction, largest \( F(2, 28) = 3.24 \) (Greenhouse–Geisser corrected \( p \) value = .07).

The results of the choice tests and group means for consumption of each fluid, shown separately for subjects given reexposure to sucrose and those not given reexposure, are presented in Figure 3. As the top panel shows, the difference in consumption of Fe and Fne was small and was not influenced by reexposure to the US. An ANOVA with flavor (Fe or Fne) and group (reexposed or not) as the variables revealed no significant effects, all \( F_s < 1 \). The results of the flavor versus water tests (presented in the two lower panels of Figure 3) show, with one exception (the choice between Fne and water in reexposed subjects), a small preference for the flavor over water. But, as before, the differences were small and not significant. An ANOVA with flavor and group as the variables for the comparison of Fe versus water produced no significant effects, all \( F_s < 1 \); for the comparison of Fne and water, largest \( F(1, 14) = 2.24, p = .15 \). An ANOVA of the preference ratios, .58 (reexposed) and .56 (nonreexposed) for Fe, and .43 (reexposed) and .63 (nonreexposed) for Fne, did not detect any significant main effect or interaction, largest \( F(1, 14) = 1.30, p = .27 \). To facilitate interpretation of these null findings, we computed Bayesian \( t \)-tests (see, e.g., Jones, Dwyer, & Lewis, 2015). JZS-values were \( B_{10} = \)...

![Figure 3](image-url)

**Figure 3.** Experiment 2: group mean consumption on choice tests of water (Wt), an extinguished flavor (Fe), and a nonextinguished flavor (Fne) for subjects given reexposure to sucrose (Reexp) or not (Non Reexp). Vertical bars represent standard errors of the mean.
0.20, 0.20, and 0.62, for Fe versus Fne, Fe versus water, and Fne versus water consumption tests, respectively in the nonreexposed group. For reexposed animals, the equivalent JZS-values were $B_{10} = 0.21, 0.24,$ and $0.25$. Comparing between-groups preference-ratios, JZS-values for Fe versus Fne, Fe versus water, and Fne versus water were $B_{10} = 0.26, 0.26,$ and $0.60,$ respectively. Generally, these scores provide evidence in favor of the null hypothesis that, both when the animals are reexposed to the US and when they are not, there is no effect of the extinction procedure on test consumption.

The results for the nonreexposed subjects confirm those of Experiment 1B and extend their generality, by using different flavor concentrations. They show that, for nonhungry rats, the extinction procedure does not generate a preference for Fne over Fe on the choice test. The new results are for the subjects given reexposure to sucrose prior to the test. They provide no support for the hypothesis that this treatment will allow an effect of extinction to be seen. As was the case for the subjects not given reexposure, there was no reliable difference in any of the comparisons of the responses governed by Fe and Fne. It is worth reporting that we have carried out two further experiments investigating the effects of sucrose reexposure in which we varied the number of reexposure trials, their spacing over days, and the amount of sucrose available on each presentation. In no case did we find evidence that rats consumed significantly more of the Fne than of the Fe.

The results of this experiment do not encourage the view that test performance in our procedure is influenced by changes in adaptation level produced by exposure to sucrose. It is quite possible, however, that adaptation level effects will be evident with other procedures. In particular, Albertella, Harris, and Boakes (2008) demonstrated that these effects show context-dependence—that the expectation of a given level of sweetness is evoked by the context in which sucrose has been presented. They demonstrated this effect in a study in which treatments were given in separate drinking chambers, distinct from the home cages. In our experiment all treatments were given in the home cage, an environment that may be so familiar that it may fail to support the learning responsible for adaptation level effects. However this may be, it should be noted that in Delamater’s (2007) experiments, as in ours, the rats remained in their home cages throughout. The difference in outcome between his experiment and ours is thus not to be explained in terms of a difference in sensitivity to adaptation level effects.

**Experiment 3**

Experiments 1B and 2 have failed to find an effect of the extinction procedure in nonhungry rats, in spite of using the within-subject test procedure employed by Delamater (2007). Experiment 2 further showed that including a phase of postextinction exposure to the sucrose US does not allow an effect to emerge. We conclude that to obtain the effect found by Delamater, that is, a shift in preference between Fne and Fne, it is necessary to insert a phase of US-devaluation before the test. Why this might be so will be taken up in the final Discussion. The purpose of the present experiment was to confirm that, with our procedures, we can replicate the finding of Delamater (see also Harris et al., 2004), showing that devaluing the sucrose US, by pairing it with an injection of LiCl, would result in a preference for Fe over Fne; also that subjects in the control condition (given sucrose and LiCl unpaired) would show a preference for Fne over Fe.

**Method**

The subjects were 16 naïve male Wistar rats (Janvier, France) with a mean body weight of 292 g at the start of the experiment. They were housed in individual home cages in a colony room at the Cartuja Center for Animal Experimentation of the University of Granada, and were maintained as in Experiment 1B. The flavors and sucrose concentrations were the same as described for Experiment 2.

The experimental procedures followed those described for Experiment 2 with the exceptions stated below. After the water-derivation schedule had been established, all subjects received four conditioning trials with each of the flavors, followed by six extinction sessions with Fe. The rats were then assigned to two equal-sized groups for the sucrose revaluation phase, which consisted of three 2-day cycles of training. Rats in the Suc-Li group were given 10-min access to 20 ml of sucrose solution on the first day of the cycle, and to 20 ml of water during the second. Rats in the Suc/Li group had 10-min access to 20 ml of water during the first day and to 20 ml of sucrose during the second. Animals were given an intraperitoneal injection of 0.15 M LiCl at 20% of body weight immediately after consuming the relevant fluid during the first day of the two first cycles. (As sucrose consumption was nearly absent in the Suc-Li group on the first day of the final cycle, no further injections were given.) Thus the sucrose solution was devalued in Group Suc-Li but was unpaired with illness in Group Suc/Li. All subjects then received Fe versus Fne choice tests. Following Delamater’s procedure, the test was given twice, on consecutive days. Finally, they received choice tests of Fe versus water and of Fne versus water, as in previous experiments.

**Results and Discussion**

Overall means for consumption of the flavor-sucrose compound on the conditioning trials (i.e., pooling scores for the groups that were not treated differently at this stage) were 4.9, 5.7, 5.6, and 5.6 g, for Fe, and 5.7, 5.2, 5.5, and 5.7 g, for Fne. As in the previous experiments, consumption was lower on the early trials (in particular on the first Fe trial in this case) but steady thereafter. An ANOVA with flavor and trial as the variables revealed no significant main effect of flavor, $F < 1$. Both the effect of trial, $F(3, 45) = 5.16, p = .01, \eta^2_g = 0.26$, and the interaction, $F(3, 45) = 14.64, p < .01, \eta^2_g = 0.49$ were significant. Holm-Bonferroni tests showed that consumption on the first encounter with the flavors (first Fe trial) was lower than on the rest (largest $p < .01$); the consumption on the second Fne trial also differed from the first and the last one (largest $p < .01$); the rest of the trials did not differ among themselves (smallest $p = .06$; corrected $p$ value $= .01$).

Overall means for consumption of the Fe flavor on the six trials of the extinction phase were 12.1, 12.2, 11.6, 11.9, 11.5, and 11.9 g. Thus, as in previous experiments, there was no sign of an extinction effect by this measure. An ANOVA confirmed that the effect of trial was not significant, $F < 1$.

Consumption of sucrose during the devaluation phase decreased in the Suc-Li group (means 16.1, 3.4, and 0.6 g), but not in the Suc/Li group, which showed a modest increase in consumption (13.7, 14.7, and 15.9 g). An ANOVA with group and trial as
factors confirmed these impressions, yielding main effects of both group, \(F(1, 14) = 152.83, p < .01, \eta_p^2 = .92\), and trial, \(F(2, 28) = 93.29, p < .01, \eta_p^2 = .87\), as well as a significant Group \(\times\) Trial interaction, \(F(2, 28) = 152.61, p < .01, \eta_p^2 = .91\). Post hoc Holm-Bonferroni testing revealed that consumption decreased over the three trials in the Suc-Li group (largest \(p < .01\)). Consumption in the Suc/Li group increased significantly from the first to the third trial (\(p < .01\)).

The data of most interest were those from the Fe versus Fne tests. Figure 4 (upper panel) presents group daily means over the two days of the test. They show, replicating the result of Delamater (2007), that Fne was preferred over Fe in subjects in the group (Suc/Li) given unpaired presentations of sucrose and LiCl. Although the size of the effect was somewhat less, the reverse was true for subjects for whom sucrose had been devalued (the Suc-Li group); that is, Fe was preferred over Fne, an effect shown by Delamater (2007) and by Harris et al. (2004). An ANOVA with group and flavor as the variables found no significant main effect of flavor (\(F < 1\)), but there was a significant main effect of group, \(F(1, 14) = 5.70, p = .03, \eta_p^2 = .29\), and, importantly, a significant interaction between the variables, \(F(1, 14) = 10.36, p < .01, \eta_p^2 = .42\). Holm-Bonferroni testing confirmed that Fne was consumed significantly more than Fe in Group Suc/Li (\(p = .01\)), but that the difference between the flavors fell short of significance in the Suc-Li group (\(p = .11\)). The between-groups comparisons, however, were both significant: Fne was consumed more in Group Suc/Li than in Group Suc-Li, \(p = .03\), whereas, Fe was consumed more in Group Suc-Li than in Group Suc/Li, \(p < .01\).

The results from the tests of the flavors against water (shown in the two lower panels of Figure 4) confirm this picture, with subjects in the Suc/Li group showing a more positive response to Fne than to Fe whereas those in the Suc-Li group showed, at least by some measures, a reversed pattern. As the lower panel of Figure 4 shows, neither group showed a preference when given the choice between Fe and water; an ANOVA, with group and fluid (flavor or water) as the variables, showed no significant effects or interaction, largest \(F(1, 14) = 2.52, p = .13\). However, when given the choice between Fne and water (central panel of Figure 4), the Suc/Li group showed a small preference for the flavor, whereas the Suc-Li group showed a strong preference for water. An ANOVA yielded no significant main effects, largest \(F(1, 14) = 2.19, p = .16\), but there was a significant Group \(\times\) Fluid interaction, \(F(1, 14) = 12.53, p < .01, \eta_p^2 = .47\). Post hoc analysis showed that Group Suc-Li consumed less of the Fne flavor than water (\(p < .01\)), although the reversed difference for Group Suc/Li was not significant (\(p = .14\)). The between-groups comparisons were both significant; Group Suc-Li consumed less of Fne (\(p = .04\)) and more water (\(p < .01\)) than Suc/Li. The results of these tests may be conveniently summarized by the preference ratios (flavor consumption/total consumption) shown in Figure 5. The Suc/Li group shows a strong preference for Fne but not for Fe; the response of the Suc-Li group to Fe is similar to that of the other group, but preference for Fne is much lower. An ANOVA with group and flavor as the variables produced no significant main effects, largest \(F(1, 14) = 3.01, p = .10\), but the interaction between the variables was significant, \(F(1, 14) = 6.12, p = .03, \eta_p^2 = .30\). Post hoc Holm-Bonferroni tests revealed no difference between the groups in preference ratios for Fe (\(p = .66\)), but a difference with respect to Fne (\(p = .01\)). The two preference ratios were significantly different in the Suc/Li group (\(p = .04\)), but not in the Suc-Li group (\(p = .23\)). We again computed Bayesian t-tests in order to facilitate the analysis. For Group Suc-Li, JZS-values for paired samples t-tests regarding Fe versus Fne, Fe versus water, and Fne versus water were \(B_{10} = 0.51, 0.19\), and 2.43, respectively. For Group Suc/Li the values were \(B_{10} = 5.44, 0.27\), and 1.72, respectively. Regarding between-groups comparisons, JZS-values for Fne versus Fne, Fne versus water, and Fne versus water preference ratios were \(B_{10} = 10.07, 0.46\), and 5.07, respectively. Therefore, and in contrast with the results of Experiments 1B and 2 that found support for the null hypothesis, here we found substantial evidence in favor of the alternative hypothesis when using the within-subject Fe versus Fne test in Group Suc/Li.

\[ \text{Figure 4. Experiment 3: group mean consumption on choice tests of water (Wt), an extinguished flavor (Fe), and a nonextinguished flavor (Fne)} \]

During the US devaluation phase, rats in the Suc-Li group received paired presentations of sucrose (Suc) and lithium chloride (Li); rats in the Suc/Li group received unpaired presentations. Vertical bars represent standard errors of the mean.
errors of the mean. A group received unpaired presentations. Vertical bars represent standard presentations of sucrose (Suc) and lithium chloride (Li); rats in the Suc/Li flavor/total consumption) for a nonextinguished flavor (Fne) and an extinguished flavor (Fe) when extinction is followed by what is intended to be a control procedure (unpaired presentation of sucrose and LiCl) for the devaluation treatment given to the Suc-Li group. What we can now conclude, however, is that this control treatment is not a neutral procedure; the results of Experiments 1B and 2 demonstrate that when it is omitted there is no extinction effect, that Fe and Fne remain equally preferred. Thus, just as the preference shown in the Suc-Li group depends on the aversion established by pairing sucrose with LiCl, so the shift in preference seen in the Suc/Li group seems to depend on what has been learned as a result of the unpaired treatment.

Information on this matter comes from a study by González, García-Burgos, de Brugada, and Gil (2010). In their Experiment 2, they gave nonhungry rats pairings of sucrose and a nonpreferred flavor (Kool-Aid unsweetened cherry-flavored solution). When tested against water, the preference ratio for the flavor remained low (around .30). In order to investigate whether an association between the taste of the Kool-Aid and that of sucrose had been formed during conditioning, a US-devaluation procedure was used. It was found for subjects given pairing of sucrose and LiCl that the preference ratio decreased significantly (to a mean of about .15), suggesting that the flavor was indeed associated with the now-devalued sucrose. Unexpectedly, however, preference for the flavor increased dramatically (to a mean of about .70) in the control subjects given unpaired presentation of sucrose and LiCl. González et al. concluded that this treatment must have increased the value of the sucrose, perhaps because the unpaired arrangement, with the flavor signaling the absence of illness, allows for inhibitory learning (see, e.g., Baker, 1977).

This interpretation allows a simple summary of the findings of the experiments reported here. When rats are not hungry, nonreinforced presentations of a conditioned flavor have no effect on the preference for that flavor, even when tested with a sensitive procedure that provides a choice between Fe and Fne (Experiments 1B and 2). An effect of the extinction procedure becomes evident, however, when the sucrose US is revalued. When it is devalued, an extinguished flavor is preferred over a nonextinguished flavor; when its value is enhanced, the nonextinguished is preferred over the extinguished. We now discuss possible interpretations of these findings.

General Discussion

When the rats are hungry, a preference established by pairing a flavor with sucrose shows the extinction effect to be expected of a CR, that is, repeated presentation of the flavor in the absence of sucrose (the US) leads to a reduction in the preference. This effect was demonstrated in Experiment 1A, using the within-subject design advocated by Delamater (2007), in which two flavors undergo initial conditioning and the test phase involves a choice between the extinguished flavor (Fe) and a flavor that has not undergone extinction (Fne). Harris et al. (2004; see also Harris et al., 2000) have argued that when the subjects are hungry, the preference depends on an association between the flavor and the nutritional properties of sucrose, and that this association, like those responsible for the CRs in standard Pavlovian conditioning procedures, is sensitive to effects of extinction.

When the rats are not hungry, a conditioned preference resists the effects of extinction, and persists in spite of extensive experience of the flavor in the absence of the US (e.g., Albertella & Boakes, 2006; Harris et al., 2004). Confirmation of this result was provided by the present Experiments 1B and 2. Delamater (2007) has suggested that the failure to find an effect of extinction might be a consequence of the insensitivity of the test procedure used in the earlier experiments. Our results show that this is not the case. Our experiments failed to find an effect of extinction in nonhungry rats despite using the within-subject procedure and a choice test between Fe and Fne. This is not to say, however, that the extinction procedure is without effect in nonhungry animals, as is shown by use of US revaluation procedures. It is known that devaluing the sucrose US that has been used in conditioning a flavor preference (by pairing the sucrose with LiCl) will normally result in a reduced preference. This reduced preference is not obtained, however, when the devaluation has been preceded by the extinction procedure (Harris et al., 2004; Delamater, 2007). This result was confirmed by the present Experiment 3, which also went on to show that a procedure capable of enhancing the value of sucrose had the opposite result. It was shown that unpaired presentations of sucrose and LiCl enhanced the preference for Fne over Fe.

The original observation of the persistence of a preference in nonhungry animals prompted the conclusion that this preference was a product of a form of learning that (unlike the flavor-nutrient association though to operate in hungry animals) was not susceptible to the effects of extinction. Harris et al. (2004) suggested, initially, that preference in sated rats depends on an association between the flavor and the sweet taste of sucrose, and adopted the
assumption that this association does not extinguish. A less-arbitrary version of the same notion emerges from Pearce’s (2002) configural theory of learning. According to this theory the simultaneous presentation of a flavor (F) and sucrose (S) will establish a configural unit (FS), activated initially by the presentation of both substances, but which subsequently can be activated by either of the cues presented alone. Connections between input units and the configural unit are assumed to be bidirectional so that presentation of F will be able to activate S by way of FS (and vice versa). In this way, presentation of F alone is able to activate the representation of S and to evoke responses normally produced by S. The theory holds that once an input becomes connected to a configural unit, the strength of that connection will not be affected by subsequent experience. Thus the tendency to consume the flavor will not be reduced by the extinction procedure.

The sucrose-devaluation results challenge both of these accounts. Harris et al. (2004) assumed that reduction in the strength of a sucrose-based preference after pairing sucrose with LiCl had its effects by way of the associative chain: flavor → sweet taste → nausea. Thus, the fact that extinction protected a preference from the effect of this devaluation seems to imply that the first link in the chain must have been weakened—that the flavor-taste association is, in fact, susceptible to extinction. Similarly, in Pearce’s (2002) version, devaluing sucrose will change the properties of S, and the consequent reduction in preference for F should occur both in subjects that have previously undergone extinction and, as extinction does not weaken the ability of F to contact S, in subjects that have not. In general, the fact that extinction does influence sensitivity to sucrose devaluation means that either our account of the source of the persistence of flavor preference is wrong, or that our understanding of the process of devaluation is wrong.

Harris et al. (2004) took up the first of these possibilities. They suggested that conditioning will establish not just a flavor-taste association, but also an association between the flavor and the hedonic reaction evoked by ingesting sucrose. It is this latter association that is assumed to be resistant to extinction. But this interpretation, too, has proved unsatisfactory. Dwyer et al. (2009) have examined the effect of extinction on conditioned flavor preference using a measure of the palatability of the stimulus, and thus of the hedonic reaction to it. Specifically, the pattern of licking that rats show when consuming fluids that differ in palatability (e.g., different concentrations of sucrose) is sensitive to these differences. Dwyer et al. demonstrated that after flavor-preference conditioning the conditioned flavor initially evoked a licking pattern indicative of high palatability. Although consumption on a choice test remained high over repeated nonreinforced presentations of the flavor, the pattern of licking associated with high palatability declined. Thus, the hedonic response acquired by the flavor appears to extinguish, and cannot be responsible for the persistence seen in the consumption measure.

Given this last result, it is worth considering the alternative hypothesis, which is that it may be a mistake to assume that sucrose devaluation has its effect by way of an association between the taste of the sucrose and the state induced by the revaluation treatment. Perhaps revaluing sucrose by pairing it with an aversive event (or the omission of an aversive event) is mediated by associations involving the hedonic reaction to sucrose rather than, as previously assumed, its sensory properties. If so, then the extinction procedure, which reduces the ability of a conditioned flavor to evoke this reaction, will reduce the sensitivity of the response to the effects of sucrose revaluation, the result obtained in our Experiment 3 (and by Harris et al., 2004, and Delamater, 2007). In this way, the effects of revaluation could be accommodated while maintaining the position that learning involving the flavor and the taste of sucrose is impervious to the effects of extinction.

We acknowledge that this hypothesis seems to run counter to what has been asserted on the basis of studies of US revaluation in evaluative conditioning (e.g., Baeyens, Eelen, Van den Bergh, & Crombez, 1992; Walther, Gawronski, Blank, & Langer, 2009). In both of these studies, the fact that acquired preferences in evaluative conditioning are sensitive to revaluation of the US was taken as evidence that the conditioning procedure establishes a stimulus-stimulus (S-S) link rather than a stimulus-response (S-R) link. But our hypothesis does not contradict this view. It allows that conditioning will establish an S-S (flavor-taste) association that is resistant to the effects of extinction; but it suggests that, in addition, the second of these stimuli will evoke a hedonic reaction that can become associated with the first. We assume that, in the US-devaluation procedure, it is predominantly the hedonic response that becomes associated with nausea, and that the conditioned flavor becomes less preferred because it accesses this state by way of the chain: flavor → hedonic response → nausea. Finally, we assume that the S-R (flavor-hedonic response) link is subject to extinction, so that the effects of this procedure will be evident only when the experiment incorporates a US-revaluation procedure.

This account is speculative and needs to be more tightly specified if it is to deal with revaluation effects more generally. We should, however, address one problem that has direct relevance to the present data. It is that we have proposed that pairing a flavor with sucrose will establish both an association between the flavor and the taste of sucrose (which is not affected by extinction) and an association between the flavor and the reaction to sucrose (which is). It might be supposed that both forms of learning would contribute to performance on a test of preference, so that extinction, by removing the component based on the hedonic response, would be expected to at least reduce, even if it would not eliminate, the preference. The experimental results show no such effect, suggesting, rather surprisingly, that what we have called the hedonic response does not contribute to performance on the preference test. But although this seems counterintuitive, we may note that it is just the experimental result reported by Dwyer et al. (2009) when they showed that the licking pattern indicative of palatability declined over the course of extinction testing, whereas the preference measured by consumption did not.

References


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