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US-preexposure effects in flavor-preference and flavor-aversion learning with nonnutritive USs

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ABSTRACT

In two experiments, rats received exposure to either a saccharin or quinine solution followed by conditioning with a solution of almond as the conditioned stimulus (CS) and either saccharin or quinine as the unconditioned stimulus (US). In Experiment 1, rats received preexposure and conditioning using saccharin as the US; in Experiment 2 quinine was the US. In both cases the magnitude of the conditioning effect (an enhanced preference for the CS in Experiment 1; a reduced preference in Experiment 2) was reduced by preexposure to the US. The results provided confirmation of the occurrence of the US-preexposure effect in the flavor-preference procedure and demonstrate that the effect can be obtained with nonnutritive USs that lack significant post-oral consequences. The implications of these results for theories of the US-preexposure effect are discussed.

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1. Introduction

Prior exposure to the event to be used as the unconditioned stimulus (the US) in classical conditioning can retard subsequent learning (the US-preexposure effect). The effect is particularly well established for the case of nausea-induced flavor-aversion learning (Hall, 2009; Riley and Simpson, 2001). Rats given a series of injections of a nausea-inducing substance such as lithium chloride (LiCl) show retarded acquisition of the aversion to a novel flavor when this flavor is paired with LiCl in a conditioning procedure (and the same is true of a range of other substances normally capable of supporting aversion learning; Riley and Simpson, 2001). The effect is not confined to aversive learning—for example, Gil et al. (2011) have shown that preexposure to sucrose will reduce the effectiveness of sucrose to function as a reinforcer in establishing a preference for a previously neutral flavor.

The US-preexposure effect is of special interest, as it seems to imply the existence of a learning process that modifies the effectiveness of this stimulus and thus modulates the operation of standard associative mechanisms. Randich and Lolordo (1979), in their review of the effect, acknowledged this possibility by including habituation of the US as a possible mechanism. They also pointed out that a standard associative process (blocking) could be responsible—that cues present during preexposure (such as those supplied by the experimental context) could become associated with the US and that their presence during conditioning could block the acquisition of associative strength by the event designated by the experimenter as the conditioned stimulus (the CS). There is evidence that this latter mechanism operates in the flavor aversion learning procedure (although the cues critical for blocking are not those supplied by the environmental context, but those associated with the process of giving an injection; De Brugada et al., 2004). There is little evidence to support the notion that habituation to the US plays a role (see De Brugada et al., 2005).

In order to investigate the US-preexposure effect in a procedure in which initial presentations of the US would not be accompanied by salient cues, Gil et al. (2011) turned to flavor-preference conditioning. In this procedure pairing of a neutral flavor with a valued substance such as sucrose, will establish a preference for that flavor, an outcome that we will assume to depend on the formation of an association between the flavor as a CS and some aspect of the sucrose US. Preexposure to the US can easily be arranged simply by giving access to a sucrose solution; rats will consume

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this readily, thus eliminating the injection-related cues involved in aversion conditioning. Using this procedure, Gil et al. showed that the size of the conditioned preference was reduced by preexposure to sucrose (see also Harris et al., 2004) They also showed that this US-preexposure effect was fully evident when the environmental context used for conditioning was different from that used for preexposure, thus ruling out the possibility that this effect might depend on blocking by associative strength acquired by contextual cues during the preexposure phase. They tentatively concluded that the effect obtained in their experiments was the consequence of a habituation process that reduced the effectiveness of sucrose as a reinforcer.

Although the results reported by Gil et al. (2011) are not to be explained in terms of blocking by contextual (or injection-related) cues, another possible source of blocking, when sucrose is the US needs to be considered. This arises from the fact that sucrose has both sensory (a sweet taste) and post-consumption (nutritive) properties, and that both of these may play a role in flavor-preference learning (e.g., Sclafani and Ackroff, 1994; Sclafani et al., 1993). In terms of an associative analysis, pairing a flavor with sucrose will establish both flavor-taste and flavor-nutrient associations (e.g., Harris et al., 2000) and normally both will contribute to the preference seen on test. The situation may be different, however, if preexposure to sucrose is given. Prior exposure would allow the subject to experience the novel taste of sucrose followed by its nutritional effects and thus a taste-nutrient association could be formed in rats given US preexposure. Such an association could block the formation of a flavor-nutrient association during conditioning trials. The flavor-sweet taste association would still be formed, but the preexisting association between taste and nutrition would prevent the flavor-nutrient association from acquiring strength. The reduced preference in preexposed subjects would thus reflect that it was generated only by the flavor-taste association, with no contribution from flavor-nutrient learning.

The experiments to be reported here are intended to seek evidence for a US-preexposure effect using as the US a substance without major post-consumption consequences. With such a US, any change in the preference for a flavor paired with it will be the consequence solely of the formation of an association between the flavor and the taste of the US. In these circumstances, a US-preexposure effect could not be attributed to the blocking process just described. In Experiment 1 we used saccharin as the US; in Experiment 2 we used quinine (for which conditioning is evidenced by a reduction in the preference for the flavor associated with the US). In both experiments we used a solution of almond flavoring as the CS. This is likely to be detected primarily as an odor, but since it may also have some taste- (particularly at higher concentrations) we will refer to it as a flavor (acknowledging the possible presence of both taste and odor). Saccharin and quinine are taken to be tastes.

2. Experiment 1

In this experiment we gave rats exposure to presentations of a solution of saccharin prior to conditioning trials in which saccharin was presented in compound with almond. Preference for this flavor on test was compared with that shown by control subjects given conditioning but no preexposure. A lesser preference in the subjects given preexposure would be evidence of a US-preexposure effect. As the ability of saccharin to support the acquisition of a flavor preference depends critically on the exact conditions of training (e.g., Fanselow and Birk, 1982; Holman, 1975), we conducted a preliminary study, in which no US preexposure was given, in order to confirm that a conditioning effect could be obtained with our experimental parameters and procedures. In this we compared the preference generated by our pairing procedure, with that shown by rats given equivalent exposure to saccharin and almond, but on separate occasions. This study is reported as Experiment 1a; the effect of US preexposure as Experiment 1b. The experimental designs are summarized in Table 1.

Table 1
Experimental designs.

Experiment 1a		
Group	Conditioning	Test
SIM	4 A + Sacc	A vs. W
UNP	4 A/Sacc	

Experiment 1b			
Group	Preexposure	Conditioning	Test
PRE	8 Sacc	4 A + Sacc	A vs. W
CON	8 Water		

Experiment 2a		
Group	Conditioning	Test
SIM	4 A + Quin	A vs. W
UNP	4 A/Quin	

Experiment 2b			
Group	Preexposure	Conditioning	Test
PRE	8 Quin	4 A + Quin	A vs. W
CON	8 Water		

Note: Sacc: sodium saccharin solution; Quin: quinine sulphate solution; A: almond solution; W: water; SIM: paired presentations; UNP: unpaired presentations; PRE: preexposed: CON: nonpreexposed control.

2.1. Method

2.1.1. Subjects and apparatus

The subjects in Experiment 1a were 16 male hooded Lister rats (from Charles River Laboratories) with a mean free-feeding weight of 482 g (range: 473–492 g). They had previously served as subjects in an experiment using the conditioned suppression paradigm but were naïve to all aspects of the current stimuli and procedures. Experiment 1b used 16 male naïve Wistar rats (obtained from the University of Seville Laboratories) with a mean free-feeding weight of 285 g (range: 256–312 g). The rats were housed individually in home cages measuring $35 \times 22 \times 18$ cm, and made of translucent white plastic with wood shavings as bedding. They were maintained on a 12-h light/12-h dark cycle (lights on at 8:00 a.m.). All experimental procedures were conducted in the home cages. The stimuli used were, as the CS, a 1% (v/v) almond solution (almond flavoring supplied by Supercook, Leeds, UK), a 4 g/l sodium saccharin solution (US), and a compound of almond and saccharin made up so as to preserve these concentrations. All solutions were made with tap water and given to the animals in inverted 50-ml centrifuge tubes equipped with stainless steel, ball-bearing-tipped spouts in the home cages. Fluid consumption was measured by weighing the tubes before and after fluid presentations.

2.1.2. Procedure

To initiate a schedule of water deprivation, the standard water bottles were removed overnight; over the next two days, access to water was restricted to two 30-min sessions per day (starting at 11 a.m. and 4:30 p.m.). Fluids continued to be given at these times throughout the experiment. For Experiment 1a, the rats were assigned to two equal-sized groups. Over the next four days, the simultaneous group (SIM in the table) received a daily presentation of 10 ml of the compound solution in one of the

Table 2
Test consumption (ml).

Experiment 1a						
Group	Almond			Water		
SIM	11.65 (0.78)			2.43 (0.69)		
UNP	6.27 (1.31)			8.36 (1.90)		
Experiment 1b						
Group	Almond			Water		
	Trial 1	Trial 2	Mean	Trial 1	Trial 2	Mean
PRE	6.76 (0.89)	5.22 (1.00)	5.99 (0.83)	4.01 (0.57)	6.84 (0.74)	5.43 (0.44)
CON	7.80 (0.73)	7.87 (1.02)	7.83 (0.69)	3.74 (0.80)	2.96 (0.79)	3.35 (0.67)
Experiment 2a						
Group	Almond			Water		
SIM	7.07 (1.56)			6.99 (1.60)		
UNP	12.08 (1.37)			2.83 (1.23)		
Experiment 2b						
Group	Almond			Water		
	Trial 1	Trial 2	Mean	Trial 1	Trial 2	Mean
PRE	8.51 (1.72)	7.88 (1.31)	8.19 (0.95)	6.10 (1.34)	5.19 (1.31)	5.65 (0.80)
CON	4.15 (1.81)	5.48 (1.83)	4.81 (1.20)	13.11 (0.90)	7.05 (1.28)	10.08 (0.80)

Note: SIM: paired presentations; UNP: unpaired presentations; PRE: preexposed: CON: nonpreexposed control. Scores are group means (SEMs in parentheses).

drinking sessions (10 ml of water being available on the other session). The other, unpaired, group (UNP in the table) received four presentations of almond and four of the saccharin solution (10 ml of each per session). The presentation of the stimuli over conditioning sessions was counterbalanced, with half of the subjects in each group receiving the sequence X–Y–Y–X, and the other half of the animals receiving Y–X–X–Y; where X means almond + saccharin (group SIM) or saccharin (group UNP) and Y means water (group SIM) or almond (group UNP). Presentations were counterbalanced over the morning and afternoon sessions. After this cycle was completed, all subjects received a 30-min preference test in which two bottles were presented concurrently, one containing 30 ml of almond and one 30 ml of water. The left-right position of the bottles was counterbalanced within each group, and the position for each rat was swapped after 15 min of the test. The test was carried out during the morning session.

The subjects for Experiment 1b were assigned to two equal-sized groups, one given preexposure to the US (PRE in the table), and one not (CON). After the schedule of water deprivation had been established, the next eight days constituted the preexposure phase. One each of these days, animals in the preexposed group received 15 ml of saccharin solution in the morning sessions whereas animals in the control group received the same amount of water. Ad lib water was presented in the afternoon sessions. The next four days constituted the conditioning phase in which all subjects were given access to 15 ml of the almond + saccharin compound for 30 min (water again being presented in the afternoon). Preference tests were given in the morning sessions of the next two days, the procedure being as described for Experiment 1a. All the experimental procedures are in accord with the ethical guidelines of the University of Granada.

2.1.3. Results and discussion

2.1.3.1. Experiment 1a. Neophobia was seen during the conditioning phase in that the rats showed an initial reluctance to consume the saccharin solution. On Trial 1, the SIM group consumed a mean of 1.41 ml of the compound solution and the UNP group a mean of 1.76 ml of the saccharin solution. On Trial 2, these scores rose to 7.02 ml and 7.23 ml, respectively. On the final two trials, all subjects drank all the fluid that was available.

During the test session, the SIM group drank the almond solution readily and rather little water; the UNP group drank approximately the same total amount, and approximately equal amounts of both. Mean consumption scores for this session are shown in Table 2. These consumption scores were converted to preference ratios (volume of almond/volume of almond + volume of water) for statistical analysis. The ratio for the SIM group was .83; that for the UNP group was .45. An analysis of variance (ANOVA) of the preference ratio data showed there to be a significant difference between the groups, $F(1,14) = 11.25$. (For this and all subsequent analyses a significance level of $p < .05$ was adopted.) These results confirm that the procedure used for group SIM was effective in establishing a flavor preference using saccharin as the US.

2.1.3.2. Experiment 1b. The results of the preexposure phase (mean amounts consumed in the morning sessions) are shown in the top panel of Fig. 1. Group CON consumed water steadily throughout this phase. Group PRE showed an initial reluctance to drink the saccharin solution, but after two sessions they drank almost all the 15 ml available on each trial. An ANOVA showed there to be a significant change over trials, $F(7,49) = 64.33$; simple contrasts showed that Trials 1 and 2 differed significantly from the last trial, Fs (1,7) = 85.83, and 11.99, respectively.

The lower left panel of Fig. 1 shows the mean consumption during the conditioning phase. Control subjects, experiencing a saccharin solution for the first time, drank rather little on Trial 1, but were consuming the compound as readily as the PRE group by the last trial. An ANOVA with group and trial as the variables revealed significant effects of group, $F(1,14) = 43.97$, of trial, $F(3,42) = 98.23$, and a significant interaction, $F(3,42) = 46.96$. Analysis of simple main effects showed that the groups differed on Trials 1 and 2, $F(1,14) = 73.30$ and 8.20, respectively, but not on subsequent trials (Fs < 1).

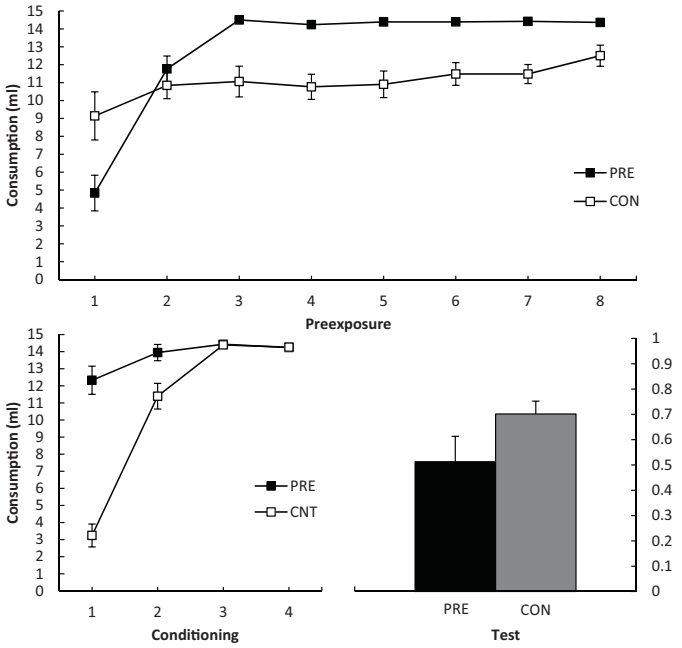

Fig. 1. Experiment 1b. Top panel: mean consumption of water (CON group) or of saccharin (PRE group) during preexposure. Lower left panel: mean consumption of saccharin + almond compound during conditioning. Lower right panel: group mean preference ratios (consumption of almond/total consumption); average of two test trials.

Group means for consumption of almond and water, for both sessions of the test are shown in Table 2. In general, the two groups drank roughly the same total amount, but whereas the CON group drank more almond than water, the PRE group showed no such preference. Group mean preference ratios averaged for the two sessions of the test phase are shown in the lower right panel of Fig. 1. It is clear that a preference for the almond solution was present only in group CON. An ANOVA conducted on the preference-ratio data with group and trial as the variables showed there to be a significant difference between the groups, $F(1,14) = 6.61$; there was no difference between trials, $F(1,14) = 1.90$, and no interaction between the variables, $F(1,14) = 3.74$. It may be noted that the lesser preference in the PRE group was observed in spite of the fact that they consumed significantly more of the compound than the CON group during the conditioning phase.

These results demonstrate that preexposure to saccharin prior to conditioning with saccharin as the US results in a reduced CR; that is, the US-preexposure effect was found with a preferred yet nonnutritive substance as the US. This example of the effect is difficult to explain in terms of the blocking-by-taste account previously suggested as an explanation for the effect obtained with sucrose. Since saccharin lacks any post-oral nutritive consequences, there is no possibility, with this US, of the formation of an association between its taste and nutritional effects. Although this form of learning could produce blocking for the sucrose case, it cannot be the whole story as an explanation of the US-preexposure effect. A different explanation for the saccharin case is needed—perhaps the habituation process evident in the loss of neophobia also produces a reduction in the effectiveness in the US as a reinforcer for conditioning.

Before turning to a non-associative explanation, however, an alternative version of the blocking hypothesis must be considered. Saccharin is a complex substance, with both sweet and bitter-tasting components (Dess, 1993). And if it is supposed that it is the sweet-tasting component of the saccharin that is the effective US supporting conditioning, then it may still be possible to apply the blocking account as an explanation of the US-preexposure effect found with this substance. Specifically, it is possible that preexposure to saccharin allows the establishment of an association between its bitter and sweet tastes. This association could then serve to block the acquisition of a subsequent flavor-taste association between the CS and the sweet taste of saccharin that is responsible for the conditioned flavor preference. This possibility cannot be ruled out on the basis of the present data; accordingly, in Experiment 2, we turned to the use of a different nonnutritive substance as the US, one for which this version of the blocking account does not apply.

3. Experiment 2

Quinine can serve as a reinforcer for flavor preference (aversion) learning; rats allowed to consume a compound of a novel flavor (or odor) and a quinine solution will show a reduced preference for the flavor (e.g., Fanselow and Birk, 1982; Harris and Westbrook, 1998). This instance of flavor-taste learning is taken to depend on the bitter taste of quinine. Quinine is the prototype of the bitter taste, having no other reported sensory attributes, and this simplicity precludes the possibility of associations among its components (such as we have postulated for the bitter-sweet compound of saccharin). It is a possible complication that quinine can have post-consumption effects, but these should not be evident at the doses used in our experiment. Specifically, although ingestion of quinine by rodents has been shown to result in a depression of food consumption and a decline in body weight (e.g., Colley et al., 1989; Kratz and Levitsky, 1978) these effects are observed only with prolonged exposure to relatively high doses. Colley et al. (1989) reported no effect in rats given quinine hydrochloride at 60 mg per day for 13 weeks, a dose much higher than that used in the present experiments (rats given preexposure in our experiment received approximately 1 mg per day for 8 days).

The purpose of the present experiment was to look at the effects of preexposure to quinine on the acquisition of an aversion with quinine as the US. As with the saccharin US of Experiment 1, conditioning may be assumed to depend on the formation of a flavor-taste association, ruling out an explanation of the US-preexposure effect in terms of blocking of learning about post-consumption consequences. And the use of a US with a simple bitter taste rules out an explanation in terms of associations formed among components of the US, as might apply for saccharin. This formed Experiment 2b. As in Experiment 1, we performed a preliminary study (Experiment 2a) to confirm that our conditioning procedure would be effective in establishing an aversion to almond.

3.1. Method

3.1.1. Subjects and apparatus

The subjects in Experiment 2a were 16 naïve Wistar rats (obtained from the University of Seville Laboratories) with a mean free-feeding weight of 287 g (range: 265–332 g). A further 16 were used in Experiment 2B. These had been used in a previous study that involved exposure to a strawberry odor, but they were naïve with respect to the flavors and procedures used in this experiment. They had a mean weight of 384 g (348–436 g) at the start of the experiment. The solutions used consisted of quinine (0.1 g/l of quinine sulphate), almond (a 4% solution of almond flavoring), and a compound of quinine and almond, made up so as to preserve these concentrations. The concentration of the almond solution was increased in this experiment to match that used by Fanselow and Birk (1982), who successfully obtained conditioning with quinine as the US using this concentration.

3.1.2. Procedure

After the schedule of water deprivation had been established, the rats in Experiment 2a were divided into two groups of eight. Group SIM received presentations of the compound solution; group UNP received separate presentation of almond and of quinine (Table 1). The schedule of training was the same as that used in Experiment 1a. All subjects were then given a two-bottle preference test for 30-min, the procedure being the same as that used in Experiment 1a.

For Experiment 2b, the rats were assigned to one of two equal-sized groups (Table 2), given US preexposure (group PRE) or no preexposure (group CON) prior to conditioning and the preference tests. The treatment given to these subjects matched that given to the equivalent groups of Experiment 1b, except that the quinine solution was used as the US and the 4% almond solution was used as CS. Since rats consume a quinine solution less readily than saccharin, the volume of fluid available on each trial during preexposure was reduced to 10 ml in this experiment. Other procedural details not specified here were identical to those described for the previous experiments.

3.1.3. Results and discussion

3.1.3.1. Experiment 2a. The almond solution evoked some neophobia. When it was first presented the UNP group drank a mean 5.6 ml, but thereafter they consumed almost all that was made available. Neophobia to the quinine solution was more profound. Over the first three trials of training, neither the UNP nor the SIM group drank more than a mean 2.9 ml of the solutions containing quinine. On the fourth trial, however, both groups drank a mean of 6.8 ml (of the compound for group SIM; of quinine alone for group UNP). This training resulted in a difference between the groups in the preference test. As Table 2 shows, the groups drank the same total amount, but the control subjects drank more almond than water, yielding a mean preference ratio of .81 for the UNP group. This preference was abolished in the SIM group that drank almond and water equally, producing a mean ratio of .51. (Data for one subject from group PRE were not available for analysis, and the mean for this group is based on the remaining seven subjects.) These scores differed significantly, $F(1,13) = 5.77$.

Although these results are consistent with the notion that pairing almond with quinine established a conditioned aversion in the SIM group (sufficient to negate the preference shown by the UNP group), another possibility must be acknowledged. Although subjects in the SIM group experienced almond in compound with quinine during training, they experienced almond alone for the first time on the preference test. Their lower consumption of almond on the test might thus reflect the occurrence of a neophobic response (that would have been habituated in the UNP group, which experienced almond alone during training). We will consider this possibility further when the results of Experiment 2b have been described.

3.1.3.2. Experiment 2b. The upper panel of Fig. 2 shows group means for consumption during the preexposure phase. Data from Trial 1 for the control group were lost, but consumption of water remained steady and high during the rest of the phase. The PRE group showed an initial reluctance to drink quinine, but consumption increased over successive presentations. An ANOVA showed there to be a significant change over trials, $F(7,49) = 10.80$; simple contrasts showed that Trials 1–3 differed significantly from the last trial, $Fs(1,7) = 40.95$, 12.65, and 7.26, respectively.

Consumption over the course of the conditioning phase is shown in the lower left panel of Fig. 2. Control subjects that experienced a quinine solution for the first time in this phase, drank less than the PRE group on Trials 1 and 2, but were consuming the compound as readily as the PRE group by the last two trials. An ANOVA

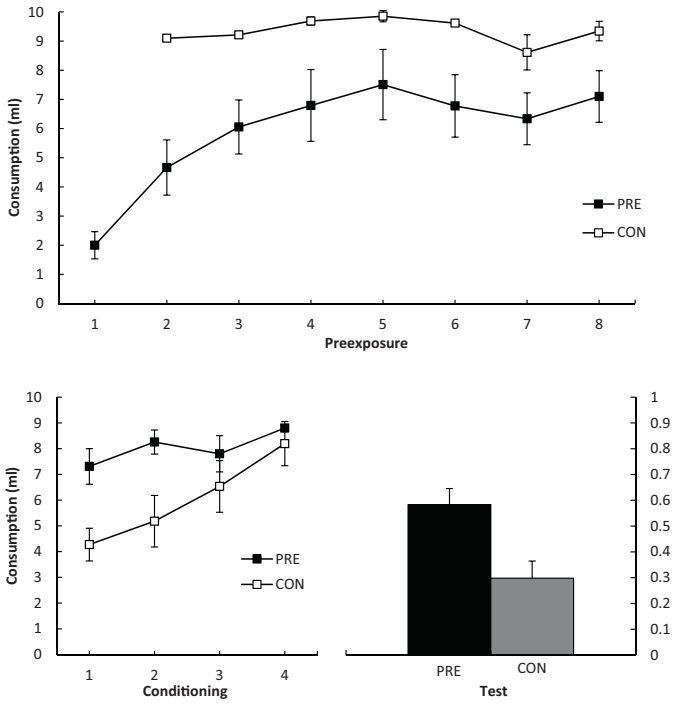

Fig. 2. Experiment 2b. Top panel: mean consumption of water (CON group) or of quinine (PRE group) during preexposure. Note that data for trial 1 of group CON were lost. Lower left panel: mean consumption of quinine + almond compound during conditioning. Lower right panel: group mean preference ratios (consumption of almond/total consumption); average of two test trials.

with group and trial as the variables revealed significant effects of group, $F(1,14) = 5.69$, of trial, $F(3,42) = 9.73$, and a significant interaction between these variables, $F(3,42) = 3.02$. Analysis of simple main effects showed that the groups differed on Trials 1 and 2, with $F(1,14) = 10.45$ and 7.74, respectively, but not on Trials 3 and 4 ($Fs \leq 1$).

Absolute consumption scores for the test are shown in Table 2 and preference ratios in the lower right panel of Fig. 2. The CON group drank less of almond than of water, resulting in a mean preference ratio of less than .5. The conditioning procedure was thus more effective in this experiment than in Experiment 2a, where the formally equivalent training given to the SIM group produced only a reduction in the preference for almond rather than a reversal of that preference. This outcome might be expected, however, on the basis that the CON group drank the almond + quinine compound more readily in this experiment than did the SIM group of Experiment 2a. This relative lack of neophobia (possibly a consequence of their having experienced a novel odor prior to the start of this study) meant that the CON subjects had more extensive exposure to the compound during the conditioning trials. However, this may be the aversion was less profound in the PRE group. Subjects in this group drank approximately the same total amount as those in the CON group (see Table 2) but they drank more almond than water resulting in preference ratios above .5. The ratio scores for the two groups differed significantly, $F(1,14) = 9.76$, but there was no significant effect of trial, $F(1,14) = 1.01$, or interaction ($F < 1$). If the aversion to almond shown by the CON group reflects a conditioning effect, the almond consumption for the PRE group suggests that this effect is attenuated by prior exposure to quinine.

These results are readily explained by the proposal that preexposure to quinine reduces its effectiveness as a US; but again, an alternative in terms of the habituation of neophobia may be possible. The conditioning procedure involved four trials in which the almond was presented (in compound with quinine). Habituation of

the neophobic response to almond might be expected to occur on these trials, and, since the PRE group drank more of the compound than did the CON group, there was more opportunity for habituation to occur in that group. The difference between the groups in consumption of almond on test might thus reflect a difference in the degree of habituation. What argues against this interpretation, however, is the fact that consumption of the almond (plus quinine) solution was low in the CON group only on the first two trials of conditioning—on the last two trials these animals drank as readily as those of the PRE group. To the extent that this change in the CON group represents a loss of neophobia to almond (and a change in the response to quinine will also play a role) we may conclude that this loss was complete by the end of the conditioning phase. The difference between the groups must be attributed to some other source. We return, therefore, to the interpretation that the aversion to almond on the test depends on the formation of an almond–quinine association and that preexposure to quinine reduces its effectiveness as a US.

4. General discussion

The experiments reported here demonstrate that the preference for (or aversion from) a previously neutral flavor that is generated by pairing that flavor with a palatable (or aversive) substance can be reduced by giving rats preexposure to the latter. If we adopt an associative analysis that treats the neutral flavor as the CS and the added substance as the US, this constitutes an example of the US-preexposure effect in this form of conditioning. The effect has been obtained previously in this form of learning, by Gil et al. (2011), but in their experiments the US used was sucrose, which is capable of generating a preference not only on account of its palatability, but also by way of its nutritive properties. This leaves open an interpretation in terms of associative blocking. If, during preexposure, the rat forms an association between the sweet taste of sucrose and its post-consumption consequences, that association might act to block the formation an association between the CS flavor and these consequences, resulting in a reduced CR. This analysis applies less readily to the results of the present experiments, in which non-nutritive substances were used as the USs so that the changes in preference produced may be assumed to be a consequence solely of shifts in palatability. (Although a version of the blocking account can be contrived for Experiment 1, which used saccharin as the US, it is difficult to do the same for the results of Experiment 2, which used quinine.)

The most widely considered alternative to blocking as an explanation for the US-preexposure effect has been in terms of habituation of the effectiveness of the US. This was the explanation (referred to by him as adaptation) offered by Kamin (1961) for his original demonstration of the effect in the conditioned suppression procedure (but see also Brimer and Kamin, 1963). The results reported here for exposure to quinine are consistent with this interpretation; with repeated presentation of quinine the initial rejection wanes and quinine is consumed more readily. If this change implies a reduction in the effective salience of this initially aversive substance, then a reduction in its ability to serve as reinforcer for conditioning might be expected. The equivalent results for saccharin are more problematic. There is certainly evidence consistent with some form of habituation, in that consumption increases over the course of preexposure. But since this change seems to indicate an increase in the palatability of saccharin, one might expect preexposure to enhance the ability of saccharin to support the development of a flavor preference—the opposite of the result obtained.

We should acknowledge, however, that the change in consumption recorded in our experiments might be a poor index of change in palatability. The low initial level could be mediated by the animal's response to novelty per se (see e.g., Braveman and Jarvis, 1978; Miller and Holtzman, 1981); the increase over trials would reflect habituation of neophobia, and would thus not provide an accurate index of changes in the hedonic properties of the substances presented. Direct evidence from other measures of palatability have given mixed results. Studies of the sweet taste of sucrose have shown that habituation can be obtained (although this depends on the exact conditions of the training procedure; see Schifferstein and Frijters, 1992). Thus Fisher and Fisher (1969) recorded the galvanic skin response evoked in human participants by a flow of fluid over the tongue. Sucrose produced a greater response than plain water, but with repeated presentation, habituation was observed. Quinine evoked an even greater response than sucrose, but this too habituated after extended training. On the other hand studies of behavior patterns in rats that are thought to index the strength of the hedonic reaction to a flavor have not provided evidence of habituation. The orofacial responses of rats evoked by infusions of quinine (Breslin et al., 1990) and of saccharin (Neath et al., 2010) do not appear to change with experience; indeed by one measure (the pattern of licking at a drinking spout), the palatability of saccharin appears to increase over successive presentations (Lin et al., 2012).

A version of an habituation account, based on adaptation-level theory (e.g., Helson, 1964), has been put forward by Boakes et al. (2007) to deal with their results on the effects of exposure to the US given after the conditioning phase in flavor-preference learning. In their experiments, rats given exposure to sucrose alone, after conditioning with a sucrose–almond compound, showed a reduced preference for almond on an almond vs. water preference test. They suggested in explanation that the phase of exposure to sucrose produces an adaptation effect that reduces the perceived sweetness of substances presented subsequently (see also Kawai and Nakajima, 1997). The preference for almond on test, which is assumed to depend on acquired sweetness, is accordingly reduced. Boakes et al. acknowledge that this adaptation process can be equated with a more traditional account of habituation in which the reduced effectiveness of a US is understood in terms of an increase in the threshold of activation of the US's central representation (e.g., Rescorla, 1973).

The similarity of the effects of US preexposure and US postexposure encourages us to look for a common explanatory mechanism; but there are reasons to doubt that the same habituation process is responsible for both. Specifically, in a follow-up study to that of Boakes et al. (2007), Albertella et al. (2008) have investigated contextual control of adaptation-level effects. They showed that their US-postexposure effect is obtained only when the preference test is given in the same context (the same distinctive cage) as was used for exposure to the US. They concluded that the rats' adaptation level was specific to the training context. This contrasts with the finding that the US-preexposure effect in flavor-preference conditioning using sucrose as the US, does not show context dependence: Gil et al. (2011) found the effect to be just as powerful in subjects that switched contexts after the preexposure phase as in subjects that remained in the same context. This effect of US preexposure may well be a consequence of some form of habituation or adaptation, but it is a form that shows different properties from that responsible for the postexposure effect.

The discussion so far has been based on the assumption that the conditioning effects obtained in our experiments depend on the formation of an association between the almond flavor and the taste of the US. It has frequently been suggested, however, that flavor preferences may be acquired by a learning process that does not involve the formation of an orthodox CS–US association (e.g., Capaldi and Hunter, 1994; Dwyer et al., 2011; Higgins and Rescorla, 2004; Pearce, 2002). Although different authors express it differently, the central idea common to these accounts is that experience

of a compound such as almond + saccharin allows the formation of a configural representation of these elements, combining the properties of both. Subsequent presentation of almond alone is able to activate this configural representation and thus evoke a response that is appropriate to the sweet taste of saccharin. This approach can readily accommodate the effects of preexposure to the event that we have hitherto referred to as the US, if it is assumed that separate presentations of one of the elements interferes with the formation of the configural representation (Rescorla and Durlach, 1981; Rescorla and Freberg, 1978). It is difficult to discriminate between this account and one that attributes the effects of preexposure to a decline in the ability of the US to support the formation of a CS–US association.

These theoretical uncertainties cannot be resolved on the basis of the present results, and we conclude by returning to the positive conclusions that can be drawn. Our results show that the US-preexposure effect, previously observed in flavor-preference conditioning only with sucrose as the US, can be obtained using USs that lack significant post-consumption consequences (saccharin and quinine). An explanation for the results with sucrose in terms of associative blocking cannot apply to the effects obtained with saccharin and quinine, encouraging the view that these effects are a consequence of some form of habituation that reduces the effectiveness of the US as a reinforcer. This habituation process will also, presumably, contribute to the US-preexposure effect observed with more orthodox conditioning procedures (Randich and LoLordo, 1979). It might also be expected to occur during the course of conditioning itself (which, after all, usually involves repeated presentations of the US), and the possible implications of this deserve investigation.

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References

Albertella, L., Harris, J.A., Boakes, R.A., 2008. Acquired flavor preferences: contextual control of adaptation-level effects. Q. J. Exp. Psychol. 61, 227–231.
Boakes, R.A., Albertella, L., Harris, J.A., 2007. Expression of flavor preference depends on type of test and on recent drinking history. J. Exp. Psychol.: Anim. Behav. Processes 33, 327–338.
Braveman, N.S., Jarvis, P.S., 1978. Independence of neophobia and taste aversion learning. Anim. Learn. Behav. 6, 406–412.
Breslin, P.A.S., Davidson, T.L., Grill, H.J., 1990. Conditioned reversal of reactions to normally avoided tastes. Physiol. Behav. 47, 538.
Brimer, C.J., Kamin, L.J., 1963. Disinhibition, habituation, sensitization, and the conditioned emotional response. J. Comp. Physiol. Psychol. 56, 508–516.
Capaldi, E.D., Hunter, M.J., 1994. Taste and odor in conditioned flavor preference learning. Anim. Learn. Behav. 22, 355–365.
Colley, J.C., Edwards, R.H., Purser, D., 1989. Toxicity studies with quinine hydrochloride. Toxicology 54, 219–226.
De Brugada, I., González, F., Gil, M., Hall, G., 2005. The role of habituation of the response to LiCl in the US-preexposure effect. Learn. Behav. 33, 363–370.
De Brugada, I., Hall, G., Symonds, M., 2004. The US-preexposure effect in lithium-induced flavor-aversion conditioning is a consequence of blocking by injection cues. J. Exp. Psychol.: Anim. Behav. Processes 30, 58–66.
Dess, N.K., 1993. Saccharin's aversive taste in rats: evidence and implications. Neurosci. Biobehav. Rev. 17, 359–372.
Dwyer, D.M., Haslegrove, M., Jones, P.M., 2011. Cue interactions in flavor preference learning: a configural analysis. J. Exp. Psychol.: Anim. Behav. Processes 37, 41–57.
Fanselow, M., Birk, J., 1982. Flavor-flavor associations induce hedonic shifts in taste preference. Anim. Learn. Behav. 10, 223–228.
Fisher, G.L., Fisher, B.E., 1969. Differential rates of GSR habituation to pleasant and unpleasant sapid stimuli. J. Exp. Psychol. 82, 339–342.
Gil, M., Symonds, M., Hall, G., de Brugada, I., 2011. Analysis of the US-preexposure effect in flavor acceptance conditioning. Learn. Motiv. 42, 273–281.
Hall, G., 2009. Preexposure to the US in nausea-based aversion learning. In: Reilly, S., Schachtman, T.R. (Eds.), Conditioned Taste Aversion: Behavioral and Neural Processes. Oxford University Press, New York, NY, pp. 58–73.
Harris, J.A., Gorissen, M.C., Bailey, G.K., Westbrook, R.F., 2000. Motivational state regulates the content of learned flavor preferences. J. Exp. Psychol.: Anim. Behav. Processes 26, 15–30.
Harris, J.A., Shand, F.L., Carroll, L.Q., Westbrook, R.F., 2004. Persistence of preference for a flavor presented in simultaneous compound with sucrose. J. Exp. Psychol.: Anim. Behav. Processes 30, 177–189.
Harris, J.A., Westbrook, R.F., 1998. Retroactive revaluation of an odor-taste association. Anim. Learn. Behav. 26, 326–335.
Helson, H., 1964. Current trends and issues in adaptation-level theory. Am. Psychol. 19, 26–38.
Higgins, T., Rescorla, R.A., 2004. Extinction and retraining of simultaneous and successive flavor conditioning. Learn. Behav. 32, 213–219.
Holman, E.W., 1975. Immediate and delayed reinforcers for flavor preferences in rats. Learn. Motiv. 6, 91–100.
Kamin, L.J., 1961. Apparent adaptation effects in the acquisition of a conditioned emotional response. Can. J. Psychol. 15, 176–188.
Kawai, N., Nakajima, S., 1997. US postexposure effect on conditioned flavor preference in the rat. Psychol. Rec. 47, 499–518.
Kratz, C.M., Levitsky, D.A., 1978. Postingestive effects of quinine on intake of nutritive and nonnutritive substances. Physiol. Behav. 21, 851.
Lin, J-Y., Amodeo, L.R., Arthurs, J., Reilly, S., 2012. Taste neophobia and palatability: the pleasure of drinking. Physiol. Behav. 106, 515–519.
Miller, R.R., Holtzman, A.D., 1981. Neophobias and conditioned taste aversions in rats following exposure to novel flavors. Anim. Learn. Behav. 9, 89–100.
Neath, K.N., Limebeer, C.L., Reilly, S., Parker, L.A., 2010. Increased liking for a solution is not necessary for the attenuation of neophobia in rats. Behav. Neurosci. 124, 398–404.
Pearce, J.M., 2002. Evaluation and development of a connectionist theory of configural learning. Anim. Learn. Behav. 30, 73–95.
Randich, A., Lolordo, V.M., 1979. Associative and nonassociative theories of the UCS preexposure phenomenon: implications for Pavlovian conditioning. Psychol. Bull. 86, 523–548.
Rescorla, R.A., 1973. Effect of US habituation following conditioning. J. Comp. Physiol. Psychol. 82, 137–143.
Rescorla, R.A., Durlach, P.J., 1981. Within-event learning in Pavlovian conditioning. In: Spear, N.E., Miller, R.R. (Eds.), Information Processing in Animals: Memory Mechanisms. Lawrence Erlbaum Associates, Hillsdale, NJ, pp. 81–111.
Rescorla, R.A., Freberg, L., 1978. The extinction of within-compound flavor associations. Learn. Motiv. 9, 411–427.
Riley, A.L., Simpson, G.R., 2001. The attenuating effects of drug preexposure on taste aversion conditioning: generality, experimental parameters, underlying mechanisms and implications for drug use and abuse. In: Mowrer, R.R., Klein, S.B. (Eds.), Handbook of Contemporary Learning Theories. Lawrence Erlbaum Associates, Mahwah, NJ, pp. 505–559.
Schifferstein, H.N.J., Frijters, J.E.R., 1992. Sweetness does not habituate during a sip-and-spit experiment. Physiol. Behav. 51, 331–336.
Sclafani, A., Ackroff, K., 1994. Glucose- and fructose-conditioned flavor preferences in rats: taste versus postingestive conditioning. Physiol. Behav. 56, 399–405.
Sclafani, A., Cardieri, C., Tucker, K., Blusk, D., Ackroff, K., 1993. Intragastric glucose but not fructose conditions robust flavor preferences in rats. Am. J. Physiol. 265, R320–R325.