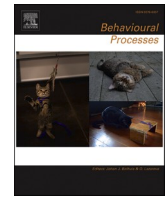
Behavioural Processes 191 (2021) 104462

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc



Motivational factors controlling flavor preference learning and performance: Effects of preexposure with nutritive and nonnutritive sweeteners

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ARTICLE INFO

Keywords:
Flavour preference
Motivational factors
Saccharin
Sucrose
Exposure

ABSTRACT

In three experiments thirsty rats were given exposure to a sweet solution (saccharin in some experiments, sucrose in others) prior to consuming a compound of the sweet substance and almond flavoring. Preference for that flavor, in a choice test of almond vs. water, was then assessed. In some cases the rats were hungry, in others they were not. When the sweetener used was saccharin, preexposure reduced the magnitude of the preference obtained on test in both hungry and nonhungry rats. When the sweetener was sucrose, preexposure had this effect only when the rats were hungry. The effects produced after preexposure to saccharin are interpreted as being the result of habituation to its sensory features that reduces the ability of these features to engage in subsequent learning. These effects will occur whether the animal is hungry or not. The results for sucrose are interpreted in terms of the fact that it possesses both sensory and nutritional properties, the role of the latter being dependent on the motivational state of subject. It is suggested that the sensory features of sucrose do not undergo habituation, but that an effect of preexposure can be obtained in hungry rats when the source of the learned preference will depend on learning about the nutritive consequences of the sucrose.

1. Introduction

Rats allowed to consume a solution of sucrose to which a distinctive flavor such as almond or mint has been added will demonstrate an increased readiness to consume unsweetened water containing that flavor.[1] This effect has been attributed to a conditioning process in which the previously neutral flavor (the conditioned stimulus, CS) becomes associated with an unconditioned stimulus (US) that includes the nutritional, caloric, properties of the sucrose (see Fedorchak, 1997, for a review). In addition to its nutritional consequences, sucrose has a sweet taste, and this in itself, is capable of generating a learned flavor preference, as is demonstrated by the effectiveness of a nonnutritive sweetener such as saccharin in generating such a preference (e.g., Holman, 1975; Fanselow and Birk, 1982). The source of the preference generated after experience of a flavor together with a sweet taste (whether this be nutritive or not) has been debated. One interpretation treats it as an orthodox stimulus-stimulus association of the type used in standard explanations of the phenomenon of sensory preconditioning (e.g., Fanselow and Birk, 1982). An alternative proposes that the effect depends on the two stimuli forming a unitary compound (Rescorla, 1981) or configure (Pearce, 2002) that allows one of the elements to activate the sensory properties of the flavor with which it has been paired.

The role of these various possible associations in flavor preference learning has been investigated in experiments in which the subjects were given exposure to the substance to be used as the US prior to, or intermixed with, the conditioning trials. Thus, Gil et al. (2011) gave rats eight presentations of a sucrose solution followed by four trials in which the sucrose solution was flavored with mint. On a final test session (prior to which the animals were put on a schedule of restricted access to food)

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[1] We refer to these substances as flavors given that they may have a discriminable taste in addition to their distinctive odors.

https://doi.org/10.1016/j.beproc.2021.104462
Received 19 March 2021; Received in revised form 12 July 2021; Accepted 16 July 2021
Available online 17 July 2021
0376-6357/

the subjects were given access to the mint solution, without the sucrose. Consumption was significantly reduced in comparison with subjects not given preexposure to the sucrose. Gil et al. offered an explanation in terms of associative blocking. They suggested that preexposure to sucrose would allow the formation of an association between its sweet taste and its nutritive consequences (“calories”). This would then act to block the formation of the equivalent association with the mint CS during the conditioning phase, reducing the ability of mint on its own to evoke responding in hungry rats in the test phase.

Harris et al. (2000) reported an extensive set of experiments using this general procedure (with almond as the CS and sucrose as the US) in which they investigated the effects of changing the motivational state of the subjects, and of using nonnutritive saccharin in the preexposure phase. Importantly, they found that the effects of separate presentations of saccharin on the flavor preference obtained by compounding almond with sucrose depended on the motivational state of the rats. For rats that were food-deprived prior to the test, the conditioned preference for almond was strong; but rats that were not hungry for the test showed a reduced preference for almond. They also found that rats that had been hungry during the training phase showed a loss of preference when they were given free access to food prior to the test. Their interpretation of this pattern of results was that associations that may be summarized as “flavor-sweet” and “flavor-calories” will be established by pairing almond with sucrose and that both can contribute to a conditioned flavor preference. In hungry animals learning and performance is likely to be dominated by the latter association, that is, by the nutritional associate of the flavor. But in animals that are not hungry the flavor-sweet association will control performance, and if this form of learning has been weakened by separate presentations of sweetness alone (i.e., by experience of saccharin) the size of the measured preference will be reduced.

Harris et al. (2000) used saccharin only in the preexposure phase of the experiments just described, with sucrose being used as the US for conditioning; but their interpretation has implications for the standard procedure used in demonstrating the US-preexposure effect, in which the preexposed stimulus is the same as that used as the US in conditioning. If saccharin were used throughout in this procedure, the motivational state of the subject should be irrelevant. Conditioning to a flavor CS paired with saccharin will depend solely on the flavor-sweet association, which may be assumed to be formed whether or not the animal is hungry; and similarly, the effects of exposure to this sweet taste are not taken to be dependent on motivational state. This issue was addressed in the current Experiment 2 which looked at the effect of exposure to saccharin on conditioning with a saccharin US in both hungry and nonhungry rats. Using a matching design and procedure, Experiment 3 examined the effects produced using sucrose as the US throughout. A necessary preliminary is to demonstrate that the basic effect of US preexposure can be obtained using saccharin as the US. Experiment 1 (which made use of a procedure employed by Gil et al., 2014) was designed to provide this.

2. Experiment 1

Gil et al. (2014) provided evidence of a US-preexposure effect, with saccharin as the US, in rats that were not food-deprived at any stage in the experiment. The present experiment followed their procedure but differed in that the rats were food-deprived throughout, as a preliminary to a direct test, in Experiment 2, of the effects of deprivation level on this version of the US-preexposure effect.

The design of the experiment is shown in the upper section of Table 1. Two groups of rats received conditioning trials in which they consumed a solution of saccharin (Sacc) to which a novel flavor (almond; A in the table) was added. Rats in the PRE group had received preexposure to saccharin; rats in the CON (control) group had not. Preference was assessed in a final test allowing choice between water flavored with almond and plain water. The subjects were food-deprived (H for hungry, in the table) throughout.

Table 1
Experimental Designs.

Experiment 1			
Group	Preexposure	Conditioning	CR Test
PRE-Suc-H	8 Sucrose	4 A + Sucrose	A vs Water
CON-Suc-H	8 Water		
PRE-Sacc-H	8 Saccharin	4 A + Saccharin	
CON-Sacc-H	8 Water		
Experiment 2			
PRE-Sacc	8 Saccharin	4 A + Saccharin	A vs Water
CON-Sacc	8 Water		
PRE-Sacc-H	8 Saccharin		
CON-Sacc-H	8 Water		
Experiment 3			
PRE-Suc	8 Sucrose	4 A + Sucrose	A vs Water
CON-Suc	8 Water		
PRE-Suc-H	8 Sucrose		
CON-Suc-H	8 Water		

Note. Suc refers to a 20 % sucrose solution; Sacc refers to a 0.4 % saccharin solution; A refers to a 1% almond solution; PRE: preexposed groups: CON: non-preexposed control groups; H (hungry): animals were food deprived.

Two further groups were included in this experiment, using sucrose as the US in order to allow a direct comparison between nutritive and the non-nutritive USs. These animals (the Suc groups in the table) received exactly the same treatment as those in the Sacc groups except that the US was the sucrose solution used in previous demonstrations of the effect (Gil et al., 2011).

2.1. *Method*

2.1.1. *Subjects and apparatus*

The subjects were 32, experimentally naïve, male hooded Lister rats (obtained from Charles River Laboratories). They had a mean free-feeding weight of 427 g (range: 416−548 g) at the start of the experiment. They were housed individually in home cages measuring 35cm × 22cm × 19 cm, 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.). The unconditioned stimuli (USs) were a 20 % (w/v) sucrose solution, and a 0.4 % solution of sodium saccharin. The conditioned stimulus (CS) was a 1% (v/v) solution of almond flavoring (supplied by Supercook, Leeds, UK). The compounds presented during conditioning were made up so as to preserve these concentrations. All the solutions were made with tap water and given to the animals in 50-ml graduated tubes fitted with rubber stoppers and stainless steel ball-bearing tipped spouts. Fluid intake was measured by weighing tubes before and after sessions.

2.1.2. *Procedure*

The animals were assigned to four equally sized groups and were water and food deprived at the beginning of the experiment. The schedules of water and food deprivation and maintenance conditions were established so that the animals had 30-min access to water (or a flavored solution) in a morning session (from 11 a.m.) and had access to water and food during an afternoon session for 90 min (from 4:30 p.m.). All the experimental treatments were conducted during the morning sessions. The preexposure phase lasted eight days. One each of these days, animals in the preexposed groups received either 15 mL of the sucrose solution (Group PRE-Suc-H) or a 15 mL of the saccharin solution (Group PRE-Sacc-H); animals in the control groups received 15 mL of unflavored water on these sessions. Over the following four days all the animals in groups PRE-Suc-H and CON-Suc-H received 15 mL of a compound of almond and sucrose; animals in groups PRE-Sacc-H and CON-Sacc-H received 15 mL of a compound of almond and saccharin. For the final test all the subjects received access to two bottles with one tube containing 30 mL of almond and the other containing 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.

2.2. Results and discussion

Fig. 1 shows consumption of fluid during preexposure. Consumption of water remained steady for the control groups over this phase. Subjects given a sweetened solution, particularly those given saccharin, showed some neophobia, but consumption increased steadily over the course of this phase and both PRE groups were drinking more than the CON groups by the end of preexposure. This description was confirmed by statistical analysis. An analysis of variance (ANOVA) conducted on these scores with preexposure condition (PRE and CON), US condition (Suc or Sacc), and trial as the variables yielded significant main effects of trial, $F(7, 189) = 22.92$, $p < 0.001$, $\eta_p^2 = .104$, of preexposure condition, $F(1, 27) = 64.43$, $p < .001$, $\eta_p^2 = 0.327$, and of US condition, $F(1, 27) = 15.85$, $p < .001$, $\eta_p^2 = .080$. There were significant interactions between preexposure condition and US condition, $F(1, 27) = 14.09$, $p < .001$, $\eta_p^2 = 0.071$, trial and preexposure condition, $F(7, 189) = 30.46$, $p < .001$, $\eta_p^2 = 0.138$, and between trial and US condition, $F(7, 189) = 2.36$, $p = .025$, $\eta_p^2 = .011$. Analysis of simple effects showed there to be a difference between trials 1 and 8 in preexposed groups, $F(7, 105) = 37.17$, $p < .001$, $\eta_p^2 = .712$, but not in the control groups, $F(7, 98) = .08$, $p = .37$, $\eta_p^2 = .0720$. There was a significant effect of US condition on trial 1 for preexposed animals, $F(1, 14) = 125.46$, $p < .001$, $\eta_p^2 = .90$, but not for control animals, $F < 1$, and significant differences between preexposed and control animals in the last trial of the preexposure phase, $F(1, 29) = 109.12$, $p < .001$, $\eta_p^2 = .79$.

Consumption of the CS-US compound during the conditioning trials is shown in Fig. 2. Neophobia to sweetened solutions was again evident, as the CON groups drank less that the PRE groups on the early trials. This effect was particularly marked in subjects given saccharin, but a difference between PRE and CON groups was also seen on Trial 1 in subjects given sucrose. No differences were evident by the final conditioning trial. An ANOVA with preexposure condition, US condition, and trial as the variables revealed significant effects of trial, $F(3, 81) = 39.31$, $p < .001$, $\eta_p^2 = 0.178$, of US condition, $F(1, 27) = 108.74$, $p < .001$, $\eta_p^2 = 0.212$, and of preexposure condition, $F(1, 27) = 70.52$, $p < .001$, $\eta_p^2 = .138$. There were significant interactions between preexposure condition and US condition, $F(1, 27) = 20.94$, $p < .001$, $\eta_p^2 = .041$, between trial and preexposure condition, $F(3, 81) = 23.87$, $p < .001$, $\eta_p^2 = .108$, and between trial and US condition, $F(3, 81) = 23.54$, $p < .001$, $\eta_p^2 = .106$. Analysis of simple effects showed there to be a significant effect of US condition on the first conditioning trial, $F(1, 27) = 4.31$, $p = .04$, $\eta_p^2 = .132$, but no differences between preexposed and control animals and no interaction (*Fs* <1).

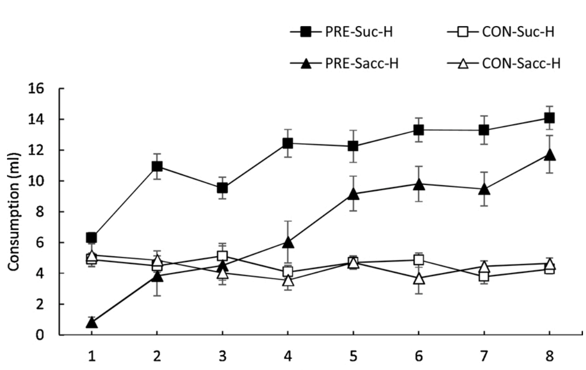

Fig. 1. Experiment 1: Mean consumption scores during preexposure for the preexposed (PRE) and control (CON) groups. Animals in the PRE groups received sucrose (Suc) or saccharin and (Sacc); those in the CON groups received access to water. Vertical bars represent SEMs.

The results of the test are shown in Fig. 3. The upper panel shows group means for intake of the almond solution and of water. All animals drank more of the almond solution than of water; and, for both the saccharin and sucrose groups, the preference for almond was less in the preexposed than in the control subjects. Individual consumption scores were converted to preference ratios (volume of almond/volume of almond + volume of water consumed) for statistical analysis. Group mean ratio scores are shown in the lower panel. Overall levels of preference were higher with sucrose than with saccharin, but for both the preference was less in the prexposed than in the control subjects. An ANOVA with preexposure condition and type of US as the variables revealed a significant difference between preexposed and control conditions, $F(1, 27) = 4.34$, $p = .04$, $\eta_p^2 = .118$, and a significant difference between the sucrose and saccharin conditions, $F(1, 27) = 5.38$, $p = .02$, $\eta_p^2 = .146$, but there was no significant interaction between these variables, $F < 1$.

These results have successfully confirmed the occurrence of the US-preexposure effect in flavor-preference learning for hungry rats trained with sucrose as the US (Gil et al., 2011). They have further shown that the effect can be obtained, under the same motivational conditions, with saccharin as the US. Saccharin is, at least initially, consumed less readily than sucrose, but exposure produces a reduction in neophobia so that consumption readily comes to exceed that of plain water (Fig. 1). Neophobia was clearly evident on the first conditioning trial in the control group given saccharin as the US (Fig. 2); but in spite of the fact that they experienced less of the compound during conditioning, these subjects showed a stronger preference on test than was shown by the preexposed subjects. And although levels of consumption of the almond solution on test were somewhat lower in animals trained with saccharin, these showed a US-preexposure effect closely similar to that seen in the sucrose-trained groups.

3. Experiment 2

This experiment examined the effect of motivational state on the US-preexposure effect obtained with saccharin as the US. It employed the general procedures shown to be effective in Experiment 1. There were four groups of subjects (see Table 1). The treatment given to groups PRE-Sacc-H and CON-Sacc-H matched that of the equivalent groups in Experiment 1. Two further groups (PRE-Sacc and CON-Sacc) received equivalent training except that access to food was maintained throughout the experiment. The proposal that preference learning with saccharin as the US is generated solely by its sweet taste implies that the US-preexposure effect would be obtained with this US, regardless of motivational state of the animals.

3.1. Method

The subjects were 32 experimentally naïve male Lister rats (obtained from Charles River Laboratories). They had a mean free-feeding weight of 320 g at the start of the experiment. They were housed and maintained under the same conditions as those described for Experiment 1. The rats were assigned to one of four equal-sized groups. The treatment given to the preexposed-hungry (PRE-Sacc-H) and control-hungry (CON-Sacc-H) groups exactly matched that given to the equivalent groups in Experiment 1. Groups PRE-Sacc and Con-Sacc differed only in that they had full access to food throughout the experiment. Any procedural details not specified here were identical to those described for Experiment 1.

3.2. Results and discussion

It is commonly observed (e.g., Bolles, 1961) that overall levels of fluid consumption tend to be lower in hungry animals than in those not food-deprived. This effect was seen during the preexposure phase (see

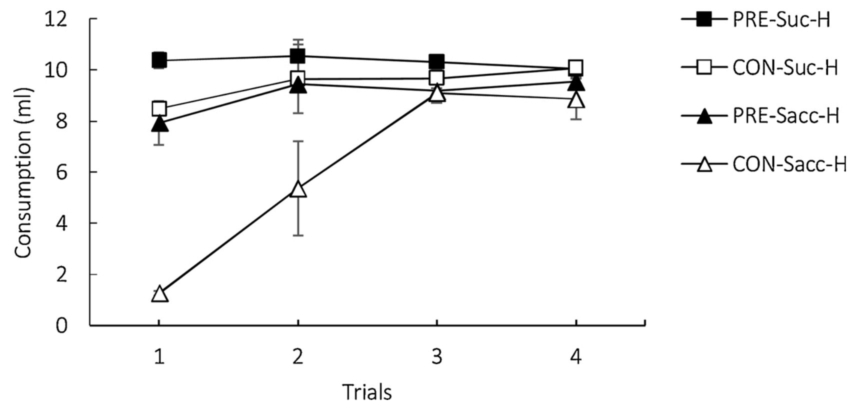

Fig. 2. Experiment 1: Group means for consumption of the almond-sucrose (Suc groups) or the almond-saccharin (Sacc groups) compound solution during the conditioning phase for animals in the preexposed (PRE) and the control (CON) groups. Vertical bars represent SEMs.

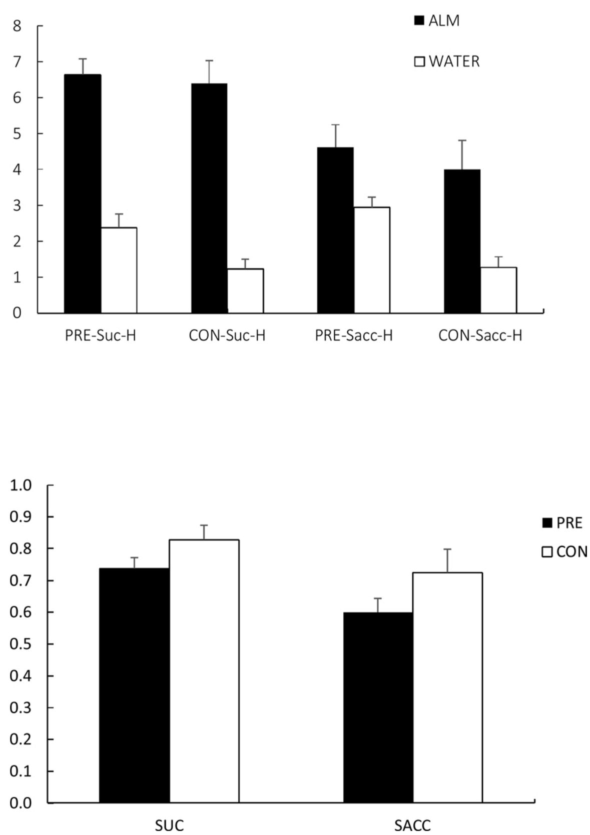

Fig. 3. Experiment 1: Group means for consumption of almond (ALM) and water during the test for animals in the preexposed (PRE) and the control (CON) groups (upper panel), and the mean ratio of almond intake over total intake (lower panel) for groups given sucrose (Groups PRE-Suc and CON-Suc) and saccharin (Groups PRE-Sacc and CON-Sacc) as the US. Vertical bars represent SEMs.

Fig. 4). As in Experiment 1, rats given preexposure to saccharin drank less on the initial trials of preexposure than on later trials; consumption of water in the control subjects remained fairly constant so that by the end of the phase, the rats given saccharin were drinking more than those given water. An ANOVA with preexposure condition, motivational state, and trial as the variables yielded significant main effects of trial, $F(7, 196) = 76.46$, $p < .01$, $n_p^2 = .214$, of motivational condition (hungry or not), $F(1, 28) = 180.49$, $p < .01$, $n_p^2 = .436$, and a significant interaction between these variables, $F(1, 196) = 8.06$, $p < .01$, $n_p^2 = .023$. Analysis of simple effects showed there to be a difference between Trials 1 and 8 in the preexposed subjects, $F(7, 105) = 68.11$, $p < .01$, $n_p^2 = .820$, but not in the control subjects $F(7, 105) = 2.00$, $p = .06$, $n_p^2 = .118$.

An initial reluctance to drink saccharin was again evident during the conditioning phase in which the control groups, that had not received preexposure to saccharin, showed low levels of consumption on the first trial (Fig. 5). There were, however, no differences among the groups in the amount of the almond-saccharin compound they consumed on the last trial of conditioning. An ANOVA conducted on the results for this trial, with preexposure condition and motivational state as the variables, revealed no significant effects for the preexposure variable, $F < 1$, or of motivational state, $F(1, 28) = 1.41$, $p = .24$, $n_p^2 = .045$, and no interaction between these variables, $F(1, 28) = 2.25$, $p = .014$, $n_p^2 = .071$.

The results of the final test are shown in Fig. 6. As before, the total amount consumed was less in the hungry animals than in those not food deprived. However, all groups showed a preference for almond over water, and a US-preexposure effect was evident in all in that preexposed subjects showed a lesser preference for the almond flavor than was shown by control subjects. An ANOVA with preexposure condition and motivational state as the variables showed there to be a significant effect of the preexposure, $F(1, 28) = 6.81$, $p = .014$, $n_p^2 = .182$, but there was no significant effect of motivational state, $F(1,28) = 1.52$, $p = .228$, $n_p^2 = .041$, and no significant interaction between these variables, $F(1, 28) = 1.02$, $p = .32$, $n_p^2 = .027$.

It is to be expected that rats given free access to (dry) food will drink more readily that those that are food-deprived and the resulting difference in absolute levels of fluid consumption complicates direct comparison between the hungry and non-hungry groups in this experiment. It is clearly the case, however, that the US-preexposure effect can be obtained in rats that are not food-deprived and there is no evidence that the size of this preference is different from that shown by hungry rats. These findings thus lend support to the proposal that the preference established using saccharin as the US depends on learning about the sweet taste of the US, something that is independent of motivational state. The effect of motivational state on the effect of US-preexposure when sucrose is used as the US is taken up in the next experiment.

4. Experiment 3

The study by Harris et al. (2000) included an examination of the effects of motivational state and preexposure to a sucrose US in experiments that involved a change of motivational state from training (i.e., from the phases of US-preexposure and of flavor-sucrose pairing) to the test phase. In one experiment (their Experiment 6) the rats were hungry during training, and in others (Experiments 4 and 7) they were not food-deprived during training. In all experiments some animals were tested when food deprived and others were tested when satiated. In all the experiments these subjects showed a lesser preference than control subjects not given preexposure to sucrose, but in no experiment was

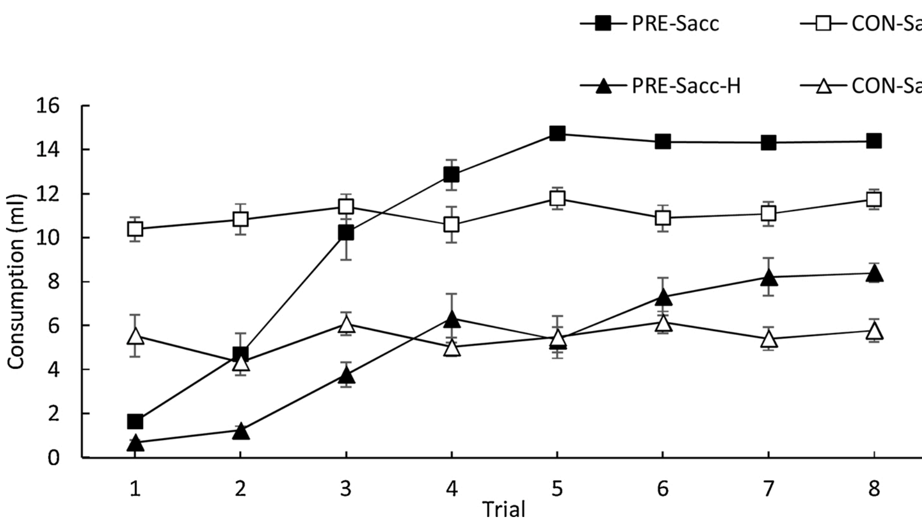

Fig. 4. Experiment 2: Mean consumption scores during preexposure for the preexposed (PRE) and control (CON) groups. Animals in the PRE groups received saccharin (Sacc); those in the CON groups received access to water. H (hungry) animals were food-deprived. Vertical bars represent SEMs.

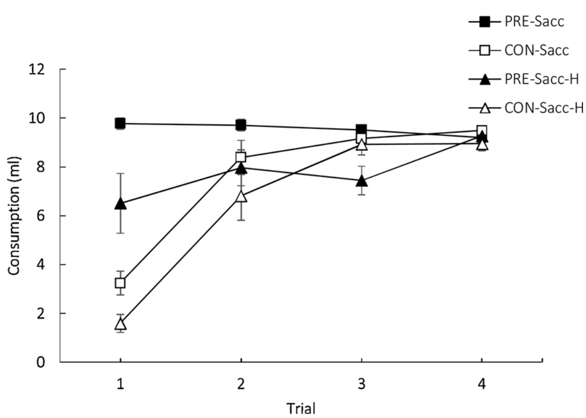

Fig. 5. Experiment 2: Group means for consumption of the almond-saccharin compound solution during the conditioning phase for animals in the preexposed (PRE) and the control (CON) groups. H (hungry) animals were food-deprived. Vertical bars represent SEMs.

there any reliable difference between the preexposed subjects tested hungry and those tested satiated. Our experiment examined the effects of motivational state on preexposure to a sucrose US, using a simpler design (see Table 1), matching that of Experiment 2. In this, one pair of groups was hungry throughout and the other pair was given free access to food. The only difference from Experiment 2 was that sucrose was used as the US. We know from Experiment 1 that the US-preexposure effect will be found in rats that are hungry (that is, in groups PRE-Suc-H and CON-Suc-H, which match those of Experiment 1). What effect will be obtained in the remaining two groups (PRE-Suc and CON-Suc), that were given free access to food throughout?

4.1. Method

The subjects were 32 experimentally naïve male Lister rats. They had a mean free-feeding weight of 330 g at the start of the experiment. They were housed and maintained under the same conditions as those described for Experiment 1. The rats were assigned to one of four equal-sized groups. The treatment given to the preexposed-hungry (PRE-Suc-H) and control-hungry (CON-Suc-H) groups was the same as that that given to the equivalent groups in Experiment 1; that is they were food-deprived, given preexposure (to sucrose for the PRE group, but only to water for the CON group), followed by four conditioning trials with a

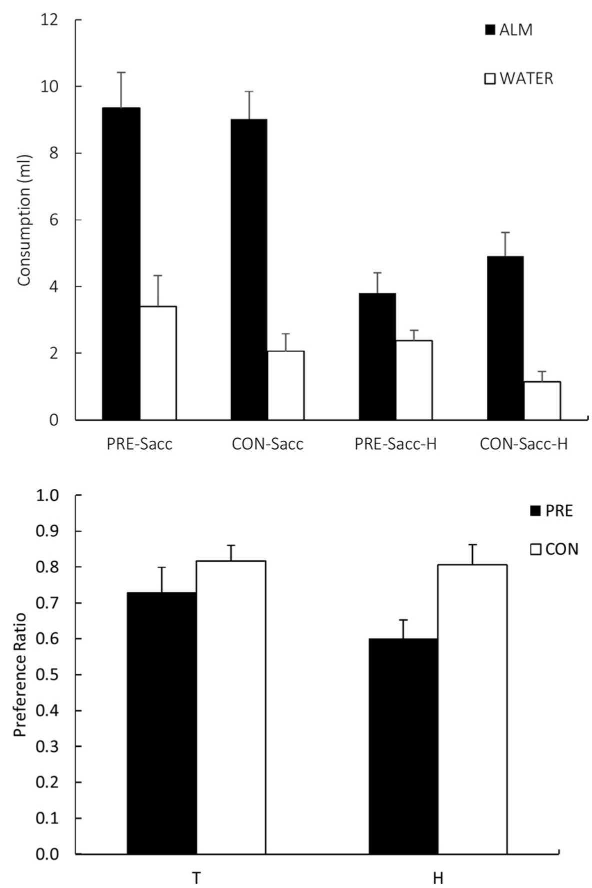

Fig. 6. Experiment 2: Upper panel: Group means for consumption of almond (ALM) and water during the test for animals in the preexposed (PRE) and the control (CON) groups. H (hungry) animals were food-deprived; others were thirsty (T) but not food deprived. Lower panel: mean ratio of almond intake over total intake. Vertical bars represent SEMs.

compound of almond and sucrose. In the final choice test they were given access to almond and to water. Groups PRE-Suc and Con-Suc differed from the H groups only in that they had full access to food throughout the experiment. Any procedural details not specified here were the same as those described for Experiment 1.

4.2. Results and discussion

Results for the preexposure phase are shown in Fig. 7. Consumption of water in the control groups remained steady across the phase; as in Experiment 2, the level for hungry subjects was lower than for non-hungry animals. Both preexposed groups showed neophobia on the initial preexposure trials, but the consumption of sucrose increased steadily over the trials, and there was no clear effect of motivational condition by the end of the phase. An ANOVA with preexposure condition, motivational state, and trial as the variables yielded significant main effects of trial $F(7,196) = 22.90$, $p < .001$, $\eta_p^2 = .117$, of preexposure condition, $F(1,28) = 15.61$, $p < .001$, $\eta_p^2 = .053$, and of motivational state, $F(1,28) = 91.91$, $p < .001$, $\eta_p^2 = .311$. There were significant interactions between trial and preexposure condition, $F(7,196) = 25.13$, $p < .001$, $\eta_p^2 = .129$, and between preexposure condition and motivational state, $F(1,28) = 40.85$, $p < .001$, $\eta_p^2 = .138$.

During the conditioning phase, subjects in all groups drank almost all of the solution that was available (Fig. 8), and, apart from the first trial when animals in the control groups encountered sucrose for the first time and drank slightly less than those in the preexposed condition, there were no differences among the groups. An ANOVA with preexposure condition, motivational state, and trial as the variables revealed significant main effects of trial, $F(3, 84) = 2.72$, $p = .049$, $\eta_p^2 = 0.048$, of preexposure condition, $F(1, 28) = 6.12$, $p = .020$, $\eta_p^2 = .051$, and an interaction between these variables, $F(3,84) = 8.31$, $p < .001$, $\eta_p^2 = 0.147$. An analysis of simple effects showed a significant difference between the preexposed and control groups on the first conditioning trial, $F(1,28) = 9.55$, $p = .004$, $\eta_p^2 = .249$.

The mean intake of the almond solution and of water on test by the rats in each group is shown in the upper panel of Fig. 9; preference ratios are shown in the lower panel. As in Experiment 1, a US-preexposure effect was evident in the hungry animals, with the preference for the almond flavor being less in the preexposed than in the control group. The subjects that were not food-deprived, however, showed no such effect. A preference for the conditioned flavor was present in both of the non-hungry groups, and was, if anything, slightly greater in the preexposed than in the control subjects. An ANOVA conducted on the preference ratios revealed no significant main effect of preexposure condition, $F(1, 28) = 2.69$, $p = .112$, $\eta_p^2 = .072$, or of motivational state, $F < 1$, but there was a significant interaction between these two variables, $F(1, 28) = 6.55$, $p = .016$, $\eta_p^2 = .176$. An analysis of simple main effects showed there to be a significant difference in the preference ratio scores between the preexposed and control groups that were hungry, $F(1, 14) = 20.37$, $p < .001$, $\eta_p^2 = .593$, but no effect in the groups that were not food-deprived, $F < 1$. The difference between the hungry and non-hungry conditions in their absolute levels of consumption makes it problematic to compare their ratio scores directly. The critical finding, therefore, is that the US-preexposure effect is clearly demonstrated in hungry animals and is quite absent in those that were not hungry.

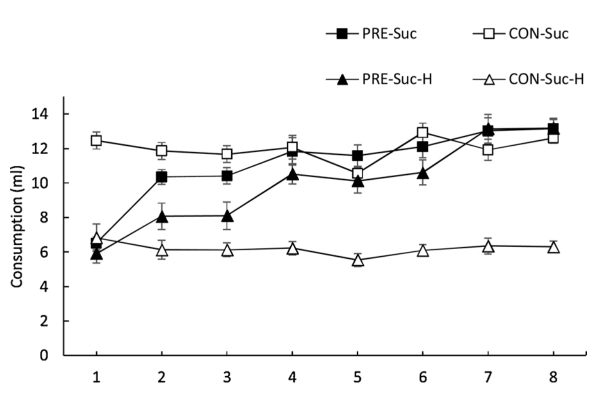

Fig. 7. Experiment 3: Mean consumption scores during preexposure for the preexposed (PRE) and control (CON) groups. Animals in the PRE groups received sucrose (Suc); those in the CON groups received access to water. H (hungry) animals were food-deprived. Vertical bars represent SEMs.

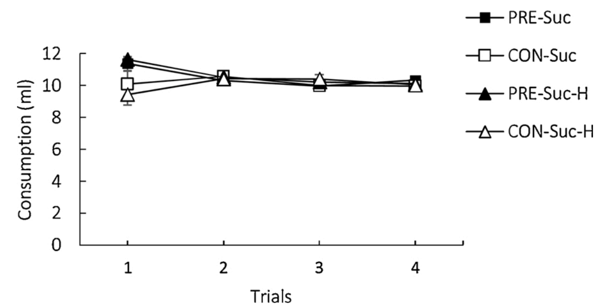

Fig. 8. Experiment 3: Group means for consumption of the almond-sucrose compound solution during the conditioning phase for animals in the preexposed (PRE) and the control (CON) groups. H (hungry) animals were food-deprived. Vertical bars represent SEMs.

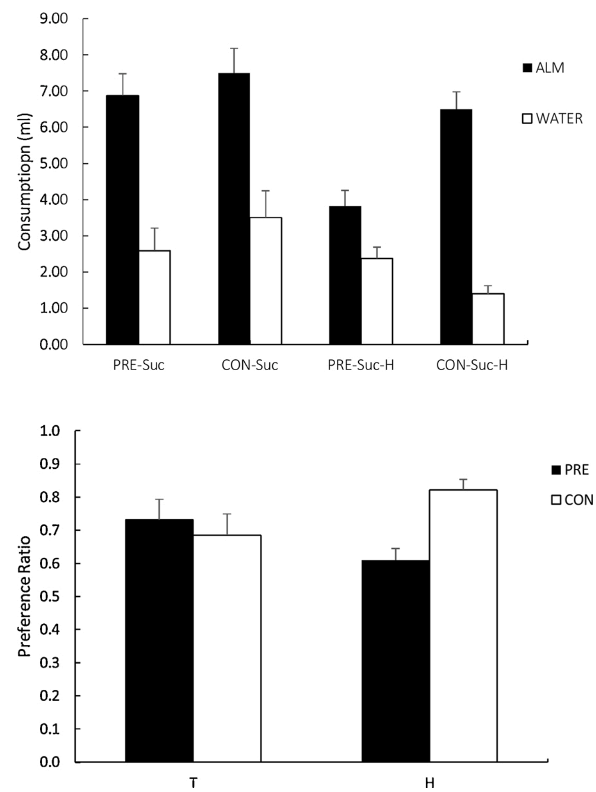

Fig. 9. Experiment 3: Upper panel: Group means for consumption of almond (ALM) and water during the test for animals in the preexposed (PRE) and the control (CON) groups. H (hungry) animals were food-deprived; others were thirsty (T) but not food deprived. Lower panel: mean ratio of almond intake over total intake. Vertical bars represent SEMs.

The experiments reported by Harris et al. (2000), and described above, have shown that a US-preexposure effect with sucrose as the US, can be obtained if the animals are hungry throughout, or at any stage of, the procedure (i.e., it is found in subjects that are food-deprived just in training or just at test). Our results indicate that the effect is absent, however, when access to food is maintained throughout the experiment. We should acknowledge, however, that two of the studies reported by Harris et al. (their Experiments 4 and 7) included animals that were not food-deprived at any stage, and that these showed evidence of a US-preexposure effect. We cannot fully resolve this discrepancy, but would note the possible role of the phenomenon of "latent hunger"—

hunger consequent on the inhibition of feeding that can be induced by the state of thirst (e.g., Rolls and McFarland, 1973). It is possible, then, that water-deprived rats may be in a self–imposed state of hunger even though food is available. The schedule of access to water in our experimental procedure was relatively generous making latent hunger unlikely. The schedule of water deprivation used by Harris et al. (which was certainly more severe than ours in the case of their Experiment 4) might have resulted in the rats being in a state of hunger throughout the procedure. If so, the conclusion prompted by the present results, that the US-preexposure effect with sucrose is not to be obtained in non-hungry animals, would remain valid.

5. General discussion

The principal findings of these experiments can be summarised briefly. The preference for an initially neutral flavor that is established by pairing it with a sweetener can be reduced by giving prior exposure to the sweet substance. When nonnutritive saccharin is used as the sweetener, this version of a US-preexposure effect is found whether or not the subjects are hungry. When the sweetener is sucrose, however, the US-preexposure effect is obtained when the animals are hungry, but not when they are not. These results have implications for our accounts of the source of the US-preexposure effect and of the nature of the learning that underlies the acquisition of a flavor preference.

5.1. Effects with saccharin

The absence of an effect of motivational state when saccharin is used as the US may be anticipated on the basis of the fact that this substance possesses only sensory properties and has no nutritional consequences. What is learned during pairings of saccharin and a flavor such as almond, whether this be the formation of an almond-sweet association or the formation of a configurational representation of the compound, will depend solely on these sensory properties and the motivational state would thus be irrelevant. The processes responsible for the US-preexposure effect should therefore operate both in hungry and non-hungry rats.

This process can be expected to differ from that responsible for the US-preexposure effect obtained when sucrose is the US. The explanation offered for the effect obtained with sucrose has been in terms of blocking. It is argued (see Gil et al., 2011) that preexposure to sucrose allows the formation of an association between the sweet taste of sucrose and the metabolic change ("calories") that follow. The existence of this sweet-taste to calories link could then act to block the formation of a flavor-calories link during subsequent conditioning trials with the added flavor. The results obtained with non-nutritive saccharin in Experiments 1 and 2 show that a US-preexposure effect can be obtained when this form of learning, and of blocking, is not possible; an alternative explanation is therefore required for the effects of preexposure to this US.

The alternative, suggested by Gil et al. (2014), has been in terms of habituation. The preexposure procedure in these experiments, repeated presentation of an event without any scheduled consequence, is essentially an habituation procedure. The most obvious effect of this procedure is the waning of any unconditioned response evoked by the stimulus (demonstrated as a reduction in neophobia in our experiments). But Hall and Rodriguez (2017, 2020) have argued that the loss of the unconditioned response produce by simple exposure to a stimulus reflects a more general reduction in its salience. An exposed stimulus that is less salient than a novel one will be less able to modify what occurs in the next stage of training, whether this is the formation of a configural representation of a flavor-saccharin compound or the formation of an association between these cues.

5.2. Effects with sucrose

In contrast to the results obtained with saccharin, the effects of preexposure to a sucrose have been found, in an experiment using procedures that exactly match those of the study with saccharin, to depend on the animal's motivational state. Such a difference between the two sweeteners might be expected on the basis of studies using the basic flavor-preference procedure.

Studies of flavor-preference conditioning in rats have demonstrated that the state of hunger can determine what is learned and/or what controls performance when sucrose is the US, whereas no such effect is found with a nonnutritive US. Thus, Fedorchak and Bolles (1987) found that the preference for a flavor paired with sucrose was enhanced when the rats were food-deprived prior to the test, whereas a preference established by training with saccharin was not affected. Capaldi et al. (1994) demonstrated equivalent results for the effect of food-deprivation during training.

On the basis of these and their own results, Harris et al. (2000) concluded that when rats are food-deprived a preference conditioned with sucrose depends chiefly, even exclusively, on an association between the flavor and calories, and that learning about the sweet taste is irrelevant. Given this analysis, a US-preexposure effect can be expected in hungry rats trained with sucrose. It will not be the result of an habituation process of the sort postulated for saccharin (we may assume that the metabolic consequences of consumption of sucrose do not habituate as sensory cues will), but it might be expected on the basis of blocking. Preexposure to sucrose will establish a sweet taste as a signal for these consequences, giving this cue the ability to block the acquisition of associative strength by the added flavor when it comes to the conditioning phase.

What remains to be explained is the absence of a US-preexposure effect in nonhungry animals trained with sucrose. As Experiment 3 has shown, pairing of a flavor with sucrose establishes a clear preference in such animals, the size of which is not influenced by preexposure to the US. The evidence just cited implies that, in contrast to the case in which the animals are hungry, this preference will be based on the sweet taste of the sucrose rather than on its nutritive properties. We need to explain, therefore, why there is no effect of US-preexposure in this case when such an effect is clearly evident when saccharin serves as the US – that is, in a procedure that is also thought to depend on learning about the sensory rather than the nutritive properties of the US. If the effectiveness of the sweet taste of saccharin as a US can habituate over the course of preexposure, why not also that of the sweet taste of sucrose in the nonhungry animals?

5.3. Factors affecting habituation to sweet tastes

A possible answer to this question comes from consideration of factors affecting the course of habituation. As we have noted, the account offered by Hall and Rodriguez (2017, 2020) holds that repeated exposure to a stimulus on its own not only reduces the overt response it evokes but also reduces its effective salience more generally. The critical factor in producing habituation is that the stimulus is followed by no consequent event. This is true of exposure to the sweet taste of saccharin – that is, this sweet taste is followed by no obvious consequences, and exposure to it should always result in habituation. After exposure to nutritive sucrose, however, metabolic consequences will occur; thus, in this case, the sweet taste will be followed by other, presumably salient, physiological changes. Our account suggests that this would be effective in preventing habituation. In the absence of such habituation, associative learning involving the sweet taste of sucrose can be expected in animals given preexposure to sucrose, just as for subjects for whom the sucrose is novel.

Evidence relevant to this analysis can be found in studies of the human response to foodstuffs. The idea that habituation might control the human response to food has been reviewed and developed by Epstein et al. (2009), but their account has been concerned principally with the short-term, within-session, effects that might be responsible for cessation of eating a given meal. Our rat subjects received their sucrose presentation at daily intervals, meaning that we require information on long-term habituation effects. Some effects have been obtained in studies of people given the same meal day after day. These have shown a decline in acceptance and intake of that food (e.g., Meiselman et al., 2000) and in willingness to work for it (Epstein et al., 2011), although whether these changes are a consequence of habituation has been disputed (Møller and Köster, 2012).

The picture may be different for a highly preferred substance like sucrose. Hetherington et al. (2002) examined the effects of giving subjects a daily session of access to chocolate (for a period of 22 days in one of their experiments), and although the rated pleasantness of the chocolate declined, there was no decline in the amount consumed, implying that some aspects at least, are not subject to habituation. Appleton et al. (2018) have provided a systematic review of explicit studies of the response to sweetness (often supplied in the form of sucrose). They considered seven population studies and 14 controlled trials; the procedures employed were very varied, as were the measures taken, leading to caution about the general conclusions. These were, however, that repeated exposure to a sweet taste can produce a reduced preference in the short term, but that there was little indication of such an effect in the long term. This conclusion is consistent with our account which requires that habituation training with spaced trials will not attenuate the effectiveness of sucrose for rats. It may be noted that our account requires that the metabolic effect of sucrose in attenuating habituation to its taste must be effective even when the rats are not food deprived, as in our Experiment 3. It is relevant therefore to note that in many of the studies reviewed by Appleton et al. (2018) the subjects were well-fed individuals.

Author statement

Marta Gil: Conceptualization, Experimental design, Data collection and analyses, and writing the original draft. **Geoffrey Hall:** Conceptualization, Experimental design, Reviewing and Editing. **Isabel de Brugada**: Conceptualization, Experimental design, Reviewing and Editing.

Acknowledgements

This work was supported by project PGC2018-095965-B-I00 FEDER/ Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación.

We thank M. R. Yeomans for helpful discussion.

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