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Does drinking saccharin weaken an association of sweet with calories? Pre-exposure effects in flavor preference learning.

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Highlights

Rats can learn flavor-flavor and flavor-nutrients associations

The expression of conditioned taste preferences depends on the motivational state.

Saccharin exposure does not weaken the formation of a sweet-calorie association

Abstract

The main aim of this experiment was to examine the claim that exposure to non-nutritive sweeteners weakens the formation of a sweet-calorie association. Three groups of food-deprived rats received training in which they drank an almond-flavored maltodextrin and saccharin solution. A final test phase assessed their preference for almond. The groups differed in preexposure prior to training. One was pre-exposed to saccharin, one to saccharin plus maltodextrin, and the third, control condition, received only water at this stage. When the rats continued under food deprivation for the test phase, the group exposed to the compound (saccharin plus maltodextrin) showed a weaker preference than the other two groups, while those pre-exposed to saccharin showed as strong a preference as the controls. When the test was conducted with the rats no longer food-deprived, only the water group showed a strong preference. These results support the proposal that rats can form both flavor-flavor and flavor-nutrient associations, expression of which will depend on motivational state. They did not find support for the suggestion that prior exposure to a non-nutritive sweetener can enhance subsequent learning about the nutritive properties of a sweet food.

Keywords: *preference learning, flavor-flavor, flavor-nutrient, US-preexposure effect, saccharin, extinction*

1. Introduction

Rats will exhibit a preference for an initially neutral flavor after it has been paired with a palatable taste such as sucrose or glucose. This acquired preference is an instance of classical conditioning with the flavor as the conditioned stimulus (CS) and the sugar as the unconditioned stimulus (US) [1] but see [2,3] . This preference could be a consequence of learning about the palatable sensory properties of the US (and thus reflect a flavor-flavor association) (e.g., [4, 5, 6, 7]) and/or of learning about the nutritional content of the US (reflecting a flavor-nutrient association) (e.g., [8, 9, 10, 11]). Evidence for flavor-flavor and flavor-nutrient learning has been obtained from a variety of procedures. These have included the use of non-nutritive sweeteners as US (e.g., [7, 12, 13]), manipulating the hunger/satiety state (e.g., [4, 14]), applying sham feeding procedures [5], devaluation procedures (e.g., [6, 15]), or using nutrients as US through intragastric infusions (e.g., [9, 16]).Thus, these two types of associations that potentially underly a flavor preference are independent (e.g. [1, 17] and can be acquired simultaneously (e.g., [18]) or even interact (e.g., [19, 20]). For example, acquired preferences following conditioning by intragastric infusions of nutrients are greater when accompanied by a palatable taste cue (saccharin) than when no such taste signal is received [20].

Acquisition of a conditioned preference can be prevented or hindered by exposure of the US prior to training, the US-preexposure effect (e.g., [7]). Pre-exposure to sucrose which has both a sweet taste and nutritive properties can weaken subsequent learning of both flavor-flavor and flavor-nutrient associations [18, 21]. But what is the effects of preexposure to a sweetener such as saccharin, which has no nutritional properties? This question is of particular interest in the context of the current "obesity epidemic." There has been some controversy over the claim that artificially sweetened beverages provide a healthy alternative to sugar-sweetened beverages (e.g., [22, 23] [24, for a recent metanalysis]). A controversial argument against this claim has been made following experiments that appear to show that rats given access to saccharin gained more weight than controls [25, 26] (but see [27]). In this context Davidson et al. [28] suggested that one possible effect of pre-exposure to saccharin is that such exposure weakens sweetness as a signal for predicting calories. This suggestion assumes that there is an innate or perinatally acquired association between sweetness and calories(e.g., [29, 30]). Since no calories result from the intake of non-nutritive

sweeteners, a process of extinction will result in a weaker sweet-calorie association [31]. According to this view, because a sweet taste normally acts as a CS+ that predicts the occurrence of nutrients (US), it blocks or overshadows other flavoured cues from entering into association with nutrients during a conditioning process. However, the authors ague that, if a preestablished relationship between sweetness and calories is weakened, sweetness will not compete so effectivelywith other flavors. As a consequence, pre-exposure to saccharin is expected to enhance subsequent flavor-nutrient learning when a sweet and caloric nutrient is used as the US.

To test this prediction Davidson et al. [28] gave one group of rats pre-exposure to a saccharin solution, while a control group received only water. All subjects then underwent a training procedure consisting of pairings of one flavor (CS_{GLU}+) with glucose and of another flavor with polycose (CS_{POLY}+). Polycose is a form of maltodextrin that has approximately the same caloric qualities as glucose but lacks its sweet taste [5]. The rats had free access to food in this phase of training. After 24 h of food deprivation, responses to the CS flavors were assessed in preference tests. The rats were given two tubes, one containing the CS_{GLU}+ flavor, the other the CS_{POLY}+ flavor. Rats pre-exposed to saccharin showed a higher level of consumption of the glucose-paired flavor than that shown by the control group. This result encouraged the claim that the extinction of the sweet-calorie relationship leads to excessive intake of sweet foods. As sweetness will no longer be a predictive cue for food energy supply, the physiological responses responsible for initiating satiety responses and inhibiting appetite are not initiated. In favour of their hypothesis, in a second experiment the authors found that rats on a high-fat diet sweetened with glucose but also supplemented with saccharin gained more weight and consumed more calories. This pattern did not occur in other non-sweet high fat diets [28].

The results from the experiment reported by Davidson et al. [28] contrast with those reported by Harris et al. [18]. This study also investigated the effects of preexposure to saccharin on subsequent flavor conditioning using a sugar as the US. In this study just one flavor (almond) was used. In the conditioning phase almond was added to sucrose. The test procedure consisted of a 2-bottle choice between the almond solution and plain water. Over the course of several experiments, they found that saccharin pre-exposure resulted in a weakened almond preference (a version of the US-preexposure effect) when the subjects were trained and tested under non-food

deprivation conditions. However, when tested in a state of hunger, pre-exposed subjects expressed a strong conditioned preference for almond, one similar to that of controls. These authors interpreted the results as indicating that, when animals are not food deprived, their behavior is controlled by a flavor-flavor association, but that, when they are hungry, behavior is mainly governed by a flavor-nutrient association. In the present context the critical finding from these experiments is that, when the animals are hungry on test (the procedure used by Davidson et al. [28]), subjects given preexposure did not show the US-preexposure effect; that is, they consumed as much of the test flavor as the control subjects. They did not, however, consume *more* than the controls, as might be expected on the basis of the results of Davidson et al. [28]. The failure of the study by Harris et al. [18] to find the effect later reported by Davidson et al. [28] prompted the present experiment.

This experiment used the same basic training procedures as those employed by Harris et al. [18]; that is, just one flavor was employed during conditioning, and the test procedure pitted this flavor against water. Although we slightly increased the amount of training given at each stage in our experiment (in comparison to Harris et al. [18]) the procedure used in these experiments differs from that of Davidson et al. [28] in that they gave very extensive training at each stage of their experiment. We gave subjects in the experimental group pre-exposure to saccharin, whereas control subjects received just water at this stage. This was followed by conditioning trials in which a novel flavor (almond) was paired with a nutrient US. The latter was a compound of maltodextrin and saccharin, thus maintaining the sweet flavor that was used in the preexposure phase for the experimental group. We also included a third group that was exposed to the compound to be used as the US; thus, these rats were pre-exposed to the compound of saccharin and maltodextrin. This provided a check that the procedure was one that would produce a standard US-pre-exposure effect. In the test phase the subjects were presented with the flavor cue (almond) versus water. Preference tests were conducted both when the subjects had been food-deprived and when they had received full access to food.

This design allowed the opportunity to confirm the finding of Harris et al. [18] that, after preexposure to saccharin, the US-preexposure effect is found when tested under satiety. More critically, would subjects tested in a state of hunger express not only a preference, but a stronger preference, than that shown by the control group, the

outcome expected on the basis of the results of Davidson et al. [28]. Finally, we might expect the non-pre-exposed group to express preference in both conditions, whereas the saccharin plus maltodextrin group would show little or no preference in either motivational condition.

2. Method

2.1. Subjects and apparatus

The subjects were 64 Wistar rats, of which half were non-naïve. The non-naive rats came from a perceptual learning experiment, had no prior contact with the solutions and procedures used here and were equally distributed to the different experimental conditions. This was carried out in two batches that counterbalanced the order of the motivational states of animals during testing. Rats were divided into three groups matched for weight, and previous experience: Saccharin+Maltodextrin Group (*N*=22; Weight= 331.7 g), Saccharin Group (*N*=22 Weight= 337.8 g), Water Group (*N*=20, Weight= 331.2 g). Rats were individually housed in translucent plastic cages (35 × 12 × 22 cm) with wood shavings as bedding and were monitored daily by those responsible for animal welfare in the research center. Rats were maintained on a 12-h light/dark cycle for the whole procedure, starting the light cycle at 8:00 am.

The animals had restricted access to standard chow and water throughout pre-exposure, training, and half of the testing. The rats were reduced to 80-90% of their ad lib weights and were maintained at this level by being fed a restricted amount of food in the afternoon session. Access to the target solution and water was given in two sessions per day (morning and afternoon sessions respectively). The experimental solutions were prepared every day with tap water and presented to animals in centrifuge tubes (50 ml capacity) with stainless steel, ball-bearing-tipped spouts. All tubes were placed in the middle of the front metal cover of the cages in the sessions in which just a single bottle was presented to avoid the effects of any side preferences during the choice tests. Consumption was measured by weighing tubes before and after each procedure. The flavored solutions were composed of 0.4% sodium saccharin, 21.6% maltodextrin (dextrinomaltose, 2% monosaccharides, 7% disaccharides, 91% higher polysaccharides; Guinama Brand) and 0.05% almond flavoring (Manuel Riesgo).

The Ethics Committee for Animal Research at the University of Granada (05/11/2020/125) approved all the procedures described in this paper. These procedures were classified as low severity according to European guidelines.

2.2. Procedure

At 16.00 hrs on the day before the experiment began water was removed. Thereafter fluid access was limited to two 30-minute daily sessions: a morning session starting at 10:00 and an afternoon session starting at 16:00. On Days 1 and 2 the animals had access to water, allowing us to measure baseline consumption and allowing the rats to habituate to the schedule.

As summarized in Table 1, on Day 3 all rats started the 10-day preexposure phase. The rats were given 15 ml of either a saccharin solution (Saccharin Group), a saccharin + maltodextrin solution (Saccharin + Maltodextrin Group) or water (Water Group). The Water Group received water every day except the last day, when they received saccharin to prevent neophobia during training. On Day 13 the 4-day training phase began, in which all three groups were given 10 ml of the almond-flavored saccharin + maltodextrin mixture.

On Days 17 and 18 the rats underwent two 2-bottle training sessions prior to the final tests; this was to acclimatize them to the test procedure and also to weaken possible side preferences (for a similar procedure, see [32]). This phase involved performing the same procedure as in the tests but with both tubes containing water. The 30-min test procedure consisted of presenting two tubes, one containing 30 ml of almond-flavored water and other containing 30 ml of plain water. After 15 min the experimenter entered the room and changed tubes´ position (those that were on the left are now on the right and vice versa), thus minimizing any left/right bias. Also, during the preference tests, the initial position in which almond or water was placed was also randomised across animals and tests. After a further 15 min, all tubes were removed and consumption from each tube was measured. Animals were tested for preferences in two motivational states, so that rats in Batch 1 were tested first under food deprivation conditions and afterwards under non-food deprivation conditions, and Batch 2 was tested in the opposite order. Testing lasted 6 days (4 days under hunger and 2 days under satiation). Rats in Batch 1, when test 4 was completed, were given ad lib food so that during tests 5 and 6, preference was measured in the non-deprived state. In contrast,

rats in batch 2, the morning after finishing the two-bottle training, were given ad libitum food to perform tests 1 and 2 in a non-deprived state. When they finished the second undeprived test, food was removed again and remained in the same food-deprived condition as during pre-exposure and conditioning. Before starting the four preference tests, they spent 48 hours of deprivation in these conditions receiving water during the morning and afternoon sessions.

Table 1. *Experimental procedure*

Group	*Pre-exposure* (Dep) Days 3-12	*Training* (Dep) Days 13-16	*2-Bottle training* (Dep) Days 17-18	*Preference test* (Dep)x4 / (NoDep)x2 Days 19-24
Saccharin	S			
Saccharin + Maltodextrin	S+M	A+S+M	Water vs Water	A vs Water
Water	W*			

Note. "S" denotes saccharin, "M" is maltodextrin, "W" refers to water and "A" means almond, the flavored cue. "+" refers to a mixture of different solutes. "*" denotes that this group the last day received a familiarisation of saccharin instead of water. "vs" is refering to the simultaneous presentation of different substances. "Dep" means food deprivation, whereas "NoDep" means non-food deprivations. "x4" and "x2" refers to the number of days that rats do the test under certain motivational state. "/" denotes counterbalancing between batches when performing the order of tests.

2.3. Statistical analysis

General linear model null hypothesis testing was conducted, assuming a rejection level of $p < 0.05$, using Greenhouse–Geisser corrections for mixed factorial analysis of variance when needed. Partial eta squared, and Cohen's d tests were used to measure effect sizes. Post-hoc tests were carried out for multiple comparisons applying the Holm's correction.

Intakes during training and pre-exposure was analyzed with a repeated measures ANOVA, with Batch and Group as between-subject variables and Day as the within-subject measure.

To analyze the animals' preferences in the tests, preference ratios were calculated. These consisted of dividing consumption of the CS+ by total consumption. A score above 0.5 would indicate a preference for CS+ (preference), while a score below 0.5 would indicate a preference for water (avoidance). A score not differing from

0.5 (random) would indicate neither preference nor avoidance of flavor. To study the preference of the rats across the different motivational states, the preference ratios in each condition were averaged leaving two final ratios: satiety versus hunger preference ratio. These data were submitted to a repeated measures ANOVA with Batch and Group as between-subject measures and Deprivation as within-subject measure.

3. Results

Mean scores for consumption during pre-exposure are shown in Table 2. During this phase, one rat from the Saccharin group in Batch 1 had to be excluded because it did not drink any saccharin. A Greenhouse-Geisser correction was applied to the intake data from this phase after Mauchly´s test of sphericity revealed a violation of the sphericity assumption. Results revealed a main effect of Day $F(6.79, 387.24)= 26.44$, $p< 0.001$, $\eta_p^{2=}$ 0.31, Group $F(2,57)= 99.90$, $p< 0.001$, $\eta_p^{2=}$ 0.77 and Batch $F(1,57)= 6.96$, $p< 0.01$, $\eta_p^{2=}$ 0.10. The interaction Day*Group also reach significance $F(13.58, 387.24)= 11.23$, $p< 0.001$, $\eta_p^{2=}$ 0.28. The interactions Day*Batch $F(6.79, 387.24)= 1.37$, $p>0.05$, $\eta_p^{2=}$ 0.02, Day*Batch*Group $F(13.58, 387.24)= 1.07$, $p>0.05$ $\eta_p^{2=}$ 0.03 and Group*Batch $F<1$ were not significant. The Day*Group interaction meant that there were significant differences between groups during Pre-exposure (Saccharin+Maltodextrin > Saccharin > Water). Presumably this was because of differences in palatability. The interaction between Group and Day reflected increased intakes by both experimental groups as neophobia attenuated. The significant main effect of Batch means that the first batch consumed overall more than the second batch $t(2.6)$, $p_{holm}= 0.01$, $d= 0.3$ (see Table 2).

Table 2. *Solution intakes during pre-exposure*

Solution	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day 8	Day9	Day10
Batch 1										
Saccharin (S)										
M	3.2	7.2	6.7	7.6	8.0	9.9	9.9	9.7	11.7	11.2
SE	0.3	1.2	0.8	1.2	1.3	1.2	1.2	1.2	1.1	1.0
Saccharin + Maltodextrin (S+M)										
M	7.8	13	13.8	14.3	14.5	14.9	14.8	14.9	15.1	15.1
SE	0.9	0.9	0.6	0.5	0.5	0.5	0.8	0.5	0.4	0.1
Water (W)										
M	5.3	5.7	4.1	4.7	3.2	5.6	4.5	3.9	4.1	5.4
SE	0.3	0.5	0.4	0.7	0.8	0.4	0.8	0.8	0.4	1.2

Batch 2										
Saccharin (S)										
M	2.1	6.1	7.8	7.6	7.8	8.5	9.2	8.7	10.7	10.4
SE	0.6	1.4	1.5	1.6	1.2	1.4	1.6	1.2	1.2	1.2
Saccharin + Maltodextrin (S+M)										
M	6.2	9.8	12.4	13.2	13.7	14.0	13.6	14.4	14.6	14.8
SE	0.6	0.8	0.4	0.2	0.1	0.1	0.1	0.05	0.1	0.1
Water (W)										
M	3.9	4.3	3.6	1.9	2.9	2.3	4.8	2.9	2.0	3.2
SE	0.6	0.5	0.6	0.6	0.5	1.0	0.7	0.6	0.7	1.0

Data from training sessions were submitted to a repeated measures ANOVA. A Greenhouse-Geisser correction was again applied. The main effect of Day $F(1.59, 91.08)= 7.88$, $p= 0.002$, $\eta_p^{2=}$ 0.12, Batch $F(1,57)= 10.14$, $p= 0.001$, $\eta_p^{2=}$0.15 and Group $F(2,57)= 3.2$, $p= 0.05$, $\eta_p^{2=}$0.10 reached significance. None of the interactions reached significance: Day*Batch, Day *Group $Fs<1$, Batch*Group $F(2,57)= 1.03$, $p> 0.05$, $\eta_p^{2=}$0.03, Day*Batch*Group Batch $F(3.1, 91.08)=1.6$, $p> 0.05$, $\eta_p^{2=}$0.05. Post-hoc tests regarding the Group factor revealed that there were only significant differences when Saccharin+Maltodextrin Group was compared to the Water Group $t=2.54$, $p_{holm}=0.04$, $d=0.32$. The Saccharin Group did not differ from either the Saccharin + Maltodextrin Group $t=-1.08$, $p_{holm}>0.05$ $d= -0.3$ or from the Control Water Group $t=1.45$, $p_{holm}> 0.05$ $d=0.3$. These differences may reflect neophobia to the flavored maltodextrin plus saccharin solution in both Saccharin and Water groups. The mean intakes over all training days were: Saccharin $M=9.69$ $SE=0.08$, Saccharin+Maltodextrin $M= 9.83$, $SE= 0.109$, Water $M=9.49$, $SE= 0.1$). The factor Batch again showed a higher total consumption in the first batch than in the second batch, $t=3.1$, $p_{holm}=0.02$, $d= 0.4$, with means over all days of training: Batch 1, $M= 9.85$, $SE= 0.07$; Batch 2, $M= 9.50$, $SE= 0.79$).

The most important data, those from the test sessions, are shown in Table 3. Table 3 depicts the mean total intakes of the rats across the different tests in the food deprived or undeprived state. It can be observed that in the food deprived state, the rats drank less amount of liquid, a usual pattern for this motivational condition. These data were transformed into preference ratios to render them more comparable across conditions (See Figure 1). It may be seen that in the food-deprived condition the Saccharin and Water groups showed a greater almond preference than did the

Saccharin+Maltodextrin group, while there appears to be no difference between the Saccharin and Water groups. When tested in the absence of food-deprivation condition, only the Water group expressed a conditioned preference. Analysis of the test data revealed a significant main effect of Deprivation $F(1,57)=19.97$, $p< 0.001$, $\eta_p^{2=}0.26$, Batch $F(1,57)=7.6$, $p= 0.008$, $\eta_p^{2=}0.11$, and Group $F(2,57)=8.53$, $p< 0.001$, $\eta_p^{2=}0.23$. The interaction between Deprivation and Group was also significant, $F(2,57)=5.99$, $p= 0.004$, $\eta_p^{2=}0.17$. The remining interactions did not reach significance, all $Fs<1$.

Post hoc comparisons revealed that in the food-deprived condition there were significant differences between the Saccharin Group and the Saccharin + Maltodextrin Group, $t=3.19$ $p_{holm}<0.016$ $d=0.97$, and between the Saccharin + Maltodextrin Group and the Water Group, $t=-3.429$ $p_{holm}= 0.009$, $d=-1.06$. No significant differences were found between the Saccharin Group and the Water Group $t<1$. When the preference ratios obtained under food-deprived conditions were submitted to a one-sample t-test to be compared to the 0.5 value, the ratios for all groups reached significance: Saccharin Group $t(20)=13.87$, $p<0.001$, $d= 3.02$, Saccharin + Maltodextrin Group $t(21)=3.29$, $p=0.003$, $d= 0.7$, Water Group $t(19)=9.78$, $p<0.001$, $d= 2.18$.

As for preferences obtained in the non-deprived condition, post hoc comparisons revealed significant differences between Saccharin and Water Groups, $t=-1.14$, $p_{holm}=0.004$, $d= -1.14$, and between the Saccharin + Maltodextrin and the Water Groups, $t=-3.21$, $p_{holm}=0.016$, $d= -0.99$. No difference was found between the Saccharin and Saccharin + Maltodextrin Groups, $t<1$. When the preference ratios obtained under undeprived conditions were submitted to a one-sample t-test, only the Water Group $t(19)=7.675$, $p<0.001$, $d= 1.71$ reached significance, while Saccharin Group $t(20)=1.29$, $p>0.05$, $d= 0.28$ and Saccharin + Maltodextrin Group $t(21)=1.52$, $p>0.05$, $d= 0.21$ did not differ to 0.5 value.

Table 3. *Direct consumption during preference tests.*

	Food Deprived		Non Food Deprived	
	Almond	Water	Almond	Water
S	4.63 (0.44)	1.31 (0.14)	7.51 (0.79)	6.00 (0.41)
S+M	3.43 (0.3)	2.3 (0.58)	7.06 (0.61)	6.67 (0.85)
W	5.30 (0.46)	1.30 (0.21)	9.30 (0.54)	4.78 (0.32)

Figure 1. *Preference ratios for the CS+over total consumption*

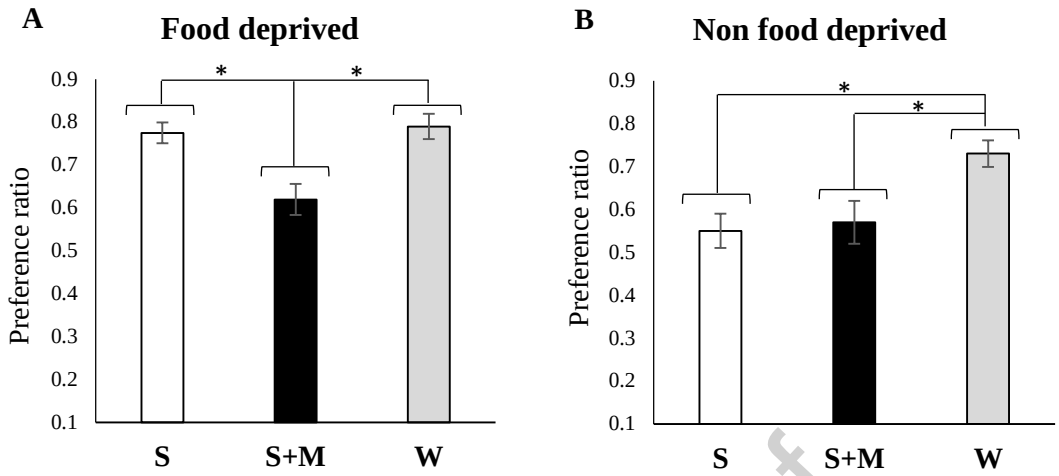

Fig 1. Data from the preference tests Mean ratio of almond consumption over total consumption across the groups Panel A: food deprived condition. Panel B: non food deprived condition.

4. Discussion

The present results are consistent with those reported by Harris et al. [18], in that they demonstrate that, when hungry, animals can learn both flavor-flavor and flavor-nutrient associations and that the expression of each association is modulated by the animals' motivational state at test.

Of particular interest were the test data from the Saccharin Group. As shown in Figure 1, when hungry on test, they revealed as strong an almond preference as the Water Group but, when tested sated, they failed to show the strong almond preference that the Water Group again showed. This may be interpreted as indicating that during training the Saccharin Group had acquired a flavor-nutrient association but not a flavor-flavor association. It does not appear, however that experience of saccharin weakens a prior sweet-calorie association. According to this proposal, preference for almond should have been greater in the Saccharin than in the Water Group -- a result consistent with that reported by Davidson et al. [28]. This predicted difference was not found in the present experiment.

As for the Saccharin +Maltodextrin group, their almond preference data were entirely consistent with the well-documented US-pre-exposure effect. According to

standard accounts of this effect (e.g., [33]), since the addition of almond in the training stage did not signal any change either to the taste or to the energy content of the already familiar compound, neither a new flavor-flavor or a new flavor-calorie association will be formed. The US-preexposure effect has been mainly explained in terms of a blocking process but also in terms of habituation [33, 34, 35]. In associative learning preparations other than conditioned preferences, it has been observed that part of the blocking effect comes from the association between the US with the context in which it is preexposed [36] and/or with relevant cues [37]. In the case of flavor preference learning, it has been observed that a change of the context does not alter the US pre-exposure effect, thus leaving little scope for this interpretation [38] (Gil et al., 2011). In the case of sugar, it has been suggested that during pre-exposure an association is generated or strengthened between its sensory properties -the sweet taste- (CS+) and its nutritional properties (US). Thus, this learning would block any subsequent association between a flavored cue and the nutritional properties of the taste (See Gil et al., [21]). This last process could explain the pre-exposure effect found in the Saccharin + Maltodextrin group, that is to say, the association between saccharin´s sweet taste and maltodextrin´s calories would block the subsequent association between almond and calories.

The account is different for the group pre-exposed to saccharin, since the latter has the sweet taste but lacks the calories. In this case, two main interpretations have been suggested. On the one hand, as mentioned in the introduction, saccharin exposure could trigger sweet-caloric extinction [39]. This process would prevent a later blocking effect, but it could also devalue its sweet properties, preventing a subsequent flavor-flavor learning. The latter claim is not supported by our data. Another possibility to explain saccharin preexposure effect comes from a non-associative source. Since exposure to saccharin is not followed by any relevant nutritional consequence - especially in a deprived state - pre-exposure to this taste could trigger a habituation process and therefore weaken its effectiveness as a US [21]. This last proposal is not novel, as it has already been used to explain US pre-exposure effects where the blocking explanation is not plausible, as for example in the case of some specific drugs like morphine [40, 41].

It is clear that both flavor-flavor and flavor-nutrient learning play a crucial role in eating behavior, developing food preferences, biasing food choice, or determinating the portion size of a meal (e.g., [42, 43]). In particular, in recent years the flavor-

nutrient phenomenon has acquired great relevance given its role in stimulating the intake of caloric foods – of particular importance in the context of today's obesogenic environments [10, 44, 45, 46]. The past few decades have provided much insight into the underlying physiological mechanisms of nutrient-based preference learning, operating by the gut´s nutrient detection and sending of reward signals to the brain (see [1, 42] for reviews). This process, now known as appetition, is the opposite of satiation, which is responsible for inhibitory intake responses following the nutrient detection [46]. While acquiring preferences for nutrient-rich foods is a very useful tool in conditions of scarcity, nowadays with the widespread availability of highly palatable, low-cost, energy-dense foods, this learning may be counterproductive to health [47]. Thus, it has been suggested that the appetition properties of certain nutrients are key to the overstimulation of intake of hypercaloric foods, and thus playing a potential role in obesity [46]. Of particular interest is the case of sugary foods and drinks, which have palatability and caloric properties, both critical for the development of food preferences. Given this situation, it is possible that non-nutritive sweeteners, lacking such appetition properties could be a suitable substitute for the sugar-based items.

The study of the beneficial/harmful effects of non-nutritive sweeteners has been the subject of much research given the ubiquity of high-calorie food products and the increasing rates of obesity in the past decades (for a review see [48, 49]).One of the possible adverse effects of the use of non-nutritive sweeteners has been the subject of this research: the presumed extinction of the sweet-calorie relationship. It has been suggested that the possible triggering of this process could be responsible for a deficit in the regulation of intake (for example, the inability to control calorie intake through conditioned satiety), ultimately leading to an increase in body weight [28]. Although we did not address this question directly in our experiment, we conclude that our results suggest that there is no such alteration of the sweetness-calorie relationship, at least when a flavor preference learning paradigm is used.

Some caveats have to be considered. First, it is possible that the failure to find a greater preference in the Saccharin group simply reflects a ceiling effect, given that, as shown in in the left-hand panel of Figure 1, the Water group displayed an almond preference approaching 80%. Secondly, there are differences in total consumption during the pre-exposure phase, with the Saccharin+Maltodextrin group consuming greater amounts than the Saccharin group. This could make it hard to interpret the US-

prexposure effect since the groups had a different amounts of exposure to the US. Also, it is also possible that the discrepancy between the present results and those reported by Harris et al. [18] and those of Davidson et al. [28], reflects differences in procedure. In particular, Davidson et al. [28] pre-exposed and trained rats for long periods of time and with unlimited access to the compounds. In contrast, the procedures used by Harris et al. [18] and in the present experiment involved much shorter conditioning times and limited access to the solutions. It is possible that differences in the amount of pre-exposure and conditioning could lead to different forms of learning. For example, prolonged pre-exposure to saccharin may be needed to weaken pre-existing sweet-calories associations, especially if, as has been suggested, the sweet-caloric association are innate. Therefore, future studies should focus on how other kind of procedures, different from those traditionally used in learning preferences (short, limited amount of exposure), might alter the content of learning and how it is expressed as a function of different motivational states.

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Transparency and Openness

This study has not been preregistered. The data of all the experiments are available in the APA´s repository on the Open Science Framework (OSF): https://osf.io/gr823 [50]

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