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Predictive learning in nutrient-based flavor conditioning

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This paper presents two experiments on nutrient-based flavor conditioning with rats as subjects and sucrose as the unconditioned stimulus (US). Experiment 1 was aimed at establishing an optimal control for conditioning, comparing simultaneous and serial presentations of a flavor and the US. The results showed that simultaneous, but not serial training, produced conditioning. Experiment 2 was designed to obtain evidence of summation as an index of both conditioned inhibition and predictive learning. Group Simultaneous received Pavlovian conditioned inhibition training during which flavor A was simultaneously paired with sucrose on excitatory trials (A+), and forming an unreinforced compound with flavor B on inhibitory trials (AB-). An independent excitor for the summation test was also trained by simultaneous pairings with sucrose (C+). In the control group (Blocked), the AB- trials were presented forming a block at the beginning of training to avoid a negative contingency-relationship with sucrose, and flavor A received serial rather than simultaneous pairing with sucrose (A → +). On the summation test, only in group Simultaneous was consumption of the CB compound lower than that of flavor C alone, suggesting that, during training, flavor A activated an expectancy of the US occurrence.

Aprendizaje predictivo en condicionamiento al sabor basado en nutrientes. Se presentan dos experimentos de condicionamiento al sabor basado en nutrientes con ratas. El Experimento 1 estableció una condición de control óptima para el condicionamiento comparando presentaciones simultáneas y seriales de un sabor y sacarosa. El procedimiento de entrenamiento simultáneo, pero no el serial, produjo condicionamiento. El Experimento 2 se diseñó para obtener evidencias de sumación como índice tanto de inhibición condicionada como de aprendizaje predictivo. El grupo simultáneo recibió entrenamiento en inhibición condicionada Pavloviana: el sabor A se presentó simultáneamente con sacarosa en los ensayos excitatorios (A+), y formando un compuesto no reforzado con el sabor B en los ensayos inhibitorios (AB-). Se entrenó también un excitador independiente para la prueba de sumación mediante emparejamientos simultáneos con sacarosa (C+). En el grupo control los ensayos AB- fueron presentados formando un bloque al inicio del entrenamiento, y el sabor A recibió emparejamientos seriales con la sacarosa en lugar de simultáneos (A → +). En la prueba de sumación, el consumo del compuesto CB fue menor que el del sabor C en solitario solo en el grupo simultáneo, sugiriendo que durante el entrenamiento el sabor A activó una expectativa de la ocurrencia de la sacarosa.

Learned flavor acceptance/preference is often established either through flavor-taste learning—pairing the target flavor with a palatable taste such as that of a saccharine solution (e.g., Fanselow & Birk, 1982; Holman, 1975)—or by flavor-nutrient learning—in which the target flavor is paired with a nutrient presented either orally or intragastrically (e.g., Capaldi, Campbell, Sheffer, & Bradford, 1987; Sclafani & Nissenbaum, 1988). Flavor preference learning has been considered as a form of Pavlovian conditioning (e.g., Rozin & Zellner, 1985) in which the target flavor acts as the conditioned stimulus (CS), and the unconditionally preferred taste or the nutrient act as unconditioned stimuli (USs).

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Two different learning mechanisms have been proposed to account for conditioned flavor preference (CFP). One of them is *affective learning* or *evaluative conditioning* in which there is a shift in the evaluation of the CS in the direction of the evaluation of the US. The CS recovers a representation of the US but not an expectation of its occurrence (Baeyens, Crombez, De Houwer, & Eelen, 1996). This mechanism affects consumption of the CS flavor activating a hedonic reaction. Thus, it is based on the learned sensory-affective properties of the flavor (Rozin & Zellner, 1985). The second mechanism is called *inference* or *contingency learning* by which the CS becomes a predictor for the US. Once the CS-US association is formed, the CS not only activates a representation of the US, but also an expectancy of its occurrence. This allows prediction for significant events such as caloric intake when subjects are hungry. In this case, the main determinants for food selection are the anticipated consequences of consumption (Rozin & Zellner, 1985), thus it may be described as predictive learning. However, it has been suggested that a «transfer of affect», rather than an «expectancy of receiving some reinforcement», underlies

flavor-nutrient learning (e.g., Capaldi, 1996; Capaldi & Privitera, 2008), due in part to the resistance to extinction using a nutrient as US. Nonetheless, there is evidence suggesting that learning about the nutrient consequences of the consumption of a flavored solution (i.e., flavor-nutrient learning) might be predictive —based on the anticipation of the US occurrence— whereas learning about the sensory-affective properties of its consumption (i.e., flavor-flavor learning) might not. Evidence comes, precisely, from studies on extinction of CFP.

It has been suggested that CFP can be extremely persistent (e.g., Capaldi, Myers, Campbell, & Sheffer, 1983). However, it is worth noting that persistence or resistance to extinction has often been demonstrated using differential training, in which a flavor is reinforced (CS+) whereas the alternative flavor is not (CS-), and a two-bottle choice tests (i.e., CS+ vs. CS-) during the extinction phase. This particular procedure has not been without criticism (e.g., Boakes, Colagiuri, & Mahon, 2010), as rats avoid the CS- both after differential and unpaired training procedures (Boakes et al., 2010; see also Harris, Gorrisen, Bailey, & Westbrook, 2000); therefore, this kind of test might confound preference for the CS+ with avoidance for the CS-, which would not extinguish (Zimmer-Hart & Rescorla, 1972). However, other studies have found resistance to extinction using more conventional training and test procedures. For instance, when thirsty rats are given a simultaneous compound of a hedonically neutral flavor and a palatable nutrient such as sucrose, they show a preference for the flavor over plain water when subsequently given a two-bottle test. Repeated testing does not produce a decrease in conditioned preference (e.g., Harris, Shand, Carrol, & Westbrook, 2004; Expts. 1A & 1B).

Interestingly, resistance to extinction of a nutrient-based conditioned preference is affected by motivational state during training and testing. Consider the study by Harris et al., (2004). Rats trained and tested thirsty will show resistance to extinction (Expts. 1A & 1B), but preference will decrease if animals trained thirsty are next tested hungry (Expt. 2B). Harris et al., (2000) established that rats' preference for a flavor was based solely on the flavor-taste association if the rats were not food deprived, but preference among food-deprived rats was based exclusively on a flavor-calorie association. Therefore, the dissociation between thirsty and hungry animals on extinction performance found in the experiments by Harris et al., (2004) suggests that two kinds of association are learned during training, and that motivational state selects which one controls performance at the time of testing, one of them showing conventional extinction (flavor-calorie) when animals are hungry.

Taken together, these studies suggest that flavor-calorie association might be an instance of predictive learning, based on the value of the CS as a predictor of the occurrence of the US (inference or contingency learning), whereas flavor-flavor learning might not (affective learning or evaluative conditioning). Accordingly, the US representation repeatedly activated by the flavor CS in absence of the nutrient US during the extinction phase might be in the base of the decrease in preference.

The main goal of this study was to further examine whether learning accruing to a flavor simultaneously paired with a nutrient, in this case sucrose, may be considered an instance of predictive learning using the summation test for conditioned inhibition (Experiment 2). The aim of Experiment 1 was to establish an optimal control group for excitatory conditioning avoiding the problems of both the unpaired and the differential training procedures.

From some theoretical accounts, conditioned inhibition occurs whenever a cue signals the absence of an otherwise expected US (e.g., Rescorla & Wagner, 1972). Therefore, we considered conditioned inhibition as a tool to examine whether activation of the US expectancy occurred during training using a Pavlovian conditioned inhibition procedure in which stimulus A (CS+) is always reinforced when presented alone, but unreinforced when presented forming a compound with stimulus B. Under these conditions B becomes a conditioned inhibitor (CS-) signaling the absence of an otherwise expected US (i.e., the US expectation activated by stimulus A). An independent second excitor (C+) was also trained for the summation test. The rationale underlying Experiment 2 is as follows. If during training flavor A activates an expectancy of the US occurrence, flavor B should become a conditioned inhibitor as the US does not occur in its presence; therefore consumption of flavor C should decrease when presented forming a simultaneous compound with stimulus B in the summation test (i.e., C vs. BC). Because flavor preference among food-deprived rats is based exclusively on flavor-calorie association (Harris et al., 2000), rats were food deprived on test. Performance on the summation test was compared with that of a control group in which no activation of the US occurrence by flavor A was expected (i.e., consequently flavor B should not became an inhibitor). Experiment 1 was designed with the purpose of establishing such a control condition.

Experiment 1

The goal of Experiment 1 was to set up an optimal control condition for excitatory conditioning to be used in Experiments 2, due to the problems raised by both the unpaired and differential training procedures mentioned above. Several unpublished experiments conducted in our lab showed that serial presentations of the target flavor followed a few seconds later by sucrose did not produce evidence of a conditioned preference in thirsty rats measured through a two-bottle test (flavor vs. water) when tested food deprived. Accordingly, previous research using serial or sequential presentations of flavors have reported either absence of flavor-flavor learning using an interstimulus interval longer that 9 sec (Lavin, 1976) or more successful conditioning with simultaneous than with sequential presentations in flavor-nutrient learning (Higgins & Rescorla, 2004). Thus, we thought that a serial group might be used as a control for CFP.

Method

Subjects and solutions

Sixteen naïve female Wistar rats at least 110 days old at the start of the experiment were housed in individual home cages and kept in a large colony room with a 12-hour light/12-hour dark schedule. Training sessions took place daily in the home cages during the light cycle at approximately 09:30 am. Rats were water deprived and had continuous access to food (Global Diet 2014 Chow; Harlan, Barcelona, Spain) throughout the experiment, with the exceptions mentioned below. Fluids were administered in 50-ml plastic tubes with a rubber stopper fitted with a stainless steel ball-bearing tipped spout. Fresh solutions were made daily with tap water and administered at room temperature. Consumption was estimated by weighing the tubes before and after fluid presentation to the

nearest 0.1 g. The US was a 20% (wt/vol) sucrose solution. The target flavor, flavor A, was a 1% (vol/vol) almond solution in tap water (almond flavoring supplied by SuperCook, Leeds, UK). For this and the following experiment, all the experimental procedures were approved by the University of Granada Ethics Committee, and were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Procedure

Animals were water deprived for four days by restricting their daily access to water to 30 min. Before the start of the training phase, they were divided into two groups equated in average body weight (n= 8). The training phase consisted of four 5-min sessions (Days 1-4) in which rats in group Simultaneous received 6 ml of a flavor A-sucrose compound—6 ml were provided to guarantee a 5-ml consumption to compensate for possible fluid spillage. Afterwards, they had free access to water for 25 min. Group Serial was first exposed to 6 ml of flavor A for 5 min and, immediately afterward, to 6 ml of the sucrose solution for another 5 min. Next they had free access to water for 20 min. After the last training phase, food was removed from the home cages. The next three days (Days 5-7) rats drank water for 30 min in two bottles to habituate them to the two-bottle test used in the conditioning and extinction tests; afterwards, they had access to water and food for 90 min. This 3-day period of food deprivation was scheduled to guarantee fluid intake on test as we had previously detected a reluctance to consume unreinforced flavors immediately after food deprivation. The two-bottle conditioned preference test took place on Day 8, and was repeated on Days 9-13 to study the extinction course of the conditioned preference in group Simultaneous. In each test rats had 30-min access to two bottles, one containing 20 ml of the target flavor and the other 20 ml of water. The position of the bottles containing the flavored solution (i.e., left and right) was counterbalanced within group and between days.

Data analysis

For all the analyses, a significance level of $p<.05$ was adopted. Data were analyzed using repeated-measures analysis of variance (ANOVA) followed by simple main effects Tukey's tests, where appropriate. Two-tailed t-tests were used to evaluate data not involving multiple comparisons.

Results and discussion

Training phase. Consumption of the compound in group Simultaneous, and of both the sucrose and the flavor A solutions in group Serial, was analyzed through three repeated-measure ANOVAs with Trial as the factor. Consumption of the compound differed among trials in group Simultaneous, $F(3, 21)= 15.19$, consumption of the first day being lower than that of the others, which did not differ among themselves (means: 3.52, 5.92, 5.62, and 5.96 g). A similar pattern was found in the consumption of the sucrose solution in group Serial, $F(3, 21)= 38.11$ (means: 3.73, 5.89, 6.15, and 6.06 g). These patterns suggest that fluids containing the dense sucrose solution were affected by neophobia on the first trial in both groups. On the contrary, consumption of flavor A in group Serial proceeded smoothly with no differences among trials $F(3, 21)= 1.88$ (means: 4.25, 4.62, 4.56, and 5.25 g).

Test phase. Preference ratios [volume of A/(volume of A + volume of water)] were calculated for each two-bottle test and were analyzed through repeated-measures ANOVA with Group as the between-subjects factor and Day as the within-subjects factor (see Figure 1). There were main effects of both Group, $F(1, 14)= 12.44$, and Day, $F(5, 70)= 2.74$, and the interaction was also significant, $F(5, 70)= 2.97$. Two separate one-way ANOVAs were conducted to analyze differences among days for each group. There were no differences in group Serial, $F<1$, but preference ratios differed in group Simultaneous, $F(5, 35)= 4.81$. Post hoc Tukey's tests showed that the average preference ratio of test 6 was significantly lower than that of the tests 1-5, which did not differ among them. Regarding differences between groups in each test, the average preferences ratios of group Simultaneous were significantly higher than those of group Serial on day 1, $t(14)= 3.98$, day 2, $t(14)= 4.73$, and marginally higher on day 3, $t(14)= 1.93$, $p= 0.07$. In addition, the preference ratio on the first day (conditioning test) was significantly higher than .5 in group Simultaneous, $t(7)= 11.93$ ($p<.0001$), but not in group Serial, in which the preference ratio did not differ from .5, $t(7)= 1.75$. Therefore, it seems that conditioned preference for flavor A developed only in group Simultaneous and decreased by day 4 compared to the control group. This decrease is in agreement with the results of Harris et al. (2004), showing that resistance to extinction of nutrient-based conditioned preference is not observed when rats are repeatedly tested under food deprivation. These results replicate our previous observations and thus show that serial flavor-sucrose training constitutes a good control condition for excitatory conditioning, eliminating the flavor avoidance problems arising from both the differential and the unpaired training procedures.

Experiment 2

The results of Experiment 1 suggest that a flavor simultaneously paired with a sucrose solution during training activates a

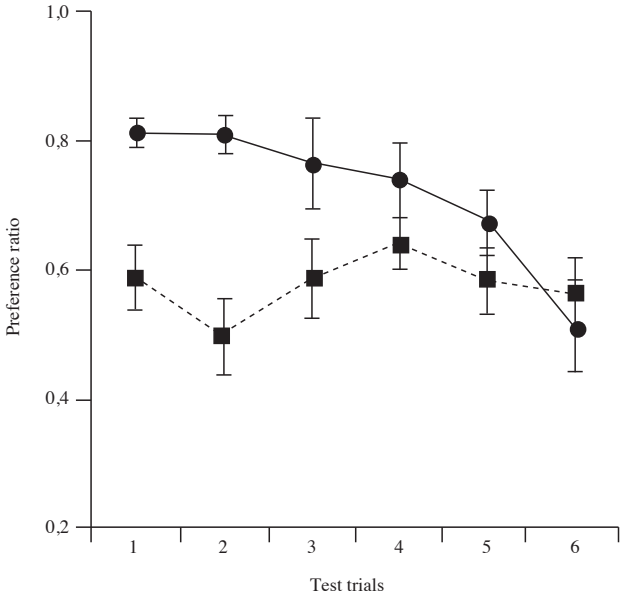

Figure 1. *Mean preference ratios on conditioning and extinction tests for groups Simultaneous and Serial in Experiment 1. Error bar represents SEM*

representation of the sucrose on test that in turn produces a preference for the flavor over plain water. However, this result does not allow us to conclude that the flavor also activates an expectancy of the nutrient during training, although the observed extinction of the conditioned preference when animals were hungry supports that hypothesis. Experiment 2 was designed with the aim of finding such evidence: if an expectancy of the US is repeatedly activated in presence of a flavor (B) and the nutrient does not occur, this flavor should become a conditioned inhibitor and pass the summation test. Two groups of thirsty rats, Simultaneous and Blocked, were trained during several daily sessions. In both groups, flavors A and B were presented unreinforced simultaneously (AB-) during six sessions. There were two critical differences between the groups: the training procedure for flavor A (simultaneous in the case of group Simultaneous and serial in the case of group Blocked); and the location of the AB- trials (presented at the beginning of training in group Blocked instead of intermixed throughout training as was the case for group Simultaneous). This latter manipulation was intended to minimize any possible negative contingency-relationship between flavor B and sucrose in the control group. Note that because Experiment 1 showed that serial A→ + presentations did not produce conditioning, the manipulation cannot be considered to produce sensory preconditioning learning to flavor B. A third flavor (C+) was trained as an independent excitor for the summation test in both groups. Taking into account the results from Experiment 1, activation of the US expectancy by flavor A is not expected during training in group Blocked and, therefore, flavor B should not acquire inhibitory properties. Consequently, flavor B should decrease consumption of flavor C in the summation test only in group Simultaneous.

Method

Subjects and apparatus

The subjects were 16 female Wistar rats at least 110 days old at the start of the experiment. They had previously participated in a conditioned flavor preference experiment with 1% (vol/vol) almond and 20% (wt/vol) sucrose, but were orthogonally assigned to both groups in order to equate experience with those stimuli. Animals were housed and maintained in a similar way as in Experiment 1. For flavor A, the US was 6 ml of a 20% (wt/vol) sucrose solution, whereas for flavor C the US was 10 ml of a 10% (wt/vol) sucrose solution. Flavor A was a 1% (vol/vol) mint solution. Flavors B and C were 1% (vol/vol) solutions flavored with either banana or vanilla, counterbalanced (mint, banana, and vanilla flavorings supplied by SuperCook, Leeds, UK). Fresh solutions were made every day using room-temperature tap water. Fluid and food deprivation are detailed below.

Procedure

Animals were water deprived by giving a daily 30-min period of free access to tap water for four days before the start of the experiment. Afterwards they were divided into two halves equated in body weight (n= 8). Training sessions took place daily in the home cages during the light cycle at approximately 09:30 am. The two testing sessions were scheduled at approximately 12:00 noon (see below). Group Simultaneous received conditioning to flavor C on days 1-3 and 14, consisting of 10-min access to 10 ml of a simultaneous compound of flavor C and sucrose solution. On days 5, 7, 9, and 11 they received 6 ml of simultaneous compound of flavor A and sucrose for 5 min. On days 4, 6, 8, 10, 12, and 13 animals had 10-min access to 10 ml of simultaneous unreinforced compound of flavors A and B (see Table 1). The training schedule for group Blocked was similar, with two important exceptions: a) on days 1-6 they received the six unreinforced AB presentations (blocked trials at the beginning of training); and b) on days 10-13, 6 ml of flavor A were presented for 5 min immediately followed by 5-min access to 6 ml of sucrose (serial A → + presentations). Flavor C was trained in a similar way to that of group Simultaneous on days 7-9, and 14. After training, both groups were treated identically. During days 15-17, animals were both water and food deprived by limiting access to both commodities to 90 min. On the afternoon of day 17, water bottles were returned to the home cages and removed on day 18 at 09:00 am, 3 h before testing began. As one-bottle tests were used, this manipulation was aimed to maintain animals hungry but not very thirsty during testing and thus increase the sensitivity of the measure; rats should drink the solution as long as it was a cue for a nutrient and not because it was a fluid. Summation testing took place on days 18-19. The order of presentation for the two tests, C or CB, was counterbalanced across the two days in each group. The first summation test took place on day 18 at 12:00 noon. At the end of the session, the animals were given free access to water and 90-min access to food. On day 19, water bottles were removed at 09:00 am, and the second session of the summation test took place at 12:00 noon. Each test session lasted 10 min.

Results and discussion

Training phase. An ANOVA conducted on the C+ compound consumption with group (Simultaneous and Blocked) and trials (1-4) as factors yielded a significant effect of trial, $F(3, 42)= 3.73$, and group × trial interaction, $F(3, 42)= 4.85$. There was no main effect of group. Post hoc Tukey tests revealed that consumption was similar along trials in group Simultaneous (means: 9.41, 9.26, 9.08, and 9.56 g). However, consumption was lower in trial 1 compared to trials 3 and 4 in group Blocked (means: 8.26, 9.42, 9.82, and 9.77 g). Comparing both groups, consumption on trial 1 in group Blocked was significantly lower than the consumption on trial 4 in group Simultaneous, but consumption levels in both groups were similar in the last three conditioning trials. Regarding consumption during the six unreinforced AB trials, and ANOVA with group and trial as factors revealed that groups did not differ

Table 1
Design of Experiment 2 (Summation test)

Groups	**Training** *Thirsty*	**Summation test** *Mildly Thirsty & Hungry*
Simultaneous	3 C+, AB-, A+, AB-, A+, AB-, A+, AB-, A+, AB-, AB-, C+	C; CB
Blocked	6 AB-, 3 C+, 4 A → +, C +	

Note. A, B and C= flavors; += US sucrose; -= non reinforcement; Simultaneous= group given flavor A-sucrose compound during training; Blocked= control condition given the six unreinforced AB at the beginning of training (blocked trials), and the sucrose solution after the consumption of flavor A (serial presentation)

between them, F<1, but there was a significant effect of trial, F(5, 70)= 2.85, as well as a group × trial interaction, F(5, 70)= 3.32. Post hoc Tukey tests showed that consumption was similar across trials in group Simultaneous (means 7.65, 8.16, 7.33, 8.82, 7.62, and 8.70 g) whereas trial 4 differ from trial 6 in group Blocked (means 7.52, 8.26, 7.83, 6.91. 8.37, and 8.78 g). No other differences were significant. Consumption of the A+ compound in group Simultaneous was similar across trials, as revealed by the ANOVA, F<1 (means 4.78, 5.17, 5.78, and 5.13 g). Similar pattern was observed in group Blocked regarding consumption of flavour A, F<1 (means 4.73, 4.47, 4.67, and 5.12 g), as well as for consumption of the sucrose solution, F(3, 21)= 1.40 (means 4.83, 5.77, 5.06, and 5.00 g).

In summary, consumption of the different solutions proceeded smoothly and similarly in both groups. The average intakes (for group Simultaneous and Blocked, respectively) were 9.33 and 9.32 g for the C+ compound, and 7.94 and 8.05 g for the AB- unreinforced compound. The mean consumption of the A+ compound in group Simultaneous was 5.21 g, whereas consumption of Flavor A and sucrose solution, respectively, were 4.75 and 5.16 g for group Blocked.

Summation test. Consumptions of both C and CB flavored solutions were transformed into acceptance scores (Biederman & Davey, 1997) in the form of intake suppression ratios according to *a/(a+b)*, where *a* and *b* are, respectively, the amounts of the CB compound flavor and flavor C consumed in the test. Acceptance score under 0.5 shows that animals are drinking less of the compound than of the excitor (i.e., summation effect). Using acceptance scores rather than flavor consumption scores did not change the pattern of results but did increase statistical sensitivity by factoring out individual differences in amount of fluid intake. The average acceptance scores and the absolute consumptions of flavor C and CB after 10 min of testing appear in Figure 2 (main figure and inset, respectively). There was a significant difference between groups in average acceptance score, *t(14)*= 2.87 (means 0.40 and 0.49, for group Simultaneous and Blocked, respectively). Regarding the ANOVA on the average absolute consumptions, there was a main effect of solution, *F(1, 14)*= 8.49, and the group × solution interaction was close to the significance level, *F(1, 14)*= 3.50, p= 0.07. The main effect of group was not significant, *F*<1. Consumption of flavor C did not differ between groups, t(14)<1, and the difference in the compound consumption fell just short of the conventional level of significance, t(14)= 1.83, p= 0.08. Interestingly, within-subjects comparisons between C and CB consumptions for each group, which reduced the impact of the variability in fluid intake among animals, showed that the difference was reliable for group Simultaneous, *t*(7)= 3.57, but not for group Blocked, *t*(7)= 0.51.

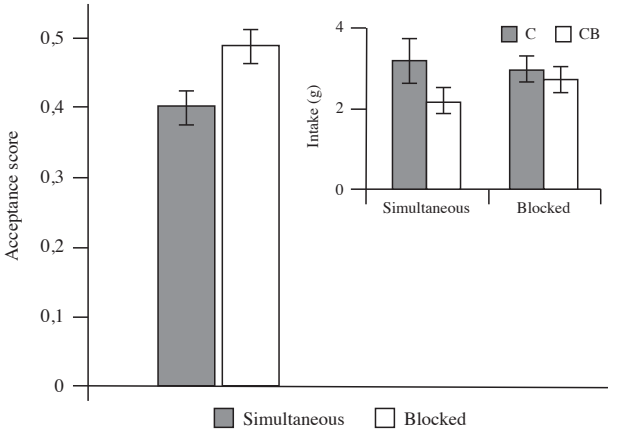

Figure 2. *Mean acceptance score on 10-min summation test for groups Simultaneous and Blocked in Experiment 2. The inset shows the average consumption of flavor C and CB flavor compound for both groups in the test. Error bar represents SEM*

Taken together these results suggest that flavor B acted as a conditioned inhibitor for the nutrient US in group Simultaneous. The acceptance score was significantly lower than that of group Blocked, which was virtually equal to 0.5, revealing that the consumption of the CB compound was lower than the consumption of the excitor C in the experimental group. The analysis of the total consumption was somewhat less sensitive, showing a marginally significant Group × Summation interaction, which once explored revealed that only in group Simultaneous was consumption of the CB compound lower than that of flavor C alone. Therefore, it seems safe to conclude that flavor B passed the summation test for inhibition in group Simultaneous.

General discussion

The main goal of the present study was to examine whether a flavor simultaneously paired with a sucrose solution activated an expectancy of calorific intake as conditioning developed throughout training (Experiment 2). To achieve this goal, we make use of the the summation test for conditioned inhibition; if the nutrient expectancy was activated during training, the absence of the nutrient in presence of a flavor should render that flavor a conditioned inhibitor.

Experiment 1 corroborated a previous observation from our lab showing that serial flavor-sucrose presentations to thirsty rats did not produce evidence of a preference over plain water when tested hungry, thus it was used as a control procedure for flavor conditioning in Experiment 2, which revealed that adding flavor B to the excitor flavor C produced a reduction in the consumption when comparing with the consumption of the excitor alone (i.e., summation effect). However this effect was only found in group Simultaneous. Using serial flavor A-sucrose pairings and arranging the unreinforced AB trials at the beginning of training in group Blocked precluded the possibility of the acquisition of inhibitory properties by flavor B. The absence of the summation effect in this group precludes an explanation of the decrease in compound consumption in group Simultaneous in terms of generalization decrement. Taken together, these results point out that animals in group Simultaneous did in fact learn about the absence of an otherwise expected US during training (i.e., predictive learning).

Boakes et al., (2010) have recently obtained evidence showing that food deprived rats both during training and testing, decrease the consumption of a flavor paired with the reduction in a nutrient using both unpaired an differential procedures —usually considered as controls— and a flavor preference test. The authors have labeled this decrease as «the missing calorie effect». The flavor becomes a signal for the reduction in an expected nutrient acquiring inhibitory properties. This is interesting in itself, because, as mentioned previously, it has been considered that flavor-nutrient learning could rely on «transfer of affect» rather than on a cognitive expectancy of receiving nutrients (e.g., Capaldi, 1996; Capaldi & Privitera, 2008). The study by Boakes et al., and this present study

may be the first examples of inhibitory learning using conditioned flavor acceptance/preference and a specific test for inhibition.

Our results complement Boakes et al. study on «missing calorie effect» using the Pavlovian conditioned inhibition procedure, in addition to that produced by unpaired and differential training. It also adds evidence of learning about the absence of a nutrient in thirsty non-food-deprived trained animals (Experiment 2). It has been established that rats can learn about a nutrient even if they have free access to food (e.g., Yiin, Ackroff, & Sclafani, 2005), and our results furthermore suggest that water-deprived rats with ad lib access to food during training can learn that a flavor signals the lack of an otherwise expected nutrient. It is important to note that water deprived animals fed with dry food eat less than rats that have ad lib access to both food and water; thus, animals can be slightly hungry under these conditions. Explicit food deprivation during training, however, might not be a necessary condition for this kind of learning. The present results suggest that non-food-deprived rats do not only learn about the hedonic properties of the US when consuming a simultaneous compound of a neutral flavor and a palatable nutrient. Rats also learn about the occurrence and absence of the nutrient properties of the US.

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