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Learned Avoidance of Flavors Signaling Reduction in a Nutrient

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Food-deprived rats learned to avoid a flavor negatively correlated with access to a rich nutrient, 20% maltodextrin (20M) solution. This avoidance in two-bottle choice tests was produced by training consisting of either an unpaired condition where sessions of unflavored 20M were intermixed with sessions of 2 or 3% maltodextrin (2M or 3M) flavored with salt (Experiment 1) or almond (Experiments 3 and 4) or a differential conditioning procedure where one flavor was mixed with 20M and another with 2M (Experiment 2). Avoidance was counter-conditioned by mixing the target flavor with 20M (Experiment 1), generalized to a neutral context (Experiment 3), and displayed strong resistance to extinction (Experiment 4). The results demonstrated that food avoidance learning can occur in the absence of an aversive unconditioned stimulus and indicated that unpaired control groups and differential conditioning procedures may be misleading in flavor preference learning research when further control conditions are absent.

Keywords: taste aversion, flavor preference learning, inhibitory learning, re-acquisition, rats

Pairing a food or flavored solution with an aversive unconditioned stimulus—typically nausea induced by lithium chloride—can result in conditioned aversion toward the food or flavor. This well-known phenomenon, conditioned taste aversion (CTA), has been intensively studied for half a century (Reilly & Schachtman, 2009). Less extensively documented are two further ways in which the hedonic properties of a food or specific flavor can change as a result of its relationship with different consequences. First, a flavor that is followed by or accompanies a nutrient or an attractive taste such as that of saccharin can acquire positive hedonic qualities—flavor preference learning (FPL; Capaldi, 1996; Sclafani, 1991). For both CTA and FPL, a flavor needs to have a positive contingent relationship with some consequence. By contrast, a further kind of flavor learning is produced by a negative relationship. Rats can acquire a preference for a flavor paired with recovery from the effects of a lithium injection or for one trained as a conditioned inhibitor with lithium serving as the U.S. (e.g., Best, Dunn, Batson, Meachum, & Nash, 1985; but see Delamater, Kruse, Marlin, & LoLordo, 1986); as indicated in Table 1, this is sometimes known as the *medicine effect* (e.g., Barker & Weaver, 1991). The phenomenon identified in the present study is indicated in the bottom right-hand cell of Table 1. The experiments reported here found that rats will learn to avoid a flavor that signals the absence of otherwise expected nutrient, an effect we term the *missing calorie effect*.

The existence of this effect has been suggested by previous research. Thus, in the course of studying effects of motivational factors on learned flavor preferences Harris, Gorissen, Bailey, and Westbrook (2000) detected avoidance of an almond flavor after it had been included in a control condition where this flavor was explicitly unpaired with either a salt-sucrose (Experiment 2) or a vanilla-sucrose solution (Experiment 5). They noted that this might be seen as a form of negative contrast (e.g., Flaherty, 1982) but did not pursue this topic.

The research reported here was prompted by results of an unpublished study that aimed to compare successive and simultaneous training of FPL in hungry rats, following Higgins and Rescorla (2004). As in their experiments, we included control groups given unpaired training: On some training days, these rats were given a high-energy drink, unflavored 20% maltodextrin (20M), and on other days a weak solution of the same hydrolyzed starch solution, 2% maltodextrin (2M), to which an almond flavor had been added. Unlike Higgins and Rescorla (2004) we introduced occasional two-bottle tests in which almond-flavored 2M was pitted against unflavored 2M. An unexpected finding from these tests was that the control rats given such unpaired training displayed avoidance of the almond flavor that appeared to be highly resistant to extinction. The experiments reported here were designed to follow up these results, including comparisons between unpaired training as the experimental treatment and various control conditions.

Experiment 1: Unpaired Versus Blocked Training

Using the design shown in Table 2, Experiment 1 compared a group, Unpaired, given training consisting of intermixed sessions of the target flavor in 2M and of unflavored 20M with a group, Blocked, designed to provide a neutral control by first giving these rats a block of sessions in which the flavor was mixed with 2M and then a block of sessions in which they were given only unflavored 20M. This control procedure was found to be satisfactory in our previous experiments involving fluid-deprived rats (Albertella & Boakes, 2006).

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Table 1
Types of Flavor-Consequence Learning

	Consequences	
Contingent relation	Nausea and other aversive events	Nutrients and other positive events
Positive contingency	Conditioned taste avoidance (CTA)	Flavor preference learning (FPL)
Negative contingency	Medicine effect	Missing calorie effect

To test for the generality of the apparent avoidance learning described above the flavor in this experiment was salt (0.5% NaCl). As shown in Table 2, for both groups the training stage was followed by excitatory training in which salt was now added to 20M (*N* + 20M). This was intended as a retardation test, the prediction being that excitatory training would more rapidly produce a salt preference in the Blocked than in the Unpaired group. Two-bottle tests between salt plus 2M (*N* + 2M) and unflavored 2M were introduced after the end of initial training (Test 1) and after six sessions of excitatory training (Test 2).

Method

Subjects. Sixteen naïve male hooded Wistar rats were approximately 200 days old at the start of the experiment when their mean weight was 388 g, range 286–465 g. They were housed in large plastic cages each containing eight rats. Allocation to the two weight-matched groups (each $n = 8$) was such that there were four rats from each home cage squad in each condition. Food restriction was progressively increased over a period of 3 days and from then on the amount of food they were given 30 min after the end of each daily session was adjusted so as to maintain them at 80% of their projected ad lib weights for the rest of the experiment. Access to water in the home cages was unrestricted, except that the water bottles were removed 30 min before the start of a session; they were returned immediately after each session.

Apparatus. The eight drinking chambers were clear acrylic cages, measuring 33 × 21 × 19 cm, with floors covered with a layer of kitty litter. The lids of the cages were made of a stainless steel grill through which the steel spouts of 200-ml drinking bottles could be inserted. The solutions were 1% natural almond essence (Queen Fine Foods Pty. Ltd., Queensland, which when undiluted contains 48% ethyl alcohol) in tap water, 2 or 20M (DE17 hydrolyzed corn starch, Myopure, Australia; www.myopure.com.au) and unflavored maltodextrin at these two concentrations.

Procedure. All sessions lasted 10 min. In the initial 12-session training stage Group Unpaired were given unflavored 20M on Days 1, 2, 5, 6, 9, and 10 and 0.5% saline in 2M (*N* + 2M) on the remaining 6 days. Group Blocked were given *N* + 2M on Days 1 through 6 and unflavored 20M on Days 7 through 12. On Days 13 and 14 all rats were given 2M in two bottles to familiarize them with the test procedure and as a check against strong (i.e., >80%) position preferences. Over the next 2 days (Test 1) the rats were given a choice between *N* + 2M and unflavored 2M, with the salt flavored solution in the left-hand bottle on Day 15 and in the right-hand bottle on Day 16.

After 2 days when the rats remained in their home cages but food restriction was maintained (Days 17 and 18), both groups were given 6 days of excitatory training. On Days 19, 23, and 24 they were given unflavored 2M and on the other 3 days they were given *N* + 20M. Test 2 followed exactly the same procedure as Test 1, with 2 days of 2M in both bottles followed by a choice between *N* + 2M and unflavored 2M (Days 25 and 26).

Analysis of test data in this and all subsequent experiments started with an ANOVA applied to total intakes. The results of such preliminary analyses are reported only when significant differences in total intakes were detected between groups. Where no such differences were found, subsequent ANOVAs or *t* tests were applied to percent preferences for almond calculated by dividing total intake of the almond-flavored solution over pairs of test sessions by total fluid intake over these sessions.

Table 2
Designs of Experiments 1–4

Experiment 1				
Groups	Training	Test 1	Excitatory training	Test 2
Unpaired	6 × N + 2M vs. 6 × 20M	N + 2M vs. 2M	3 × N + 20M vs. 3 × 2M	N + 2M vs. 2M
Blocked	6 × N + 2M, then 6 × 20M			
Experiment 2				
Groups	Training	Test		
Diff Almond+	6 × A + 20M vs. 6 × N + 2M	A + 6M vs. 6M and N + 6M vs. 6M		
Diff Salt+	6 × N + 20M vs. 6 × A + 2M			
Nondiff	6 × A + 2M vs. 6 × N + 2M			
Experiment 3				
Groups	Stage 1: Inhibitory training	Stage 2: Context training	Generalization tests	
Unpaired	6 × A + 3M vs. 6 × 20M	3 × High context: 20M vs. 3 × Low context: 3M	High context vs. Low context × Hungry vs. Thirsty	
Blocked	6 × 20M, then 6 × A + 3M			
Experiment 4				
Stage 1: Inhibitory training	Stages 2 and 4: Context training	Test 1	Stage 3: Extinction	Test 2
6 × A + 3M vs. 6 × 20M	3 × High context: 20M vs. 3 × Low context: 3M	High context: A + 3M vs. 3M	12 × A + 3M or 12 × 3M	High context: A + 3M vs. 3M

Note. A = 1% Almond essence; N = 0.5% NaCl (Salt); 2M = 2% maltodextrin; 3M = 3% maltodextrin; 6M = 6% maltodextrin; 20M = 20% maltodextrin.

Results and Discussion

As shown in the upper panel of Figure 1, in Test 1 the Unpaired group avoided the salt-flavored solution, whereas the Blocked group displayed a slight preference (i.e., >50%) for this solution, $t(14) = 2.63$, $p = .02$. In Test 2, given after three sessions of excitatory training, there was a group difference in overall intake, $t(14) = 2.35$, $p = 0.034$. As can be seen from the lower panel of Figure 1, this difference resulted from a greater intake of the salt solution by the Blocked group. Although both groups showed a strong preference (>90%) for this solution, the Unpaired group's salt preference was significantly lower than the Blocked group's, $t(14) = 2.75$, $p = .02$.

In summary, this experiment confirmed the suggestion from our previous unpublished study that unpaired training can produce avoidance of a flavor that is negatively correlated with 20M. It also showed that such training produces some retardation of subsequent excitatory conditioning produced by mixing the flavor with this nutrient, an effect that is generally accepted as one of the basic markers for inhibitory learning (Hearst, 1972; Rescorla, 1969).

Experiment 2: Differential Conditioning Involving Two Flavors

Many experiments on flavor preference learning have employed a within-subject design based on a differential conditioning procedure whereby a target flavor, F_T, serves as a CS+ by pairing it with a rich nutrient, and a control flavor, F_C, serves as the CS− by pairing it with either a weak nutrient or the absence of any nutrient. If an animal shows a preference for F_T over F_C, then it has usually been assumed that the animal had acquired a positive preference for F_T and that F_C had remained neutral (e.g., Capaldi, Hunter, & Lyn, 1997; Elizalde & Sclafani, 1990).

Some experiments of this kind have followed differential conditioning with an extinction procedure consisting of repeated presentation of F_T in the absence of any nutrient and have then given a repeat test between F_T and F_C. It has been usually observed that the preference for F_T over F_C has remained unaffected by the extinction procedure (Capaldi, Myers, Campbell, & Sheffer, 1983; Drucker, Ackroff, & Sclafani, 1994; Elizalde & Sclafani, 1990; but see Delamater, 2007). Such results have pointed to the conclusion that a conditioned preference for the flavor, F_T, is unusually resistant to extinction. However, this generally accepted interpretation was challenged by Harris, Shand, Carroll, and Westbrook (2004). They suggested that inhibitory learning could occur to F_C and that some failures to detect any effect of the extinction procedure—whether this consisted of F_T alone or presentation of both F_T and F_C—could be understood in terms of persistent avoidance of F_C resulting from inhibitory learning (but see Myers, 2007; Perez, Lucas, & Sclafani,1998). The results from Experiment 1 provided strong support for this possibility. This was tested di-

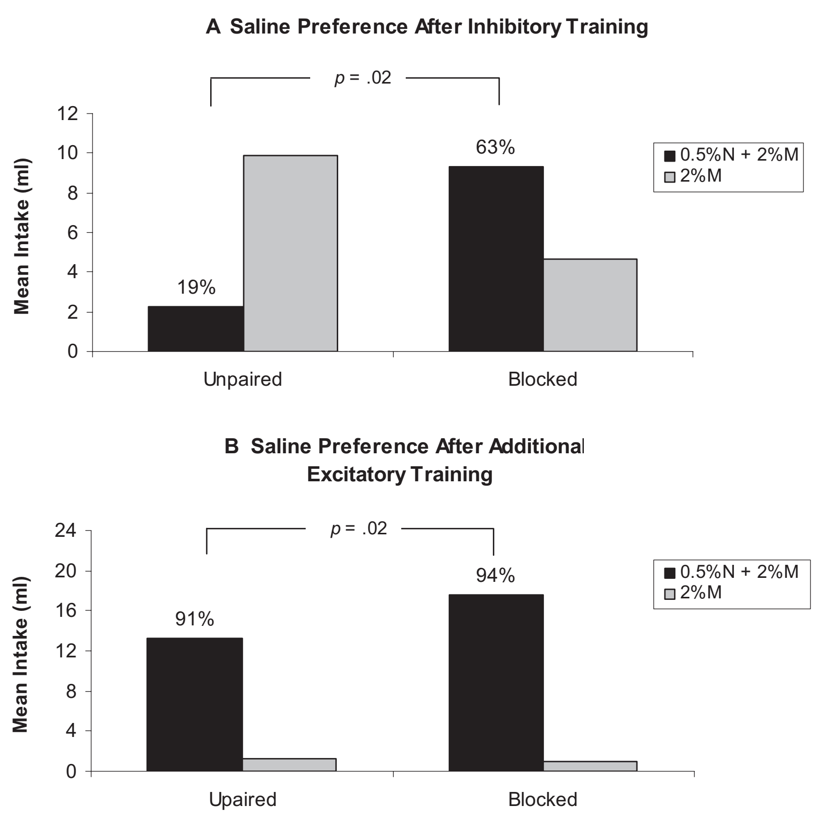

Figure 1. Experiment 1. Test intakes of 0.5% sodium chloride in 2M (N + 2M) and unflavored 2M immediately after the training stage in which one group were given Unpaired training and the control group was given Blocked exposure to N + 2M followed by blocked sessions of unflavored 20M (A: Test 1); and following excitatory training consisting of three sessions of N + 20M and three of unflavored 2M for both groups (B: Test 2).

rectly in Experiment 2 where the critical question was whether rats would avoid a flavor that had served as a CS− in a differential conditioning procedure.

The design of the experiment is shown in Table 2. A combination of the two groups given differential training, Group Almond+ and Group Salt+, can be viewed as a standard within-subject design in which allocation of the two flavors—almond and salt in this case—as CS+ or CS− has been counterbalanced. The relatively novel aspect was to include a third group, Group Nondiff, which was given equivalent exposure to the two flavors when they were separately mixed with the weak nutrient, 2M. This group thus provided a control against which to assess both excitatory and inhibitory learning in subjects given differential training.

Method

Subjects. Twenty-eight female hooded Wistar rats, with ages between 130 and 230 days, weighed a mean of 285 g, range 192–364 g, at the start of the experiment. They were housed in three groups of eight and one of four in large plastic cages. Eight rats were allocated to each of the two differential groups and the remaining 12 to the Nondifferential group, in way that matched body weight across the three groups. Progressive food restriction was introduced 3 days before the start of the experiment and from then on, they were maintained at 80% of their projected ad lib weights, as in Experiment 1. Water was continuously available in the home cages, except for 30 min before each daily session.

Apparatus. This was the same as in Experiment 1.

Procedure. As previously, all sessions lasted 10 min. During the 12-day training stage Days 1, 2, 5, 6, 9, and 10 were "almond days" in that Group Almond+ was given access to 1% almond in 20M (A + 20M), while Group Salt+ and Group Nondiff were given 1% almond in 2M (A + 2M). The remaining 6 days were "salt days" when Group Salt+ was given 0.5% NaCl in 20M (*N* + 20M) and the other two groups were given 0.5% NaCl in 2M (*N* + 2M).

In the test stage, as shown in Table 1, 6% maltodextrin (6M) was used as the common test solution instead of the 2% used in Experiment 1. This change was intended to reduce intake variability across rats. On Days 13 and 14 all rats were given unflavored 6M in two bottles and over the next 4 days were given choice tests between the flavors added to 6M and unflavored 6M. Group Almond+ and half the rats in Group Nondiff were given the almond solution in the left bottle and unflavored solution in the right bottle on Day 15 and the same solutions, but in the reverse positions, on Day 18. For these rats, the left bottle contained the salt solution on Day 16 and the right bottle contained this solution on Day 17. A similar sequence was used for Group Salt+ and the remaining six rats in Group Nondiff, except that their preferences for salt were tested on Days 15 and 18 and for almond on Days 16 and 17.

Results and Discussion

Preference values were calculated across the two test sessions for each flavor as in Experiment 1. During training four rats in the Nondiff condition drank far less than all other rats, consuming an average of less than 2 ml per session of either the almond- or salt-flavored 2M solution, so that their exposure to the two flavors was relatively limited. Consequently, planned contrasts comparing the groups' test preferences were carried out both when these four rats' results were included and when they were excluded. The latter data are shown in Figure 2. Planned contrasts confirmed that the almond preferences shown in the upper panel were greater in Group Almond+ than in Group Nondiff, $F(1, 21) = 18.9$, $p < .001$, and greater in Group Nondiff than in Group Salt+, $F(1, 21) = 4.55$, $p = .04$. The lower panel of Figure 2 shows the equivalent pattern from the salt tests. Planned contrasts confirmed that preferences for salt were stronger in Group Salt+ than in Group Nondiff, $F(1, 21) = 24.2$, $p < .001$, and the latter were stronger than salt preferences in Group Almond+, $F(1, 21) = 7.12$, $p = .01$. The same analysis carried out without excluding any rats showed a similar pattern: Group Almond+ preferred almond more than did Group Nondiff, $F(1, 25) = 17.41$, $p < .001$, and the latter preferred almond more than did Group Salt+, $F(1, 25) = 7.03$, $p = .014$, whereas Group Salt+ preferred salt more than did Group Nondiff, $F(1, 25) = 36.81$, $p < .001$, but the difference between the latter and Group Almond+ just failed to reach significance, $F(1, 25) = 4.02$, $p = .056$.

It can be concluded from this experiment that the differential conditioning design still used very widely in the study of flavor

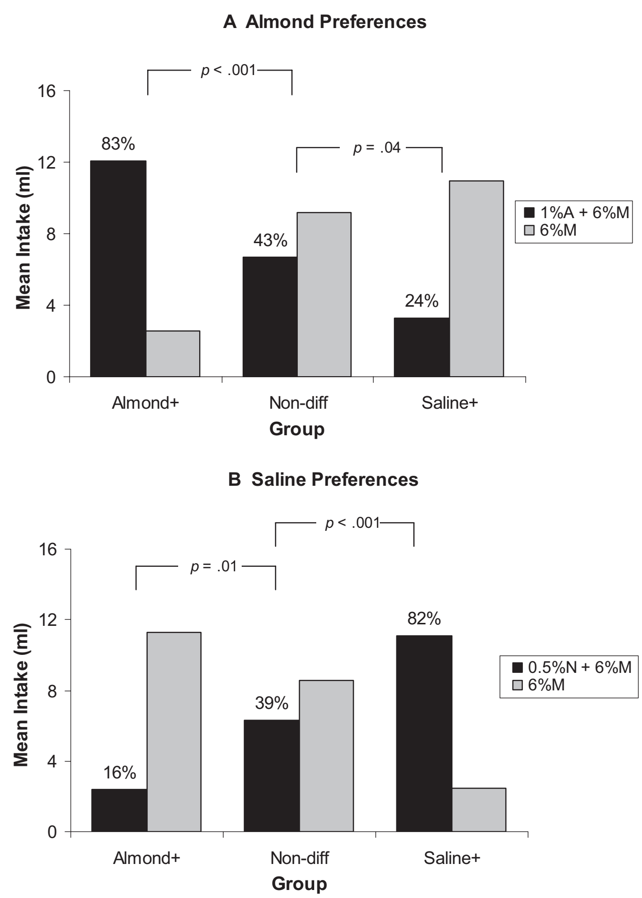

Figure 2. Experiment 2. Test intakes following differential conditioning in which the Almond + group was given almond in 20M alternating with salt in 2M, the Salt + group was given the reverse combination, and the Nondiff group was given alternating exposure to the flavors both in 2M. The top panel (A) shows intakes in tests between almond in 6M and unflavored 6M and the bottom panel (B) shows intakes in tests between salt in 6M and unflavored 6M.

preference learning, as in many other conditioning preparations, is one that can produce both excitatory conditioning to the CS+ flavor and inhibitory conditioning in the form of avoidance of the CS− flavor. The latter possibility is commonly ignored (e.g., Dwyer, 2008) but in future it should clearly be taken seriously in the light of the present results.

Experiment 3: Generalization of Inhibitory Flavor Learning

This experiment examined generalization of the kind of flavor avoidance found in Experiments 1 and 2. The first question we asked was whether avoidance of the target flavor depends on the rat being hungry. Because flavor *preferences* acquired by hungry rats for example, after drinking a flavor-sucrose solution, are reduced when the rats are no longer hungry (e.g., Harris et al., 2000), it seemed possible that hunger-based avoidance might be similarly reduced. The second question was whether the target flavor is avoided only in a context where the rat might otherwise expect high calories, for example, the training context. Possible attenuation of the effect by testing in a more neutral context is suggested by studies of conditioned inhibitors of fear indicating that inhibition might be a "slave" process (Fowler, Kleiman, & Lysle, 1985).

Initial training was similar to the unpaired condition used in Experiment 1. One change was to use 3% maltodextrin (3M) as the weak solution instead of the 2M used in the previous experiments, because, as noted earlier, some rats consistently drank very little of the 2% solution in Experiment 2. Another change was to give the Blocked control group its 20M in a block of six trials *before* six trials of A + 3M; this is the reverse of the sequence given to the Block group in Experiment 1. The main reason was again to try to increase consumption of the dilute solution. A possible risk posed by this change was that the 20M->A + 3M transition might itself produce weak avoidance of almond in a subsequent test, that is, an example of negative contrast (Flaherty, 1996), and therefore provide too conservative a control condition. However, this risk seemed small, because previously we had failed to detect such a contrast effect in an unpublished experiment. A third change was to employ a different rat strain, Sprague–Dawleys, as suitable Wistars were unavailable at the time.

The design of the experiment is shown in Table 2. The rats were food-deprived throughout the two successive training stages: Flavor Inhibitory training and Context Discrimination training. Inhibitory training was carried out in a "High" context, one in which 20M was available on half the sessions. Context Discrimination training was designed to teach the rats to associate 20M with the High context and 3M with the Low context. Subsequent two-bottle tests asked whether almond avoidance produced by unpaired training would generalize (1) from the High to the Low context; and (2) from hunger to thirst.

Method

Subjects. Nineteen male Sprague–Dawley rats were obtained from the University of Sydney Psychology breeding colony and were group housed as previously, but with five rats in three of the squads and four in the fourth squad. At the start of the experiment, they were approximately 230 days old and weighed a mean of 485 g, range 381–545 g. Initially, 10 rats were allocated to the Unpaired group and nine to the Block group, so as to approximate matching of body weights in the two groups, but with the restriction that within a squad at least two rats were allocated to each group. During the course of training two rats had to be removed from the experiment because of illness, leaving nine Unpaired and eight Block rats to undergo the final testing stage.

Restriction on food access was progressively increased over a period of 4 days, so that body weights were at approximately 80% of ad lib level at the start of the experiment. They were maintained throughout training at 80% of their projected ad lib weights by giving an adjusted amount of food 30 min after they returned to the colony room after a drinking session. During training (Stages 1 and 2) water was continuously available in the home cages, except for 30 min before a session. During some parts of the test phase, ad lib access to food was restored, while fluid-access was restricted, as detailed below.

Apparatus. Two sets of drinking chambers were located in separate laboratories. One set were the eight acrylic cages used in Experiments 1 and 2. The second set consisted of stainless steel cages, measuring 13 × 19 × 18 cm, with floors of steel mesh, side walls of steel sheet, a rear wall consisting of a steel access door and a front wall made of vertical steel rods. Fluids were delivered in both kinds of cages via 100-ml plastic bottles (Hagen) fitted with steel spouts.

Procedure. All sessions consisted of access to a single drinking bottle, except where noted below, and lasted 10 min, except on Day 1 (20 min) and Day 2 (15 min). The first stage of training (Inhibitory Flavor training) was carried out only in the High context; for half the rats this was an acrylic cage and the other half a steel cage. Following the similar schedule for unpaired training used in Experiment 1, on Days 1, 2, 5, 6, 9, and 10 the Unpaired group was given unflavored 20M and on Days 3, 4, 7, 8, 11, and 12 was given almond mixed with 3M. On Days 1–6 the Blocked group was given unflavored 20M and on Days 7–12 was given A + 3M.

After the initial training stage, the two groups were treated identically. The second stage consisted of Context Discrimination training using unflavored maltodextrin. On Days 13, 14, and 17 all rats were given 3M in their second set of drinking chambers, the Low Context, and on Days 15, 16, and 18 were given 20M in the High Context where they had received initial training. On the last two sessions of this stage (Days 17 and 18) two bottles containing maltodextrin were given in each session, that is, 2 bottles of 3M on Day 17 and 2 bottles of 20M on Day 18, to accustom the rats to the 2-bottle test procedure.

On return to the colony room after the Day 18 session half the rats in each subgroup were given ad lib access to food and their water bottles were removed 30 min later. The sequence of conditions for the subsequent tests—deprivation condition X context—was completely counterbalanced. In each pair of Test Sessions A + 3M was given in the left-hand bottle and unflavored 3M in the right-hand bottle in one session and with the positions of the solutions reversed in the other session. While remaining in a given deprivation condition one pair of tests took place in one context and the other pair in the other context (Days 19–22). After these four sessions the deprivation conditions were reversed: Unlimited food access was restored to those previously food deprived and water access was now restricted to 30 min per day, whereas unlimited access to water was restored to those previously fluid deprived and food access now restricted as during training. The same sequence of tests was now given under the new deprivation condition (Days 23–26).

Results and Discussion

Initial ANOVA applied to total test intakes revealed that the rats drank more when fluid-deprived than when food-deprived, $F(1, 15) = 28.24$, $p < .001$; there were no other main effects and no interactions, largest $F(1, 15) = 3.36$, $p > .05$. Consequently almond preferences when the rats were tested hungry were analyzed separately from preferences when thirsty.

The results obtained from tests under food deprivation, shown in the left-hand panel of Figure 3, were very clear. As suggested by this figure, relative to the Blocked rats, the Unpaired rats showed the same degree of avoidance of almond in the Low context as in the High context in which they had been trained. This was confirmed by a mixed ANOVA that found a main effect of Group, $F(1, 14) = 13.30$, $p < .01$, but neither a main effect of Context or a Group × Context interaction, both Fs < 1. It may be noted that an experiment by Best et al. (1985), one that had suggested to us that flavor avoidance might not generalize to a neutral context, compared flavor preferences in the experimental context with those displayed in the rats' home cages. It thus remains possible that, if we had tested our rats in their home cages, some generalization decrement may have been detected. On the other hand, the complete transfer to a neutral context found here is consistent with results indicating strong generalization of conditioned inhibition across contexts in preparations using auditory and visual stimuli (Bouton & Nelson, 1994; Nelson & Bouton, 1997).

The results obtained from the tests under fluid deprivation, shown in the right-hand panel of Figure 3, were less conclusive. There was again a main effect of Group, $F(1, 14) = 4.98$, $p < .05$, and again neither a main within-subjects effect of Context or a Group × Context interaction, largest $F(1, 15) = 3.16$, $p = .10$. Although this analysis suggests that the Unpaired rats also avoided almond when thirsty and did so as much in the Low as in the High context, inspection of the right-hand panel of Figure 3 shows that these rats yielded an almond preference of over 40% (a value we would normally expect from control groups) as compared to the approximate 25% when hungry. The data suggest that the difference between the groups may have arisen, not because Unpaired rats avoided almond when thirsty, but because the Blocked rats surprisingly displayed a preference for almond when tested thirsty in the High context. The latter could have been a freak result; it is certainly not at all obvious why it occurred.

Experiment 4: Extinction Test

Ever since Zimmer-Hart and Rescorla (1972) most experiments have found that, after a conditioned inhibitor (e.g., X produced by A + vs. AX-training) has been subjected to a standard extinction treatment, that is, repeated presentation of X in the absence of any US (unconditional stimulus), no decrease of its inhibitory property can be detected. Thus, it has been generally concluded that inhibitors are highly resistant to extinction. As mentioned in the Introduction, our previous unpublished experiment suggested that such resistance is also shown by flavor avoidance produced by Unpaired training but it did not include appropriate controls. The first aim of the present experiment was to test for extinction under standard conditions whereby the extinction treatment is carried out in the training context in comparison with a control condition that lacked repeated exposure to the flavor.

Following the experiment by Best et al. (1985; Experiment 1) mentioned above, it seemed possible that less resistance to extinction might be found if an extinction treatment is carried out in a neutral context, one in which the animal has never experienced the

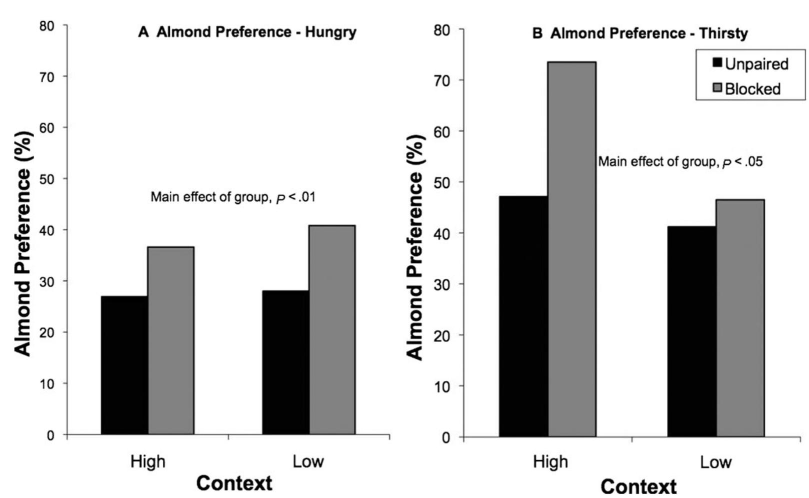

Figure 3. Experiment 4. Following unpaired training, in an Extinction stage two groups were given 12 sessions in a context associated with a High concentration of maltodextrin (20M), receiving either Almond + 3M (Expose-Hi group) or unflavored 3M (No Expose-Hi group), and two groups were given 12 sessions in a context associated with a Low (3M) concentration, again receiving either Almond + 3M (Expose-Lo group) or unflavored 3M (No Expose-Lo group). This figure shows almond preferences in 2-bottle tests carried out both before the Extinction stage (Pre-Extinction: Test 1) and after this treatment (Post-Extinction: Test 2).

US. The second aim of the present experiment was to test this possibility under more completely controlled conditions than those used by Best et al. (1985).

As shown in Table 2, all rats were first given Unpaired training in their High context and then Context Discrimination training, exactly as for the Unpaired group in Experiment 3 and indeed run at the same time. The results from a subsequent two-bottle test in the High context (Test 1) were used to allocate the rats to four groups matched for almond preferences in Test 1, according to a 2 × 2 design with High versus Low Context as one factor and Exposure versus No exposure as the other factor. During the Extinction stage Expose rats were given repeated exposure to 1% almond in 3M, whereas No Expose rats were given unflavored 3M.

Following the extinction stage, all rats were given further context training, so that the next test (Test 2) was completely identical to Test 1, that is, preceded by context training and carried out in the High context. The main dependent variable was the within-subject change in preference from Test 1 to Test 2. Finally, the same test, Test 3, was given in the Low context.

Method

Subjects. Sixteen male Sprague–Dawley rats from the same source as those in Experiment 3 were group housed as four squads, each of four rats. At the start of the experiment, they were approximately 200 days old and weighed a mean of 443 g, range 372–505 g. Deprivation conditions throughout were exactly as for the training stages of Experiment 3.

Apparatus. This was exactly as in Experiment 3.

Procedure. Inhibitory flavor training and subsequent context training were exactly as for the Unpaired group in Experiment 3 such that all rats were placed in their High context (for eight rats this was a steel cage and for the other rats an acrylic cage) and were given unflavored 20M on 6 days and A + 3M on the other 6 days in a double alternation sequence (Days 1–12). They were then given Context Discrimination training (Days 13–18) in which unflavored 20M was given their High context on 3 days and unflavored 3M on the other 3 days. Rather than the single bottle used previously, two bottles containing the same solution were used in the final two sessions of context training (Days 17 and 18) to prepare rats for the choice tests that followed. Testing (Test 1) between A + 3M and unflavored 3M took place in the High context on Days 19 and 20.

During the Extinction stage (Days 21–32) half the rats were placed in their High context for each of these 12 sessions and half in their Low context, receiving A + 3M every session if allocated to the Expose-High group ($n = 4$) and unflavored 3M if in the No expose-High group ($n = 4$). The remaining rats were placed in their Low context for each of these sessions and similarly received either A + 3M (Expose-Low group; $n = 4$) or unflavored 3M (No expose-Low group; $n = 4$). These allocations were counterbalanced so that within each group for two rats, a steel chamber served as the High context and for the other two an acrylic chamber served this role.

Context Discrimination training was repeated (Days 33–40), following the same procedure as before, and this was followed by a final test stage. Test 2 (Days 41–42) was given in High context and followed by Test 3 (Days 43–44) given in the Low context. The procedures for these tests were identical to those of Test 1. Unless already described here, procedural details were the same as in Experiment 3.

Results and Discussion

To check that rats in the Expose condition did indeed get sufficient exposure to the almond flavor and to test the possibility that intakes might vary across contexts, we examined intakes in the 12 sessions of the Extinction stage. These ranged between 9 and 16 ml per session and ANOVA revealed a general increase in intake over these sessions, linear trend $F(1, 12) = 12.84$, $p < .01$, but no main effect of group or group × trend interaction, largest $F(1, 12) = 2.33$, $p > .10$. Thus, there was no indication that the rats given almond-flavored 3M on these days drank any less than those given unflavored 3M and no indication that intakes varied across the two contexts.

The important data were obtained from the choice tests given before (Test 1) and after (Test 2) the Extinction stage, when almond preference shown by the four groups ranged between 12% and 32%, as shown in Figure 4. This experiment did not include a blocked control group but in the context of the preference values obtained in Experiments 1–3 these figures strongly suggest that all four groups learned to avoid almond. A mixed ANOVA applied to these data, with Test (Test 1 vs. Test 2) as a within-subject factor, found a main between-subjects effect of Context, $F(1, 12) = 205.80$, $p < .001$, indicating that the two groups that were placed in the Low context during the extinction stage, avoided almond more (when all rats were tested in the High context) than the groups placed in the high context throughout, and an interaction between Context and Exposure, $F(1, 12) = 6.87$, $p < .05$. Inspection of Figure 4 indicates that this interaction arose because the Context effect was greater in the groups that were not exposed to almond during the extinction stage. Most importantly, there was no main effect of Test and no interaction between this factor and Exposure, or any other interaction, largest $F(1, 12) = 2.64$, $p > .10$.

The final test (Test 3) was given in the Low context and this failed to find any group differences that might indicate an extinc-

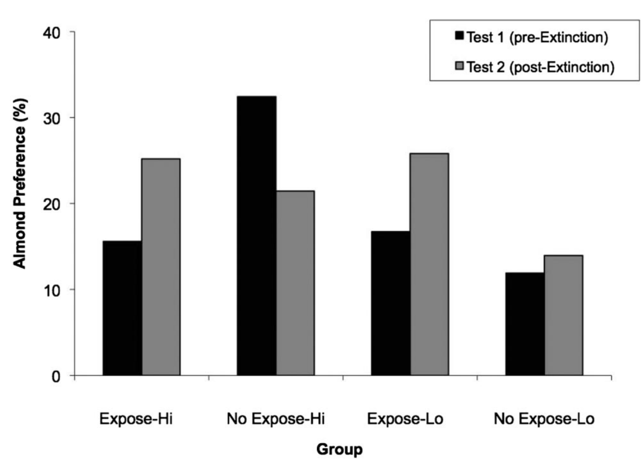

Figure 4. Experiment 3. Almond preferences following either unpaired or blocked training when tested either in a context previously associated with a High concentration of maltodextrin (20M) or in one associated with a Low concentration (3M) and either food-deprived (A, left-hand panel) or fluid-deprived (B, right-hand panel).

tion effect, with an almond preference of 35% in the Expose-High group compared to 37% in the No expose-High group and a preference of 38% in the Expose-Low group compared to 25% in the No expose-Low group; the effect of Context found in Tests 1 and 2 was no longer significant, largest $F(1, 12) = 3.44$, $p = .09$.

In summary, all four groups showed avoidance of almond following inhibitory training and this did not change even after the two Exposure groups had been given 12 further sessions of A + 3M, a result that is consistent with the results of other studies that have examined the effect of repeated exposure to a conditioned inhibitor (e.g., Zimmer-Hart & Rescorla, 1972). The analysis was powerful enough to detect an unanticipated (and probably meaningless) interaction between the context and exposure factors, so it seems unlikely that even a major increase in the size of the groups would have led to detection of an extinction effect. One cannot, of course, conclude that exposure has no effect on avoidance of a flavor trained as an inhibitor, but this present experiment suggests that any such effect is at best slight.

General Discussion

In summary, the main result from this study were that in all four experiments rats learned to avoid a flavor added to a solution containing very little nutrient when there was a negative contingency between this solution and one containing a rich nutrient, namely, 20M. This result was found both when salt was used as the target flavor, in comparison with either a blocked control group (Experiment 1) or a nondifferential group (Experiment 2), and when almond was used (Experiments 2, 3, and 4). Because the averted flavor in Experiment 1 passed the retardation test and both averted flavors in Experiment 2 can be seen as passing a summation test in that they suppressed intake of a relatively novel solution, 6M, it seems reasonable to categorize the target flavors in these experiments as conditioned inhibitors (Rescorla, 1969). Furthermore, this type of flavor avoidance learning was found to be robust in that it generalized completely to another context, one that had never been associated with high concentration of maltodextrin (Experiment 3), and showed a high degree of resistance to extinction (Experiment 4).

Complementary results have been obtained in two flavor experiments reported by Garcia-Burgos and González (in preparation). These researchers used conditioned inhibition discrimination training between A+ and AB− and then tested for an inhibitory effect of B in a summation test, BC versus C, where C was a third flavor trained as an excitor (C+); their main dependent variable was intake in a one-bottle test rather than preference in a two-bottle test of the kind used in the present experiments. In the summation test intake of BC was found to be less than that of C alone in the group given conditioned inhibition training, but not in a blocked control group (Garcia-Burgos & González, in preparation; Experiment 2). This and the present study may be the first examples of learning involving a taste to pass the standard tests for conditioned inhibition, since in what appears to be the only previous attempt to submit a taste to such tests the evidence was equivocal (Delamater et al., 1986).

If flavor avoidance in the present set of experiments is a result of inhibitory learning, then one question it poses has to do with the underlying associative process. One possibility is suggested by the similarity between the unpaired training procedure used in Experiments 1, 2, and 4 and that developed by Baker (1977) whereby unsignaled shocks were delivered in some sessions and a neutral stimulus presented in alternating shock-free sessions. He found that this procedure was effective in establishing the neutral stimulus as a conditioned inhibitor. Applied to the present experiments, this suggests that in 20M sessions rats learn to associate the drinking chamber with a high calorie consequence and that the almond flavor becomes associated with the very much reduced calorie intake on A + 2M or A + 3M sessions. An alternative account makes no appeal to context conditioning but instead assumes stimulus generalization based on the stimulus properties, flavor, of maltodextrin solutions, probably in terms of both their taste (Sclafani, 1987) and viscosity (Davidson & Swithers, 2005). This alternative proposes that the flavor of 20M becomes associated with a high calorie intake and this generalizes to some extent to 3M and 2M. When almond is combined with such low concentration solutions, the calorie intake is much less than expected; hence, almond becomes an inhibitor. This second account can be seen as proposing that the present effect results from a variant of conditioned inhibition training, A+ versus A*X−, in which A is the flavor of 20M that generalizes to A*, the flavor of 3M or 2M, and X is the added target flavor. One way of testing the first alternative is to arrange that the 20M and almond plus 3M solutions are given in different contexts. A result showing that this weakened flavor avoidance learning would indicate the involvement of context conditioning, without, of course, ruling out a role for the second, flavor generalization, account. Such an experiment has not yet been tried.

Turning to the resistance to extinction of flavor avoidance found in Experiment 4, Zimmer-Hart and Rescorla (1974) were the first to report that an extinction treatment consisting of repeated nonreinforced exposure to the inhibitor, X, had no detectable impact on its inhibitory properties that had been produced by conditioned inhibition training, A+ versus AX− (see also Devito & Fowler, 1986; Williams & Overmier, 1988). Williams (1986) also reported high resistance to extinction of an inhibitor produced by an explicitly unpaired procedure similar to that used in the present experiments, but with shock as the US, and Rescorla (1982) found such resistance in an autoshaping procedure using food as the US. As is consistent with these various studies, in Experiment 4 the almond aversion established by unpaired training was unaffected by the extinction treatment consisting of 12 sessions in which this flavor was presented in 3M.

As implied in the above discussion and by the provisional label, the *missing calorie* effect, we have assumed throughout that the crucial difference between 20M and 3M or 2M solutions lies in their calorie content. However, these solutions also differ in palatability (Sclafani, 1987) so that it is possible that inhibitory learning occurs because the low concentration maltodextrin solutions are less preferred, in which case a label such as *disappointing taste* might be more appropriate. The reason we have assumed that differences in palatability play at best a minor role is because, in several previous experiments using unpaired training as a control procedure, but with fluid-deprived rats given unrestricted access to food and given sucrose rather than maltodextrin solutions, we have not detected any flavor avoidance of the kind repeatedly obtained in the present experiments (e.g., Albertella & Boakes, 2006; Experiment 2). In these experiments, the difference in palatability between 8% sucrose and almond-flavored water was arguably far greater than that between 20M and 3M. Nevertheless, two other reports of flavor avoidance were based on experiments that gave unpaired (Harris et al., 2000) or conditioned inhibition training (Garcia-Burgo & González, in preparation) using sucrose. Clearly, the relative roles of palatability and calorie content in the present kind of flavor avoidance learning require further examination.

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