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Retardation and summation tests after extinction: The role of familiarity and generalization decrement

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In four conditioned taste aversion experiments with rats as subjects, the effects of extinguished or pre-exposed flavors on retardation and summation tests was compared. Experiment 1 showed that when steps were taken to ensure similar exposure to the target flavor in all conditions, acquisition after pre-exposure and reacquisition after extinction proceeded at a similar rate and was slower than acquisition in a new stimulus control condition. In Experiment 2, reacquisition occurring 2 days after extinction was again slower than acquisition to a new stimulus, but this retardation disappeared when extinction and reacquisition were separated by a 21 days interval. Experiment 3 showed that both an extinguished and a pre-exposed flavor produced a similar summation effect, attenuating the aversion to a previously conditioned flavor. Finally, Experiment 4 showed that this attenuation was also produced by a new flavor. These results suggest, first, that retarded acquisition after extinction of a conditioned taste aversion might be the result of latent inhibition produced by extended experience with the flavor during extinction and, second, that attenuation of aversion to a test excitor on a summation test might not reflect any specific learning process but be simply due to stimulus generalization decrement.

In the terminology of associative learning, extinction is the process by which the behavioral effects of the association between an antecedent event and a consequence are eliminated by repeated presentation of the antecedent alone. In Pavlovian, or classical, conditioning, this involves repeated exposure to the conditioned stimulus (CS) alone after an acquisition phase in which it has been paired with an unconditioned stimulus (US). While there have been several influential models aimed at explaining the basic mechanisms of acquisition of associative learning (e.g., Rescorla & Wagner, 1972; Pearce & Hall, 1980; Wagner, 1981), extinction has received much less attention and there is not yet a generally accepted theoretical explanation of this apparently simple learning phenomenon. A central issue in the explanation of extinction is wether it is simply an "unlearning" process or wether it involves some active learning process through which the subject learns a new, negative or

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inhibitory association, between the antecedent and the consequence (e.g., Bouton,1993). In the case of Pavlovian conditioning, a strict interpretation of this view means that extinction would endow the CS with the behavioral properties of a conditioned inhibitor.

Classical theories of associative learning, such as the Rescorla and Wagner model (Rescorla and Wagner, 1972) conceive extinction of Pavlovian conditioning as a process by which the CS reaches an asymptote of zero associative strength, not leaving room for active inhibitory learning. However, there have been some recent reports apparently showing that extiguished flavor CSs after conditioned taste aversion have behavioral properties similar to those of a conditioned inhibitor, namely slow reacquisition and summation (e.g., Calton, Mitchell & Schachtman, 1996; Schachtman, Threlked & Meyer, 2000). But Aguado, Brugada & Hall (2001) have obtained results that cast considerably doubt on the interpretation of these data as showing the conditioned inhibiting power of an extinguished flavor CS. These authors showed that, when the proper control conditions are included, the results of the traditional inhibition tests do not lend themselves to an interpretation of extinction in terms of conditioned inhibition. Specifically, Aguado *et al.* (2001) showed, first, that when a non-reinforced preexposure control condition is included, reacquisition after extinction of conditioned taste aversion is slower than acquisition to a new flavor but faster than acquisition to a preexposed one, replicating previous similar results with the conditioned suppression procedure (Bouton & Swartzentruber, 1989). Following the suggestion by Bouton (1986), Aguado et al. reasoned that, in order to show the retardation effect, the proper comparation is not between reacquisition and conditioning to a completely new stimulus, but between reacquisition and conditioning to a preexposed stimulus. Given that extinction involves non-reinforced exposure to the CS, the extinction-reacquisition sequence is identical to the conditions that give rise to latent inhibition, which is precisely a retardation of learning caused by non-reinforced preexposure (Lubow & Moore, 1959), usually interpreted as caused by a loss of associability (e.g., Pearce & Hall, 1980).

A second relevant result from the Aguado *et al*. (2001) report, also found by Schachtman *et al*. (2000), is that if a retention interval intervenes between extinction and reacquisition, reconditioning to the extinguished CS proceeds at a similar rate to that of a new stimulus, thus abolishing the retardation effect. In the Aguado *et al*. report (Exp.2) a retention interval of 15 days between extinction and reacquisition virtually abolished the retardation effect. Moreover, replicating previous findings (Aguado et al., 1997; Kraemer & Roberts, 1984) a similar interval between preexposure and conditioning attenuated the latent inhibition effect in a preexposed group. Though this result does no by itself prove that latent inhibition and slow reacquisition after extinction are mediated by the same mechanism (loss of associability), the fact that there is manipulation that produces exactly the same effect in both cases is perfectly consistent with this interpretation. Though less parsimoniuos, other interpretations are of course plausible and

Schachtman *et al.* (2000) take this result as evidence of forgetting of extinction in the spirit of Bouton´s interference theory (Bouton, 1993)

The second classical test of Pavlovian conditioned inhibition is summation, where a conditioned inhibitor counteracts the behavioral effects of an excitatory CS. While some studies using the conditioned taste aversion procedure have shown that an extinguished CS apparently passes this summation test (e.g., Calton *et al.*, 1996; Schachtman *et al.*, 2000), this result is by no means conclusive. In the Schachtman et al. report, for example, the addition of the extinguished flavor sligthly reduced the aversion shown to the excitatory CS (Exps. 2 and 3), but given that a control condition was not included to control for a possible generalization decrement, this result cannot unambiguously be interpreted as a true summation effect. Moreover, Aguado *et al.* (2001, Exp.4) found a similar reduction of aversion to the compound in an extinction group and in a control for generalization decrement, where the stimulus added to the excitatory CS during the compound test had been simply preexposed.

The present experiments are an attempt to resolve the inconsistencies concerning the behavioral properties of extinguished CSs in retardation and summation tests with the conditioned taste aversion procedure. Experiments 1 and 2 introduce some procedural variations in order to avoid some interpretative problems of our previous results (Aguado *et al.*, 2001; see Denniston & Miller, 2003) concerning reacquisition after extinction. Experiment 3 is a new attempt to test for the ability of an extinguished CS to reduce responding to an excitatory CS in comparison with a preexposed stimulus and Experiment 4 is an explicit attempt to evaluate the potential contribution of generalization decrement to the results of the summation test.

EXPERIMENT 1

This experiment attempted to replicate previous findings of retarded reacquisition after extinction, using the conditioned taste aversion procedure. As we have previously mentioned, Aguado *et al.* (2001) found that reacquisition of an extinguished flavor aversion was retarded with respect to the performance shown by rats for whom the flavor was novel. By contrast, reacquisition after extinction was faster than acquisition in a group of animals who had been given non-reinforced preexposure to the flavor. That is, the reacquisition rate after extinction was intermediate to the rate of acquisition in the novel stimulus and preexposed groups (see also Bouton & Swartzentruber, 1989). However, in the study by Aguado *et al.* (2001), the groups differed in their experience with the target flavor before reacquisition (extinction group) or first acquisition (preexposed group). Given that on the first extinction trials the conditioned flavor still retains considerably excitatory power, the animals in the extinction group would always tend to consume less of that flavor than those of the preexposure control, for whom the flavor is simply new. In fact, subjects in the extinction condition consumed less fluid even on the final extinction session than those in the preexposure condition.

This difference might be of relevance to our interpretation of delayed reacquisition after extinction as a case of latent inhibition. Given that latent inhibition is directly dependent on the degree of exposure to the stimulus (e.g., Fenwick, Mikulka & Klein, 1975; Franchina, Domato, Patsiokas & Griesemer, 1980; Lubow, 1973) and that in taste aversion learning the degree of exposure is determined by the consumption of the fluid, extinction subjects might have shown faster acquisition than the pre-exposed subjects simply because they had received less exposure to the flavor during extinction. Thus, it might be that if the animals in the extinction and preexposure conditions were equated as to their level of exposure to the target flavor, both groups would acquire the aversion at exactly the same rate. To test for this possibility, in this experiment we equated the groups in their experience with the target flavor during extinction or preexposure.

Table 1 summarizes the experimental design of this experiment. In Phase 3 (reacquisition phase) of the experiment, three groups of rats received pairings of the target flavor (A) with the illness produced by an injection of lithium chloride (LiCl). For one of these groups (group Extinction), the target flavor had been paired in Phase 1 (acquisition phase) with LiCl and then presented alone, without consequence, for 10 extinction trials (Phase 2). In group Control, another flavor (B) was paired with illness in the first phase and extinguished in the second phase. Group Pre-exposed received reinforced trials with flavor B in Phase 1 and then received 10 trials of exposure to flavor A in Phase 2. Thus all groups were equated in their experience of LiCl-induced illness, and groups Extinction and Pre-exposed in the number of non-reinforced presentations of the target flavor. Moreover, in order to guarantee that the Extinction and Pre-exposure groups were similarly exposed to the target flavor, subjects in groups Pre-exposed and Control received each day an exposure to the fluid equivalent to the mean intake of the group Extinction. Given these conditions, only if group Extinction shows a more profound retardation than group Pre-exposed would be necessary to conclude that some process in addition to latent inhibition, possibly conditioned inhibition, is operating in the former group.

Table 1: Design of Experiment 1.

Group	Phase 1	Phase 2	Phase 3
Extinction	2 A +	10 A -	2 A+; 1 A -
Pre-exposed	2 B +	10 A -	2 A +; 1 A -
Control	2 B +	10 B -	2 A +; 1 A -

Note: A and B: flavors; +: injection of LiCl: -: non-reinforcement

METHOD

Subjects and apparatus. The subjects were 30 male Wistar rats, 90 days old at the start of the experiment and with a mean free-feeding weight of 306 g (range 294-402 g). They were housed in individual standard home cages with free access to food. The experiment was conducted at the same time each day during the light portion of a 12:12-h light-dark cycle. All treatments took place daily in the home cages. Fluids were presented on these sessions by means of 50-ml polycarbonate centrifuge tubes equipped with stainless steel ball-bearing tipped spouts that protruded into the cages. Fluid consumption was measured by weighing the tubes before and after fluid presentation and recording to the nearest 0.5 g. The fluids were a decaffeinated coffee solution (1% w/v) and a solution of vinegar (1% v/v). The fluids were counterbalanced throughout the experiment. Half of the subjects received exposures to the decaffeinated coffee, while the other half of the subjects received the vinegar solution.The unconditioned stimulus (US) for the aversive conditioning trials was an injection of 0.15 *M* LiCl administered intraperitoneally at 10 ml/kg of body weight (this is a dose inferior to that used by Aguado et al., 2001, who injected animals with 0.15*M* LiCl /20 ml/kg).

Procedure. Initially, the rats were adapted to a water deprivation schedule for 4 days. During this period, the rats were allowed free access to water in the drinking tubes for 30 min on each morning. Supplementary water (presented in the standard water bottles) was given for 30 min each afternoon throughout the experiment. The rats were then assigned to three equal-sized groups approximately matched in term of the amount of water they consumed in this stage.

In Phase 1 of training (see Table 1) all subjects received two conditioning trials on which, after receiving access to 20 ml of the appropriate solution for 30 min, they were injected with LiCl. On these days rats in group Extinction received exposures to the target solution, whereas those in groups Pre-exposed and Control were given the alternative solution. The two days of conditioning were separated by a recovery day on which the rats had free access to water during the 30-min drinking sessions.

Phase 2 was an extinction phase for animals in groups Extinction and Control. Subjects in these groups had free access to the flavor conditioned in Phase 1 for 30 min each morning for 10 consecutive days. Supplementary water was provided from the standard water bottles for 30 min on the afternoon. During this phase, subjects in group Pre-exposed were given 10 sessions of access to the target flavor. In order to guarantee that all rats had the same exposure to the fluids during this phase, subjects in group Pre-exposed received each day an exposure to the fluid equivalent to the mean intake of the other two groups. This phase was followed by a recovery day on which the rats received access to water for 30 min on the morning and supplementary water on the afternoon. In Phase 3 all subjects received three trials consisting of free access to the target flavor for 30 min. The first two

trials ended with an injection of LiCl, while the third trial was a non-reinforced test trial. A recovery day followed each reinforced trial.

RESULTS AND DISCUSSION

At the end of the water deprivation period, there were no differences between groups in mean intakes of water. A single-factor ANOVA conducted on the last day deprivation data revealed no effect of Group ($F < 1$). During conditioning phase (Phase 1), flavor consumption decreased between the first and second conditioning trials, revealing that all three groups formed strong aversions to the fluids with which they were conditioned. One subject of group Control was eliminated from the study on this phase because it became ill and died. An analysis of variance (ANOVA) performed on the consumption data for this phase, with two between-subjects factors (Group and Flavor) and one within-subjects factor (Day), revealed no significant effects of Group [F (2,23) = 1.32; $p = 0.28$] and Flavor ($F < 1$), but an effect of Day [F (1,23) = 123.74; $p < 0.001$]. There were no significant interactions between the within-subjects factor and the Group or the Flavor factors ($F < 1$, in each case). The interaction Group X Flavor was no significant [F (2,23) = 1.33; $p = 0.28$].

During Phase 2, fluid consumption, suppressed by the aversion established during Phase 1, recovered over the course of extinction in groups Extinction and Control. The mean consumption on the last trial of this phase was, for group Extinction, 19.3 ml; and for group Control, 18.9 ml. Subjects in group Pre-exposed drank the target flavor readily during this phase, consuming 19.2 on the final session. A comparison of consumption during this phase demonstrated a significant effect of Day [F (9,207) = 263.62; $p < 0.001$], revealing increased consumption over these trials. There were no significant effects of Group, of Flavor, and their interaction ($F < 1$ in all cases). The analysis also revealed no significant interactions between the within-subjects factor (Day) and the Group ($F < 1$) or the Flavor factors [F (9,207) = 1.39; $p = 0.19$].

Figure 1 presents the results of central interest, group mean intake of target flavor during Phase 3, which included two reacquisition trials and a final, non-paired, test trial. As can be seen in the figure, group Control drank less than each of the other two groups over the four trials, showing a stronger aversion than the other two groups, Extinction and Pre-exposed, that showed higher levels of consumption and thus a weaker aversion. It is important to note that these two groups did not differ over trials, revealing that reacquisition occurred equally slowly after extinction and after preexposure. This description of the results was confirmed by statistical analysis.The ANOVA performed on the consumption data for this phase, with Group and Flavor as between-subjects factors and Day as within-subjects factor, revealed significant effects of Group [F (2,23) = 6.21; $p = 0.007$] and Day [F (2,46) = 97.27; $p < 0.001$], as well as a Group X Day interaction [F (4,46) = 3.67; $p = 0.01$]. Neither the effect of Flavor nor any interactions involving the other factors were significant ($Fs < 1$). One-way analyses carried out trial by trial

yielded a significant effect of Group on day 2 [F (2,26) = 7.57; p = 0.003] and day 3 [F (2,26) = 4.05; p = 0.02], but not on day 1 (F < 1). Post hoc Newman-Keuls comparisons (p < 0.05) revealed that, whereas the groups Extinction and Preexposed did not differ, each of these groups differed from the control group .

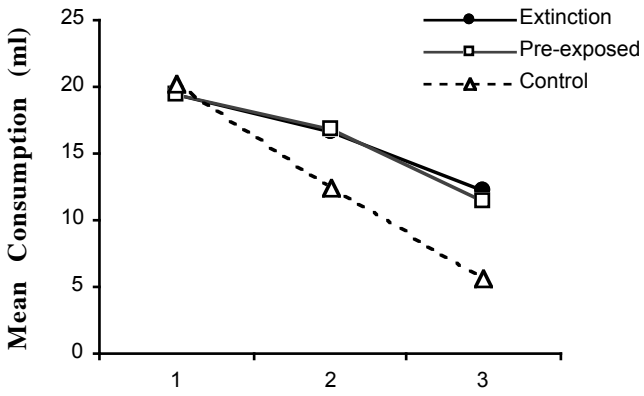

Figure 1. Group mean consumption of the target flavour on the Phase 3 conditioning trials in Experiment 1. The first two trials of the phase were followed by an injection of LiCl; the final trial was non-reinforced.

This experiment replicated previous results with the conditioned taste aversion procedure, showing that reacquisition after extinction is retarded compared to conditioning to a new flavor. In the Aguado *et al.* report (2001; Exp. 1), however, the rate of reacquisition in the extinction group was faster than acquisition in a group that had only been previously exposed to the flavor. This result might be taken as a demonstration that the extinguished flavor not only was not inhibitory but retained, in fact, some excitatory power. However, in the present experiment we controlled for the possibly confounding role of differences in consumption of the target flavor during extinction and preexposure. When steps were taken to ensure a similar level of exposure to the target flavor in the extinction and preexposure groups, both acquired the aversion at a similar rate during phase 3. This suggests that, whether the flavor has been extinguished or simply preexposed before conditioning, the critical variable determining the rate of acquisition is the level of prior exposure to the flavor. This simple variable might thus explain by itself the retardation effect after extinction. It might be said, then, that in the present experiment the target flavor acted simply as a familiar stimulus both after extinction and after preexposure. From this in can be inferred that in order to explain retarded reacquisition when an extinction group and a new stimulus group are compared, there is no need to invoke new inhibitory properties acquired by the flavor during extinction.

EXPERIMENT 2

The aim of this experiment was to investigate whether or not a retention interval following extinction reduces the slow reacquisition otherwise seen with an extinguished CS. The results of Experiment 1 are consistent with the suggestion that the retardation effect obtained following extinction treatment was a consequence of the occurrence of latent inhibition. Support for this suggestion might be obtained by demonstrating that a variable known to modulate the degree of retardation produced by stimulus preexposure is also effective in the extinction condition. Aguado *et al*. (2001; Exp. 2; see also Schachtman *et al*., 2000) adopted this strategy and observed that extinction showed the same sensitivity to the effects of a retention interval as did latent inhibition. Given the potential theoretical importance of the sensitivity of the effects of extinction to the passage of time, the present experiment is an attempt to replicate the main result of an attenuation of the effects of extinction when a long retention interval is interpolated between extinction and reacquisition phases.

The design of Experiment 2 is presented on Table 2. Two groups of rats were given conditioning (Phase 1), extinction (Phase 2), and reacquisition (Phase 3) with the target flavor (A). For one group, Ext-Short, the interval between the last extinction trial and reacquisition was 2 days (as in Experiment 1), whereas for the other, group Ext-Long, that interval was 21 days (this is a longer retention interval than that used in Aguado *et al*. (2001; Exp.2), who used a 15 days retention interval). In a third group, Control, an alternative flavor (B) was first conditioned and extinguised, and then conditioning trials with the target flavor were given. We anticipated, on the basis of previous studies (e.g., Aguado, Symonds & Hall, 1994; Aguado *et al*., 2001; Kraemer & Roberts, 1984), that slow reacquisition effect after extinction observed in Experiment 1 will be reduced by the long retention interval given to group Ext-Long.

Table 2: Design of Experiment 2.

Group	Phase 1	Phase 2	Interval	Phase 3
Ext-Long	2 A +	10 A -	21 days	2 A +; 1 A -
Ext-Short	2 A +	10 A -	2 days	2 A +; 1 A -
Control	2 B +	10 B -	2 days	2 A +; 1 A -

Note: A and B: flavours; +: injection of LiCl: -: nonreinforcement

METHOD

Subjects and apparatus. This experiment used 27 male Wistar rats. The body weights of the rats averaged 358 g (ranging from 297 to 418 g). The rats were maintained under the same conditions as in the Experiment 1 with food freely available in their home cages. Experimental treatments were given daily, in the morning, in the same cages.

Procedure. The procedure for this experiment is shown in Table 2. Details not specified here were identical to those described for Experiment 1. After an initial period of water deprivation, the animals were divided into three equal-size groups, matched for baseline levels of water consumption. In Phase 1, all subjects received two conditioning trials in which they were given access to 20 ml of a flavor for 30 min immediately followed by an injection of 0.15 *M* LiCl (10 ml/kg of body weight). For animals in groups Ext-Long and Ext-Short the solution was the target flavor (A); for animals in group Control it was the alternative solution (B).The flavors were counterbalanced along all the experiment. A recovery day followed each conditioning session. In Phase 2, all the three groups received 10 extinction trials consisting of daily 30-min exposures to the flavor conditioned in Phase 1. A retention interval, of 2 days for groups Ext-Short and Control, and of 21 days for group Ext-Long, intervened between Phase 2 and Phase 3. On each day during this interval, the animals were given free access to water for 30 min on the morning and supplementary water on the afternoon. In Phase 3 all subjects were given three trials consisting of free access to the target flavor for 30 min. The first two trials ended with a LiCl injection and the third trial was a nonreinforced trial. There was a recovery day following each reinforced trial.

RESULTS AND DISCUSSION

There were no differences between groups in mean intakes of water on the end of deprivation period ($F < 1$). All groups showed a decrease in consumption during the initial conditioning phase (Phase 1). The ANOVA performed on the consumption data for this phase revealed only a significant main effect of Day [F (2,20) = 447.62; $p < 0.001$], confirming that aversion increased over conditioning days. Neither the main effects of Group and Flavor [F (2,20) = 1.80; $p = 0.19$, and $F < 1$, respectively) nor interactions involving these two factors and Day approached significance ($F < 1$ in each case).

Extinction (Phase 2) proceeded uneventfully as well; consumption of flavor conditioned during Phase 1 recovered over the course of extinction in all three groups. One subject from group Control was dropped from the study on Day 2 of this phase because it failed to drink at least 2 ml of the target flavor on this day. The mean consumption on the last extinction trial for the various group were: group Ext-Long, 20.82 ml; group Ext-Short, 22.16 ml; group Control, 19.33 ml. The analyses carried out on the data for this phase revealed a significant effect of Day [F (9,180) = 109.8; $p < 0.001$], but no

significant effects of Group [F (2,20) = 2.44; p = 0.11] and Flavor [F (1,20) = 3.28; p = 0.08], and no interactions betweeen these two factors (F < 1).

Consumption on phase 3, which included two reacquisition trials and a final, non-paired, test trial, is represented in Figure 2. As can be seen in the figure, rats in groups Ext-Long and Control showed a decrease in consumption over the trials, indicating that aversion occurred readily in these groups. By contrast, the group (group Ext-Short) that experienced a short retention interval between extinction and reacquisition phases drank more over the course of this phase, indicating slow reacquisition of the aversion.This description of the results was confirmed by the ANOVA performed on the consumption data for this phase, with Group and Flavor as between-subjects factors and Day as within-subjects factor. The analyses revealed significant effects of Group [F (2,20) = 14.11; p < 0.001] and Day [F (2,40) = 67.20; p < 0.001], as well as a Group X Day interaction [F (4,40) = 3.50; p = 0.01]. Neither the effect of Flavor nor any interactions involving the other factors were significant (Fs < 1). One-way analyses carried out trial by trial yielded a significant effect of Group on day 2 [F(2,23) = 11.43; p < 0.001] and day 3 [F (2,23) = 13.68; p < 0.001], but not on day 1 [F (2,23) = 1.70; p = 0.2]. Post hoc Newman-Keuls comparisons (p<0.05) revealed that, whereas the groups Ext-Long and Control did not differ, each of these groups differed from the group Ext-Short.

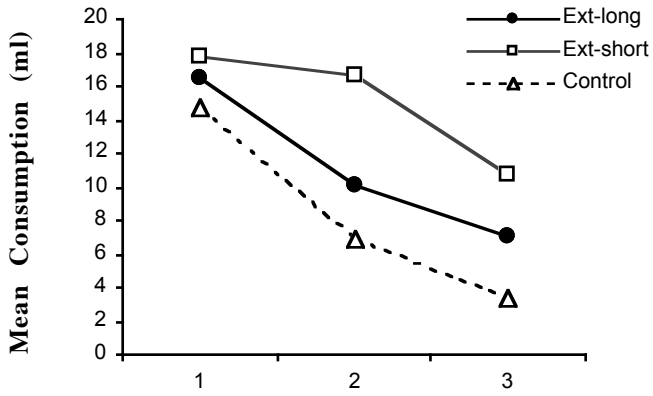

Figure 2. Group mean consumption of the target flavour on the Phase 3 conditioning trials in Experiment 2. The first two trials of the phase were followed by an injection of LiCl; the final trial was non-reinforced.

Slower reacquisition in group Ext-Short than in group Control replicates the effect found in Experiment 1, retarded acquisition after extinction with respect to a control condition in which the target flavor is novel. In addition, the absence of any difference between group Control and group Ext-Long indicates that the slow reacquisition after extinction can be

virtually abolished by interposing a long retention interval between extinction and reacquisition phases. This pattern of results is similar to that obtained by Aguado *et al* (2001), who also showed an attenuation of the retardation effect when reacquisition took place 15 days after extinction. These authors directly compared the effect of the 15 days retetion interval after preexposure and after extinction and found in both cases a similar attenuation of the effect. Though this result admittedly does not definitively prove that slow reacquisition after extinction results from latent inhibition, it is perfectly consistent with this interpretation.

EXPERIMENT 3

Experiment 3 sought to determine the potential of both an extinguished flavor and a preexposed flavor to pass a summation test. Previous studies not including a preexposed stimulus control condition (e.g., Calton *et al*., 1996; Schachtman *et al*., 2001) have apparently found that extinction causes a CS to pass such a test. However, we (Aguado *et al*., 2001) have shown that an extinguished flavor produces exactly the same summation effect as a preexposed flavor and this effect cannot thus be taken as proof of conditioned inhibition by the extinguished flavor. In this experiment, we attempted to provide new evidence on this issue, comparing again the effects of preexposure and extinction through the summation test. Table 3 provides a description of the procedure of Experiment 3. All animals received initial conditioning and extinction of a flavor (A), followed by conditioning of a second flavor (B) which served as the test excitor. During Phase 2, all animals also received non-reinforced exposures to a third flavor (C). During testing, subjects in group Extinction received the test excitor presented in compound with the conditioned and extinguished flavor (AB), while those in group Pre-exposed had access to the excitor in compound with the pre-exposed flavor (CB). All subjects also received a test trial with B to assess the aversion governed by the test excitor. We expected to find, on the basis of the results of Experiment 1, that an extinguished flavor is equally effective in alleviating the aversion to the test excitor than a pre-exposed flavor. This result would support the view that similar processes may underlie the two phenomena (extinction and latent inhibition).

Table 3: Design of Experiment 3.

Group	Phase 1	Phase 2	Phase 3	Test
Extinction	2 A +	10 A -, 10 C-	1 B +	AB -, B-
Pre-exposed	2 A +	10 A -, 10 C -	1 B +	CB -, B -

Note: A , B , C: flavours; +: injection of LiCl: -: nonreinforcement

METHOD

Subjects and apparatus. The subjects were 18 male Wistar rats, with a mean free-feeding weight of 450 g (ranging from 378-511 g) at the start of the experimeent. Except where otherwise stated, apparatus and other procedural details were the same as those described before.

Procedure. The procedure for this experiment is shown in Table 3. After a period of water deprivation, the animals were assigned to two equal-size groups matched in terms of the amount of water they consumed in this stage. In Phase 1, all subjects received two conditioning trials in which they were given access to 20 ml of a flavor (A) for 30 min immediately followed by an injection of 0.15 *M* LiCl (10 ml/kg of body weight). Each conditioning trial was followed by a rest day on which animals received water in their home cages.

Following conditioning, extinction phase (Phase 2) started. This phase lasted 20 days. All subjects received, on alternative days, presentations of the flavor conditioned in Phase 1 (Flavor A), and presentations of a second flavor (C in this case). In order to equate experience with the flavors during this phase, subjects were given 30-min sessions of free access to the flavor undergoing extinction but restricted access to the alternative flavor. On each of these days, rats received an exposure to the Flavor C equivalent to the group mean consumption of Flavor A in the previous day; thus, all subjects had the same experience with the conditioned and then extinguished flavor and with the pre-exposed flavor. The flavors, A and C, were counterbalanced along all the experiment. Phase 3 consisted of a single presentation of a solution of 8 % sucrose (Flavor B, the test excitor) for 30 min followed by an injection of 0.15 *M* LiCl at 10 ml/kg of body. Following a recovery day, all subjects received a first test consisting of free access to the Flavor B for 30 min. After a recovery day, subjects in group Extinction received a second test consisting of free access to the AB compound for 30 min, whereas those in group Pre-exposed received free access to the CB compound for 30 min.

RESULTS AND DISCUSSION

Analysis conducted on the water intake on the day before the first conditioning trial found no significant group differences ($F < 1$). During the initial acquisition phase (Phase 1), the two groups exhibited a decrease in flavor consumption. There were no differences between the groups on the ANOVA conducted on the intake scores from the two conditioning trials ($F < 1$). This analysis also revealed no effect of Flavor nor a significant Group x Flavor interaction ($F < 1$ in both cases), but a significant effect of Day [$F(1,14) = 55.03$; $p < 0.001$].The interactions involving the two between-subjects factors and the Day factor were not significant ($Fs < 1$).

The conditioned aversion to Flavor A during the acquisition phase decreased as expected across the ten extinction trials. There were no differences between the groups during this phase. The mean consumption on

the last extinction trial was, for group Extinction, 18.8 ml; and for group Pre-exposed, 17.9 ml. The ANOVA carried out on Flavour A consumptions from the phase revealed a significant main effect of Day [F (9,126) = 47.08; p < 0.001]. Neither the effects of Group and Flavor nor the interactions between these two factors and Day were significant (F < 1 in each case). A similar analysis was carried out on consumptions of Flavor C during this phase. Mean consumptions on the final session for group Extinction and group Pre-exposed were 19.8 ml, and 20.3 ml, respectively. This analysis revealed a significant effect of Day [F (9,126) = 125.85; p < 0.001]. The pattern indicates that the Day effect was evident regardless of Group and Flavor [Fs (9,126) < 1.79]. Finally, a paired t test analysis conducted on consumptions of Flavor A and Flavor C on the end of this phase revealed that there were no differences in each group (ts < 1). An analysis of sucrose consumption (the known excitor for the subsequent summation test) on the conditioning trial revealed no group differences (F < 1). The mean intake of sucrose on this conditioning trial was, for group Extinction, 22.3 ml; and for group Pre-exposed, 23.8 ml.

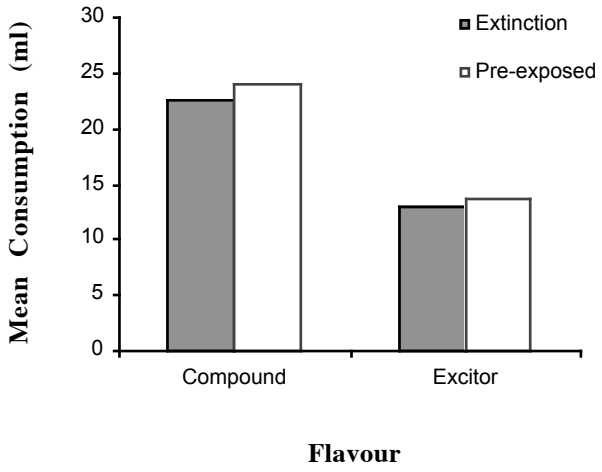

Figure 3. Group mean consumption on the test trials in Experiment 3.

Flavor consumption during the test trials is presented in Figure 3. This figure shows that the groups did not differ in the amount they consumed of the test excitor B, and that both groups drank more of the compound than of the test excitor presented alone. An ANOVA conducted on consumption during these test trials with Group and Trial as the factors revealed a no significant effect of Group (F < 1), but a significant effect of Trial [F (1,16) = 71.94 ; p < 0.001]. There was no significant interaction between these two factors (F < 1). An analysis of simple main effects showed that groups did not differ on the compound trial, and on the B trial (Fs < 1). The mean consumption on the compound trial was, for group Extinction, 22.5 ml; and

for group Pre-exposed, 24 ml. The mean consumption on the test with B was, for group Extinction, 12.9 ml; and for group Pre-exposed, 13.6 ml.

In short, the above pattern of results offers no support for the proposal that an extinguished CS in conditioned taste aversion acts as a conditioned inhibitor in a summation test with a newly established flavor CS. Such an extinguished CS acts rather in the same way as a preexposed stimulus. The more parsimonious explanation of this similarity is that both extinguished and preexposed flavors attenuate the aversion to the new CS due to their familiarity. If this interpretation is correct, then the effects of an extinguished flavor CS on a summation test might as well be interpreted as resulting from simple stimulus preexposure, or latent inhibition, ocurred during extinction.

EXPERIMENT 4

Experiment 3 showed that an extinguished flavor passed a summation test for conditioned inhibition, but no more so than did an equivalently pre-exposed flavor. This finding might be interpreted at least in two different ways. First, it might be that both extinction and preexposure give the stimulus a similar new associative property that is reflected in the summation test. For example, it might be that attenuation of the aversion to an excitatory flavor by extinguished and preexposed flavors would be due to their association to safety or, even more simply, to their familiarity. However, a plausible alternative explanation would be in terms of generalization decrement. Stimulus generalization decrement or stimulus change, due to the compounding of the excitatory flavor and the test flavor (extinguished or preexposed), might largely be responsible for the summation effect after both extinction and preexposure.

Another potential source of confounding is that in Experiment 3 extinction of the target flavor was interspersed with exposure to the neutral flavor during the extinction phase. This procedure resembles a differential inhibition procedure, which might have resulted in the acquisition of conditioned inhibition by the neutral flavor, thereby allowing it to pass a summation test (see Denniston & Miller, 2003). With the aim of obtaining summation test data that are free from these problems, in Experiment 4 we did not include non-reinforced presentations of the neutral flavor during the extinction phase. Moreover, in order to properly evaluate the possible contribution of generalization decrement, the present experiment included test conditions in which the subjects received the test excitor in compound with either a familiar CS (that is, the previously extinguished or pre-exposed flavor) or a novel, nonfamiliar flavor. The design of this experiment is outlined in Table 4.

There were two treatment groups. As in Experiment 3, group Extinction received conditioning and then extinction with flavor A. Group Pre-exposed was given unpaired exposure to flavor A and LiCl, followed by exposure to A. Then, a new excitor, B, that would serve as the excitatory flavor for the summation test, was established in both groups. Finally, the test phase

explored the effects of compounding the excitor B independently with A, the extinguished or preexposed flavor, and with C, the novel flavor. In this way, the relative contribution of familiarity to the flavor produced by extinction and preexposure and of generalization decrement, might be evaluated.

Table 4: Design of Experiment 4.

Group	Phase 1	Phase 2	Phase 3	Test
Extinction	1 A +	10 A -, 1 C-	1 B +	B-, AB -, CB-
Pre-exposed	1 A / +	10 A -, 1 C-	1 B +	B-, AB -, CB-

Note: A , B , C: flavours; +: injection of LiCl: -: nonreinforcement

METHOD

Subjects and apparatus. The subjects were 20 experimentally naive, female Wistar rats with a mean free-feeding weight of 281 g (ranging from 250-314 g) at the start of the experiment. They were maintained on the same food and water regime as in our previous experiments. Except where otherwise stated, the apparatus and the general procedure employed were the same as those described for Experiment 3. The flavors, A and C, were a coffee solution (1% w/v) and a vinegar solution (1.5% v/v). These two fluids were counterbalanced throughout the experiment. The flavor B (the test excitor) was a solution of 8% sucrose.

Procedure. After a schedule of water deprivation had been established, the rats were assigned to two groups (n=10) matched in terms of the amount of water they consumed in this stage. In Phase 1, subjects in group Extinction received a single conditioning trial consisting of exposure to 20 ml of a flavor (A) for 30 min, followed immediately by injection of 0.15 *M* LiCl (10 ml/kg of body weight). Rats in group Pre-exposed were given access to the flavor A followed by an injection of an equivalent volume of 0.15 *M* NaCl. On the day following conditioning, group Extinction received a non-contingent (that is, not paired with the consumption of any flavor) injection of NaCl, while group Pre-exposed received a non-contingent injection of LiCl. This was done to ensure equivalent exposure to LiCl. All rats, therefore, had equivalent exposure to the flavor and lithium, the only difference being whether the two stimuli were paired. The third day following conditioning served as a recovery day. On this day, rats were maintained on their regular food and water regimen and given no injections.

On Phase 2, all rats received nonreinforced presentations of the flavor A (the flavor experienced in Phase 1) for 10 consecutive days. In order to ensure equivalent exposure to the flavor during this phase, rats in group Extinction were given 20 ml of the flavored solution on each session, while those in group Pre-exposed received an exposure to the flavor equivalent to the mean consumption of the extinguished animals on each of these sessions. On the

next day, all animals received a presentation of 20 ml of the flavor C for 30 min in order to familiarize them with the novel flavor to be used on summation testing.

On Phase 3, all animals received a single presentation of the sucrose solution (flavor B, the test excitor) for 30 min, followed immediately by an injection of 0.15 *M* LiCl (10 ml/kg i.p.). After a recovery session with water, the testing phase, which lasted three days, started. On the first day, rats received a test trial with flavor B to assess aversion to the test excitor. The summation test was given over the next two days. All rats were given free access to the BA or BC compounds for 30 min on two successive sesions. Order of presentation of the compounds was counterbalanced. On the first day, half of the animals on each group received the test excitor presented in compound with the familiar flavor (BA), while the remaining subjects had access to the excitor in compound with the novel flavor (BC). The second day, the rats received the alternative solution.

RESULTS AND DISCUSSION

An initial *t* test analysis conducted with the water intakes on the last deprivation day found no significant differences between the two groups [t (18) = 1.76; p = 0.30]. Phase 1 training successfully established an aversion to flavor A in group Extinction. The comparison of mean consumptions of A for this group on the conditioning trial and on the first day of Phase 2 showed a significant difference between these scores [t (9) = 4.79; p = 0.001]. Consumption of A was, however, reestablished on this group over the ten extinction trials (Phase 2). The mean consumption on the last extinction trial (8.7 ml) was similar to that on the conditioning trial (8.4 ml). These scores did not differ reliably [t (9) = 0.22; p = 0.82]. By this measure, then, extinction was complete. During Phase 2, group Pre-exposed drank the flavor A readily, consuming 7.8 ml on the last trial of this phase. On the familiarization session with the flavor C, the novel flavor, the groups did not differ in the amount they consumed of this solution [t (18) = 1.05; p = 0.30]. The mean amount consumed on this session was, for group Extinction, 5.9 ml; and for Group Pre-exposed, 5.6 ml. On the conditioning trial with flavor B, the test excitor (Phase 3), there were no differences between the mean consumptions of the two groups [t (18) = 1.50; $p > 0.05$].

The results of the test phase are presented in Figure 4. This figure shows that the groups did not differ in the amount they consumed of the test excitor B [t (18) = 0.72; p = 0.47], and that both groups drank more of the compound BA (the extinguished or pre-exposed flavor compounded with the excitor) than of the test excitor presented alone [ts (9) > 4,22; $ps < 0.002$). A similar analysis showed that consumption of the compound BC (the novel flavor with the excitor) was indeed greater than consumption of B alone in the two groups [ts (9) > 3.16; $ps < 0.01$). An additional *t* test comparison showed that the two groups consumed similar amounts on BA trial [t (18) = 1.05; p = 0.30], and on BC trial [t (18) = 0.57; p = 0.57]. Finally, the statistical analysis showed that the consumption of the two compounds (BA and BC) was similar

in group Extinction [t (9) = 1.19; p = 0.26], and in group Pre-exposed [t (9) = 1.38; p = 0.19]. The mean consumption on the BA trial was, for group Extinction, 12.5 ml; and for group Pre-exposed, 14 ml. The mean consumption on the test with BC was, for group Extinction, 11.3 ml; and for group Pre-exposed, 12.2 ml. The mean consumption of the test excitor, B, was for group Extinction, 8.9 ml; and for group Pre-exposed 8.1 ml.

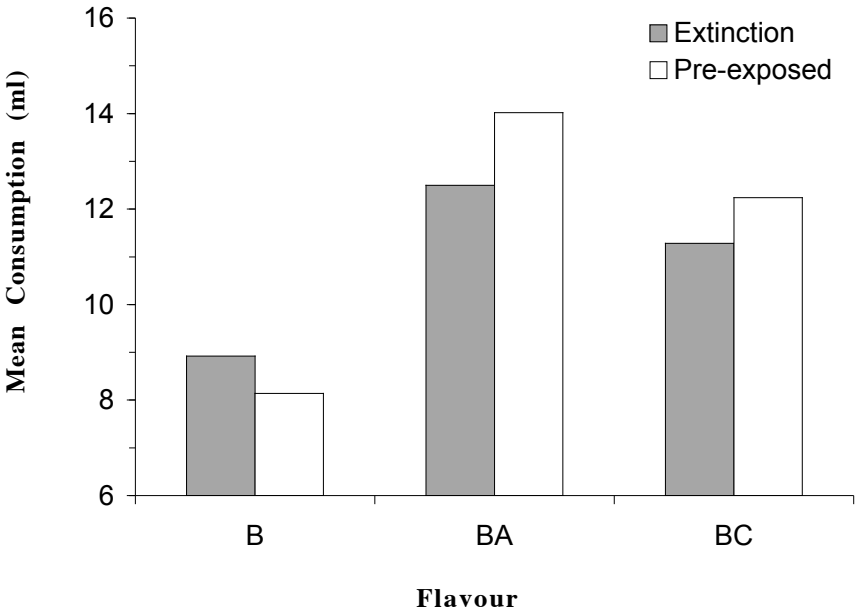

Figure 4. Group mean consumption on the test trials in Experiment 4.

In summary, the present experiment replicated the results of Experiment 3, showing that a similar attenuation of the aversion to a test excitor on a summation test is produced by an extinguished and a preexposed flavor. As we have already mentioned, this result was previously obtained by Aguado, Brugada & Hall (2001) and has been more recently replicated by Brooks, Bowker, Anderson & Palmatier (2003, Exp. 2). Moreover, the present experiment shows that this attenuation is similar to that produced by a non-familiar flavor. Thus, the most parsimonious explanation of these results is that a common simple mechanism, generalization decrement, is responsible of the attenuation of the aversion to the test excitor by an extinguished, a preexposed or a new flavor.

GENERAL DISCUSSION

In the present series of experiments we studied the ability of an extinguished flavor CS in conditioned taste aversion to "pass" the traditional tests of Pavlovian conditioned inhibition, namely retardation and summation (Rescorla, 1969). Replicating the findings of Aguado, Brugada & Hall (2001), we found, first, that reacquisition in an extinguished group was slower than acquisition in a new flavor group, but proceeded at a similar rate than acquisition in a group that had been pre-exposed to the flavor (Exp.1). Given that in this experiment measures were taken to ensure that both extinguished and pre-exposed animals received equivalent exposure to the flavor before the final, reacquisition phase, the more straigthforward interpretation of these result is that one and the same mechanism is acting in both groups, latent inhibition. This interpretation is strengthened by the results of Experiment 2, where we found that retardation of acquisition after extinction was considerably attenuated when reacquisition took place 21 days after extinction. This result also replicates, with a more complete design, what we had previously found (Aguado *et al*., 2001; see also Schachtman *et al*., 2000). This result is of special relevance, given that latent inhibition is also attenuated when a long retention interval is interposed between preexposure and conditioning (e.g., Aguado *et al*., 1994).

Experiment 3 was a new attempt at evaluating the effects of an extinguished flavor CS on a summation test. Again, an extinguished and a pre-exposed group were compared and again aversion to a newly conditioned flavor was attenuated in both groups and to a similar extent. Given that previous reports of summation after extinction of conditioned taste aversion did not include a pre-exposed control condition (Calton et al., 1996, Exp. 2; Schachtman *et al*., 2000), we believe that they cannot be taken as evidence for the interpretation of extinction in terms of conditioned inhibition. However, it should be recognized that Calton et al. included a preexposure control group in their Experiment 3 and observed a slight attenuation of aversion to a former excitor in the extinction group but not in the preexposed control. However, this difference was obtained because in the preexposure control the summation of the preexposed flavor and the former excitor did not produce any attenuation of aversion. This is in itself a somewhat unexpect result, because in our studies we have routinely found that when summated to a former excitor, a preexposed flavor significantly attenuates aversion to it, most probably due to generalization decrement. In fact, Experiment 4 of the present report showed that a new flavor attenuated aversion to a test excitor in a summation test and that it did so to a similar extent than an extinguished or a preexposed flavor.

In the present report we have presented evidence concerning the possible development of conditioned inhibition during extinction of Pavlovian conditioning with the conditioned taste aversion paradigm. We have looked at the effects of extinguished flavors on two traditional tests for inhibition, retardation and summation and we believe that a different explanation shoud be proposed for each. First, although not proving this interpretation, the

results of Experiments 1 and 2, together with those of Aguado, Brugada & Hall (2001, Exps. 1 and 2), are consistent with an explanation of retarded acquisition after extinction in terms of latent inhibition. We are not proposing that extinction can be reduced to latent inhibition. Pavlovian extinction results in a decrement of the conditioned response (CR) or, in conditioned taste aversion, a reduction of the aversion to the flavor, and so it must obviously involve some process that counteracts in some way the effects of conditioning, for example a response inhibition process, as has been suggested by Rescorla (1993). But CR decrement is not the only behavioral effect of extinction. An extinguished CS also is slowly re-conditioned (at least in some paradigms, as conditioned taste aversion) and sometimes can attenuate the CR to a newly established CS. The first of these effects seems to be easily explained as due to extended exposure during extinction. To make our proposal more explicit, our results lead us to think that the retardation effect does not reflect any properties specific of an extinguished CS and is, instead, common to repeatedly exposed, or familiar, stimuli. Whether the specific mechanism by which repeated exposure leads to retarded acquisition is loss of associability, learned unattention or learned safety, cannot be decided based in our results and this is a theoretical issue that is beyond our present purposes.

Second, results of Experiments 3 and 4 showed that attenuation of aversion to an already existing excitor on a summation test is not specific either to extinguished flavors. A similar effect was produced both by a preexposed and a new flavor. This result replicates and extends the results previously obtained by Aguado, Brugada & Hall (2001) and by Brooks et al. (2003, Exp.2). Thus, there seems to be no convincing evidence that the summation test after extinction of a conditioned taste aversion shows the conditioned inhibitory power acquired by the flavor during the course of extinction. A straigthforward interpretation of the results of the summation test is simply in terms of stimulus change or generalization decrement.

To sum up, the results here reported suggest that the effects of an extinguished flavor on the summation and retardation tests with the conditioned taste aversion paradigm might not reflect any mechanism acting specifically during extinction, for example the development of conditioned inhibition. These tests might, instead, reflect the action of more general mechanisms, namely latent inhibition and generalization decrement.

RESUMEN

Pruebas de retraso y sumación después de la extinción: El papel de la familiaridad y el decremento de generalización. En cuatro experimentos de aversión al sabor con ratas se compararon los efectos de estímulos extinguidos o preexpuestos en las pruebas de retraso y de sumación. El Experimento 1 mostró que cuando el grado de exposición al sabor crítico es igual en todas las condiciones, la adquisición tras la preexposición y la readquisición después de la extinción se producen con la misma tasa y más lentamente que la adquisición en una condición de control con un estímulo nuevo. En el Experimento 2, la readquisición 2 días después de la extinción fue, de nuevo, más lenta que la adquisición con un nuevo estímulo, pero este retraso desapareció cuando mediaba un intervalo de 21 días entre la extinción y la readquisición. El Experimento 3 mostró que un sabor extinguido y otro preexpuesto producen un efecto de sumación comparable, atenuando por igual la aversión a un sabor previamente condicionado. Finalmente, el Experimento 4 mostró que una atenuación similar de la aversión es producida también por un estímulo nuevo. Estos resultados sugieren, primero, que el retraso de la adquisición observado con un sabor extinguido podría deberse a la inhibición latente producida por la experiencia repetida con el sabor durante la extinción y, segundo, que la atenuación de la aversión a un excitador en la prueba de sumación podría reflejar decremento de la generalización y no un proceso específico de aprendizaje.

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