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Tests for inhibition after extinction of a conditioned stimulus in the flavour aversion procedure

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In four experiments, rats received flavour aversion conditioning followed by extinction. The flavour was then subjected to retardation and summation tests. Experiment 1 showed that reacquisition of an extinguished flavour aversion was retarded with respect to the performance shown by rats for whom the flavour was novel. No retardation was found, however, with respect to a control group that had been given non-reinforced pre-exposure to the flavour. Experiment 2 demonstrated that extinction showed the same sensitivity to the effects of a retention interval as did latent inhibition, consistent with the view that the retardation effect was a consequence of the occurrence of latent inhibition during extinction. An extinguished stimulus was also found to alleviate the response governed by a separately trained excitor in a summation test (Experiments 3 and 4), but the size of this effect did not exceed that produced by a control stimulus when the procedure used ensured an equivalent aversion to the test excitor in the two cases. These results challenge the proposal that extinction can turn a stimulus into a net inhibitor.

Extinction in classical conditioning (discontinuation of presentation of the unconditioned stimulus, US, with continued presentation of the conditioned stimulus, CS) is commonly held to involve some form of inhibitory learning process. In the influential model proposed by Rescorla and Wagner (1972) this inhibitory process is the symmetrical opposite of the excitatory process that produces increments in associative strength during initial conditioning. Extinction is conceived as a process of "unlearning" in which the associative strength of the CS is decremented trial by trial, until a level is reached (zero strength) appropriate to the

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absence of reinforcement. As the authors of the model were well aware, however, this account must be regarded as an oversimplification, given the evidence (some of it provided by Pavlov, 1927/1960, himself) that the initial association is unlikely to be unlearned as a consequence of the extinction procedure. Current accounts of extinction have usually adopted some form of the notion (proposed, e.g., by Konorski, 1948) that the inhibitory association formed during extinction coexists with the excitatory association formed during conditioning and does not eliminate it. (For an exposition of this view see Bouton, 1993; see also Delamater, 1996; Rescorla, 1993.)

Neither of these accounts supposes, however, that extinction will convert the CS into a net inhibitor, by which is meant a stimulus having properties antagonistic to those possessed by an excitatory CS. According to these accounts, extinction will do no more than render the CS associatively neutral, either because the process of unlearning is complete or because the newly acquired inhibitory link matches in its effects those produced by the previously established excitatory association. It is of substantial theoretical importance, therefore, that recently published experiments (e.g., Calton, Mitchell, & Schachtman, 1996; Hart, Bourne, & Schachtman, 1995) have generated results consistent with the possibility that an extinguished CS can come to function as a net inhibitor. These experiments examine the properties of such a CS when it is subjected to the retardation and summation tests that are routinely regarded as being diagnostic of inhibition (Rescorla, 1969).

Although it has been widely asserted that reacquisition after extinction occurs with particular rapidity, Bouton (1986) has pointed out the inadequacy of the designs of many of the early experiments that have been taken to show such an effect. He went on to show, in experiments using the conditioned suppression procedure (see also Bouton & Swartzentruber, 1989), that a CS that has undergone a large number of extinction trials will reacquire the conditioned response (CR), when reinforcement is reinstated, less readily than will a novel stimulus acquiring the CR for the first time at this stage. Although not found in all training paradigms (see Napier, Macrae, & Kehoe, 1992; Ricker & Bouton, 1996, for a discussion of the reasons for the discrepancy), this retardation effect has been repeatedly confirmed in studies using the flavour aversion learning procedure (Calton et al., 1996; Danguir & Nicolaidis, 1977; Hart et al., 1995; but see also Revusky & Coombes, 1979). The study by Calton et al. is of particular interest in this context in that is also included a summation test. In this it was demonstrated that an extinguished CS was capable of alleviating the suppression of consumption evoked by a separately trained excitor when it was presented in compound with that excitor. It appears that an extinguished CS can "pass" both of the classic tests (retardation and summation) for conditioned inhibition, and Calton et al. concluded, on these grounds, that extinction will render the stimulus inhibitory.

These results could have important implications for our understanding of the nature of extinction. If extinction produces a CS that is a net inhibitor, then it will be necessary to rethink several of our current theories of the phenomenon. It seemed worthwhile, therefore, to conduct further experiments designed to assess the reliability of the effects reported for the flavour aversion paradigm and to explore the validity of possible alternative interpretations of these effects. The first two experiments reported here investigated the retardation test result, the final two experiments the summation test result. They confirm the reproducibility of both the retardation and the summation test results, but they also suggest that neither effect is likely to be a consequence of inhibition as it is usually conceived. Rather they may best be interpreted

as showing that the retardation result is a consequence of a version of the latent inhibition effect and that the result obtained on the summation test reflects various generalization (and generalization decrement) effects.

EXPERIMENT 1

Calton et al. (1996) in their Experiment 1 gave rats a conditioning trial with a saccharin flavour as the CS followed by nine extinction trials. For these subjects, further reinforcement of saccharin produced a negligible suppression of consumption; in contrast, control subjects that had received acquisition and extinction with a different flavour in the initial phases of the experiment acquired an aversion to saccharin quite readily. The present experiment included two groups of subjects (groups extinction and control) intended to allow a replication of this retardation effect.

It has long been appreciated (see, e.g., Rescorla, 1969) that retarded acquisition (or in this case, reacquisition) is not in itself enough to demonstrate that a stimulus has acquired associative inhibition—simple exposure to a stimulus will produce the same result (the latent inhibition effect)—and the mechanism responsible could well reflect, not an associative change, but a change in the associability of the stimulus (e.g., Pearce & Hall, 1980). Given that extinction involves non-reinforced presentations of the CS, it might be that slow learning after extinction is simply a manifestation of latent inhibition and not a consequence of whatever specific learning process takes place during extinction. Bouton and Swartzentruber (1989) made just this point and included in their experiments a control condition in which rats were given the same number of nonreinforced trials as the extinction group but without any initial reinforced trials. Reacquisition of conditioned suppression occurred more rapidly in the extinction group than in this control group—that is, this comparison revealed no evidence of the retardation effect in the group given the extinction treatment.

Accordingly the present experiment included a third group of subjects (group pre-exposed) given the same number of non-reinforced trials with saccharin as the extinction group, but given initial conditioning with a different flavour (in this case, vinegar). These animals can be expected to show latent inhibition—slow acquisition to saccharin in the final stage of the experiment. Only if group extinction shows a more profound retardation than group pre-exposed will it be necessary to conclude that some process in addition to latent inhibition is operating in the former group.

The experimental design is summarized in Table 1. In Phase 3 of the experiment, all three groups of rats received pairings of the target flavour (saccharin) and illness produced by an injection of lithium chloride (LiCl). For one of these groups (extinction), saccharin had been paired in Phase 1 with LiCl and then presented without consequence for 10 extinction trials (Phase 2). In group control, another flavour (vinegar) was paired with illness in the first phase and extinguished in the second. Group pre-exposed received reinforced trials with vinegar in Phase 1 and then received 10 trials of exposure to the target flavour 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 saccharin.

TABLE 1
Experimental design: Experiment 1

Group	*Phase 1*	*Phase 2*	*Phase 3*
Extinction	2 sac+	10 sac–	2 sac+,1 sac–
Pre-exposed	2 vin+	10 sac–	2 sac+,1 sac–
Control	2 vin+	10 vin–	2 sac+,1 sac–

Method

Subjects and apparatus

The subjects were 18 male Wistar rats, with a mean free-feeding weight of 320 g at the start of the experiment. They were housed singly, with food freely available and under a 12-hr light/12-hr dark cycle (lights on at 8:00 a.m.). Experimental treatments were given daily, in the morning, in the home cages. Fluids were presented on these sessions by means of a 50-ml centrifuge tube equipped with a metal spout that protruded into the cage. The flavoured solutions used during training were 0.1% saccharin and 1% vinegar. Supplementary water (presented in the standard water bottles) was given for 30 min each afternoon.

Procedure

Before the start of the experiment, the rats were introduced to a schedule of water deprivation and allowed to habituate to the novel drinking tubes, water being presented in these tubes for a 30-min period on each morning for 3 days. The rats were then assigned at random to one of three equal-sized groups.

In Phase 1 of training (see Table 1) all animals received two conditioning trials on which, after drinking 10 ml of a flavoured solution over 30 min, they received an intraperitoneal injection of 0.15 M LiCl (at 20 ml/kg body weight). For group extinction the solution was saccharin; for groups pre-exposed and control it was vinegar. The two days of conditioning were separated by a recovery day on which the rats had free access to unflavoured water during the 30-min drinking sessions.

Phase 2 was an extinction phase for animals in groups extinction and control. Animals in group extinction had free access to the saccharin solution for 30 min each morning for 10 consecutive days (extinction of the target flavour); those in group control were given the vinegar solution (extinction of the alternative flavour). Group pre-exposed was given 10 sessions of access to saccharin (pre-exposure to the target flavour). In Phase 3 all animals received three trials consisting of free access to saccharin for 30 min. The first two trials were reinforced by a injection of LiCl. A recovery day followed each reinforced trial. Fluid consumption was assessed (to the nearest 0.5 ml) by weighing the drinking tubes before and after each trial.

Results and discussion

During Phase 1, all three groups formed strong aversions to the flavours with which they were conditioned. During Phase 2, group pre-exposed drank the saccharin solution readily, consuming 26.5 ml on the final session of this phase. Consumption of saccharin was reestablished on group extinction, although the level of consumption (19.2 ml) on the final session of the phase remained lower than that shown by group pre-exposed. This outcome—reduced consumption of a previously conditioned flavour even after prolonged extinction—has previously been reported by Rosas and Bouton (1996) and by Bevins, Jensen, Hinze, and Besheer (1999).

Bevins et al. (1999) suggest that the effect may reflect the growth of a preference for saccharin in the control (pre-exposed) condition, although the possibility that the CS retained some excitatory strength in group extinction cannot be ruled out on the basis of these data. Group control resumed drinking the vinegar solution during Phase 2 and consumed 14.1 ml on the final extinction session.

Figure 1 presents the results of central interest—group means for consumption of saccharin in Phase 3. Group control drank less than the other two groups on the first conditioning trial, presumably reflecting a neophobic reaction to saccharin, which was experienced by these animals for the first time on this trial. That group extinction drank somewhat less than group pre-exposed accords with the difference between these groups that was evident at the end of Phase 2. All groups showed a suppression of consumption as a consequence of the conditioning trials. Suppression was profound in group control, slight in group pre-exposed, and occurred at an intermediate level in group extinction.

This description of the results was confirmed by statistical analysis. An analysis of variance with group and trial as the variables showed there to be a significant effect of group, $F(2, 15) = 67.64$, of trial, $F(2, 30) = 61.60$, and their interaction, $F(4, 30) = 4.94$. In this and subsequent analyses a significance level of $p < .05$ was adopted. One-way analyses carried out trial by trial yielded a significant effect of group on each trial: $F(2, 15) = 9.75, 41.30$, and 85.20, for Trials 1, 2, and 3, respectively. Paired comparisons for each trial with Tukey's test gave significant differences for all comparisons, except on Trial 1, where only the difference between groups pre-exposed and control was significant.

The comparison of groups extinction and control confirms that reacquisition after extinction is retarded when comparison is made (as, e.g., in the experiments by Calton et al., 1996, and by Hart et al., 1995) with a control condition for which the CS is novel at the start of the test. But also, as in the experiment by Bouton and Swartzentruber (1989), reacquisition is found to be rapid when the comparison is made with a condition (group pre-exposed) in which the group has not undergone conditioning and extinction but has had extensive prior exposure

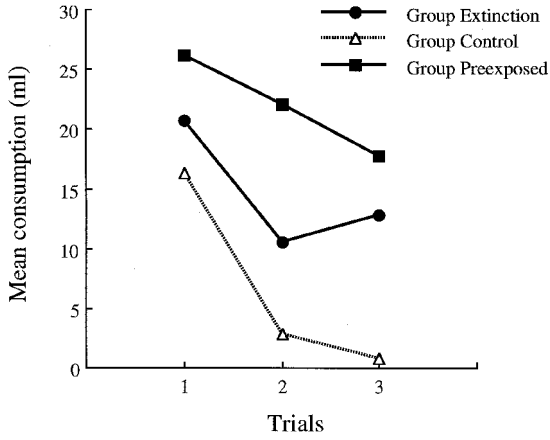

Figure 1. Experiment 1: Group mean consumption of saccharin solution during Phase 3. The first two trials of the phase were followed by an injection of LiCl; the final trial was non-reinforced. Group extinction had previously received conditioning with saccharin followed by extinction; group pre-exposed had received pre-exposure to saccharin; for group control, saccharin was novel.

to the CS. This pattern of results does not require the conclusion that extinction renders the CS inhibitory. Rather it can be readily accommodated by the proposal that the latent inhibition process assumed to be responsible for retarding Phase-3 acquisition in group pre-exposed also operated during the extinction trials in which group extinction received non-reinforced exposure to the CS. That group extinction acquired the aversion in Phase 3 more readily than did group pre-exposed is to be expected if 10 extinction trials did not completely eliminate the excitation established by Phase 1 training in the former group. Alternatively (or additionally), the opportunity for latent inhibition to occur would have been restricted to some extent in group extinction given that their Phase 1 training meant that (unlike group pre-exposed) they drank less than the full amount of saccharin offered on the early trials of Phase 2.

EXPERIMENT 2

The results of Experiment 1 are consistent with the suggestion that latent inhibition is responsible for slow reacquisition after extinction, but they do not prove the point. Although both group extinction and group pre-exposed acquired the aversion in Phase 3 less readily than did group control, it is possible that they did so for different reasons. Support for the suggestion that latent inhibition is responsible in both cases might be obtained, however, by demonstrating that a variable known to modulate the degree of retardation produced by stimulus pre-exposure is as effective in the extinction condition as in the pre-exposure condition. This was the strategy adopted by Bouton and Swartzentruber (1989) with their demonstration that a change of context alleviates the retardation effect in both cases. In this experiment we applied the same logic by trying to see if a factor known to influence latent inhibition, its diminution with the passage of time, also works for the slow reacquisition effect after extinction.

Several studies have found that latent inhibition is conditioned taste aversion is attenuated when pre-exposure and conditioning are separated by a long retention interval (e.g., Aguado, Symonds, & Hall, 1994; Kraemer & Roberts, 1984). If retardation of reacquisition after extinction in conditioned taste aversion results, at least in part, from mere exposure to the flavour during extinction (that is, to latent inhibition), then this effect should be reduced or even abolished if extinction and reacquisition are separated by a long retention interval. In order to assess this possibility, the present experiment made use of two groups, both of which were given conditioning (Phase 1; Table 2), extinction (Phase 2), and reacquisition (Phase 3) with saccharin. For one group, however (group ext-short), the interval between the last extinction trial and reacquisition was 2 days, whereas for the other (group ext-long) that interval was 15 days. (These intervals were the same as those used in our previous study of retention interval effects in flavour aversion learning: Aguado, de Brugada, & Hall, 1997.) In a third, control group, an alternative flavour was first conditioned and extinguished, and then conditioning trials with saccharin were given.

We anticipated, on the basis of the results of Experiment 1, that group ext-short would acquire the Phase 3 aversion less readily than would group control. The question of central interest was whether this retardation might be reduced or even abolished by the long retention interval given to group ext-long. Two further groups (the pre groups of Table 2) were included in order to confirm that the retention interval and training procedures used here were effective in producing an attenuation of latent inhibition. These groups received the same treatment as the ext groups except that their Phase 1 conditioning used a flavour other

TABLE 2
Experimental design: Experiment 2

Group	*Phase 1*	*Phase 2*	*Retention interval*[a]	*Phase 3*
Ext-short	2 sac+	10 sac–	2	2 sac+,1 sac–
Ext-long	2 sac+	10 sac–	15	2 sac+,1 sac–
Pre-short	2 vin+	10 sac–	2	2 sac+,1 sac–
Pre-long	2 vin+	10 sac–	15	2 sac+,1 sac–
Control	2 vin+	10 vin–	2	2 sac+,1 sac–

Note: ext: extinction; pre: pre-exposed; sac: saccharin solution; vin: vinegar solution; + indicates an injection of LiCl; – indicates non-reinforcement.
[a]In days.

than saccharin. Phase 2 thus constituted a latent inhibition treatment—non-reinforced pre-exposure to the flavour used as the CS in Phase 3. For group pre-short the interval between pre-exposure and conditioning was 2 days; for group pre-long the interval was 15 days. We expected to find that conditioning would occur more readily in the latter group.

Method

Subjects

The subjects were 39 male Wistar rats with a mean weight of 321 g at the start of the experiment and maintained under conditions similar to those described for Experiment 1. The animals were divided into four groups of eight and one (group control) of seven.

Procedure

In Phase 1, all animals received two conditioning trials in which drinking of 10 ml of a flavoured solution was followed by an injection of 0.15 M LiCl (at 20 ml/kg of body weight). For animals in groups ext-long and ext-short the solution was of 0.1% saccharin; for animals in group control and in groups pre-long and pre-short it was 1% vinegar. A recovery day followed each conditioning day. In Phase 2, groups control, ext-short, and ext-long received 10 extinction trials, consisting of daily 30-min exposures to the conditioned flavour. Groups pre-long and pre-short received daily 30-min exposures to saccharin during this phase. A retention interval, of 2 days for groups control, ext-short, and pre-short, and of 15 days for groups ext-long and pre-long, intervened between Phase 2 and Phase 3. On each day during this interval, the animals were given access to unflavoured water for 30 min. Phase 3 consisted of two conditioning trials, on which animals had free access to saccharin for a 30-min period prior to injection with LiCl, followed by a final non-reinforced saccharin test trial. Phase 1 training was started 13 days earlier for groups ext-long and pre-long, than for the other three groups, so that all subjects entered Phase 3 on same day. Details not specified here were identical to those described for Experiment 1.

Results and discussion

Fluid consumption, suppressed by Phase 1 training, recovered over the course of extinction so that on the last trial of Phase 2, group ext-long drank 20.3 ml of saccharin, and group ext-short drank 20.2 ml. Group pre-long drank 21.6 ml of saccharin on this trial, and group pre-short drank 26.1 ml. Group control drank 25 ml of the vinegar solution.

Figure 2 shows group means for consumption of saccharin during Phase 3. It can be seen that the two groups that experienced a short retention interval between the last exposure to saccharin and conditioning to it (groups ext-short and pre-short) showed high levels of consumption throughout the test, indicating rather poor acquisition of the aversion. Acquisition occurred readily, however, in the groups for which conditioning to saccharin took place after the 15-day retention interval (groups ext-long and pre-long). At both retention intervals, suppression of consumption was somewhat more marked in the ext than in the pre condition, and, indeed, group ext-long reacquired the aversion to saccharin at the same rapid rate as did the control group not previously exposed to saccharin.

This description of the results was largely confirmed by statistical analysis. An analysis of variance performed on the consumption data for Phase 3, with group and trial as the variables, revealed significant effects of both variables and their interaction: For group, $F(4, 34) = 26.23$; trials, $F(2, 68) = 239.09$; and for the interaction, $F(8, 68) = 28.16$. One-way analyses performed on the data for each trial gave significant differences of the group factor on all three trials: $F(4, 34) = 6.12$, 53.48, and 27.95, for Trials 1, 2, and 3, respectively. The significant effect on Trial 1 was solely due to the low score shown by group ext-short. Pairwise comparisons using Tukey's test showed that this group differed from each of the others, which did not differ among themselves. We are not able to explain this low level of consumption in group ext-short, and it is not an effect that has been replicated in other (unpublished) work of ours employing the same general design.

Of more importance for our present purposes is the pattern of differences observed on Trials 2 and 3. Tukey's test showed that on both these trials group ext-short differed both

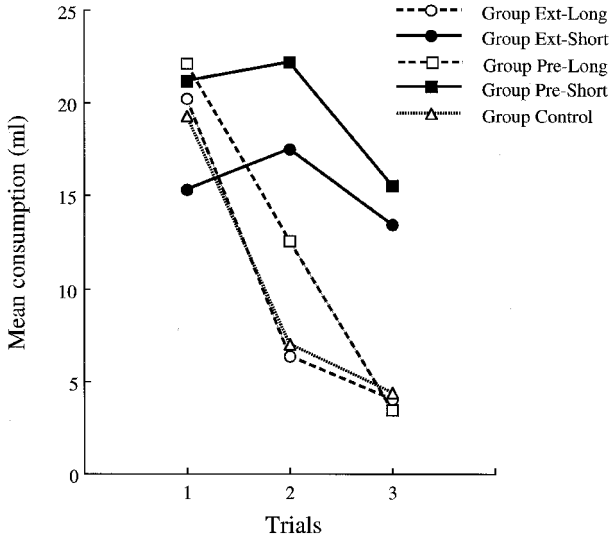

Figure 2. Experiment 2: Group mean consumption of saccharin solution during Phase 3. The first two trials of the phase were followed by an injection of LiCl; the final trial was non-reinforced. The ext groups had previously received conditioning with saccharin followed by extinction; the pre groups had received pre-exposure to saccharin. Short indicates an interval of 2 days between extinction (or pre-exposure) and the start of Phase 3; long indicates an interval of 15 days. For group control, saccharin was novel at the start of Phase 3.

from group control and from group ext-long and that the latter group did not differ from group control. Slower Phase-3 acquisition in group ext-short than in group control replicates the effect found in Experiment 1—retarded acquisition after extinction when comparison is made with a control condition in which the CS is novel. The absence of any difference between group control and group ext-long, a group that had also received the extinction procedure, indicates that the slow reacquisition effect can be abolished by interposing a long retention interval between extinction and reacquisition of a conditioned aversion. The results for the Pre groups show a parallel effect of the retention interval for the latent inhibition case, with group pre-short consuming more (i.e., acquiring the aversion less readily) than groups pre-long and control. Finally, it is of interest that group ext-long differed significantly from group pre-long on this trial, with the latter group showing a lesser aversion to saccharin. This suggests that even after a long retention interval and similar exposure to the target flavour, reacquisition after extinction is faster than acquisition after only pre-exposure, a result than can be interpreted as showing a savings effect on reacquisition even under these conditions.

Our principal result, that extinction and latent inhibition show a similar sensitivity to the effects of a long retention interval, is reminiscent of a result previously reported by Kraemer and Spear (1992). Specifically, they found that pre-exposure to a flavour, A, followed by conditioning to another flavour, B, resulted in an attenuation of the aversion to B by generalization of latent inhibition from A to B, but that this effect was eliminated if the test with B was delayed for 21 days. Similarly, using a generalized extinction procedure, they found that conditioning to A followed by exposure to B resulted in an attenuated aversion to A, and again that this effect was absent if the test with A was delayed.

One important difference between Kraemer and Spear's (1992) results and those obtained in the present experiment is that we looked at the effect of extinction on reacquisition and not on "spontaneous" changes in the aversiveness of the flavours over time. In this experiment there was no sign of spontaneous recovery in the ext-long group; indeed, on the first trial of the test phase, suppression of consumption was less marked in the group given the retention interval (group ext-long) than in group ext-short. Although spontaneous recovery of an extinguished CR is well established for some conditioning procedures (e.g., Rescorla, 1997), its failure to appear here is consistent with previous work on taste aversion. In their investigation of the spontaneous recovery using this procedure, Rosas and Bouton (1996) obtained the effect (after an 18-day retention interval) after three but not after eight extinction trials. It is thus not surprising that spontaneous recovery was not found after 10 extinction trials in the present experiment.

A further difference between our experiment and that of Kraemer and Spear (1992) is that we obtained our effects using the same target flavour for pre-exposure and conditioning and for extinction and reacquisition, instead of the two flavours used in the generalized latent inhibition or extinction procedure in Kraemer and Spear's experiments. (Kraemer and Spear failed to obtain their effects when the same flavour was used throughout.) A question still to be resolved is why in Kraemer and Spear's experiments the effect depended critically on using different flavours in each phase and why direct, not generalized, latent inhibition and extinction did not show any sensitivity to retention interval.

Kraemer and Spear (1992) interpreted their results as indicating that extinction and latent inhibition involve a shared process. The most radical interpretation of this suggestion is that the formation of a representation that the stimulus is followed by no event is solely responsible

for both phenomena and that they differ only procedurally in that, in the case of extinction, a conditioning phase precedes non-reinforced exposure whereas in latent inhibition conditioning follows. Our results are perfectly consistent, however, with the less radical position that both extinction and pre-exposure produce a change in the associability of the stimulus (latent inhibition). It is not our purpose to attempt to resolve this issue here. It is enough to note that the existence of the latter possibility means that slow reacquisition after extinction cannot be unambiguously attributed to the acquisition of net inhibitory strength by the extinguished CS; rather it is more parsimoniously interpreted as being an example of a version of latent inhibition.

EXPERIMENT 3

The notion that extinction brings about a reduction in stimulus associability can accommodate the results described so far. It now becomes critical, therefore, to examine the proposal that an extinguished CS can "pass" a summation test, an effect not to be explained in terms of latent inhibition. Evidence to support this proposal comes from Calton et al. (1996). In their Experiment 3B, they gave rats in the experimental group conditioning with saccharin followed by nine extinction trials. A test excitor (vinegar) was then established, and the rats were tested with the vinegar–saccharin compound. That they drank more on test than did control subjects given the same initial training but tested with vinegar alone is perhaps not surprising—it may simply mean that the addition of saccharin renders vinegar more palatable. More critical is the comparison made with a further control group. Animals in this group received initial conditioning with a different flavour (coffee), nine non-reinforced trials with saccharin, and conditioning to vinegar before the final test with the saccharin–vinegar compound. These subjects too drank significantly less than the experimental subjects suggesting that, for the latter, the treatment given to saccharin (conditioning followed by extinction) had endowed this flavour with inhibitory properties.

Our first step in investigating this intriguing finding was to attempt to replicate its essential features using our own experimental procedures. The experimental design is summarized in Table 3. There were two groups of subjects. Those in the experimental group received conditioning and then extinction with Flavour A. Flavour B was then reinforced to established a test excitor, and the critical test consisted of measuring consumption of the AB compound. Comparison was made with a control group given the same treatment except that initial conditioning was to a different flavour, C. All subjects received a final test trial, in which B was presented alone, to assess the aversion governed by the test excitor. It is necessary to include

TABLE 3
Experimental design: Experiment 3

Group	*Phase 1*	*Phase 2*	*Phase 3*	*Test*
Experimental	1 A+	9 A–	1 B+	AB–, B–
Control	1 C+	9 A–	1 B+	AB–, B–

Note: A and C represent sucrose and saline solutions (counterbalanced); B represents a solution of dilute acid; + indicates an injection of LiCl; – indicates non-reinforcement.

such a test (one not available in Experiment 3B of Calton et al., 1996), as any difference between the groups in their response to the AB compound can only be interpreted easily if we are confident that the groups do not also differ in the degree of aversion controlled by B. For reasons presented later, we were concerned that this experimental design might generate group differences in the strength of B.

Method

Subjects

The subjects were 24 male hooded Lister rats, with a mean free-feeding weight of 438 g at the start of the experiment. They had previously served in a study of shock-reinforced conditioning but were naive with respect to the stimuli and procedures used in the present experiment. They were housed in individual cages in a colony room, illuminated between 8:00 hours and 20:00 hours each day. After a schedule of water deprivation had been established, as in the previous experiments, they were assigned at random to one of two equal-sized groups.

Procedure

On the first day of training they were given access to 10 ml of Flavour A (experimental subjects) or Flavour C (control) subjects, and consumption of the flavour was followed by an injection of 0.3 M LiCl at 10ml/kg of body weight. For half the rats in each group, the conditioning flavour was a 10% sucrose solution, and for half it was a 1% solution of NaCl. After a recovery day, Phase 2 began. On each of the next 9 days, all subjects were given free access for 30 min to Flavour A (the conditioned flavour for the experimental subjects, the alternative flavour for the control subjects). Phase 3 consisted of a single presentation of 5 ml of a 0.01 M solution of HCl (Flavour B) followed by an injection of 0.3 M LiCl at 10ml/kg of body weight. On the first test trial all groups received free access to the AB compound for 30 min. It had been intended that the next day should consist of a test trial with Flavour B but, because of an error on the part of the experimenters, all subjects received a further compound trial, half of each group receiving the AB compound as before, and half the AC compound. The test with B was thus given on the day following this unscheduled extra test trial. Procedural details not specified here were the same as those described for the previous experiments.

Results and discussion

Phase 1 training successfully established an aversion to Flavour A in the experimental group. On the first day of Phase 2, the mean consumption of A for this group was 1.6 ml whereas that for the control group was 7.8 ml. These means differed reliably, $t(22) = 6.79$. Consumption of A increased in both groups over the course of Phase 2, consistent with the occurrence of extinction in the experimental group and the habituation of neophobia in the control group. By the final day of this phase the group mean consumption scores were 18.1 ml for group experimental and 20.8 ml for group control. These scores did not differ significantly, $t(22) = 1.84$.

The results of the test phase (group means for consumption on each of the test trials) are shown in Figure 3. Both groups drank more of the AB compound than of B presented alone. This difference need not be taken to imply that A has acquired inhibitory properties—it may be that the AB compound is intrinsically more palatable than B; or the addition of A might produce generalization decrement so that B presented in compound is discriminated to some

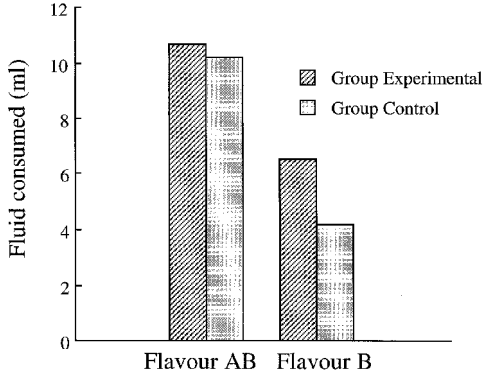

Figure 3. Experiment 3: Group mean consumption in the test phase of Flavour B (acid) and of an AB compound (A = saline or sucrose). All subjects had previously received a reinforced trial with B. Group experimental had received conditioning and extinction with A; group control had received non-reinforced pre-exposure to A.

extent from B presented alone. The theoretically important comparison is between the two groups in their consumption of AB, and here group experimental drank marginally more than group control. Although small, this difference is consistent with the proposal that A has acquired inhibition in group experimental. Interpretation of this difference is complicated, however, by the fact, also revealed by the figure, that the groups differed in their consumption of B, with group experimental again drinking more than group control. An analysis of variance with group and stimulus type as the variables showed there to be a significant effect of stimulus type, $F(1, 22) = 67.59$, and of group, $F(1, 22) = 5.09$. The interaction between these variables was not significant, $F(1, 22) = 2.30$.

It will be recalled that, intervening between the test trials, half the animals in each group received a further presentation of AB, and half received, in error, a trial with the novel compound AC. This error appeared to be without important consequence. A further analysis of the data for the test with B, which included as a variable the type of compound that the subject had experienced on the preceding trial, revealed no significant effect of this variable ($F < 1$) and no interaction between this variable and group ($F < 1$). The main effect of group (experimental vs. control) was, however, reliable, $F(1, 20) = 8.50$.

As we have said, the difference between the groups in their consumption of AB accords with the suggestion that A had acquired inhibition in group experimental. But to adopt this conclusion would be unwise in view of the fact that the groups also differed in their consumption of B. The pattern of results shown in Figure 3 can equally be interpreted as showing that the aversion to the test excitor was somewhat less profound in group experimental and that this was revealed both in the direct test with B and the test with the AB compound. In fact, there are good reasons for expecting that the experimental design used here (and in the experiment by Calton et al., 1996) might result in B being more aversive in group control than in group experimental. It seems likely that there will be some degree of generalization between the flavour trained in Phase 1 and the test excitor B. For group control, therefore, the aversion governed by B in the test phase would depend on the aversion directly conditioned to B plus a component from generalization of the aversion established to the Phase 1 flavour. For group experimental, on the other hand, the generalized component would be lacking, given that

Phase 2 allowed the extinction of the aversion trained in Phase 1. In order to obtain summation test data that are free from this problem, it is necessary to devise an experimental design that ensures that the aversion governed by the test excitor is matched in the critical groups. Experiment 4 attempted to achieve this.

EXPERIMENT 4

The design of Experiment 4 is outlined in Table 4. There were two treatment groups. As in Experiment 4, group experimental received conditioning and then extinction with Flavour A followed by a test of the effects of compounding A with the test excitor B. Group control received initial conditioning with Flavour C followed by non-reinforced presentations of A in Phase 2. But Phase 2 for this group also included non-reinforced presentations of C, allowing extinction of the aversion established in Phase 1 and thus equating the groups in the extent to which generalization from Phase 1 training might influence the aversion controlled by the test excitor. The experimental group was matched to the control group in that these subjects received trials in Phase 2 with the alternative stimulus (C in this case). A further refinement was that an effort was made to ensure that the total amount of exposure to the two flavours was equated for each of the groups. By giving 19 Phase 2 trials with the flavour undergoing extinction (A for the experimental group and C for the control group) but only 12 trials with the alternative flavour, we were able to compensate for the fact that relatively little of the conditioned flavour was consumed on the early trials of this stage.

With the experimental design used here, the groups are matched in the extent to which generalization from the aversion established in Phase 1 contributes to the aversion controlled by the test excitor B in the final test phase. We may hope, then, that the groups will show the same degree of aversion to B, thus providing the possibility of a less ambiguous test of A's effect on B than that allowed by Experiment 3. In these circumstances, the observation that A is more effective in alleviating the aversion to B in group experimental than in group control would support the view that conditioning and extinction of A in group experimental had turned A into a net inhibitor. Alternatively if an extinguished stimulus still retains some measure of excitatory strength (as might be concluded on the basis of the results of Experiments 1 and 2), A will be less effective at alleviating the aversion to B in group experimental than in group control.

TABLE 4
Experimental design: Experiment 4

Group	*Phase 1*	*Phase 2*	*Phase 3*	*Test*
Experimental	2 A+	19 A–, 12 C–	2 B+	AB–, B–
Control	2 C+	12 A–, 19 C–	2 B+	AB–, B–

Note: A and C represent sucrose and saline solutions (counterbalanced); B represents a solution of dilute acid; + indicates an injection of LiCl; – indicates non-reinforcement.

Method

Subjects

The subjects were 24 experimentally naive, male hooded Lister rats with a mean free-feeding weight of 380 g at the start of the experiment. They were maintained as in Experiment 3, and the general procedures employed were the same as those described for Experiment 2.

Procedure

After a schedule of water deprivation had been established, the rats were divided at random into two equal-sized groups. Both then received two Phase 1 conditioning trials in which access to 10 ml of Flavour A (experimental group) or Flavour C (control group) was followed by injection of 0.15 M LiCl at 10 ml/kg of body weight. A recovery day followed each of these trials. For half the rats in each group the flavour used in Phase 1 was saline, and for half it was a sucrose solution; for the remainder the arrangement was reversed.

On each of the next 12 days all animals received two 30-min drinking sessions daily, one in the morning and one in the afternoon. On each session 10 ml of a flavoured solution was presented. For half the animals in each group, Flavour A was presented in the morning and Flavour C in the afternoon; the reverse arrangement held for the other subjects. For the next 7 days, a flavoured solution was presented only in the morning (unflavoured water being made available in the afternoons). On these sessions, group experimental received Flavour A, and group control received Flavour C.

In Phase 3 all subjects received two trials (each followed by a recovery day) in which access to Flavour B (an HCl solution) was followed by an injection of 0.15 M LiCl at 10 ml/kg of body weight. On the day following the second recovery day, all subjects received a first test consisting of free access to the AB compound for 30 min. On the following day all received a similar test with Flavour B alone.

Results and discussion

Phase 1 conditioning established an aversion to the flavour reinforced in that stage. On the first day of Phase 2, the mean amount consumed of this flavour (A for the experimental group and C for the control group) was 0.3 ml. The mean consumption of the alternative flavour was 7.8 ml. All individuals drank more of the latter than of the former. Over the subsequent 11 trials on which the alternative flavour was presented, all animals drank almost all of the full amount offered. Consumption of the conditioned flavour increased steadily over the extinction trials, so that by Trial 14 all that was offered was consumed; this situation continued for the remaining five presentations of this flavour. In consequence the rats drank approximately the same amount of each of the flavours during Phase 2. Group mean consumption, totalled over all trials of this phase was 108.2 ml for the flavour conditioned in Phase 1 and 104.2 ml for the alternative flavour. These scores did not differ significantly, $t(23) = .22$.

The results of the test phase are presented in Figure 4. The groups did not differ in the amount they consumed of the test excitor B. Both drank more of the AB compound than of B, but the alleviation of suppression was much more marked in group control than in group experimental. An analysis of variance with group and trial type as the variables showed there to be a marginally significant effect of group, $F(1, 22) = 3.89$, $p < .01$, a significant effect of trial type, $F(1, 22) = 80.67$, and a significant interaction between these variables, $F(1, 22) = 7.22$. An analysis of simple main effects showed that the groups differed on the AB trial, $F(1, 22) = 6.29$, but not on the B trial ($F < 1$).

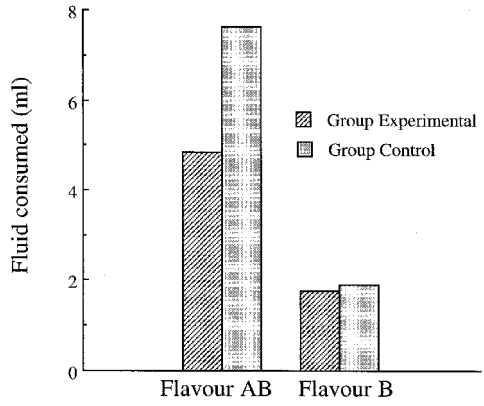

Figure 4. Experiment 4: Group mean consumption in the test phase of Flavour B (acid) and of an AB compound (A = saline or sucrose). All subjects had previously received reinforced trials with B. Group experimental had received conditioning and extinction with A and non-reinforced pre-exposure to C; group control had received non-reinforced pre-exposure to A and conditioning and extinction with Flavour C.

These results supply no support for the suggestion that extinction will render a CS inhibitory as assessed by a summation test. Consumption of AB was indeed greater than consumption of B, but this was true of both groups and probably reflects the occurrence of generalization decrement in both. The critical comparison of AB consumption in the two groups showed that the alleviation of suppression to B produced by the addition of A was less marked in group experimental than in group control. This outcome is perfectly consistent with the suggestion that an extinguished CS may still retain some residual amount of excitation.

This last observation raises the possibility that extinction might have been incomplete in group experimental, even after 19 Phase-2 trials, and that had further extinction training been given, excitation would have been replaced by inhibition. Alternatively, the phase of conditioning with B might have produced a reinstatement effect (e.g., Rescorla & Heth, 1975; but see also, Bouton, 1982), restoring the excitation governed by A and obscuring such inhibition as it might have acquired during the extinction phase. In order to evaluate these possibilities, all subjects were given a further phase of training immediately after completion of the test just described.

Over the course of the next 8 days they received, on alternate days, four further presentations of A and four of C. On each of these trials 30 ml of fluid was offered (more than a rat will normally drink in a 30-min session), allowing a more accurate assessment of the extent of extinction of the flavour conditioned in Phase 1. On the final trials of this phase the mean amount consumed of the flavour that had been conditioned in Phase 1 (A for the experimental subjects and C for the control subjects) was 17.0 ml; the mean amount consumed of the alternative flavour was 18.3 ml. These scores did not differ reliably. By this measure, then, extinction was complete. Further tests then followed, without further conditioning to B, thus precluding the possibility of reinstatement of the aversion to A. All subjects received a test trial with a compound of B and the conditioned flavour, followed by a trial with a compound of B and the alternative flavour, and finally a trial with B presented alone. The mean amounts consumed were 8.3 ml of B plus the conditioned flavour, 10.3 ml of B plus the alternative flavour,

and 4.1 ml of B alone. An analysis of variance, with trial type as a within-subject variable and Phase 1 group assignment (experimental or control) as a between-subject variable, revealed no significant effect of the latter ($F < 1$) but a significant effect of trial type, $F(2, 44) = 40.41$. Subsequent pairwise comparisons using Tukey's test showed that the score for each trial type differed significantly ($p < .05$) from each of the others. Thus the aversion governed by B was alleviated both by the presence of the conditioned flavour and also by the presence of the alternative flavour; but even after very extensive extinction, the flavour that had been conditioned in Phase 1 still remained more excitatory than the alternative flavour. These results thus lend no support to the proposal that extinction will endow a CS with inhibition; rather, by some measures, such a CS will still retain some excitatory properties, even after apparently complete extinction of the initial excitation.

GENERAL DISCUSSION

The experiments reported here were designed to assess the proposal that prolonged extinction can turn an excitatory CS into a net inhibitor. They successfully confirmed that such a stimulus can indeed pass both retardation and summation tests for inhibition. In each case, however, further investigation suggested an alternative explanation for the results. In the case of the retardation test, relatively slow reacquisition by the trained and extinguished CS was found only when comparison was made with a control group for whom the stimulus used in the test phase was novel (Experiment 1). This prompted the suggestion that the slow learning shown by the extinguished group is a product of the occurrence of latent inhibition during the nonreinforced trials of extinction (see also Bouton, 1986). Support for this suggestion came from the demonstration (Experiment 2) that extinction shows the same sensitivity to the effects of a retention interval as does latent inhibition itself.

The ability of an extinguished CS to pass a summation test was found only in circumstances in which the training procedure also produced a reduction in the magnitude of the CR governed by the test excitor with which it was compounded (Experiment 3). When this factor was controlled for (in Experiment 4), no inhibitory properties were evident; rather evidence of residual excitation was found even in a stimulus that had undergone very extensive extinction training.

The impact of these results is largely negative; that is, they serve chiefly to undermine support for the theoretically intriguing proposition that extinction might be able to generate a net inhibitor. On the positive side, however, they help to clear the way for the full development of those theories of extinction that suppose that the inhibitory effects of extinction could match, but never exceed, the excitatory effects produced by initial training.

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