

BRIEF COMMUNICATIONS

Factors Determining the Effects of Associative Activation on Habituation

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In 2 experiments, rats received flavor-aversion conditioning with two flavors, B and C, to which they had been preexposed. In both experiments, C was preexposed in compound with another flavor in a block of CX trials. In Experiment 1, B was presented in compound with Y, and BY trials were alternated with presentations of Y alone. In Experiment 2, B was presented in compound with X, and BX trials were alternated with presentations of X alone. No difference was detected in Experiment 1 between B and C in the ease with which they conditioned, but in Experiment 2 it was found that B conditioned more readily than C. This latter result is consistent with the hypothesis that experience with the associate of a target stimulus can act to maintain the effective salience of that stimulus; however, the results of Experiment 1 challenge this interpretation or indicate the operation of other factors that limit the effectiveness of this salience modulation process.

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Blair, Wilkinson, and Hall (2004) reported in their Experiment 3b the effects of preexposure to the stimuli on flavor-aversion conditioning. The subjects, rats, were given nonreinforced preexposure to three compound flavors, AX, BX, and CX (A, B, and C were unique, distinguishing features of the compounds and X was a component present in all of them). Preexposure was scheduled so that AX and BX were presented in alternation, whereas CX was presented on a separate block of trials. After this experience, separate groups of rats were given flavor-aversion conditioning with either B or C as the conditioned stimulus (CS). Acquisition occurred more readily in the group given B as the CS. Blair et al. interpreted this result in terms of the notion of salience modulation offered by Hall (2003; see also Hall, Blair, & Artigas, 2006). According to this account, exposure to a stimulus results in a loss of effective salience. This proceeds unopposed for stimulus C on the CX trials, but for stimulus B the intermixed presentations of its associate X (on the AX trials) are held to activate a process that reverses this loss. Conditioning will thus occur more readily for the salient stimulus B than for the less salient stimulus C.

Dwyer and Honey (2007) recently published a study that calls this account into question. Although the detailed procedure used in their experiments differed in several ways from that used by Blair et al. (2004), the logic was essentially identical. Their rats received presentations of two compound stimuli, CX and BY, along with separate presentations of the associate of one of them, Y in this case. (Their nomenclature was slightly different, but we retain the

system in which B and C are the critical stimuli to facilitate comparison with the results of Blair et al.) They then gave conditioning to the compound BC. According to the theory proposed by Hall (2003), the separate presentations of Y during preexposure should act to maintain the salience of B, and B should condition more readily than C. But when B and C were tested after the compound conditioning trial, no difference was seen; indeed, in their Experiment 2, Dwyer and Honey found that the aversion was somewhat greater for C than for B.

The results reported by Dwyer and Honey (2007) constitute a challenge to the theoretical view proposed by Hall (2003). As a first step in attempting to meet this challenge, it is necessary to identify, empirically, the source of the discrepant results, and that was the aim of the experiments that follow. The source of the discrepancy must lie in some aspect of the procedural differences mentioned above. These include the following: The flavors used as stimuli by Dwyer and Honey were different from those used by Blair et al. (2004); the exact schedules of preexposure were different in the two reports; Dwyer and Honey paired B with a different stimulus (Y) in preexposure, whereas Blair et al. used X as the common element throughout; Dwyer and Honey gave conditioning to the BC compound followed by tests with the elements, whereas Blair et al. conditioned B and C separately; and in the study by Blair et al., presentations of the associate of B consisted of trials in which it was compounded with another stimulus (i.e., AX trials), whereas in Dwyer and Honey the associate (Y) was presented on its own.

It is difficult to know which of these factors is critical. Our intuition was that the use of the compound conditioning procedure might be so—that the presentation of B and C in compound on the conditioning trial in the Dwyer and Honey (2007) procedure might have induced generalization decrement effects that obscured the effects produced by differences in salience. Accordingly, in Experiment 1 we examined the condition in which the subjects were conditioned with the BC

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compound, but we also included separate groups conditioned with either B or C as the CS. We followed Dwyer and Honey in their choice of flavors, in giving preexposure to CX and BY (rather than BX), and in giving separate presentations of Y alone (rather than in compound with some other stimulus). The schedule of stimulus presentation was, however, analogous to that used by Blair et al. (2004).

Experiment 1

In this experiment, all the subjects received preexposure, consisting of trials with BY and Y presented in alternation, and a separate block of trials on which CX was presented. One group of rats (the compound group) then received two trials of conditioning in which consumption of the BC compound was followed by an injection of lithium chloride (LiCl); separate tests with B and C assessed the aversion acquired by each element. Other rats (the element groups) received aversion conditioning with either B or C as the CS. (The experimental design is summarized in Table 1.) If the salience modulation mechanism postulated by Hall (2003) operates in these circumstances, we would expect to see a more profound aversion to B than to C on the test for the compound group and over the course of acquisition for the element groups.

Method

Subjects and apparatus. The subjects were 24 experimentally naive male hooded Lister rats (*Rattus norvegicus*), with a mean ad lib weight of 310 g at the start of the experiment. The rats were singly housed with continuous access to food in a colony room that was artificially lit from 8 a.m. to 8 p.m. each day. Access to water was restricted as detailed below.

The solutions used as experimental stimuli were administered in the home cages at room temperature in 50-ml plastic centrifuge tubes, each equipped with a rubber stopper to which was fitted a stainless steel ball-bearing-tipped spout. We used the flavors used by Dwyer and Honey (2007): sucrose, 2% (weight/weight); saline, 0.9% (weight/weight) sodium chloride; quinine, 0.00006 M quinine sulfate; and acid, 0.01 M hydrogen chloride. For half the subjects, stimulus B was sucrose and stimulus C was saline; for the remainder, the reverse applied. For half of the subjects in each of these conditions, X was quinine and Y was acid; for the remainder,

the reverse applied. Consumption was measured by weighing the tubes before and after trials to the nearest 0.5 g. The unconditioned stimulus for the conditioning trials was an intraperitoneal injection of 0.15 M LiCl at 10 ml/kg of body weight.

Procedure. Water deprivation was initiated by removing the standard water bottles overnight. On each of the following 4 days, access to water was restricted to two daily sessions of 30 min at 1 p.m. (the afternoon session) and 6 p.m. (the evening session). Presentation of fluids continued to be given at these times throughout the experiment.

The rats were randomly assigned to one of three equal-sized groups, the compound group or one of the elements groups (see Table 1), before the start of the preexposure phase. This phase lasted 6 days. All subjects received four presentations of each of the flavors CX, BY, and Y. Half of the rats in each group were first given 4 days of alternating trials of BY and Y, with 10 ml of one being presented during the afternoon session and 10 ml of the other being presented during the evening session. For half of these rats, BY was the afternoon stimulus and Y was the evening stimulus; for the rest, the arrangement was reversed. The next 2 days consisted of a block of presentations of CX in which 10 ml of this flavor was made available in both afternoon and evening drinking sessions. The remainder of the subjects in each group were treated identically except that they received the blocked presentations of CX on the first 2 days of the phase, followed by 4 days of BY and Y.

After completion of the preexposure phase, the compound group received two conditioning trials with the compound flavor BC as the CS. The first conditioning trial was given in the afternoon session the day after preexposure ended. It consisted of a 10-ml presentation of the flavor for 30 min, followed by an injection of LiCl. All rats were given free access to water in the evening session. The next day was a recovery day on which rats had unrestricted access to water for 30 min during both afternoon and evening sessions. The second conditioning trial was given in the afternoon session of the next day. It was identical to the first except that the rats were given free access to the flavor for 30 min before the injection. Water was available for the rats in the evening session after this conditioning trial, and a further recovery day followed. In the next afternoon session, rats in the compound group received the first of two 30-min test sessions. Half of the rats were given free access to B, and the remainder were given free access to C. Water was made available in the evening session. On the next afternoon session, rats that had been tested with B the previous day were given a test trial with C and vice versa.

The element groups received three conditioning trials—one group, the element (B+) group of Table 1, with B as the CS and the other, the element (C+) group, with C as the CS. As before, only 10 ml was available on the first trial, and each conditioning trial was followed by a recovery day.

Results and Discussion

During preexposure, the compound group drank a mean of 8.5 ml CX per trial, the element (B+) group drank 8.6 ml per trial, and the element (C+) group drank 8.9 ml per trial. The equivalent means for consumption of BY were 8.5 ml, 8.0 ml, and 8.9 ml. Consumption of Y alone was somewhat less in all groups. The mean scores were 7.7 ml for the compound group, 7.5 ml for the element (B+) group, and 7.9 ml for the element (C+) group. An

Table 1
Experimental Designs

Group	Pre-exposure	Conditioning	Test
Experiment 1			
Compound	BY/Y and CX	CB+	C and B
Element (C+)	BY/Y and CX	C+	—
Element (B+)	BY/Y and CX	B+	—
Experiment 2			
Compound	BX/X and CX	CB+	C and B
Element (C+)	BX/X and CX	C+	—
Element (B+)	BX/X and CX	B+	—

Note. C, B, X, and Y refer to flavors. During pre-exposure, stimuli separated by a forward slash (/) were presented on alternate trials; + refers to the administration of LiCl.

analysis of variance (ANOVA) was conducted on these scores, the variables being group, flavor, and order of trials in preexposure (whether the block of CX trials occurred on the first or the last 2 days of the preexposure phase). There was a significant effect of flavor, $F(2, 36) = 5.11$ (here, and throughout, a significance level of $p < .05$ was adopted). No other main effect or interaction was significant ($F_s < 1.94$, $p_s > .17$). Pairwise comparisons using t tests showed that consumption of both CX and BY was greater than consumption of Y.

The compound group drank a mean of 8.6 ml on the first conditioning trial and 11.8 ml on the second conditioning trial. This increase in consumption is to be expected given that rats were given a fixed amount (10 ml) of the BC compound on the first trial, but free access to it on the second. These trials were successful in establishing an aversion, as consumption was reduced to some extent on the test trials (in the absence of conditioning, we would expect rats to drink about 15 ml of sucrose or of saline). Group means for consumption of B and C are shown in the left panel of

Figure 1. The results are essentially identical to those reported by Dwyer and Honey (2007): There was no substantial difference between the two flavors in the amount consumed, but, if anything, the aversion to B (the stimulus whose associate had been presented on separate trials in preexposure) was somewhat less than that to C. An ANOVA with flavor (B or C) as a within-subjects variable and preexposure order (i.e., CX first or last) as a between-subjects variable produced no significant effects (all $F_s < 1$).

The results for the groups conditioned with the elements do nothing to support our hypothesis that the failure to find a difference in the compound group might be a consequence of generalization decrement produced by the compound conditioning procedure. The right panel of Figure 1 shows the mean amount consumed by each group on each of the conditioning trials and the final test trial. As before, the amount consumed increased on the second trial, when free access was given, but then declined. The group conditioned with B as the CS showed marginally less consumption on the second trial than that conditioned with C, but

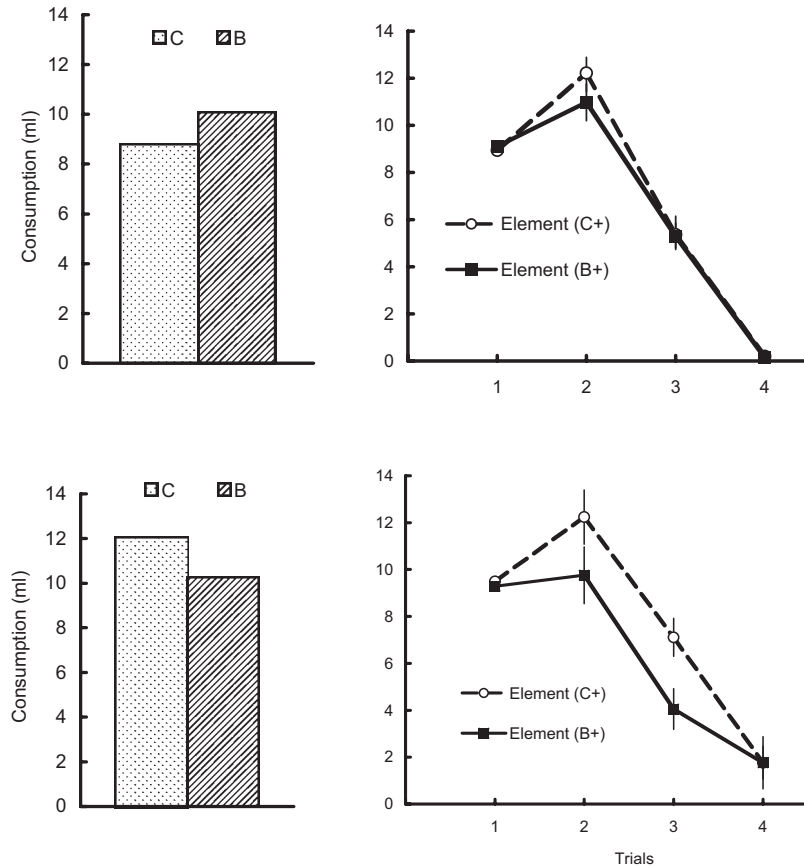


Figure 1. Top: Experiment 1. Left panel: Group mean consumption of the flavors B and C for the subjects given aversion conditioning with the BC compound. Right panel: Group mean consumption during three conditioning trials and a test trial for groups conditioned with B or with C as the conditioned stimulus. All subjects had received preexposure to a block of CX trials and to trials with BY and Y presented in alternation. Bottom: Experiment 2. Left panel: Group mean consumption of the flavors B and C for the subjects given aversion conditioning with the BC compound. Right panel: Group mean consumption during three conditioning trials and a test trial for groups conditioned with B or with C as the conditioned stimulus. All subjects had received preexposure to a block of CX trials and to trials with BX and X presented in alternation. Vertical bars represent the standard errors of the means (SEMs).

an ANOVA with group, trial, and preexposure order as the variables showed there to be no significant difference between the groups. There was significant main effect of trial, $F(3, 36) = 210.01$, but neither the main effect of group ($F < 1$) nor that of order, $F(1, 12) = 1.11$, was significant. None of the interactions was significant; largest $F(1, 36) = 2.98$, for the interaction of order and group.

The null results of this experiment allow us to reach some positive conclusions. The failure of Dwyer and Honey (2007) to find a reliable difference between (their equivalents of) stimuli B and C is not (or is not solely) attributable to their use of the compound conditioning procedure, and the success of Blair et al. (2004) in finding a difference between B and C is not a direct consequence of the particular preexposure schedule they used—the same schedule used here failed to produce a reliable difference. Armed with this information, our next experiment turned to the investigation of another feature that distinguished the two experiments.

Experiment 2

In the experiment by Blair et al. (2004), the same common element was used throughout; that is, the subjects were exposed to intermixed presentations of BX and AX and to a block of CX trials. In the experiments by Dwyer and Honey (2007), each of the critical cues had a different partner; that is, the subjects experienced BY trials (and separate presentations of Y) and CX trials in preexposure. Experiment 1 reproduced this feature of Dwyer and Honey's procedure. From one point of view, the arrangement of using separate associates (X and Y) allows a cleaner test of the hypothesis under consideration—in the procedure used by Blair et al., associative activation of both B and C can be expected to occur in the subgroup given the CX trials in the first phase of preexposure, a feature that would be expected to limit the chances of finding a difference between these two stimuli. However, the use of separate X and Y stimuli introduces an unwanted complication. With this procedure, the associate of B is presented twice as often as the associate of C. Conditioning to B and C on test may be influenced in some way by the number of times their associates have been presented in preexposure. In the present experiment, therefore, we opted to eliminate this factor. The design of the experiment was essentially identical to that of Experiment 1 (see Table 1) except that presentations of BY and Y during preexposure were replaced by presentations of BX and X.

Method

The subjects were 24 experimentally naive male hooded Lister rats (*Rattus norvegicus*), with a mean ad lib weight of 430 g at the start of the experiment. In the preexposure phase, all subjects received intermixed presentations of BX and X and a separate block of CX trials. Eight subjects received conditioning with the BC compound, followed by tests with B and C; two further groups of eight rats received conditioning with B or with C. The flavors used were those described in Experiment 1. Sucrose and saline served as the B and C stimuli (counterbalanced); for half the subjects, the X stimulus was quinine, and for half it was acid. In details not specified here, the procedure was the same as that described for Experiment 1.

Results and Discussion

During the preexposure trials, all groups drank almost all of each of the fluids made available (group mean scores ranged between 9.0 ml and 9.4 ml). An ANOVA paralleling that conducted on the preexposure data for Experiment 1 yielded no significant effects (all $F_s < 2$).

The compound group drank a mean of 9.3 ml on the first conditioning trial and 11.4 ml on the second conditioning trial when free access was given. The results of the test on which the flavors were presented separately are shown in the lower left panel of Figure 1. In contrast to Experiment 1, the rats now drank somewhat less of flavor B than of flavor C, a result consistent with the suggestion that the effective salience of B might be higher than that of C. The difference, however, was not statistically significant; an ANOVA with flavor and preexposure order as the variables yielded $F(1, 6) = 1.04$ for the effect of flavor (other $F_s < 1$).

The lower right panel of Figure 1 shows mean amounts consumed by the element groups across the three conditioning trials and the final test trial. The groups did not differ on the first trial on which a fixed amount of fluid was given, nor did they differ on the final test trial when consumption was almost totally suppressed in both groups. However, the acquisition of the aversion proceeded more rapidly in the element (B+) group than in the element (C+) group. An ANOVA with group, trial, and preexposure order as the variables confirmed this impression. The main effect of trial was significant, $F(3, 36) = 73.18$, as was the interaction between group and trial, $F(3, 36) = 3.61$. A simple main effects analysis confirmed that the groups differed reliably both on the second conditioning trial, $F(1, 48) = 4.75$, and on the third $F(1, 48) = 7.27$, but did not differ on the other trials ($F_s < 1$). No other effects were significant, although the main effect of order, $F(1, 12) = 3.36$, and the triple interaction of group, trial, and order, $F(3, 36) = 2.47$, approached significance ($p < .08$ in both cases; other $F_s < 1$). The possible implications of these results are taken up in the General Discussion.

General Discussion

According to the account offered by Hall (2003), repeated presentation of a stimulus (such as C in the CX compound of these experiments) will lead to a reduction in its effective salience, but presenting a stimulus in alternation with presentations of its associate (as was the case for B given the BX/X procedure in Experiment 2) will attenuate this loss of salience. If B is more salient than C, it should function more effectively as a CS, and conditioning should occur more readily to B than to C. Such a result was obtained in Experiment 2. The effect was small and not significant when the test procedure involved conditioning in which B and C were presented as a simultaneous compound; we have suggested, however, that generalization decrement effects might be expected to attenuate the difference between the stimuli with this procedure. When B and C were trained independently, a clear difference was evident, with B conditioning more rapidly than C (thus replicating the essence of the findings reported by Blair et al., 2004).

Consideration of the effect of order of stimulus presentation during preexposure revealed effects consistent with an interpretation of the results of Experiment 2 in terms of associative activation. Each of the experimental groups in that experiment was made up

of two subgroups, one that received a block of CX trials before the BX/X trials and one that received the CX trials after exposure to BX/X. The proposal is that presentation of X alone will produce associative activation of its associate and that such activation tends to reverse any loss of salience that the associated stimulus may have suffered on the occasions on which it was actually presented. When the CX trials come last in the preexposure sequence, only stimulus B can benefit from this effect, and the difference between B and C can be expected to be substantial. However, when the CX trials come first, both B and C will be activated on the X-alone trials and the effect on C will be less than that on B only to the extent that the XC association might extinguish during the X-alone trials (the XB association being maintained by the intermixed BX trials). In this subgroup, the difference between B and C on test can be expected to be attenuated. Further analysis of the Group \times Trial \times Order interaction, reported above for the elements groups of Experiment 2, reveals just this pattern of results. In neither of the subgroups was there a difference between B and C in the amounts consumed on the first conditioning trial or on the final test trial (the subjects drank all that was available on the first trial, and scarcely any on the last); however, for the subgroups given CX last during preexposure, there was a clear difference in the response to B and C on the other trials. These subjects drank 14.2 ml C on the second conditioning trial but only 9.4 ml B on that trial, a significant difference by a simple main effects analysis, $F(1, 12) = 5.56$. On the third trial, the subjects trained with C drank 8.3 ml, whereas those trained with B drank 4.6 ml, $F(1, 12) = 3.24$, $p < .1$. Critically, however, there was no real difference between the subgroups given CX trials first during preexposure. On conditioning Trial 2, the mean scores were 10.3 ml C and 10.1 ml B ($F < 1$), and on Trial 3 the scores were 5.9 ml C and 3.5 ml B, $F(1, 12) = 1.52$. This attenuation of the difference between B and C in subjects given CX first during preexposure is just what is predicted by the associative activation account.

Whatever the merits of this account as an explanation for the results of Experiment 2, it faces real problems in dealing with the fact that the procedure used in Experiment 1 (modeled on that used by Dwyer & Honey, 2007) failed to generate a difference between B and C on the test—and this was true not only when they were conditioned as a compound but also when they were conditioned separately. The only feature of Experiment 1 that distinguished it from Experiment 2 was that the stimulus compounded with B during preexposure (Y) was different from that (X) compounded with C. The assumptions underlying the salience modulation account lead to the expectation this arrangement should enhance the likelihood of finding a difference between B and C. As we have just discussed, this account predicts that the difference between B and C should be reduced in subjects given CX as the first block of trials during preexposure when, as in our Experiment 2, X-alone trials are given later in preexposure. Using a different associate for B should eliminate this problem and allow a sizable salience modulation effect to emerge both in the subgroup given CX first in preexposure and in the subgroup given CX second. From this point

of view, the absence of a difference between B and C in Experiment 1 is especially puzzling.

We have no ready solution to this puzzle at this stage and can offer only speculation. If we wish to maintain the view that the results of Experiment 2 (and also those reported by Blair et al., 2004) are a consequence of the operation of the salience modulation mechanism described above, it is necessary to suppose that the procedure used in Experiment 1 introduced some other process that opposed the effects of this mechanism. Perhaps the critical factor is that in Experiment 1, the associate of C was experienced only on compound (CX) trials, whereas the associate of B was experienced twice as often, on BY trials and on Y-alone trials. Is it possible that the extra latent inhibition that might be expected to accrue to Y would influence, and retard, conditioning when Y's associate B was used as a CS? If so, the enhancement of the effective salience of B produced by the Y-alone trials might be offset by a loss of associability more marked that suffered by stimulus C. By contrast, no difference in associability is predicted for the procedure used in Experiment 2 in which B and C shared the same associate, allowing the effects of a difference in effective salience to be observed.

The fact that exposure to a stimulus can produce changes in associability and the changes in salience under investigation here complicates the attempt to assess the latter by means of conditioning procedure—acquisition of associative strength will be influenced by both parameters. It was for this reason that Blair et al. (2004) and Hall et al. (2006) made use of a range of other measures of stimulus effectiveness (e.g., the magnitude of the unconditioned response elicited by the stimulus in question or its ability to interfere with the conditioned response controlled by another cue). These other measures (unlike those used by Dwyer & Honey, 2007, and in Experiment 1) consistently supported the view that associative activation of a stimulus can restore its lost salience.

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