Journal of Experimental Psychology:
Animal Behavior Processes
2002, Vol. 28, No. 2, 190–199

0097-7403/02/$5.00 DOI: 10.1037//0097-7403.28.2.190

Perceptual Learning With a Sodium Depletion Procedure

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Rats in a state of salt need prefer a flavor that has previously been paired with saline (Experiment 1). In Experiments 2 and 3, rats exposed to 2 saline concentrations, presented either concurrently or on separate trials, and each paired with a different flavor, showed a preference for the flavor that had been associated with the stronger saline. This effect was substantial, however, only in those rats that had experienced the concurrent exposure schedule. This effect cannot be attributed to a difference in the strength of within-compound associations produced by the 2 preexposure schedules (Experiment 4). It is suggested that concurrent preexposure can engage a learning process that enhances the discriminability of the preexposed stimuli.

It is now well documented that preexposure to two stimuli can enhance the extent to which subjects can subsequently discriminate between them. Examples of this perceptual learning effect have frequently been obtained in experiments that make use of the flavor-aversion learning procedure (e.g., Honey & Hall, 1989; Mackintosh, Kaye, & Bennett, 1991). In the first stage of training, rats are given preexposure to two flavored solutions, A and B (e.g., saline and sucrose). Their subsequent ability to discriminate between these flavors is then assessed by conditioning an aversion to one of them (A) and measuring the extent of generalization to the other (B). It has been routinely found that the effect of such preexposure is to reduce the extent to which such generalization occurs. This outcome is consistent with the suggestion that preexposure enhances the ease with which the flavors can be discriminated, but it is also open to an interpretation in terms of latent inhibition. Preexposure to A can be expected to restrict the readiness with which this flavor will acquire aversive properties during the conditioning phase. The weakness of the response controlled by B might thus indicate not so much a reduction in generalization between A and B as the fact that the strength acquired by A is insufficient to support much generalization.

In an attempt to address this issue, Symonds and Hall (1995; see also Bennett & Mackintosh, 1999; Honey, Bateson, & Horn, 1994) made use of a procedure comparing two conditions, both of which involved preexposure to the critical flavors. In the intermixed condition animals received presentations of A and B on alternate trials; in the blocked condition they received a block of A trials followed by a block of B trials (or vice versa). It was suggested that the former schedule would increase the likelihood that the animals would be able to compare the stimuli and might thus enhance the discriminability of the stimuli in a way that the latter schedule would not. And indeed, Symonds and Hall found that generalization from A to B was less profound in the intermixed than in the blocked condition. Because the amount of exposure given to A was matched in the two schedules, it was argued that differences in latent inhibition, and thus in the associative strength acquired by A, could not be responsible for the test result.

It will be noted that the argument just advanced rests on the assumption that the extent of latent inhibition suffered by the A stimulus will be determined by the total amount of exposure given—the argument would lose its force if it were thought that latent inhibition was sensitive to the schedule of preexposure. Direct assessment of the rate at which conditioning proceeds after intermixed and blocked preexposure has not revealed any reliable difference between the two conditions (e.g., Symonds & Hall, 1995), but this result cannot be decisive in that it tells us about the properties of the A stimulus as a whole, whereas generalization from A to B will be determined by the strength acquired by those features that the A stimulus holds in common with the B stimulus. This matter may be investigated by adding an explicit common feature to the critical stimuli—by giving intermixed or blocked preexposure to the compounds AX and BX (where X represents a common element, present on all trials). After conditioning with AX, a test in which X is presented alone will give information about the associative strength acquired by a feature common to the two preexposed stimuli. Experiments using this design have given mixed results (see Bennett & Mackintosh, 1999), but in at least one case (Mondragón & Hall, in press) it has been found that the X element acquired less strength after intermixed than after blocked preexposure. This difference between the groups could explain the finding made by Mondragón and Hall that the intermixed group also showed less of an aversion to BX than did the blocked group. It remains to be explained why the groups should have differed in the amount they learned about X. Mondragón and Hall (in press) offered an explanation in terms of Gibson's (1969) differentiation theory, which suggests that intermixed preexposure will enhance the perceptual effectiveness of features that distinguish between

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This work was supported by a grant from the United Kingdom Biotechnology and Biological Sciences Research Council. We thank C. Bonardi and E. Mondragón for helpful discussion.

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the stimuli and reduce that of common elements (such as X). It is also consistent, however, with the proposal that latent inhibition of the X element was more profound in subjects given intermixed preexposure than in those given blocked preexposure. In the absence of a fully specified theory of the nature of the latent inhibition effect, this proposal cannot be excluded.

The experiments reported in this article attempted to address this issue by looking for evidence of a perceptual learning effect in a training procedure that does not involve a conditioning phase and in which, therefore, latent inhibition cannot play a part. To do this we gave rats either intermixed or blocked preexposure to two different concentrations of a saline solution and then made use of the fact that it is possible to induce a state of salt need in rats by means of a single simple injection. Rats that have received this injection may, with appropriately chosen concentrations, show a preference for a stronger saline solution over a weaker solution. For the rats to show such a preference they must, of course, be able to discriminate between these two, presumably rather similar, stimuli. The question of interest was how such a preference might be influenced by preexposure. If intermixed preexposure enhances the discriminability of the two different concentrations, then subjects given this form of preexposure might be expected to show a reliable preference for the stronger solution rather than the weaker solution. Subjects given blocked preexposure, on the other hand, may find the discrimination more difficult and thus show a less reliable preference. The logic underlying this experimental design is formally equivalent to that used in demonstrating the perceptual learning effect in flavor aversion. That is, in both procedures, the rats were given intermixed or blocked preexposure to two similar flavors, followed by a treatment designed to endow the flavors with different affective values to allow a test of their ability to discriminate between the flavors. In the flavor-aversion procedure, however, this change in affective value is achieved by means of a conditioning procedure (thus allowing the possibility that latent inhibition will play a role in any effect obtained). In the present experiments the change in affective value was achieved without the use of a conditioning procedure.

In the form just described, the experimental design envisages giving the animals a preference test between weak and strong saline solutions. We were concerned, however, that such a test might provide a rather insensitive measure of the ability of the animals to discriminate the two concentrations on the basis of their sensory or perceptual properties. Specifically, the consumption of a saline solution will have important metabolic effects for rats in a state of sodium need, effects that are likely be more substantial when the solution is strong rather than weak. It is possible that a sensitivity to these metabolic effects could allow a rat to show a preference for the stronger solution over the weaker one, even if its ability to discriminate the two concentrations on the basis of their immediate sensory properties was poor. To avoid this problem we devised a test in which the discrimination required was not between the two saline solutions themselves but between flavor cues that had been associated with them. To do this we made use of the version of the sensory preconditioning procedure devised by Fudim (1978).

In Fudim's (1978) procedure rats were given initial exposure to two compound stimuli, a saline solution to which a distinctive flavor (X) had been added and a sucrose solution to which a different flavor (Y) had been added. When tested with X and Y after a state of salt need had been induced, the rats showed a preference for X. This result was interpreted as showing within-event learning, in which the formation of an X–saline association allowed X to generate a response appropriate to its associate after a treatment that endowed that associate with motivational significance. The experiments reported here adopted the same general procedure, except that Flavor X was paired with a strong concentration of saline and Flavor Y was paired with a weak concentration of saline during preexposure. We anticipated that, with appropriately chosen parameters, animals with a salt need might show a preference for X over Y when these flavors were presented in the absence of saline on test. They would only be able to do so, however, to the extent that they are able to discriminate strong from weak and X from Y. If intermixed preexposure enhances the discriminability of the preexposed stimuli, then the preference will be more marked in animals given this schedule during preexposure than in animals given a blocked schedule. This issue was investigated in Experiments 2 and 3, and in Experiment 4 we went on to test alternative explanations for the effect obtained in those experiments. Experiment 1 was conducted to establish appropriate parameters for the demonstration of within-event learning in this training preparation.

Experiment 1

The procedures used in this experiment were modeled on those described by Westbrook et al. (1995) in their study of within-event learning using the sodium-depletion procedure. All of the animals received initial exposure to a saline solution to which another flavor had been added. (Because subsequent experiments require the use of two flavors, we trained half of the animals with vanilla as the added flavor and the rest with almond, to confirm the efficacy of both.) Half of the subjects were then given an injection of furo–doca (FD; see *Subjects and apparatus*) to establish a state of salt need; half were injected only with the vehicle (physiological saline). All were then given a two-bottle choice test between plain water and water containing the flavor with which they had been trained. We anticipated that rats given the FD injection would show a preference for the flavored water. The design of Experiments 2 and 3 calls for the use of two saline concentrations, both of which need to be effective in generating the preference. Accordingly, in this experiment we gave half of the rats in each condition initial training with a strong saline solution and half training with a weaker solution and hoped to show, in animals given the FD injection, a preference for the flavor associated with saline in both cases. There were thus four groups of subjects: The strong–FD and weak–FD groups were given the FD injection after initial training with strong and weak saline, respectively, and the strong–VEH and weak–VEH groups were given equivalent initial training but were injected with vehicle (VEH), rather than FD.

Method

Subjects and apparatus. The subjects were 32 experimentally naive male hooded (Lister) rats, approximately 14 weeks old at the start of the experiment. They were housed in pairs in a colony room that was lit from 8:00 a.m. to 8:00 p.m. each day. Experimental treatments were given in distinctive cages, located in a different room on the laboratory. These cages measured 35 × 22 × 19 cm, had walls and floor made of transparent plastic, and a roof of wire mesh through which drinking bottles could be inserted. Inverted 50-ml centrifuge tubes equipped with stainless steel ball-bearing-tipped spouts were used to present fluids in these cages. The solutions used as the target stimuli in this experiment were measured quantities of weak saline (0.5% [wt/vol] NaCl), strong saline (1% [wt/vol] NaCl), almond (2% [vol/vol] almond essence, from Supercook, Leeds, United Kingdom) and vanilla (1% [vol/vol] Supercook vanilla essence). When these flavors were presented in compound form, they were mixed to maintain these individual concentrations. The treatment used to induce a sodium appetite was a subcutaneous injection of .5 ml of a mixture of 10 mg of furosemide (furo) and 5 mg of deoxycorticosterone acetate (doca) dispersed in 20 ml of distilled water with 1 drop of the dispersant Tween 80.

Procedure. The rats were weighed and randomly allocated to four equal-sized groups. The standard water bottles were removed, and over the next 2 days a schedule of deprivation was established in which access to water was given for 30 min in each of 2 daily sessions. These sessions were initiated at 10:00 a.m. and 4:00 p.m. The next 4 days constituted the preexposure phase of the experiment. On each of these days, the subjects were transferred to the experimental cages at 1:00 p.m. for 20 min, where they received access to 12 ml of a flavored solution. For half of the subjects (the weak–VEH and weak–FD groups) this was the weak saline solution; for the remainder (the strong–VEH and strong–FD groups) it was the strong saline solution. For half of the animals in each of these groups the added flavor was vanilla and for half it was almond.

Three hours after exposure to the fluid on Day 4, subjects in the weak–FD and strong–FD groups received an injection of FD; subjects in the weak–VEH and strong–VEH groups received an injection of physiological saline. The food was then removed from the home cages in the colony room, and the subjects were given access to distilled water overnight. On the next day, the distilled water was removed from the cages 3 hr prior to the test session. The test was initiated at noon and consisted of a 20-min session in which the subjects received a presentation of the pretrained flavor (almond or vanilla) in one drinking tube and plain water in the other. The position of the tubes that contained either the flavor or water was counterbalanced across the groups. The amount consumed was determined by weighing each of the tubes before and after the session.

Results and Discussion

During preexposure, the rats readily drank almost all of the solution that was offered. Thus on the last day of preexposure, the strong–FD group consumed a mean of 10.6 ml, the weak–FD group consumed a mean of 11.2 ml, the strong–VEH group consumed a mean of 10.7 ml, and the weak–VEH group consumed a mean of 10.3 ml.

Figure 1 shows group mean scores for consumption of plain water and flavored water during the test session. Inspection of the data revealed no obvious difference between animals trained with vanilla and those trained with almond, and the results for these two subgroups are pooled in the figure. It is evident from the figure that animals showed relatively low levels of fluid consumption on the test trial (this presumably is a result of the fact that the water bottles were removed only 3 hr prior to the test). The findings of central interest, however, are that animals given an injection of the vehicle (the weak–VEH and strong–VEH groups) drank approximately equal amounts of plain water and flavored water, whereas those given an injection of FD (the weak–FD and strong–FD groups) drank more of the flavored water than plain water. The strong–FD group drank more of the flavored water than any other group, although it should be noted that this group also drank more plain water than the other groups.

An analysis of variance (ANOVA) was conducted on these data with the between-subjects variables of drug (FD or VEH) and strength of salt presented during compound preexposure (weak or strong) and the within-subjects variable of test fluid (water or flavored water). This analysis revealed a significant effect of drug, $F(1, 28) = 15.76$, salt strength, $F(1, 28) = 4.03$, and a significant interaction between these two factors, $F(1, 28) = 4.39$. (Here and elsewhere a significance level of $p < .05$ was adopted.) There was also a significant effect of test fluid, $F(1, 28) = 12.67$, and a significant interaction between test fluid and drug, $F(1, 28) = 8.66$. To determine the source of these interactions, the data from the VEH groups and FD groups were subjected to separate analyses. The data for the VEH groups showed no significant effects of salt strength, the nature of the test fluid, or the interaction between these two variables (all Fs < 1). The data for the FD groups produced a significant effect of the nature of the test fluid, $F(1, 14) = 5.55$, a significant difference between the groups trained with different salt strengths, $F(1, 14) = 13.36$, but no significant interaction between these two factors ($F < 1$).

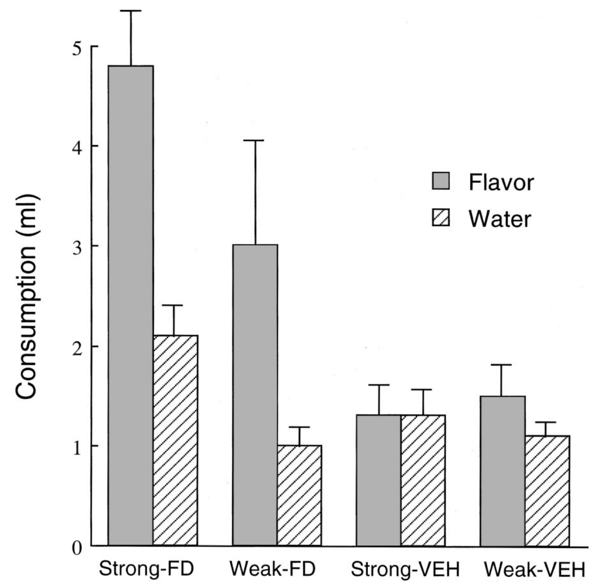

Figure 1. Experiment 1: Mean amount of fluid consumed on a two-bottle test with a water and a flavored solution. FD groups had received an injection of furo–doca, and VEH groups an injection of vehicle. Animals in the strong groups had previously experienced the test flavor in compound with a 1% saline solution; animals in the weak groups had previously experienced the test flavor in compound with a 0.5% saline solution.

To make patterns of preference more easily visible, the scores summarized in Figure 1 were converted to a ratio measure: intake of flavored water over total intake. Group mean ratio scores are shown in Figure 2. It is clear from the figure that neither of the VEH groups showed any real preference but that both the FD groups did. The score for the strong–FD group was only slightly higher than that for the weak–FD group. An ANOVA conducted on these data with injection (FD or VEH) and salt strength (weak or strong) as the variables revealed a significant effect of the injection, $F(1, 28) = 6.87$, but no effect of salt strength and no interaction between the variables (both Fs < 1).

The results of this experiment indicate that experience of a flavor in compound with a saline solution, followed by the induction of a sodium appetite, results in subjects showing a preference

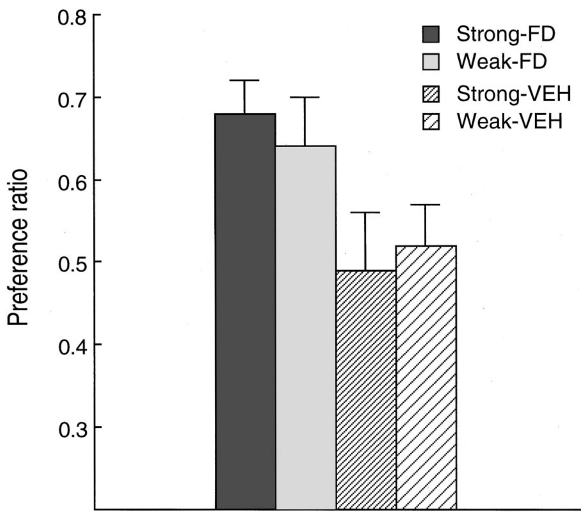

Figure 2. Experiment 1: Group mean ratio scores (intake of flavored water over total intake) for the test phase. FD groups had received an injection of furo–doca, and VEH groups an injection of vehicle. Animals in the strong groups had previously experienced the test flavor in compound with a 1% saline solution; animals in the weak groups had previously experienced the test flavor in compound with a 0.5% saline solution.

for the salt-associated flavor over water. It should be acknowledged that this experiment does not include the control condition—specifically, one in which animals receive unpaired presentations of the flavor and of the saline—that would guarantee that the preference shown by the groups given the FD injection is indeed the consequence of their having experienced the flavor and saline together during the initial phase of training. With this caveat, we may conclude, however, that these training procedures are capable of producing the within-event learning effect that is required by the design of our subsequent experiments. (It may be added that the design used in these experiments supplies confirmation of the assumption that our training procedures generate within-event learning.) The effect was found for both of the saline concentrations used. There was some indication that the strong saline solution produced a bigger preference than the weak solution, but the difference between the two groups was small and not statistically reliable. This outcome encourages the view that rats might find it difficult, but not impossible, to discriminate between the two concentrations of saline, a combination that makes them ideal for the experiments that follow. The next two experiments assessed the ability of rats to make the discrimination after exposure to both saline concentrations and sought to determine whether the ease of this discrimination can be modified by the schedule of presentation during preexposure.

Experiment 2

In this experiment all the rats received preexposure to two flavored compounds, one of which consisted of Flavor X (e.g., vanilla) mixed with the strong (S) salt solution (XS) and one of which consisted of Flavor Y (e.g., almond) mixed with the weak (W) salt solution (YW). All then received an injection of FD to induce a sodium appetite, followed by a choice test in which they were given access to the X and Y flavors. To the extent that the rats can discriminate weak from strong and X from Y, a preference for X on the test may be expected.

During the preexposure phase, the rats were divided into two groups. One of these (Group I) experienced an intermixed schedule of preexposure, in that the two compound flavors were presented simultaneously in separate bottles throughout preexposure. The other group of subjects (Group B) received the same total amount of preexposure but according to a blocked schedule in which both bottles contained one of the compounds for the first block of trials and the other compound on the second block of trials. If the opportunity for comparison provided by the intermixed schedule enhances the ease with which the relevant stimuli can be discriminated, then we might expect that the preference shown for X on the test would be stronger in Group I than in Group B.

Method

The subjects were 32 experimentally naive male hooded (Lister) rats, approximately 12 weeks old at the start of the experiment. They were housed and maintained in the same way as the subjects in Experiment 1. The cages used for the experimental sessions were the same as those used in Experiment 1. The solutions used were S and W saline flavored with almond or vanilla (Flavors X and Y), the concentrations being the same as those described for Experiment 1.

After the schedule of water deprivation had been established, the subjects received, over the next 10 days, a regime of preexposure in which they were presented with the two target compounds. On each day of this phase, all subjects were moved to the experimental cages at noon where they received access for 1 hr to two bottles, each containing a compound flavor. The two bottles that were used to present these flavors were separated by a distance of approximately 10 cm. For the subjects in Group I, these flavors were the compounds XS and YW; for subjects in Group B, both of the bottles contained only one of these compound flavors for the first 5 days of the phase, the second compound being presented during the next 5 days of the phase. For half of the animals in this group, XS was presented during the first part of the preexposure phase and YW during the second part; for the remaining animals, this arrangement was reversed. On each day the subjects also received supplementary water in their home cages for 1 hr beginning at 5:00 p.m. Approximately 3 hr after the final preexposure session, the subjects were injected with FD in the same way as in the previous experiment, the food was removed, and the subjects were given overnight access to distilled water. On the next day, the water bottles were removed 3 hr prior to the test session. On the test, the rats were presented with a choice of two bottles, one containing Flavor X and the other containing Flavor Y. The duration of the test was 20 min. For half of the animals in each group, Flavor X was vanilla and Flavor Y was almond; for the remainder, X was almond and Y was vanilla.

Results and Discussion

During preexposure the animals readily consumed both of the compound flavors. On the last day of preexposure for animals in Group I, the mean amount of XS consumed was 7.4 ml and the mean amount of YW consumed was 8.0 ml. On the last session of preexposure to XS, animals in Group B consumed a mean of 15.7 ml, and on the last session of preexposure to YW, they consumed a mean of 14.5 ml. (The Group B scores are the total consumption from the two bottles containing the same solution that were presented on the trial; they are thus approximately double those recorded for each of the separate bottles presented to subjects in Group I.)

Figure 3A shows the group mean intakes of the X and Y flavors on the test session. It is clear that both groups drank more of X than Y and that the difference in response to the two flavors was more marked in Group I than in Group B. Statistical analysis of the data summarized in the figure confirmed only the first of these points. An ANOVA with group (I or B) and test flavor (X or Y) as the variables showed a significant effect of test flavor, $F(1, 30) = 5.5$, but neither the main effect of group ($F < 1$) nor the interaction between these two variables ($F < 1.5$) was found to be significant. A reliable difference between the groups was obtained, however, in a further analysis that made use of a ratio measure, intake of Flavor X over total intake. Group mean ratios are presented in Figure 3B. This shows that Group I had a higher preference ratio than Group B, indicating that the former group showed a stronger preference for X (the flavor associated with strong salt) over Y than did the latter group. An ANOVA conducted on the data summarized in the figure confirmed this impression, revealing a significant difference between the two groups, $F(1, 30) = 6.37$.

On the basis of the results of Experiment 1, we expected that the rats in Experiment 2 would show a preference for the flavor that had, in preexposure, been associated with the stronger saline solution. To show a preference with the procedures used in Experiment 2, the rats needed to be able to discriminate between the two test flavors and the two saline concentrations presented during preexposure. The fact that subjects in Group I showed a stronger preference than subjects in Group B accords with the suggestion that the preexposure procedure used for Group I (which allowed the possibility of making a direct comparison between the XS and YW compounds) was particularly effective in enhancing the discriminability of the relevant stimuli.

We should, however, consider a possible alternative account of these results. For all subjects in the present experiment, the test consisted of presenting the animals with a choice between two different flavors. For subjects in Group I, this arrangement was similar to that in which the stimuli were presented during the preexposure phase. For subjects in Group B, on the other hand, the conditions of testing were quite different from those of preexposure. For the latter group, the test trial was the first occasion on which they encountered the simultaneous presentation of two different flavors. This factor may be enough to explain why subjects in Group B failed to show a clear preference for X over Y—for instance, subjects in this group might, having become accustomed to receiving only one type of flavor in both bottles, have a reduced tendency to sample the flavor from more than one bottle, a tendency that would clearly interfere with the expression of any preference. Experiment 3 was conducted to investigate the role of this factor.

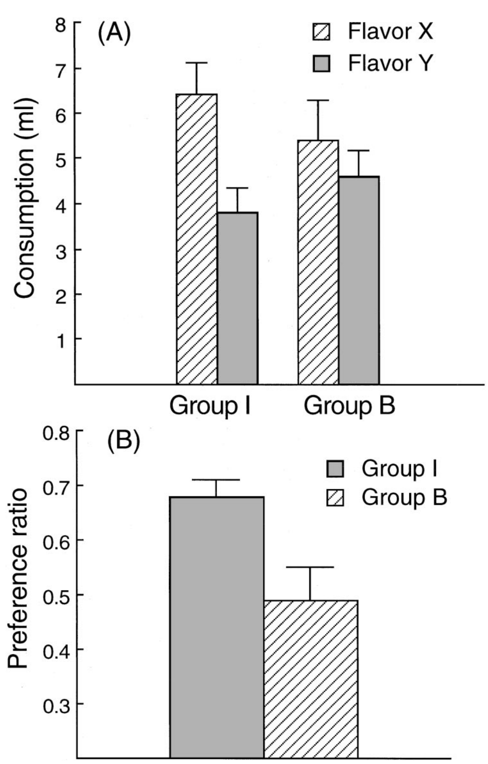

Figure 3. Experiment 2. A: Mean amounts of fluid consumed by rats given a choice between Flavor X and Flavor Y (almond and vanilla, counterbalanced). For all animals, X had previously been experienced in compound with a strong saline solution and Y had previously been experienced in compound with a weak saline solution. Group I experienced X and Y on the same trial in preexposure; Group B experienced them on separate blocks of trials. B: Mean preference ratios (intake of X over total intake).

Experiment 3

The aim of Experiment 3 was to replicate the finding of Experiment 2 but with the use of a slightly modified test procedure. As in Experiment 2, we compared the effects of giving intermixed and blocked preexposure to the XS and YW compounds on the subsequent intakes of X and Y following sodium depletion. The critical difference, however, was that the subjects in Experiment 3 were not presented with a choice between X and Y during the test phase but instead were tested on separate occasions for their intakes of each of the two flavors. Thus the way in which the flavors were presented on test differed from the arrangement used in preexposure, both for Groups B and I. If the results of Experiment 2 were a consequence of the fact that the novel test arrangement disrupted the ability of Group B to show a preference, then such a disruption would be expected for both groups under the current test conditions. If, however, the results of Experiment 2 reflect a genuine enhancement of the discriminability of the stimuli in Group I, then it should be possible to obtain evidence of a more marked preference for X over Y in Group I than in Group B, even with a procedure in which the intakes of Y and X are tested on separate sessions.

Method

The subjects were 32 experimentally naive male hooded (Lister) rats, housed and maintained in the same way as was described for Experiments 1 and 2. They were approximately 16 weeks old at the start of the experiment. All details of the water deprivation schedule, flavor stimuli, and experimental cages were identical to those described for Experiment 2.

There were two groups of subjects, given either intermixed (Group I) or blocked (Group B) preexposure to the XS and YW compounds, as in Experiment 2. They then received an injection of FD to induce a sodium appetite. On the next day the subjects received two separate tests. The first of these tests was initiated at 11:00 a.m., and for all subjects this consisted of a 20-min presentation of two bottles, one containing water and the other containing a flavor. For half of the subjects in each group this flavor was X and for the remaining half this flavor was Y. In the second test given 3 hr later, the subjects again received two bottles, one containing water and the other containing the flavor not presented on the first test. Procedural details not specified here were identical to those described for Experiment 2.

Results and Discussion

As in Experiment 2, the animals readily consumed the fluids offered during the preexposure phase. On the last day of preexposure for animals in Group I, the mean amount of XS consumed was 9.1 ml and the mean amount of YW consumed was 8.9 ml; on the last session of preexposure to XS, animals in Group B consumed a mean of 19.3 ml and on the last session of preexposure to YW, they consumed a mean of 17.0 ml.

Figure 4A shows the mean amount of the water and the flavored solution consumed by each group on the test sessions. There were no differences in the amount of water consumed, either between the groups or as a function of whether the other bottle contained X or Y; but both groups, on each of their test sessions, drank more of the flavor than water, indicating that our training procedure was effective in establishing a preference for the salt-associated flavor. The groups differed in their consumption of the test flavors. Group I drank substantially more of X than Y, whereas Group B showed no such preference and in fact drank slightly more of Y than X.

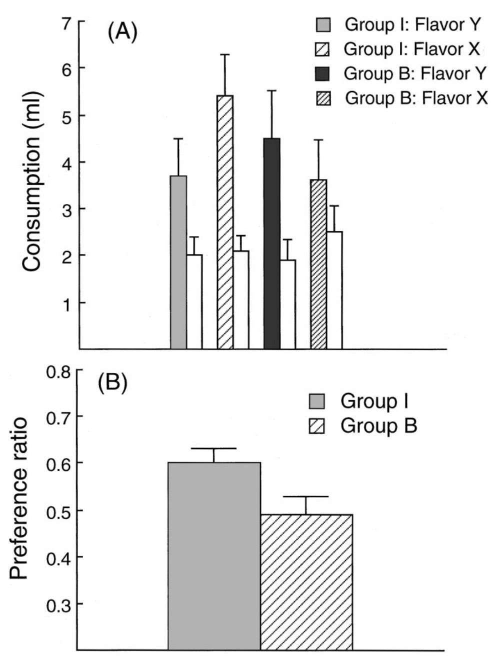

Figure 4. Experiment 3. A: Mean amounts of fluid consumed by rats given a choice (on separate trials) between Flavor X and water and between Flavor Y and water (almond and vanilla, counterbalanced). White bars indicate water consumption. All animals had previously experienced X in compound with a strong saline solution and Y in compound with a weak saline solution. Group I experienced X and Y on the same trial in preexposure; Group B experienced them on separate blocks of trials. B: Mean preference ratios (intake of X over total intake of X and Y).

Statistical analysis confirmed this description of the results. An ANOVA was conducted on the data summarized in the figure, the variables being group (I or B), trial type (whether the flavor presented was X or Y), and fluid consumed (water or the flavored solution). This revealed a significant effect of the type of fluid, $F(1, 30) = 14.89$, as would be expected, given the general preference for the flavored solution over water. No other main effects or interactions were significant (all Fs < 2), apart from that for the Group × Trial type interaction, where $F(1, 30) = 3.33$, and the three-way interaction of Group × Trial × Flavor, $F(1, 30) = 5.24$.

Further analyses were conducted to determine the source of the three-way interaction. An analysis of the water consumption data, with group and trial type as the variables, yielded no significant effects (all Fs < 1). An equivalent analysis conducted on the data for consumption of the flavored solutions produced no main effects of group or trial type (Fs < 1), but there was a significant interaction between these factors, $F(1, 30) = 6.07$. An analysis of simple main effects demonstrated that the scores for consumption of X and Y differed significantly in Group I, $F(1, 30) = 5.15$, but not in Group B, $F(1, 30) = 1.47$.

To allow a comparison with the results presented in Experiment 2, the flavor consumption data were also expressed as preference ratios by calculating intake of Flavor X over the total intake of X + Y. These scores are presented in Figure 4B. It is clear that Group I had a higher preference ratio than Group B. Although the difference between these scores fell just short of statistical significance, $F(1, 30) = 3.72$, $p = .06$, these results are consistent with those of the previous experiment and, combined with the outcome of the analysis of the absolute consumption scores just presented, permit the conclusion that Group I showed a preference for X over Y, whereas Group B did not.

The results of Experiment 3, like those of the previous experiment, suggest that rats exposed to both strong and weak saline solutions, each with its own added flavor (the compounds XS and YW) will consume more of Flavor X than Flavor Y when tested in a state of salt need. The outcome of particular interest was that this preference is dependent on the subjects having experienced the two compounds in an intermixed fashion during preexposure, a finding that might be regarded as an instance of perceptual learning in which the intermixed preexposure affords the subjects the opportunity to discriminate between the compound stimuli in a way in which the blocked schedule does not. It is, however, plausible that quite a different mechanism is responsible for these results, and this possibility is examined in Experiment 4.

Experiment 4

In Experiments 2 and 3, subjects given intermixed preexposure to XS and YW, when tested in a state of salt need, showed a greater preference for Flavor X over Y than did subjects given the blocked preexposure schedule. Although our interpretation of these findings has rested on the assumption that intermixed training is particularly effective in promoting discrimination between these compounds flavors, we should acknowledge that the degree of this preference will also depend on another important factor. Specifically, for rats to show a preference on test in this procedure, it is common ground that they will need to form an association between the flavor presented on test and the (now valued) saline. The degree of this preference will be determined by the strength of the flavor–saline association, and, it might be argued, the strength of this association could well differ between Group I and Group B, being stronger in the former than in the latter.

One possibility is suggested by the finding of Rescorla and Freberg (1978) that within-event learning will be attenuated if one of the events is experienced, on some occasions, outside the compound. In our procedure, animals given XS also experienced saline (admittedly, it was of a different concentration) on other trials. The failure of Group B to show a marked preference for X on test might reflect the fact that the X–(strong) saline association formed on the first block of exposure trials had been broken down during the Y–(weak) saline association trials of the second block. It is equally possible that presenting the Y–(weak) saline association during the first block of trials also could retard the formation of the X–(strong) saline association during the second block of trials. If this breakdown of within-compound associations operates less readily when the stimuli are presented in an intermixed fashion during preexposure, then this may be all that is needed to explain the present findings.

Another possibility arises from the fact that the intermixed and blocked groups differed in the way in which their exposures to the critical stimuli were spaced. In particular, for subjects in the intermixed condition, each flavor (X and Y) was paired with each concentration of salt once a day for 10 days, but for subjects in Group B, X was paired with strong salt (on two trials) for 5 consecutive days and Y was paired with weak salt for 5 days. In essence, intermixed training allows the stimuli to be presented in a more spaced schedule than does the blocked arrangement. It is possible then that the greater trial spacing afforded by the intermixed procedure resulted in the formation of stronger within-compound associations in Group I than in Group B.

Given these arguments, Experiment 4 set out to examine the possibility that (for whatever reason) intermixed preexposure to two compound flavors produces stronger within-compound associations than does blocked exposure to these stimuli. Two groups of subjects were, as before, given either intermixed or blocked exposure to the compounds AN (almond–salt) and VN (vanilla–salt); in this experiment, however, the same concentration of saline was used throughout. A salt need was then induced, and the animals were given a test in which they were allowed to choose between A (or V) and water. If the intermixed preexposure schedule produces stronger within-compound associations than the blocked schedule, then the preference for the flavored water should be greater in Group I than in Group B. On the other hand, if the results of Experiments 2 and 3 are entirely a consequence of enhanced discrimination between the two saline concentrations in the intermixed condition, then there could be no difference between Groups B and I in this procedure in which only one saline concentration is experienced.

Method

The experiment was run in two identical replications with 16 experimentally naive male hooded (Lister) rats in each. The rats in the first replication were 16 weeks old at the start of the experiment; those in the second replication were 28 weeks old. They were housed and maintained in the same way as the subjects in Experiments 1–3. All details of the water deprivation schedule, flavor stimuli, and experimental cages were identical to those described for Experiments 2 and 3.

The rats were assigned at random to two equal-sized groups. Group I received, over the course of 10 days, intermixed exposure to the compound Flavors AN (almond + saline) and VN (vanilla + saline), whereas those in Group B received the blocked schedule of exposure to the compounds. Both concentrations of saline (0.5% and 1%) were used, but each animal experienced just one, half of each group receiving weak throughout and half receiving strong. All subjects then received an injection of FD to induce a sodium appetite and on the next day were given a test trial in which they were permitted to drink from two bottles, one containing a test flavor and the other containing water. For half of the subjects, the test flavor was almond and for half it was vanilla. For half of each of these subgroups, the test flavor had been paired with strong saline during preexposure and for half it had been paired with the weaker saline. Procedural details not specified here were identical to those described for Experiment 3.

Results and Discussion

As in the previous experiments, the subjects readily consumed the flavors that were offered during the preexposure phase. On the final day of preexposure, subjects in Group I consumed a mean of 10.2 ml of AN and 9.9 ml of VN. For subjects in Group B, those given AN on the final preexposure session consumed a mean of 19.7 ml and those given VN consumed 19.8 ml. There was no systematic effect of the concentration of saline on consumption during preexposure.

Figure 5A shows the mean amounts of water and the flavored solution consumed by each group on the test session. Inspection of the data revealed no systematic difference between subgroups given A or V on the test or between those given weak or strong saline during preexposure, and the data presented are collapsed

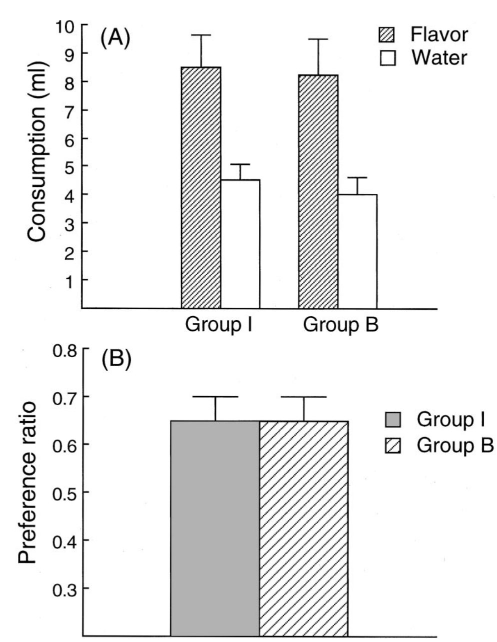

Figure 5. Experiment 4. A: Mean amounts of fluid consumed on a test trial in which rats were given a choice between a flavor and water. For all animals the flavor had been presented in compound with saline during preexposure, either in a block of trials (Group B) or intermixed among trials with another flavor (Group I). B: Mean preference ratios for this test (intake of flavor over total intake).

over these factors and over the factor of replication. The figure shows that both Group I and Group B consumed more of the test flavor than water, but there was no sign of any difference between the groups in their consumption of either the flavor or the water. An ANOVA was conducted on these data with group (I vs. B), test fluid (flavor vs. water), and replication as the variables. This revealed a main effect of the nature of the fluid, $F(1, 28) = 18.20$, but no significant effect of group ($F < 1$) and no interaction between these two variables ($F < 1$). The older (and larger) animals used in the second replication drank more than those used in the first, particularly of the flavored solution (for the two Group Is the mean amounts of flavor and water consumption were 5.8 ml and 4.5 ml in the first replication and 11.3 ml and 4.5 ml in the second; the corresponding scores for the two Group Bs were 5.1 ml and 3.4 ml for the first replication and 11.3 ml and 4.7 ml for the second). Consequently the analysis showed a main effect of replication, $F(1, 28) = 27.50$, and of the interaction between replication and test fluid, $F(1, 28) = 7.46$, but, critically, the three-way interaction of group, replication, and test fluid was not significant ($F < 1$).

Preference ratios were also computed for each subject (flavor intake over total intake), and the group mean scores are presented in Figure 5B. It is clear that neither group showed a strong preference for the flavored water and that the groups did not differ in terms of their preference ratios. An ANOVA with group and replication as the variables yielded no significant effects: for replication, $F(1, 28) = 3.29$ (other Fs < 1).

The results of Experiment 4 were clear cut. Exposure to almond and vanilla, each in compound with a saline solution, produces a small preference for the flavored solution (over water) in animals in a state of salt need. Although the controls needed to prove the point were not included in this experiment, it may be assumed, on the basis of the results already presented, that this preference is a consequence of the formation of a flavor–salt association. That the preference was not very marked may be seen as consistent with demonstrations that within-event learning can be disrupted by presenting one of the critical elements (in this case, the salt) outside of the compound (Rescorla & Freberg, 1978). More important for our present purposes, however, is the failure to obtain any difference between the intermixed and blocked conditions in their tendency to choose the test flavor over water. The results obtained in Experiments 2 and 3 critically depend on the fact that two different saline concentrations were used during preexposure. Possible explanations of this effect are discussed next.

General Discussion

Experiment 1 confirmed the previously established finding that animals in a state of salt need will show a preference for a flavor that had previously been experienced in association with saline. It further suggested that the magnitude of this preference might be sensitive to the strength of the saline with which the flavor had been associated. Experiment 2 provided a within-subject demonstration of this effect. Rats given intermixed exposure to both strong and weak saline solutions, each with its own added flavor (the compounds XS and YW), showed a preference for X over Y when tested in a state of salt need. Rats in Group I in Experiment 3 showed an equivalent preference. This result can be explained in terms of standard notions of within-event learning (e.g., Fudim, 1978; Rescorla & Cunningham, 1978; Westbrook et al., 1995). If we assume that separate representations exist for weak and strong saline, exposure to the compounds will establish two associations that we may symbolize as X–S and Y–W. On test, X and Y will each activate its own associate, and giving value to these associates by inducing the salt need will enhance the likelihood that X and Y will be consumed. Assuming that the stronger saline is more valued than the weaker saline explains the preference for X over Y.

For this outcome to be obtained it is, of course, necessary for the rats to be able to discriminate X from Y and S from W. In particular, it seems likely that generalization between S and W, two different concentrations of the same substance, would be extensive, although some generalization might also be expected between the specific flavors used as X and Y. Any procedure that reduces such generalization would tend to enhance the extent to which the rats would be likely to show a preference for X over Y. In Experiments 2 and 3 we used a preexposure schedule, which, on the basis of previous work (e.g., Symonds & Hall, 1995), we thought might enhance the discriminability of the preexposed stimuli. Specifically we gave animals in the intermixed condition preexposure in which XW and YS were presented concurrently. If the opportunity for stimulus comparison provided by this arrangement increases the discriminability of the preexposed stimuli, then the preference for X might be expected to be especially marked in this condition. This was the result obtained.

The outcome of Experiment 4 served to rule out the possibility that this result was a consequence of quite a different mechanism. In particular, we allowed that the subjects in the intermixed condition might show a more marked preference for X over Y, not because this arrangement enhanced discriminability of the preexposed stimuli but because the intermixed procedure generated stronger within-compound associations than those formed when the stimuli were presented in a blocked schedule. The results of Experiment 4 showed that this was not the case—when the same saline concentration was used throughout, the degree of preference for the saline-associated flavor was the same after intermixed as after blocked preexposure. Our central finding of Experiments 2 and 3 might therefore be best regarded as an instance of a perceptual learning effect, in which intermixed exposure to differing concentrations of saline can facilitate discrimination between them in a way that is not possible when the same events are presented in a blocked schedule.

One of the reasons for choosing this experimental design was the assumption that the discrimination between two saline concentrations might be intrinsically rather difficult and thus might be likely to show a substantial benefit from a preexposure procedure that allowed the animal to compare them. It is apparent, however, that the procedure used for the intermixed condition also allowed the animals the opportunity to compare Flavors X and Y. The effect seen on test could just as plausibly be attributed to a change in the discriminability of X and Y as to a change in the discriminability of S and W. Our experimental results do not allow a choice between these possibilities (and, in fact, both may have occurred). But given that our central aim was simply to demonstrate a perceptual learning effect in this experimental paradigm, this is not a matter for concern.

The demonstration that a perceptual learning effect can be obtained in these conditions allows us to draw certain conclusions about the mechanisms involved. First, it shows that the effect does not depend on a mechanism involving latent inhibition. As outlined at the beginning of this article, most previous examples of perceptual learning have made use of a procedure in which, after preexposure to the critical stimuli, animals received conditioning with one of them, and generalization to the other was assessed. The outcome of this procedure, reduced generalization to the test stimulus, is open to an interpretation in terms of latent inhibition—that is, it could be argued that some feature of the preexposure arrangement promotes the development of latent inhibition to elements that the two stimuli hold in common and that the poor generalization observed on test reflects the fact that these elements acquired little associative strength during conditioning. The training procedure used here, which does not involve a conditioning phase, rules out such an interpretation.

The results of these experiments also argue against the associative account of perceptual learning offered by McLaren, Kaye, and Mackintosh (1989; see also McLaren & Mackintosh, 2000). According to this account, alternating presentations of two stimuli that share a common element (AX and BX) will establish mutual inhibitory links between the unique features A and B. To the extent that generalization between AX to BX depends on the ability of the X element to activate a representation of the unique feature belonging to the other compound, then generalization will be reduced after this form of preexposure, as the existence of the inhibitory link will suppress such activation. This account is certainly plausible as an interpretation of the sort of perceptual learning effect demonstrated, for example, by Symonds and Hall (1995). In their procedure, the rats in the intermixed condition received separate trials with AX and BX, these compounds being presented according to an alternating schedule. Standard accounts of associative learning readily predict the development of inhibitory associations between A and B in these conditions. Experimental results recently reported by Dwyer, Bennett, and Mackintosh (2001), which incidentally also made use of the salt-need induction procedure, provide evidence that such associations do in fact form.

To establish the reality of inhibitory associations between A and B is one thing; to show that they are the sole, or even the major, source of the perceptual learning effect is another. Although an analysis in terms of inhibitory associations can accommodate the results reported by Symonds and Hall (1995), it applies much less well to the intermixed procedure used in the present Experiments 2 and 3, in which the compound flavors were presented concurrently during preexposure. In this case, the close temporal and spatial contiguity of the flavors might be expected to promote the establishment of excitatory associations between them. Evidence that this does in fact occur comes from a study by Alonso and Hall (1999). In their experiments, rats were given access to two bottles concurrently (as in the present experiments), one containing saline and one sucrose. After such preexposure it was found that an aversion established to one flavor generalized readily to the other. Alonso and Hall interpreted this result as being a version of sensory preconditioning, with the excitatory association between saline and sucrose, formed during preexposure, mediating generalization between them. (Similar considerations may explain the finding, reported by Bennett and Mackintosh, 1999, that the perceptual learning effect is not obtained when AX and BX are presented serially but in close temporal proximity.) Given the similarity between the preexposure procedure used by Alonso and Hall and that used in Experiments 2 and 3, it seems reasonable to conclude that excitatory associations were formed between the preexposed flavors in the intermixed groups of the experiments reported here. The fact that these groups showed a clear discrimination between the X and Y flavors attests to the operation of some other process that is able to overcome the influence of these excitatory associations. One possible candidate for such a process will be considered next.

According to Gibson's (1969) notion of stimulus differentiation, exposure to stimuli, at least if it is arranged to allow the possibility of comparison, will result in a change in the way in which they are perceived—features that the stimuli hold in common will lose effectiveness, whereas the effectiveness of unique features will be enhanced. The stimuli used in the present experiments, XS and YW, may be construed as consisting of the elements *x*, *s*, *n*, *c* and *y*, *w*, *n*, *c*, where *w* and *s* refer to the weak and strong salt components, *x* and *y* to the unique features of almond and vanilla. The symbol *n* refers to the element of saltiness held in common by W and S, and *c* represents the features that X and Y may hold in common with them. Animals given the blocked schedule of preexposure (which does not allow for comparison to occur) can be expected to perceive all of these elements and to form associations among all of those in a given set. On test with X and Y, therefore, *xc* and *yc* would be perceived and both stimuli would be able to activate *n*, *s*, and *w*. These animal would have no reason to choose one of the test stimuli over the other. The preexposure given to animals in the intermixed condition, on the other hand, would mean, in the limiting case, that these animals would perceive only the unique features, *x*, *y*, *s*, and *w*. The associations formed during preexposure would thus primarily be between *x* and *s* (which are presented together in the same bottle) and between *y* and *s* (similarly presented). When presented with *x* and *y* on test, after experiencing a procedure designed to enhance the value of the strong saline solution, these animals would tend to choose the former over the latter.

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Received May 23, 2001
Revision received December 3, 2001
Accepted December 10, 2001 ■