Title

Immediate and Delayed Flavour-Calorie Learning: Can Rats Do It?

Journal

International Journal of Comparative Psychology, 1(1)

ISSN

0889-3675

Author

Simbayi, Leickness C.

Publication Date

1987

DOI

10.5070/P411010006

The International Journal of Comparative Psychology, Vol. 1, No. 1, Fall, 1987

IMMEDIATE AND DELAYED FLAVOUR-CALORIE LEARNING: CAN RATS DO IT?

Leickness C. Simbayi

ABSTRACT: Four experiments which investigated the ability of rats to associate the flavour of a food with the later release of calories are reported. In Experiments 1 and 2, which involved immediate reinforcement, rats were trained to discriminate between two flavours (e.g. cinnamon and wintergreen), one of which was mixed with a solution of glucose which provided many calories on some days and the other with a solution of saccharin which did not yield any calories on other days. In subsequent two-bottle tests between the two flavours mixed with the same type of substrate, all rats displayed large shifts in preferences for the flavour previously paired with glucose compared to the second flavour previously paired with saccharin. Experiment 2 further showed that the conditioned effects extinguished very easily. In Experiments 3 and 4, which involved delayed reinforcement, rats were trained to discriminate between the two flavour cues, both dissolved in saccharin, one of which was reinforced with food after a long delay on some days and the other with nothing on other days. In Experiment 3 glucose was delivered after a 30 min. delay whereas in Experiment 4 various kinds of food were used and the delay was reduced to 20 min. In subsequent preference tests between the flavour cues in Experiment 3, only a small, but significant, increase in preference for the paired flavour was detected. Similarly in Experiment 4 some evidence for discrimination learning was again found with glucose, but there was no evidence that rats could associate a flavour with starch solution or solid chow over the 20 min. delay. Overall these results show that rats can easily form flavour-calorie associations under immediate reinforcement conditions but they do so with great difficulty when long delays are involved.

INTRODUCTION

Omnivores are confronted with the difficulty of relating the delayed results of digestion with the flavour signals that are available during intake. One explanation is that they have a learning mechanism that not only allows them to avoid food which makes them ill but also to seek out food that provides calories. Whereas there is considerable evidence for the

---

Request for Reprints to: Department of Psychology, University of Zambia, P.O. Box 32379, Lusaka, Zambia.

This research was carried out in the Laboratory of Experimental Psychology of the University of Sussex, Brighton, England, U.K. as part of a Ph.D. project. It was partially financed by a U.K. Medical Research grant to Dr. M. J. Burton and also by a University of Zambia Staff Development Fellowship of which the author was a recipient from August, 1979 to July, 1986. The author wishes to acknowledge, with deep gratitude, the immense help received during the study from Drs. Burton and R. A. Boakes, both of the Laboratory of Experimental Psychology at Sussex University, who jointly served as supervisors for this project. Their constant encouragement throughout my sojourn at Sussex University is especially appreciated.

former in the form of conditioned taste aversions (for recent reviews, see Braveman & Bronstein, 1985), there is at best only sparse evidence for learning about the positive effects of food such as calories and the flavours associated with them. This second type of learning is important in that, in addition to offering an explanation of how animals learn about the caloric values of different foods, it has also been implicated in the regulation of food intake (for recent discussions of this issue, see Booth, 1985; Deutsch, in press; Le Magnen, 1984).

One of the best demonstrations that animals are capable of flavour-calorie learning appears to be that of Holman (1975). In this series of experiments rats were given daily access to either cinnamon—or wintergreen-flavoured solutions and only one of the flavours was followed by access to a reward solution; conditioning effects were monitored both by measuring consumption of the solutions and by giving a two-bottle choice test in which subjects were given simultaneous access to both flavours. Holman found that a flavour followed immediately by a nonnutritive sweet solution (saccharin) came to be preferred over the nonreinforced flavour, whereas, if the saccharin was delayed, no change in relative preferences was observed. However, if a flavour was reinforced with a nutritive sweet solution, namely glucose, an acquired preference was obtained even with a 30 min. delay (see Experiment 5). Holman concluded that his subjects had learned to associate the flavour serving as the positive stimulus with the subsequent arrival of calories from the glucose. Apart from demonstrating delayed flavour-calorie learning, Holman's results are also important because they provide an example of animals learning to associate events separated by a 30 min. interval which is unique outside the conditioned taste *aversion* literature; the closest alternative example is that of Lett (1975) who used special priming procedures to obtain delayed reinforcement learning in a T-maze.

In view of the importance of these results, the series of experiments reported in the present paper was undertaken in an attempt to explore further the effects reported by Holman (1975). Under immediate reinforcement conditions (see Experiments 2 and 3), Holman was able to detect quite a large shift in flavour preferences even though he had used a strong saccharin solution (0.32%) as a reinforcer. Because saccharin is inert, Holman's findings were most probably as a result of flavour-flavour rather than flavour-calorie associations. Thus, there was still a need to demonstrate flavour-calorie learning under such conditions. Both Experiments 1 and 2 reported in the present paper attempted to do this by using glucose, a substance which is rich in calories, as a reinforcer instead of the strong saccharin solution. In addition, Experiment 2 also measured the persistence of such an effect. The final two experiments tested for the ability of rats' to acquire delayed flavour-calorie learning using procedures similar to those of Holman's Experiments 4 and 5. Experiment 3 in the present paper simply asked whether rats could associate a specific flavour with the arrival of a glucose solution 30 min. later, whereas, Experiment 4 compared the ability of different types of reinforcers to support flavour-calorie learning over a 20 min. delay.

EXPERIMENT 1

In this experiment, rats were given one flavour mixed with saccharin on some days and the second flavour mixed with glucose on the remaining days. Unlike in Holman's experiments, however, no quinine was added to glucose in order to equate its palatability with the bitter after-taste normally associated with the ingestion of saccharin. This should ensure that the glucose solution used here was a more effective reinforcer than that in Holman's experiments. The concentrations of the solutions were such that from pilot studies it was known that the glucose was highly preferred to the saccharin solution when both were unflavoured. Hence a strong conditioned preference for the flavour paired with glucose was anticipated.

METHOD

Subjects and Maintenance

The subjects were 16 naive adult male rats aged about 90 days and weighing 265-385 g. at the beginning of the experiment. As in all experiments reported in this paper, the rats used were of the Lister hooded strain bred in the animal house of the School of Biological Sciences at the University of Sussex and were individually housed in plastic cages (North Kent Plastics, Dartford, Kent, England) with metal grill lids. The experimental room in which the animals were housed was temperature controlled at 20 +/-3 degrees C, with a fixed 12 h light-dark cycle (lights on at 0600 hrs.). Neither in this nor any other experiment in this paper was a reversed light-dark cycle used.

Materials

Flavour extracts consisted of 2.0% cinnamon oil (Sigma London Chemical Company Limited, Poole, Dorset, England) and 2.0% oil of wintergreen (methyl salicylate), (Sigma), both dissolved in ethanol, volume by volume (v/v). Reinforcers consisted of 20% alpha-D(+)-glucose (dextrose or corn sugar), (Sigma) dissolved in water, weight by volume (w/v), and 0.065% sodium saccharin (British Drug Houses, now M.W. Scientific Limited, Poole, Dorset, England) also dissolved in water w/v. Cinnamon-flavoured solutions contained 0.5% (v/v) cinnamon extract and wintergreen-flavoured solutions 1.0% (v/v) wintergreen extract.

Habituation

As was the case in the majority of experiments reported in this paper, unless specified otherwise, subjects were given a minimum of two weeks to habituate to the experimental room and maintenance conditions prior to the start of the experiment. This was intended to minimize the effects of novel and stressful stimuli because feeding behaviour in the rat is a process which is easily disrupted by disturbance in the laboratory environment. During the habituation period in this experiment, the subjects were fed only 16 g. of standard laboratory chow pellets (Spratt's Expanded Rodent Diet, Spiller's Limited, Newmarket, Suffolk, England) daily at 1700 hrs. in order to habituate them to the feeding regimen which was maintained throughout the duration of the experiment. Similarly, they had continuous access to water, except from 0930 to 1700 hrs. the time during which water was not made available and also training solutions were presented for 30 min., beginning at 1200 hrs. each day.

Procedure

Unflavoured saccharin and glucose solutions were offered for familiarization for four consecutive days before training began. All rats first received 40 ml. of unflavoured saccharin solution overnight, i.e. between 1700 and 0930 hrs. on the following day, and another 40 ml. for three hours in the afternoon (between 1200 and 1500 hrs.) on the next day, then 40 ml. of unflavoured glucose solution overnight and another 40 ml. for three hours in the afternoon on the next day.

Following familiarization, the rats were assigned to two groups (n=8 each), namely Groups C and W, matched on the basis of their consumption of the two unflavoured solutions during familiarization. The actual experiment consisted of a single 8-day training period which was followed by a single test day. During training, Group C received cinnamon flavour mixed with glucose solution on some days and wintergreen flavour mixed with saccharin solution on other days, whilst Group W received the same treatment as Group C, except that flavour-reinforcer pairings were reversed. The flavour of the cue solution on any given day was varied according to a double-alternation schedule: cinnamon on the first day, wintergreen on the next two days, cinnamon on the next two days, and so on. Thus, during the single 8-day conditioning cycle each subject received four flavour-reinforcer pairings.

The testing on Day nine involved a two-bottle "free choice" procedure (cinnamon versus wintergreen), whereby using a counterbalanced design all rats were offered 40 ml. of the two flavours in both substrates, i.e. in one condition both flavours were presented side-by-side simultaneously in saccharin solution and in the other in glucose. These two conditions were given for ten min. test periods separated by a 30 min. interval. During the initial test period, half the animals in each group received flavours mixed with saccharin solution, while the other half received flavours mixed with glucose solution. The substrates were reversed for the second test period. In order to minimize any positional biases half the animals receiving flavours in each substrate were offered cinnamon on the left and wintergreen on the right, whilst the other half received the two flavours in reversed positions. Furthermore, after the initial five min. the positions of the bottles were switched, i.e. those on the right were moved to the left side and vice versa.

Relative preferences for cinnamon flavour in the choice tests were then calculated in terms of percentages of total fluid consumption by each subject during each test as follows:

$$\text{Preference for cinnamon (\%)} = 100 \times \frac{\text{intake of cinnamon}}{\text{intakes of both cinnamon and wintergreen}}$$

RESULTS

During training, Group C consumed significantly more cinnamon flavour (M=7.5 ml. per 30 min. period) than Group W (M=3.3 ml.), while Group W consumed more wintergreen (M=9.3 ml.) than Group C (M=5.3 ml.). These data were assessed using an analysis of variance (ANOVA) with flavours (cinnamon vs. wintergreen) and groups (C vs. W) as factors. This analysis revealed a significant main effect of flavours, $F(1,28) = 6.23$, $p < 0.05$, indicating an overall preference for wintergreen over cinnamon. There was a significant interaction between flavours and groups, $F(1,28) = 4.41$, $p < 0.05$.

Figure 1

Mean preferences (%) for cinnamon flavour during two-bottle tests in Experiment 1 (n=8). During training cinnamon and wintergreen were mixed with plain glucose in Groups C+ and W+ respectively. In separate tests the flavours were presented either in a saccharin or in a glucose substrate. Bars represent standard errors.

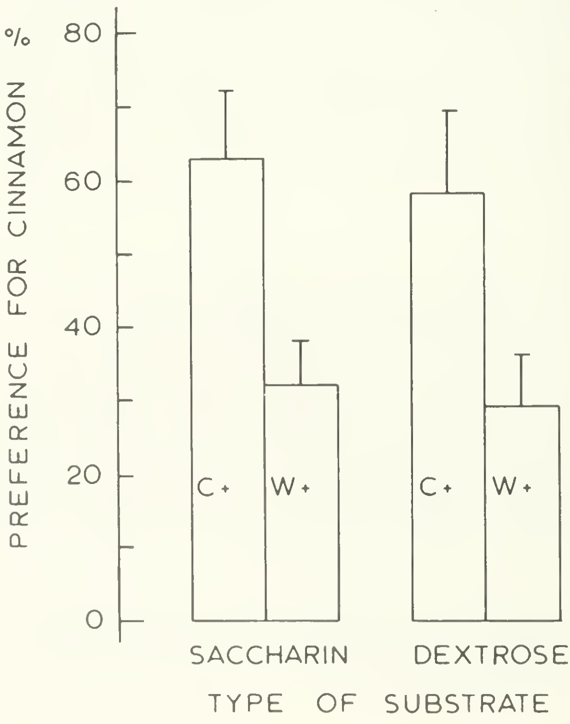


Figure 1 shows the relative preferences for cinnamon flavour obtained during the two conditions of testing in this experiment. These data were assessed using ANOVA with groups and types of test substrate as factors. This analysis revealed only a significant main effect of groups, $F(1,28) = 12.17$, $p < 0.01$; that is, Group C had a significantly higher preference for cinnamon flavour than Group W irrespective of the type of substrate, as clearly shown in Figure 1.

DISCUSSION

Experiment 1 showed that immediate differential reinforcement with saccharin and glucose can support strong flavour-calorie learning in rats. The flavour associated with glucose rapidly came to be preferred over the flavour associated with saccharin, irrespective of the type of test substrate used. Thus, the rats were clearly capable of recognizing the flavour previously paired with glucose equally well in the two test conditions. This finding replicates a recent one by Mehiel and Bolles (1984).

EXPERIMENT 2

Experiment 1 demonstrated the robustness of the basic phenomenon of flavour-calorie learning based on immediate reinforcement. The purpose of Experiment 2 was to determine how long such an effect persists.

METHOD

Subjects

The subjects were 16 naive male rats of the same strain and origin as in Experiment 1. They were about 90 days old and weighed 260-470 g.

Materials and Procedure

The materials and procedure were the same as in Experiment 1, except that three successive extinction choice tests were given on days 9 to 11.

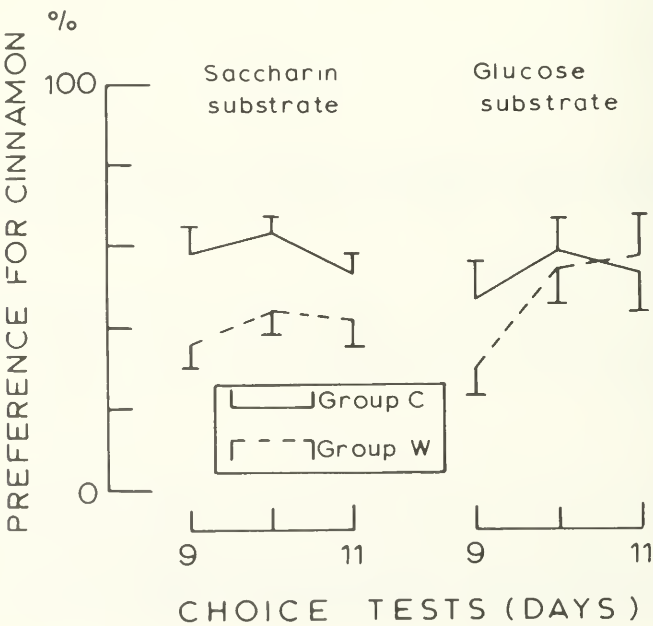
RESULTS

During training, Group C consumed more cinnamon solution (M=8.3 ml.) than Group W (M=5.3 ml.), while the opposite was true for wintergreen consumption, i.e., Group W consumed more wintergreen (M=8.6 ml.) than Group C (M=6.1 ml.). These data were assessed using an ANOVA as was done for similar data in Experiment 1. Although none of the main effects were significant, the interaction between flavours and groups was significant, $F(1, 28) = 14.66$, $p < 0.001$.

Figure 2 shows test preferences for cinnamon flavour during the successive extinction choice tests in this experiment. An overall ANOVA (with groups, types of test substrate and test sessions as factors) failed to reveal any significant effects, although the main effects of both groups ($CF(1, 28) = 2.52$) and test sessions ($CF(2, 56) = 2.74$) were approaching significance, both $0.05 <$ ps. < 0.10. Since there was some indication of conditioned preferences when saccharin was used as the test substrate in that Group C showed higher preference for cinnamon relative to wintergreen than Group W, as can be seen in Figure 2, the between-group differences were further assessed using unrelated t-tests. The analysis confirmed this expectation by showing that the differences between the two groups were significant during both the first and second test sessions when test flavours were presented in saccharin solution only, ts. $(14) = 2.29$ and 2.71, respectively, both ps. < 0.05. However, there was no signifi-

Figure 2

Mean preferences (%) for cinnamon flavour during successive two-bottle tests in Experiment 2 (n=8). The training and test procedures were the same as in Figure 1, except that testing was repeated on three consecutive days. Bars represent standard errors.



cant difference between the two groups' preferences in Test 3 under the same condition. Unlike saccharin, none of the between-group comparisons were significant on any test when glucose was the test substrate. Thus, as can be clearly seen in Figure 2, the conditioning effects were more clear when saccharin was used as the test substrate than when glucose was used.

DISCUSSION

Two things are clear. The first, demonstrated by the partial replication of Experiment 1 (e.g. see data for Day 9 in Figure 2), flavour-calorie learning based on immediate reinforcement is both a very robust and consistent phenomenon. The second point is that the effects are extremely transient in that they extinguish very easily. Following a single 8-day conditioning cycle (i.e. four flavour-reinforcer pairings), large increases in preferences for glucose-paired flavours were observed for two consecutive days if a nonnutritive test substrate (saccharin) was used but the effects were much less pronounced if a nutritive test substrate (glucose) was used. Thus, stronger flavour-calorie learning was exhibited when test flavours were mixed with saccharin than with glucose. Similar results have also been reported for other types of positive flavour preferences learning such as those based on flavour-*hunger* associations. For example, Capaldi and her colleagues (e.g. Capaldi and Myers, 1982; Capaldi, Myers, Campbell & Sheffer, 1983) have recently reported that rats given saccharin solution developed a stronger flavour preference than those given sucrose solutions. Perhaps this discrepancy is due to overshadowing of the flavour CSs by the flavour of glucose during consecutive test days.

EXPERIMENT 3

Both Experiments 1 and 2 relied on immediate reinforcement, whereby distinct flavours were directly mixed with various types of reinforcers which yielded either many or no calories. Although this situation most closely resembles that which prevails during normal ingestion of food in nature and therefore is ecologically valid, food is not usually available in simple forms such as glucose which can be utilized immediately by the organism but rather in more complex forms such as starch (a common carbohydrate), proteins and fat. Before the calories contained in these foods can be released, the food has first to be broken down (i.e. digested) by enzymes in the GI and this takes some time. In fact, it is surprising that even the absorption of calories from glucose has been estimated to take from 15 min. to over 1 h. (e.g. Cori, 1925; Kohn, Dawes & Duke, 1965; Magee & Reid, 1935; Reynell & Spray, 1953, 1956; although cf. Pilcher, Jarman and Booth's (1974) and Booth's (1979) estimate that the absorption of calories from glucose and starches takes 5-30 min. Because of this inherent delay between actual ingestion and the final realization of the energy contained in a food in the form of calories, it appears that an experimental situation involving delayed, rather than immediate, differential reinforcement would provide some very interesting information as regards the optimal conditions under which flavour-calorie learning occurs. Therefore, the purpose of the last two experiments reported in this paper were aimed at establishing the optimal US-CS delay conditions under which flavour-calorie learning will occur.

The aim of Experiment 3 was to get rats to learn that a given flavour would be followed 30 min. later by access to glucose. The method used was Holman's flavour-tracking procedure and the experiment was essentially identical to his Experiment 5, except for the following changes: a) a control group given a pseudo-discrimination procedure was added in order to assess the consequences of long-term exposure to the two flavours when neither reliably predicted subsequent glucose; b) no quinine was added to the glucose solution to make it less palatable than saccharin; this change was introduced in the belief that plain glucose solution used would be a more effective reinforcer than that in Holman's experiment; and c) a total of 24 training sessions was given, with a test session interspersed after each block of eight sessions, instead of the total of 20 training sessions followed by a single test used by Holman; this change was introduced in order to monitor the course of conditioning.

METHOD

Subjects

The subjects were 24 rats of the same sex, strain and origin as in previous experiments. They were aged about 90 days and weighed 265-385 g. at the beginning of the experiment. The rats had previously been used in an omission conditioning experiment (Wilson, 1983), but had no previous experience with either the two sweet solutions (i.e. saccharin and untainted glucose) used as differential reinforcers or the two flavours (i.e. wintergreen and cinnamon) used as CSs in this experiment. The subjects were housed and maintained in conditions similar to those in both previous experiments. They also had continuous access to water, except from 1300 to 1430 hrs. when training solutions were presented.

Materials

All materials were the same as in the two earlier experiments, except for one minor change, namely, 1.0% cinnamon flavour extract was used instead of 0.5% to flavour the cinnamon solutions.

Procedure

The rats were first habituated to the experimental conditions for two weeks during which they were fed only six pellets (approx. 12 g.) of standard laboratory chow daily at 1530 hrs. The same feeding regimen was maintained throughout the duration of the experiment. During the last four consecutive days of the two-week habituation period, i.e., before the actual experiment commenced, the animals were familiarized with both plain (i.e. unflavoured) saccharin and untained glucose solutions as in previous experiments.

The main part of the experiment consisted of three 8-day training periods, each of which was followed by a single test day. On each training day, the rats were offered 40 ml. of cue solution for 30 min. at 1300 hrs. while the reinforcement solution (glucose) was offered 30 min. after the removal of the cue solution to appropriate groups. The flavour of the cue solution on any given day was varied according to a double-alternation schedule as in previous experiments. The rats were divided into three groups (n=8 each) which were treated as follows: Group C+ was offered 40 ml. of reinforcement solution for 30 min. after the removal of the cinnamon cue solution, and nothing after the wintergreen solution; Group W+ was offered the reinforcement solution after wintergreen and nothing after cinnamon; Control Group was offered both glucose and no reinforcement after either cinnamon or wintergreen on a four-day cycle, e.g., on the first day, cinnamon was followed by glucose; on the second day, wintergreen was followed by nothing; on the third day, wintergreen was followed by glucose; on the fourth day, cinnamon was followed by nothing, and so on.

As in previous experiments, the one day tests following each eight day training cycle involved the two-bottle procedure, whereby all the rats were offered 40 ml. of each cue solution side-by-side simultaneously for 30 min. Relative preferences for cinnamon flavour in the tests which followed each eight day cycle were then calculated as before.

RESULTS

The amounts of cue and reinforcer solutions drunk during training sessions are summarized in Table 1. Cue solution consumption data were assessed using an ANOVA with groups (C+ vs. control vs. W+), type of flavour cue (cinnamon vs. wintergreen), and the duration of training (Cycles 1 vs. 2 vs. 3) as factors. This analysis revealed significant main effects for all three factors: groups, $F\ (2,42) = 4.04$, $p < 0.05$; flavour cue, $F\ (1.42) = 5.79$, $p < 0.05$; and conditioning cycles, $F\ (2,84) = 77.88$, $p < 0.001$. The interaction between flavours and conditioning cycles was also significant, $F\ (2,84) = 7.14$, $p < 0.01$. From Holman's Experiment 5 we expected Group C+ to drink more cinnamon and Group W+ to drink more wintergreen. This would have been confirmed by an interaction between groups and flavours, but no such effect was detected in the analysis or suggested by the data shown in Table 1. Thus, for example, although wintergreen consumption doubled in the course of training, by Cycle 3 it was no greater in Group W+ than in Group C+.

Table 1
Mean Consumption (ml.) of Training Solutions During the Three Conditioning Cycles of Experiment 3 (n=8)

Group C+ was reinforced with plain glucose 30 min. after the removal of cinnamon (C) but received no reward after wintergreen (W); Group W+ received reversed flavour-reinforcer pairings to Group C+; the control group was treated like Group C+ for half of the time and like Group W+ for the other half, i.e. flavours did not predict glucose. R=reinforcer, i.e. glucose. *Note:* Both flavours used for training and testing were dissolved in 0.065% saccharin solution.

	Cycle 1			*Cycle 2*			*Cycle 3*		
	C	W	R	C	W	R	C	W	R
C+	9.4	11.0	18.3	14.3	16.6	20.9	19.0	22.3	20.5
W+	10.1	11.6	17.5	12.3	17.0	21.3	15.3	22.0	21.9
Control	5.5	8.4	17.4	10.7	14.8	22.4	14.6	18.0	20.8

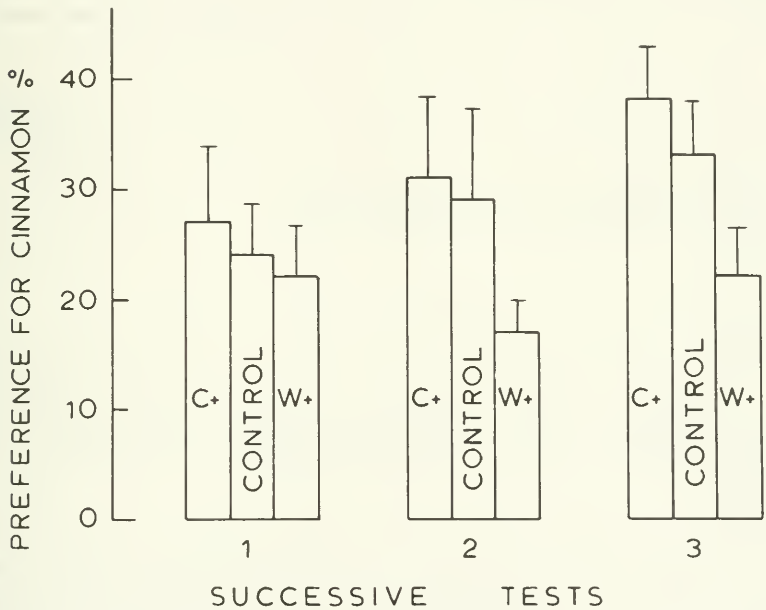
The relative consumption of the two flavours on each individual test session is shown in Figure 3 in terms of preference for cinnamon. As indicated by this figure, Group C+ tended to show higher preferences for cinnamon than the control group, which in turn showed higher preferences than Group W+, and these differences tended to increase with training. Although no main effects or interactions were detected by an ANOVA (with groups and duration of training as factors), a trend analysis using orthogonal polynomials (Ferguson, 1981) established that by the final cycle there was a reliable trend for cinnamon preferences to increase from Group W+ to control to Group C+, $F(1,21) = 5.32$, $p < 0.05$.

DISCUSSION

The only indication that animals had learned the relationship between a specific flavour and the later arrival of glucose came from the test data where a significant difference was found in terms of relative measures between the three groups on the third test. The close similarity between the results for Group C+ and the control group, as shown in Figure 3, suggests that the consistent pairing of cinnamon with glucose had little effect, but that the group difference arose mainly as a result of increased preference for wintergreen, the more preferred flavour, when it was paired with glucose.

Figure 3

Mean preferences (%) for cinnamon flavour during two-bottle tests in Experiment 3 (n=8). During training Group C+ was reinforced with glucose 30 min. after the removal of cinnamon but with nothing after wintergreen; Group W+ received reversed flavour-reinforcer pairings to Group C+; the Control group was treated like Group C+ for half of the time and like Group W+ for the other half, i.e. flavours did not predict glucose. During testing only the two flavour cues, cinnamon and wintergreen, which were both mixed with a saccharin substrate, were used. Bars represent standard errors.



The results differed from those of Holman's Experiment 5 in two main ways. First, there was no detectable associative effect on the consumption of cue solutions during training sessions, i.e. when only one flavour was available at a time. Second, the change in relative preference as measured during the choice tests was of the order of half that reported by Holman. The mean relative preferences that can be estimated from his published data give a cinnamon preference of 56% for Group C+ and of 31% for Group W. This difference of 25% obtained after 20 training days compares with a difference between Groups C and W of only 16% on Test 3 following 24 training days in this experiment.

EXPERIMENT 4

One possible explanation for the small amount of learning detected in Experiment 3 is that the glucose solution was an ineffective reinforcer, and in particular may have had partly aversive effects due to its hypertonic properties (Booth, Lovett & McSherry, 1972; although cf. Fitsimons,, 1961; Jacobs, 1961, 1962, 1963). It was decided to test the use of starch as a reinforcer in order to obtain stronger flavour-calorie learning, since it is as nutritive as glucose, but has no complications such as those associated with the consumption of glucose. In additon, it was also decided to test flavour-calorie learning using solid food in the form of pellets as a reinforcer, since, if this proved to be as effective a reinforcer as liquid starch, it would have been generally more convenient to use in subsequent experiments. Thus, the aim of Experiment 4 was to compare the effectiveness of these three reinforcers: glucose, starch solution and solid food.

The procedure was identical to that of the previous experiment, except that the delay between removal of cue solution and presentation of the reinforcer was reduced from 30 to 20 min. and the strength of the cinnamon solution was decreased. Both changes were intended to increase the size of any conditioning effects.

METHOD

Subjects

The subjects were 24 naive rats of the same age, sex, strain and origin as in Experiment 3. They were housed and maintained in similar conditions.

Materials

The cue and reinforcement solutions were exactly the same as in Experiment 3.1, except that the concentration of the cinnamon cue solution was decreased from 1% to 0.5% in order to make its palatability closer to that of the wintergreen solution. In addition to 20% glucose solution the following two reinforcers were also used: 20% Snowflake (a low-glucose maltodextrin derivative of starch), (CPC [U.K] Ltd.) dissolved in water and standard dry laboratory chow pellets (Spratts Expanded Rodent Diet). The starch was sweetened during Cycle 3 by adding 0.1% sodium saccharin.

Procedure

Familiarization was carried out as in Experiment 3, but only overnight and not in the afternoons. In addition, several parametric changes were made to the training procedure. The rats had access to cue solutions limited to five min. only. Then, 20 min. after removal of the cue solutions, half the groups received appropriate reinforcers for 30 min. Training was given in the mornings at 1000 hrs. to ensure greater separation than in Experiment 3 between the effects of ingestion of reinforcers and those of ingesting the maintenance food at 1700 hrs.

The experiment consisted of three 8-day conditioning cycles, each followed by a test day. The rats were divided into six equal groups ($n=4$ each) which received cinnamon- and wintergreen-flavoured cue solutions on a double-alternation schedule. Half of the groups were reinforced with one of the three reinforcers following cinnamon, namely, Groups C-D (Cinnamon-Dextrose, i.e. glucose), C-S (Cinnamon-Starch), and C-Ch (Cinnamon-chow), while the other groups were reinforced following wintergreen, namely, Groups W-D (Wintergreen-Dextrose, i.e. glucose), W-S (Wintergreen-Starch), and W-Ch (Wintergreen-chow). Animals were given 40 ml. of cue and reinforcement solutions during training and testing, but in the case of solid food ten pellets (approximately 20 g.) were given during training. During the final cycle sweetened starch was used to reinforce Groups C-S and W-S. Daily consumption of training and test solutions was measured and the test procedure was exactly as in Experiment 3.

RESULTS

The consumption of cue solutions during training is summarized in Table 2. These data were assessed using an ANOVA with flavours, amount of training, type of reinforcer and the type of reinforcement contingency (C+ vs. W+, i.e. whether cinnamon or wintergreen was reinforced) as factors. This analysis revealed that the only significant main effect was that of training, $F(2,72) = 74.29$, $p < 0.001$. There were significant interactions between type of reinforcer and conditioning cycle, as well as between flavours, type of reinforcement contingency and type of reinforcer, Fs. $(4,72) = 3.32$ and 2.61, both ps. < 0.05. The former of these interactions appeared to reflect the slightly greater increase in overall consumption of cue solution across training cycles in the two groups given the solid food reinforcer. The latter interaction indicated that the difference between comparable C+ and W+ groups was greatest for glucose reinforcement than for the other reinforcers.

Assessment of relative preferences for cinnamon during the three tests using an ANOVA showed significant main effects for all three factors: reinforcement contingency, $F(1,18) = 9.25$, $p < 0.01$; type of reinforcer, $F(2,18) = 12.85$, $p < 0.001$; and conditioning cycles, $F(2,36) = 7.14$, $p < 0.01$. In addition the interaction between reinforcement contingency and type of reinforcer was also significant, $F(2,18) = 4.63$, $p < 0.05$. The interesting aspects of these results are best discussed with respect to the data displayed in Figure 4. This graph shows for each test and reinforcer type the difference in preference for cinnamon between comparable C+ and W+ groups. A positive score indicates that the group given the reinforcer following cinnamon had a higher preference for this flavour than the group given the same reinforcer following wintergreen.

Table 2
Mean Consumption (ml.) of Training Solutions During the Three Conditioning Cycles in Experiment 4 (n=4)

Type of reinforcer	*Reinforcement contingency (Group)*	*Cycle 1* C	*Cycle 1* W	*Cycle 2* C	*Cycle 2* W	*Cycle3* C	*Cycle3* W
Glucose	C+ (C-D)	5.6	5.0	5.5	5.1	6.4	5.7
	W+ (W-D)	5.8	5.4	6.7	6.6	6.9	7.1
Starch	C+ (C-S)	5.6	5.6	6.2	5.8	7.0	7.0
	W+ (W-S)	6.1	5.5	5.9	6.4	7.3	7.9
Laboratory Chow	C+ (C-CH)	5.2	4.9	6.2	6.5	7.5	6.9
	W+ (C-Ch)	5.0	4.9	6.1	5.8	6.4	6.1

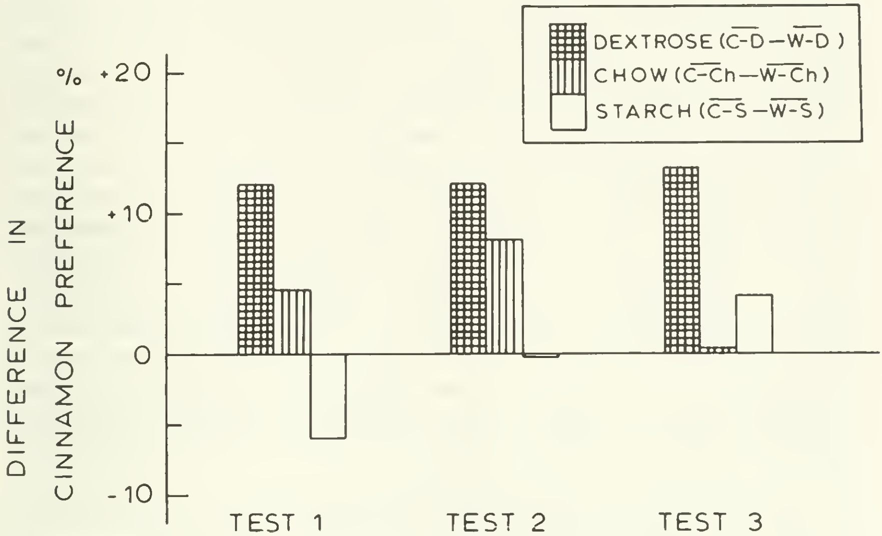
For each reinforcer, the C+ contingency involved presenting that reinforcer 20 min. after the removal of cinnamon (C) but nothing after the removal of wintergreen (W), whereas in the W+ contingency flavour-reinforcer pairings were reversed. D=dextrose, i.e. glucose; S=starch; CH=lab chow.

It can be seen from this figure that, of the results of the analysis given above, the interaction between contingency and type of reinforcer indicates that the C+ - W+ difference was greatest for glucose (+12% over 3 tests) and least for starch (-1%). The absence of any interaction involving the cycle factor indicates that there was no evidence of any change in the effect of the contingency with further training. As can be seen in Figure 4, in the glucose groups the C+ - W+ difference remains essentially constant across tests. In other words, conditioning in these two groups seems to be complete by the end of the first 8-day cycle and not to have taken place at all in the remaining four groups.

Planned comparisons between pairs of groups indicated that only Group C-D had significantly higher preferences for cinnamon than Group W-D during Tests 1 and 2, $t(6) = 2.25$ and 3.08 respectively, both ps. < 0.05, but not on Test 3. However, since this analysis involved multiple comparisons, significance strictly required an alpha-level of less than 0.02 and so orthogonal comparisons were carried out. These showed that on all three tests, the difference between Groups C-D and W-D were marginally significant, respectively $F(1,18) = 3.79$, 4.08 and 3.93, $0.10 > p < 0.05$. No reliable

Figure 4

Differences in mean preferences (%) for cinnamon flavour during two-bottle tests in Experiment 4. Different groups of rats (n=8) were given one of three reinforcers, i.e., glucose, starch or lab chow, 20 min. after the presentation of flavour cues. See text for additional details.



differences were obtained when similar comparisons were made for the other two pairs of groups which had received either starch solution (Groups C-S vs. W-S) or solid laboratory chow (Groups C-Ch vs. W-Ch) as reinforcers.

DISCUSSION

These results showed that the glucose solution was a better reinforcer of flavour-calorie learning than either solid laboratory chow or starch solution. Consequently they rule out the possibility that glucose is a particularly inappropriate reinforcer for learning over a delay. In fact there was no strong evidence that animals in the chow or starch groups learned the flavour-reinforcer relationship, even when the starch was sweetened during the final training cycle.

The main procedural differences between this and Experiment 3 were the use of more dilute cinnamon and the reduction in delay from 30 to 20 min. The results from the glucose groups suggest that these changes may have led to more rapid acquisition, in that there was a clear difference between Groups C-D and W-D on Test 1 following only eight training sessions and no subsequent increase. However, the size of the difference in

Experiment 4 (12%) remained, if anything, smaller than that reached after 24 sessions in Experiment 3 (16%).

GENERAL DISCUSSION

The interpretation of the results obtained from both Experiments 1 and 2 is complicated by the fact that consumption was not equal during conditioning trials. Thus, the possibility exists that this differential consumption contributed to the direction of the flavour-calorie learning. Arguing against this differential consumption hypothesis are data presented by Holman (1975), who reported that a flavour paired with saccharin and available 60 min. per day was not preferred over another flavour paired with saccharin and available only 5 min. per day (see Experiment 1). In other words, amount of exposure had no effect on positive flavour learning. Futhermore, as indicated earlier, in Holman's Experiments 2 and 3 he showed that, in a two-bottle test, a flavour paired with a high concentration of saccharin is preferred over a flavour paired with a dilute concentration of saccharin. Thus, we can rule out the possibility that a CTA to the saccharin-paired flavour is responsible for the present results. In support, Mehiel and Bolles (1984) have recently looked specifically for such an effect and failed to find it.

Another aspect of the results deserving further comment is the apparent very low persistence of flavour-calorie learning effects as shown in Experiment 2. In contrast, other types of the same phenomenon have been shown to be incredibly persistant. For example, Capaldi et al. (1983) found flavour preferences based on hunger during original flavour consumption to persist through 28 test days and Revusky (1974) found similar flavour preferences based on thirst to persist throughout ten days of testing after which they were no longer significant. Therefore, the persistence of the flavour-calorie learning effects compares very much less favourably with other types of the same phenomenon. This makes a lot of sense in that both rapid and very persistant learning, such as that which is normally seen in CTA learning, is not required in flavour-calorie learning because compensating for the consequences of caloric loss can be spread over a number of subsequent meals whereas when poisons are involved a mistake might otherwise prove to be fatal.

The apparent failure to obtain any flavour-calorie learning in Experiment 4 when both starch and ordinary laboratory chow served as reinforcers whereas only glucose was effective seems very interesting. This could reflect the somewhat slower digestion of these foods so that the effective flavour-calorie delay is longer than with glucose. Another possibility is that it might be because glucose is less familiar than the other foods. It is however unclear from the present data which of these two variables might be more crucial. Therefore, more research is needed to resolve this issue.

Another important issue concerns whether the association is really between flavours and the postingestive consequences of ingesting glucose such as calories, i.e. flavour-calorie associations, or it is rather between the flavours and the orosensory properties (i.e. palatability) of glucose, i.e. flavour-flavour associations. Holman's conclusion that the former were important was based on the contrast between the absence of any conditioning when saccharin was used (Experiment 4) and the large effect when glucose was used over the same delay (Experiment 5). Supporting evidence has been obtained from experiments using solid diets (e.g. Bolles, Hayward & Crandall, 1981; Booth, 1972; Hayward, 1983) and others using ethanol (e.g. Crawford & Baker, 1982; Deems, Oetting, Sherman & Garcia, 1986; Sherman, Hickis, Rice, Rusiniak & Garcia, 1983). In contrast, flavour-flavour learning appears difficult to obtain over long delays in that Lavin (1976) failed to find any sign of association between two novel flavours when a delay of ten sec. or more intervened between their presentation in a sensory preconditioning procedure. Thus, it seems likely that the rats were learning to associate, say, cinnamon with the later release of calories from the glucose, especially under delayed reinforcement conditions. Whilst it is worth noting the possibility that flavour-flavour associations may have overshadowed flavour-calorie associations under immediate reinforcement conditions in the experiments reported in the present paper, recent experiments carried out in our laboratory which examined the effects of tainting glucose with various concentrations of quinine in order to make it less palatable than saccharin did not support the idea (Simbayi, Boakes & Burton, in preparation).

If rats are indeed capable of delayed flavour-calorie learning, the second issue raised by all results obtained in the present study concerns whether the rather weak effects in these results were due to the insensitivity of the conditioning method used. In particular, the following question may be asked: Could it be that the flavour of the dextrose itself which actually intervenes between the presentation of the flavour CSs (i.e. cinnamon and wintergreen) and the later release of calories may actually have overshadowed these "artificial" flavour cues? Thus, the possibility is worth noting that more overshadowing of this kind may have occurred in the present experiments than with the quinine-tainted glucose used by Holman (1975). This seems reasonable given evidence from CTA literature that if rats experience two novel solutions prior to being made ill they are more likely to associate the aversive interoceptive US with the solution drunk in closer temporal proximity to that US (Revusky, 1971). However, recent results obtained in our laboratory from experiments which compared the ability of quinine-tainted glucose (as Holman did) and non-tainted glucose (as in the present paper) when both were matched against saccharin solution did not strongly support this "overshadowing" argument although the effects were generally in the predicted direction (Dr. V. Garcia, personal communication, 1985; Clelia Rossi-Arnaud, personal communication, 1985-1986).

In conclusion, rats appear to be capable of learning flavour-calorie associations under both immediate and delayed reinforcement conditions. Although immediate flavour-calorie learning is robust, it extinguishes rather easily. In contrast, delayed flavour-calorie learning appears to be very weak.

REFERENCES

Bolles, R. C., Hayward, L. & Crandall, C. (1981). Conditioned taste preferences based on caloric density. *J. Exp. Psychol.: Anim. Behav. Processes, 7*, 59-69.

Booth, D. A. (1972). Conditioned satiety in the rat. *J. Comp. Physiol. Psychol., 81*, 457-471.

Booth, D. A. (1979). Metabolism and control of feeding in man and animals. In K. Brown & S. C. Cooper (Eds.), *Chemical influences on behaviour.* London: Academic Press.

Booth, D. A. (1985). Food-conditioned eating preferences and aversions with interoceptive elements: Conditioned appetites and satieties. In N. S. Braveman & P. Bronstein (Eds.), Experimental assessments and clinical applications of conditioned food aversions, *Ann. N.Y. Acad. Sci., Vol. 443.*

Booth, D. A. (in press). Conditioning and the control of feeding. In R. A. Boakes, D. A. Popplewell & M. J. Burton (Eds.), *Eating habits.* Chichester: John Wiley.

Booth, D. A., Lovett, D., & McSherry, G. M. (1972). Postingestive modulation of sweetness preference gradient in the rat. *J. Comp. Physiol. Psychol., 78*, 485-512.

Braveman, N. S., & Bronstein, P. (Eds.) (1985). Experimental assessment and clinical applications of conditioned food aversions. *Ann. N.Y. Acad. Sci., Vol. 443.*

Capaldi, E. D., & Myers, D. E. (1982). Taste preference as a function of food deprivation during original taste exposure. *Anim. Learning Behav., 10*(2), 211-219.

Capaldi, E. D., Myers, D. E., Campbell, D. H., & Sheffer, J. D. (1983). Conditioned flavour preferences based on hunger level during original flavour exposure. *Anim. Learning Behav., 11*(1), 107-115.

Cori, C. F. (1925). The fate of sugar in the animal body. I. The rate of absorption of hexoses and pentoses from the intestinal tract. *J. Biol. Chem., 66*, 691-715.

Crawford, D. & Baker, T. B. (1982). Alcohol dependence and taste mediated learning in the rat. *Pharmacol. Biochem. Behav., 16*, 253-261.

Deems, D. A., Oetting, R. L., Sherman, J. E., & Garcia, J. (1986). Hungry, but not thirsty, rats prefer flavours paired with ethanol. *Physiol. Behav., 36*(1), 141-144.

Deutsch, J. A. (in press). Signals determining meal size. In R. A. Boakes, D. A. Popplewell & M. J. Burton (Eds.), *Eating habits*, Chichester: John Wiley.

Ferguson, G. A. (1981). *Statistical analysis in psychology and education* (5th ed.) Tokyo: McGraw-Hill Kogakashu.

Fitsimons, J. T. (1961). Drinking by nephroctomized rats injected with various substances. *J. Physiol.* (Paris)., *155*, 563-579.

Hayward, L. (1983). The role of oral and postingestional cues in the conditioning of taste preference based on differing caloric density and caloric outcome in weanling and mature rats. *Anim. Learning Behav., 11*, 325-331.

Holman, E. W. (1975). Immediate and delayed reinforcers for flavour preferences in rats. *Learning and Motivation, 6*, 91-100.

Jacobs, H. L. (1961). The osmotic postingestion factor in the regulation of glucose appetite. In M. R. Kare & B. P. Halpern (Eds.), *Physiological and behavioral aspects of taste.* Chicago: University of Chicago Press.

Jacobs, H. L. (1962). Some physical, metabolic, and sensory components in the appetite for glucose. *Amer. J. Physiol., 203*, 1043-1054.

Jacobs, H. L. (1963). Effects of intragastric hypertonic glucose loads on AC brain resistance in rats. *Proc. Soc. Exp. Biol. Med.*, *114*, 657-659.
Kohn, S. W., Dawes, E. D., & Duke, J. W. (1965). Absorption of carbohydrates from the intestine of the rat. *Biochimica et Biophysica Acta*, *107*, 358-362.
Lavin, M. J. (1976). The establishment of flavour-flavour associations using a sensory preconditioning training procedure. *Learning and Motivation*, *7*, 173-183.
Le Magnen, J. (1985). *Hunger. Problems in the behavioural sciences.* Cambridge, U.K.: Cambridge University Press.
Lett, B. T. (1975). Long delay learning in the T-maze. *Learning and Motivation*, *6*, 80-90.
Magee, H. E., & Reid, E. (1931). The absorption of glucose from the alimentary canal. *J. Physiol.* (Paris), *73*, 163-173.
Mehiel, R. & Bolles, R. C. (1984). Learned flavour preferences based on caloric outcome. *Anim. Learning Behav.*, *12*, 421-427.
Pilcher, C.W.T., Jarman, S. P., & Booth, D. A. (1974). The route of glucose to the brain from food in the mouth of the rat. *J. Comp. Physiol. Psychol.*, *87*, 56-61.
Revusky, S. (1971). The role of interference in association over a delay. In W. Honig & H. James (Eds.), *Animal memory.* New York: Academic Press.
Revusky, S. (1974). Retention of a learned increase in the preference for a flavoured solution. *Behav. Biol.*, *11*, 121-125.
Reynell, P. C., & Spray, G. H. (1956a). The simultaneous measurement of absorption and transit in the gastrointestinal tract of the rat. *J. Physiol.*, (Paris), *131*, 452-462.
Reynell, P. C., & Spray, G. H. (1956b). The absorption of glucose by the intact rat. *J. Physiol.*, (Paris), *134*, 531-537.
Sherman, J. E., Hickis, C. F., Rice, A. G., Rusiniak, K.W., & Garcia, J. (1983). Preferences and aversions for stimuli paired with ethanol. *Anim. Learning Behav.*, *11*, 101-106.
Simbayi, L. C., Boakes, R. A., & Burton, M. J. (In preparation). Acquired preferences for flavours mixed with nutritive and nonnutritive sweet solutions. University of Sussex.
Wilson, P. N. (1983). *The inhibition of instrumental responding under omission training.* Unpublished D. Phil. thesis, University of Sussex.