Partial reinforcement and resistance to extinction in honeybees

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Previous experiments have shown the partial reinforcement effect in honeybees under conditions which permit an interpretation in terms of sensory carryover. In the five experiments reported here, the effect was sought under conditions which would require an interpretation in terms of associative reinstatement. Since it is not feasible to train honeybees in widely spaced trials, several different interpolated-trials procedures were employed which had in common the feature that nonrewarded response to a stimulus never was followed by rewarded response to the same stimulus. Implications of the negative results for the interpretation of the overlearning-extinction effect and successive negative contrast in honeybees are considered.

Experiments on learning in honeybees show a wide range of phenomena known from work with vertebrates (Abramson, 1986; Abramson & Bitterman, 1986; Bitterman, Menzel, Fietz, & Schäfer, 1983; Couvillon & Bitterman, 1980, 1982, 1984; Couvillon, Klosterhalfen, & Bitterman, 1983; Klosterhalfen, Fischer, & Bitterman, 1978; Sigurdson, 1981a, 1981b). Among them is successive negative incentive contrast or SNC (reduced interest in a less preferred reward due to prior experience with a preferred reward), a phenomenon discovered early in rats by Elliott (1928) which provided the first clear indication that their instrumental performance is influenced by remembered reward. The fact that SNC occurs in honeybees (Couvillon & Bitterman, 1984) is of particular significance because it does not seem to be a general phenomenon of vertebrate learning, having failed repeatedly to appear in work with descendants of certain older vertebrate lines (Bitterman, 1975; see Flaherty, 1982, for a recent review, and Couvillon & Bitterman, 1985b, and Schmajuk, Segura, & Ruidiaz, 1981, for work conducted after that reviewed by Flaherty). Where we find in honeybees what does seem to be a general phenomenon of vertebrate learning, we must at least consider the possibility of a common mechanism evolved in some remote common ancestor (necessarily a simple mechanism in view of the presumptive structural simplicity of the common ancestor), but our immediate suspicion in the case of SNC is of convergence.

In the experiments now to be reported, we turn to a companion phenomenon of vertebrate learning, the par-

tial reinforcement effect or PRE (greater resistance to extinction after training with intermittent than with consistent reinforcement). Just as successive contrast points to the influence of remembered reward, so the PRE suggests control of performance by remembered nonreward. The general idea is that resistance to extinction is increased in intermittently rewarded animals because they are rewarded for responding while in some sense remembering nonreward. Where training is massed (that is, where intertrial intervals are relatively brief), a simple carryover interpretation is sufficient (Hull, 1952; Sheffield, 1949); the intermittently rewarded animals may be assumed to be rewarded for responding to stimuli correlated with nonreward, or to traces or short-term memories of those stimuli (continuously decaying afferent residuals), that persist from one trial to the next. Where, however, the training is spaced, with intertrial intervals of 1 day or longer (Rashotte & Surridge, 1969; Weinstock, 1954), some sort of reinstatement must be conceived, whether as associative memory (Capaldi, 1971) or as conditioned frustration (Amsel, 1958). Two different ways in which the consequences of response on one trial (S_2) may exercise discriminative control of response (R_1) to the conditioned stimulus (S_1) on a subsequent trial are schematized in Figure 1. In the carryover case, S_2 persists during the intertrial interval; in the reinstatement case, S_2 is evoked by S_1 , with which it has been associated.

The validity of the distinction between carryover and reinstatement is supported by work with goldfish and African mouthbreeders, fishes of rather different species, which show the PRE in massed trials (Gonzalez & Bitterman, 1967; Gonzalez, Eskin, & Bitterman, 1963) but not in widely spaced trials (Gonzalez, Behrend, & Bitterman, 1965; Longo & Bitterman, 1960; Schutz & Bitterman, 1969). In work with turtles, too, the PRE has been found in massed trials (Murillo, Diercks, & Capaldi, 1961) but not in widely spaced trials (Pert & Bitterman, 1970; Pert & Gonzalez, 1974). The validity of the distinction is supported also by the results of interpolated-

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Figure 1. Two ways in which the consequences of response on one trial (T) can exercise discriminative control of responding on the next trial (T + 1). S_1 , conditioned stimulus; R_1 , conditioned response; S_2 , consequences of response. In the carryover case, S_2 persists during the intertrial interval; in the reinstatement case, S_2 is evoked by S_1 with which it has been associated.

trials experiments, which are conventional massed-trials experiments—one group consistently rewarded and another intermittently rewarded for response to some specified stimulus—with an important exception: Trials with distinctly different stimuli are interpolated between trials with the specified stimulus to interfere with carryover. Under such conditions, the PRE appears in pigeons (Couvillon, Brandon, Woodard, & Bitterman, 1980), which show it as well in widely spaced trials (Roberts, Bullock, & Bitterman, 1963), but not in goldfish (Couvillon, 1984). Control by carryover and control by reinstatement are distinguished also by experiments on double alternation in goldfish (Couvillon & Bitterman, 1981).

Greater resistance to extinction in intermittently rewarded than in consistently rewarded honeybees has been reported in four experiments, all under conditions that make it unnecessary to assume control by remembered (in the sense of associatively reinstated) nonreward. The experiments were rather different. One was a free-operant experiment with free-flying subjects (Grossmann, 1973); the second was a field study with marked bees (Robacker & Ambrose, 1978); in the third, temporarily confined subjects were trained in massed discrete trials (Sigurdson, 1981a); and in the fourth, again with free-flying subjects, reward was delayed substantially on half the training visits (Couvillon & Bitterman, 1980), a technique long known to increase resistance to extinction in rats (Crum, Brown, & Bitterman, 1951). In all cases, however, nonrewarded response to a stimulus soon was followed by reward for response to that stimulus, permitting an interpretation of the results in terms of carryover. Our purpose in the present experiments was to look for a PRE under conditions that would rule out such an interpretation.

In work with free-flying bees, widely spaced trials cannot be used, because the interval between visits is controlled by the subject, which shuttles back and forth between the hive and the laboratory every few minutes of its own accord. Furthermore, there can be no non-rewarded visits; if the bee is not fed to repletion on a given visit, it forages elsewhere and may not return to the laboratory for hours. Accordingly, we found it necessary to develop some new procedures, adaptations of the interpolated-trials strategy used in work with vertebrates. There are, of course, various ways of implementing the interpolated-trials strategy in work with honeybees, and our purpose here was to explore some likely possibilities.

EXPERIMENT 1

In this experiment, we studied extinction of response to a distinctive target (labeled with an orange disk and the scent of jasmine) that was presented to the animal at the outset of each of a series of training visits. For the Consistent group, the target contained a 20- μ l drop of 50% sucrose solution; for the Partial group, it contained a 20- μ l drop of 50% sucrose solution on half the visits and a 20- μ l drop of tap water (unacceptable to the animals and distinguishable from the sucrose only by taste) on the remaining visits. The orange-jasmine target then was replaced by a gray, unscented target containing a large drop of 20% sucrose solution, from which the animal fed to repletion before returning to the hive. For the Partial group, therefore, unrewarded experience with the orange-jasmine target was separated from rewarded experience with that target at least by the time taken to feed to repletion on the gray target and to make a subsequent trip to and from the hive. It should be noted that bees which have recently tasted 50% sucrose do not readily accept 20% sucrose for a short time, a phenomenon readily understandable in terms of sensory adaptation (Bitterman, 1976). The use of 20% sucrose on the gray target meant that unrewarded experience with the orange-jasmine target would not be followed immediately by reward. By the same token, adaptation to the 20% sucrose presented on the gray target was expected to increase the reward-value of the drop of 50% sucrose subsequently encountered on the orange-jasmine target, which was another reason for using the lower concentration. In spaced-trials experiments with rats, large reward seems to be necessary for the appearance of the PRE (Gonzalez & Bitterman, 1969; Hulse, 1958; Wagner, 1961).

Method

Subjects. The subjects were 16 foragers, all experimentally naive. They came from our own hives, which were set up in the vicinity of the laboratory.

Procedure. Individual bees were pretrained to fly from the hive to the laboratory and to drink to repletion from a large drop of 50% sucrose solution on a target that was set on the sill of an open window. An animal was selected at random from a group of foragers at a feeding platform equipped with a large jar of 12%-15% su-

crose solution, carried in a matchbox to the laboratory, set down on the target, permitted to drink its fill of the 50% solution (during which time it was marked with a spot of colored lacquer), and then allowed to fly to the hive. Typically, the animal returned to the laboratory of its own accord in a few minutes and continued thereafter to shuttle back and forth between the hive and the laboratory as long as food was available. If the marked bee did not return to the laboratory after the first placement, it was picked up again at the feeding platform (where it usually could be found), carried back to the laboratory in the matchbox, and set down on the target once more. This procedure was repeated (with more than two placements rarely found to be necessary) until the bee returned to the laboratory of its own accord.

The targets were covered petri dishes of clear plastic, 5.5 cm in diameter. In each cover, which was sprayed with flat gray paint, eight equally spaced holes, .5 cm in diameter, were drilled at the outer circumference. One set of targets (OJ) was labeled with an orange color and the scent of jasmine: Fixed to the center of each cover was a 2.5-cm disk of the same orange plastic (O) used in our previous studies, and the dishes themselves contained wads of cotton that were impregnated with the jasmine scent (J) used in earlier work. The second set of targets (G) had plain gray covers without colored disks and contained unscented cotton wool. The covers were washed and exchanged for others in the same set after each visit in order to balance extraneous stimuli, any given cover being used only with a dish of the same scent category (jasmine or unscented) to prevent contamination.

Pretraining for all animals began with an initial placement and feeding to repletion on an OJ target that contained a large drop of 50% sucrose solution. The first time the bee returned to the laboratory of its own accord, it was permitted to drink about 5 μ l of the 50% sucrose solution on OJ, after which the experimenter captured it in a matchbox, substituted G for OJ, and placed the bee on G, where it drank to repletion from a large drop of 20% sucrose solution. After that, the animals were assigned in quasi-random fashion to two groups of 8 animals each, a Consistent group and a Partial group, which were given 12 training visits to OJ followed by G, which always contained a large drop of 20% sucrose solution. For the Consistent group, OJ always contained a 20- μ l drop of 50% sucrose solution (OJ+) and was replaced by G as soon as the bee finished feeding. For the Partial group, there were six such visits mixed with six others on which OJ contained a 20- μ l drop of tap water (OJ-); OJ- was exposed until 30 sec after the bee's first contact with it, whereupon it was replaced by G. The sequence of rewarded (R) and nonrewarded (N) OJ visits was RRNRNN RNNRR.

After these 12 training visits, there was an extinction test. Upon its return to the laboratory, the bee found a fresh OJ target with 20 μ l of water. A 10-min extinction timer was started 30 sec after the animal was first sighted by the experimenter or when it first landed on the target, whichever occurred first. All contacts with the target during the extinction period were recorded by the experimenter, who pressed a button that activated a counter programmed to print stored frequencies at 30-sec intervals. In acquisition, contacts with OJ during each unrewarded 30-sec presentation were recorded in the same way as in extinction.

Results

After OJ+ presentations in training, the animals of both groups showed transient interruption of feeding on G. They would walk or fly away from the 20% drop of sucrose several times before settling down to steady feeding. After OJ- presentations, as expected, the Partial animals showed no such interruptions of feeding on G, because their most recent prior experience was with 20%

sucrose. During the 30-sec OJ- presentations, the Partial animals would taste the water, walk or fly away, and then return to the target repeatedly. The number of contacts with the target on each visit fluctuated about a mean of 6.3, with no systematic change over successive presentations of OJ, although it may be that the 30-sec period was too short to show any increase in persistence that might have been produced by the training.

In Figure 2, the course of extinction in the two groups is plotted in terms of the mean number of responses in successive 30-sec intervals. Again in extinction, the animals would land on the target, taste the water, walk or fly away, return to the target, leave again, and so forth, with absences increasing in length and returns becoming less frequent, a quite familiar pattern (Couvillon & Bitterman, 1980). As the curves show, the Consistent group responded a bit more rapidly at first than the Partial group, and then somewhat more slowly, giving rise to what seems to be the familiar partial-reinforcement crossover. Analysis of variance fails, however, to yield either a significant groups effect ($F < 1$) or a significant interaction of groups $\times$ 2.5-min intervals [$F(3,42) = 1.91, p = .1421$].

It is possible that the interaction might have been significant had we used a larger number of subjects in each group, although in our previous bee experiments groups of the same size have proved to be sufficient for the clear demonstration of a variety of vertebrate effects. It is possible, too, that a larger number of training visits with a larger number of N-to-R transitions might have generated a significant partial reinforcement effect, although bees generally learn very rapidly under these circumstances; in recent modeling work, learning-rate parameters on the order of .25 were found to be necessary for good fits to the data (Couvillon & Bitterman, 1985a). It should be noted, as well, that 12 visits were enough to show a substantial effect of intermittently delayed reward on resistance to extinction in free-flying bees trained under conditions that permitted a carryover

Figure 2. The course of extinction in the Partial and Consistent groups of Experiment 1, plotted in terms of the mean number of responses in successive 30-sec intervals.

interpretation (Couvillon & Bitterman, 1980). Another possibility to be considered is that the large difference between the training and extinction conditions employed here produced a generalization decrement in both groups, serving to mask the effects of differential experience with non-reward, and it was this possibility that we chose to examine in the second experiment.

EXPERIMENT 2

In this experiment, the acquisition procedure was almost the same as in the first experiment, but the extinction procedure was changed to make it more similar to the acquisition procedure in order to minimize the possibility of a masking generalization decrement. The 10-min extinction test was abandoned in favor of a discrete-trials test, each trial consisting of a 1-min exposure to OJ— followed by feeding to repletion on G. Visits of this kind were permitted until the bee failed to make any contact with OJ— during the 1-min period. Under these conditions, the feeding on G continued to produce reliable return patterns, while response to OJ— extinguished. The only change in the acquisition procedure involved the sequence of OJ+ and OJ— trials, which now provided four N-to-R transitions instead of only three.

Method

Subjects. The subjects were 16 foragers, all experimentally naive, from our laboratory hives.

Procedure. The pretraining procedure and, as already noted, the training procedure were the same as in the previous experiment except that the sequence of OJ+ and OJ— visits for the Partial group was RNNRRRRNRRNRR. After 12 training visits, the series of extinction visits began. Each visit consisted of a 1-min exposure to OJ— followed by G, which contained a large drop of 20% sucrose, as it had in training. All contacts with OJ— were recorded as before. The experiment ended when, on any visit, the animal made no response to OJ—.

Figure 3. The course of extinction in the Partial and Consistent groups of Experiment 2, plotted in terms of the mean number of responses on each of the first twenty extinction visits.

Results

As might be expected from the similarity of the training procedures, the acquisition performance of the two groups in this experiment was almost identical to the performance of the corresponding groups in the previous experiment. In Figure 3, the extinction performance is plotted in terms of the mean number of responses to OJ— on each visit for the first 20 visits, by which time all but three of the animals (one Consistent and two Partial) had reached the criterion of extinction. The curves again show greater initial response strength on the part of the Consistent animals, although with less suggestion of a crossover than in the first experiment. Analysis of variance yields no significant groups effect ($F < 1$) but does yield a significant interaction of groups $\times$ 5-visit blocks [$F(3,42) = 6.16, p = .0014$], the main source of which clearly is the large difference in initial level.

EXPERIMENT 3

The general plan of this experiment, considerably simpler than the previous two, was as follows: All animals were trained with two highly discriminable stimuli, A and B. On half the visits, A was presented with a large drop of 50% sucrose solution and the subject fed to repletion. On the remaining visits, intermixed with those of the first kind, the subject found A, which contained a large drop of tap water; after a time, A was replaced by B, which contained a large drop of 50% sucrose solution, from which the subject fed to repletion. (A nonrewarded visit to A always was separated from a rewarded visit to A at least by a rewarded visit to B and a trip to the hive.) In extinction, one group of animals—the Partial group—was tested with A, and a second—the Consistent group—was tested with B. What we have here, of course, is a variant of the within-subjects design that has failed in many vertebrate experiments to yield a PRE, presumably because the effects of intermittent reinforcement may generalize (Brown & Logan, 1965; Pavlik, Carlton, & Hughes, 1965; Rashotte & Amsel, 1968). In the present case, the A— $\rightarrow$ B+ training may reward the animal for response to the sensory aftereffects of nonreward per se, and so increase resistance to extinction independently of target properties. We elected, nevertheless, to do the experiment on the ground that a PRE, if it occurred, could be traced unmistakably to nonrewarded experience with A and probably, therefore, to reinstatement, although it still would be necessary to rule out the possibility of control by a trace-compound—carryover from A and non-reward.

Two other features of the present experiment may be noted. One is that the number of rewards for response to the partially and consistently rewarded stimuli rather than the number of visits to each of them was the same—an equated-reinforcements design rather than the more common equated-trials design. In early massed-trials experiments with African mouthbreeders, the partial reinforce-

ment effect appeared more readily with equated reinforcements (Gonzalez, Eskin, & Bitterman, 1962), although both goldfish (Schutz & Bitterman, 1969) and the mouthbreeders (Gonzalez et al., 1965) fail to show the effect in widely spaced trials even with equated reinforcements. Another feature of the present experiment is that the total number of training visits was doubled in order to increase the opportunity for learning about non-reward.

Method

Subjects. The subjects were 16 foragers, all experimentally naive, from our laboratory hives.

Procedure. For half the animals in each group, A targets were labeled with an orange disk and the scent of jasmine and B targets were labeled with a yellow disk and the scent of lemon; the opposite was true for the remaining animals. In pretraining, the animals were placed on special targets labeled with all four components, after which there were two visits, with A and B presented successively on each and the animals taking sucrose from both. (The sucrose concentration was 50% throughout.) Then came the 24 training visits as already described. The sequence of rewarded and non-rewarded visits to A was: RNNRNNRNNR, repeated. On the first six N visits, the duration of A- exposure (before replacement with B+) was 2 min; on the last six N visits, the duration of A- exposure was increased to 3 min. All contacts with A- were recorded in the usual manner. On the 25th visit, there was a 10-min extinction test either with A, the partially rewarded stimulus, or with B, the consistently rewarded stimulus; the Partial group was extinguished with A, and the Consistent group with B. The target used in each case contained a large drop of tap water, and all contacts with it were recorded. The subjects were assigned to the two groups quasi-randomly.

Results

The performance of the animals on unrewarded A visits in acquisition continued to show a sharp decline in number of contacts with the target from the first to subsequent 30-sec intervals. Rather than even a hint of an increase in resistance to extinction during the course of training, there was somewhat the reverse, and no reason, therefore, to expect a PRE in the final extinction test. The performance of the Consistent and Partial groups in that test is plotted in Figure 4 in terms of the mean number of responses in successive 30-sec intervals, and indeed there is no suggestion of a PRE, but only an initial difference in rate of responding that favors the Consistent group. Analysis of variance yields no significant main effect [$F(1,14) = 2.16, p = .1636$], but does yield a significant interaction of groups $\times$ 2.5-min intervals [$F(3,42) = 25.45, p < .0001$], that seems simply to reflect the initial difference in level of responding.

EXPERIMENT 4

If the results of Experiment 3 are to be explained on the assumption of a strong generalized carryover effect that obscured a stimulus-specific reinstatement effect, it should be possible to show a PRE with a Partial group trained as in Experiment 3 (one stimulus partially rewarded, the other consistently rewarded) and a Consistent group for which both stimuli are consistently re-

Figure 4. The course of extinction in the Partial and Consistent groups of Experiment 3, plotted in terms of the mean number of responses in successive 30-sec intervals.

warded, since generalized and specific influences should combine to produce greater resistance in the Partial group. Experiments 1 and 2 provide such a comparison, of course, but it may be that the transitions from 50% to 20% sucrose on OJ+ visits produced a generalized increase in the resistance of both groups and so obscured the influence of differential treatment: As the animals readapted to 20% sucrose on each visit, the initially nonrewarding 20% sucrose became acceptable, rewarding the animals for response to the sensory aftereffects of nonreward in much the same way as in the earlier work on partial delay of reward (Couvillon & Bitterman, 1980).

In the present experiment, the Partial group was trained very much as were the animals of Experiment 3, with 12 visits to A containing sucrose and 12 intermixed visits on which A containing water was replaced after a time by B containing sucrose. Here, however, there was a Pure Consistent group—given 12 visits to A containing sucrose and 12 intermixed visits to B containing sucrose—that had no unrewarded experience at all. In extinction, both groups found A containing water. The manner in which the stimuli were presented in this experiment was somewhat different from that used in Experiment 3; the change, described below, was made in the hope of accentuating experience with the two targets and their contents.

Method

Subjects. The subjects were 16 foragers from our laboratory hives.

Procedure. For half the animals in each group, A was an orange target (not, as before, a gray target labeled with an orange disk) that was scented with jasmine, and B was a gray target scented with

peppermint; the opposite was true for the remaining animals in each group. Placement (with 50% sucrose) in pretraining was on a target that was half orange, half gray, and labeled with both scents, after which there was one visit to each of the two training targets (order balanced over animals) with feeding to repletion on each. In training, the sequence of the 12 rewarded and 12 nonrewarded visits to A for the Partial group was RNNRRNNRRNNR, repeated. On R visits, A was presented with a 20- μ l drop of (50%) sucrose, replaced when that sucrose was taken with another A containing 20 μ l of sucrose, and so forth, until the animal was replete and returned to the hive; three presentations usually were necessary. On the first six N visits, A was presented with 20 μ l of water, replaced after 1 min with another A containing 20 μ l of water, and then after 1 min with a series of B targets (typically, three), each containing 20 μ l of 50% sucrose, until the animal was replete and returned to the hive. On the last six N visits, there were three 1-min presentations of A with water before a series of B target presentations with sucrose. The training of the Pure Consistent group was the same as that of the Partial group, except that there were no unrewarded presentations of A. On visits corresponding to the N visits of the Partial group, the animals of the Pure Consistent group were given a series of B targets with sucrose from the outset. On the 25th visit—the extinction visit—all animals had a series of 20 1-min presentations (trials) of A targets containing water. The number of contacts with each target was recorded.

Results

The performance of the Partial animals on unrewarded A visits in acquisition was much like that in the previous experiment, giving no hint of increasing resistance to extinction and no reason, therefore, to expect a PRE. The performance of the Partial and Pure Consistent animals in extinction is plotted in Figure 5 in terms of the mean number of responses on successive trials, and again there is nothing that can be taken with any confidence to indi-

Figure 5. The course of extinction in the Partial and Pure Consistent groups of Experiment 4, plotted in terms of the mean number of responses on successive trials.

cate greater resistance in the Partial animals. The groups effect is insignificant [$F(1,14) = 1.92, p = .1873$], and the significant interaction of groups $\times$ 5-trial blocks [$F(3,42) = 7.22, p = .0005$] seems primarily to reflect a somewhat higher initial level of response in the Pure Consistent animals. These results are interesting because they show neither a general nor a stimulus-specific effect of nonreward on resistance to extinction, either of which alone might have produced a PRE.

EXPERIMENT 5

In the experiments reported thus far, the critical stimulus was rewarded on some visits and nonrewarded on others, with feeding on a different stimulus and a trip to the hive intervening between nonrewarded and subsequently rewarded presentations. The procedure used in this fifth experiment eliminated intervening trips to the hive by scheduling both nonrewarded and subsequently rewarded presentations on the same visit, separating them only by rewarded presentations of a different stimulus. The purpose was to inquire into the possibility of control by memory of more recent nonreward. That recency of nonreward is important is suggested by interpolated-trials experiments with pigeons (Couvillon et al., 1980), which show patterning effects attributable to the memory of recent but not of more remote nonreward. In general outline, Experiment 5 is a replication of Experiments 3 and 4. Two groups were trained with A partially rewarded and B consistently rewarded, after which one of the groups (the Partial group) was extinguished with A and the other (the Consistent group) was extinguished with B. A third group (the Pure Consistent group) was trained with both A and B consistently rewarded, then extinguished with A.

Method

Subjects. The subjects were 24 foragers from our laboratory hives.

Procedure. For half the animals in each group, A was an orange target scented with jasmine and B was a gray target scented with lemon; the opposite was true for the remaining animals in each group. The pretraining was done with targets that were half orange, half gray, and labeled with both scents, each animal being placed with a large drop of 50% sucrose and permitted to drink to repletion. When the animal first returned of its own accord, it found a complex target containing only a 10- μ l drop of 50% sucrose, which was replaced, as soon as the animal took the sucrose and flew off the target, by another such target, and so on until the animal was replete and returned to the hive (six such trials were typically required). After another such pretraining visit, there were 24 training visits. For the Pure Consistent group, there was a sequence of rewarded A and B trials on each of these visits. A targets and B targets were presented equally often in balanced order, for example, ABBABA...; half began with A, and half began with B. Each target contained 10 μ l of sucrose and as soon as the sucrose was taken was replaced by the next target in the sequence. For the Partial group and the Consistent group, the sequences consisted not only of rewarded A and B trials, but also of unrewarded A trials (on which the target would be put down for 1 min with 10 μ l of water instead of sucrose). These sequences were constructed with the restrictions that A- trials never be followed immediately by A+ trials (but only by B+ trials) and that there be equal numbers

of A+, A-, and B+ trials (an equated-reinforcements design): for example, B+, A-, A-, B+, A+, A+, A-, B+, A+.... The orders were such that the frequency requirements would be satisfied over visits even if a given animal tended to take only 5 drops of sucrose per visit or, which was quite unusual, only 4. If an animal was not replete (did not return to the hive) after 6 drops, again unusual, there would be another A+ or B+ target in an order that was balanced over visits. On the 25th visit, the Partial animals and the Pure Consistent animals were extinguished in a series of 20 1-min presentations of A trials containing water, and the number of contacts with each target was recorded. The Consistent animals were extinguished in the same way with B targets.

Results

In acquisition, the performance of the Partial and Consistent groups, to which animals were assigned in quasi-random fashion, was very much the same. The mean number of responses on A- trials was 10.4 for the Partial animals and 10.7 for the Consistent animals. Neither group showed any marked tendency to increase or to decrease the frequency of response on A- trials as training continued, the means for the four successive 6-visit blocks being 9.4, 11.2, 10.6, and 10.5 for the Partial animals and 10.0, 11.3, 10.9, and 10.7 for the Consistent animals.

The course of extinction in the three groups is plotted in Figures 6 and 7. Figure 6, which compares the Partial and Consistent groups, shows a difference in initial level of responding—higher in the Consistent group—

Figure 6. The course of extinction in the Partial and Consistent groups of Experiment 5, plotted in terms of the mean number of responses on successive trials.

Figure 7. The course of extinction in the Partial and Pure Consistent groups of Experiment 5, plotted in terms of the mean number of responses on successive trials.

that soon disappears. These results are much like those of Experiment 3 (Figure 4). Figure 7, which compares the Partial and Pure Consistent groups, shows no clear difference between them, either at the outset or later on. Analysis of variance based on the data for all three groups yields a significant Groups effect [$F(2,21) = 5.02$, $p = .0165$] and an insignificant interaction of groups $\times$ 5-trial blocks [$F(6,63) = 1.90$, $p = .0951$]. Subsequent analyses show a significant difference in responding between the Consistent group and the Partial group [$F(1,14) = 5.10$, $p = .0405$] and between the Consistent group and the Pure Consistent group [$F(1,14) = 8.37$, $p = .0118$] but not between the Partial group and the Pure Consistent group ($F < 1$). Given the fact that all three groups were trained at the same time and under comparable conditions, it is possible to conclude that the relatively high level of initial responding in the Consistent group (which we saw earlier in Experiment 3) is not due to consistent reward per se but to concurrent intermittently rewarded experience with another stimulus—particularly, perhaps, to the A- $\rightarrow$ B+ sequence. Interesting, too, is that the A- $\rightarrow$ B+ sequence fails again in this experiment to retard extinction of response to A. Only A- $\rightarrow$ A+ training seems to do that, as in the earlier work on partial delay (Couvillon & Bitterman, 1980), although a formal comparison of the two procedures has not yet been made.

DISCUSSION

Five experiments designed to preclude an interpretation in terms of carryover as distinct from associative reinstatement failed to show a reliable PRE in honeybees. The experimental designs may seem at first to be overly complicated, but the constraints imposed by the nature of the animal are substantial, and the data are orderly enough

to be considered seriously. The negative results need not, of course, be taken to mean that a PRE based on remembered nonreward is not to be found in honeybees, and it is possible that we will be able to find the effect after we have acquired a better understanding of the determinants of performance in our experimental situations.

Our failure here to find the PRE is reminiscent of earlier work with goldfish and African mouthbreeders, in which the conditions important for the appearance of the effect, even in massed trials without control of carryover, came to be appreciated only gradually (Gonzalez & Bitterman, 1967; Gonzalez et al., 1962; Wodinsky & Bitterman, 1959, 1960). Our failure here is reminiscent also of the difficulty of finding the PRE in classical conditioning experiments with vertebrates, where it often does not appear at all, or may be suggested only by crossover in the extinction curves, although a clear difference in overall level of responding does on occasion appear (Mackintosh, 1974). Despite the formal instrumental contingency in the present experiments with free-flying bees—the animals must land on the target to obtain food—the effective contingency may well be Pavlovian, which is to say that the landing may better be treated as a classically conditioned approach to a stimulus associated with food (cf. Grossmann, 1971). An understanding of the results for classical conditioning in vertebrates, still to be achieved, may help us to understand the results for honeybees (or the other way round).

These speculations aside, the possibility should be considered that there is no reinstatement-based PRE in honeybees, although that may seem unlikely in view of the fact that the same animals show SNC, which led us to look for it in the first place. The two phenomena, long thought to have much in common, actually have already been "dissociated" in work with preweanling rats that shows the PRE appearing at an earlier age than SNC (Chen, Gross, & Amsel, 1981). Furthermore, although it may be possible, as Chen et al. suggest, to explain their results (PRE without SNC) in terms of frustration theory (Amsel, 1958, 1962), the theory clearly permits a more straightforward explanation of the opposite results (SNC without PRE) obtained in honeybees: SNC requires only appetitive conditioning and conditioned frustration, whereas the PRE requires counterconditioned frustration as well. This argument, made by Chen et al. themselves (p. 157), is persuasive enough in our opinion to warrant concern about the role of carryover in their experiments. In any case, SNC, as it has been found in honeybees, does not require even conditioned frustration, but only unconditioned (primary) frustration generated during the (single) test. The same is true of the overlearning-extinction effect, or OEE, as found in honeybees (reduced resistance to extinction following prolonged training with sucrose of high concentration), the discovery of which prompted a search for SNC, with the full realization, of course, that the OEE might be due to reduced appetite rather than to frustration (Couvillon & Bitterman, 1980, 1984). The question of whether what looks like frustration in honey-

bees (disrupted feeding produced by downshift in concentration of sucrose) can be conditioned might well be examined independently of further work on partial reinforcement.

There even is some possibility that we can dispense entirely with the concept of frustration in honeybees defined as emotional response to unrealized expectation of reward. The OEE may prove, after all, to be a product of reduced appetite—due, that is, not to prolonged training with a highly concentrated sucrose solution, but to the feeding involved in that training. To control appetite, it may be necessary in future experiments to turn to a within-subjects procedure patterned after that used by Coate and Gardner (1965) to equate general experience in their work on the overlearning-reversal effect. The demonstration of SNC in honeybees does not suffer from failure to control nutritive level, since both the contrast and control groups were trained in exactly the same way, with a series of visits to 50% sucrose on target A alternating with visits to 20% sucrose on target B, and then tested (after the last training visit to 20% on B) either with 20% on A (contrast) or with 20% on B (control). Although the transient disruption of feeding shown by the contrast animals certainly suggests emotional response to unrealized expectation, it may have a simpler explanation. Consider a honeybee that tastes 20% sucrose soon after having tasted 20% sucrose; the new sucrose is taken readily. If, however, the honeybee has just tasted 50% sucrose, the 20% sucrose is not acceptable for a time—there is some disturbance before the animal settles down to uninterrupted feeding—which we may explain on the assumption that the 50% sucrose has changed the adaptation level (Bitterman, 1976). Finally, we come to the case of the animal that tastes 20% sucrose just after having tasted 20% sucrose, but now in the presence of a stimulus previously associated with 50% sucrose. Here, again, there is some transient disturbance, which may be due, not to comparison of the new taste with an expected taste or to any expectation of taste whatsoever, but to a direct effect of the stimulus on the adaptation level that is mediated by its association with the strong sucrose taste. Questions of this sort might not have arisen had we been able to find a reinstatement-based PRE in honeybees, but now that they have, it may be well to try to deal with them before inquiring further into the effects of partial reinforcement.

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