Short Communication

Perseveration in the Color Choices of Honeybees

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INTRODUCTION

In the course of recent efforts to develop suitable techniques for the study of short-term memory in honeybees, we have obtained some results that bear on the problem of "flower constancy," the familiar disposition of individual foragers to confine their visits to flowers of the same plant species (Grant, 1958). Where an animal has experience with the currently available alternatives, it may make an "informed" choice on the basis of such properties as caloric worth, probability of reward, or ease of handling (Waddington, 1983). In other circumstances, choice may be "uninformed," continuing to be governed by a preference that precludes experience with the alternatives. Consider, for example, the results of some experiments by Wells and Wells (1983), who studied the behavior of individual foragers given unrestricted access to artificial patches of yellow and blue flowers: From the very outset, some of their animals persistently chose yellow and some persistently chose blue, whether the rewards provided by the flowers of the two colors were the same or whether they were substantially different in quantity, quality, or probability; as the authors noted, differences in profitability could not be expected to influence the behavior of animals foraging only on flowers of one color. Such preferences may well be determined by the structure of the animal, or by its previous experience, or both, antedating exposure to the experimental situation and influencing the very first choice. Our results point to a reward mechanism that favors persistence even in the absence of an antedating preference.

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METHOD

The subjects were 16 foraging honeybees (*Apis mellifera*), all experimentally naive, from our own hives situated near the laboratory. They were assigned to two groups of eight animals each, a pilot group and an alternation group. The animals were trained individually and sequentially, each of them in a single experimental session of several hours duration.

The training situation is diagrammed in Fig. 1. It was a resined plywood enclosure, 58 cm wide, 58 cm high, and 46 cm deep, set into a laboratory window. The enclosure was open to the outside, and it was fitted with a pair of sliding Plexiglas panels through which the observer had access from the inside. The targets used were plastic cones like that sketched in Figure 2, 6.2 cm in the widest diameter, 2 cm high, and 28 ml in volume. Their inside surfaces were colored either yellow or blue. The center-to-center distances from A to B and from A to C were 27 cm; that from B to C, 16 cm.

Each subject was captured in a small matchbox on its arrival at a feeding station providing 10–15% sucrose solution, carried to the laboratory, and set down at a large drop of 50% sucrose solution in a single target at location A of the diagram. The subject was marked with a spot of colored lacquer as it fed to repletion, after which it was permitted to leave for the hive. Adapted to 50% sucrose and finding the lower concentration at the feeder less acceptable, the animal usually would come back to the laboratory after a few minutes, continuing thereafter to shuttle between the hive and the training situation as long as sucrose was provided there. If the marked animal did not return after its first placement, it was carried again to the training situation from the feeding station, where it usually could be found. When the animal did return of its own accord, there was another baited target at location A from which it fed to repletion, and the same was true on its second and third returns. The color of the placement target and of the target found on the first return was yellow for half the animals
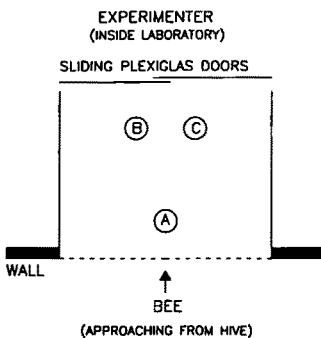

Fig. 1. Diagram of the experimental situation showing the locations (A, B, and C) at which targets were placed during training. The enclosure (58 cm in wide, 58 cm high, and 46 cm deep) was open to the outside, and it was fitted with a pair of sliding Plexiglas panels through which the observer had access from the inside. The center-to-center distances from A to B and from A to C were 27 cm; that from B to C, 16 cm.
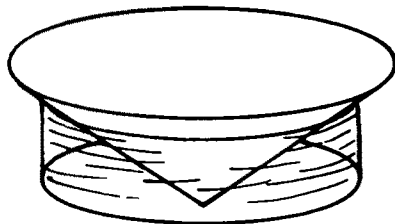
Fig. 2. Sketch of a target (6.2 cm in its widest diameter, 2 cm deep, and 28 ml in volume). Each target was a plastic cone, gray on the outside, either yellow or blue on the inside, and set in a clear cylindrical base.

(in quasi-random sequence) and blue for the others. On the second and third returns, the opposite colors were found. The targets used on each of these pretraining visits and on each of the subsequent training visits were drawn from two large sets of otherwise identical yellow and blue targets to which they were returned after washing with water at the conclusion of the visit; the purpose of the procedure (a standard one in this laboratory) is to randomize extraneous stimuli.

Alternation Group. We came to this work with an interest in what is known in the vertebrate literature as alternation or "win-shift" training—that is, in the possibility of training honeybees in a series of trials with two colors *to choose on each trial the nonrewarded color of the immediately preceding trial.* Arriving from the hive on each of 24 training visits, a subject of the alternation group found a single target—the *sample*—at location A of the diagram. The sample was yellow on 12 of the visits and blue on the rest (in two balanced, quasi-random sequences) and contained 10 μl of 50% sucrose solution. As the animal was ingesting the sucrose, two additional targets, one yellow and the other blue, were introduced at locations B and C, the geometry of the situation being such that the animal could not see the new targets or the movements of the experimenter while it was feeding. The new target of the *same* color as the sample, which was as likely to be at location B as at location C, contained a 10-μl drop of water (unacceptable to the animal and distinguishable from the sucrose only by taste), while the new target of the *opposite* color contained a 10-μl drop of 50% sucrose solution. (If the sample was yellow, the new blue target contained sucrose; if the sample was blue, the new yellow target contained sucrose). After ingesting the sucrose in the sample target, the animal would leave it to choose between the new targets, and in the event of error was free at once to correct its choice (that is, to go to the target containing sucrose). Error was defined as a full landing on the inside surface of the target containing water.

Then the first target was withdrawn, the new target containing sucrose with the animal feeding in it was moved to location A, where it served as the sample for the second trial—a technique suggested by the work of Waddington and Gottlieb (1990). Two fresh targets were placed at locations B and C, one yellow,

the other blue, with each color as likely to be in either location; the target of the same color as the second sample contained 10 μl of water, and the target of the opposite color contained 10 μl of the sucrose solution. After the animal found the target containing sucrose and was feeding in it, the target was moved to location A, where it served as the sample for the third trial, and fresh yellow and blue targets were placed at locations B and C. The training continued in this way until the animal was replete (that is, after it had taken 50 or 60 μl of sucrose) and left of its own accord for the hive, returning 4–5 min later for another series of trials. There were usually four to six trials per visit, the actual number being determined by the animal itself.

   Pilot Group. In an experiment that preceded the alternation experiment, we trained a pilot group of eight animals under the same conditions except that one of the two colors (blue for four animals selected at random, yellow for the rest) was always rewarded. If the sample target was blue, choice of blue was rewarded, after which the blue target was moved to location A, blue and yellow targets were placed at location B and C, with the blue alternative again containing sucrose. In effect, the animals were trained in a simple color discrimination under the special conditions used in the alternation experiment, the purpose being to inquire into the efficacy of those conditions. For different animals, the pilot training continued for at least three and for as many as seven visits.

RESULTS AND DISCUSSION

   The pilot experiment showed the strong color constancy to be expected from the fact that for any given subject only one of the two colors was rewarded. What was surprising, however, was that the constancy was evident from the very outset. On the first three visits for the eight subjects, with the number of trials ranging from 112 to 144, there were only three errors altogether—an error by one subject on its first trial, by a second on its second trial, and by a third subject on its fifth trial—and four subjects permitted three or four additional visits (64–72 additional trials) made no errors at all on those visits. An explanation of these results in terms of initial preferences is unlikely, because the subjects were randomly assigned to the two subgroups for which one or the other color was consistently rewarded; with seven of the eight subjects choosing correctly on the very first trial, the probability of chance correspondence between initial preferences (if any) and group assignments is less than 0.036. Instead, the preferences displayed by the animals must have developed very quickly during training, the rewarded experience with the sample preceding the first choice apparently playing a critical role. Whatever the mechanism at work here, there is little reason to suppose that it does not operate also in the field wherever there are no strong preexisting preferences.

   Performance in the alternation experiment was rather poor by comparison.
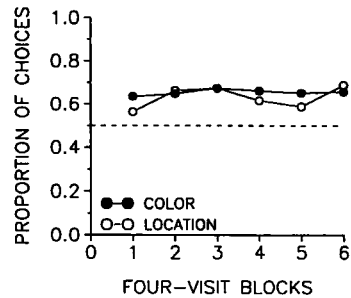
Fig. 3. Mean proportion of perseverative color choices (choices of the most recently rewarded color) and perseverative location choices (choices of the location at which reward was given most recently) in successive four-visit blocks of alternation training.

Although the animals were rewarded on each trial for choosing the nonrewarded color of the preceding trial, they showed instead a persistent preference for the rewarded color. Plotted in Fig. 3 is the mean proportion of perseverative (erroneous) color choices in each block of four visits. The proportion of such choices was greater than chance at the outset of training and showed no tendency to decline as training continued. The mean overall proportion for the eight subjects was 0.65, which differs significantly from 0.50 ($t = 6.26$ with 7 df, $P < 0.01$, two tails), and analysis of variance gives no intimation of significant change over blocks of visits [$F(5,35) < 1, P > 0.05$]. Again, we see a powerful effect of rewarded experience with the sample that is especially clear here because the perseveration in the choice of color to which it led, which was consistently rewarded in the pilot experiment, was consistently nonrewarded in this one. The perseveration is interesting because it does not seem open to conventional interpretation in terms of associative learning; experience in standard discrimination experiments (with longer intertrial intervals) suggests that preference should not be influenced so markedly by a single rewarded encounter with one of two colors after both have been frequently rewarded (Fischer *et al.*, 1993; Menzel, 1969). It is tempting to propose instead a supplementary mechanism that enhances the attractiveness of a rewarded color for a brief period following the reward— a recency or short-term memory effect.

The subjects of the alternation experiment showed substantial perseveration also in the choice of location, with respect to which neither perseveration nor alternation was differentially rewarded. Plotted in Fig. 3 along with the data for color is the mean proportion of trials in each block of four visits on which the animals went first to the location at which reward had been found on the immediately preceding trial. The proportion was greater than chance at the outset of training and showed no tendency to decline as training continued. The mean overall proportion of perseverative location choices for the eight subjects was 0.63, which differs significantly from 0.50 ($t = 6.01$ with 7 df, $P < 0.01$, two tails), and analysis of variance gives no indication of significant change over blocks of visits [$F(5,35) < 1, P > 0.05$]. The two perseverative tendencies

(for color and location) appeared to summate (increasing the probability of error) when, on any trial, the two colors were in the same locations as on the preceding trial and to counteract each other (decreasing the probability of error) when the locations of the two colors were interchanged. It is interesting to note that there have been many studies of short-term memory in rats in circumstances that capitalize on the long-known reluctance of rats to return to the most recently visited location (Wingfield and Dennis, 1934), and a like reluctance in honeybees has recently been reported by Brown and Demas (1994), whose experiments, however, are open to serious criticism (Burmeister *et al.*, 1995). We have found the opposite—a preference for the most recently visited location.

Perseveration in the choice of location is not observed in the field, where the alternatives are greater in number and less well defined. Foraging honeybees tend to move from one flower to the next in a straight line (Waddington, 1980) and return to a recently visited location is discouraged in any case by the presence of the depleted flower, which may be marked with an aversive scent (Giurfa and Núñez, 1992; Núñez, 1967); possible influences of marking were avoided here by the use of fresh targets on each choice trial. Whether or not the location effect plays any role at all in the field, it does provide an interesting parallel to the color effect, which it puts into useful perspective. In this experiment, the color rewarded on any given trial remained after the reward. As far as we now can tell, therefore, the color effect may depend entirely on the continued presence of the sample color (as a stimulus) at the time of choice, but the location effect—with the animal away from the previously rewarded place at the time of choice—seems indisputably mnemonic. For further work on short-term memory for color, it will be necessary to modify the training situation in such a way as to remove the sample before the choice can be made and to permit (in location as well as in color experiments) exact control of the time interval between the experience to be remembered and the opportunity for choice.

ACKNOWLEDGMENT

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