Delayed Alternation in Honeybees (Apis mellifera)

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Previous experiments with honeybees (Apis mellifera) failed to show learned control of performance by short-term memory. In this study, honeybees were trained with an improved technique to choose 1 of 2 colors that was either the same as a recently rewarded sample (perseveration) or different (alteration). Because any increase in associative strength stemming from the sample experience would tend to promote perseveration and contravene alteration, the equal difficulty of the 2 tasks suggests that the role played by the sample was primarily discriminative. The animals remembered on each trial the immediately preceding experience with reward and learned to use that information appropriately. These new results extend the list of what may well be fundamental similarities in the learning of vertebrates and honeybees.

The performance of honeybees in learning experiments patterned after experiments with vertebrates has been found to be similar in many respects to the performance of the vertebrates (Bitterman, 1988, 1996). Some of the similarities have been probed in further experiments, with results that point (despite the remoteness of the evolutionary relation and the vast differences in brain size and structure) to the operation of common functional principles. For example, the overlearning–extinction effect in honeybees (Couvillon & Bitterman, 1980) seems to be a special case of negative-incentive contrast (Couvillon & Bitterman, 1984; Shiroyama & Bitterman, 1987), attributable (as in rats) to frustration generated by unrealized anticipation of reward (Amsel, 1958). There are also some differences in the results for honeybees and vertebrates that, like the similarities, call for further analysis. The experiment reported here follows up on recent failures to find evidence in honeybees of the learned control of performance by short-term memory known in a variety of vertebrate species.

In experiments by Brown and Demas (1994; Demas & Brown, 1995), honeybees were studied in an analogue of the radial maze designed by Olton and Samuelson (1976) for work on short-term memory in rats. Confronted on each trial with an array of six baited targets distinguished by adjacent colored markers, the honeybees showed a tendency to avoid targets visited earlier in the trial, from which it was concluded that the control of performance by short-term or working memory had been found also in honeybees. The conclusion was difficult, however, to accept in the light of the substantial shortcomings of the technique used, which are considered in detail by Burmeister, Couvillon, and Bitterman, (1995, pp. 373–374). In subsequent experiments not open to the same criticisms, Burmeister et al. used arrays of three or four targets and found only some rather marked position biases that might conceivably have masked any short-term memory effects. They suggested that subsequent work be done in even simpler situations, analogues of the two-choice situations in which the reluctance of rats to return to recently visited locations was first clearly established (e.g., Glanzer, 1953; Montgomery, 1952).

Ohyama, Couvillon, and Bitterman (1995) trained individual foragers with two targets different in color, rewarding them on each trial for choosing the nonrewarded color of the immediately preceding trial. The training situation was a modified laboratory window, on the sill of which colored plastic targets were presented in a simple triangular pattern like that diagrammed in Figure 1. Arriving from the hive on each of 24 training visits, the subject found a single target—the sample—at Position A. The sample target, either yellow or blue, contained a 10-μl drop of 50% (w/v) sucrose solution. As the subject was ingesting the sucrose, two additional targets, one yellow and the other blue, were introduced at Positions B and C; the geometry of the situation and the structure of the (conical) targets were such that the subject could not see the new targets or the movements of the experimenter while it was feeding in the sample target. The new target of the same color as the sample, which was as likely to be at Position B as at Position C, contained a 10-μl drop of water, whereas the new target of the opposite color contained a 10-μl drop of 50% sucrose solution. The purpose of the water (unacceptable to the animal and distinguishable from the sucrose only by taste) was to make it impossible for the animal to choose the correct target on the basis of the drop of sucrose it contained. The inability to discriminate except by taste is well documented by the results of a variety of experiments; among them, for example, is an experiment on probability learning by Fischer, Couvillon, and Bitterman (1993) in which honeybees continued for many trials to perform at the chance level in a 50–50 problem, each of two targets containing a drop of sucrose on a random half of the trials and a drop of water on the rest.

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 (i.e., to go to the target containing sucrose). Then the sample target was withdrawn.
and the new target containing sucrose with the animal feeding in it was moved to Position A, where it served as the sample for the second trial—a technique suggested by the work on amount of reward by Waddington and Gottlieb (1990). Two fresh targets were placed at Positions B and C, one yellow and 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 Position A, where it served as the sample for the third trial, and fresh yellow and blue targets were placed at Positions B and C. The training continued in this way until the animal was replete (i.e., after it had ingested about 100 µl of sucrose solution and the other containing 10 µl of water. After the animal found the sucrose in one of the targets, the target with the animal feeding in it was moved to Position A, and fresh blue and yellow targets were introduced at B and C for a second choice trial; the correct target now providing enough sucrose solution to fill the crop and prompt return to the hive. The locus of reward on the second trial, which constituted a test of the subject’s ability both to retain that information in the short interval between trials and to learn to use it appropriately. Here again, small (summate) tendencies to persevere in the choice of color and position were evident at the outset and unaltered by differential reward, whether for perseveration or for alternation.

As noted by Ohayma et al. (1995), a limiting feature of the procedure used in these experiments is the continued presence of the target in which the subject has just been rewarded as it makes each choice. When response to color is differentially rewarded, there need be no memory of color at all but only of the preceding reward in that color, although when response to position is differentially rewarded, as by Isnec et al. (1997), memory of position is required because the sample is not in the previously rewarded position. It is necessary also to consider the possibility that the mere movement of the target from B or C to the sample position is disruptive. A first step toward improving the technique was taken by Brown et al. (1998), who used, not colored targets, but clear plastic petri dishes set on the surface of a horizontal video monitor and illuminated from below with computer-generated colors; each trial began with the illumination of the sample, which was turned off as the animal left it, and the two alternatives only then turned on. Even so, the results were the same as before: Whether rewarded for perseveration or for alternation, the animals showed a small but significant tendency to perseverate in the choice of color that was unaltered over 50 training trials.

In the experiment reported here, we continued to explore the possibilities of the video technique, which we modified by eliminating the cumbersome petri dishes and training the animals to choose between disks of light on the horizontal surface of a flat monitor. We also modified the training procedure. Brown et al. (1998) used a noncorrection method, following each erroneous choice with another trial after a 10-s interval, which meant that some intertrial intervals were long (after each correct choice, the subject, having fed to repletion on the large drop of sucrose solution given as reward, would return to the hive to deposit it) and others were short (a new trial beginning 10 s after each incorrect choice). Out of interest in minimizing proactive interference, we used a pure spaced-trials procedure (a single trial per visit). After an incorrect choice, the subject was free to go to the correct alternative and feed to repletion, which meant that a visit to the hive separated each trial from the next one; each choice directly followed experience with a sample found on the animal’s return.

***Figure 1.*** Diagram of the experimental situation. The enclosure (58 cm wide × 58 cm high × 56 cm deep), set into a laboratory window, was open to the outside. It was fitted with a pair of sliding Plexiglas panels through which the observer had access from the inside. Set horizontally in the floor of the enclosure was a 15-in. NEC flat-screen video monitor. Disks of computer-generated colored light, 6 cm in diameter, that served as targets could be introduced at Positions A, B, and C. The (center-to-center) distances were 18.5 cm between A (the sample) and B or C and 19 cm between B and C.
from the hive. Some animals were rewarded for perseverance and others were rewarded for alternation.

**Method**

**Subjects**

The subjects were 33 foraging honeybees (Apis mellifera), all of them experimentally naive, from our own hives situated near the laboratory. They were selected at random for a perseveration group, two alternation groups, and a control group. There were 8 animals in each group, except the first alternation group, in which there were 9. The animals in each group were trained individually and sequentially, each in a single session of several hours duration.

**Apparatus**

The training situation, diagrammed in Figure 1, was a resined plywood enclosure, 58 cm wide, 58 cm high, and 56 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 to the enclosure from inside the laboratory. Set into the floor of the enclosure and level with its surface was a 15-in. NEC flat-screen video monitor (NEC, Tokyo, Japan) on which the 6-cm disks of computer-generated colored light that served as targets could be introduced at Positions A, B, and C. The (center-to-center) distances were 18.5 cm between A (the sample) and B or C and 19 cm between B and C. The colors (the same used by Brown et al., 1998) were green (VGA No. 2) and violet (No. 5).

**Pretraining**

Each animal was captured in a matchbox on its arrival at a nearby feeding station providing 10%–15% sucrose solution, carried to the laboratory, and set down at a large (100-μl) drop of 50% sucrose solution centered on a target projected at Position A. One half (180°) of the target was green, and the other was violet. The animal was marked with a spot of colored lacquer as it fed to repletion, after which it returned to the hive to deposit the sucrose. Normally, the animal would come back to the laboratory several minutes later, continuing thereafter to shuttle between the hive and the training situation as long as sucrose was provided there. If it did not come back after its first placement, it was carried again to the hive and the training situation as long as sucrose was provided there. If the subject went first to the target containing water, it was free at once to make the drop of solution first tasted by the animal; if the first contact was with the incorrect color, the sample contained water and the other contained sucrose; that is, choice of the color different from the sample color was consistently rewarded. For the control group, neither perseverance nor alternation was differentially rewarded; again, the sample was green on half the trials and violet on the rest, with choice of the same color rewarded quasi-randomly on half the trials and the alternative color on the rest.

As in our previous experiments with plastic targets, we began with two measures of choice on each trial. One was based on the target with which the subject first made contact; if the first contact was with the incorrect target, a target error was recorded. The second measure was based on the drop of solution first tasted by the animal; if the first contact was with the water—contact with the water elicits a distinctive withdrawal response—a drinking error was recorded. It soon became evident, however, that the target measure was not useful in the video situation.

**Results**

**Perseveration Group**

In the left-hand portion of Figure 2, the performance of the perseveration group is plotted in terms of the mean proportion of initially correct choice in each of five successive blocks of 4 training visits. The curve based on the drinking measure provides clear evidence of learning to perseverate, but the curve based on the target measure does not. Analysis of variance (ANOVA) yielded a significant measure effect, $F(1, 7) = 29.88, p < .05$ (the alpha level used throughout), with an insignificant block effect ($F < 1$) and a significant Measure × Block interaction, $F(4, 28) = 3.74$. The proportion of correct choice in the 20 training trials was significantly greater than chance for the drinking measure. .71, $t(7) = 6.30, SE = 0.03$, but not for the target measure, .49, $t(7) = 0.21, SE = 0.03$. Improvement with continued training is suggested by an analysis based on drinking errors alone, which yielded a significant block effect, $F(4, 28) = 3.24$.

In our previous experiments with plastic targets—not only experiments on short-term memory but many other discrimination experiments in which choice training was given—the target and drinking measures were highly correlated, and our standard prac-
tice was simply to report the first of them; target errors, which reflected the animal’s first evaluation of the stimuli on each choice trial, were easier to describe, and intuitively somewhat more meaningful. Behavior in the video monitor situation was different, however, than it was when plastic targets were used. In the short-term memory experiments with plastic targets, the animal would fly up from the sample after ingesting the small drop of sucrose solution it provided and then hover for a time over the alternatives, as though making a comparison, before landing on one of them. In the monitor situation, the animal did not fly up from the sample but would walk rapidly away on the surface of the monitor in a seemingly random direction, giving no indication that the alternatives were being compared. Variations of the technique designed to encourage flying up from the sample before choice were unsuccessful; when, for example, a barrier was introduced between the locations of the sample and the alternatives, the animal would walk up over it and down the other side. The behavior suggested that only after making contact with one of the alternatives did the animal decide whether or not to approach the drop of solution it contained. In any case, evidence of discrimination is provided here only by the drinking measure.

**Alternation Groups**

In the right-hand portion of Figure 2, with drinking error as the criterion, the performance of the two alternation groups is plotted in terms of the mean proportion of initially correct choice in each of five successive blocks of 4 training visits. The results for the first group give clear evidence of learning to alternate; an ANOVA yielded a significant block effect, $F(4, 32) = 3.79$, and the proportion of correct choice in the 20 training trials was significantly greater than chance, $t(8) = 2.83, SE = 0.03$. The results for the second group—which was added because of the novelty and special importance of the positive results for the first group and because the exclusive reliance on drinking errors was not contemplated at the outset of the work—replicate the results for the first; an ANOVA yielded a significant block effect, $F(4, 28) = 2.84$, and the proportion of correct choice in the 20 training trials was significantly greater than chance, $t(7) = 4.62, SE = 0.03$.

**Control Group**

Although the hypothesis that our animals could distinguish between drops of sucrose and water without tasting them had been rejected repeatedly in work with plastic targets, it seemed a sensible precaution here to test the hypothesis anew with transilluminated drops presented on the surface of a video monitor. In Figure 3, with drinking error as the criterion, the performance of the control group is plotted in terms of the proportion of initially correct choice on each of the 20 training visits. An ANOVA, again over five 4-visit blocks, yielded an insignificant block effect, $F(4, 28) = 1.77$, and the proportion of correct choice in the 20 trials did not differ significantly from chance, $t(7) = 1.32, SE = 0.04$. For purposes of comparison, the trial-by-trial performance of the perseveration group and the pooled alternation groups is plotted in Figure 4. With differential reward either for perseveration or for alternation, performance began at about the chance level and improved gradually in much the same way in the two problems as training continued.

**Discussion**

These new results differ from the earlier ones in the absence of an initial tendency to perseverate. At the outset of training, the animals showed no preference here either for the most recently rewarded color or for the alternative color. Another difference is that the animals could be trained to choose either the most recently encountered color or the alternative color, the equal difficulty of the two problems presumably reflecting the absence of an initial tendency to perseverate or to alternate. The results for both problems point to learned control of performance by short-term memory, but those for alternation are especially instructive. Although increase in the associative strength of the rewarded sample color would tend to facilitate perseveration and to contravene alternation (cf. Brown et al., 1998, on perseveration in honeybees, and Bailey & Mazur, 1990, on “positive recency” in pigeons), the honeybees learned to do both with equal facility, which suggests that the role played by the sample experience was primarily discriminative.

A simple associative theory of both perseveration and alternation in honeybees is suggested by work on conditional discrimination (Couvillon & Bitterman, 1987, 1988). Honeybees can be trained, for example, to choose a green rather than a blue target.

![Figure 3. Performance of the control group plotted in terms of the proportion of initially correct choice on each of the 20 training visits (drinking measure).](image1)

![Figure 4. Performance of the perseveration group and the pooled alternation groups plotted in terms of the proportion of initially correct choice on each of the 20 training visits (drinking measure).](image2)
when both targets are scented with geraniol but a blue instead of a green target when both are scented with peppermint, and what the animals seem to learn in such training, whether with odor or color as the conditional dimension, is to respond differentially to the four color–odor compounds. The performance of the animals in such experiments has been modeled quantitatively and with considerable precision on two commonplace assumptions. First is that the attractiveness of each alternative is given by the strength of its association with sucrose (which increases with reward and decreases with nonreward), and second is that choice is a function of relative associative strength. With traces or short-term memories of the sample colors in the conditional role, learning to choose between the offset of the sample and the opportunity for choice, a purpose that might be served by restraint in a small compartment between the offset of the sample and the opportunity for choice, a purpose that might be served by restraint in a small compartment such as used by Grossmann (1971) to study delay of reward. It would be desirable as well to find a way to focalize the process of choice as it is focalized when plastic targets are used, perhaps in renewed efforts to encourage flying up from the sample. Honeybees can be trained to fly from a sample on a monitor in one window to choose between two colors on a monitor in an adjacent window, but the delays are long and unacceptably variable. Delays in previous work with plastic targets also were longer than in the present experiment, and it is conceivable that the difference in delay was at least partially responsible for the difference in the results obtained. For further progress, it may be reasonable to work with foragers confined temporarily in situations like those developed by Sigurdson (1981). In any case, the technique even as developed thus far seems to have uncovered another fundamental similarity in the learning of honeybees and vertebrates.


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