Evidence for Spatial Working Memory in Honeybees (Apis mellifera)

Michael F. Brown and Gregory E. Demas

Spatial working memory (the ability to represent multiple locations in a flexible, dynamic manner) has been studied in a range of vertebrate species. The results of 3 experiments indicate that this ability also exists in at least one invertebrate (honeybees; Apis mellifera).

Individual honeybees collected sugar solution from a matrix of 6 locations. They avoided revisits to locations previously depleted of solution more accurately than expected by chance. The results rule out several nonmemorial explanations for this ability, and it is therefore best explained by a spatial working memory system that allows discrimination of previously visited locations from those not yet visited. These results substantially expand the range of species in which spatial working memory has been demonstrated.

According to Baddeley (1986), the term working memory, as it has been used in the study of human memory, "implies a system for the temporary holding and manipulation of information during the performance of a range of cognitive tasks" (p. 34). In the animal memory literature, working memory has been similarly defined as memory for information that is temporary in use and changing in content, such as the memory used in discrete-trials memory paradigms (Honig, 1978). In vertebrate animals an ability for high-capacity, robust working memory has been found in the context of spatial food-gathering tasks. Rats (Olton & Samuelson, 1976), several species of birds (Kamil & Balda, 1985; Roberts & Van Veldhuizen, 1985; Sherry, 1984; Shettleworth & Krebs, 1986; Sperelakis & Edwards, 1986), and Siamese fighting fish (Roitblat, Tham, & Golub, 1982), among others, have been found to discriminate locations where food has already been depleted from locations where food remains to be gathered by using working memory. The seminaturalistic experiments that we report present evidence for working memory ability in honeybees.

The most extensive investigations of animal working memory in the context of spatial tasks have been carried out with rats in the radial-arm maze paradigm (Olton & Samuelson, 1976). Rats gather a small amount of food from each of a number of spatial locations (typically 8 or 12). They rapidly acquire the ability to avoid revisiting locations that they have already visited by using working memory for the visual cues that correspond to the locations (Suzuki, Augerinos, & Black, 1980; Zoladek & Roberts, 1978). This spatial working memory has been shown to have a duration of at least several hours (Beatty & Shavalia, 1980) and is surprisingly resistant to retroactive and proactive interference manipulations that are very disruptive in most animal and human memory paradigms (Cook & Brown, 1985; Olton, 1978; Roberts, 1981). Physiological manipulations and measures indicate that the hippocampus is critically involved in spatial working memory performance in rats (Kesner, 1986; McNaughton, 1989; O’Keefe & Nadel, 1978; Olton & Pappas, 1979).

The flexibility required by a working memory task is one of its defining features. Olton (1978) found that rats performed very accurately in eight successive trials on the same radial-arm maze. That is, the rat had to choose from among the eight arms and, after the arms were rebaited by the experimenter, choose from among them again, with this process repeated for a total of eight trials. The fact that rats were able to avoid revisits within each trial yet return to arms from one trial to the next, led Olton to propose that working memory was reset at the beginning of each trial. Considerations like these have led to the view that working memory is a memory structure that is separate from long-term or reference memory. The function of working memory is to temporarily hold a small amount of information, the utility of which is limited to the behavior (or cognitive task) that is currently being carried out (e.g., Honig, 1978).

Among invertebrates the learning and memory abilities and mechanisms of bees have probably received at least as much attention as any group. Extensive investigations of associative learning in honeybees by Couvillon and Bitterman have indicated a number of important commonalities with vertebrates (e.g., Bitterman, 1988; Couvillon & Bitterman, 1982, 1986, 1987). A number of behavioral biologists have also studied learning and memory in honeybees. These have included investigations of form (shape) learning (Gould, 1985, 1986b), color learning (Menzel, 1979), memory for spatial relations among objects (i.e., landmarks) and food sources (Cartwright & Collett, 1982; Gould, 1987), and the nature of the representations that control large-scale spatial performance (Dyer, 1991; in press; Gould, 1986a).
Menzel has provided extensive data about short-term memory in bees (Menzel, 1979, 1990; Menzel, Hammer, Braun, Mauelshagen, & Sugaw, 1991). These studies have been concerned with the dynamic changes in behavioral effects that occur after experience and the memory-consolidation processes that underlie those changes. Particularly relevant for our research is a recent report by Greggers and Menzel (1993), who studied the behavior of honeybees foraging from among four artificial “flowers.” The flow rate of the sucrose solution provided by the four feeders, among other variables, was manipulated in order to determine the extent to which choice of feeder was matched to its flow rate. Greggers and Menzel found that feeders with higher flow rates were visited more often than those with lower flow rates. The theoretical model that they developed to explain the behavior of their bees included two memory processes, one that represents the associative strength of each feeder and a second that represents the associative strength of the patch of all the feeders. Thus, the theoretical conclusions of Greggers and Menzel rest on the foundation of associative learning processes and involve feeder-specific memories that consist of values of associative strength that change when a feeder is visited. On the other hand, our concern is with a working memory structure conceived of as holding discrete representations of multiple locations, wherein the identity of those representations varies according to the identity of locations that have been visited at any particular point in an experimental trial. Thus, although the experimental procedure and some aspects of the theoretical analysis used by Greggers and Menzel are similar to those used in our experiments, the analytic techniques used by them to interpret their data are aimed at a different set of questions. As a result, it is unclear whether their bees avoided revisits to recently visited locations.

Thus, no previous investigation with honeybees (or any other invertebrate) has been explicitly concerned with the existence of a flexible, dynamic, multiple-item memory system of the sort corresponding to the working memory that has been studied in vertebrates. On the other hand, the ecology of honeybee foraging suggests that such a working memory system is of functional value. Honeybees visit hundreds of flowers during each of numerous daily foraging trips (Seeley, 1985; Winston, 1987). Furthermore, when a nectar-rich patch of flowers is encountered during a foraging trip, bees tend to change flight directions from flower visit to flower visit, thereby producing the effect of lingering in the profitable patch (Heinrich, 1979; Pyke, 1978; Waddington, 1980; Waddington & Heinrich, 1981). Given that individual flowers tend to be depleted of nectar during a single visit (Pleasants, 1981), it is to bees’ advantage to avoid revisits to recently visited flowers. Similar logic motivated an earlier study of spatial working memory for flower visits in a nectar-feeding bird (Kamil, 1978).

Experiment 1

The first experiment was designed as a seminaturalistic analog of radial-arm maze studies. Individual bees repeatedly visited an apparatus of six cells baited with sugar solution. Because cells were depleted of solution by bees during a single entry, the contingencies encouraged bees to enter each cell only once during each visit to the apparatus. This arrangement was designed to correspond to the rat’s visiting each arm of a radial-arm maze only once during each trial. The goal of Experiment 1 was to determine whether bees, like rats, avoid revisits to locations that have been depleted.

It has clearly been shown that rats avoid such revisits in the radial-arm maze by using a flexible, dynamic, multiple-location memory system (i.e., spatial working memory). A number of nonmemorial explanations for the avoidance of revisits have been repeatedly ruled out. The design of Experiment 1 allowed us to test two such alternatives for any avoidance of revisits by bees. First, our estimate of chance performance, to which the bees’ performance was compared, controls for avoidance of revisits that is based on the movement pattern of the bee as it travels from location to location. Systematicity in the movement patterns of bees during natural foraging has been shown to affect their foraging efficiency (Heinrich, 1979; Pyke, 1978; Waddington, 1980; Waddington & Heinrich, 1981) and may similarly affect the probability of revisits in this experimental situation. Second, it is possible that bees may simply perceptually discriminate (on the basis of sight or odor) the presence of sugar solution in the cells. This was tested in probe trials during which only a subset of the cells were baited with solution.

Method

Subjects. The subjects were 15 honeybees (Apis mellifera) from two standard beehives maintained on the Villanova University campus.

Apparatus. The apparatus consisted of the 2 × 3 matrix of holes in a 28 × 28 cm (6 mm thick) plywood surface shown in the top panel of Figure 1. The holes were 1.1 cm in diameter. The two columns of three holes were separated by 14.2 cm (center to center); the columns were 22.7 cm long. The middle holes were staggered, with one closer to the topmost hole and the other closer to the bottom hole; each was 9.8 cm from the closest hole. The plywood surface was painted violet. Underneath each hole was a clear polyurethane cylindrical cell (one cell from a Corning [Corning, NY] 25820 tissue culture tray; 18 mm deep and 15 mm in diameter), open end up. Six irregularly shaped and colored objects, intended to serve as spatial landmarks, were located on the surface as shown in Figure 1.

This apparatus was located inside a 34.4 cm wide × 18.8 cm high × 52.5 cm long clear Nalgene container with a removable clear Flexiglas lid. This allowed us to control access to the apparatus, so that only 1 bee interacted with the apparatus at a time. The apparatus was located approximately 13 m from the two hives. It was 5 m away from a feeding station, which was sometimes baited with 50% (volume/volume) sugar solution and was painted the same violet color as the apparatus surface. Cells were baited with either a microsyringe or an eyedropper. Trials were videotaped.

Procedure. The bees were initially recruited to the apparatus by transporting them to it from the feeder and allowing them to feed to repletion on the apparatus surface. During this first visit to the apparatus, each bee was marked with paint to allow identifi-
analyses). When these conditions were met, the lid was opened, and the bee left the apparatus. Such a sequence of cell visits constituted a trial. Typically, a bee left the apparatus for approximately 5 min before returning for the next trial.

Each bee was allowed to return to the apparatus for up to 30 completed trials. After these 30 trials, the 6 bees that completed the experiment last were allowed to return to the apparatus for five additional probe trials. Before each of these trials, a randomly selected set of three of the six cells were baited. Other aspects of these probe trials were identical to the earlier trials.

It required from 1 to 3 days (Mdn = 2) for each bee to complete the experiment. Data were collected in this and the following experiments during late July, August, and September, when bees are active but there is a dearth of natural nectar sources in the Villanova, Pennsylvania, area.

Results and Discussion

Fifteen bees that completed at least 26 trials were considered to have completed the experiment and were included in the data analysis. Figure 2 shows mean performance over the course of three blocks of 10 trials each (for the 8 bees that completed fewer than 30 trials, means for the last block are computed for fewer than 10 trials). Performance is measured in terms of the number of correct choices (visits to previously unvisited cells) during the first six choices and the number of choices made before all six cells had been visited. These performance measures correspond to those typically used in studies with the radial-arm maze.

Two estimates of chance performance are shown for comparison. The strict estimate assumes random selection from among the six alternatives during each choice. The modified estimate is designed to take into account the possibility that systematicity in the cell-to-cell movement pattern of a bee may lower the probability of revisits, just as it has been argued that the movement patterns of foraging bees in nature reduce the probability of flower revisits (Pyke, 1978; Waddington, 1980; Waddington & Heinrich, 1981). To test this, the data for individual bees in each block of trials were used to construct matrices of transition probabilities. Given that a bee was in each of the six cells or had just started a trial and not yet visited a cell, the probability of moving from that cell or from trial entry to each of the six cells was determined. The overall transition probability matrix is shown in Table 1. The matrices for individual bees in each block of trials were used in Monte Carlo simulations in which cells were chosen with the same transition probabilities. Any tendency to avoid revisits to cells beyond what is shown by these simulations cannot be accounted for by systematicity in the cell-to-cell movement patterns of the bees.

The algorithm used to implement the Monte Carlo simulations chose randomly from among six alternatives (strict estimate) or used the cell-to-cell transition probabilities of individual subjects during individual trial blocks (modified estimate) until all six had been chosen. The strict estimate is based on 1,000 simulations. The modified estimates are based on 1,000 simulations based on each bee's transition matrix during each of the three trial blocks. If systematicity

**Figure 1.** The apparatus used in Experiment 1 (top panel) and one of two identical apparatus used in Experiments 2 and 3 (bottom panel). (For comparison with the data in Table 1, the holes are numbered starting with the top row, from left to right. The colors of the landmarks on the apparatus used in Experiments 2 and 3 are blue, green, orange, red, yellow, and white for the landmarks adjacent to Holes 1–6, respectively.)
in the movement patterns of bees can account for the tendency to avoid revisits without memory for the identity of previously visited cells, then the simulations ought to perform as well as the bees. The modified estimates of chance shown in Figure 2 reflect the mean performance of the simulations.

Two Data Source (bees vs. simulations) × Trial Block repeated measures analyses of variance (ANOVAs) were used to compare the performance of the bees with the modified estimates of chance provided by the simulations. In terms of the number of correct visits made during the first six choices, the bees chose more accurately than expected on the basis of their individual transition matrices, F(1, 14) = 41.8, p < .001. Furthermore, the difference between the number of correct visits made by the bees and chance performance increased over trial blocks, as shown by a significant interaction term, F(2, 28) = 5.1, p < .01. Similarly, in terms of the number of visits required to visit all six cells, the bees required fewer visits than expected, F(1, 14) = 133.6, p < .001. The difference between the number of visits required by the bees and chance performance increased over trials, F(2, 28) = 4.2, p < .05.

We note that the comparison of bees' performance to these simulations may result in a conservative estimate of the contribution of working memory to the avoidance of cell revisits. This is because any tendency to avoid a revisit to a just-visited cell is included in the transition probability matrix and is therefore reflected in the modified estimate of chance performance. It is possible, however, that at least part of such a tendency is based on working memory.

One explanation for the above-chance choice accuracy is that the bees simply perceptually discriminate baited and unbaited cells (on the basis of odor or sight). This possibility was tested during the five probe trials that followed the primary experiment. If an ability to directly discriminate the presence of sugar solution in unvisited cells was responsible for performance in the primary experiment, then the unbaited cells ought to have been visited later in the choice sequence than the baited cells. For each probe trial, the initial visit to each cell was ranked according to its ordinal position among the six initial cell visits. If unbaited cells can be discriminated and are therefore less likely to be visited, the mean rank of unbaited cells ought to be greater than the expected chance value of 3.50. The mean rank for unbaited cells was 3.35. Thus, there is no evidence that the physical presence of sugar solution increased the tendency of the bees to visit cells.

Experiment 2

The results of Experiment 1 demonstrate that honeybees avoid revisits to locations recently depleted of sugar solution and that this avoidance cannot be explained by systematicity in the movement of bees from location to location.

<table>
<thead>
<tr>
<th>Previous location</th>
<th>Destination cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Begin trial</td>
<td>18.2</td>
</tr>
<tr>
<td>Cell 1</td>
<td>5.0</td>
</tr>
<tr>
<td>Cell 2</td>
<td>28.6</td>
</tr>
<tr>
<td>Cell 3</td>
<td>29.2</td>
</tr>
<tr>
<td>Cell 4</td>
<td>12.6</td>
</tr>
<tr>
<td>Cell 5</td>
<td>16.9</td>
</tr>
<tr>
<td>Cell 6</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Note. Each row shows the percentage of transitions (cell-to-cell movements) to each of the six cells, given the previous location of the bee (either the cell chosen last or initial entry into the apparatus for a new trial). Data are collapsed across bees and trial blocks.
or by an ability to perceptually discriminate the presence of the solution. However, a remaining possibility is that a physical remnant of a bee’s visit to a cell can be used as a discriminative cue that the cell has been previously visited. The most obvious version of this general explanation is that an odor left by the bee allows previously visited cells to be discriminated from those that have not been visited.

There is evidence that bees leave an odor mark on flowers during natural foraging. Such odors have most often been thought to serve as attractants (Ferguson & Free, 1979; Free & Williams, 1983; von Frisch, 1967). Attractant odors, of course, may work against the phenomenon demonstrated by Experiment 1. However, there has also been discussion of a so-called footprint pheromone that may serve as a repellent to mark flowers already depleted of nectar (Corbet, Kerslake, Brown, & Morland, 1984, for bumblebees; Free & Williams, 1983; Giurfa & Núñez, 1992; Wetherwax, 1996; see Free, 1987, for a review).

The clearest evidence for this possibility comes from the recent experiment by Giurfa and Núñez (1992). They studied bees foraging on an artificial patch of flowers similar to the apparatus used in these experiments. They used an air extraction system to remove odor from the artificial flowers and found that bees avoided revisits to a greater extent when the system was not activated than when it was.

A repellent odor mark of the sort indicated by Giurfa and Núñez’s (1992) data may fully or partially explain the results of Experiment 1. Experiment 2 was undertaken to determine if the results of Experiment 1 could be replicated under conditions that did not allow any influence of odor marks on performance. To this end, a bee was first allowed to visit three cells of an apparatus very similar to that used in Experiment 1, and then it was captured. The apparatus was replaced with a second, identical one baited just as the first apparatus was baited after a bee’s third cell visit. Choices made on this second apparatus could not be affected by any odor left during the first choice visits. Performance measures were designed to measure any tendency of the bees to avoid revisits to cells corresponding to the three cells visited on the first apparatus.

**Results and Discussion**

Unlike bees in Experiment 1, the bees in this experiment often failed to visit all the baited cells before ceasing to visit cells. Specifically, bees failed to make a second choice on the second apparatus during 19% of the trials, and failed to make a third choice during 40% of the trials. Although we cannot determine the explanation for this difference, one possibility is that the vial containment procedure disrupted behavior in a manner that resulted in fewer cell visits.

As in Experiment 1, choice accuracy was measured in relation to a measure of chance, provided by Monte Carlo simulation, which controlled for any systematicity in the cell-to-cell movement patterns of the bees. However, in Experiment 2, the measure was restricted so that only the ability of bees to avoid revisits to cells visited on the first apparatus during choices made on the second apparatus was considered. In addition, the measure had to accommodate the fact that bees did not always visit all baited cells.

The data were analyzed in two blocks of 12 trials each to allow changes in performance over the course of trials to be detected. As in Experiment 1, the data for individual bees in each block of trials were then used to construct matrices of transition probabilities. The transitions included were only those that occurred on the second apparatus. Given that the bee was in each of the six cells (or the apparatus had just been switched and the bee was starting from the vial), the probability of moving from that cell (or from the vial) to each of the six cells was determined. For each trial of each bee, 100 simulated trials were used to estimate the choice accuracy expected on the basis of these transition probability matrices. In each simulated trial the algorithm first visited the same three cells visited by the bee on the first apparatus. The algorithm then chose from among cells, by using the same transition probabilities as the bee during the relevant trial block, until the same number of choices made by the bee were made by the algorithm.
Figure 3 shows the mean probability of a correct choice during the first three choices on the second apparatus for the bees and the algorithm. A correct choice is defined as a visit to a cell on the second apparatus that corresponded to one that was not visited on the first apparatus. Revisits to cells already visited on the second apparatus were not included in the calculation of these probabilities (for the bees or the simulations), and therefore any tendency to avoid revisits to cells where an odor mark may have been present is not included in the analysis. Performance measures were restricted to the first three choices made on the second apparatus because additional choices were very rare among some of the bees. The data are collapsed across the two trial blocks because, as we show later, there was no evidence that trial block had any effect on choice accuracy.

A Data Source (bees vs. simulations) × Choice Number × Trial Block repeated measures ANOVA was used to compare the performance of the bees to the estimate of chance provided by the simulations and to detect any differences in performance as a function of choice number or trial block. The critical result is a reliable difference between the choice accuracy of the bees and the simulation, $F(1, 9) = 22.9, p < .001$. There were no reliable effects of trial block, $F(1, 9) < 1$, or choice number, $F(2, 18) < 1$. In addition, none of the interaction terms approached significance.

These results provide evidence that bees avoid revisits to cells under conditions that cannot be explained in terms of either an odor mark or systematicity in the cell-to-cell movement patterns. Unfortunately, the results of the probe trials included at the end of the experimental procedure render the data difficult to interpret. The probe trial data for Experiment 2 were analyzed somewhat differently than they were in Experiment 1. Specifically, because the bees did not always visit all six cells during the probe trials, cells that were not visited during a trial were given a rank of 7. Other cells were given ranks corresponding to the ordinal position of the initial visit to the cell, as in Experiment 1. The mean rank of the randomly chosen cells that had been baited before the trial were compared with the mean rank of the cells not baited. The mean rank (across bees) of the baited cells was 2.9 and of the unbaited cells, 4.4. These values are reliably different, $t(9) = 3.1, p < .01$, and indicate that the presence of sugar solution could be directly perceived by the bees in Experiment 2. Thus, the ability of the bees to avoid visits to unbaited cells on the second apparatus may have been due, at least in part, to this perceptual discrimination ability rather than working memory.

**Experiment 3**

Experiment 3 was designed as a replication of Experiment 2, with one change in procedure designed to prevent the possibility of perceptual discrimination of baited and unbaited cells. Specifically, cells on the second apparatus that corresponded to those that had been visited on the first apparatus were baited with water. It is widely believed that honeybees cannot detect the presence of sugar in solution from any odor of the sugar. Therefore, it seems most likely that the apparent ability to discriminate baited cells in Experiment 2 was based on visual cues. The procedure in Experiment 3 was intended to eliminate this cue. With a series of probe trials, similar to those used in the previous experiments, we tested whether this procedure was successful in eliminating any perceptual discrimination ability.

**Method**

**Subjects and apparatus.** The subjects were 5 bees from a full-sized observation hive located inside a campus building; bees could pass through a tunnel that connected the hive and a window. The hive was three floors above the laboratory where data were collected. The apparatus was the same as that used in Experiment 2 and was located on a laboratory window ledge (with the window open).

**Procedure.** A bee was collected either from a feeder on the building roof near the window leading to the hive or by netting it as it left the window. It was taken in a vial to the laboratory, and the vial was placed (open end down) over a baited cell on the first apparatus so that the bee could enter the cell. Entry into the first cell was sometimes facilitated by placing an opaque cover over the vial. The bee was then allowed to feed from among the cells, and it was marked during this initial visit. It then flew out of the laboratory through the window. If a bee returned to the apparatus consistently, it was considered a subject in the experiment.

In all respects except the following, the procedure was identical to that of Experiment 2. In addition to baiting three cells of the second apparatus with sugar solution, 4–5 μL of water was placed in the cells on the second apparatus corresponding to those visited.
on the first apparatus. Approximately 10–12 min elapsed between trials. During the three probe trials that followed the 24 experimental trials, three randomly selected cells were baited with sugar solution, and the remaining three cells were baited with water.

Results and Discussion

As in Experiment 2, bees did not always complete the task; that is, they did not visit all three cells baited with sugar solution on the second apparatus. Thus, the same performance measures and analytic techniques used in Experiment 2 were used to analyze the results of Experiment 3. Figure 4 shows the mean probability of a correct choice during the first three choices on the second apparatus for the bees and the algorithm. As in Experiment 2, these probabilities were calculated only for visits to cells that had not already been visited on the second apparatus. Again, the data are shown collapsed across the two blocks of 12 trials each.

The data were analyzed in an ANOVA analogous to that used for the data from Experiment 2. The critical result is that the bees chose more accurately than expected on the basis of the simulations, $F(1, 4) = 10.3, p < .05$. There was no reliable effect of trial block, $F(1, 4) = 1.3$, but the main effect of choice number approached the standard criterion for statistical reliability, $F(2, 8) = 3.5, p = .08$, as did the interaction between choice number and data source, $F(2, 8) = 4.2, p = .06$. There was no evidence for any other interactions among the effects of the variables.

During the three probe trials, the mean rank (across bees) of the cells that contained sugar solution was 3.84 and that of the cells that contained water, 3.77. Thus, there was no evidence that bees chose cells on the basis of perceptual cues of baited cells in this experiment.

These results clearly indicate that, as in the previous experiments, the bees avoided revisits to cells more accurately than expected on the basis of their cell-to-cell transition tendencies. In Experiment 3, as in Experiment 2, this ability cannot be explained by odor marks left as the bee forages. Finally, the results of probe trials confirm that the presence of sugar solution in cells could not be discriminated from the presence of water. Thus, we infer that memories produced by cell visits on the first apparatus influenced choices made from among cells on the second apparatus.

Although the relevant statistical interaction was not reliable, it appears that the difference between empirical and simulated performance was restricted to choices made somewhat late on the second apparatus. Given the rather small number of subjects in Experiment 3 (and the resulting low level of statistical power), the possibility that this effect existed but was not detected must be considered. One explanation for higher levels of accuracy during choices made later is that a disruptive effect of the vial containment procedure was more effective during the choices made immediately after that procedure than during choices made later. If so, the estimate of working memory ability provided by these results is reduced by the disruptive effect of trapping bees in a vial for several seconds.

General Discussion

These data indicate that honeybees can discriminate the location of previously depleted food sources from those not yet depleted by using working memory. It is important to emphasize that this memory requires the simultaneous representation of multiple locations and is flexible and dynamic in content. The set of visited locations changes from one trial to the next, as well as over the course of the choice sequence within each trial.

The results from Experiments 1 and 2 are open to nonmemorial explanations. In Experiment 1, bees may have used odor marks to discriminate previously visited cells. In Experiment 2, there was evidence that bees could discriminate baited and unbaited cells on the basis of a perceptual cue. It is not clear why this ability was exhibited during Experiment 2 but not during Experiment 1. However, when considered in the context of the results of Experiment 3, in which both odor marks and perceptual cues are ruled out, our results provide compelling evidence that memory produced by previous cell visits affects later choices. This behavior is directly analogous to the ability of rats and other vertebrates to avoid revisits to locations depleted of food on the basis of working memory.

We must note that the level of choice accuracy displayed by the bees in these experiments is not high when compared with the levels of accuracy shown by rats and other verte-
brates in the analogous tasks. However, the fact that above-chance levels of choice accuracy were statistically reliable in experiments with rather small numbers of subjects indicates that bees were very consistent in choosing cells according to a process that enhanced choice accuracy. It may, of course, be that higher levels of working memory ability can be seen under slightly or substantially different experimental procedures. Initial work examining spatial working memory in pigeons indicated low levels of ability (Bond, Cook, & Lamb, 1981), but later studies showed that changes in experimental procedures resulted in greatly improved levels of choice accuracy (Roberts & Van Veldhuizen, 1985; Spetch & Edwards, 1986). It may be that the ability of bees is similarly constrained by some unknown aspect of the present procedures. Alternatively, it may be that the spatial working memory ability of honeybees is in fact impoverished in relation to that of the vertebrates in which it has been studied earlier. This study ought to be considered a preliminary demonstration of working memory ability in honeybees, which we hope will stimulate more extensive work directed at this critical comparative issue.

The demonstration of working memory ability adds substantially to the range of behavioral phenomena that have been identified in bees. This spatial working memory ability may be used in the context of natural foraging by honeybees, although that remains to be determined. Other potential mechanisms for the avoidance of flower revisits are available, specifically the influence of systematic movement patterns (Pyke, 1978; Waddington, 1980; Waddington & Heinrich, 1981; but see Zimmerman, 1979), the footprint or flower-marking pheromone (Corbet et al., 1984; Free, 1987; Giurfa & Núñez, 1992; Wetherwax, 1986), and perceptual discrimination of nectar or physical changes produced by earlier visits (Heinrich, 1979; Thorp, Briggs, Estes, & Erikson, 1975). However, at least under these experimental conditions, evidence for avoidance of visits to previously depleted locations can be demonstrated when these alternative mechanisms are ruled out. On the other hand, the results of the probe trials in Experiment 2, together with the data of Giurfa and Núñez (1992), indicate that future investigators of working memory in bees need to carefully consider the influence of these alternative mechanisms.

Perhaps the most important implication of our results is that working memory ability can be demonstrated across a wide phylogenetic range. This ability has been studied in detail in the handful of animal species favored in psychological laboratories (rats, pigeons, and several primate species). However, as reviewed earlier, it also appears to exist in a wide range of other vertebrate species. Our data show that working memory ability can also be demonstrated in an invertebrate. This ability can therefore be supported by a nervous system that is very different from those in which it has been previously studied. Our understanding of the physiological systems that are necessary and sufficient for working memory must accommodate this finding. Of course, it will require additional work to determine the similarity of the memory structures and processes responsible for working memory ability in bees and those responsible for working memory ability in the more well-known vertebrate preparations. However, our results encourage the view that a number of fundamental psychological mechanisms that have been characterized as general among vertebrates (e.g., Macphail, 1982) are even more general.

References


Received October 18, 1993
Revision received March 5, 1994
Accepted March 8, 1994

Acknowledgment of Ad Hoc Reviewers

The Editor thanks the following persons for reviewing manuscripts. (Numbers in parentheses indicate assistance with more than one manuscript.)

Charles Abramson (2)
John J. B. Ayres
Thomas J. Daniels
Robert Fagen
Lynn A. Fairbanks (2)

Kevin J. Flannelly
Roy P. Fontaine
Harry Frank
Ronald Holden
Martin Kavaliers

Dale M. Madison
William A. Mason
H. Lyn White Miles
Robert W. Mitchell
William Timberlake

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.