

Honeybee Memory: Navigation by Associative Grouping and Recall of Visual Stimuli

S. W. Zhang,* M. Lehrer,† and M. V. Srinivasan*

**Centre for Visual Science, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra, ACT 2601, Australia;* and †*Department of Neurobiology, Zoological Institute, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland*

Studies of navigation in bees and ants are beginning to reveal that foraging insects traveling repeatedly to a food source navigate by using a series of visual images of the environment acquired en route (Collett, 1996; Collett et al., 1993; Judd & Collett, 1998; Wehner et al., 1990, 1996). By comparing the currently viewed scene with the appropriate stored image, the insect is able to ascertain whether or not it is on the correct path and make any necessary corrections. If a bee happens to forage at more than one site, then she needs not only to memorize a separate set of images for each route that she has learned but also to retrieve the set of images that is appropriate to each route. Here we examine the bee's capacity to learn and later retrieve from memory two different sets of visual stimuli. Bees were trained to fly through a compound Y-maze where they were presented alternately with two different sequences of visual stimuli on their route to a food reward. We find that bees can indeed store two different sequences of images simultaneously. Furthermore, the trained bees are able to classify the memorized images into two groups, one pertaining to each three-stimulus set. Exposure to any of the images pertaining to one set triggers recall of all of the other images associated with that set. Associative grouping and recall of visual stimuli, demonstrated here for the first time in honeybees, provide an effective means of retrieving the appropriate navigational information from memory. © 1999 Academic Press

Key Words: honeybee; vision; navigation; learning and memory; associative grouping and recall; maze learning; sequence learning.

INTRODUCTION

There is much experimental evidence showing that bees, as well as some species of ants, navigate to food sources and back to their homes by memorizing a sequence of visual images experienced en route (Collett, 1996; Collett et al., 1993; Wehner et al., 1990, 1996). If an insect is to make use of such stored images for navigation, it must know, at each stage of its journey, which image to expect next: a comparison of the next stored image with what is actually experienced would then help determine whether the insect is "on course" and

We thank Hong Zhu for assistance with the experiments. Thanks to Jochen Zeil as well as the two anonymous referees for several helpful suggestions for improving the manuscript. This research was partly supported by the International Human Frontier Science Program (Grant RG-84/97).

Address correspondence to Dr. S.W. Zhang, Centre for Visual Science, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra, ACT 2601, Australia. Fax: +61-2 6249-3808. E-mail: swzhang@rsbs.anu.edu.au.

would provide information for making course corrections, if necessary. Several studies (Collett et al., 1993; Collett, 1996; Wehner et al., 1990, 1996) suggest that bees and ants indeed take note of salient landmarks encountered on the way, as well as the sequence in which the landmarks occur.

The problem of navigating by using stored mental images becomes more complex when an insect forages at more than one site. Bees can easily be trained to forage at two different sites (e.g., Collett & Kelber, 1988; Dyer, 1991; Menzel et al., 1996; Wehner et al., 1990), and it is likely that they often do so even under natural conditions. In such situations the insect must recall from memory only those images that are appropriate to the route that it is currently taking. Can insects retrieve such navigational information from memory in a selective way?

Here we explore this question by training bees to two different "routes" in a maze, where each route is specified by a distinct set of visual stimuli. We ask whether the trained bees are capable of learning the visual images as belonging to two distinct groups, one corresponding to each route. We also ask whether the trained bees, when presented with one of the images associated with one of the routes, can recall all of the other images that are associated with that route. This type of learning and recall, which we term "associative grouping and recall," would, if extant, enable the bee to organize and retrieve navigational information pertaining to multiple routes.

MATERIALS AND METHODS

General

The experiments were carried out in an All Weather Bee Flight Facility at the Australian National University's Research School of Biological Sciences. The facility consisted of a modified glasshouse in which the internal temperature was regulated by a computer to maintain $24 \pm 5^\circ\text{C}$ during the day and 17°C at night. A beehive, mounted on the wall of the facility, had two entrances, one allowing bees access to the inside of the facility and the other to the outside. Bees foraging indoors obtained sugar water from feeders in the facility. For each experiment, about 10 bees were marked individually and trained to visit the experimental apparatus described below. The trained bees flew regularly between the hive and the apparatus.

Apparatus

The experimental apparatus (Fig. 1a) was a compound Y-maze consisting of a series of interconnected cylinders, each of diameter 25 cm and height 25 cm, covered by a transparent Perspex lid. Bees could fly from one cylinder to the next through a hole, 3 cm in diameter, positioned halfway up the wall of the cylinder, i.e., 12.5 cm above the ground. The first cylinder carried two holes arranged diametrically opposite to each other. Bees arriving from the hive entered this cylinder through the entrance hole and moved to the second cylinder through the opposite hole, which was marked by a visual stimulus. The second cylinder, as well as the third one, carried three holes, one serving as the entrance (coming from the previous cylinder), and two others, 90° apart, serving as exits leading to the next cylinder. Each of the exits carried a visual stimulus, between which the bees had to choose. A bee, having made a correct

choice in the second as well as in the third cylinder, arrived in a fourth cylinder where she found a feeder with sugar solution. Thus, the second and the third cylinder acted as decision stages: at each of these cylinders the bee had to choose between two stimuli. The single stimulus in the first cylinder served as an "Indicator" stimulus, which determined the choices that the bees had to make in the subsequent decision stages.

Visual Stimuli and Training Procedures

Each stimulus was presented as an 18 cm \times 18 cm square centered on an exit which led to the next cylinder. The stimulus presented in the first cylinder (i.e., the Indicator stimulus) was a black-and-white grating (period 4 cm) oriented either horizontally (Stimulus A) or vertically (Stimulus A'), respectively. The second cylinder (first decision stage) offered a choice between a blue square (Stimulus B) and a green one (Stimulus B'), and the third between a sectored disk (sector angle, 30°) (C) and concentric rings (ring width, 2 cm) (C') (Fig. 1b). When the first cylinder presented the horizontal grating, the feeder could only be reached if the bee chose blue in the second cylinder and the sectored disk in the third. However, when the vertical grating was placed in the first cylinder, the bee could reach the reward only if she chose green in the second cylinder and the ring pattern in the third. A bee that made a wrong choice arrived in a "dead-end" cylinder which offered neither a reward nor another choice of stimuli. Bees entering such "dead-end" cylinders were released by raising the Perspex lid briefly. Such bees sometimes flew back to the hive, but usually they reentered the first cylinder through the entrance hole to seek the reward afresh. Throughout the training, the Indicator stimulus (A or A') was changed every 20–30 min (ca. 6 visits per bee, on average). Thus, the bees had to learn, simultaneously, two stimulus sequences, "horizontal-blue-sectors" (ABC) and "vertical-green-rings" (A'B'C') (Fig. 1b). The positions of the stimuli at the first decision stage (left, right) were swapped every 20–30 min (ca. 6 visits per bee), in order to ensure that the bee's decisions were based on the visual stimuli that they encountered, and not on the preference for a particular exit. This swap was done at the midpoints of the periods of presentation of the Indicator stimuli (horizontal or vertical grating). The positions of the stimuli at the second decision stage (left, right) were swapped every 10–15 min (ca. 3 visits per bee), for similar reasons. In all, this procedure used eight different configurations, spanning a cycle that lasted between 80 and 120 min depending upon how frequently the bees visited.

Each bee that had negotiated the maze successfully and collected a reward in the fourth cylinder was released from this cylinder to fly home without having to trace her way back through the maze.

A bee choosing between B and B' in the second cylinder (first decision stage) could not see whether the third cylinder contained the patterns C and C' or was a dead-end cylinder, because a 6 cm \times 6 cm baffle was placed behind the entrance to the third cylinder (with a 1-cm clearance, to enable the bee to pass). Similarly, a bee choosing between C and C' in the third cylinder (second decision stage) could not see whether the next cylinder contained the feeder or was a dummy, because the feeder was placed on the floor just under the entrance and was thus invisible to the bee from the entrance.

Tests

The bees were tested not only on the training sequences ABC and A'B'C' (learning tests), but also in transfer tests which represented all possible permutations of the training sequences (five different ones for each of the two sequences), as illustrated in Figs. 2, 3, and 4. The reward continued to be offered during all tests, to prevent bees from losing their motivation to visit the apparatus. The transfer tests, however, were conducted for short periods at a time (10–15 min or 3 visits per bee, on average). Between tests, the bees were trained further for a period of at least 20–30 min using at least two of the eight different training configurations. Although the left-right configurations of the stimuli were not swapped during a test, successive tests of a given kind used different configurations, to cancel out the effects of any positional bias. These measures ensured that the bees did not learn the unfamiliar sequences (see also *Controls for Stability of Performance*, below). It took about 10 h to carry out one cycle of the 10 different sequences of transfer tests, interspersed with training segments. This cycle of testing was repeated 3–4 times to collect sufficient data for analysis.

Analysis of Performance

In the tests, the first choice made by each bee at each of the decision stages (first and second stage) was noted. (If a bee made a wrong choice at the first decision stage, she was released from the apparatus to allow her to enter it afresh.) Performance at each stage was measured in terms of the percentage of correct choices. Each decision made by a bee was treated as a statistically independent event, and all decisions (from all bees) were pooled to obtain a total sample size, n . This procedure is justified for several reasons. First, performance was evaluated by using only the first decision made by each bee at each decision stage. Secondly, bees had to reenter the apparatus to make a fresh set of decisions. Earlier studies (e.g., Srinivasan and Lehrer, 1988) indicate that successive choices made by bees under such conditions are statistically independent. Thirdly, since transfer test durations were relatively short (involving at most 3 visits per bee) and were interspersed with longer training periods, as explained above, learning effects during such a test were negligible and the choices made by a bee during its first flight in a particular transfer test did not influence its choice behavior in the subsequent (at most 2) flights in the same test. Fourthly, no particular transfer test was repeated using exactly the same left-right configuration of stimuli: the configurations were different for each repetition of a test, as explained above under *Visual Stimuli and Training Procedures*. Our procedure for evaluating bee performance in mazes is standard practice (e.g., van Hateren et al., 1990; Collett et al., 1993; Horridge & Zhang, 1995).

If the bees have not learned to make any associations during the training, then the probability of making a correct choice in the first decision stage would be 50%, as would be the probability of a correct choice in the second decision stage. Thus, the probability of the bee arriving at the reward simply by choosing randomly at both of the decision stages would be 25%. A χ^2 test was used to examine whether the experimentally observed choice frequencies were significantly different from the random-choice levels, and $2 \times 2 \chi^2$ tests were

carried out to examine whether changing the order of appearance of the stimuli in the tests affected the bees' choice performance.

Controls for Stability of Performance

A typical experimental series can run for several days and it is important to ensure that the learning state of the bees remains constant during this protracted period. To control for this, we compared the results obtained in learning tests starting from the fourth day (before the first transfer test was commenced) and going through to the eighth day (when the last transfer test was completed).

The performance in five learning tests was analyzed, the first at 12:38 PM on Day 4, the second at 9:15 AM on Day 5, the third at 9:20 AM on Day 6, the fourth at 9:10 AM on Day 7, and the fifth at 10:20 AM on Day 8. There was no significant difference in the bees' performance between any of these tests (see Table 1). This indicates that the learning state of the bees was constant during this period and had plateaued before the first test was commenced.

A similar analysis showed that performance was also stable in the transfer tests. For example, analysis of performance in the first decision stage in 3 transfer tests for sequence ACB yielded the following results: Test 1, on Day 4, 76.0% ($n = 25$); Test 2, on Day 5, 76.7% ($n = 30$); Test 3, on Day 8, 74.1% ($n = 27$). There was no significant difference between these choice frequencies ($p > .80$ in all cases). This observation is supported by data from an earlier study of honeybee navigation in mazes, where we have shown that learning does not occur during such transfer tests (see Table 5 of Zhang et al., 1996).

RESULTS

Can Bees Memorize Two Different Sets of Stimuli Simultaneously?

The results obtained with the bees trained simultaneously on the stimulus sequences ABC and A'B'C' are shown in Fig. 1c. It is clear that the bees learned and distinguished the stimuli belonging to the two sequences. When they were confronted with the horizontal grating (A) in the first cylinder they showed a preference for blue over green at the first decision stage, and a preference for sectors over rings at the second decision stage. Conversely, when they encountered the vertical grating (A') in the first cylinder, the bees preferred green over blue at the first decision stage, and rings over sectors at the second decision stage. In each case, the choice frequency for the relevant stimulus was significantly better than the random-choice level of 50%. Furthermore, the bees' ability to make two correct choices in sequence (i.e., horizontal grating to blue to sectors, Fig. 1c, bar 5, and vertical grating to green to rings, bar 6) was also significantly better than the random-choice level of 25%. The results of statistical tests for significance are given in Fig. 1c. Clearly, then, viewing the Indicator stimulus (horizontal or vertical grating) triggers recall of the appropriate stimuli that are expected to follow.

Can Bees Correctly Distinguish between Stimuli Belonging to the Two Training Sets When Their Sequence of Presentation Is Altered?

We have seen above that bees can distinguish between the stimuli belonging to two different sets, ABC and A'B'C', when they are presented in the same

TABLE 1

Statistical Tests to Control for Stability of Performance for Sequences ABC and A'B'C', from Day 4 to Day 8

	Day 4	Day 5	Day 6	Day 7	Day 8
A to B	58/81 = .72	35/53 = .66	23/32 = .72	31/46 = .67	13/18 = .72
Day 4		$\chi^2 = .240$	$\chi^2 = .0004$	$\chi^2 = .088$	$\chi^2 = .056$
58/81 = .72		$p > .50$	$p > .99$	$p > .70$	$p > .80$
Day 5			$\chi^2 = .010$	$\chi^2 = .005$	$\chi^2 = .037$
35/53 = .66			$p > .90$	$p > .95$	$p > .80$
Day 6				$\chi^2 = .029$	$\chi^2 = .091$
23/32 = .72				$p > .80$	$p > .70$
Day 7					$\chi^2 = .006$
31/46 = .67					$p > .90$
B to C	44/58 = .76	31/47 = .66	15/22 = .68	26/31 = .84	10/13 = .77
Day 4		$\chi^2 = .80$	$\chi^2 = .170$	$\chi^2 = .370$	$\chi^2 = .078$
44/58 = .76		$p > .30$	$p > .50$	$p > .50$	$p > .70$
Day 5			$\chi^2 = .008$	$\chi^2 = 2.20$	$\chi^2 = .170$
31/47 = .66			$p = .90$	$p > .10$	$p > .50$
Day 6				$\chi^2 = 1.02$	$\chi^2 = .028$
15/22 = .68				$p > .30$	$p > .80$
Day 7					$\chi^2 = .014$
26/31 = .84					$p > .90$
A' to B'	55/72 = .76	52/69 = .75	47/68 = .69	44/62 = .71	21/26 = .81
Day 4		$\chi^2 = .003$	$\chi^2 = .603$	$\chi^2 = .265$	$\chi^2 = .034$
55/72 = .76		$p > .95$	$p > .30$	$p > .50$	$p > .80$
Day 5			$\chi^2 = .391$	$\chi^2 = .137$	$\chi^2 = .081$
52/69 = .75			$p > .50$	$p > .70$	$p > .70$
Day 6				$\chi^2 = .001$	$\chi^2 = .76$
47/68 = .69				$p > .95$	$p > .30$
Day 7					$\chi^2 = .475$
44/62 = .71					$p > .30$
B' to C'	33/55 = .60	34/52 = .65	37/47 = .79	28/44 = .64	13/21 = .62
Day 4		$\chi^2 = .14$	$\chi^2 = 3.30$	$\chi^2 = .026$	$\chi^2 = .012$
33/55 = .60		$p > .70$	$p > .05$	$p > .80$	$p > .90$
Day 5			$\chi^2 = 1.56$	$\chi^2 = .001$	$\chi^2 = .0001$
34/52 = .65			$p > .20$	$p > .95$	$p > .99$
Day 6				$\chi^2 = 1.85$	$\chi^2 = 1.33$
37/47 = .79				$p > .10$	$p > .20$
Day 7					$\chi^2 = .019$
28/44 = .64					$p > .80$

Note. The subtables A to B and B to C show performance in the first and second decision stages, respectively, for the ABC sequence; similarly for the sequence A'B'C'. The numerator and denominator in each fraction denote the numbers of correct and total choices, respectively.

sequence as in the training (Figs. 1b and 2a). Can bees trained on the sets ABC and A'B'C' correctly distinguish between the stimuli belonging to the two sets even when the stimuli appear in a different succession? To investigate this we tested the trained bees on the same sets of stimuli but in which the second and

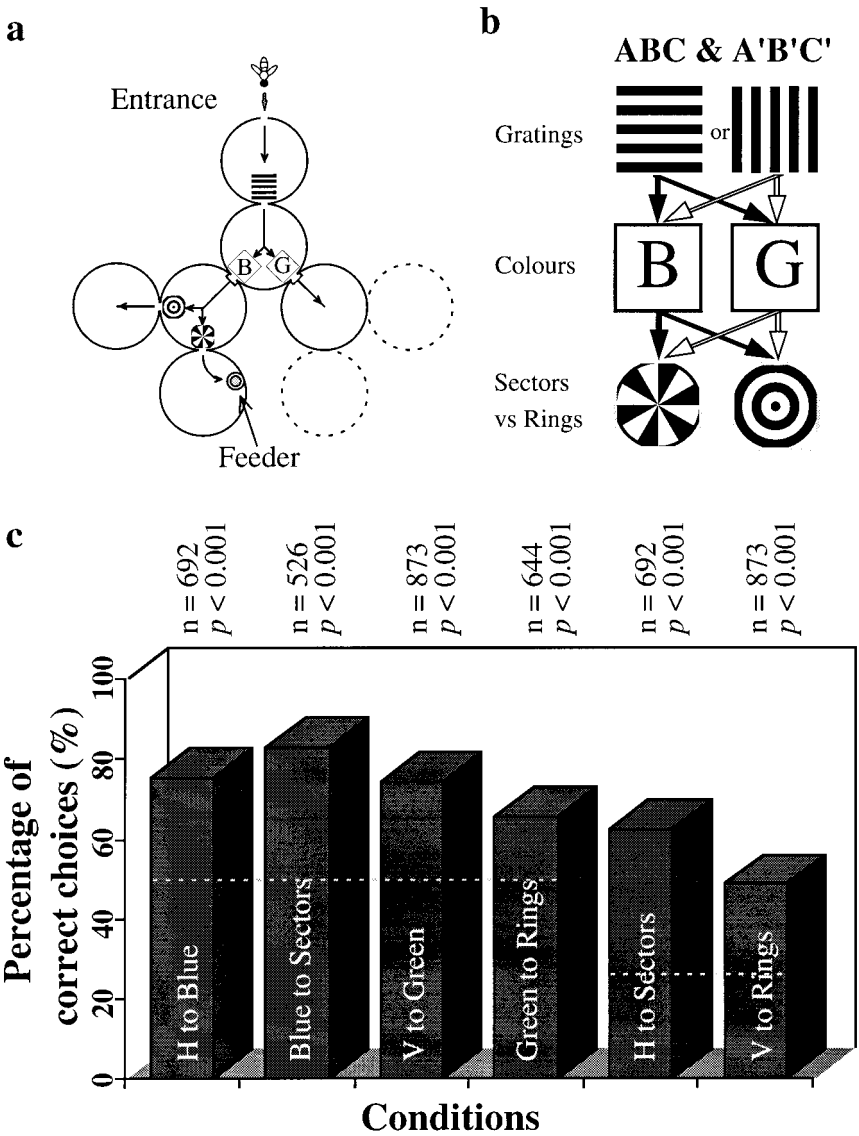


FIG. 1. (a) The compound Y-maze used in the experiments. (b) The two sequences of visual stimuli used in the training, ABC and A'B'C'. The dark arrows show possible choices when the Indicator stimulus is A, and the light arrows when it is A'. (c) Choice behavior of bees trained on these sequences at each of the decision stages. The first two bars represent the bees' choice frequencies in favor of the correct stimulus at the first and second decision stages, respectively, for the first stimulus sequence (ABC). The next two bars give corresponding data for the second sequence (A'B'C'). The fifth bar depicts the frequency of correct choices in the first as well as the second decision stage for the first sequence, and the sixth bar shows corresponding data for the second sequence. Dashed lines: random choice level. H, horizontal grating; V, vertical grating; n, total number of choices. p results of statistical tests for significance (χ^2).

third stimuli had been interchanged, i.e., using the sequence ACB and A'C'B'. The Indicator stimuli (A and A') remained unaltered. The results (Fig. 2b) reveal that the trained bees continued to choose the stimuli appropriate to the Indicator stimulus even when they appeared in an unfamiliar sequence. Thus,

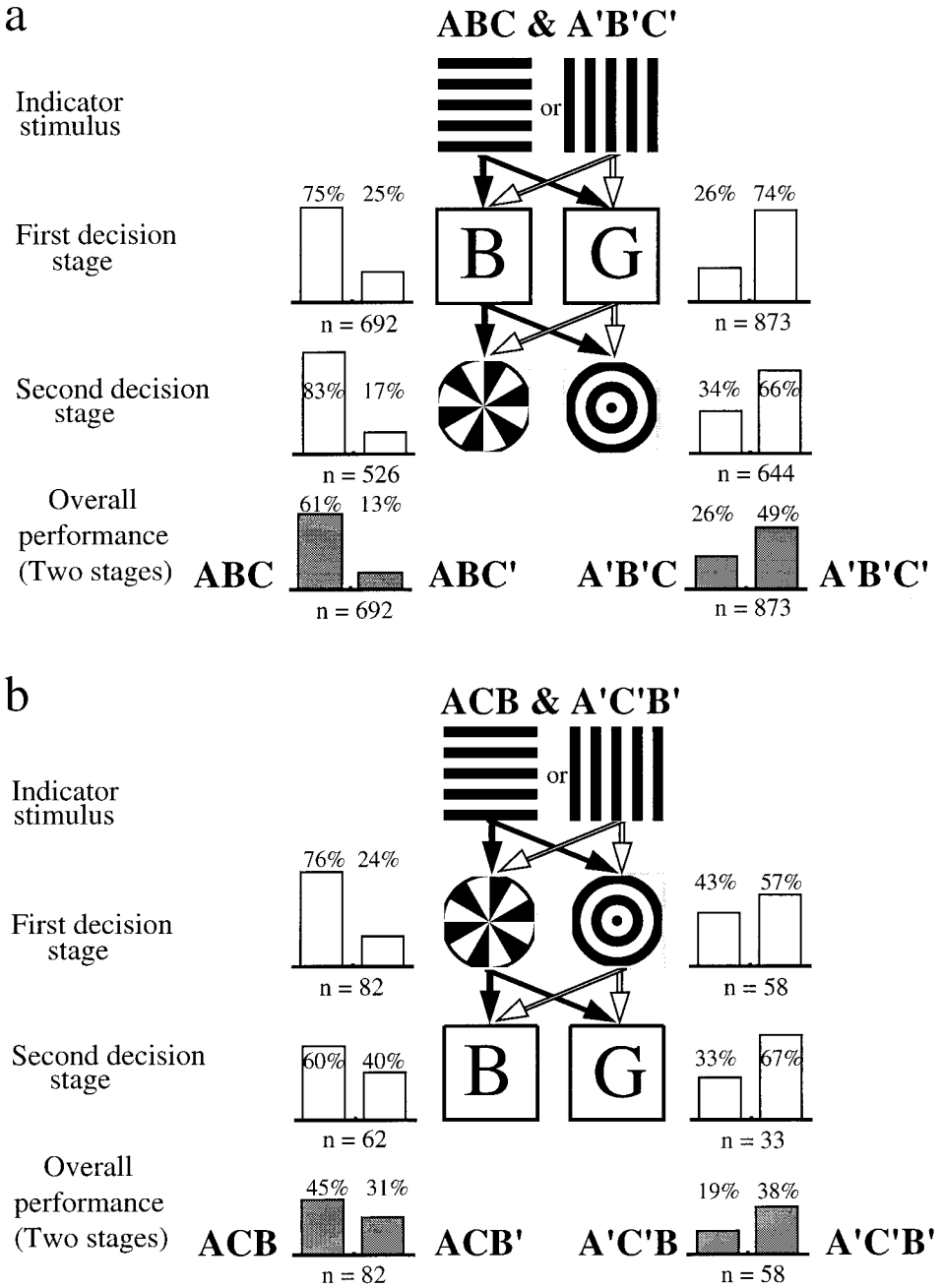


FIG. 2. Choice behavior of bees, trained simultaneously on sequences ABC and A'B'C', in subsequent tests where they encounter the training sequences (a) or altered sequences (b). In each panel, the top set of bars shows the percentage of choices in favor of the two stimuli in the first decision stage, and the middle set the percentage of choices in favor of the two stimuli in the second decision stage. In the bottom set (dark bars), the outer bars show the percentage of flights that met with overall success (i.e., arrival at the reward), and the inner bars the overall percentage of flights in which bees chose correctly in the first decision stage, but incorrectly in the second. Note that the percentages corresponding to each set of dark bars do not sum to 100. This is because the dark bars represent only those flights in which the correct stimulus was chosen in the first decision stage. For example, in (b), the left-hand set of dark bars sums to 76%, which is the percentage of bees that chose the sectored pattern after encountering the horizontal grating as an Indicator stimulus. Arrows as in Fig. 1b. *n*, total number of choices.

bees encountering the horizontal grating in the first cylinder continued to prefer the sectors over the rings even when these stimuli appeared at the first decision stage, and blue over green even when the color stimuli appeared at the second decision stage.

Next, we examined the performance of the trained bees in unfamiliar sequences where even the Indicator stimulus was different from that encountered during training. Figures 3a and 3b show the results of transfer tests in which the bees encounter the pair of sequences BAC and B'A'C' and the pair BCA and B'C'A'. In these tests, the Indicator stimulus was a color rather than a grating. Figures 4a and 4b show the results of tests in which bees encounter the pair of sequences CAB and C'A'B' and the pair CBA and C'B'A'. In these tests the Indicator stimulus was a sectored or a ring pattern rather than a grating. The results show that, in all of the tests using altered sequences, the bees continued to choose the appropriate stimulus at each stage of the maze. These findings indicate that, in general, exposure to any member of the trained stimulus sets ABC or A'B'C' is sufficient to trigger associative recall of all of the other stimuli belonging to that set. In all of the tests, changing the Indicator stimulus (from A to A', B to B', or C to C') causes the bees to change (and reverse) their preference for the stimuli that they encounter at subsequent stages of the maze. This change in preference is statistically significant in all cases ($p < .05$, $2 \times 2 \chi^2$ test).

It should be noted that, in this experiment, the bees are not specifically trained to distinguish between A and A', which are the Indicator stimuli in the training. Nevertheless, the bees distinguish between them in the transfer tests because they associate them with the stimulus sets ABC and A'B'C', respectively. It is also clear from this set of tests that the bees are capable of treating the stimulus pairs (B, B') and (C, C') as Indicator stimuli, even though these are never encountered as Indicator stimuli in the training. However, the bees' success in arriving at the reward (i.e., in choosing correctly in the first as well as the second decision stages, see Figs. 2–4, bottom set of histograms in each panel) was greatest when the sequences were as in the training situation (ABC and A'B'C', Fig. 2a). Performance was also good with the exactly reversed sequences (CBA and C'B'A', Fig. 4b).

Does Associative Recall of Stimuli Depend upon Where the Stimuli Are Encountered in the Maze?

When bees are trained to the stimulus sets ABC and A'B'C', is the ability to recognize, say, B as belonging to the first set better when the pair of stimuli B and B' are encountered in the first decision stage (as in the training) than when they are encountered in the second decision stage? To examine whether the accuracy of associative recall depends upon where in the maze the stimulus is encountered, we have analyzed the bees' preferences for either member of the pairs (A, A'), (B, B'), and (C, C') when these pairs are presented at various stages in the maze.

Consider first the preference for B over B' when bees encounter the stimulus set (A, B, C). This analysis is shown in the upper half of Table 2. A comparison of the sequences ABC and ACB reveals that the preference for B over B' is significantly stronger when this pair is presented in the first decision stage rather than in the second decision stage, that is, when it is presented at the

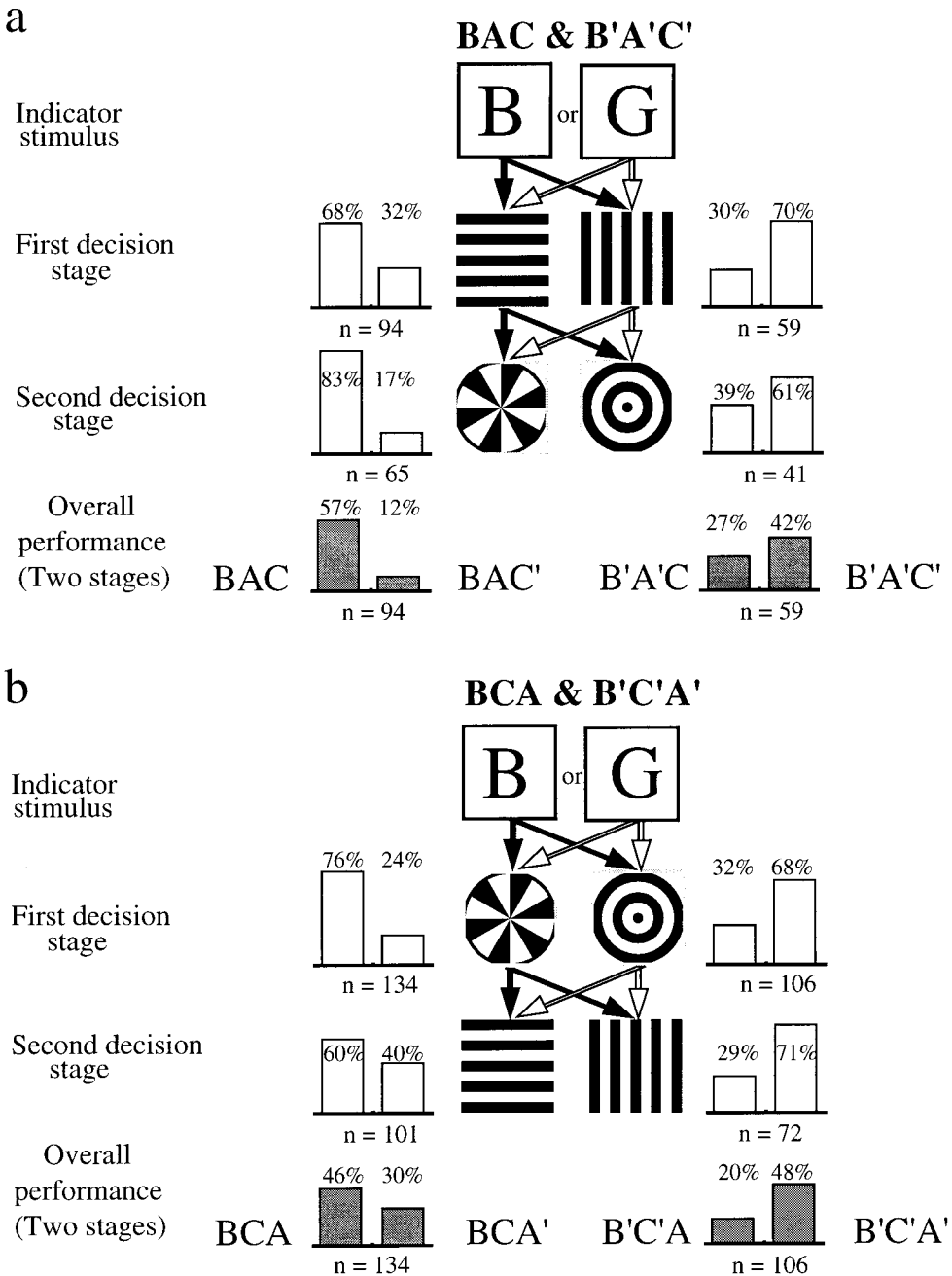


FIG. 3. Choice behavior of bees, trained simultaneously on sequences ABC and A'B'C', in subsequent tests where they encounter altered sequences (a, b). Other details as in Fig. 2.

same stage in the maze as in the training. However, this is not the case when one compares the preference for B over B' in the sequences CBA and CAB. Consider now the preference of C over C'. A comparison of sequences ACB and ABC reveals that the preference for C over C' is not significantly different when this pair is presented in the second decision stage than in the first

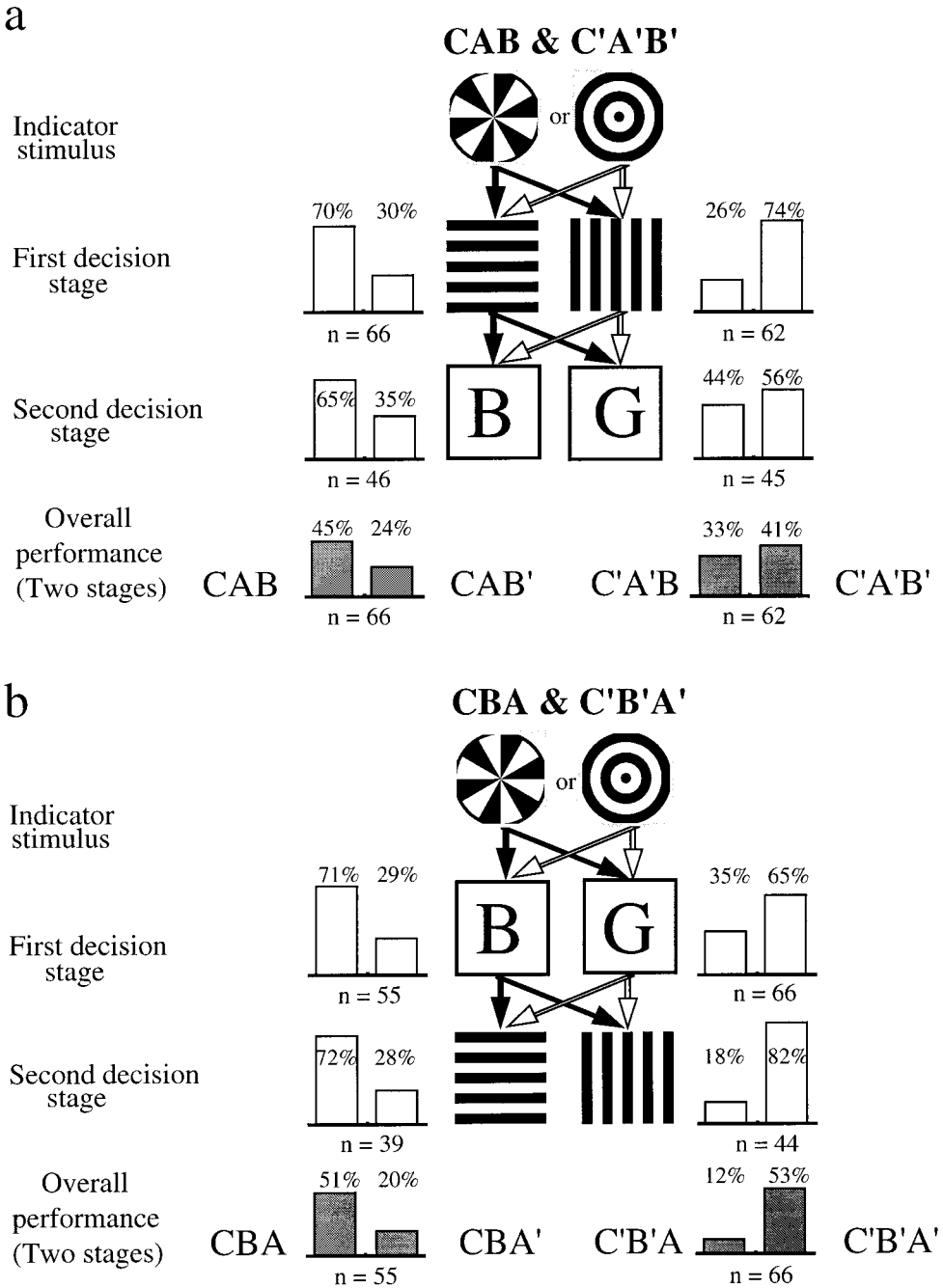


FIG. 4. Choice behavior of bees, trained simultaneously on sequences ABC and A'B'C', in subsequent tests where they encounter altered sequences (a, b). Other details as in Fig. 2.

decision stage, that is, when it is presented at the same stage in the maze as in the training. This is also the case when one compares the preference for C over C' in the sequences BCA and BAC. Consider, finally, the preference for A over A'. Since these function as Indicator stimuli in the training, the bees have

TABLE 2

Analysis of the Performance of Bees, Trained as Shown in Fig. 1b, When They Encounter the Stimulus Sets ABC and A'B'C' Presented in Various Sequences

A		Stimulus	B versus B': choice frequencies in favour of B		C versus C': choice frequencies in favour of C		A versus A': choice frequencies in favour of A			
		Position								
B	Performance in first decision stage	Sequence ABC 75.4%	$p > 0.50$	Sequence CBA 70.9%	Sequence ACB 75.6%	$p > 0.98$	Sequence BCA 76.1%	Sequence BAC 68.0%	$p > 0.98$	Sequence CAB 69.7%
	Performance in second decision stage	$p < 0.02$	$p > 0.50$	$p > 0.10$	$p > 0.30$	$p > 0.30$	$p > 0.98$			
C	Performance in first decision stage	Sequence ACB 59.7%	$p > 0.50$	Sequence CAB 65.2%	Sequence ABC 83.1%	$p > 0.99$	Sequence BAC 83.1%	Sequence BCA 60.4%	$p > 0.20$	Sequence CBA 71.8%
	Performance in second decision stage	$p > 0.30$	$p > 0.30$	$p > 0.20$	$p > 0.50$	$p > 0.95$	$p > 0.30$			
A'		Stimulus	B' versus B: choice frequencies in favour of B'		C' versus C: choice frequencies in favour of C'		A' versus A: choice frequencies in favour of A'			
		Position								
B'	Performance in first decision stage	Sequence A'B'C' 74.3%	$p > 0.10$	Sequence C'B'A' 65.2%	Sequence A'C'B' 56.9%	$p > 0.20$	Sequence B'C'A' 67.9%	Sequence B'A'C' 69.5%	$p > 0.70$	Sequence C'A'B' 74.2%
	Performance in second decision stage	$p > 0.30$	$p > 0.30$	$p > 0.20$	$p > 0.50$	$p > 0.95$	$p > 0.30$			
C'	Performance in first decision stage	Sequence A'C'B' 66.7%	$p > 0.30$	Sequence C'A'B' 55.6%	Sequence A'B'C' 65.7%	$p > 0.50$	Sequence B'A'C' 61.0%	Sequence B'C'A' 70.8%	$p > 0.20$	Sequence C'B'A' 81.8%
	Performance in second decision stage									

Note. The large boxes show the choice frequencies in favor of a particular stimulus, for various stimulus sequences and at various stages in the maze (first or second decision stage). The small boxes show p values associated with the results of χ^2 tests to examine whether the differences in the choice frequencies in the neighboring large boxes (left versus right, or top versus bottom) are statistically significant.

not been trained to choose between them and have never encountered them in the first or second decision stages during the training. Nevertheless, the bees discriminate A from A' in the tests because they have learned to group them associatively with the stimulus sets ABC and A'B'C', respectively. Here again, when we compare the sequences BAC and BCA (or CAB and CBA), we find that the preference for A over A' does not depend significantly upon whether this pair is presented in the first or the second decision stage.

A similar conclusion emerges when one examines the bee's choice preferences with respect to the stimulus pairs (A, A'), (B, B'), and (C, C') when bees encounter the stimulus set A'B'C', as shown in the lower half of Table 2. With this set, B' is consistently preferred over B, C' over C, and A' over A. However, in no case does the preference for the appropriate stimulus of a pair vary significantly with the stage in the maze at which the stimulus is encountered. In summary, we conclude that associative recall of stimuli is largely independent of the stage at which the stimuli are encountered in the maze.

We can also ask whether the preference for a given member of a stimulus pair depends upon whether the stimulus pair that precedes it is the same as in the training or is different. Consider, first, the sequences ABC and CBA in the upper half of Table 2. We see that when the pair (B, B') is encountered in the

first decision stage, the preference for B over B' is not significantly stronger when the preceding stimulus is A, as in the training, than when it is C. Considering next the sequences ACB and CAB, we see that a similar observation holds for the case where (B, B') is encountered in the second decision stage. In fact, this result applies to all observed preferences for all stimulus pairs, as shown in Table 2. We conclude from this analysis that the ability of the trained bees to choose the appropriate member of a stimulus pair in the maze does not depend upon the stimulus pair that precedes it.

In a new experiment we trained bees on a different sequence of stimuli, BCA and B'C'A'. Here the Indicator stimulus was a color (blue or green), the stimuli encountered in the first decision stage were the sectored and ring patterns, and the stimuli encountered in the second decision stage were the horizontal and vertical grating. The bees learned this task and performed just as well as they did in that of Fig. 1 (data not shown). Thus, bees are capable of grouping and recalling visual stimuli associatively regardless of whether the Indicator stimulus that they experience during training is a color or a pattern.

Finally, we compared the performance of individual bees in the learning and transfer tests carried out in this study. In most cases, the choice frequencies shown by individual bees in any given situation were not significantly different from each other, thus justifying pooling of the data from all bees. While space constraints prevent us from listing the choice frequencies of each bee in each situation, Table 3, showing data from two individual bees, demonstrates consistent performance.

DISCUSSION

Our results show, for the first time, that bees can learn and recall, by association, two different sets of stimuli. Exposure to any member of one of the sets triggers recall of the other two members of that set.

We also find that the associative recall of the stimuli is largely independent of the sequence in which they are encountered during the tests. In our experiments, the trained bees do not hesitate to enter the apparatus even when the Indicator stimulus, seen upon entering the maze, is different from the one they normally encounter. They proceed readily through the entire maze even when the stimulus at the entrance is that which they normally encounter at the very end of the maze during training. This suggests that, in our experiments, the bees are not associating each pair of stimuli with a particular stage of the maze. Perhaps the nature of the training, which requires the bees to classify the stimuli that they encounter into two sets by association with the Indicator stimulus seen in the first cylinder, makes them insensitive to the sequence in which they encounter the stimuli. Another reason why the bees behave as though they ignore the stimulus sequence may be that, since the stimuli appear in rapid succession while flying through the maze, the bees treat the entire maze as a single, large "flower" in which all of the stimuli are encountered more or less simultaneously, rather than as a sequence of landmarks on the way to a food source. In any case, the bees do not need to know the sequences of the stimuli in order to make the necessary associations.

The associative learning and recall that we have studied here is much more intricate than the well-investigated ability of animals to associate a particular stimulus with a reward. It represents the ability to group a number of stimuli

TABLE 3
Choice Behavior of Two Individual Bees at Each Decision Stage
in All of the Stimulus Sequences Tested in Figs. 2-4

Indicator stimulus	Stimulus sequence	Bee No. 21		Bee No. 42	
		First decision stage	Second decision stage	First decision stage	Second decision stage
A	ABC	10/14 = 71.4%	8/10 = 80.0%	10/10 = 100%	7/10 = 70.0%
A'	A'B'C'	9/10 = 90.0%	5/9 = 55.6%	12/19 = 63.2%	7/12 = 58.3%
A	ACB	3/4 = 75.0%	3/3 = 100.0%	11/13 = 84.6%	7/11 = 63.6%
A'	A'C'B'	3/8 = 37.5%	2/3 = 66.7%	10/16 = 62.5%	5/10 = 50.0%
B	BAC	12/19 = 63.2%	10/12 = 83.3%	7/8 = 87.5%	5/7 = 71.4%
B'	B'A'C'	6/7 = 85.7%	6/6 = 100.0%	5/5 = 100.0%	4/5 = 80.0%
B	BCA	7/8 = 87.5%	5/7 = 71.4%	11/16 = 68.8%	8/11 = 72.7%
B'	B'C'A'	6/7 = 85.7%	5/6 = 83.3%	10/16 = 62.5%	7/10 = 70.0%
C	CAB	6/6 = 100.0%	4/6 = 66.7%	10/13 = 76.9%	10/10 = 100.0%
C'	C'A'B'	8/11 = 72.7%	4/7 = 57.1%	3/4 = 75.0%	3/3 = 100.0%
C	CBA	5/6 = 83.3%	5/5 = 100.0%	4/4 = 100.0%	3/4 = 75.0%
C'	C'B'A'	6/10 = 60.0%	5/6 = 83.3%	7/12 = 58.3%	5/8 = 62.5%

Note. The numerator and denominator in each fraction denote the numbers of correct and total choices, respectively.

together, and to recall all of the stimuli belonging to that group when one member is presented. It is important to note that the recall that we observe in our experiments must involve associative memory, because the design of the maze is such that the bee no longer sees the Indicator stimulus when she makes her decisions in subsequent stages of the maze.

Collett et al. (1993) examined the ability of bees to learn to correctly choose a single sequence of visual stimuli. Bees entering a maze had to learn, for example, to prefer "white" over "black" in the first stage, "blue" over "yellow" in the second stage, and a vertical grating over a horizontal one in the third. The authors found that bees could learn this task well. They then tested the bees by confronting them with combinations of positive stimuli. When the trained bees encountered "white" versus "vertical" in the first stage, they preferred "white"; but when they encountered the same combination in the last stage, they preferred "vertical." This result and related findings (Collett et al., 1993) suggest that the bees were learning not only the appropriate choice of stimulus at each stage but also the sequence in which the stimuli occurred.

Our experimental paradigm is not designed to examine sequence learning. It explores a different question, namely, whether bees can learn and recall visual stimuli by grouping them associatively. Having said this, there is some evidence for sequence learning, albeit weak, even in our experiments. The bees' overall success in negotiating the maze (i.e., in reaching the reward) is slightly better with the training sequences (ABC and A'B'C', see Fig. 2a) and with the exactly reversed sequences (CBA and C'B'A', see Fig. 4b) than with the other sequences, although the differences are not always significant. Under natural conditions, the trained and the reversed sequences would correspond to the route to the food source and from there back to the hive, respectively. Our bees did not have to trace the way back through the maze on their way home: they were released by raising the lid of the destination cylinder after they had

collected their reward. Therefore, the observed capacity for “backward chaining” in our experiments must be an inherent property of the way in which memories for landmarks are organized, and not a consequence of experiencing the reversed sequence on the way back to the hive. It is possible that the learning process that operates on the way to the food source “prepares” the bee for the sequence in which the cues should occur on the home-bound route as well. This possibility is consistent with other findings in which bees are able to use information learned primarily on the way to a food source, to navigate on the way back. For example, bees learn the distance to a food site based on visual cues encountered primarily on the way to the site, but they use this information on the homebound journey as well (Srinivasan et al., 1997).

Collett and Kelber (1988) showed that a bee’s memory can be “primed” by contextual cues: a bee can be trained to find food at one location when it encounters a particular configuration of landmarks, and at another location when it encounters a different configuration. Zhang et al. (1996, 1998) showed that bees can learn to negotiate a maze by using color signals as “signposts,” one color signaling a left turn and another a right turn. The present study shows that bees can learn to use information provided by the Indicator stimulus in one scene to determine their decisions in two further scenes that they encounter subsequently, and that they can do this despite the fact that the “priming” scene (the scene containing the Indicator stimulus) is no longer visible when the decisions are made.

Our data reveal interesting differences in the trained bees’ responses to the sectored and the ring stimuli: they tend to make more correct choices when the correct stimulus is the sectored pattern than when it is the ring pattern. Thus, in Fig. 1, bar 2 is significantly greater than bar 4 ($p < .001$) and bar 5 is significantly greater than bar 6 ($p < .001$). This trend is also evident when one inspects the second major column of Table 2. The preferences for C over C’ when bees encounter sequences of the stimulus set (ABC) are consistently and in some cases significantly greater than the preferences for C’ over C when bees encounter corresponding sequences of the stimulus set (A’B’C’). That is, in Table 2, $ACB > A’C’B’$ ($p < .05$), $ABC > A’B’C’$ ($p < .001$), $BCA > B’C’A’$ ($p > .10$), and $BAC > B’A’C’$ ($p < .02$). Although bees can be trained to prefer sectored or ring stimuli equally well in a dual-choice training paradigm (Horridge & Zhang, 1995), naive bees do exhibit a strong spontaneous preference for sectors over rings, presumably because the former pattern closely resembles a flower (Lehrer et al., 1995). This spontaneous preference might be unmasked when bees are trained on a task such as ours, which challenges their ability to recall stimuli associatively.

How accurate, or effective, is the ability of a bee to make associations between the various stimuli that she encounters in our experiments? We attempt to quantify this in terms of a model of associative recall, described below.

Consider bees that have learned to group the stimuli A, B, and C as belonging to one set, and the stimuli A’, B’, and C’ as belonging to another. In a learning test, a bee entering the maze must first determine whether the Indicator stimulus that she encounters is A or A’ (horizontal or vertical grating). This discrimination is crucial in determining whether she chooses B or B’ in the first decision stage. We can estimate the probability of a trained bee correctly distinguishing between A and A’ from previously published data

(Horridge, 1996a, b; Lehrer et al., 1985; Srinivasan et al., 1994) on the ability of bees to learn to discriminate between vertical and horizontal gratings in a Y-maze. The average probability of correct choices in such training experiments is .85. This discrimination capacity is roughly symmetrical; that is, it is approximately the same regardless of which orientation is rewarded (Srinivasan et al., 1994). We may thus denote this probability by $P(A) \cong P(A') = .85$. (Note that, in our experiment, the bee does not have to *choose* between A and A': she encounters only one of the Indicator stimuli at any given time, but she has to *determine* which one it is. The probability of making this discrimination correctly is estimated to be .85.) Once the bee has identified the Indicator stimulus, she has to recall, associatively, the other stimuli that belong to the same group. (If the Indicator stimulus is A, the relevant stimuli are B and C; if it is A', the relevant stimuli are B' and C'.) The bee then enters the first decision stage, where she encounters two stimuli, B and B' (blue and green colors). Here she must choose the stimulus which belongs to the group that she has recalled. In order to do this, the bee must be able to discriminate B and B'. In separate, unpublished training experiments using a Y-maze, we have established that bees trained to discriminate between the two colors used in our experiments choose the correct color with a symmetrical probability of $P(B) \cong P(B') = .93$. From published data, again using Y-mazes (Horridge, 1996b; Horridge & Zhang, 1995) we estimate that bees trained to discriminate between sectored and ring patterns choose the correct pattern with a symmetrical probability of $P(C) \cong P(C') = .85$. When the bee leaves the first decision stage, she has attempted to (a) identify the Indicator stimulus (A or A'), (b) decide, by associative recall, which other stimuli (B and C, or B' and C') are associated with the Indicator stimulus, and (c) choose appropriately between B and B'. The probability that the bee chooses correctly in the first decision stage therefore depends upon how well she performs in each of the above subtasks.

We define an "Association Coefficient," α , which assumes a value of unity when the probability of correct associative recall is 1.0, and a value of zero when the probability of correct associative recall is .5, i.e., when the bees show no association. It can be shown (see Appendix) that the association coefficient can be estimated from the known values of $P(A)$ and $P(B)$, and the experimentally measured probabilities of correct choices in the first decision stage, as follows.

The association coefficient α_{AB} , which describes the accuracy with which the Indicator stimulus A triggers recall of the associated stimulus B, is given by

$$\alpha_{AB} = \{a \cdot [P_{AB}(\text{corr}) - e] - b \cdot [P_{A'B'}(\text{corr}) - e] - .5 \cdot (a^2 - b^2)\} / [.5 \cdot (a^2 - b^2)], \quad (1)$$

where $a = P(A) \cdot [2 \cdot P(B) - 1]$, $b = [1 - P(A)] \cdot [1 - 2 \cdot P(B)]$, and $e = P(A) \cdot [1 - P(B)] + P(B) \cdot [1 - P(A)]$.

In the above expression $P_{AB}(\text{corr})$ is the experimentally measured probability with which the bee (correctly) chooses stimulus B in the first decision stage when the Indicator stimulus is A, and $P_{A'B'}(\text{corr})$ is the experimentally measured probability with which the bee (correctly) chooses stimulus B' in the first decision stage when the Indicator stimulus is A'. $P(A) = P(A') = .85$ is the probability of correctly discriminating a horizontal grating from a vertical one,

and $P(B) = P(B') = .93$ is the probability of correctly discriminating between a blue and a green disk.

Similarly, the association coefficient $\alpha_{A'B'}$, which describes the accuracy with which the Indicator stimulus A' triggers recall of the associated stimulus B' , is given by

$$\alpha_{A'B'} = \{a \cdot [P_{A'B'}(\text{corr}) - e] - b \cdot [P_{AB}(\text{corr}) - e] - .5 \cdot (a^2 - b^2)\} / [.5 \cdot (a^2 - b^2)]. \quad (2)$$

Analogously when the bee flies through a sequence ACB in a transfer test, the association coefficients pertaining to her choice behavior in the first decision stage can be estimated as follows:

$$\alpha_{AC} = \{a \cdot [P_{AC}(\text{corr}) - e] - b \cdot [P_{A'C'}(\text{corr}) - e] - .5 \cdot (a^2 - b^2)\} / [.5 \cdot (a^2 - b^2)] \quad (3)$$

and

$$\alpha_{A'C'} = \{a \cdot [P_{A'C'}(\text{corr}) - e] - b \cdot [P_{AC}(\text{corr}) - e] - .5 \cdot (a^2 - b^2)\} / [.5 \cdot (a^2 - b^2)], \quad (4)$$

where $P_{AC}(\text{corr})$ is the experimentally measured probability of (correctly) choosing C in the first decision stage when the Indicator stimulus is A , and $P_{A'C'}(\text{corr})$ is the measured probability of (correctly) choosing stimulus C' in the first decision stage when the Indicator stimulus is A' . Here $a = P(A) \cdot [2 \cdot P(C) - 1]$, $b = [1 - P(A)] \cdot [1 - 2 \cdot P(C)]$, and $e = P(A) \cdot [1 - P(C)] + P(C) \cdot [1 - P(A)]$.

Analogous expressions can be obtained for the association coefficients corresponding to the stimulus sequences BAC , $B'A'C'$, BCA , $B'C'A'$, etc.

Note that the association coefficient, as defined here, is a measure of the bee's ability to link visual stimuli together perceptually. It is quite different from the term "associative strength" which is simply a measure of the efficacy with which animals learn to associate a stimulus with a reward (e.g., Dickinson, 1980).

An approach similar to that described above can be used to model the bees' performance at the second decision stage, but there the analysis is complicated by the possibility that a bee's decision at the second stage is influenced not only by the Indicator stimulus, but also by her decision in the first stage. As this effect is not easily modeled, we have restricted our attention to performance in the first decision stage.

The values of the association coefficient, α , calculated using equations like (1) through (4) to model the bees' performance for various sequences at the first decision stage, are shown in Table 4. We see from this table that all 12 values of the association coefficient are greater than zero, indicating that the bees were displaying associative recall in all of the sequences that were tested. All of the values (except one) are greater than .5, six are greater than .70, and two are greater than .90. This implies that the ability of the trained bees to associate each stimulus they encounter with the appropriate set is, on the whole, quite good. In the cases where the value of α is close to unity, we infer

TABLE 4

Values of the Association Coefficient, α , Estimated as Explained in the Text from the Bees' Performance in the First Decision Stage for Various Stimulus Sequences

Type of test	Pattern sequence	Association coefficient α	Pattern sequence	Association coefficient α
Learning test	ABC	$P_{AB}(\text{corr}) = .754$ $\alpha_{AB} = .838^{\#}$	A'B'C'	$P_{A'B'}(\text{corr}) = .743$ $\alpha_{A'B'} = .813^{\#}$
Transfer test 1	ACB	$P_{AC}(\text{corr}) = .756$ $\alpha_{AC} = .930^*$	A'C'B'	$P_{A'C'}(\text{corr}) = .569$ $\alpha_{A'C'} = .396^*$
Transfer test 2	BAC	$P_{BA}(\text{corr}) = .680$ $\alpha_{BA} = .602^{\#}$	B'A'C'	$P_{B'A'}(\text{corr}) = .695$ $\alpha_{B'A'} = .644^{\#}$
Transfer test 3	BCA	$P_{BC}(\text{corr}) = .761$ $\alpha_{BC} = .848^{\#}$	B'C'A'	$P_{B'C'}(\text{corr}) = .679$ $\alpha_{B'C'} = .614^{\#}$
Transfer test 4	CAB	$P_{CA}(\text{corr}) = .697$ $\alpha_{CA} = .832^*$	C'A'B'	$P_{C'A'}(\text{corr}) = .742$ $\alpha_{C'A'} = .960^*$
Transfer test 5	CBA	$P_{CB}(\text{corr}) = .709$ $\alpha_{CB} = .660^{\#}$	C'B'A'	$P_{C'B'}(\text{corr}) = .652$ $\alpha_{C'B'} = .533^{\#}$

* Pattern-pattern associations.

Color-pattern associations.

that the bees' performance in choosing the correct stimulus is limited primarily by errors in discriminating the stimuli *visually*, rather than by errors in associative recall. The sequence for which α has the lowest value (sequence A'C'B') involves the stimulus C' (rings), which, in accordance with our observations (see above), is a stimulus for which the bees tend to show a relatively low preference.

On the whole, the trained bees seem to be just as accurate at establishing associations between patterns, as between patterns and colors. The mean value of α in tests that measure pattern-pattern associations (marked by * in Table 4) is not significantly different ($p > .4$) from that in tests that measure color-pattern associations (marked by # in Table 4). However, if one ignores the unusually low value of α obtained for the sequence A'C'B', then pattern-pattern associations are, on average, significantly better than color-pattern associations ($p < .02$). This question, however, needs further investigation.

The ability to group and recall visual stimuli associatively could be important when a bee forages at more than one location, because the routes to the two sites will probably present different sets of landmarks. When a bee commences her flight to one of the sites, the first salient landmark that she encounters on this route would trigger recall of all of the other landmarks that she can expect to encounter on the way. A "content addressable" memory of this nature would provide the bee with a simple and effective means of organizing and recalling the appropriate items from the numerous "snapshots" that she has stored in memory for navigational purposes. It is also likely (from the results of previous studies (Collett, 1996; Collett et al., 1993; Judd and Collett, 1998) that, during the journey, the appearance of each landmark triggers recall of the next one to be expected, although our own experiments did not address this particular question.

Our work so far suggests that bees can group, in an associative way, two sets of three landmarks, where each set would represent a different route under

natural conditions. These landmarks are stored in memory in such a way that those belonging to a given set are associated closely with each other. Further investigation is needed to examine how many different sets of landmarks can be remembered in this way, and the number of landmarks that can be grouped and recalled within each set.

APPENDIX

Here we explain how performance in the first decision stage can be modeled and used to quantify the bee's capacity to recall stimuli associatively.

Consider the sequences ABC and A'B'C' and a test in which the Indicator stimulus is A. By the time the bee leaves the first decision stage, she has attempted to (a) identify the Indicator stimulus (A or A'), (b) decide, by associative recall, which other stimuli (B and C, or B' and C') are associated with the Indicator stimulus, and (c) choose appropriately between B and B'.

Let $P_{AB}(\text{ass})$ denote the probability that the bee correctly recalls, by association, the stimulus B when she encounters the Indicator stimulus A. $P_{AB}(\text{ass}) = 1.0$ represents perfectly accurate associative recall; $P_{AB}(\text{ass}) = .5$ denotes no associative recall whatsoever (random recall). Values of $P_{AB}(\text{ass})$ that are lower than .5 would represent a tendency for the bee to make the wrong association more often than the right one. $P_{AB}(\text{ass}) = .0$ implies that the bee always makes the wrong association. Similarly, let $P_{A'B'}(\text{ass})$ denote the probability that the bee correctly recalls, by association, the stimulus B' when she encounters the Indicator stimulus A'. $P_{AB}(\text{ass})$ and $P_{A'B'}(\text{ass})$ are measures of the bee's accuracy in recalling the stimuli B and B' associated with A and A', respectively. We wish to estimate their values from the data.

We denote by $P_{AB}(\text{corr})$ the (measured) probability of the bee choosing the correct stimulus (B) at the first decision stage when the Indicator stimulus is A. We propose that this probability is given by the sum of four terms, each representing a different contingency:

(i) The bee identifies the Indicator stimulus correctly to be A (this has a probability $P(A)$), makes the appropriate association, B (this has a probability $P_{AB}(\text{ass})$), and, finally, chooses the stimulus that is appropriate to this association in the first decision stage (this has a probability $P(B)$). Assuming that these are independent events, the probability of this contingency is $P_{AB}(\text{ass}) \cdot P(A) \cdot P(B)$.

(ii) The bee identifies the Indicator stimulus correctly to be A (this has a probability $P(A)$), but makes the wrong association, B' (this has a probability $[1 - P_{AB}(\text{ass})]$), and, finally, chooses the stimulus that is not appropriate to this association, B, in the first decision stage (this has a probability $[1 - P(B)]$). This contingency will also lead to the correct stimulus being chosen in the first decision stage, because the bee has made two opposite errors. The probability of this contingency is $[1 - P_{AB}(\text{ass})] \cdot P(A) \cdot [1 - P(B)]$.

(iii) The bee identifies the indicator stimulus incorrectly to be A' (with a probability $[1 - P(A)]$), makes the appropriate association, B' (this has a probability $P_{A'B'}(\text{ass})$), and, finally, chooses the stimulus that is not appropriate to this association, B, in the first decision stage (this has a probability $[1 - P(B)]$). This contingency will also lead to the correct stimulus being chosen at the first decision stage, because the bee has again made two opposite errors. The probability of this contingency is $P_{A'B'}(\text{ass}) \cdot [1 - P(A)] \cdot [1 - P(B)]$.

(iv) The bee identifies the indicator stimulus incorrectly to be A' (this has a probability $[1 - P(A)]$), makes the wrong association, that is, it recalls B rather B' (this has a probability $[1 - P_{A'B'}(\text{ass})]$), and, finally, chooses the stimulus that is appropriate to this association, B , in the first decision stage (this has a probability $P(B)$). This contingency will also lead to the correct stimulus being chosen at the first decision stage, because the bee has made two opposite errors. The probability of this contingency is

$$[1 - P_{A'B'}(\text{ass})] \cdot [1 - P(A)] \cdot P(B).$$

We may therefore write

$$\begin{aligned} P_{AB}(\text{corr}) &= P_{AB}(\text{ass}) \cdot P(A) \cdot P(B) + [1 - P_{AB}(\text{ass})] \cdot P(A) \cdot [1 - P(B)] \\ &\quad + P_{A'B'}(\text{ass}) \cdot [1 - P(A)] \cdot [1 - P(B)] \\ &\quad + [1 - P_{A'B'}(\text{ass})] \times [1 - P(A)] \cdot P(B). \end{aligned} \quad (\text{A1})$$

Similarly, consider the situation when the Indicator stimulus is A' . Using an argument similar to that outlined above, it can be shown that $P_{A'B'}(\text{corr})$, the (measured) probability of the bee choosing the correct stimulus (B') at the first decision stage when the Indicator stimulus is A' , can be expressed as

$$\begin{aligned} P_{A'B'}(\text{corr}) &= P_{A'B'}(\text{ass}) \cdot P(A) \cdot P(B) + [1 - P_{A'B'}(\text{ass})] \cdot P(A) \cdot [1 - P(B)] \\ &\quad + P_{AB}(\text{ass}) \cdot [1 - P(A)] \cdot [1 - P(B)] \\ &\quad + [1 - P_{AB}(\text{ass})] \times [1 - P(A)] \cdot P(B). \end{aligned} \quad (\text{A2})$$

In Eqs. (A1) and (A2), $P(A)$ and $P(B)$ are known (see above), while $P_{AB}(\text{corr})$ and $P_{A'B'}(\text{corr})$ are given by our data (see Table 4). Equations (A1) and (A2) therefore represent two linear equations in the two unknowns, $P_{AB}(\text{ass})$ and $P_{A'B'}(\text{ass})$, that we wish to determine. They can be solved to yield

$$P_{AB}(\text{ass}) = \{a \cdot [P_{AB}(\text{corr}) - e] - b \cdot [P_{A'B'}(\text{corr}) - e]\} / (a^2 - b^2) \quad (\text{A3})$$

and

$$P_{A'B'}(\text{ass}) = \{a \cdot [P_{A'B'}(\text{corr}) - e] - b \cdot [P_{AB}(\text{corr}) - e]\} / (a^2 - b^2), \quad (\text{A4})$$

where $a = P(A) \cdot [2 \cdot P(B) - 1]$, $b = [1 - P(A)] \cdot [1 - 2 \cdot P(B)]$, and $e = P(A) \cdot [1 - P(B)] + P(B) \cdot [1 - P(A)]$.

For convenience, we define an "Association Coefficient," α_{AB} , which is related to $P_{AB}(\text{ass})$ by $\alpha_{AB} = [P_{AB}(\text{ass}) - .5] / .5$. The association coefficient is, intuitively, a better way of expressing the bee's ability to recall stimuli associatively than is the probability of correct associative recall. This is because the association coefficient assumes a value of unity when the probability of correct associative recall is 1.0, a value of zero when the probability of correct associative recall is .5, i.e., when the bees show no association, and negative values when the bees make wrong associations more often than correct ones. Inserting the expres-

sion for $P_{AB}(\text{ass})$ from (A3) into the above relationship, we obtain an expression for the association coefficient α_{AB} :

$$\alpha_{AB} = \{a \cdot [P_{AB}(\text{corr}) - e] - b \cdot [P_{A'B'}(\text{corr}) - e] - .5 \cdot (a^2 - b^2)\} / [.5 \cdot (a^2 - b^2)]. \quad (\text{A5})$$

α_{AB} describes the accuracy with which the Indicator stimulus A triggers recall of the associated stimulus B.

Similarly, we can obtain an expression for the association coefficient $\alpha_{A'B'}$, which describes the accuracy with which the Indicator stimulus A' triggers recall of the associated stimulus B':

$$\begin{aligned} \alpha_{A'B'} &= [P_{A'B'}(\text{ass}) - .5] / .5 \\ &= \{a \cdot [P_{A'B'}(\text{corr}) - e] - b \cdot [P_{AB}(\text{corr}) - e] - .5 \cdot (a^2 - b^2)\} / [.5 \cdot (a^2 - b^2)]. \end{aligned} \quad (\text{A6})$$

The values of α_{AB} and $\alpha_{A'B'}$ can be estimated from the known values of $P(A)$ and $P(B)$ and the measured values of $P_{AB}(\text{corr})$ and $P_{A'B'}(\text{corr})$ (probabilities of correct choice of B and B' at the first decision stage when the Indicator stimulus is A or A', respectively).

Analogous expressions for α_{AC} and $\alpha_{A'C'}$ can be derived for the stimulus sequences ACB and A'C'B', etc. [see, for example, Eqs. (3) and (4) under Discussion]. The values of association coefficient calculated for all of the sequences are given in Table 4.

REFERENCES

- Collett, T. S. (1996). Insect navigation en route to the goal—multiple strategies for the use of landmarks. *Journal of Experimental Biology*, **199**, 227–235.
- Collett, T. S., Fry, S. N., & Wehner, R. (1993). Sequence learning by honeybees. *Journal of Comparative Physiology, Series A*, **172**, 693–706.
- Collett, T. S., & Kelber, A. (1988). The retrieval of visuo-spatial memories by honeybees. *Journal of Comparative Physiology, Series A*, **163**, 145–150.
- Dickinson, A. (1980). *Contemporary animal learning theory* (pp. 123). Cambridge University Press.
- Dyer, F. C. (1991). Bees acquire route-based memories but not cognitive maps in a familiar landscape. *Animal Behaviour*, **41**, 239–246.
- van Hateren, J. H., Srinivasan, M. V., & Wait, P. B. (1990). Pattern recognition in bees: Orientation discrimination. *Journal of Comparative Physiology, Series A*, **167**, 649–654.
- Horridge, G. A. (1996a). Pattern vision of the honeybee (*Apis mellifera*)—The significance of the angle subtend by the target. *Journal of Insect Physiology*, **42**, 693–703.
- Horridge, G. A. (1996b). The relation between pattern and landmark vision of honeybee (*Apis mellifera*). *Journal of Insect Physiology*, **42**, 373–381.
- Horridge, G. A., & Zhang, S. W. (1995). Pattern vision in honeybees (*Apis mellifera*)—Flower-like patterns with no predominant orientation. *Journal of Insect Physiology*, **41**, 681–688.
- Judd, S. P. D., & Collett, T. S. (1998). Multiple stored views and landmark guidance in ants. *Nature*, **392**, 710–714.
- Lehrer, M., Horridge, G. A., Zhang, S. W., & Gadagkar, R. (1995). Shape vision in bees—Innate preference for flower-like patterns. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, **347**, 123–137.

- Lehrer, M., Wehner, R., & Srinivasan, M. V. (1985). Visual scanning behaviour in honeybees. *Journal of Comparative Physiology, Series A*, **157**, 405–415.
- Menzel, R., Geiger, K., Chittka, L., Joerges, J., Kunze, J., & Mueller, U. (1996). Bees travel novel homeward routes by integrating separately acquired vector memories. *Journal of Experimental Biology*, **199**, 141–146.
- Srinivasan, M. V., & Lehrer, M. (1988). Spatial acuity of honeybee vision and its spectral properties. *Journal of Comparative Physiology, Series A*, **162**, 159–172.
- Srinivasan, M. V., Zhang, S. W., & Bidwell, N. (1997). Visually mediated odometry in honeybees. *Journal of Experimental Biology*, **200**, 2513–2522.
- Srinivasan, M. V., Zhang, S. W., & Witney, K. (1994). Visual discrimination of pattern orientation by honeybees—performance and implications for cortical processing. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, **343**, 199–210.
- Wehner, R., Bleuler, S., & Shah, D. (1990). Bees navigate using vectors and routes rather than maps. *Naturwissenschaften*, **77**, 479–482.
- Wehner, R., Michel, B., & Antonsen, P. (1996). Visual navigation in insects: Coupling of egocentric and geocentric information. *Journal of Experimental Biology*, **199**, 129–140.
- Zhang, S. W., Bartsch, K., & Srinivasan, M. V. (1996). Maze learning by honeybees. *Neurobiology of Learning and Memory*, **66**, 267–282.
- Zhang, S. W., Lehrer, M., & Srinivasan, M. V. (1998). Eye-specific learning of routes and “signposts” by walking honeybees. *Journal of Comparative Physiology, Series A*, **182**, 747–754.