The binding and recall of snapshot memories in wood ants (*Formica rufa* L.)

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Summary

Insects can locate spatial goals by means of 2-D retinotopic views of the surrounding landmarks, which they memorise from the vantage point of the goal. Wood ants acquire such snapshot memories while fixating conspicuous landmarks with frontal retina, and their snapshots extend horizontally at least 120° into the periphery. Are spatially separate items within such an extended snapshot bound together so that a snapshot is recalled as a whole, or are its components recognised individually?

We approached this question by training ants to find food midway between two upright black cylinders of different sizes and then examined where they searched when they were given two cylinders of the same size. If the ants know which cylinder replaces the small cylinder and which the large, they should search at a position where the two equal-sized cylinders subtend the same angles as do the training cylinders when viewed from the feeder. Ants conformed to this prediction under one condition, searching at a shorter distance from the substitute for the large cylinder than from the substitute for the small cylinder. But, under another condition, ants were unable to distinguish between the two equal-sized cylinders. Ants failed when white curtains completely surrounded the platform on which the cylinders were placed. They succeeded when one side of the platform had a patterned curtain.

We suggest that ants take two snapshots at the feeding site, one when facing the small cylinder and one when facing the large cylinder, and that each snapshot includes the patterned curtain, if it is there. Ants will view the patterned curtain with the lateral retina of one eye when facing the small cylinder and with the lateral retina of the other eye when facing the large cylinder. Our data suggest that there may be associative links between these spatially separate components of the snapshot, which cause the memory of the small cylinder or the large cylinder to be recalled according to which eye sees the curtain. It seems that an extended snapshot not only enhances the accuracy of localisation but can also increase the reliability of snapshot recall, provided that the components of a snapshot are bound together.

Key words: wood ant, landmark guidance, snapshot, binding, configural learning, navigation.

Introduction

Much evidence (for reviews, see Collett and Zeil, 1998; Collett and Collett, 2002; Zeil et al., 2003) suggests that insects pinpoint a place by means of two-dimensional (2-D) retinotopic memories of the surrounding landmarks viewed from the place – a snapshot. Insects can store several snapshots at once and so must ensure that they recall the appropriate snapshot. Correct recall must often be hard to accomplish because of the similarity of natural objects used as landmarks when filtered through an insect's low acuity retina (Land, 1997). In the present paper, we make use of recent results on wood ants (Durier et al., 2003) to analyse how ants might enhance the reliability of snapshot recall. Our current understanding of snapshot use by wood ants is that an ant stores one or more snapshots at a food site while fixating conspicuous landmarks. These snapshots encode the angular size of the fixated landmark from the vantage point of the food site. They also extend horizontally beyond the fixated landmark to

include features at least as far as 120° into the periphery (Durier et al., 2003). In order to return to the food site, the ants fixate and approach landmarks that look smaller than their apparent size in their snapshots. The site is regained when both the ant's current view of the landmark matches the stored angular size of the frontally viewed landmark and peripheral landmarks assume their stored retinal positions.

Contextual cues like the surrounding panorama, or perhaps an insect's motivational state, seem to prime the recall of appropriate snapshots (reviewed by Collett and Collett, 2002). The problem we investigate here is how ants might select between snapshots within a single context. Does the recall of a snapshot just depend on recognising the landmark that is fixated, or do other regions of the snapshot contribute to recall? And are the different spatial regions of a snapshot linked together so that each component of a snapshot can prime the recall of other components? We have approached these

questions by analysing the behaviour of ants that are trained to a food site that lies midway between two cylinders of different sizes and then tested with two cylinders of the same intermediate size. Since it is unlikely (Zollikofer et al., 1995) that the second cylinder is visible when the ant fixates the first cylinder, questions arise as to whether ants store independent views of the large and small cylinders, and, if so, what cues are employed to recall the correct memory when ants face a particular cylinder. In tests with similar-sized cylinders, accurate recall cannot be accomplished by relying solely on the appearance of the cylinder. Other cues are required. This experiment was therefore performed under two conditions. In the first condition, the rectangular arena on which the experiment was conducted was entirely surrounded by white curtains, thus minimising extra array cues. In the second condition, the curtains on one side were patterned, so as to provide a strong additional cue.

Materials and methods

Ants

Queen-right colonies of wood ants (*Formica rufa* L.) were housed and maintained in 670-litre plastic tanks. For experiments, groups of foragers (~50 ants) were collected from the nest and taken in a plastic box to an arena where they could forage for sucrose. Those ants that found sucrose rapidly were marked individually with drops of coloured paint. Training continued with this smaller group (~12 ants). Ants were returned to the nest after feeding, whereupon they regurgitated the sucrose to nest mates and could soon be collected for another trial. Ants made about three foraging trips per hour.

Apparatus

Experiments were performed in a 2.7 m×4.8 m rectangular arena with a white plastic floor that was illuminated by banks of high-frequency fluorescent lights fixed above a false translucent plastic ceiling. The position on the floor of a glass microscope slide with a drop of sucrose on it was marked by two upright black cylinders (Fig. 1). The arena was completely surrounded by floor-to-ceiling curtains. These were either all white or one side was patterned with randomly arranged black shapes, each of which subtended at least 5° when viewed from the starting point. The patterned curtain covered the side of the arena that the ants approached so that when ants were close to the food the cylinders were always viewed against a white background (Fig. 1).

Training and testing

Ants were placed individually in a small triangular starting pen (S in Fig. 1) placed in a fixed position relative to the cylinders. They could leave the pen through an exit hole in the corner nearest to the array. Ants eventually found the slide, ingested the sucrose and usually returned close to the starting pen. To speed up training, another ant was released as soon as the previous ant had arrived at the feeder. For the first few training trips, ants were corralled by rectangular
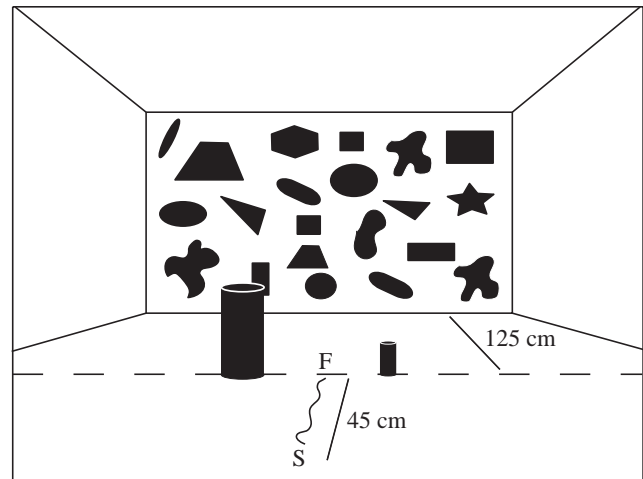

Fig. 1. Sketch of apparatus with the patterned curtain in place along one side of the arena. Between each training trial, cylinders, starting pen (S) and feeder (F) were shifted *en bloc* to a new position along a 2.5 m line indicated by the broken line. The patterned curtain was only fixed in place during the experiment illustrated in Fig. 5. For the other experiments, all the curtains surrounding the arena were white.

blocks of Perspex that surrounded the array. Between foraging trips, the starting pen, the array and the feeder were moved *en bloc* along an imaginary line connecting the landmarks (Fig. 1), so that the feeding site was specified as far as possible by the array rather than by other room cues. The arena floor was cleaned periodically with water and with alcohol before each test. Tests in which individual ants searched for food with no slide present were first given after about 20 foraging trips. Three to five further training trips preceded each subsequent test.

When ants were trained with cylinders of unequal sizes, two groups of ants were used. One group had the smaller cylinder on their left and the other had it on their right. When there was a similar asymmetry in tests, two sets of tests were given with the smaller cylinder to one side in one test and to the other in the second test. Data from the two experiments or test types have been made compatible and then pooled.

Video recording

The ants' trajectories in tests were tracked with a fixed camera hidden in the false ceiling 3 m above the centre of the arena. It was convenient to move the cylinder array to one of three standard positions on the arena floor before each unrewarded test. The test positions were not used in training. The camera (Sony EVI-D30) has movable optics, allowing a high-resolution image of any part of the arena to be captured. The camera is controlled by a PC (Pentium II 233 MHz) running custom software (Fry et al., 2000). The system extracts the ant's position and longitudinal orientation at 50 Hz. Before analysis, the output was smoothed by taking a moving average with a window size of nine frames. The ant's path was recorded for 6 min or until the ant approached so close to one of the cylinders that the tracking system 'lost' the ant.

Data analysis

The results are mostly presented as two-dimensional distributions in which individual data points are not independent. For statistical tests between distributions, each individual test trial from an ant was summarised to give a single data point, and comparisons were made between populations of these data points. Search distributions were compared by recording the mean position of an ant along the line connecting the landmarks for each test trial, using these mean 'X positions' as independent data points. Note this test does not provide statistical information about the position of the peaks of the distributions. The relative fixation of the two cylinders during each of the approaches of Fig. 5 was calculated as the number of frames in which the ant fixated ($\pm20^\circ$) one cylinder divided by the number of frames in which the ant fixated the other cylinder.

Results

Ants trained with equal-sized cylinders

We start by analysing in more detail a previously published experiment (Durier et al., 2003) to illustrate the precision of the wood ants' search behaviour when a single snapshot can guide the ants to a feeding site. Ants were trained to find food midway between two cylinders of the same size (7.5 cm in diameter and 22.5 cm high) placed 70 cm apart. The ants' search was then recorded when the feeder was absent, either using the training cylinders (Fig. 2A) or a test configuration (Fig. 2B) in which one cylinder was smaller (5 cm×15 cm)
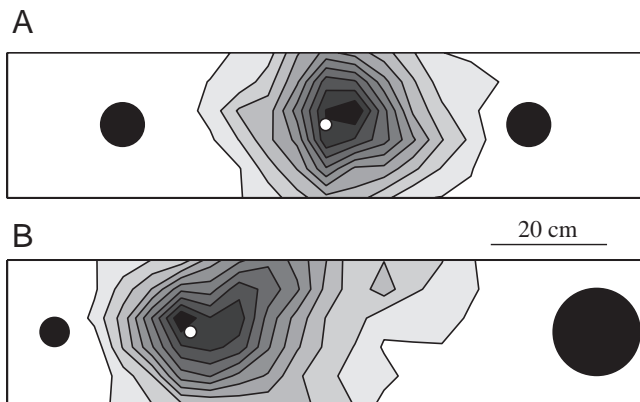

Fig. 2. Where ants search for an absent feeder that is normally placed between two cylinders. Results pool data from two experiments in which the starting pen was either 40 cm or 100 cm from the feeder. (A) Tests with training cylinders of equal size. (B) Tests in which one cylinder is smaller and one larger than in training. Here, and in the remaining figures, the position of the feeder predicted by the snapshot is shown by a white circle. The contour plot represents the percentage of time the ants spent in each area; the darker the grey, the higher the percentage. The darkest area represents 9.8% of the total search in the training condition (*N*=71 paths) and 3.81% in the test condition (*N*=93 paths). The mean X positions (see Materials and methods) of the two distributions differ significantly (Wilcoxon two-sample test; *P*<0.001, *t*=8.04, d.f.=162).

than in training, the other was larger (15 cm×45 cm) and the separation between them was increased to 93.4 cm. In both types of test, ants searched mostly where the angular size of each cylinder matched the angular size of the training cylinders viewed from the food site.

Evidence that ants facing a particular cylinder position themselves relative to that cylinder comes from a test in which each cylinder indicated a different search position. In training, the food was midway between two cylinders of the same size (15 cm in diameter and 45 cm high) separated by 70 cm. Ants searched accurately when tested with this configuration (Fig. 3A). Tests were then given with cylinders of half the size (7.5 cm×22.5 cm) separated by 70 cm. If ants used the apparent size of each cylinder to control their search, they should search in two positions, at 17.5 cm from each cylinder, which is roughly what they do (Fig. 3B). The data of Fig. 3C,D show that the peak associated with each cylinder is generated primarily when the
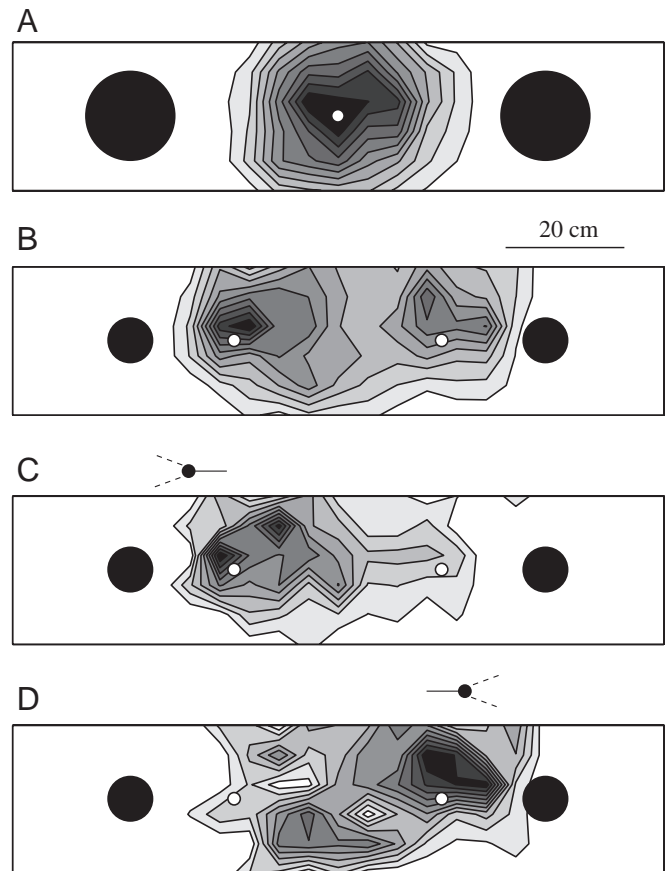

Fig. 3. Search distributions for an absent feeder that is normally placed between two cylinders. Starting pen was 70 cm from the feeder. (A) Tests with training cylinders of equal size. (B) Tests with two smaller cylinders. The darkest area represents 18.98% of the search in the training condition (*N*=41 paths) and 4.32% in the test condition (*N*=42 paths). (C,D) Search distribution with small cylinders, when ants were fixating within $\pm20^\circ$ of the centre of one or other cylinder, as shown by the schematic ant above each distribution. The mean X positions of the two distributions differ significantly (Student's *t*-test; *P*=0.005, *t*=2.91, d.f.=72).

ants face that cylinder, further supporting the hypothesis (Judd and Collett, 1998; Nicholson et al., 1999; Durier et al., 2003) that ants record snapshots when facing a landmark. Since the two training cylinders have the same angular size when viewed from the goal, it is not clear whether the ants have acquired a separate snapshot for each cylinder or whether they have a single snapshot that they can apply to either cylinder.

Ants trained with a large and a small cylinder

What happens when the two cylinders viewed from the food site have different angular sizes? Do ants learn a separate snapshot for each cylinder? We trained ants to a food site that lay midway between a small cylinder (5 cm in diameter and 15 cm high) and a large cylinder (15 cm in diameter and 45 cm high) that were 93.4 cm apart. Two separate experiments were performed with this arrangement of cylinders. In one experiment, the curtains surrounding the rectangular experimental arena were all white. In the other experiment, a black-and-white, patterned, floor-to-ceiling curtain was hung along the wall that the ants faced as they approached the cylinders (Fig. 1), so that when an ant turned to face the cylinder on its left, the curtain would be seen by its right eye, and *vice versa*. Ants were tested both with the training configuration and with cylinders of intermediate size (7.5 cm×22.5 cm) separated by 93.4 cm.

In the experiment with white curtains, ants tested with the training array searched in roughly the predicted position (Fig. 4A). In tests with medium-sized cylinders, the peak of the search distribution shifted a little way towards the medium-sized cylinder that replaced the large cylinder (Fig. 4B). But the peak was still a long way from the predicted site.
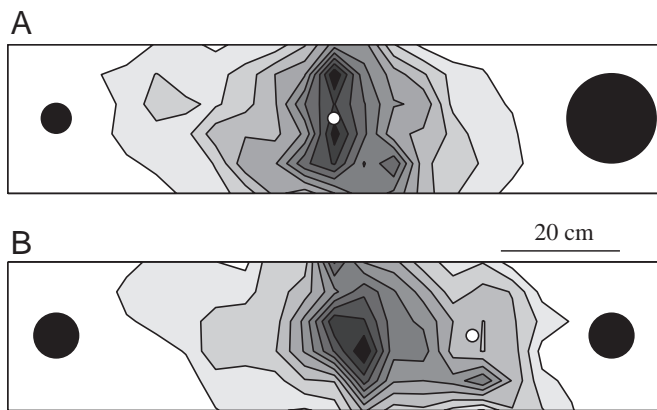

Fig. 4. Search distributions for an absent feeder that is normally placed between two cylinders. A white curtain surrounded the experimental arena. Starting pen was 45 cm from the feeder. (A) Tests with one small and one large training cylinder. (B) Tests with two equal-sized cylinders. The darkest area represents 7.97% of the search in the training condition (N=53 paths) and 3.99% in the test condition (N=57 paths). The mean X positions of the two distributions differ significantly (Student's t-test; P=0.002, t=3.24, d.f.=108) from each other and from the predicted food sites (Student's t-test; A: P=0.005, t=2.96, d.f.=52; B: P<0.001, t=10.43, d.f.=56).

These results cast doubt on several hypotheses. (1) Ants learn the apparent size of only one cylinder viewed from the goal, which they try and match to both the large and the small cylinders. If this were the case, ants searching between the two medium-sized cylinders should generate two search peaks rather than one. (2) Ants acquire a single snapshot that mixes the two cylinders (e.g. a superposition of two attractors, producing a minimum that is located in an intermediate position). According to this hypothesis, the peak of the search distribution should not be close to the predicted feeding site in tests with the training cylinders. (3) Ants learn two snapshots, one for each cylinder, and recall them appropriately. Had this been the case, the search peak should have been in the predicted location in tests with the medium-sized cylinders. (4) Ants learn the appearance of the two cylinders correctly but cannot identify them accurately in tests with either pair of cylinders. According to this hypothesis, the search peak would have been incorrectly positioned in tests with both the training and the medium-sized cylinders. One hypothesis that is consistent with the results of Fig. 4 is a variant of hypothesis (4) above: ants learn the two cylinders correctly and can usually identify them correctly in the training conditions in a direct approach from the starting point, when the cylinders can be recognised by their size. But ants are more likely to misidentify the cylinders when they are the same size, so that extra supporting cues are needed for their recognition.

With the white curtains, too little information may have been available for disambiguating the two test cylinders. The additional cue given by the patterned curtain dramatically changed the position of the ants' search peak. The ants now searched in the expected position with the training cylinders (Fig. 5B) and they searched close to the predicted position when tested with the medium-sized cylinders (Fig. 5D). These results are consistent with the ants recognising the cylinders correctly.

The ants' search behaviour when trained with the patterned curtain is reflected in their approaches to the food site. In tests with the training cylinders, ants walked relatively straight to the goal and tended to fixate the two cylinders with roughly equal frequency (Fig. 5A). Ants fixated the larger cylinder more than the smaller cylinder in approximately half of the approaches (28 out of 61 approaches, P>0.1, sign test). When approaching the medium-sized cylinders (Fig. 5C), ants fixated the cylinder that replaced the large cylinder more often (64 out of 91 approaches, P<0.01, sign test) than that replacing the small cylinder. This behaviour is consistent with the ants tending to fixate landmarks that are smaller than their expected size as viewed from the feeding site (Durier et al., 2003).

Likewise, the overall direction of the ants' approach was aimed at the feeding site in tests with the training cylinders. In tests with the medium-sized cylinders, the approach was biased slightly, but significantly, towards the cylinder that replaced the large one. The mean of the distribution of points at which each trajectory intersected a line parallel to and 5 cm in front of the line between the cylinders was almost midway between the training cylinders (mean ± s.D.=0.07±10.4 cm, N=59, where 0 cm is the midpoint). With the medium-sized cylinders, the
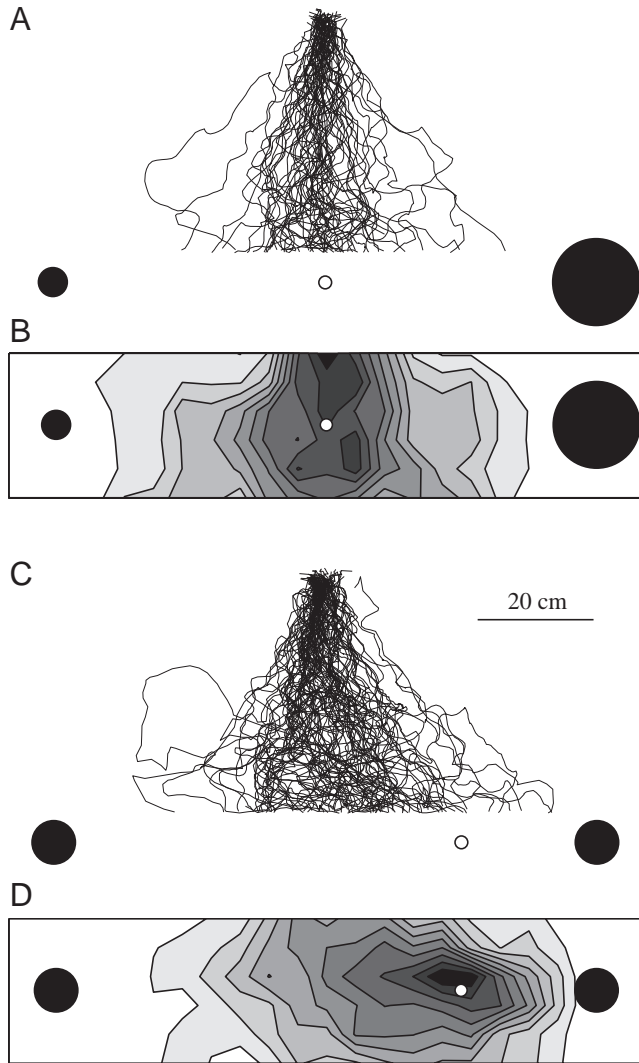
Fig. 5. Approaches to an absent feeder (A,C) and search distributions between the two cylinders (B,D). The curtain on one side of the arena is patterned. Starting pen was 45 cm from the feeder. (A,B) Tests with small and large training cylinders. (C,D) Tests with two medium-sized cylinders. The darkest area represents 5.79% of the search in the training condition (*N*=61 paths) and 6.36% in the test condition (*N*=91 paths). The mean X positions of the two distributions differ significantly (Student's *t*-test; *P*<0.001, *t*=5.14, d.f.=150). Because of the long tail of the distribution, the mean X position of the distribution in D differs significantly from the predicted food site (Student's *t*-test; *P*<0.001, *t*=12.80, d.f.=90), while the mean X position of the distribution in B does not differ from the predicted food site (Student's *t*-test; *P*=0.324, *t*=0.99, d.f.=60). Distribution in D differs significantly from the distribution of Fig. 3B (Student's *t*-test; *P*=0.03, *t*=2.194, d.f.=146).

intersection points shifted towards the substitute for the large cylinder (mean ± S.D.=5.8±12.6 cm, *N*=90). The two distributions differ significantly (Wilcoxon test, *t*=3.00, *P*<0.01).

Taken together, the searches and approaches suggest that (1) ants identified the two training cylinders, (2) they knew which medium-sized cylinder corresponded to which training cylinder, and (3) on their approach to the medium-sized cylinders they fixated preferentially the cylinder that appeared smaller than its expected size when viewed from the feeder (Durier et al., 2003).

Ants trained with the white curtains behaved somewhat differently. Ants approaching the training cylinders fixated the small cylinder significantly more frequently than the large cylinder (37 out of 53 approaches, *P*<0.01, sign test), suggesting that without the patterned curtain they often misidentified the training cylinders. However, in tests with the equal-sized cylinders, they approached the medium-sized cylinder replacing the large training cylinder more than the other medium-sized cylinder (43 out of 57 approaches, *P*<0.01, sign test), indicating that, to some degree, these ants do know which cylinder is which.

Discussion

Ants that were trained to find food at a point that is defined by two cylinders and tested with cylinders of different sizes searched where the test cylinders have the same apparent size as the training cylinders viewed from the feeding site (Wehner et al., 1996; Durier et al., 2003). We have shown here that the behaviour of ants trained to find food midway between two cylinders of different sizes and tested with cylinders of the same intermediate size varied according to what they saw with their peripheral retina. They searched in the predicted location when a patterned curtain covered one wall of the arena during training and testing. Ants failed to search correctly in similar tests when the arena was surrounded entirely by white curtains during training and testing. The patterned curtain helped the ants identify which medium-sized cylinder corresponds to the large training cylinder and which to the small.

There are three rather different ways in which ants might use the patterned curtain to identify the medium-sized cylinders correctly. The first is to store two snapshots at the feeder site, one for each cylinder, and to use the retinal position of the image of the curtain to help decide which snapshot to recall. The second is to store just one snapshot when fixating one cylinder and ignore the other cylinder, again using the retinal position of the curtain to distinguish between the cylinders. The third is to store one snapshot and to use the patterned curtain as a polarising or compass cue that tells the ants what orientation they should adopt when they search close to the food site (c.f. Åkesson and Wehner, 2002; Collett and Baron, 1994). The data of Figs 3 and 4 suggest that in some situations both cylinders do contribute to the ants' search. The evidence that both cylinders contribute to the ants' behaviour in the experiment with the patterned curtain is no more than circumstantial. It comes from the roughly equal fixation of the two cylinders in approaches to the training array, which suggests that each cylinder is identified correctly. There is also no evidence that, when the ants search close to the usual feeder site, they fixate one cylinder more than the other or that their pattern of fixation differs with the presence or absence of the patterned curtain. In principle, direct evidence of two snapshots could be obtained by separating the medium-sized

cylinders as in Fig. 3C,D. The hypothesis would then be supported if individual ants searched at a different distance from each cylinder. Unfortunately, the search behaviour of individual ants was too erratic for this test to be feasible.

What we can say with certainty is that the curtain was essential for ants to identify the medium-sized cylinders correctly. By fixating one or other cylinder, the ant determines the retinal positions of other visual features in the room. We suggest that these simultaneously viewed features, with retinal position as one parameter, are bound together into a single snapshot. Specifically, when facing and learning the appearance of one cylinder, the ant also learns the retinal position of the patterned wall and forges associative links between these two components of the snapshot. The view of the patterned wall in a particular retinal position when ants face one or other cylinder can then help them recall the correct memory and identify the cylinder that they are facing. The failure of ants to search correctly when the surroundings are all white and the landmarks are identical supports the hypothesis of such an associative link between memories of the peripherally viewed wall and the centrally viewed cylinder. In general, associative links between spatially separated parts of a snapshot will make recognition more robust. In recent years, there have been a number of studies showing that bees can form associative links between different visual stimuli (Srininvasan et al., 1998; Zhang et al., 1999; Giurfa et al., 2001) and that the same visual stimuli can be bound together in different combinations in different contexts (Fauria et al., 2000). The present study indicates that associative links may be formed between items viewed simultaneously by different regions of the retina. We suggest that learning an extended, as opposed to a narrow, snapshot can have two somewhat separate benefits. First, it improves an insect's precision in locating a goal, as demonstrated earlier (Cartwright and Collett, 1983; Wehner et al., 1996; Durier et al., 2003). Secondly, an extended snapshot, with binding between its components, enhances the reliability of snapshot recall.

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