The learning of a sequence of visual patterns by the ant *Cataglyphis cursor*

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We used a maze to explore the ability of *Cataglyphis cursor* to store multiple visual patterns presented in a fixed sequence. Ants were trained individually to negotiate a linear maze that consisted of four boxes connected by tunnels and through which an ant travelled from a sucrose feeder back to its nest. Each box had one entrance and two possible exits. One exit led to a blocked tunnel and the other to an open tunnel leading to the entrance of the next box. The open and closed exits in each box were labelled by different solid, black shapes that were specific to each box. Ants learnt to negotiate the maze using the shapes for guidance rather than a fixed motor strategy. Trained ants could not only discriminate positive from negative shapes, but had also learnt which positive shape belonged to which box. For example, when the positive shape appropriate to box 1 (1+) was pitted against that appropriate to box 3 (3+), ants preferred 1+ to 3+ in box 1, but chose 3+ over 1+ in box 3. We conclude that ants can identify individual positive shapes and expect to encounter them in the correct order independently of extra-maze cues.

Keywords: shape discrimination; visual sequences; context learning; navigation; ants; *Cataglyphis cursor*

1. INTRODUCTION

Ants, bees and wasps tend to follow stereotyped routes when travelling between their nest and a foraging site. In many cases their path is guided by visual landmarks (Baerends 1941; Thorpe 1950; Janzen 1971; Rosengren 1971; Heinrich 1976; Collett *et al.* 1992; Wehner 1992; Wehner *et al.* 1996; Thomson *et al.* 1997). The complexity, length and constancy of an individual's route suggest that insects may store many visual patterns that differ only subtly from each other, and that are recalled at appropriate points along the route. It is hard to test such ideas in a natural environment. Mazes, however, provide a more controlled situation for analysing this aspect of route learning.

Ants and bees have been found to be skilled at learning complex mazes to reach a food source (Schneirla 1929; Weiss 1953; Zhang *et al.* 1996). Not only do bees learn to distinguish between three very different pairs of visual stimuli (patterns and colours) when negotiating a maze, but they also seem to recognize each stimulus in its correct spatial context within the maze (e.g. Collett *et al.* 1993; Zhang *et al.* 1998). We report here a study that examined the ability of the ant *Cataglyphis cursor* to negotiate a maze which required that the insect discriminates between four different pairs of black shapes. We asked whether the ants had learnt the different shapes and the positions in which they were encountered.

2. MATERIALS AND METHODS

(a) *Animals*

The Mediterranean ant, *Cataglyphis cursor*, forages individually, without recruitment, over distances ranging from a few centi-

metres to about 20 m (Lenoir *et al.* 1990). We collected a queen-right colony of about 3000 workers in the south of France (Aude), which was kept in an experimental room in the University of Sussex. The colony was housed in a darkened, plastic container at a temperature of about 25 °C, with high humidity, and foraged under a 12:12 h light:dark cycle.

(b) *The maze and the shapes*

A diagram of the training and testing apparatus is shown in figure 1. The bottom part was a transparent Perspex tunnel, 122 cm long, which led the ants from a vestibule outside their nest to a box containing a sucrose feeder. The upper part was the maze, also 122 cm long, through which ants could be trained individually to return to their nest without interference from other foraging ants in the tunnel below. We trained ants on the return trip because they are then strongly motivated to return to their nest. The maze consisted of a series of four identical, clear Perspex boxes coated on the inside walls with fluon and connected by transparent tunnels (figure 1a). Each box had one entrance and two exits. The exits were labelled by two solid, black shapes that were specific to that box (figure 2b). One shape indicated that the exit directly below led to the next compartment, and the other shape that the associated exit led to a blocked tunnel.

The eight shapes used were printed by a laser printer onto white paper. The maximum angular width or height of the shapes ranged from 14° to 20° when viewed from the box entrance, and they were matched to have the same area. Preliminary studies with a somewhat simpler maze had established that ants do not have strong spontaneous preferences for any of these stimuli and that they can learn to discriminate between them (Pastergue-Ruiz & Beugnon 1994, unpublished data). In

Figure 1. Top view (a) and side view (b) of the set-up. Filled arrows indicate the traffic of unmarked ants, and open arrows indicate the homeward paths of marked ants through the maze.

Figure 2. (a) Arrangement of visual patterns for the two training conditions. (b) Diagram of a box (length, 9 cm; width, 6 cm; height, 4 cm) showing the visual patterns belonging to the fourth box. Each box had one entrance and two exits, one of which was blocked. (c) Arrangement of visual patterns in tests with 1+ versus 4+ in boxes 1 and 4, and with 1+ versus 3+ in boxes 1 and 3. The test-box was always located in the normal training position of the fourth box in the experimental room.

box 1, the positive shape was a round disk, in box 2 it was a star, in box 3 it was a rectangle, and in box 4 a diamond (figure 2a). For convenience, we call these shapes 1+, 2+, 3+ and 4+, respectively, and the corresponding negative partners 1−, 2−, 3− and 4−.

There were two conspicuous directional cues outside the maze: a large sheet of black card to one side of the maze and a 150 W bulb at one end of the room, which was aligned with the nest-tunnel axis.

(c) Training regime

The nest was connected to the tunnel one week before the experiments began to give workers time to become accustomed to foraging at the sucrose feeder. Once a week, frozen crickets were provided in the vestibule and the water reservoir was refilled. After experiments had begun, access to the feeder was restricted to the daily experimental period, which lasted from about 8.30 to 16.30 (corresponding to the main foraging time in the field). Experiments were performed over 50 consecutive

days, from 23 February to 13 April 1998, and 15 consecutive days, from 1 to 15 July, which enabled 520 hours of observation in total.

Some foragers were collected at the feeder and marked individually with dots of coloured paint on their thorax or gaster. Once an ant had been selected and marked, it was only allowed to reach home through the maze. To ensure that this condition was met, the feeder box was frequently scrutinized and all marked ants seen on the feeder were collected and placed in a waiting box provided with a second feeder. After a period in the waiting box (ranging from 5 min to 1 h), an ant was released singly at the start of the maze and its homing path was recorded on videotape. We noted its choice of exits, its total travel time and the time at which it entered each box. On average, the marked ants performed between three and ten foraging trips a day.

Some ants took a very long time to travel through the maze and we evolved criteria for eliminating those ants from the experiment. Ants were removed from the maze if they did not

enter the first box within 10 min, or did not reach the end of the maze within 30 min, or if they remained stationary in one spot for more than 5 min. Ants that failed in any of these ways were returned to the vestibule. If an ant failed on two successive trials, it was placed in the waiting box until the end of the experimental period. If this ant failed on its next trial the following day, it was removed from the experiment and allowed to forage freely in the lower tunnel.

Out of 56 marked ants, 11 were eliminated in this way. Of these 11 ants, eight died the day after their removal. The remaining three died within the next 4 days. Among the ten other experimental ants that died, three had failed once and three had failed twice. It thus seems likely that most of the ants that failed to negotiate the maze were unhealthy.

To prevent ants from developing a side preference in any of the boxes, the negative and positive shapes were switched between sides to give two training conditions (1 and 2 in figure 2*a*), which alternated from trial to trial for each ant. This training procedure gave ants the opportunity to solve the maze by learning only the first pair of patterns in box 1 and then alternating sides as they moved from box to box (figure 2*a*). Tests showed that ants did not adopt this strategy.

Cataglyphis ants are said not to lay chemical trails (Harkness 1977) and to deposit chemical markers only in the immediate vicinity of the nest (Mayade *et al.* 1993). Nonetheless, we wished to exclude the possibility that the maze could be negotiated by following odour cues. Therefore, we ensured that the two exits and the tunnels attached to them were used equally so that any odour cues would be uniformly distributed. Thus, when one tunnel was open, the other tunnel was blocked with a sliding door 5 cm from the exit (figure 1*a*). On the subsequent trial, when the shapes switched sides, the previously blocked tunnel was opened and the previously open tunnel was shut.

Our criterion for successful learning of the maze was that ants negotiated the maze on two successive trials without error; that is, without entering a blocked exit. Once an ant met this criterion, it was given a single test in which it encountered a new arrangement of shapes. After each test, training continued until the ant reached the same criterion again when it was tested once more. Each ant was used no more than twice in each type of test.

(d) Tests

The principal question probed by the tests was whether ants associated a particular shape with a particular box. In experimental terms, we asked whether ants prefer 1+ to 3+ in box 1, and 3+ to 1+ in box 3. Similarly, is 1+ preferred to 4+ in box 1, and 4+ preferred to 1+ in box 4?

To avoid the influence of extra-maze cues on the test results, all four types of test were conducted in the last box of the maze, and both exits were then open. For this box to act as box 1, boxes 1 to 3 were removed, and the first long tunnel connected directly to it (figure 2*c*). When the last box was to represent box 3, just one of the preceding boxes was removed.

We also wished to prevent ants from encountering one of the tested shapes (1+, 3+, 4+) before they reached the test-box, in case their choices there were biased by their reaction to the same pattern earlier in the sequence. Therefore, the sequence used in the tests was 2+ versus 2– in both boxes 1 and 2 (figure 2*c*).

Finally, we had to ensure that the ants' choices were not influenced by the left–right alternation that they experienced during training. Thus, for tests in box 1, 1+ was sometimes on the same side as it had been in the immediately preceding training trial,

and sometimes on the opposite side. For half of the tests in box 3 and 4, 3+ and 4+ were on the same side as the positive pattern in the previous box and for the other half the sides were reversed. We call a test with a pattern in its expected position in the sequence a 'normal' test and the other type of test a 'reversed' test. The ants' choices in boxes 3 or 4 were not included if ants made errors in the previous box, but such errors were rare (figure 3*c,d*).

A subsidiary question was whether ants could distinguish a positive shape from its negative partner in unexpected positions in the sequence. This question was approached in two ways. First, in further tests, ants were confronted with 3+ and 3– in box 1. Second, we analysed the choices between 2+ and 2– in the first and second boxes when ants were given a choice between 1+ and 3+ or between 1+ and 4+ in boxes 3 or 4.

The statistical significance of all the choices was assessed using the χ^2 test with Yates correction for continuity, treating each choice as independent.

3. RESULTS

The major question of this study is whether ants learn to associate the positive shapes with their position in the sequence. The results of two series of tests show that they do.

When ants were confronted with patterns 1+ and 4+ in box 1, they preferred 1+ to 4+, whereas given the same choice in box 4, they preferred 4+ to 1+ (figure 3*a*). Similarly, ants chose 1+ over 3+ in box 1, but chose 3+ over 1+ in box 3 (figure 3*b*). These results show that the ants' choices are strongly influenced by context. They prefer the pattern that they would normally encounter in the test-box.

Importantly, the pattern of choice was the same in normal and reversed tests (figure 3*a–c*). If we pool the test types in box 3 and 4, then the preferred pattern was chosen in 83% of normal tests and in 81% of reversed tests. Similarly, for tests in box 1, the preferred pattern was chosen in 90% of normal tests and 83% of reversed tests. The results are therefore not biased by the alternation procedure used during training, thus demonstrating that the ants' choice of pattern during tests was not influenced by possible motor strategies.

The ants' choices also show that patterns are recognized in unexpected contexts. Thus, 3+ is chosen over 3– in box 1, where it was not usually encountered, as well as in box 3 (figure 3*c*). We also analysed the preference for 2+ over 2– in boxes 1 and 2, during tests when 2+ and 2– were present in both boxes. The shape 2+ was preferred over 2– in box 1 and also in box 2 (figure 3*d*), although the same patterns had already been viewed in box 1. Thus, repetition does not markedly change the ants' preference.

4. DISCUSSION

The results presented here show the power of the maze as a tool for investigating pattern recognition. When ants were forced to encounter an ordered sequence of four shapes, they associated them with their position in the sequence even though the linkage was not essential for solving the maze. The sensitivity of the ants to context showed that they could discriminate between different positive shapes, a result that would otherwise be hard to obtain.

Figure 3. Choice of visual pattern. (a) Test of 1+ versus 4+: ants chose the circle (1+) over the diamond (4+) in box 1 ($\chi^2_{(1)} = 7.70, p < 0.01$, tests on 19 ants) and the diamond over the circle in box 4 ($\chi^2_{(1)} = 5.41, p < 0.03$, 20 ants). (b) Test of 1+ versus 3+: ants chose the circle over the rectangle (3+) in box 1 ($\chi^2_{(1)} = 7.70, p < 0.01$, 21 ants) and the rectangle over the circle in box 3 ($\chi^2_{(1)} = 6.41, p < 0.03$, 14 ants). (c) Tests of 3+ versus 3-: ants chose the rectangle over the triangle (3-) in box 1 ($\chi^2_{(1)} = 5.41, p < 0.03$, 25 ants) and box 3 ($\chi^2_{(1)} = 14.26, p < 0.001$, 21 ants). (d) Tests of 2+ versus 2-: ants chose the star (2+) over the square (2-) in box 1 ($\chi^2_{(1)} = 6.78, p < 0.01$, 27 ants) and box 2 ($\chi^2_{(1)} = 9.99, p < 0.003$, 27 ants).

Ants encountered a total of eight shapes. The results show formally that 2+ is discriminated from 2-, 3+ from 3-, 1+ from 3+, and 1+ from 4+ (figure 3). The fact that ants can successfully negotiate the maze without error, and that preference scores are uninfluenced by the alternation procedure used in training, indicates that ants also discriminate 1+ from 1- and 4+ from 4-. Thus, in all likelihood *Cataglyphis cursor* can identify individually all four positive shapes. These results reinforce the suggestion from more naturalistic studies of route guidance that Hymenoptera are able to store a large number of visual patterns. Although recall is strongly influenced by contextual cues, patterns are still recognizable out of sequence (see also Zhang *et al.* 1998).

Several previous studies on honeybees have emphasized the importance of context in pattern recognition (e.g. Collett & Kelber 1988; Chittka *et al.* 1995; Menzel *et al.* 1996; Collett *et al.* 1997; Srinivasan *et al.* 1998). It is striking how powerful the present contextual effects are. The preferences for 1+ over 3+ in box 1 and for 3+ over 1+ in box 3 are at least as strong as that for 3+ over 3- in box 1. Ants have learnt the position of each shape as being at the beginning, or in the middle, or at the end of the maze. Our results show that they can remember such a visual sequence without the aid of a fixed motor strategy.

It is crucial for navigation that the correct visual cues are recalled at the appropriate point along a route.

Linking recognition to context, so that the ant arrives at a location expecting to see a particular shape, helps ensure that this happens. The use of non-local, contextual cues also makes it easier to distinguish between similar landmarks in different places.

What contextual information do ants link to the positive patterns so that they recognize them in the correct sequence independently of any extra-maze cues? It clearly need not be a visual stimulus that impinges on the ant while it is making its decision. Thus, 1+ and 3+ presented in the same box, with the same view of the room outside, evoked different responses according to the position of the box within the sequence. In the experiments described here, the contextual signal must come from previous events in the sequence and be stored internally. However, the experiments do not tell us whether insects store the whole sequence, or whether most of the sequence resides in the outside world and internal linkages extend only one step back in the chain (a minimal memory model using the terminology of Ballard *et al.* (1992)). We also do not know what provides the context. Is it the preceding visual pattern(s) (Zhang *et al.* 1998), the number of boxes encountered (Chittka & Geiger 1995), the distance walked, or is it some combination of these cues?

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