



Nest mark orientation in desert ants *Cataglyphis*: what does it do to the path integrator?

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(Received 15 December 2004; initial acceptance 20 January 2005;
final acceptance 15 March 2005; published online 7 November 2005; MS. number: 8393R)

In desert ants, path integration is the fundamental system of long-distance navigation. Normally, the path integrator is reset to the zero state whenever the ant returns to the starting point of its foraging journey, the nest. We investigated whether the landmark panorama characterizing the nest entrance suffices to reset the path integrator. We designed an experimental paradigm in which the position where the ants' path integrator was at the zero state (PI position) did not coincide with the position of the nest as marked by the familiar landmark panorama (LM position). Once the ants displaced from the training area to a distant test field had arrived at the PI position, the artificial landmark array defining the nest site was installed at the LM position. The ants immediately headed towards the LM position and searched there persistently. However, upon removal of the landmarks they returned to the PI position. Hence, they ignored information from their path integrator while they concentrated their search at the landmark-defined nest position, but they again relied on their continually updated path integrator as soon as the landmarks had been removed. Although their concentrated search at the LM position showed that the ants had used the landmarks as reliable cues for the position of their nest, this landmark-induced search for the nest entrance did not reset the ant's path integrator. In general, this might mean that geocentric visual cues in themselves are not sufficient to recalibrate the egocentric path integrator.

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Path integration is the predominant means of navigation in the desert ant *Cataglyphis fortis*. By relying on a skylight compass and some kind of odometer, the ant continually updates a homebound path integration vector that informs it about its position relative to the nest. Therefore, the position of the nest is defined as the point where the state of the path integrator is zero. Owing to the egocentric nature of this kind of orientation, path integration has an error rate that increases with the length of a foraging trip, that is, the distance the ant has ventured out from the nest (the latter two parameters are correlated, see Wehner et al. 2004). Therefore, in addition to path integration, route-defining landmarks are used as geocentric navigational aids that help to compensate for the accumulating errors of the path integrator (Wehner et al. 1996; Australian desert ants, *Melophorus bagoti*: Kohler & Wehner 2005). Experiments in which ants could use the path integrator or information gained by familiar route marks

revealed that ants follow the route marks even when their path integrator is at the zero state (Bisch-Knaden & Wehner 2003b; Andel & Wehner 2004). The ants seem to link snapshots of the route marks to so-called local vectors that guide them home (Collett et al. 1998). However, when the route marks to which the ants have been trained are manipulated during an ant's foraging journey so that their information conflicts with that of the path integrator, the latter keeps running as a backup system. Even though the ants are redirected by the route marks, they later either turn back to the nest (Sassi & Wehner 1997) or, after an experimental displacement, go to the fictive position of the nest as defined by the path integrator (Collett et al. 2003; Andel & Wehner 2004).

The nest entrance itself is defined by certain marks, which are used by the ants to pinpoint the position of the nest entrance rather than the direction towards it. The learning of nest-defining landmarks can easily be demonstrated by releasing an ant with its path integrator in the zero state close to a landmark array to which it has been trained. The ants head directly for the position at which the nest entrance has been relative to the array during training and make a dense search pattern (Wehner &

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Räber 1979). In the natural environment, the position of the nest defined by the path integrator and the position defined by the nest marks usually correspond well. The question then arises whether landmarks defining the position of the nest are sufficient to reset the path integrator when, owing to a particular experimental paradigm, the ant's path integrator is not at the zero state when the ant reaches the nest-defining landmark array.

In our experimental set-up we trained ants to find a feeder, while their nest entrance was marked by a square array of cylindrical landmarks. After several training runs, the ants were displaced from the feeder to a distant test field. Upon release they ran along their path integration (home) vector and searched at the fictive position of the nest. While the ants were searching at this position, that is, the position defined by the path integrator (PI position), we installed the landmark array at some distance from the PI position. If they ignored the information provided by their path integrator, the ants would immediately head for the landmark array and search consistently at the nest position as defined by the landmarks (LM position). Would this intensive search at the LM position reset the path integrator that would have kept running while the ants were guided by the landmarks (see e.g. Sassi & Wehner 1997; Andel & Wehner 2004)? We tried to answer this question by quickly removing the landmarks and further recording the ants' trajectories. If the ants then returned to the PI position, their path integrator would not have been reset by the landmark array defining the position of the nest. However, if the ants continued to search at the LM position, their path integrator would have been reset.

METHODS

We carried out the experiments within a salt-pan area between Maharès and Chaffar in southern Tunisia. Ants were trained to find food (a piece of watermelon) 5 m to the south of the nest entrance. A landmark array of four black cylinders (each 20 cm wide and 40 cm high) was

placed at the corners of a $2 \times 2\text{-m}^2$ square with the nest in its centre (Fig. 1a). Ants reaching the feeder were marked on the gaster with a date-specific colour dot and were tested (at the earliest) 1 day after training, that is after they had made on average 30 foraging runs (Akesson & Wehner 2002).

We tested 35 ants under each of two conditions with each ant being tested in either condition not more than once. When single ants were tested in both conditions ($N = 20$), the order of the test conditions was alternated, and at least 1 day of training separated the two tests. In condition 1 we caught the ants at the feeding site and released them on a remote test field (Fig. 1b). By using a white grid painted on the flat ground we recorded the ants' trajectories for 8 min each (for details of recording paradigms see Wehner 1982). After the first 2 min of recording (phase 1) a four-landmark array identical to that used in the training situation was installed on the test field in such a way that the fictive position of the nest as defined by the landmark array (the LM position) was located 3 m to the west of the fictive position of the nest as defined by the path integrator (the PI position). After putting the landmarks in place, we recorded the ants' trajectories for another 1-min period (Fig. 1c, phase 2). Then we quickly removed the landmarks and recorded the ants' trajectories for a further 5-min period (Fig. 1d, phase 3). In test condition 2 we again displaced the ants from the feeder to the test field, but now recorded their trajectories for 8 min without any landmarks being present (for search examples of conditions 1 and 2 see Fig. 2).

Finally, the ants' trajectories were digitized and, by using Gedit for Windows, we computed search density plots. In addition, the search densities within $3 \times 3\text{-m}^2$ squares around the LM position and the PI position were determined and compared in order to test the following hypotheses. (1) The path integrator is not reset by the landmark array. (2) The path integrator is reset by the landmark array.

Means are given \pm SDs.

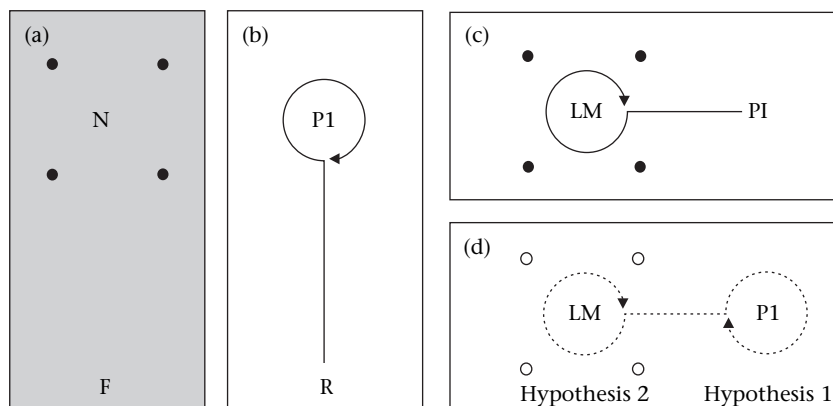


Figure 1. (a) Training situation; (b) phase 1: ant released within the landmark-free test field and recorded for 2 min; (c) phase 2: landmarks added 3 m west of the PI position, and the ant recorded for another 1 min; (d) phase 3: landmarks removed again and the ant recorded for another 5 min. Circular arrows depict the sites at which the ant searched. Dashed lines depict the sites at which the ant was expected to search according to hypothesis 1 (path integrator not reset by the landmark array) and hypothesis 2 (path integrator reset by the landmark array). N: nest; F: feeder; R: point of release; PI: fictive position of the nest as defined by the path integrator; LM: fictive position of the nest as defined by the landmark array; filled circles: landmarks present; open circles: landmarks removed. Grey shading: training area (a) 30 m from the test area (b).

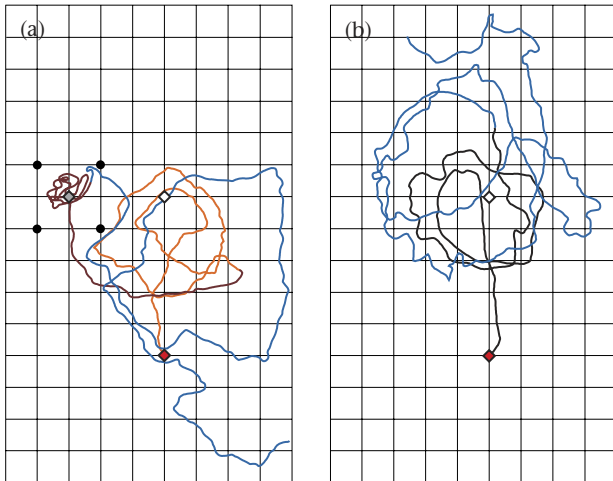


Figure 2. Search examples under (a) condition 1 and (b) condition 2. (a) Red diamond: point of release; white diamond: fictive nest defined by the path integrator; grey diamond: fictive nest defined by the landmark array; orange line: homebound run and search (2 min) in phase 1 (no landmarks present); brown line: search (1 min) in phase 2 (landmarks present); blue line: search (5 min) in phase 3 (landmarks removed again). (b) Search (8 min) when no landmarks were present. The blue line depicts the last 5 min of the search that were compared with phase 3 of condition 1.

RESULTS

When released within the test field, the ants ran directly along their home vector and started a systematic search (Fig. 3a). However, in phase 2 when the landmarks were added at a position (LM position) to the side of the fictive position as defined by the path integrator (PI position), the ants headed straight towards the landmarks (Fig. 4) and concentrated their searches very narrowly around the LM position (Fig. 3b). In phase 3, when the landmarks were removed again, the ants could have returned to the PI position (hypothesis 1, Fig. 1d) or they could have continued to search around the LM position (hypothesis 2, Fig. 1d). The latter result would have been expected if the ants, having persistently searched within the landmark array for 1 min, had reset their path integrator to the LM position. This, however, was not the case. After the landmark array had been removed, that is, in phase 3, the ants' search trajectories shifted back to the fictive position of the nest as defined by their non-calibrated path integrator. This can be demonstrated best by comparing for each ant the search densities within $3 \times 3\text{-m}^2$ squares around the PI and LM positions: $19.9 \pm 10.7\%$ of the trajectories were located within the PI-defined square, whereas the LM-defined square contained only $14.3 \pm 10.0\%$ of the ants' total trajectories (Fig. 3c, Wilcoxon signed-ranks test: $Z = 2.334$, $N = 35$, $P < 0.02$). Furthermore, a centred search around either the PI position or the LM position would imply that the search density profiles around any preferred position were symmetrical along the north-south axis. This was clearly the case for the PI position ($48.4 \pm 21.1\%$ west of the fictive nest; Wilcoxon rank-sum test: $Z = 0.278$, $N = 35$, $P = 0.82$), but not for the LM position ($15.3 \pm 13.4\%$ west of the fictive nest; $Z = 5.168$,

$N = 35$, $P < 0.0001$). The result was clear-cut. Although the ants concentrated their searches within the landmark array as long as it was present, they did not reset their path integrator by the 1-min presentation of the nest-defining landmarks.

Even if the landmarks did not cause the ants to reset their path integrator, however, they could have had at least some slight effect on the ants' subsequent searches. To test for such effects, we compared the ants' search densities in phase 3 of condition 1, when the landmarks were already taken away, with the search density during the last 5 min in condition 2 (blue line in Fig. 2b), in which no landmarks were present at all. The two search densities determined within the PI-defined $3 \times 3\text{-m}^2$ squares did not differ (condition 1: $19.9 \pm 10.7\%$, $N = 35$; condition 2: $18.7 \pm 10.1\%$, $N = 22$; Mann-Whitney U test: $U = 350$, $P = 0.5$). In addition, there were no differences between the search densities within the former LM-defined $3 \times 3\text{-m}^2$ squares in condition 1 with the equivalent squares in condition 2 (condition 1: $14.3 \pm 10.0\%$, $N = 34$; condition 2: $13.4 \pm 8.3\%$, $N = 22$; $U = 367$, $P = 0.77$). Furthermore, the search densities west and east of the PI position did not differ between conditions 1 and 2 (condition 1: $48.4 \pm 21.1\%$ west of the fictive nest, $N = 34$; condition 2: $43.0 \pm 15.4\%$ west of the fictive nest, $N = 22$; $U = 327$, $P = 0.34$). In conclusion, even while the ants were narrowly searching around the fictive position of the nest within the landmark array, after the landmarks were removed, no information about their previous positions remained and the centre of the search switched back to the position defined by the path integrator (Fig. 5).

DISCUSSION

In the present study, we tested whether the ants' path integrator can be reset to zero when the ants reach an area that corresponds visually to their nest site. In the experimental paradigm, the current state of the ants' home vector did not match the stored reference state for the nest (both differed by 3 m), but the actual visual landscape was identical to the one they had learned at the nest entrance. In such a conflict situation a possible resetting of the path integrator by the learned snapshot would have resulted in ants continuing the search around the snapshot-defined position, even after the landmarks had been removed.

None of the 35 ants tested in this experimental paradigm did search that way (Fig. 3c). As soon as the landmarks had been removed, the ants, which had concentrated their search within the landmarks, headed back towards the position of the nest as defined by their path integrator. This result clearly shows that the ants' path integrator keeps running even when the ants persistently search at a different location at which the landmark surroundings completely mimic the ones present at the nest site. Ants do not centre their search within landmarks to which they have not been trained (Loch & Wehner 1996). Therefore, the fact that the ants moved directly towards that site and searched persistently at the very location of the fictive nest is clear proof that they immediately

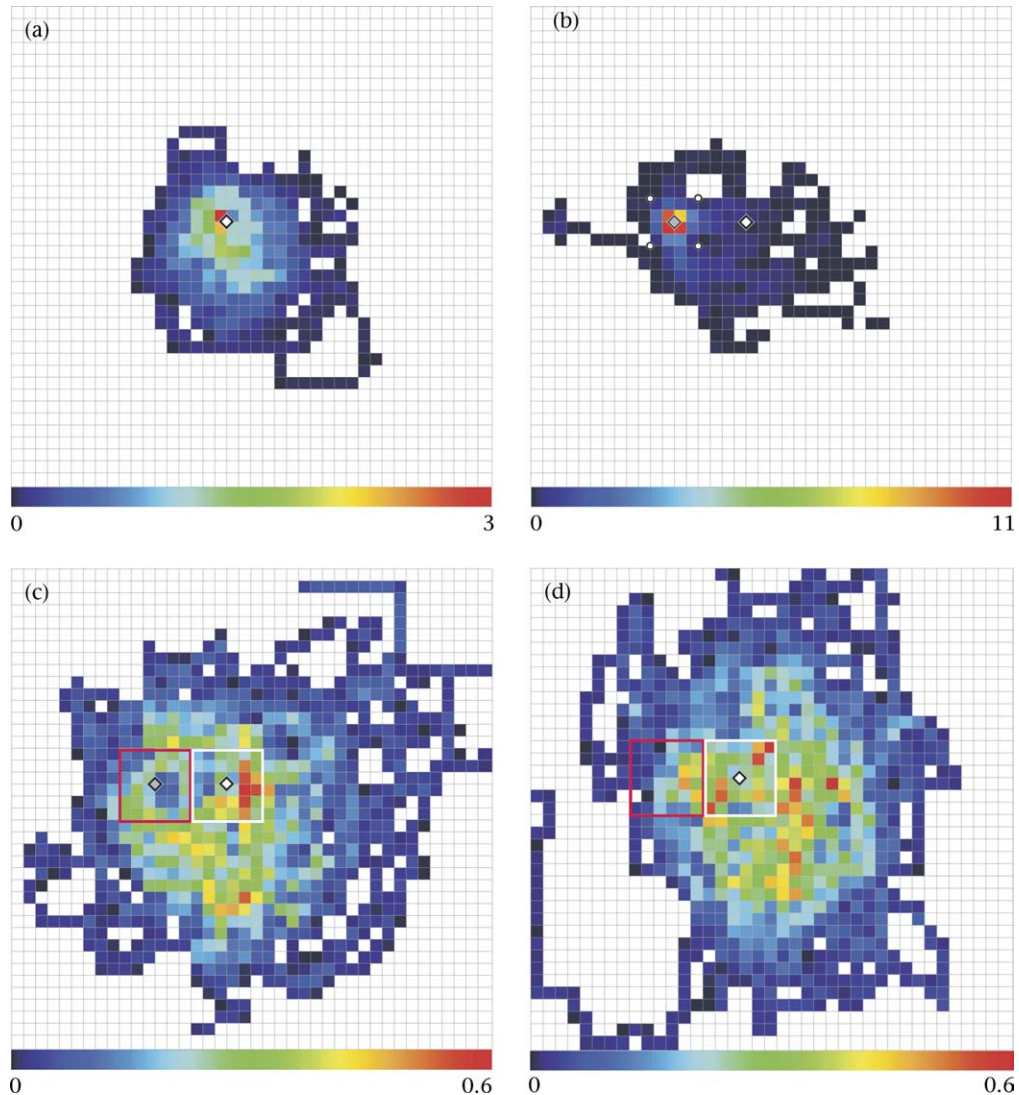


Figure 3. Search density plots. (a–c) Condition 1. Ants ($N = 35$) were taken from a feeder and displaced to a remote test field (for training setup see Fig. 1a). (a) Ants were recorded for 2 min after having run along their path integration vector. (b) Landmarks were added 3 m west of the fictive nest and the ants recorded for another 1 min. (c) Landmarks were removed again and the ants were recorded for another 5 min. (d) Condition 2. Similarly trained ants ($N = 35$) were recorded for 8 min without any landmarks (the search density diagram focuses on the last 5 min of the recording). Small white circles in (b): landmark array; white diamond: fictive nest defined by the path integrator (PI); grey diamond: fictive nest defined by the landmark array. The numbers below the colour gradient depict the relative search densities (%) per square ($50 \times 50 \text{ cm}^2$) of the grid. The open white (open red) squares give the $3 \times 3 \text{ m}^2$ around the fictive nest defined by the PI (landmarks) that were used for the statistical comparison of the search densities (see Results).

recognized that place as their nest site. Therefore, we could reject hypothesis 2 that the path integrator is reset by the landmark array.

Other authors have shown that the ants' path integrator is continually updated even if particular experimental paradigms cause the ants to deviate from their global vector courses by following landmark-coupled local vectors (Sassi & Wehner 1997; Collett et al. 1999; Andel & Wehner 2004). Collett et al. (2003) trained ants to travel along an L-shaped channel system in which the feeder at the end of the second leg of the route was marked with landmarks. In the test situation, Collett et al. extended the first part of the L-shaped route during one outbound run and displaced the ants from the feeder to a test field.

If the landmarks at the end of the second leg of the channel had recalibrated the ants' path integrator, the homeward trajectories should have pointed in the same direction as they did in the training situation. On the other hand, if the path integrator had not been recalibrated, the trajectories should have differed from those of the controls and should have pointed towards the actual position of the nest. As the latter was the case, Collett et al. (2003) argued that landmarks did not recalibrate the path integrator. However, their experimental paradigm did not guarantee that the ants had actually learned the landmarks as a cue defining the feeding site. Furthermore, the observed differences between the ants' heading directions and those of the controls were rather small and

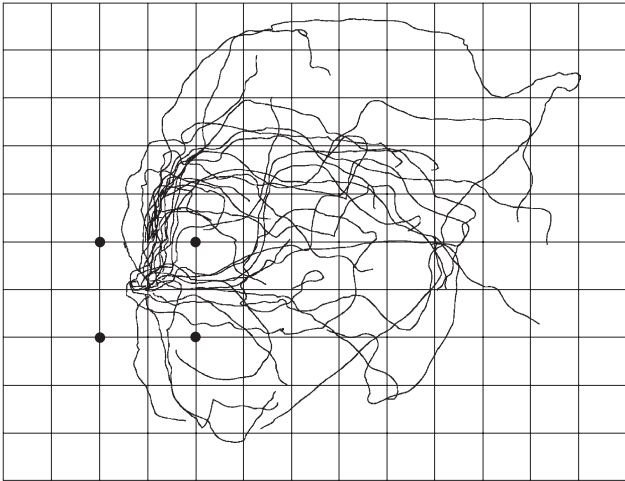


Figure 4. Ants' trajectories towards the landmark-defined (LM) position. Landmarks (black circles) were added after a 3-min search around the path integrator-defined position. Black lines: part of the trajectories from when the landmarks were added to when the ants reached the LM position the first time; grid has a square width of 1 m.

pointed in the direction predicted from the systematic errors observed by Müller & Wehner (1988) in ants performing L-shaped outbound runs.

In our experiments, however, the ants' pronounced search peaks occurring within the landmark array that had been presented temporarily during the test situation indicated that the ants had indeed learned the landmarks and had taken the location marked by them to be their nest site.

In a number of previous experiments the path integrator has been set in conflict with route-defining landmarks. However, route- and nest-defining landmarks differ in their robustness against memory decay in so far as nest marks are well stored even when the ants have not seen

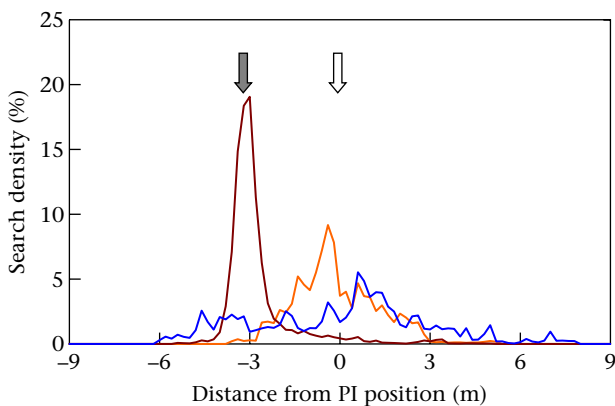


Figure 5. Search density plot of condition 1 along an east–west oriented corridor (2 m wide, 18 m long) with the path integrator-defined (PI) position in the centre. White arrow: PI position; grey arrow: landmark-defined position; orange line: no landmarks present; brown line: landmark array added 3 m west of the fictive nest; blue line: landmarks removed again. Search densities were calculated relative to the total search within the corridor during each situation.

them during their previous homeward run (Bisch-Knaden & Wehner 2003a). Furthermore, Ziegler & Wehner (1997) showed that the time courses of memory decay differ substantially depending on whether the information is provided by the path integrator or by the nest-defining snapshot memories. Even after having been kept in the dark for 20 days, the ants recognized the nest marks extremely well, whereas any information about the path integration vector was gone. Hence, in our experiments one could have expected that landmarks defining the nest site would have been able to reset the ants' path integrator. However, as our results show, such was not the case. Even during their continued and concentrated searches at the landmark-defined fictive position of the nest, the ants did not reset their path integrator. Even though information provided by nest-based landmarks clearly overrides information provided by the path integrator, it does not reset the latter.

In honeybees, *Apis mellifera*, the result of one experiment has been taken as proof for a landmark-dependent resetting of the path integrator (Srinivasan et al. 1997). In this experiment, also discussed by Biegler (2000), bees were trained to locate a feeder that was placed at various positions from the entrance of a tunnel through which the bees had to fly. In the test situation with no feeder present the narrowness of the search peak, that is, the precision with which the bees located the feeder, depended on the formerly learned position of the feeder. The longer the distances the bees had to fly within the tunnel, the broader was the search peak. However, when a conspicuous landmark, a baffle, was added within the tunnel some 2 m in front of the feeder, the width of the bees' search peak was markedly reduced, even when the bees had to fly long distances within the tunnel to reach the feeder. This improved accuracy in locating the feeder after the bees had passed the landmark was interpreted in terms of the path integrator being reset by the presence of the landmark. However, in contrast to the present study on ants, during the critical tests the landmark was not removed after the bees had passed it. Therefore, the most likely interpretation of the results described by Srinivasan et al. (1997) is that the landmark was used by the bees to trigger a local vector, which ants and bees are known to attach to landmarks (Collett et al. 1998, 2002; Bisch-Knaden & Wehner 2001, 2003b) and to use that landmark-based local vector in addition to the path integration global vector. Furthermore, for as long as the landmarks were present during the test, the bees could have just used them for pinpointing the goal (Wehner & Räber 1979; Cartwright & Collett 1983). Hence, further experiments are needed to disentangle the effects of local and global vectors in this honeybee training paradigm.

In hamsters, *Mesocricetus auratus*, Etienne et al. (2004) found that an episodic view of a learned landmark array resets the animal's path integrator. As the hamster's path integration system is based completely on idiothetic cues, path integration in hamsters is much more susceptible to cumulative errors (Mittelstaedt & Mittelstaedt 1973; Benhamou et al. 1990). Recalibration of this error-prone system by external landmarks certainly enhances the hamster's orientation back to the burrow. In contrast,

the ants' path integrator, using a compass based on external (skylight) cues, is less prone to errors. This difference in accuracy between the path integrators of hamsters and ants might favour different ways of weighting egocentric and geocentric information when both are in conflict.

In *Cataglyphis*, landmarks are important for locating the nest entrance (Wehner & R ber 1979; Cartwright & Collett 1983). The fact that the ants did not reset their path integrator while experiencing the very landmark panorama that they had learned before when departing from, and returning to, the nest makes any further resetting and recalibrating effects of landmarks on the egocentric path integration system unlikely. We are currently exploring what the nest-based cues might be that would trigger a resetting process.

Acknowledgments

We thank Christine Gutzwiler and Valerie Domanico for help with data collection and the Swiss National Science Foundation for financial support (Grant 31-61'844).

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