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Desert ants: is active locomotion a prerequisite for path integration?

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Abstract Desert ants *Cataglyphis fortis* have been shown to be able to employ two mechanisms of distance estimation: exploiting both optic flow and proprioceptive information. This study aims at understanding possible interactions between the two possibly redundant mechanisms of distance estimation. We ask whether in *Cataglyphis* the obviously minor contribution of optic flow would increase or even take over completely if the ants were deprived of reliable proprioceptive information. In various experimental paradigms ants were subjected to passive horizontal displacements during which they perceived optic flow, but were prohibited from active locomotion. The results show that in desert ants active locomotion is essential for providing the ants' odometer and hence its path integrator with the necessary information.

Keywords *Cataglyphis fortis* · Locomotion · Path integration · Optic flow · Proprioception

Introduction

During their foraging excursions desert ants of the genus *Cataglyphis* are confronted with a uniform environment that does not necessarily provide sufficient external orientation cues. Therefore, these ants rely on path integration (vector navigation) as their main means of navigation. As this strategy demands egocentric information on the animal's actual position, the home vector must be updated continually by integrating the segments of a foraging path and hence determining both direction and length of each single segment.

While the ant's walking direction is deduced from skylight cues (Wehner 2003), distance estimation seems to be based on two mechanisms: optic flow as perceived within the ventral visual fields of the eyes plays a certain role, but is dominated by additional, potentially proprioceptive, information (Ronacher and Wehner 1995) The significance of the optic flow was determined in ants that were walking on a transparent ground-floor with a moving pattern underneath. Hence the walking animals had the possibility to at least partially compensate the insufficiencies of the optic flow system by using the correct input from the proprioceptive system, e.g. a step counter as proposed by Wittlinger et al. (2006). However, how do the ants integrate optic flow information into their path integrator, if the inputs from both systems do not coincide? Does one system take over if the other fails to work properly, or does the odometer compute a mean value of both inputs? In addressing this question we disentangled the two systems completely by letting the ants experience passive displacements during which they could not walk properly, but were able to experience optic flow cues.

Materials and methods

In order to have sufficient control on the ants active and passive movement trajectories, and at the same time controlling the presence of optic flow cues we

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enclosed the ants' movements in channels. All former channel experiments performed with *Cataglyphis* ants used test channels which had been visually as homogenous as possible. The fact that in these experiments the ants were always sure about the fictive position of the nest (i.e. exhibited sharp search peaks there) shows that contrary to bees in ants the presence of optic flow is not necessary for gauging distances correctly. However, as optic flow whenever present influences distance estimation to a remarkable extent (Ronacher and Wehner 1995), we designed experiments in which the ants were either provided or not provided with optic flow during their pre-test treatment, but were never provided with optic flow in the test situation.

Training situation

Desert ants, *Cataglyphis fortis*, were trained to forage to a feeder located 1.5 m away from the nest entrance (Fig. 1). During the whole time the ants were enclosed in a channel arrangement (profile dimensions: 7 by 7 cm) depriving them from external landmark information but presenting them with directional information by skylight cues (width of strip like celestial window: 53°). Animals returning from the feeder with a food item between their mandibles were captured directly at the nest entrance and transferred to one of two different test set-ups. The training procedure assured that the ants had completely run off their (1.5-m) home vector and hence were in their zero-vector state.

Treatment 1

The zero-vector ants were released at the elevated end of an inclined channel (length: 4 m; inclination: 52°) oriented orthogonally to the training set-up (Figs. 1, 2). The channel, the interior of which was painted uniformly grey (Experiment A), was made slippery to the ants by covering its interior with a transparent plastic foil. When being released inside the channel, the ants usually directly slid down the inclined 4-m channel, and in doing so covered a ground distance of 2.4 m (Fig. 1: Treatment 1). In order to test whether they had detected the more or less passive movement, they were picked up at the bottom end of the channel and were released into a horizontal test channel that was parallel to the ground projection of the inclined channel. If the ants were able to monitor the passive displacement and by that integrate it into the ground distance covered (Wohlgemuth et al. 2001), they should have exhibited a nest search not centred on the release point but, ideally, shifted by 2.4 m in the direction opposite to the
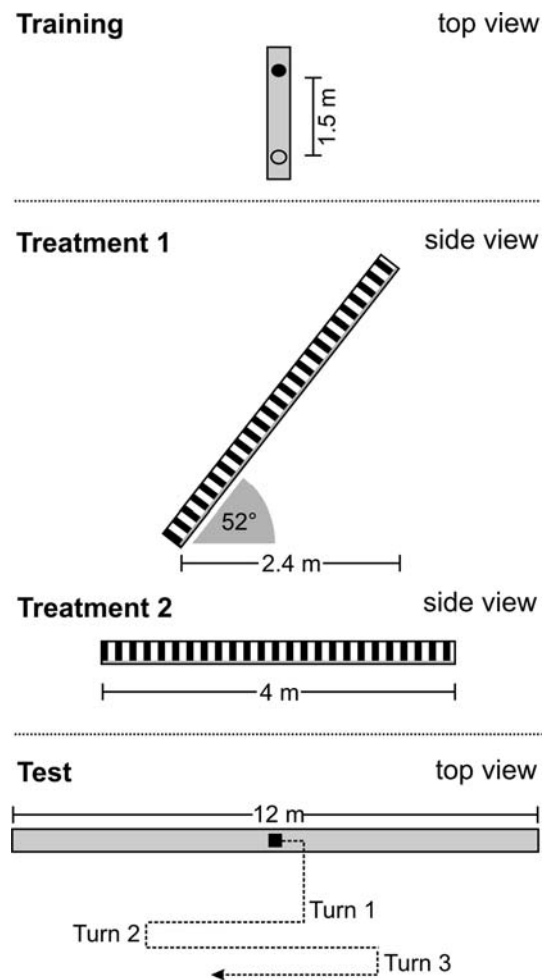

Fig. 1 Schematic set-up of Experiment 1. Ants were trained in a channel to a feeder at a very short distance. Then they were transferred either to an inclined channel with a slippery surface (Treatment 1) or to a flat channel (Treatment 2). In both set-ups they experienced a passive displacement oriented orthogonal to the training set-up. During Experiments B–E, a black and white stripe pattern ($\lambda = 4$ cm) provided full ventral and lateral optic flow to the ants. Subsequently, they were released in a test channel where each single ant's nest search was recorded. (*Filled circle* nest entrance; *empty circle* feeder; *filled rectangle* point of release after the experimental treatment.)

prior experienced displacement (compare Figs. 1 and 2). We tracked the first eight turning points of each ant's nest search behaviour linearly performed within the test channel. In order to be sure that the ants still maintained their homing motivation, ants which while being tested had lost their food crumbs were excluded from further analyses (see Results section for the success rate of each set).

In another experiment (Experiment B) we increased the optical flow within the inclined channel by providing the interior of the channel with a pattern of black and white stripes ($\lambda = 4$ cm) arranged orthogonally to
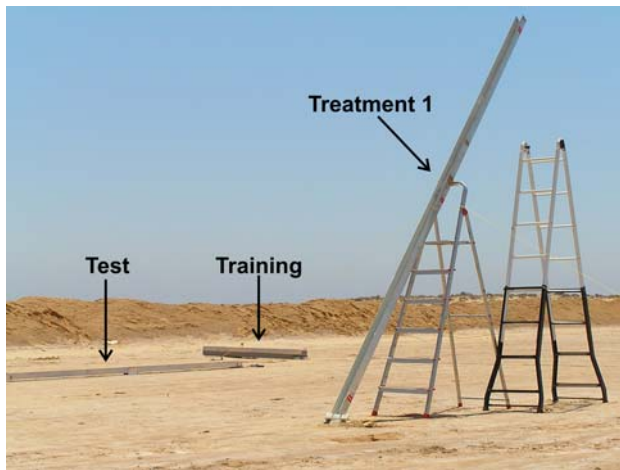
Fig. 2 Experimental set-up inlcuding training, treatment 1 and test. Both the test channel and the ground projection of the treatment channel were oriented orthogonally to the training channel

Fig. 3 Schematic set-up of a fixated ant in Treatment 2. The ant was fixated in a pipette tip (*big figure*) and then mounted on a little trolley (*small figure*) that was moved through the channel. It was ensured that the ant's head relative to the ground corresponded to the normal head position during running (≈ 44°) as observed by Wehner (1982)

Control

As a control, ants were captured at the nest entrance and transferred directly into the test channel. Once tested, they were excluded from further experiments.

Data analysis

We recorded the first eight turns of each ant's nest search. In order to determine the search density distribution of a single animal's search we divided the test channel in virtual bins of 0.1 m length and calculated how often each bin had been visited by the ant. We then normalized the obtained data for each ant by dividing the number of visits within each bin by the total number of visits of all bins. The search density distribution of an experimental set shows the mean value of each bin for all ants.

For statistical analysis the centre of each animal's search was determined by calculating the median of the visited bins. In all experiments (A–E) we tested whether the search centres differed from that of the control group by using the Kruskal–Wallis test ($\alpha = 0.05$, Kruskal and Wallis 1952) accompanied by the Dunn-Sidak multiple comparisons post-test (Hochberg and Tamhane 1987). In order to characterise the precision, with which a tested ant located its fictive nest, we determined the search ranges of each animal by following Sommer and Wehner (2005). The values obtained from each experiment (A–E) were tested against the values of the control group using the tests mentioned above.

Results

The first attempt to disentangle optic flow and active locomotion was to let the ants slide down the inclined and uniform channel. Once an ant was released with a food item it stepped into the channel and almost

the sliding movements of the ants. A third group of ants (Experiment C) was released five times consecutively in the inclined and patterned channel before the ants were transferred to the test channel. This procedure enabled us to increase the distance between the fictive position of the nest as defined by the path integrator if no movement had been detected by the ants (0 m) and the fictive position of the nest as defined by the path integrator if movement had been detected (2.4 m × 5 = 12.0 m).

Treatment 2

In a second set of experiments, zero-vector ants were again exposed to passive movements but now by clamping them into the tip of a pipette with their head peering out and the rest of the body (including the legs) being immobilized by the tip (Fig. 3). The tip was then fixed at the front of a small vehicle, so that the position of the ant's head relative to the ground corresponded to the normal head position during running (about 44°, Wehner 1982). The vehicle was moved with a speed of about 0.3 m/s (corresponding to the ants' normal running speed) within the now horizontally oriented channel (length 4 m, Fig. 1: Treatment 2), which was again provided with black and white rectangular grating (Experiment D). Thus, the immobile ants were exposed to optic flow cues as they would usually perceive them during undisturbed running. In order to increase the ants' virtual home vector we exposed the ants to five consecutive passive movements in the patterned horizontal channel before we recorded their search patterns (Experiment E). Each ant was tested only once.
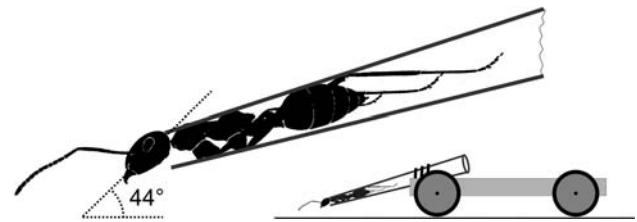
Fig. 4 Search density distributions of ants' nest searches after passive displacement with full optic flow. *A* During the experiment ants were sliding down the slippery inclined and homogenously painted channel. *B* The ants were sliding down the channel as in *A*, but this time experiencing enhanced optic flow by a black and white pattern. *C* The ants experienced the same conditions as in *B*, but the treatment was repeated five times before the test. *D* Fixated ants were experiencing directed optical flow on the retina while being moved through a horizontal channel. *E* The ants were fixated as in *D* but driven through the channel five times before being tested. (*Shaded area* control experiment of ants that were tested without passive displacement. *Solid lines* hypothetical nest entrance without integration of optic flow information. (0 m). *Dotted lines* hypothetical nest entrance with full integration of optic flow information.)

instantly lost control of its active locomotion. The horizontal component of the 4-m displacement was 2.4 m. If the ant had integrated its passively encountered movement, the centre of its search density distribution should have been located at a distance of 2.4 m (dotted line in Fig. 4A). However, all ants exhibited searches centred on the release point (Experiment A: median = 0.1 m, $n = 25$ successful runs of 27 trials). This result indicates that the ants had not compensated for their experienced travel (Fig. 4A, solid line). It does not differ (Dunn: $P > 0.05$) from the control experiment, in which the ants were tested directly after they had been picked up in the training situation (Fig. 4A–E: shaded area, median = 0.4 m; $n = 25$ of 27 trials).

In Experiment B we introduced a black-and-white stripe pattern underneath the transparent foil, changing the optic impression to the moving ant from a uniform grey to a more obvious pattern. However, although the very same pattern proved to be useful for recognition of self induced optic flow in actively running ants (Ronacher and Wehner 1995), the now prominent optic flow experienced by the sliding ants did not induce the ants to search at a position (Fig. 4B: median = 0.2 m, $n = 25$ of 25 trials) different from the control group (Dunn: $P > 0.05$), even if the sliding procedure was repeated five times (Fig. 4C: Experiment C, median = −0.15 m, $n = 4$ of 5 trials, Dunn: $P > 0.05$).

As the ants were often tumbling down the channel rather than sliding in a directed manner, the optic flow on the retina did not always move in a straight direction from cranial to caudal. In addressing this issue we fixated the ants and transferred each single ant manually through the (in this case horizontally oriented) channel with the animal's head in its natural position. Now the ants experienced an optic flow pattern similar to the one an actively running ant would perceive, but were again refrained from any active locomotion. (Fig. 4D: Experiment D, median = 0.55 m, $n = 18$ of 36

trials). As the search density distribution of the animals did not differ from the one of the control group (Dunn: $P > 0.05$), the directed optic flow had not provided the ants' path integrator with any input information. Five
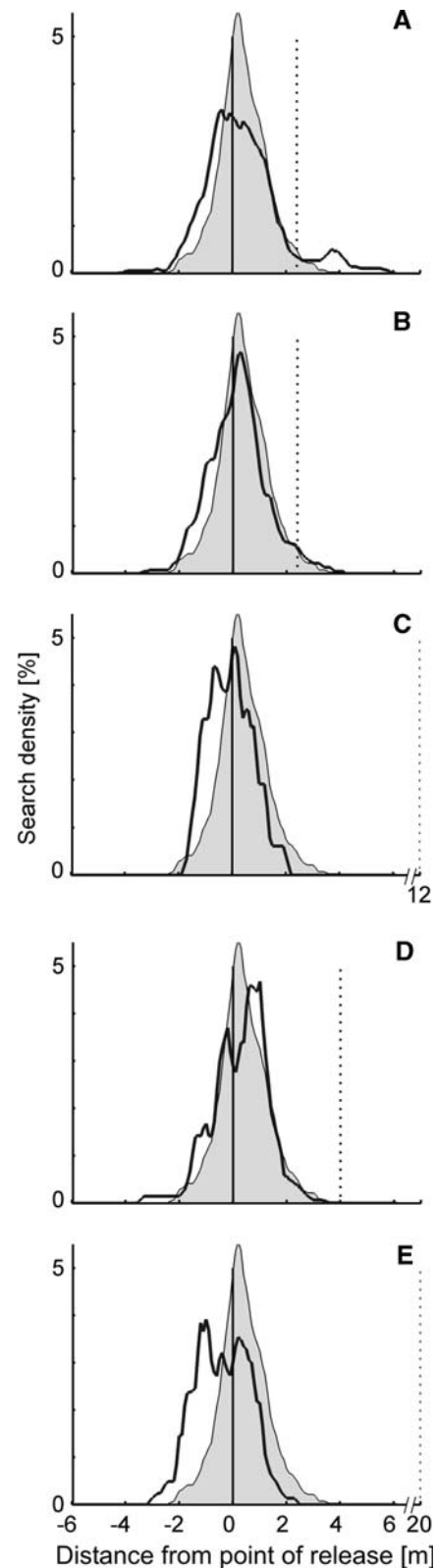
repetitions of the procedure mentioned above (Fig. 4E: Experiment E, median = −0.55 m, $P < 0.05$, $n = 15$ of 19 trials) resulted in a significant difference from the search medians of the control group (Dunn: $P < 0.05$). However, the difference is small and oriented in the wrong direction; i.e., instead of compensating for the 20-m displacement, the ants ran in the very same direction in which they had been displaced. In summary, in all experiments, apart from Experiment E, the search centres did not differ from that obtained in the control experiment in which the ants were tested without any displacement treatment.

The median search ranges, i.e. the difference between the extreme points of an ant's nest search behaviour, did not differ significantly among the various paradigms from the search ranges of the control ants (median search ranges: control: 2.9 m, A: 3.0 m, B: 3.2 m, C: 3.1 m, D: 2.3 m, E: 2.5 m; Kruskal–Wallis: $P = 0.2227$). In all experiments the ants located their fictive nest with the same precision, no matter which treatment they had encountered before.

Discussion

Distance estimation is an essential component of path integration (for a review see: Wehner and Srinivasan 2003). Flying insects use optic flow as their main source for distance estimation (honeybees: Esch and Burns 1995, stingless bess: Hrncir et al. 2003, wasps: Ugolini 1987). Using an elegant experimental paradigm Srinivasan et al. (2000) were able to calibrate the bees' odometer. By forcing the bees to fly through a patterned tunnel, the optic flow received by the bees could be quantitatively manipulated, and its influence on the bees' odometer could be quantified by analysing the distance information played out in the bees' recruitment dances. Depending not only on the distance covered but also on the bees' flying altitude (Esch and Burns 1995) and on the visual structuring of the environment (Tautz et al. 2004), distance estimation via optic flow is error prone. Contrary to flying bees desert ants walk, and do so within an optically rather homogeneous environment. Hence, as the altitude of the animals above ground and the visual structure of the ants' foraging environment are rather constant, optic flow experienced in the ventral field of view should reveal a good estimation of distance covered. Ronacher and Wehner (1995) tested ants in channels within which ground patterns could be moved either in or against the ants' running direction. Hence the optic flow perceived by the ants in the test situation was either reduced or increased. If ants were exposed to a reduced optic flow during their home-bound runs, they underestimated the distance covered, while they overestimated it, if the optic flow was unnaturally high. However, the effect of pattern movement on the distance estimation was smaller than expected if the ants had relied exclusively on the experimentally manipulated optic flow cues. Furthermore no speed trapping was observed; i.e., the ants did not adjust their running speed to the manipulated optic flow. The fact that ants whose ventral eye hemispheres had been covered with light tight paint (Ronacher and Wehner 1995) were still able to gauge distances precisely shows that optic flow is not essential for measuring distances as long as proprioceptive cues exist. In the present account, by preventing the ants from active locomotion, and hence proprioceptive input, but letting them experience optic flow field cues, we set out to test whether optic flow is sufficient for path integration, if proprioceptive cues were eliminated.

Ants are able to calculate the ground distance when they are trained in an array of uphill and downhill channels (Wohlgemuth et al. 2001, 2002). Are they still able to do so if during translocation they do not move actively, but had to rely on optic flow alone? We released ants within slippery downhill channels and by this forced them to slide down passively instead of walking actively (Fig. 4A). Within the channel the ants, when attempting to walk, fell over and then usually slid down the channel in a directed manner with their body not consistently oriented as it would be during controlled locomotion. As the search patterns subsequently performed by the ants in the horizontal test channel did not differ at all from the control ants, the tested ants had not been aware of the experienced displacement, and hence had not included this information into their path integration system. Even if the downhill channel was conspicuously patterned, the ants did not make use of the flow-field information obtained during their passive movements (Fig. 4B).

Ugolini (1987) transported wasps, *Polistes gallicus* (L.), in a transparent container to a previously unfamiliar site, allowing them to assess both optic flow and skylight cues. Upon release the wasps headed towards home, seemingly having integrated the passively experienced displacement. As the ants tested in the present account were sliding down the channel, they might not have experienced optic flow field cues coherent enough in directional (cranial–caudal) terms to trigger direction sensitive cells in their visual system (as described, for example, by Egelhaaf and Warzecha 1999). In order to improve this flow-field situation, we fixated the ants in front of a small vehicle with their eyes in the natural angular position and distance from the ground

(Fig. 3) and moved them passively through a horizontal channel. By this procedure, the immobilized ants experienced naturalistic optic flow cues as freely walking ants would have experienced them and analogous to the optic flow cues recorded and replayed to stationary flies (Kern et al. 2001). Nevertheless, the ants did not take into account the perceived displacement when calculating their position relative to the nest. While bees and wasps might completely rely on optic flow information for path integration, ants have been shown to do so only to a minor extent. However, even when this minor effect had been increased by repeating the pre-test sliding treatments five times (Experiments C and E), the ants continued to search for the nest as if they had not integrated any optic flow field cue at all.

If distance estimation via optic flow and via proprioceptive cues were part of a redundant odometric system, one would expect that the ants were able to detect their passive displacement visually and hence move the centres of their searches either 2.4 m (Experiments A and B), or 4 m (Experiment D), or even further away from their starting point (Experiment C: 12 m, Experiment E: 20 m). Such was not the case. The ants searched at the very same place as the non-displaced control ants did, and hence completely ignored the displacement which they could have deduced from the visual movement of the surroundings experienced during their passive displacements. Obviously, *Cataglyphis* ants use optic flow field cues for distance estimation (as shown by Ronacher and Wehner 1995) only if they move actively and hence simultaneously gain information from their motor control system.

Might the missing influence of the optic flow just be due to the ants' confusion caused by the experimental treatments? In this case, we would not have expected the ants to directly pick up a food item (i.e. to be still in their foraging mood). Furthermore we would have expected search patterns that differed in their spatial spread from that of control ants. As this was not at all the case, the missing integration of optic flow, rather than merely a state of confusion by the treatments seems to have been the major component responsible for our results.

In polydomus ants experienced foragers can be observed carrying indoor workers from the principal nest housing the queen to newly founded satellite nests. When the carrier ant and the carried ant get separated, and when both ants are transferred to an unfamiliar test area, the carrier continues running in the new direction, while the carried ant walks back in the direction of the original nest. Obviously, the latter ant has obtained directional information while being carried (*Cataglyphis bicolor*: Duelli 1976; *Cataglyphis*

iberica: Fourcassie et al. 2000). The carried ants even had some coarse knowledge of the distance over which they had been carried (Duelli 1976). They might have acquired this knowledge by measuring the time they had been carried, but as carrier and carried ants adjust their head inclination, and hence the tilt angle of their eyes, in exactly the same way, processing of optic flow might also have played a role in the absence of proprioceptive input. As in the current experiments proprioceptive cues were eliminated as well, this time experimentally, an influence of passively experienced optic flow-field cues could have been expected on the basis of the results obtained in ants that had been carried by nest mates. However, not the least bit of such an influence had been observed. In conclusion, whatever the contribution of visual flow-field information to the ant's odometer might be, such a contribution is present only if the ants are walking actively rather than being displaced passively.

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