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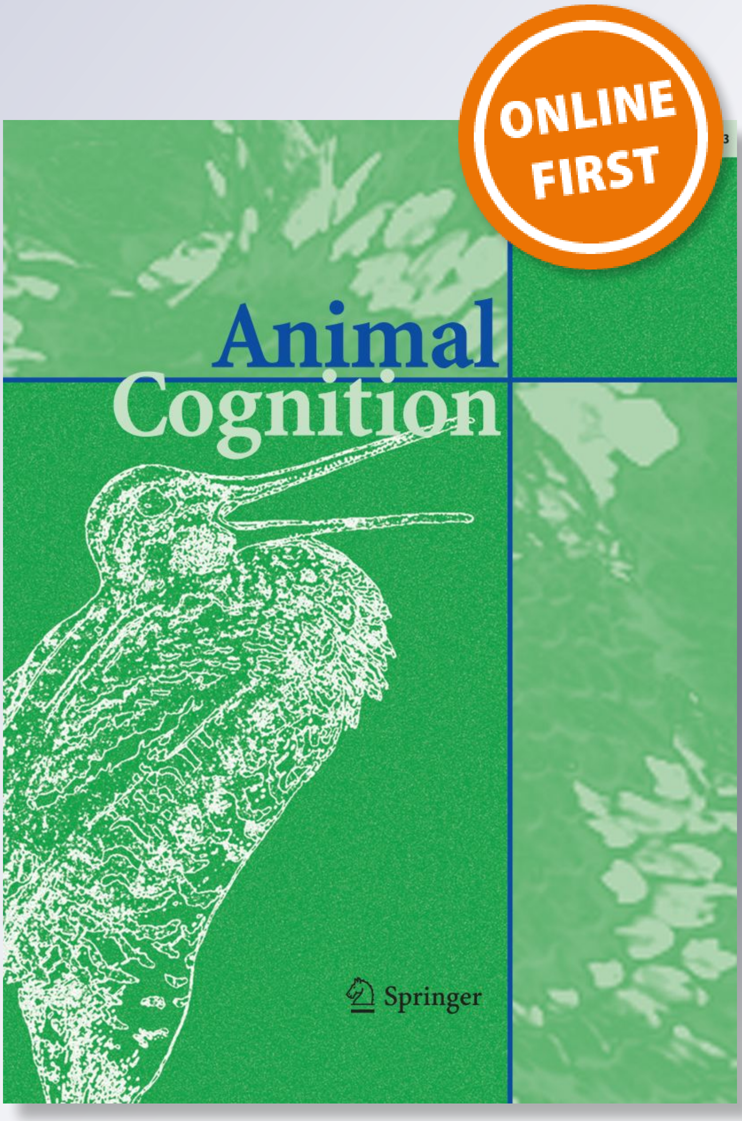

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ORIGINAL PAPER

Running paths to nowhere: repetition of routes shows how navigating ants modulate online the weights accorded to cues

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Abstract

Ants are expert navigators, keeping track of the vector to home as they travel, through path integration, and using terrestrial panoramas in view-based navigation. Although insect learning has been much studied, the learning processes in navigation have not received much attention. Here, we investigate in desert ants (*Melophorus bagoti*) the effects of repeating a well-travelled and familiar route segment without success. We find that re-running a homeward route without entering the nest impacted subsequent trips. Over trips, ants showed more meandering from side to side and more scanning behaviour, in which the ant stopped and turned, rotating to a range of directions. In repeatedly re-running their familiar route, ants eventually gave up heading in the nestward direction as defined by visual cues and turned to walk in the opposite direction. Further manipulations showed that the extent and rate of this path degradation depend on (1) the length of the vector accumulated in the direction opposite to the food-to-nest direction, (2) the specific visual experience of the repeated segment of the route that the ants were forced to re-run, and (3) the visual panorama: paths are more degraded in an open panorama, compared with a visually cluttered scene. The results show that ants dynamically modulate the weighting given to route memories, and that fits well with the recent models, suggesting that the mushroom bodies provide a substrate for the reinforcement learning of views for navigation.

Keywords Desert ant · View-based navigation · Path integration · Mushroom bodies · Cue weighting

Introduction

Sisyphus in the desert

Imagine traversing a familiar route home in the blazing red desert of Central Australia, but, when the front door looms and the comforts of home beckon, you find yourself inexplicably back where you started your journey. How would you retrace your route? This is the nightmare that we foisted repeatedly on red honey ants, *Melophorus bagoti*, expert navigators wielding and combining multiple strategies (Cheng 2012; Cheng et al. 2009, 2014; Kohler and Wehner 2005). This conundrum for the ants, facing the displacements which we call rewinding, revealed in detail what happens when their favourite strategy, following well-travelled routes based on the visual panorama, no longer reaps its usual reward of getting home. We were inspired to expand on two accounts on this theme on formicine cousins of *M. bagoti*, North African *Cataglyphis* ants (Andel and Wehner 2004; Collett 2014). We here depict in detail how the tiny brain of a desert ant adjusts its navigational toolkit when faced with the drama inflicted by rewinding, a burden for the heat loving red honey ants (Christian and Morton 1992; Muser et al. 2005) reminiscent of the task for the mythical Sisyphus, who had to push a rock up a hill only for it to roll down to the bottom again.

Individually foraging desert ants boast three major strategies for navigating robustly over long distances (Collett et al. 2013; Knaden and Graham 2016; Pritchard and Healy 2017;

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Wehner 2003): path integration (PI), the ability to keep track of the straight-line distance and direction from the starting point of travel (Collett and Collett 2000; Müller and Wehner 1988; Ronacher 2008; Wehner and Srinivasan 2003), view-based guidance relying on terrestrial visual (hereafter visual) information learnt from panoramic scenes (Collett et al. 2007; Graham and Cheng 2009; Pritchard and Healy 2017), and systematic search (Schultheiss et al. 2015; Wehner and Srinivasan 1981) to compensate for errors in navigation. Ants combine information from different strategies simultaneously and flexibly (Collett 2012; Legge et al. 2014; Narendra 2007; review: Wehner et al. 2016), perhaps even weighting the cues optimally based on their reliability (Hoinville and Wehner 2018; Legge et al. 2014; Wystrach et al. 2015). Thus, when walking in uncertain conditions, desert ants zigzag more from side to side, called meandering, and stop and scan the environment more, showing searching mixed with directed navigation (Wehner et al. 2016; Wystrach et al. 2014, 2011b). We asked how red honey ants adjust their navigational strategies when their familiar route home does not lead to their entering their abode.

Rewinding to reveal intricacies of running routes without success

Our rewinding technique was inspired by two studies tapping this theme. Andel and Wehner (2004) rewound *C. bicolor* ants as they ran home along a narrow channel decked with landmarks. In their experiment, the ants reached their nest on each trip, only to be dragged out again to re-run the channel. This was as if Sisyphus succeeded in placing the rock at the top of the hill, but then was asked to push a second rock. Rewinding built up the vector calculated by PI. When the ants were tested in a long channel without adorning landmarks—thus without the familiar visual cues defining their route—the rewound ants dashed off in the opposite, nest-to-start-point direction. Collett (2014) allowed individual North African *C. fortis* ants to develop routes across an open environment dominated by a single conspicuous black cylinder. Collett (2014) then rewound the ants, now with their PI vector indicating zero, just once. Interestingly, some ants appeared confused for a prolonged period before eventually commencing their regular route, suggesting that a single unsuccessful event reduces the trust that ants have in their visually defined direction (Graham and Mangan 2015).

We rewound red honey ants repeatedly without letting them enter their nest. We documented their meandering, scanning, and U-turning to move in the opposite direction. We predicted increases in all these behaviours as rewinding stamps its mark over repeated re-runs. A series of manipulations let us delve into three factors contributing to reduced confidence in route-running: the length of the vector in the opposite, nest-to-feeder direction built up by rewinding, the memories of the particular visual cues along the rewound portion of a route, and the structure of the visual environment. In addition, we tested whether our experimental manipulations can lead to the impacts even following long intervals, to test if long-term route memories are updated because of the rewinding process.

Methods

Animals

Thermophilic, diurnally foraging red honey ants, *Melophorus bagoti*, are found throughout semi-arid Central Australia (Christian and Morton 1992; Muser et al. 2005). Two nests at a field site on a private property ~ 10 km south of Alice Springs, Australia were used.

Experimental setup

The scenery surrounding the nests consisted of grass tussocks, mostly of the invasive buffel grass (*Cenchrus ciliaris*), bushes, trees, and a few low buildings (Fig. SI1). The test area, however, was cleared of vegetation. One of our experimental nests (Nest 1) had plants near the nest and test area, making for proximal cues in the surrounding panorama, while Nest 2 lay in an open area with a little vegetation in the vicinity of the nest.

A square-shaped feeder made of plastic ($15 \times 15 \times 9$ cm deep) was sunk into the ground 10 m from a nest. At Nest 1, an enclosure of smooth plywood low walls 10 cm high was constructed around feeder and nest (Fig. 1a, SI1). Both these walls and the walls of the feeder were too slippery for the ants to climb. The feeder was provisioned with mealworm and cookie pieces. During training, sticks were used as bridges, allowing foraging ants to climb out of the feeder.

The enclosure walls were used to form two tracks (Fig. 1a, SI1). A narrower track free of obstacles served as the outbound route for the ants, while the wider track, with obstacles along the way, served as the homebound route. Strings wound around tent pegs were used to make a grid of 1-m squares. The grid allowed experimenters to transcribe the paths of homebound ants onto similarly gridded paper (i.e., copies of Fig. 1a). For a coordinate system, we labelled the nest 0,0, while the feeder was labelled 0,− 10, with metres as units. Two sections of the homeward path were labelled Section A (from $y = -10$ to $y = -7$) and Section B ($y = -6$ to $y = -3$).

Procedure

All ants were trained to be familiar with the homeward route before being tested. An ant arriving at the feeder for the first

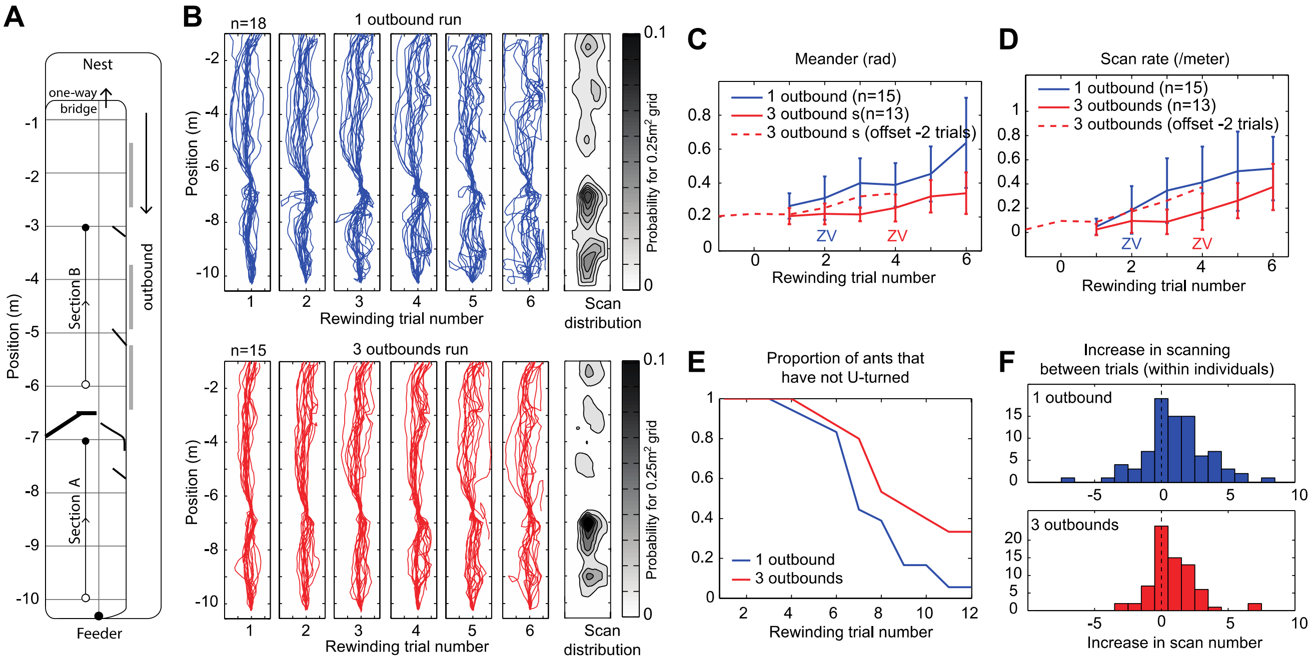

Fig. 1 Paths are more disturbed following repetition and cue conflict. **a** Schematic of the nest and feeder surrounds. A one-way system meant ants had a clear outward journey before being captured at the feeder. The return journey included two sections of interest: Section A, which was relatively clear of objects, and Section B, which was visually cluttered. Diagonal lines represent baffles that were used to make a clear distinction between Sections A and B and also to reduce wall following. The vertical light grey bars represent wooden boards placed just outside the enclosure that serve as additional landmarks along the route. Replicas of this schematic were used to transcribe the routes of ants during tests. **b** Paths of ants for their first 6 re-runs of the homeward path. Upper: ants had performed only one outward run before the start of the rewinding. Lower: ants with three outward runs before rewinding. The locations of bouts of scanning were also recorded. The spatial distribution of scans for both conditions is plotted, with dark shades representing an increasing probability that scans would have been observed within a 0.25 m^2 grid square. **c, d** For the conditions with one and three-outbound runs, Meander and Scan rate are compared, respectively. Only ants with at least six trials without U-turn were used in this comparison. Plots show means with standard deviations. Because of the different numbers of outbound runs, ants experience a zero-vector state (ZV) on different run numbers. For additional reference, the curve of mean Meander and Scan rate for the three-outbound group is also shown offset by two runs to allow a comparison adjusted for PI state (dashed line). **e** Survival curves for ants in the one- and three-outbound groups. Lines show proportion of ants still remaining, with ants removed from the groups once they have performed a U-turn on a homeward run. **f** For the one-outbound (upper) and three-outbound (lower) groups, histograms show the change in the number of scanning bouts from one run to the next. The positive skew shows that scans become more frequent with increasing number of re-runs

time was painted with one dot of enamel paint (Tamiya™ brand), in a colour that denoted the day of arrival at the feeder. Ants were tested after at least 2 days from the day of their first arrival. Except for Condition *3c*, each ant was tested on only one occasion.

Ants were tested one at a time. For tests, the sticks were removed from the feeder for the duration of the test. The test ant was captured in a dark tube and released with its food just in front of the feeder or further along the route, depending on the test. To ensure homing motivation, only ants holding on to their piece of food were tested. The test ant was allowed to run home but typically captured in a dark tube along the route or just before it entered the nest to be returned to a release point to run again, a process that we called rewinding. Similarly, to manipulate the PI state of ants prior to the start of rewinding, ants could be allowed to complete an outward run to the feeder before being captured and returned to the vicinity of the nest for another outward run. Variations on this theme made up the test conditions.

Test conditions

We investigated four questions by making ants repeat routes. (1) What is the effect on habitual route guidance of conflicting path integrator states caused by rewinding? To do this, we took ants that had run to the feeder either once or three times; thus, we had groups starting with different PI home vectors and different conflicts between visual guidance and PI at each stage in the rewinding. (2) What is the specific effect of visual repetition from re-running one portion of the route? Here, ants were manipulated, so that they only had repeated exposure to a specific portion of the route. This tests if rewinding modulates visual guidance generally, or affects the specific views encountered in the rewound section especially adversely. (3) How persistent are the effects of repeating a route? By asking whether route repetition influences navigational memories even after long periods, we can ask if rewinding changes long-term memories. (4) What is the influence of environmental structure on how route repetition influences routes? We tackled this question by asking ants to repeat visually simple or visually complex routes and asking if behavioural changes differ according to the type of visual scenery.

Each of these questions was tackled via several test conditions. Each condition was given a number/letter code (Table 1), with a short description. Full details of test conditions are given in the supplementary materials.

Data analysis

All recorded test paths were digitised with GraphClick™ for further analysis, delivering a series of coordinates describing ants' paths. We analysed scan rate, defined as the number of scanning bouts displayed per metre of path travelled (with scans as defined by Wystrach et al. 2014), and Meander, defined as the mean of the angles formed between successive 30 cm path segments (following Wystrach et al. 2011b). Most of the analysis consisted of standard parametric statistics, with condition as a between-subjects factor, and rewinding trial number as the repeated measure. However, the trial number on which an ant first performed a U-turn and travelled back in the nest-feeder direction was analysed using non-parametric statistics.

Results

The effect on visual route guidance of route repetition and conflicting path integrator states

(a) Conditions 1a and 1b: 1 or 3 outbound runs before repeating homeward route

To investigate how rewinding and PI state influence path characteristics, we allowed ants to have either one (Condition *1a*) or three foodward runs (Condition *1b*) from nest to feeder before being allowed to grab some food and beginning re-runs of the homeward route. Thus, at the start of the rewinding process, ants either had a normal PI home vector or had accumulated a vector of three times the magnitude of the normal home vector. Therefore, the group with a single outbound journey experienced a conflict between PI and visual guidance from the second rewinding run, while the group with three-outbound journeys experienced a conflict from the fourth rewinding run. Following those points in the rewinding sequence, the conflict for both groups increased

Table 1 Summary of test conditions

	Test condition	Key manipulations
1a	One-outbound re-runs	1 Outbound trip Repeated homebound trips
1b	Three-outbound re-runs	3 Outbound trips before arriving at feeder Repeated homebound trips
1c	Nine captures in a single homeward run	Captured nine times on one trip home Test after tenth capture near nest
2a	Re-run of Section A only	Ran Section A four times Test on fifth trip
2b	Re-run of Section B only	Ran Section B 4 times Test on 5th trip
3a	Re-run of Section A with delay	Ran Section A four or five times Delayed for 24 h before test
3b	Re-run of Section B with delay	Ran Section B four or five times Delayed for 24 h before test
3c	Re-run of Sections A and B with nest return	Ran Section A four times, then went home Next foraging trip; ran Section B four times Tests immediately after running Section B (as zero-vector ant) and on next trip to feeder (as full-vector ant)
4a	One-outbound re-run open (Nest 2)	1 Outbound trip (Nest 2), open Repeated homebound trips
4b	One-outbound re-run with clutter (Nest 2)	1 Outbound trip (Nest 2) with landmarks added near route Repeated homebound trips

between the ant's PI system and the direction indicated by visual cues, as the PI home vector got longer in the nest-to-feeder direction. Over six runs, both groups of ants became more variable in their paths (Fig. 1b), meandering and scanning more. Our formal measure of Meander was higher in ants that had a greater conflict between visual guidance and PI, because they had experienced only one-outbound run to the feeder (Condition *1a*) than in ants that had experienced three-outbound runs before reaching the feeder (Condition *1b*, Fig. 1c). An analysis of variance found statistical significance in all the effects: condition (one-outbound trip vs. three-trips, $F_{1,156}=56.01$, $p<0.0001$), trials ($F_{5,156}=14.57$, $p<0.0001$), and their interaction ($F_{5,156}=3.42$, $p=0.006$). Ants also scanned more frequently over repeated runs home (Fig. 1d, f), and ants with three-outbound runs scanned less often than ants with one-outbound run (Fig. 1d). An analysis of variance found significant main effects of Condition ($F_{1,156}=28.35$, $p<0.0001$) and trials ($F_{5,156}=16.07$, $p<0.0001$), but not a significant interaction ($F_{5,156}=1.53$, $p=0.184$). Scans were not evenly distributed across the length of the route home (Fig. 1b). Both ants with one-outbound run and ants with three-outbound runs scanned most in the early part of the route, especially just before the first set of barriers at -7.

We further analysed how ants lost confidence in their visually guided route by looking for the point at which the PI direction finally overrode the visually defined direction and ants U-turned and began travelling in the nest-to-feeder direction. Ants with three-outbound runs before rewinding had a higher survival curve (proportion of ants that had still not performed a U-turn by a given trial number) than ants with 1 outbound run (Fig. 1e). This difference, however, did not reach statistical significance (non-parametric test on number of trials before the first turn-back: $Z=1.68$, $p=0.092$). After U-turning, ants typically ran for a long distance away from home, that is, in the direction of their negative path integration vector, confirming that ants had, indeed, accumulated a large vector, as observed by Andel and Wehner (2004).

(b) Condition 1c: nine captures on one trip home

As a control for the repeated capturing and disruption of the homebound journey, we captured ants nine times on one journey home, resulting in a zero-vector ant being tested after the tenth capture. Repeated capturing had no notable effect on the ants' navigation (Fig. SI2). The ants performed much like zero-vector ants that had been captured only once near their nest. Ants in Condition *1c* had a closer resemblance to ants with a same PI state (Condition *1a*, second release) than to ants after the same number of captures (Condition *1a* and *1b*, tenth release, Supplementary Results SI2). While we cannot rule out that repeated captures may have some effect on paths, the changes in behaviour that arise from repeatedly running the home path must be attributed mostly to the increasing vector length of path integration in the negative (nest-to-feeder) direction and/or repeated viewing of the scenes along the route, which is examined next.

The specificity of the effect of visual repetition on route disturbance

Condition 2a and 2b: re-running Section A or Section B repeatedly

In these conditions, ants re-ran only Section A or only Section B four times and were then tested on the entire route (Fig. 2a). In comparison with ants that re-ran the entire route repeatedly, their meander and level of scanning were similar. We then examined scan rates and meander for the particular route sections (A or B) of the entire route. Ants that had re-run Section A repeatedly scanned and meandered more in Section A compared to ants that had re-run Section B (Fig. 2b, d). On the contrary, in Section B of the route, it is ants that had re-run Section B that displayed a higher Meander and Scan rate (Fig. 2c, e). The statistical analysis is combined with Condition 3 in the next sub-section.

The persistence of route repetition effects

(a) Condition 3a and 3b: re-running Section A or Section B with delay before testing

As in the two previous conditions, ants in conditions *3a* and *3b* re-ran Section A only or Section B only four times (Fig. 2). Ants were then held for 24 h before being tested on the entire route. The delay served to test if the disruption caused by repeatedly running a segment persists. Results again show a persistent increase in meander and scans specific to the route section that the ants had repeatedly re-run the day before (Fig. 2). In addition, the 24 h delay increased the disruption as compared to ants that had performed the test immediately after the four rewinding trials (Fig. 2).

For each of Meander and Scan rate on the fifth (test) run, we combined conditions *2a, 2b, 3a*, and *3b* in a three-way analysis of variance, with Condition (rewound on A or B) and delay (immediate test vs. 24-h delay) as between-subjects factors, and section (A or B) as repeated measure. For Meander, the analysis found significant main effects of all three factors (Condition: $F_{1,97}=8.64$, $p=0.0041$; delay: $F_{1,96}=41.84$, $p<0.0001$; section: $F_{1,96}=55.16$, $p<0.0001$). A significant Condition by section interaction was also found ($F_{1,96}=70.53$, $p<0.0001$), while the other interactions were not significant (Condition by delay: $F_{1,96}=0.38$, $p=0.5383$; delay by section: $F_{1,96}=2.87$, $p=0.0935$; Condition by delay by section: $F_{1,96}=1.82$, $p=0.1803$). For

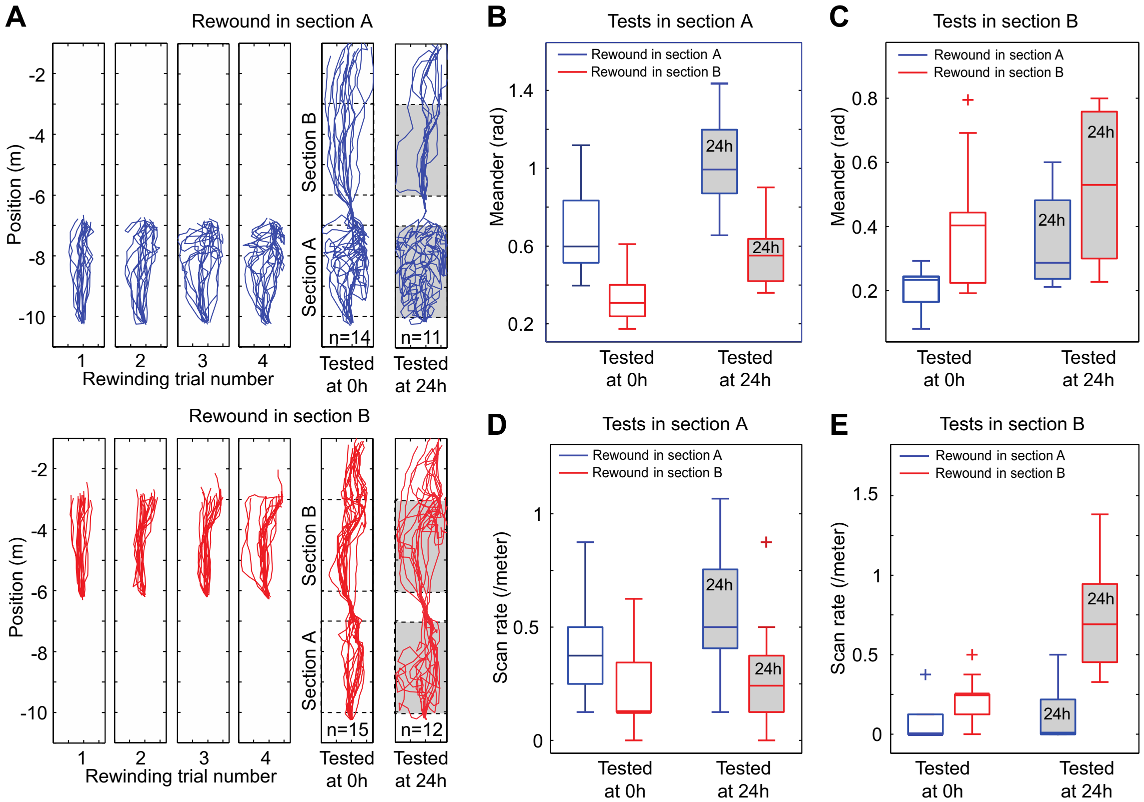

Fig. 2 Ants show more disturbance in the sections in which they were rewound. After arriving at the feeder on their first trip, ants were allowed to run the first part of the route (Section A) before being rewound to the feeder position a total of four times, or they were taken from the feeder, with their food, to the mid-point of the route and allowed to complete the Section B before being rewound to the mid-point (again for four trips). Thus, we have two groups of ants with rewinding experience in Section A or Section B only. **a** Paths of ants given rewinding in Section A (top) or Section B (bottom). For purposes of clarity, rewinding trials show paths that were selected randomly from a larger number of paths. **b, c** Meander of ants in Section A (**b**) or Section B (**c**). **d, e** Scan rates of ants in Section A (**d**) or Section B (**e**). The box plots show the median (middle line in the box), the 25th and 75th quartiles (bottom and top of the box, respectively), and 1.5 times the interquartile range (whiskers), as well as outliers (individuals beyond the range of the whiskers)

Scan rate, the analysis of variance revealed significant main effects of Condition ($F_{1,96}=8.89$, $p=0.0036$) and delay ($F_{1,96}=12.61$, $p=0.0006$), but not of section ($F_{1,96}=0.25$, $p=0.6167$). Each of the interactions was found to be significant (Condition by delay: $F_{1,96} = 6.18$, $p=0.0147$; Condition by section: $F_{1,96}=36.26$, $p<0.0001$; delay by section: $F_{1,96}=11.77$, $p=0.0009$; Condition by delay by section: $F_{1,96}=8.59$, $p=0.0042$).

(b) Condition 3c: section A re-run with return to nest

In this condition, ants re-ran Section A four times, went inside the nest, reached the feeder again, and then re-ran Section B 4 times, so that, on a test, they were in a similar path integration state to ants in conditions *2a* and *2b*. Compared with condition *2a*, in which ants did not enter their nest before the test, entering the nest did not affect the ants' behaviour in Section A, whereas having a full-vector reduced meander and scan rate (Supplementary Results, Fig. SI3).

Influence of the visual environment on route disturbance

Conditions 4a and 4b: re-running with or without proximal objects (nest 2)

These two conditions were implemented with nest 2, which lay in a rather open area. Ants re-ran the route home repeatedly without (open landscape, Condition *4a*) or with (cluttered landscape, Condition *4b*) added proximal objects around the route (Fig. SI4A). The presence of nearby proximal objects mitigated the detrimental effects of repeated route-running, or, to put it another way, having an open landscape along the route meant the effects of repeated route-running were more severe (Fig. SI4B). Ants performed their first U-turn after fewer re-runs in the open landscape (Condition *4a*), and they scanned and meandered a little more, compared with their performance in the cluttered landscape (Fig. 3). For Meander and Scan rate on the first three trials, we conducted a mixed analysis of variance with Condition (open or cluttered landscape) as the between-subjects factor and trials as repeated measure. For Meander, both main effects reached significance (Condition: $F_{1,81} = 9.84$, $p = 0.0023$; trials: $F_{2,81} = 18.45$, $p < 0.0001$), but the interaction did not reach significance ($F_{2,81} = 2.47$, $p = 0.091$). For Scan rate, only the main effect of trials reached significance ($F_{2,81} = 17.72$, $p < 0.0001$). The main effect of Condition ($F_{1,81} = 1.57$, $p = 0.214$) and the interaction ($F_{2,81} = 0.46$, $p = 0.636$) did not reach significance. For the trial on which ants first turned back, the difference between conditions was significant by a non-parametric test ($Z = 3.13$, $p = 0.0018$).

Discussion

Repeated rewinding produces a Sisyphean task for the ants, in which following their route fails to land them in their nest. The red honey ants transformed their route-running, meandering and scanning more and eventually giving up and U-turning in the opposite, nest-to-feeder direction, following the commands of path integration. We built on earlier results on this theme (Andel and Wehner 2004; Collett 2014) to elucidate factors that contribute to the ants' reduced confidence in the familiar visually defined route, normally a favoured strategy of experienced *M. bagoti* foragers (Cheng et al. 2009, 2014; Kohler and Wehner 2005; Wystrach et al. 2011b).

Factors affecting route-following

As the first factor, increasing conflict between visually guided directions and path integration leads to path degradation. Given an equivalent number of route repetitions, ants with a greater conflicting PI vector length showed more meandering and pausing to scan, and eventually performed route U-turns earlier in the rewinding sequence (Fig. 1). This shows that learnt visual guidance and PI are simultaneously active in influencing behaviour even when in 180° directional conflict (as observed in Collett 2012; Freas and Cheng 2017; Legge et al. 2014; review: Wehner et al. 2016). In addition, it shows that the weight given to PI increases with vector length (as shown in Wystrach et al. 2015).

As a second factor, viewing a portion of a route without getting home reduces confidence in that particular segment of the route. Seeing familiar scenes on the route without getting home combines elements of extinction and aversive conditioning of the visually defined route, without being fully analogous to either learning phenomenon. After repeating a section of a familiar route over and over again, and even in the absence of a strongly conflicting PI vector, ants displayed strong disorientation specifically on that section (Fig. 2). Interestingly, this specific effect is still evident in the routes of ants even after a 24 h delay, and whether or not ants had been allowed inside their nest (Fig. 2, SI3). The fluent route-following behaviour did not recover spontaneously, that is, after the mere passage of time without further relevant training. The trust in route-following also did not recover after a single success (see SI3). These results imply that the route recapitulations change long-term memory, rather than causing short-term adaptation. They also illustrate the dynamics of continuous learning in a naturalistic task, highlighting this study's brand of experimental ethology of learning (Freas et al. 2019).

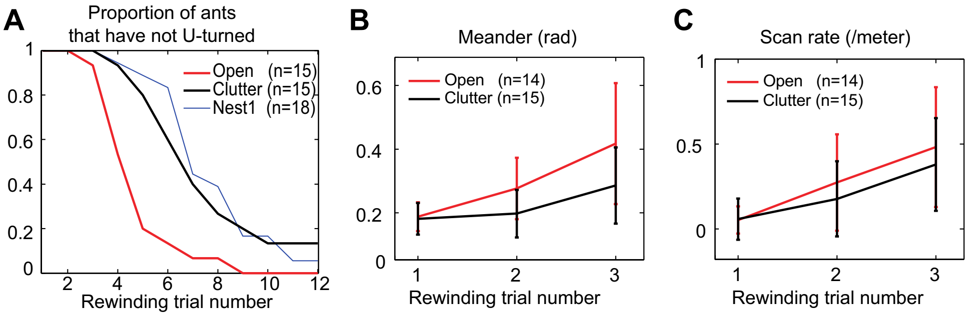

Fig. 3 Ants show more path disturbance after rewinding in an open landscape vs. a cluttered landscape. To investigate the impact of visual clutter on route changes, we repeated the basic rewinding protocol (see Fig. 1) with two groups of ants at a second nest site. The nest environs were left open or had visual clutter added. **a** The proportion of ants that have not turned back in the open and cluttered landscape of Nest 2, and in the landscape of Nest 1, which was visually cluttered (data from Fig. 1e). **b** Meander in the paths of ants rewound in the open or cluttered landscape of nest 2 (mean ± SD). **c** Scan rate in the ants rewound in the open or cluttered landscape of nest 2 (mean ± SD)

As the third factor, the course of route changes with rewinding depends on the structure of the visual environment. This was suggested by Collett (2014; see also Graham and Mangan 2015) to explain why path decrements from repetition are not observed in all experimental situations. Here, we showed that when ants were navigating in an open environment, as opposed to the same environment with additional proximal visual clutter, path disruption arose after fewer repeated runs, and thus with a weaker conflict between visual guidance and PI (Fig. 3, SI4). It could be that because aversive conditioning sets in for specific views experienced during a familiar route home, the slowly changing visual panorama along an open route provides a larger and longer window to modify the valence of specific views.

Adaptive use of information based on reliability

Ants adjust the weight given to different navigational systems based on many factors. For path integration, more weight is assigned to longer vectors (Hoinville and Wehner 2018; Merkle et al. 2006; Merkle and Wehner 2010; Stone et al. 2017; Wystrach et al. 2015), and for visual guidance, more weight is accorded to familiar views (Legge et al. 2014), unambiguous views (Huber and Knaden 2017), and more recently encountered views (Freas and Cheng 2017). All these rules of thumb make intuitive sense. Here, we show that the weight attributed to view-based route guidance also depends on the experiences of failure, which reduce the weight given to visually guided route segments. This adjustment does not depend on immediately available information, but requires the accumulation of information over time. That is, this second-order information about the reliability of a navigational system requires a form of memory.

Ants also have reduced trust in their visual route memories when travelling through open environments. Functionally, this makes sense as the perceived change in scenery while moving depends on the proximity of the surrounding objects. One can estimate one's position based on memorised views more accurately and precisely if the environment is cluttered (Schultheiss et al. 2013; Zeil et al. 2003). Thus, provided that the current view is equally familiar, visual guidance should be trusted more in cluttered than in open environments. Taken together, our results highlight nuances in the way that ants weight their navigational tools, opening up questions regarding the mechanistic basis of navigation.

The neural basis of flexible route guidance

Our findings give firm support for the role of associative learning in view-based navigation. According to the current literature, the mushroom bodies (MB) undergird associative learning in insects (Aso et al. 2014; Bazhenov et al. 2013; Cohn et al. 2015; Galizia 2014; Peng and Chittka 2017; Perry et al. 2013; Webb and Wystrach 2016), including the learning of views that can guide familiar routes (Ardin et al. 2016; Cruse and Wehner 2011; Hoinville and Wehner 2018; Webb and Wystrach 2016). MB support reinforcement learning by separating patterns in the input, and assigning positive valences to positively reinforced patterns. In travelling routes, ants move forward when experiencing familiar reinforced views, and turn more or scan when the view is not similar to reinforced views (Kodzhabashev and Mangan 2015; Lent et al. 2010; Wystrach et al. 2011a; Zeil et al. 2014). We think that rewinding reduces the valence associated with views, also making ants increase meandering and scanning. Functionally, these behaviours expose the navigator to novel views of the environment, allowing ants to explore new options for reaching home. In general, online updating of the valence of visual route memories could be adaptive in natural foraging. For instance, aversive associations with views might help ants to learn to detour around an obstacle or a trap, a form of avoidance learning (work in preparation).

Like most experimental treatments, our manipulations do not reflect conditions experienced naturally by ants. However, the manipulations enabled us to explore the mechanisms underlying natural navigation. We found support in the foraging of ants in their natural habitat for a general point regarding information processing: in general, if a cue is perceived repeatedly and independently of a reward, it is not a good predictor of that reward, and the weight attributed to it should be lowered. In addition, for navigation, a cue perceived repeatedly is not a good indicator of one's current position, and should be ignored. Using our rewinding method enabled us to clearly show that ants do, indeed, apply this principle, and start to reduce their confidence in views if these are no longer reliable.

Conclusions

The rewinding method asks ants to repeat the portions of a familiar route. By implementing rewinding, we have shown dynamic online processing in cue integration, with ants incorporating all the route experiences to update route memories and cue weightings. For cue integration, ants use more than heuristics based on immediately available information, such as the current PI vector length or the current visual familiarity. They also adjust the weight attributed to route memories based on the information accumulated over successive trials, that is, over time. Moreover, their fine-tuning depends on how informative the visual scenery is, as reflected in the number of nearby objects (cluttered vs. open environments). We think that reinforcement learning is central to all these experience-dependent modifications. Further investigation of these dynamic learning processes, in conjunction with modelling the associative learning in navigation implemented in the mushroom bodies of insects, will be particularly informative.

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Author contributions AW, SS, and PG conceived and designed the study. AW and SS carried out experimentation and digitised the paths. AW analysed the data. AW drew the figures. PG and KC drafted the manuscript. All authors revised the manuscript and agreed on the final version of it.

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Data acessibility The raw data files and a file containing explanations are in the supplementary materials.

Compliance with ethical standards

Conflict of interests The authors declare that they have no competing interests.

Ethics standards Australia has no ethical regulations regarding work with insects. The experimentation carried out was, moreover, non-invasive, and no long-term aversive effects on the nests or on the tested individuals were observed.

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