

RESEARCH ARTICLE

The interaction of path integration and terrestrial visual cues in navigating desert ants: what can we learn from path characteristics?

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ABSTRACT

Ant foragers make use of multiple navigational cues to navigate through the world and the combination of innate navigational strategies and the learning of environmental information is the secret to their navigational success. We present here detailed information about the paths of *Cataglyphis fortis* desert ants navigating by an innate strategy, namely path integration. Firstly, we observed that the ants' walking speed decreases significantly along their homing paths, such that they slow down just before reaching the goal, and maintain a slower speed during subsequent search paths. Interestingly, this drop in walking speed is independent of absolute home-vector length and depends on the proportion of the home vector that has been completed. Secondly, we found that ants are influenced more strongly by novel or altered visual cues the further along the homing path they are. These results suggest that path integration modulates speed along the homing path in a way that might help ants search for, utilise or learn environmental information at important locations. Ants walk more slowly and sinuously when encountering novel or altered visual cues and occasionally stop and scan the world; this might indicate the re-learning of visual information.

KEY WORDS: Navigation, Multimodal interactions, Walking speed, Visual guidance, *Cataglyphis*

INTRODUCTION

A vital task in the life of a scavenging desert ant is to safely return to a rather inconspicuous nest entrance after extensive foraging journeys (Buehlmann et al., 2014; Huber and Knaden, 2015). The clever combination of innate navigational strategies and the learning of information from the environment is the secret to their navigational success (Wehner, 2003; Collett et al., 2013; Knaden and Graham, 2016). *Cataglyphis* desert ants do not lay pheromone trails but individually navigate using path integration and information from the environment such as visual (Wehner et al., 1996; Huber and Knaden, 2015) or olfactory cues (Buehlmann et al., 2012, 2014, 2015). Path integration is an innate behaviour allowing exploration of unfamiliar terrain while keeping track of direction (Wehner and Müller, 2006) and distance travelled (Wittlinger et al., 2006) to maintain an estimate of the direct path back to the origin of the journey (Müller and Wehner, 1988; Collett

and Collett, 2000; Wehner and Srinivasan, 2003; Ronacher, 2008). It is essential when unfamiliar with the environment but, as a strategy, it is prone to cumulative errors (Sommer and Wehner, 2004; Merkle et al., 2006) and may have to be followed by systematic search if the nest is not located (Wehner and Srinivasan, 1981; Schultheiss and Cheng, 2011). For accurate route guidance and homing, ants complement path integration with visual information learnt from panoramic scenes (Collett et al., 2007; Graham and Cheng, 2009; Wystrach et al., 2011a; Lent et al., 2013; Buehlmann et al., 2016). Visual information can be used to pinpoint the nest (Wehner and Rüber, 1979; Wehner et al., 1996; Narendra et al., 2007b) and guide habitual routes (Collett et al., 1992; Kohler and Wehner, 2005; Collett, 2010; Wystrach et al., 2011b). Indeed, the learning of visual cues for route guidance allows ants to robustly travel between the nest and a feeding site along idiosyncratic routes (Collett et al., 1992; Wehner et al., 1996; Graham et al., 2003; Kohler and Wehner, 2005; Wystrach et al., 2011b; Mangan and Webb, 2012).

Path integration itself involves little or no learning (Narendra et al., 2007a; Merkle and Wehner, 2009) but plays an important role in the learning of visual information. Path integration can be used to guide specific exploration behaviours which facilitate the learning of visual information (Judd and Collett, 1998; Nicholson et al., 1999; Wehner et al., 2004; Müller and Wehner, 2010; Stieb et al., 2012; Fleischmann et al., 2016, 2017). Similarly, ants using path integration in unfamiliar terrain will take consistent and direct paths, potentially simplifying the learning of visual information along a route (Collett et al., 2003). So, even though visual cues can later be retrieved and utilised independently of the state of path integration (Collett et al., 1992, 2001; Kohler and Wehner, 2005; Mangan and Webb, 2012), path integration may provide a crucial scaffold for visual learning (Graham et al., 2010; Müller and Wehner, 2010).

In experienced ants, visual guidance and path integration are redundant navigational strategies, and behavioural experiments have shown that ants can make simultaneous use of multiple sources of navigational information (Narendra, 2007b; Reid et al., 2011; Collett, 2012; Legge et al., 2014), perhaps even weighting the cues optimally, based on their reliability (Wystrach et al., 2015). One way of looking at interactions between path integration and the use of terrestrial visual cues is to investigate ants' paths when the direction indicated by the path integration system is at odds with the information from visual cues. We can see situations where ants strongly weight either visual (e.g. Narendra et al., 2013a) or path integration information (e.g. Wehner et al., 1996). However, ants often chose a compromise direction when path integration and familiar visual scenes are in subtle conflict (Collett et al., 2007; Collett, 2012; Wehner et al., 2016). Likewise, we can learn from experiments where ants with a path integration home vector are displaced to a location outside of their habitual route, such that

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learnt and current visual scenes do not match. In such situations, ants' paths are less accurate and more sinuous, and ants follow path integration for only a proportion of the home vector length before starting a systematic search (Fukushi and Wehner, 2004; Beugnon et al., 2005; Narendra, 2007b; Bühlmann et al., 2011; Wystrach et al., 2011b; Cheng et al., 2012; Cheung et al., 2012; Schultheiss et al., 2016). These studies are further evidence of interesting interactions between path integration and visual guidance, although the detailed nature of such interactions is unclear.

Our aim here was to look at path characteristics of navigating ants under the influence of path integration and visual guidance. Across three experiments with a combination of traditional recordings of full paths and high-speed recordings at key locations, we analysed ants' path characteristics for different path integration states and in response to novel and familiar visual cues. As previously reported, walking speed (e.g. Zeil et al., 1996; Narendra et al., 2013b; Degen et al., 2015; Schultheiss et al., 2015), pausing behaviour (e.g. Narendra et al., 2013b; Wystrach et al., 2014; Zeil et al., 2014) and path straightness (e.g. Bühlmann et al., 2011; Wystrach et al., 2011b) can provide insight into the interaction of path integration and visual guidance. Our new findings are that (i) ants' walking speed decreases significantly along homing paths and stays lower during subsequent search paths and (ii) ants are influenced more strongly by novel or learnt visual cues the further along their homing path they are.

MATERIALS AND METHODS

Species and study site

All experiments were performed with the North African desert ant, *Cataglyphis fortis* (Forel 1902), in a salt pan (34.954897 N, 10.410396 E) near the village of Menzel Chaker, Tunisia. Experiment 1 was performed with ant foragers from only one nest while in experiments 2 and 3, multiple nests were used.

Experiment 1: walking speeds on homing paths and nest searches

We trained foragers to travel back and forth between their nest and a feeder, which was placed 5, 10 or 20 m away (Fig. 1A), using biscuit crumbs provided *ad libitum*. For test recordings, individuals were taken from the feeder and paths were recorded on a distant test field. A grid of lines (mesh width: 1 m) was painted on the ground and the ants' paths were recorded on squared paper with time marks noted every 5 s. Here and elsewhere, each ant was recorded only once. Each path was cut at the position where the ant switched from a straight homing path to systematic nest search. This switch point was found by breaking the path into 0.5 m chunks and finding the first chunk that differed by at least 90 deg from the feeder–nest direction (see also Merkle et al., 2006; Bühlmann et al., 2011; Schultheiss and Cheng, 2012). Thus, we classified path segments before the turning point as part of the homing path and segments after this point as part of the nest search and these were analysed separately. To control for speed differences due to unknown variables, such as ant body size, the size of the biscuit crumb or the temperature, we normalised walking speed during both homing and nest search to an individual ant's mean homing and search speed, respectively. For homing paths, these normalised speed values were associated with relative path positions. To do this, for each path, the distance immediately before search was set to 1 to account for individual ants having slightly different path lengths. Paths were then divided into 10 bins and the bins filled with the corresponding speed values. If an ant provided multiple values per bin because of a low walking speed or sinuous path, the median was taken. To analyse the effect of path sinuosity on walking speed during search,

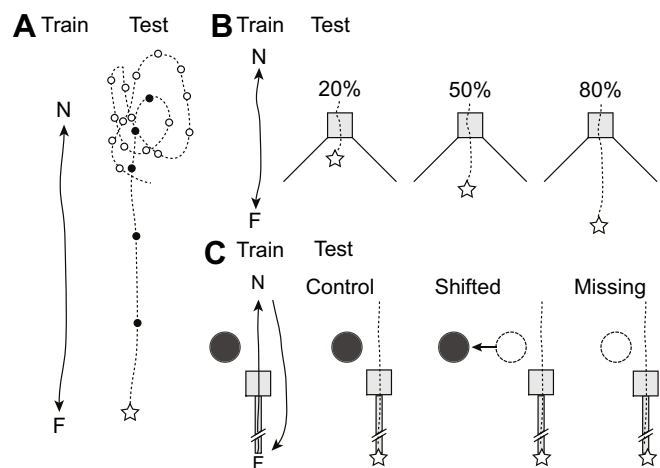


Fig. 1. Experimental configuration for training and test conditions. N, nest; F, feeder. Black solid lines with arrowheads are training paths; black dotted lines are homing paths in tests. Stars indicate the point of release in tests. (A) Experiment 1: relationship between walking speed and path integration state. Ants were trained to a feeder that was 5, 10 or 20 m away from the nest. Homing paths of ants taken from the feeder were recorded on a distant test field. Circles indicate 5 s time intervals marked simultaneously; homing (filled circles) and search paths (open circles) were analysed separately. (B) Experiment 2: response to visual changes along homing paths. Ants taken from the feeder at 5, 10 or 20 m were released on the test field such they had completed 20%, 50% or 80% of the homing path when reaching the centre of the arena (grey square). Test paths were recorded from the point of release to the edge of the arena. Visual change comes from the barriers that guided ants onto the arena and the tripod placed next to it. (C) Experiment 3: interaction between path integration and learnt visual cues along homing paths. Training distance, 10 m. During training and subsequent tests, a channel (height, 7 cm; width, 7 cm; grey rectangle) was either 1 or 6 m long, i.e. ants had completed either 10% or 60% of their homing path when reaching the exit of the channel and entering the arena (grey square). Circles represent the cylinder. There were three test conditions: control, cylinder shifted 2 m leftwards and cylinder missing. Paths were recorded from channel exit to nest.

the index of straightness (i.e. beeline/total path length) was calculated for each path segment. Path segments during search were considered as straight when their index of straightness was as high as the mean index of straightness of that ant's homing path.

Experiment 2: responses to visual novelty

As in experiment 1, paths from ants taken from a feeder at 5, 10 or 20 m from the nest were recorded on the test field. A change in the visual environment was created by adding an unfamiliar recording setup that consisted of a 0.6 m × 0.6 m wooden board (hereafter 'arena') placed on the ground with a camera tripod next to it and two barriers (each 1.5 m long, approximately 4 cm high) that were attached at 45 deg to the corners of the arena to guide the ants onto it (Fig. 1B). For the 5 m training condition, we had an extra test condition where we increased the visual mismatch by adding two black boards (each 1 m long, 0.5 m high) behind the arena (see Fig. S2D). Homing ants were released on the test field at specific locations such that they had completed 20%, 50% or 80% of their homing paths when they reached the centre of the arena. For instance, an ant taken from the feeder that was 5 m away from the nest was released 1 m (20% of path completed when crossing the arena), 2.5 m (50% of path completed) or 4 m (80% of path completed) away from the centre of the arena. Similarly, ants removed from the feeders that were 10 and 20 m away from the nest were released at the corresponding 20%, 50% and 80% locations. Paths preceding the arena were recorded on paper and once the ants

were on the arena, their paths were recorded with a high-speed camera (see below for details). As a control, paths from ants that were familiar with the setup at 20%, 50% or 80% were recorded on the training site. Because the natural spread of the paths was wider than the dimensions of the recording setup, we only considered ants in our analyses that arrived within the area enclosed by the two barriers. For the ants that entered this area, we counted those that kept walking in the homing direction and crossed the arena versus those that turned around and made a detour. If they crossed the arena, we analysed whether any U-turns occurred prior to entering the arena. For this purpose, paths were broken into 0.2 m segments and we determined whether any chunks differed by at least 90 deg from the feeder–nest direction. From the high-speed recordings, we extracted the ants' average walking speed (i.e. full path length/total time), index of straightness (i.e. beeline/full path length) and the frequency of pauses per 1 m of path. Pauses were defined as periods along the path where walking speed dropped below 0.1 m s^{-1} .

Experiment 3: interactions between path integration and visual guidance

In this experiment, the ants' food-ward and homeward training routes slightly differed. The 10 m route to the feeder was over open ground but the first section of the return to the nest was through an aluminium channel that was either 1 or 6 m long (height, 7 cm; width, 7 cm). Therefore, ants had either completed 10% or 60% of their homing path when they reached the channel exit. At the channel exit, they crossed the $0.6 \text{ m} \times 0.6 \text{ m}$ arena and passed a cylinder (height, 0.41 m; width, 0.45 m) 0.8 m to the left of the direct feeder–nest path (Fig. 1C). Ants were tested in three situations: control, cylinder shifted 2 m leftwards and cylinder missing. All tests were implemented on the familiar training ground. Paths were recorded on paper once the ants had left the channel and with the high-speed camera (mounted over the last part of the channel) to record ants crossing the arena. Walking speed, index of straightness and occurrence of pauses were extracted from the high-speed recordings as described above.

High-speed recordings and data processing

Using a Panasonic DMC-FZ200 camera, we could record portions ($60 \text{ cm} \times 60 \text{ cm}$) of ants' paths at $200 \text{ frames s}^{-1}$. The trajectories were extracted from videos using Ctrax (version 0.5.3; <http://ctrax.sourceforge.net/>) and the associated Matlab toolbox (BehavioralMicroarray; Branson et al., 2009) and corrected for tilted perspectives. When using the high-speed camera, the field of view was a large wooden board (arena) with calibration marks to enable the calibration of the camera for aspect and position. To exclude the effects of the arena edges on ants' behaviour, the outer 2 cm was omitted from analysis. Large-scale paths were recorded on paper (with time stamps) and digitised using GraphClick (version 3.0; www.arizona-software.ch/graphclick/). All data were processed and analysed in Matlab.

RESULTS

Walking speed decreases along homing path

In order to investigate the relationship between walking speed and path integration state, we recorded the homing paths of ants returning from a feeder that was 5, 10 or 20 m away from the nest (see Fig. 1A). As described many times for *C. fortis*, when released on the test ground, ants run off their path integration vector before switching into a systematic nest search with loops centred on the fictive nest position (e.g. Wehner and Srinivasan, 1981). When looking at the speed characteristics, we firstly saw a general trend

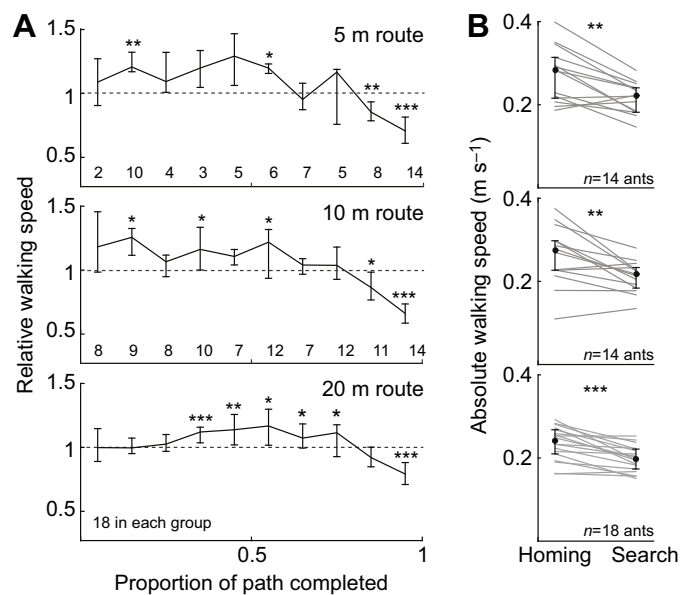


Fig. 2. Walking speed decreases along the homing path and is lower during nest search. (A) Relative walking speed along homing paths for ants with 5, 10 or 20 m homeward routes (see Fig. 1A). For each ant, walking speed was normalised to that ant's mean homing speed and each speed measurement was associated with a relative path position. For each path, the distance at the end of the homing path was set to 1 and speed data were filled into 10 equal bins. Data are plotted as medians with error bars showing the 25th and 75th percentiles. Asterisks indicate where data were significantly different from 1 (Wilcoxon tests: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Numbers below error bars show the number of ants that contributed to each bin. (B) Mean absolute walking speed during nest search (Search) compared with mean homing speed (Homing). Data are plotted as medians with error bars showing the 25th and 75th percentiles for homing and search paths, and grey lines showing changes for individual ants. Asterisks indicate significant differences using Wilcoxon matched-pairs test (** $P < 0.01$, *** $P < 0.001$).

that walking speed started high and then significantly decreased preceding the search (Fig. 2A). The ants' speed in the final path segment immediately preceding the commencement of search (medians: 5 m, $n = 14$ ants, 0.18 m s^{-1} ; 10 m, $n = 14$ ants, 0.17 m s^{-1} ; 20 m, $n = 18$ ants, 0.18 m s^{-1}) was significantly lower than the maximum speed (medians: 5 m, 0.35 m s^{-1} ; 10 m, 0.36 m s^{-1} ; 20 m, 0.32 m s^{-1}) along the homing path (Wilcoxon matched-pairs test: for all three training distances, $P < 0.001$). Ants reached this maximum walking speed at 32% (5 m: total path length, median, 5.0 m), 33% (10 m: total path length, median, 8.9 m) and 50% of their homing path (20 m: total path length, median, 18.4 m), respectively. Interestingly, when comparing the speed profiles across entire homing paths for the three groups, we saw a consistent trend that at around 85% of the homing path, ants were walking significantly slower than during the majority of the route (Fig. 2A), i.e. speed profiles seem to be independent of the absolute home-vector length.

Walking speed is lower during nest search

At the end of their straight homing paths on the test field, ants switched to a systematic nest search, and during this nest search, ants walked consistently slower than during the straight homing paths (Fig. 2B; Wilcoxon matched-pairs test: 5 and 10 m, both $n = 14$ ants, $P < 0.01$; 20 m, $n = 18$ ants, $P < 0.001$). Interestingly, the lower speed was not simply caused by paths being more sinuous. We separated search paths into straight and curved sections and the walking speed during straight portions of the search was still

significantly lower than the walking speed during the straight homing path (Wilcoxon matched-pairs test: 5 and 10 m, $P<0.01$; 20 m, $P<0.001$). As previously reported for systematic nest searches (Wehner and Srinivasan, 1981), we did not observe a change in speed across the duration of the recorded search paths (Spearman r correlation, all $P\geq 0.05$).

Tolerance for visual novelty decreases along the homing path

Having observed that ants guided by path integration reduce their walking speed along their homing path, we next looked at the ants' tolerance for visual novelty. Ants taken from the feeder were released on the test field where we had placed a small arena with barriers and a camera tripod. The visually novel setup was placed such that ants arrived at it having completed 20%, 50% or 80% of their homeward path (see Fig. 1B). We assessed the ants' response to this visual novelty by asking whether they would continue to follow their path integration indicated direction and by analysing path details. On comparing the test ants that were unfamiliar with the setup with control ants that were familiar with it, we observed significant differences in path characteristics. In the presence of visual novelty, ants less often crossed the arena and more often performed U-turns prior to crossing (Fig. 3). Moreover, in the presence of novel visual cues, ants walked slower (Fig. S1A), paused more often (Fig. S1B)

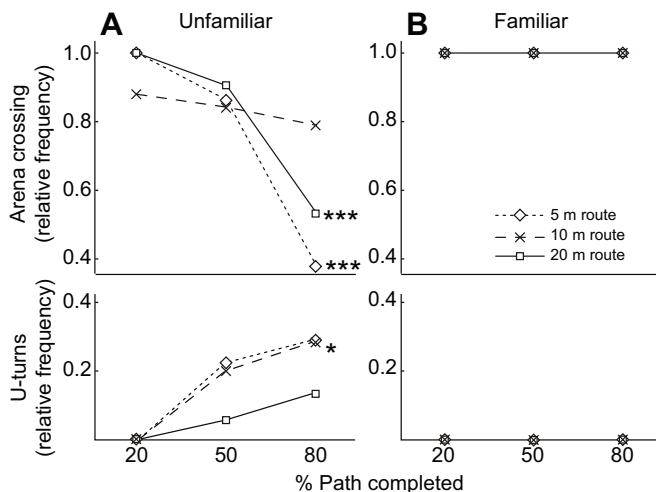


Fig. 3. Tolerance of visual novelty decreases along homing paths.

(A) Relative frequency of ants crossing the arena (top) or performing U-turns prior to crossing (bottom) when unfamiliar with the visual cues. Ants with a 5, 10 or 20 m homeward route were released such that they encountered the visual cues having completed 20%, 50% or 80% of the homing path (see Fig. 1B for experimental setup). Asterisks indicate significant differences along homeward paths using chi-squared test for trend (***) $P<0.001$, * $P<0.05$. For 5 m homeward route: 20% of path completed, $n=14$ ants, $n=14$ crossing, $n=0$ U-turns; 50% of path completed, $n=22$ ants, $n=19$ crossing, $n=4$ U-turns; 80% of path completed, $n=29$ ants, $n=11$ crossing, $n=3$ U-turns. For 10 m homeward route: 20%, $n=25$, 22, 0; 50%, $n=19$, 16, 3; 80%, $n=19$, 15, 4. For 20 m homeward route: 20%, $n=28$, 28, 0; 50%, $n=21$, 19, 1; 80%, $n=15$, 8, 1. (B) As for A but for ants that were familiar with the visual cues. For 5 m homeward route: 20%, $n=36$, 36, 0; 50%, $n=31$, 31, 0; 80%, $n=30$, 30, 0. For 10 m homeward route: 20%, $n=14$, 14, 0; 50%, $n=15$, 15, 0; 80%, $n=13$, 13, 0. For 20 m homeward route: 20%, $n=10$, 10, 0; 50%, $n=13$, 13, 0; 80%, $n=11$, 11, 0. Ants encountering visual novelty differed from control ants that were familiar with the visual cues in arena crossing (Fisher's exact test; 5 m: 20% of path completed, $P\geq 0.05$; 50% of path completed, $P\geq 0.05$; 80% of path completed, $P<0.001$; 10 m: all $P\geq 0.05$; 20 m: $P\geq 0.05$; $P\geq 0.05$; $P<0.05$) and occurrence of U-turns (Fisher's exact test; 5 m: $P\geq 0.05$; $P<0.05$; $P<0.05$; 10 m: all $P\geq 0.05$; 20 m: all $P\geq 0.05$).

and their paths were less straight (Fig. S1C). Interestingly, when increasing the visual mismatch by adding even more unfamiliar visual cues (see Fig. S2D), we saw a trend for speed and index of straightness to drop further and the frequency of pauses to further increase (Fig. S2).

We next analysed the effect of path integration state on the ants' response to visual novelty. We found that the longer ants had travelled before arriving at the novel setup, the less likely they were to cross the arena (Fig. 3A, top; chi-squared test for trend; 5 and 20 m, $P<0.001$; 10 m, $P\geq 0.05$). There was also a trend for U-turns to be more frequent with increasing distance from the release point (Fig. 3A, bottom; chi-squared test for trend; 10 m, $P<0.05$; 5 and 20 m, $P\geq 0.05$).

Response to learnt visual cues increases along the homing path

After demonstrating that ants' paths are more disturbed when modifications in the visual world are experienced further along their homeward path, we next looked at the interactions of path integration and visual guidance by learnt visual cues. Ants were trained with a cylinder located at either 10% or 60% of their homing path (for differences in walking speed, see Fig. S3A). In tests, we moved the cylinder 2 m to the left or removed it entirely (see Fig. 1C). When the cylinder was moved, the ants' paths shifted left also, both in terms of initial heading direction at the channel exit (Watson–Williams tests with Bonferroni-corrected P -value of 0.0167; 10% of path completed: Fig. 4A, control versus shifted $P<0.0167$, control versus missing $P\geq 0.0167$, shifted versus missing $P<0.0167$, control versus missing $P\geq 0.0167$, shifted versus missing $P\geq 0.0167$) and in terms of ants' lateral position when level with the cylinder (Kruskal–Wallis tests with Dunn's multiple comparison tests; 10% of path completed: Fig. 4A, control versus shifted $P\geq 0.05$, control versus missing $P<0.01$, shifted versus missing $P\geq 0.05$; 60% of path completed: Fig. 4B, control versus shifted $P<0.01$, control versus missing $P\geq 0.05$, shifted versus missing $P\geq 0.05$). At both the channel exit and cylinder level, ants that had already completed 60% of the homing path showed a greater shift than ants that had only completed 10% of their path (Mann–Whitney tests; both $P<0.05$), i.e. ants responded more strongly to changes in the learnt visual cue, the closer they were to the nest.

As we might predict from experiments 1 and 2, detailed analysis of the paths revealed that walking speed and index of straightness dropped with increasing distance along the homing path, while the number of pauses increased (Fig. S3). Looking closely at paths that contained pauses, we found that walking speed and frequency of pauses were to some extent independent (see Fig. S3D) and in around half of the paths with pauses (21 out of 46 ants), we additionally observed scanning, i.e. a rotation of the ants' body axis during a period of no translation (cf. Narendra et al., 2013b; Wystrach et al., 2014; Zeil et al., 2014). There was also a non-significant trend for changes in the visual scene to have greater impact on walking speed in ants that had completed 60% of their homing path (medians: control, 0.24 m s^{-1} , shifted, 0.21 m s^{-1} ; Mann–Whitney test: $P=0.056$) than in ants that only had completed 10% of their path (medians: control, 0.33 m s^{-1} , shifted, 0.30 m s^{-1} ; Mann–Whitney test: $P=0.934$).

DISCUSSION

We have presented information about the paths of *C. fortis* desert ants navigating by path integration, and from this we highlight two primary findings. Firstly, we observed that ants' walking speed

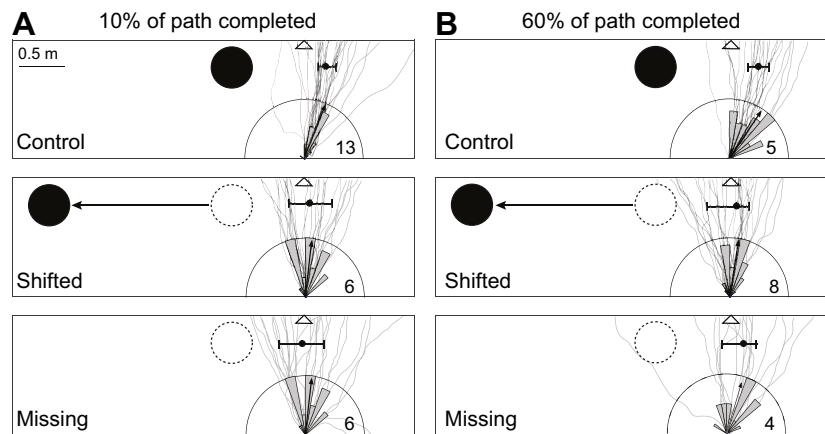


Fig. 4. Interactions between path integration and learnt visual cues. (A) In this setup (see Fig. 1C), homing ants had completed 10% of the homing path when reaching the channel exit. (B) Here, ants had completed 60% of the homing path at channel exit. Initial heading direction at 20 cm from channel exit, trajectories from individual ants, and lateral position when level with the cylinder are shown for the three test conditions: control, cylinder shifted 2 m leftwards and cylinder removed. Circles represent the cylinder and triangles the direction towards the nest. Initial heading directions are shown in circular histograms (bin size, 10 deg). The number on the circle edge indicates the scale for the circular histogram. Arrows within the histograms represent the mean vector. Note, circular histograms are not shown to the same scale as the trajectories and cylinder position. Lateral positions at cylinder level are plotted as medians with error bars showing the 25th and 75th percentiles. For 10% of path completed: control, $n=29$ ants in histogram/30 ants in error bar; shifted, $n=27/26$ ants; missing, $n=27/26$ ants. For 60% of path completed: control, $n=24/24$ ants; shifted, $n=31/31$ ants; missing, $n=17/17$ ants.

decreased significantly along their homing paths, such that they slowed down just before reaching their goal (Fig. 2A), and maintained this slower speed during their subsequent search paths (Fig. 2B). Our second result is that ants responded more strongly to novel or altered visual cues the further along the homing path they were (Figs 3 and 4). Lower walking speeds were associated with a higher frequency of pauses and more sinuous paths (Fig. 3; Figs S1, S2, S3). Low walking speeds, more pauses and meandering paths mean that ants have more time to respond to other sensory cues at locations closer to the nest. This might suggest that path integration modulates homing paths in a way that helps ants search for, utilise or learn other sensory information such as visual (Wehner and R ber, 1979; Bregy et al., 2008) or olfactory (Steck et al., 2009; Buehlmann et al., 2012) cues. As well as highlighting some of the subtle details of cue integration in navigating ants, this work also highlights the value of detailed descriptions of behaviour in naturalistic conditions.

Does the modulation of walking speed help ants to weight guidance cues?

Effective navigation is a multimodal process taking into account information from different sources (reviewed in Wehner, 2003; Collett et al., 2013; Knaden and Graham, 2016) and is tuned to the ants' sensory ecology (Fukushi, 2001; Fukushi and Wehner, 2004; Beugnon et al., 2005; Narendra, 2007a,b; Buehlmann et al., 2011; Wystrach et al., 2011b; Cheng et al., 2012; Schultheiss et al., 2016). It is well described that ants often follow visually defined routes when visual cues are at odds with path integration (Wehner et al., 1996; Kohler and Wehner, 2005; Mangan and Webb, 2012; Narendra et al., 2013a). However, we also know that navigational strategies can be used simultaneously (Narendra, 2007b; Bregy et al., 2008; Reid et al., 2011; Collett, 2012; Legge et al., 2014; Freas et al., 2017), and moreover ants might even be able to weight cues optimally based on their reliability (Legge et al., 2014; Wystrach et al., 2015). For instance, it has been shown that the weighting of the directional component of path integration gets stronger for longer path integration vectors (Wystrach et al., 2015), which matches the mathematical prediction of smaller angular variance for path

integration over larger distances (Vickerstaff and Cheung, 2010). Interestingly, we found a similar result in our experiments. Ants modulated their walking speed in such a way that they produced lower speeds near the fictive goal location (Fig. 2), i.e. where there was a shorter path integration vector. It is at these positions that ants also responded more strongly to visual cues (see Figs 3 and 4). Thus, path integration-mediated path characteristics might assist ants in adequately responding to other sensory cues at locations of importance, by allowing those other cues to act for a longer period of time. Furthermore, by reducing speed before the fictive nest, visual cues might be used before the ant overshoots the nest into less familiar areas (M ller and Wehner, 1988; Wystrach et al., 2013).

Does the modulation of walking speed allow ants to learn visual cues?

As well as interacting during navigation by experienced foragers, navigational modalities also interact during learning. Innate navigational strategies such as path integration, pheromone trails and innate responses to ecologically relevant stimuli can all facilitate learning (Voss, 1967; Collett, 1998, 2010; Heusser and Wehner, 2002; Collett et al., 2003; Graham et al., 2003; Graham and Wystrach, 2016). Learning walks are a particularly well-studied example involving path integration (Judd and Collett, 1998; Nicholson et al., 1999; Wehner et al., 2004; Graham and Collett, 2006; M ller and Wehner, 2010; Stieb et al., 2012; Fleischmann et al., 2016, 2017). During these choreographed movements, ants have ample, well-structured opportunities to learn visual scenes (Judd and Collett, 1998; Graham et al., 2010; M ller and Wehner, 2010), and there are other motor behaviours seemingly related to learning. *Melophorus* ants produce scanning behaviours along routes triggered by unfamiliarity or uncertainty (e.g. Wystrach et al., 2014), i.e. where new information is needed. Modulation of walking speed, as observed in the current study (see Fig. 2; Fig. S3), might be a similar mechanism to facilitate learning at important or novel locations. Indeed, there is ample evidence of interactions between visual learning and/or guidance and walking or flying speed. For instance, it has been reported that flight speed during learning flights increases with distance from the nest in wasps (Zeil et al., 1996) and

flight speed increases with increasing number of orientation flights in bees (Degen et al., 2015). For ants, walking speed drops when visual information is harder to extract (Narendra et al., 2013b) and ants in tandem pair walks have lower walking speeds than solitary foragers (Schultheiss et al., 2015). In all these examples, the lower walking speed seems to positively correlate with the amount of visual learning required or the difficulty of the task.

We have seen that desert ants guided by path integration modulate their speed as they travel along their homing path and search for their nest. Moreover, the further along the homing path ants are, the stronger they respond to novel and altered visual cues. Similarly, earlier work has shown that ants respond more strongly to visual or olfactory nest cues the further along the homing paths they are when encountering them (Michel and Wehner, 1995; Bregy et al., 2008; Buehlmann et al., 2012). It seems that path integration produces reduced speeds at important locations when other cues might be important and walking speed could be an indirect mechanism for weighting cues or facilitating learning. This general trend is backed up by changes of walking speed seen in *Cataglyphis* ants mounted on a track ball (Dahmen et al., 2017) and by increases in pausing and scanning in *Melophorus* and *Myrmecia* species triggered by spatial uncertainty (Narendra et al., 2013b; Wystrach et al., 2014; Zeil et al., 2014). Encountering novel or altered visual cues may trigger re-learning of route information; hence, the observed changes in the ants' movements might facilitate the required learning. Of course, as we have suggested above, modulation of path properties might also relate to cue weighting. These possibilities are not mutually exclusive; indeed, we cannot fully separate the learning and use of sensory cues. Further research is needed to gain a better understanding about cue integration and to unpick the circularity between multimodal learning and the use of multimodal cues during navigation. Finally, by providing evidence that path characteristics, like walking speed, might modulate the weighting and/or learning of environmental cues, we show the importance of looking at the fine sensorimotor details of navigating ants under naturalistic conditions.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.B., P.G.; Methodology: C.B., P.G.; Formal analysis: C.B.; Investigation: C.B., A.S.D.F.; Writing - original draft: C.B.; Writing - review & editing: C.B., P.G.

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Data availability

Data are available from the University of Sussex database SURE: <https://doi.org/10.25377/sussex.5671804>

Supplementary information

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