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1 **Running paths to nowhere: Repetition of routes shows how navigating ants**
2 **modulate online the weights accorded to cues**

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21

22 **Abstract**

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

42

43 **Key words:** desert ant, view-based navigation, path integration, mushroom bodies,
44 cue weighting

45

46 **Introduction**

47 **Sisyphus in the desert**

48 Imagine traversing a familiar route home in the blazing red desert of Central
49 Australia, but when the front door looms and the comforts of home beckon, you
50 find yourself inexplicably back where you started your journey. How would you
51 retrace your route? This is the nightmare that we foisted repeatedly on red honey
52 ants, *Melophorus bagoti*, expert navigators wielding and combining multiple
53 strategies (Cheng 2012; Cheng et al. 2009; Cheng et al. 2014; Kohler and Wehner
54 2005). This conundrum for the ants, facing the displacements we call *rewinding*,
55 revealed in detail what happens when their favourite strategy, following well-
56 travelled routes based on the visual panorama, no longer reaps its usual reward of
57 getting home. We were inspired to expand on two accounts on this theme on
58 formicine cousins of *M. bagoti*, North African *Cataglyphis* ants (Andel and Wehner
59 2004; Collett 2014). We here depict in detail how the tiny brain of a desert ant
60 adjusts its navigational toolkit when faced with the drama inflicted by rewinding, a
61 burden for the heat loving red honey ants (Christian and Morton 1992; Muser et al.
62 2005) reminiscent of the task for the mythical Sisyphus, who had to push a rock up
63 a hill only for it to roll down to the bottom again.

64

65 Individually foraging desert ants boast three major strategies for navigating
66 robustly over long distances (Collett et al. 2013; Knaden and Graham 2016;
67 Pritchard and Healy 2017; Wehner 2003): path integration (PI), the ability to keep
68 track of the straight-line distance and direction from the starting point of travel
69 (Collett and Collett 2000; Müller and Wehner 1988; Ronacher 2008; Wehner and

70 Srinivasan 2003), view-based guidance relying on terrestrial visual (hereafter
71 visual) information learnt from panoramic scenes (Collett et al. 2007; Graham and
72 Cheng 2009; Pritchard and Healy 2017), and systematic search (Schultheiss et al.
73 2015; Wehner and Srinivasan 1981) to compensate for errors in navigation. Ants
74 combine information from different strategies simultaneously and flexibly (Collett
75 2012; Legge et al. 2014; Narendra 2007; review: Wehner et al. 2016), perhaps
76 even weighting the cues optimally based on their reliability (Hoinville and Wehner
77 2018; Legge et al. 2014; Wystrach et al. 2015). Thus, when walking in uncertain
78 conditions, desert ants zig-zag more from side to side, called meandering, and stop
79 and scan the environment more, showing searching mixed with directed
80 navigation (Wehner et al. 2016; Wystrach et al. 2014; Wystrach et al. 2011b). We
81 asked how red honey ants adjust their navigational strategies when their familiar
82 route home does not lead to their entering their abode.

83 **Rewinding to reveal intricacies of running routes without success**

84 Our rewinding technique was inspired by two studies tapping this theme.
85 Andel and Wehner (2004) rewound *C. bicolor* ants as they ran home along a
86 narrow channel decked with landmarks. In their experiment, the ants reached
87 their nest on each trip, only to be dragged out again to re-run the channel. This was
88 as if Sisyphus succeeded in placing the rock at the top of the hill, but then was
89 asked to push a second rock. Rewinding built up the vector calculated by PI. When
90 the ants were tested in a long channel without adorning landmarks—thus without
91 the familiar visual cues defining their route—the rewound ants dashed off in the
92 opposite, nest-to-start-point direction. Collett (2014) allowed individual North
93 African *C. fortis* ants to develop routes across an open environment dominated by a

94 single conspicuous black cylinder. Collett (2014) then rewound the ants, now with
95 their PI vector indicating zero, just once. Interestingly, some ants appeared
96 confused for a prolonged period before eventually commencing their regular
97 route, suggesting that a single unsuccessful event reduces the trust that ants have
98 in their visually defined direction (Graham and Mangan 2015).

99

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

110

111 **Methods**

112 **Animals**

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

117

118 **Experimental set up**

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

125 A square-shaped feeder made of plastic (15 × 15 × 9 cm deep) was sunk into
126 the ground 10 m from a nest. At Nest 1, an enclosure of smooth plywood low walls
127 10 cm high was constructed around feeder and nest (Figs. 1A, SI1). Both these
128 walls and the walls of the feeder were too slippery for the ants to climb. The feeder
129 was provisioned with mealworm and cookie pieces. During training, sticks were
130 used as bridges, allowing foraging ants to climb out of the feeder.

131 The enclosure walls were used to form two tracks (Figs. 1A, SI1). A narrower
132 track free of obstacles served as the outbound route for the ants, while the wider
133 track, with obstacles along the way, served as the homebound route. Strings
134 wound around tent pegs were used to make a grid of 1-m squares. The grid

135 allowed experimenters to transcribe the paths of homebound ants onto similarly
136 gridded paper (i.e. copies of Fig. 1A). For a coordinate system, we labelled the nest
137 0,0, while the feeder was labelled 0,-10, with metres as units. Two sections of the
138 homeward path were labelled Section A (from $y = -10$ to $y = -7$) and Section B ($y =$
139 -6 to $y = -3$).

140

141 **Procedure**

142 All ants were trained to be familiar with the homeward route before being
143 tested. An ant arriving at the feeder for the first time was painted with one dot of
144 enamel paint (Tamiya™ brand), in a colour that denoted the day of arrival at the
145 feeder. Ants were tested after at least two days from the day of their first arrival.
146 Each ant was tested on only one occasion.

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

158

159 **Test conditions**

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

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

179

180 **Table 1 about here**

181

182 **Data analysis**

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

193

194

195 Table 1. Summary of test conditions

196

	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 9 times on 1 trip home test after 10th capture near nest
2a	Re-run of Section A only	ran Section A 4 times test on 5th 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 4 or 5 times delayed for 24 h before test
3b	Re-run of Section B with delay	ran Section B 4 or 5 times delayed for 24 h before test
3c	Re-run of Sections A and B with nest return	ran Section A 4 times, then went home next foraging trip; ran Section B 4 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

197

198

Fig. 1 about here

199

200 **Results**

201 **The effect on visual route guidance of route repetition and conflicting path**

202 **integrator states**

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

204 To investigate how rewinding and PI state influence path characteristics we
205 allowed ants to have either one (Condition *1a*) or three foodward runs (Condition
206 *1b*) from nest to feeder before being allowed to grab some food and beginning re-
207 runs of the homeward route. Thus, at the start of the rewinding process ants either
208 had a normal PI home vector or had accumulated a vector of three times the
209 magnitude of the normal home vector. Therefore, the group with a single outbound
210 journey experienced a conflict between PI and visual guidance from the 2nd
211 rewinding run while the group with three outbound journeys experienced a
212 conflict from the 4th rewinding run. Following those points in the rewinding
213 sequence, the conflict for both groups increased between the ant's PI system and
214 the direction indicated by visual cues, as the PI home vector got longer in the nest-
215 to-feeder direction. Over 6 runs, both groups of ants became more variable in their
216 paths (Fig. 1B), meandering and scanning more. Our formal measure of Meander
217 was higher in ants that had a greater conflict between visual guidance and PI
218 because they had experienced only one outbound run to the feeder (Condition *1a*)
219 than in ants that had experienced three outbound runs before reaching the feeder
220 (Condition *1b*, Fig. 1C). An analysis of variance found statistical significance in all
221 effects: Condition (one outbound trip vs. three outbound trips, $F_{1,156} = 56.01$, $p <$
222 0.0001), trials ($F_{5,156} = 14.57$, $p < 0.0001$), and their interaction ($F_{5,156} = 3.42$, $p =$
223 0.006). Ants also scanned more frequently over repeated runs home (Figs. 1D, F),

224 and ants with three outbound runs scanned less often than ants with one outbound
225 run (Fig. 1D). An analysis of variance found significant main effects of Condition
226 ($F_{1,156} = 28.35, p < 0.0001$) and trials ($F_{5,156} = 16.07, p < 0.0001$), but not a
227 significant interaction ($F_{5,156} = 1.53, p = 0.184$). Scans were not evenly distributed
228 across the length of the route home (Fig. 1B). Both ants with one outbound run and
229 ants with three outbound runs scanned most in the early part of the route,
230 especially just before the first set of barriers at -7.

231

232 We further analysed how ants lost confidence in their visually guided route
233 by looking for the point at which the PI direction finally overrode the visually
234 defined direction and ants U-turned and began travelling in the nest-to-feeder
235 direction. Ants with 3 outbound runs before rewinding had a higher survival curve
236 (proportion of ants that had still not performed a U-turn by a given trial number)
237 than ants with 1 outbound run (Fig. 1E). This difference, however, did not reach
238 statistical significance (non-parametric test on number of trials before the first
239 turn-back: $Z = 1.68, p = 0.092$). After U-turning, ants typically ran for a long
240 distance away from home, that is, in the direction of their negative path integration
241 vector, confirming that ants had indeed accumulated a large vector, as observed by
242 Andel and Wehner (2004).

243

244 b) Condition 1c: Nine captures on one trip home

245 As a control for the repeated capturing and disruption of the homebound
246 journey, we captured ants 9 times on one journey home, resulting in a zero-vector
247 ant being tested after the 10th capture. Repeated capturing had no notable effect

248 on the ants' navigation (Fig. S12). The ants performed much like zero-vector ants
249 that had been captured only once near their nest. Ants in Condition *1c* had a closer
250 resemblance to ants with a same PI state (Condition *1a*, 2nd release) than to ants
251 after the same number of captures (Condition *1a and 1b*, 10th release,
252 Supplementary Results S12). While we cannot rule out that repeated captures may
253 have some effect on paths, the changes in behaviour that arise from repeatedly
254 running the home path must be attributed mostly to the increasing vector length of
255 path integration in the negative (nest-to-feeder) direction and/or repeated
256 viewing of the scenes along the route, which is examined next.

257

258 **The specificity of the effect of visual repetition on route disturbance**

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

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

269

Fig. 2 about here

270

271 **The persistence of route repetition effects**

272 a) Condition 3a and 3b: Re-running Section A or Section B with delay before
273 testing

274 As in the two previous conditions, ants in Conditions 3a and 3b re-ran Section
275 A only or Section B only four times (Fig. 2). Ants were then held for 24h before
276 being tested on the entire route. The delay served to test if the disruption caused
277 by repeatedly running a segment persists. Results again show a persistent increase
278 in meander and scans specific to the route section that the ants had repeatedly re-
279 run the day before (Fig. 2). Also, the 24h-delay increased the disruption as
280 compared to ants that had performed the test immediately after the 4 rewinding
281 trials (Fig. 2).

282 For each of Meander and Scan rate on the 5th (test) run, we combined
283 Conditions 2a, 2b, 3a, and 3b in a 3-way analysis of variance, with Condition
284 (rewound on A or B) and delay (immediate test vs. 24-h delay) as between-
285 subjects factors, and section (A or B) as repeated measure. For Meander, the
286 analysis found significant main effects of all three factors (Condition: $F_{1,97} = 8.64$, p
287 $= 0.0041$; delay: $F_{1,96} = 41.84$, $p < 0.0001$; section: $F_{1,96} = 55.16$, $p < 0.0001$). A
288 significant Condition by section interaction was also found ($F_{1,96} = 70.53$, $p <$
289 0.0001), while the other interactions were not significant (Condition by delay: $F_{1,96}$
290 $= 0.38$, $p = 0.5383$; delay by section: $F_{1,96} = 2.87$, $p = 0.0935$; Condition by delay by
291 section: $F_{1,96} = 1.82$, $p = 0.1803$). For Scan rate, the analysis of variance revealed
292 significant main effects of Condition ($F_{1,96} = 8.89$, $p = 0.0036$) and delay ($F_{1,96} =$
293 12.61 , $p = 0.0006$), but not of section ($F_{1,96} = 0.25$, $p = 0.6167$). Each of the
294 interactions was found to be significant (Condition by delay: $F_{1,96} = 6.18$, $p =$

295 0.0147; Condition by section: $F_{1,96} = 36.26, p < 0.0001$; delay by section: $F_{1,96} =$
296 11.77, $p = 0.0009$; Condition by delay by section: $F_{1,96} = 8.59, p = 0.0042$).

297

298 b) Condition 3c: Section A re-run with return to nest

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

305

306 **Influence of the visual environment on route disturbance**

307 Conditions 4a and 4b: Re-running with or without proximal objects (Nest 2)

308 These two conditions were implemented with Nest 2, which lay in a rather
309 open area. Ants re-ran the route home repeatedly without (open landscape,
310 Condition 4a) or with (cluttered landscape, Condition 4b) added proximal objects
311 around the route (Fig. SI4A). The presence of nearby proximal objects mitigated
312 the detrimental effects of repeated route-running, or, to put it another way, having
313 an open landscape along the route meant the effects of repeated route-running
314 were more severe (Fig. SI4B). Ants performed their first U-turn after fewer re-runs
315 in the open landscape (Condition 4a), and they scanned and meandered a little
316 more, compared with their performance in the cluttered landscape (Fig. 3). For
317 Meander and Scan rate on the first three trials, we conducted a mixed analysis of
318 variance with Condition (open or cluttered landscape) as the between-subjects

319 factor and trials as repeated measure. For Meander, both main effects reached
320 significance (Condition: $F_{1,81} = 9.84$, $p = 0.0023$; trials: $F_{2,81} = 18.45$, $p < 0.0001$), but
321 the interaction did not reach significance ($F_{2,81} = 2.47$, $p = 0.091$). For Scan rate,
322 only the main effect of trials reached significance ($F_{2,81} = 17.72$, $p < 0.0001$). The
323 main effect of Condition ($F_{1,81} = 1.57$, $p = 0.214$) and the interaction ($F_{2,81} = 0.46$, $p =$
324 0.636) did not reach significance. For the trial on which ants first turned back, the
325 difference between conditions was significant by a non-parametric test ($Z = 3.13$, p
326 $= 0.0018$).

327 **Fig. 3 about here**

328

329 **Discussion**

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

339 **Factors affecting route-following**

340 As the first factor, increasing conflict between visually guided directions and
341 path integration leads to path degradation. Given an equivalent number of route
342 repetitions, ants with a greater conflicting PI vector length showed more

343 meandering and pausing to scan, and eventually performed route U-turns earlier
344 in the rewinding sequence (Fig. 1). This shows that learnt visual guidance and PI
345 are simultaneously active in influencing behaviour even when in 180° directional
346 conflict (as observed in Collett 2012; Freas and Cheng 2017; Legge et al. 2014;
347 review: Wehner et al. 2016). Additionally, it shows that the weight given to PI
348 increases with vector length (as shown in Wystrach et al. 2015).

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

365 As the third factor, the course of route changes with rewinding depends on
366 the structure of the visual environment. This was suggested by Collett (2014; see

367 also Graham and Mangan 2015) to explain why path decrements from repetition
368 are not observed in all experimental situations. Here we showed that when ants
369 were navigating in an open environment, as opposed to the same environment
370 with additional proximal visual clutter, path disruption arose after fewer repeated
371 runs, and thus with a weaker conflict between visual guidance and PI (Figs. 3, SI4).
372 It could be that because aversive conditioning sets in for specific views
373 experienced during a familiar route home, the slowly changing visual panorama
374 along an open route provides a larger and longer window to modify the valence of
375 specific views.

376

377 **Adaptive use of information based on reliability**

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

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

400

401 **The neural basis of flexible route guidance**

402 Our findings give firm support for the role of associative learning in view-
403 based navigation. According to current literature, the mushroom bodies (MB)
404 undergird associative learning in insects (Aso et al. 2014; Bazhenov et al. 2013;
405 Cohn et al. 2015; Galizia 2014; Peng and Chittka 2017; Perry et al. 2013; Webb and
406 Wystrach 2016), including the learning of views that can guide familiar routes
407 (Ardin et al. 2016; Cruse and Wehner 2011; Hoinville and Wehner 2018; Webb and
408 Wystrach 2016). MB support reinforcement learning by separating patterns in the
409 input, and assigning positive valences to positively reinforced patterns. In
410 travelling routes, ants move forward when experiencing familiar reinforced views,
411 and turn more or scan when the view is not similar to reinforced views
412 (Kodzhabashev and Mangan 2015; Lent et al. 2010; Wystrach et al. 2011a; Zeil et
413 al. 2014). We think that rewinding reduces the valence associated with views, also

414 making ants increase meandering and scanning. Functionally, these behaviours
415 expose the navigator to novel views of the environment, allowing ants to explore
416 new options for reaching home. In general, online updating of the valence of visual
417 route memories could be adaptive in natural foraging. For instance, aversive
418 associations with views might help ants to learn to detour around an obstacle or a
419 trap, a form of avoidance learning (work in preparation).

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

431

432 **Conclusions**

433 The rewinding method asks ants to repeat portions of a familiar route. By
434 implementing rewinding, we have shown dynamic online processing in cue
435 integration, with ants incorporating all route experiences to update route
436 memories and cue weightings. For cue integration, ants use more than heuristics
437 based on immediately available information, such as the current PI vector length

438 or the current visual familiarity. They also adjust the weight attributed to route
439 memories based on information accumulated over successive trials, that is, over
440 time. Moreover, their fine-tuning depends on how informative the visual scenery
441 is, as reflected in the number of nearby objects (cluttered vs. open environments).
442 We think that reinforcement learning is central to all these experience-dependent
443 modifications. Further investigation of these dynamic learning processes, in
444 conjunction with modelling the associative learning in navigation implemented in
445 the mushroom bodies of insects, will be particularly informative.

446

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449 letting us work on their property and providing some storage space, and the CSIRO
450 Arid Zone Research at Alice Springs for administrative support.

451

452 **Competing interests**

453 The authors declare that they have no competing interests.

454

455 **Ethics**

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

459

460 **Author contributions**

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

465

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470

471 **Data accessibility**

472 The raw data files and a file containing explanations are in the supplementary
473 materials.

474

475 **Supplementary information**

476 Supplementary materials for this article are available at

477

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597

598 **Fig. 1. Paths are more disturbed following repetition and cue conflict. A.**
599 Schematic of the nest and feeder surrounds. A one-way system meant ants had a
600 clear outward journey before being captured at the feeder. The return journey
601 included two sections of interest: Section A, which was relatively clear of objects,
602 and Section B, which was visually cluttered. Diagonal lines represent baffles that
603 were used to make a clear distinction between Section A and B and also to reduce
604 wall following. Replicas of this schematic were used to transcribe the routes of ants
605 during tests. **B.** Paths of ants for their first 6 re-runs of the homeward path. Upper:
606 Ants had performed only one outward run before the start of the rewinding.
607 Lower: Ants with three outward runs before rewinding. The locations of bouts of
608 scanning were also recorded. The spatial distribution of scans for both conditions
609 are plotted, with dark shades representing an increasing probability that scans
610 would have been observed within a 0.25m² grid square. **C and D.** For the
611 conditions with one and three outbound runs, Meander and Scan rate are
612 compared, respectively. Only ants with at least six trials without U-turn were used
613 in this comparison. Plots show means with standard deviations. Because of the
614 different numbers of outbound runs, ants experience a zero-vector state (ZV) on
615 different run numbers. For additional reference the curve of mean Meander and
616 Scan rate for the three-outbound group is also shown offset by two runs to allow a
617 comparison adjusted for PI state (dashed line). **E.** Survival curves for ants in the
618 one- and three-outbound groups. Lines show proportion of ants still remaining,
619 with ants removed from the groups once they have performed a U-turn on a
620 homeward run. **F.** For the one-outbound (upper) and three-outbound (lower)
621 groups histograms show the change in the number of scanning bouts from one run

622 to the next. The positive skew shows that scans become more frequent with
623 increasing number of re-runs

624

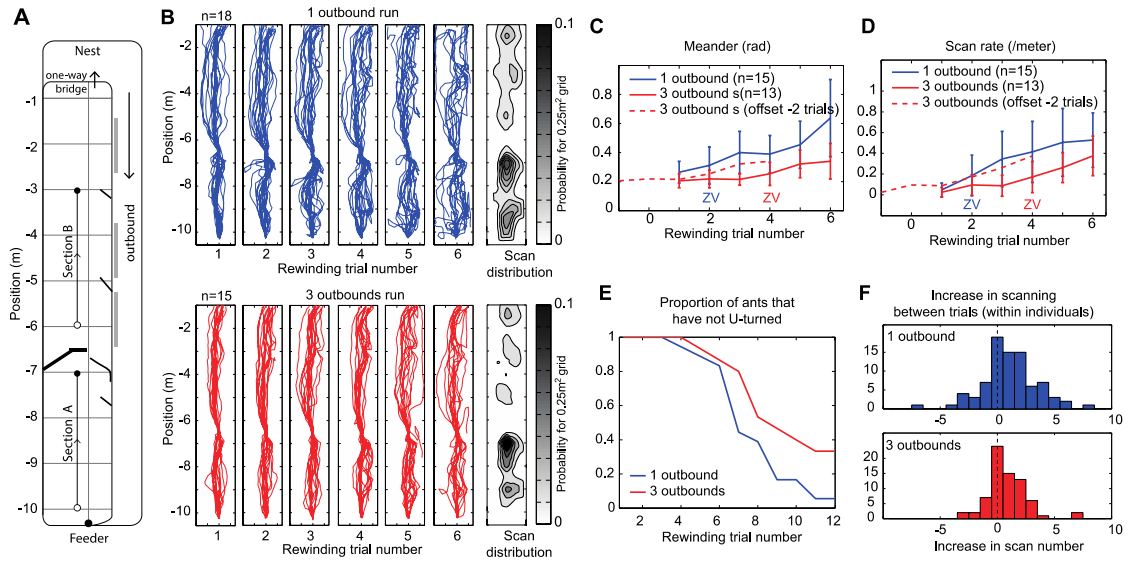
625 **Fig. 2. Ants show more disturbance in the sections in which they were**
626 **rewound.** After arriving at the feeder on their first trip ants were allowed to run
627 the first part of the route (Section A) before being rewound to the feeder position a
628 total of four times, or they were taken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

639

640 **Fig. 3. Ants show more path disturbance after rewinding in an open**
641 **landscape vs. a cluttered landscape.** In order to investigate the impact of visual
642 clutter on route changes, we repeated the basic rewinding protocol (see Fig. 1)
643 with two groups of ants at a second nest site. The nest environs were left open or
644 had visual clutter added. **A.** The proportion of ants that have not turned back in the
645 open and cluttered landscape of Nest 2, and in the landscape of Nest 1, which was

646 visually cluttered (data from Fig. 1E). **B.** Meander in the paths of ants rewound in
647 the open or cluttered landscape of nest 2 (mean \pm s.d.). **C.** Scan rate in the ants
648 rewound in the open or cluttered landscape of nest 2 (mean \pm s.d.)
649

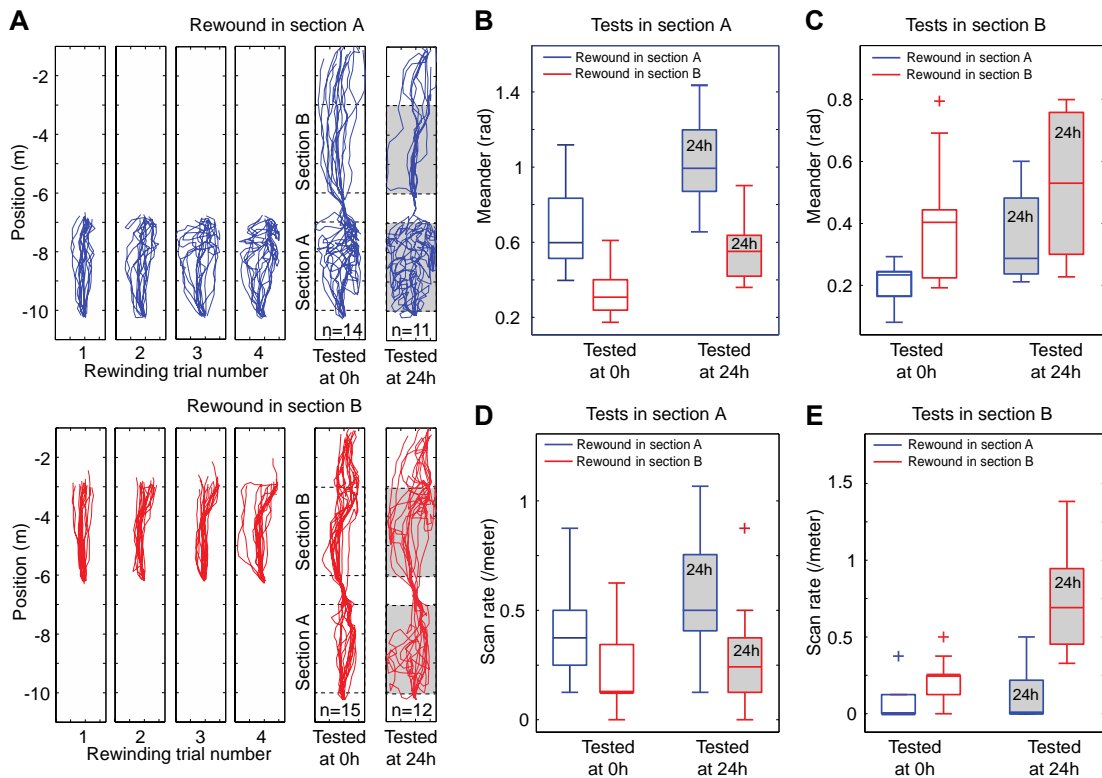
650 **Fig. 1**



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652

653 **Fig. 2**

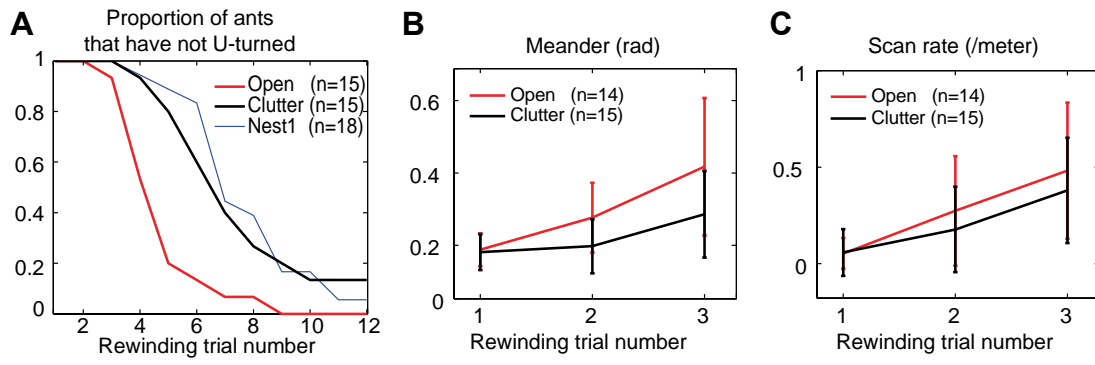


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657 **Fig. 3**



Supplementary materials

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

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Supplementary methods

Detailed procedures of each condition

1) The effect on visual route guidance of a conflicting path integrator state

1a. One outbound trip before re-runs. A trained forager was allowed to run home with a piece of food. It was captured just before entering the nest and re-released just in front of the feeder. On the next trip home, the process was repeated. Ants continued to re-run the route home until they U-turned during a homeward route or completed a maximum of 16 runs. U-turns were defined as moving in the nest-to-feeder direction for at least 1 m.

1b. Three outbound trips before re-runs. A trained forager from Nest 1 arriving at the feeder was captured before she grabbed any food. The ant was transported back to her nest and released just in front of the nest. Such a motivated forager then re-ran the route to the feeder, where it was captured once more before seizing any food. It was transported back to the nest once again, and released to run to the feeder for a third time. These ants had thus run the outbound route 3 times, and accumulated a PI home-vector of ~30 m. The ant was then treated in the same manner as Condition *1a*.

1c. Nine captures in a single homeward run. A trained forager dropping into the feeder was allowed to seize a bit of food. The ant was released to run home, and captured at 0,-9 and held in the tube for 20 s. It was released again at 0,-9, and captured again at 0,-8. After being held for 20 s, it was released again at 0,-8. Every metre of travel on the route back, the returning forager was captured, held for 20 s, and released again at the location where she was captured. At 0,-1, the test ant had been captured and released 9 times en route home. Just before the nest, it was captured once more, this time returned to the feeder (0,-10) for one last run home. On this run home, the ant had

run off the 10 m accumulated on the outbound trip, and had no home vector from path integration. In the literature, these ants are called zero-vector (ZV) ants. This condition allowed us to examine the effect of repeated captures without the large accumulation of a path integration home vector pointing in the nest-to-feeder direction.

2) The specificity of visual repetition on route disturbance

2a. Re-run of Section A only. A trained forager arriving at the feeder was allowed to grab a piece of food. The ant was released to run home, and captured at $y = -7$, the end of Section A, and returned to the start. Ants on this test ran Section A 4 times, and then were allowed to run the whole route home on the 5th trip.

2b. Re-run of Section B only. A trained forager arriving at the feeder was allowed to seize a bit of food. The ant was released to run home at $0, -6$, the start of Section B, and captured at $y = -3$, the end of Section B, and returned to the start of Section B. Ants on this test ran Section B 4 times, and then were released at the beginning of Section A and allowed to run the whole route home (Section A then B) on the 5th trip.

3) The persistence of effects from route repetition

3a. Re-run of Section A with delay. In this test condition, a forager with food re-ran Section A 4 or 5 times in the same manner as Condition 2a. On the ant's last run, however, it was captured and placed in a dark tube for 24 h. A piece of cotton wet with honey water was provided in the tube. After the delay, the ant was released with food in front of the feeder to run the whole route home once.

3b. Re-run of Section B with delay. In this test condition, a forager with food re-ran Section B 4 or 5 times in the same manner as Condition 2b. Paralleling Condition 3a, on the final run of Section B, the ant was captured and held for 24 h. After the delay, the

ants in this condition were also released with food in front of the feeder to run home once.

3c. Re-run of Sections A and B with nest return. The forager ran Section A 4 times in the manner of Condition *2a*; she was then captured and painted with additional colour for individual identification. The test ant was released with its food at the nest, and was allowed to enter the nest. On the forager's next trip to the feeder, she was captured and treated in the manner of Condition *2b*: the ant ran Section B 4 times to reach the same PI state as groups *2a* and *2b* and was released in front of the feeder and recorded along Section A. For any ants that returned after this test, it was tested as a full-vector (FV) ant by being released immediately at the feeder.

4) The influence of environmental structure on effects of route repetition

These conditions were conducted on Nest 2, either within a rather open scene or with additional proximal objects added.

4a. One outbound trip before re-runs in open environment (Nest 2). This test condition was conducted on ants from Nest 2, which had an open area around the nest, replicating the manipulations used in Condition *1a* with Nest 1.

4b. One outbound trip before re-runs in cluttered environment (Nest 2). This condition was also conducted on ants from Nest 2. It repeated the manipulations of Condition *4a*, except that experimentally provided objects were added in the vicinity of the nest to add visual clutter. Black cylinders, some of them tall, and three rectilinear bamboo baskets were strewn on the sides of the route home.

Fig. S11. Photo of test set up at nest 1.



Fig. S11. Photo of test set up at nest 1. The enclosure walls were used to form two tracks. 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. The ant's nest entrance opened onto the outbound track, forcing the outbound ants to travel on that track, at the end of which ants could drop into the feeder. The stick bridge that ants used to exit the feeder then forced ants onto the homeward track, at the end of which a one-way ramp allowed ants to drop into the nest. The roof of a tent-like pagoda stood over the central part of the track and plywood baffles (~120 cm wide and ~120 cm tall) were used to segment the route and reduce wall-following behaviour. Strings wound around tent pegs were used to make a grid of 1-m squares.

Supplementary Results

SI2. Being captured 10 times during one trip home does not disrupt route following

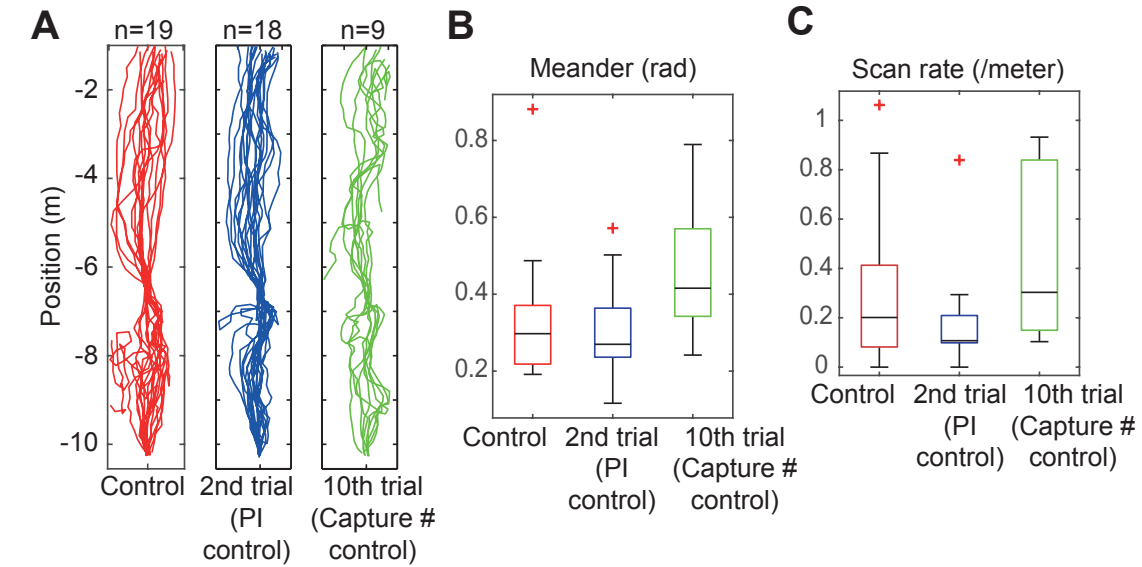


Fig. SI2. Being captured 10 times during one trip home does not disrupt route following. **A.** Paths from the control group captured 10 times during one trip home (control), compared with ants that were captured after completing the full distance to the nest on their second trip and on their 10th trip in Condition 1a. **B.** Meander in the control group captured 10 times during one trip home (control), compared with ants that were captured after completing the full distance to the nest on their second trip and on their 10th trip in Condition 1a. **C.** Scan rate in the control group captured 10 times during one trip home (control), compared with ants that were captured after completing the full distance to the nest on their second trip and on their 10th trip in Condition 1a. The box plots show the median (middle line), 25th and 75th percentiles (bottom and top of box), and 1.5 times the quartile (whiskers), and outliers beyond that range. On the second trial, the ants were equivalent to the controls in PI, while on the 10th trial, the ants were equivalent to the controls in the number of times they had been captured.

SI2 Results

We calculated an evidence ratio to investigate whether ants in Condition *1c* (control in Fig. SI2) had a closer resemblance to ants with a same PI state (Condition *1a*, 2nd release) or to ants after the same number of captures (Condition *1a and 1b*, 10th release). The evidence ratio comes from Bayesian statistics comparing the support of the data from Condition *1c* for one vs. the other hypothesis. For each hypothesis, a non-parametric test comparing Condition *1c* with another condition generated a *Z* score. The *y* value of each *Z* score ($f(Z)$) served as an estimate of the evidence for that hypothesis; the ratios of these *y* values were computed. For both Meander and Scan rate, ants in Condition *1c* resembled more closely ants with the same PI state (Meander: evidence ratio 12.93; Scan rate: evidence ratio 1.68). These ratios (both being above 1) mean strong evidence in the case of Meander and anecdotal evidence in the case of number of scans. These analyses suggest that the changes in behaviour from rewinding are mostly due to re-running the route rather than being captured repeatedly.

SI3. Entering the nest does not improve re-running after being rewound in a section

SI3 Results

We compared ants' performance in Section A across the three treatments shown in Fig. SI3. ANOVAs showed significant differences across the three groups for both Scan rate ($F_{2,24} = 11.41$; $p < 0.001$) and Meander ($F_{2,24} = 11.39$; $p < 0.001$). Tukey's multi-comparison showed no difference between ZV ants tested immediately (Fig. SI3: 4th trial) and after entering the nest (Fig. SI3: Tested after nest ZV) (Scan rate: $p = 0.382$; Meander: $p = 0.996$). However, the ants that were tested as full vector after reaching the

feeder again (Fig. SI3: Tested after nest FV) showed significantly fewer scans ($p < 0.004$) and lower Meander ($p < 0.001$) than both ZV groups.

We also compared performance in Section A of the ants in condition 3c (tested as ZV ants after previously having entered their nest) to the ants in Condition 2a (rewound on A) and to the ants in Condition 2b (rewound on B) to calculate an evidence ratio of which Condition (2a or 2b) ants in Condition 3c resembled more. For both variables, ants in Condition 3c resembled the ants in Condition 2a more (Meander: evidence ratio 731.32; Scan rate: evidence ratio 3.25). These ratios, both above 1, mean overwhelming evidence in the case of meander and moderate evidence in the case of number of scans.

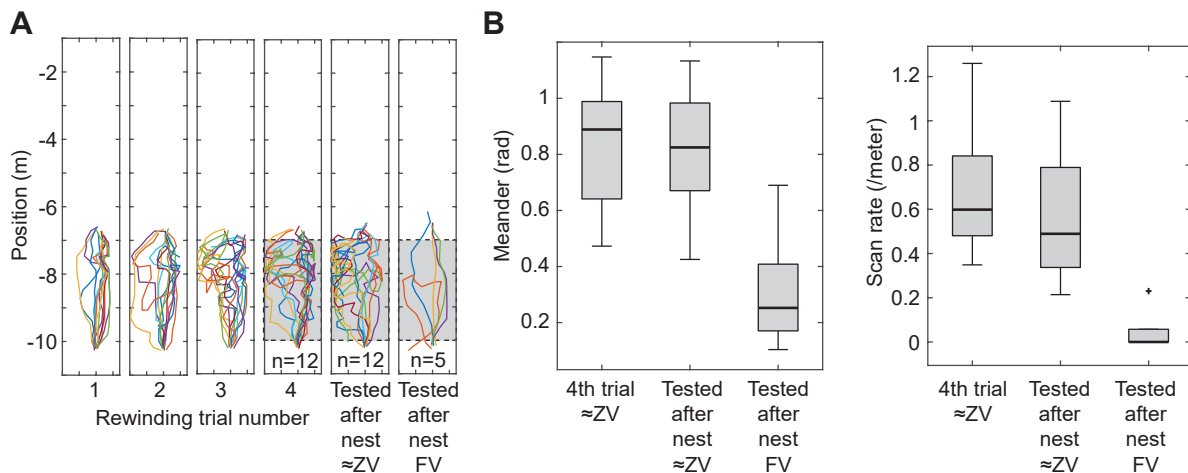


Fig. SI3. Entering the nest does not improve re-running after being rewound in a section. A. Paths of ants that re-ran Section A four times, then entered their nest, then were rewound in Section B four times on their next trip to reach a zero vector state, and then were tested on Section A either immediately (Condition 3c: Tested after nest =ZV), or on their next appearance at the feeder as full-vector ants (tested after nest FV). **B.** Meander (left) and Scan rate (right) in Section A (grey area in A) for the three treatments mentioned above. 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).

SI3 Discussion

It is clear that being in the nest did not alleviate the deleterious effects of repeated route running. However, the fact that FV ants' paths were much less affected than ZV ants' paths suggests that the path degradation observed in ZV ants resulted more from the suppression of appetitive route memories than learning new aversive visual memories that trigger turns. Indeed, given the suppression of appetitive route memories, ants should behave as in unfamiliar terrain, that is, follow their path integrator if the PI vector is large, and search around if in a ZV state, as we observed here. In the case of learning aversive memories, one would expect that the visual memories would dominate over the dictates of path integration, thus triggering turns in both ZV and FV ants. It should nonetheless be noted that the N is small, and that one of the FV ants did execute a U-turn, suggesting the existence as well of aversive memories, which may have arisen from being repeatedly captured at a specific location.

SI4. Set up and paths from Condition 4: comparing performance in an open vs. cluttered landscape

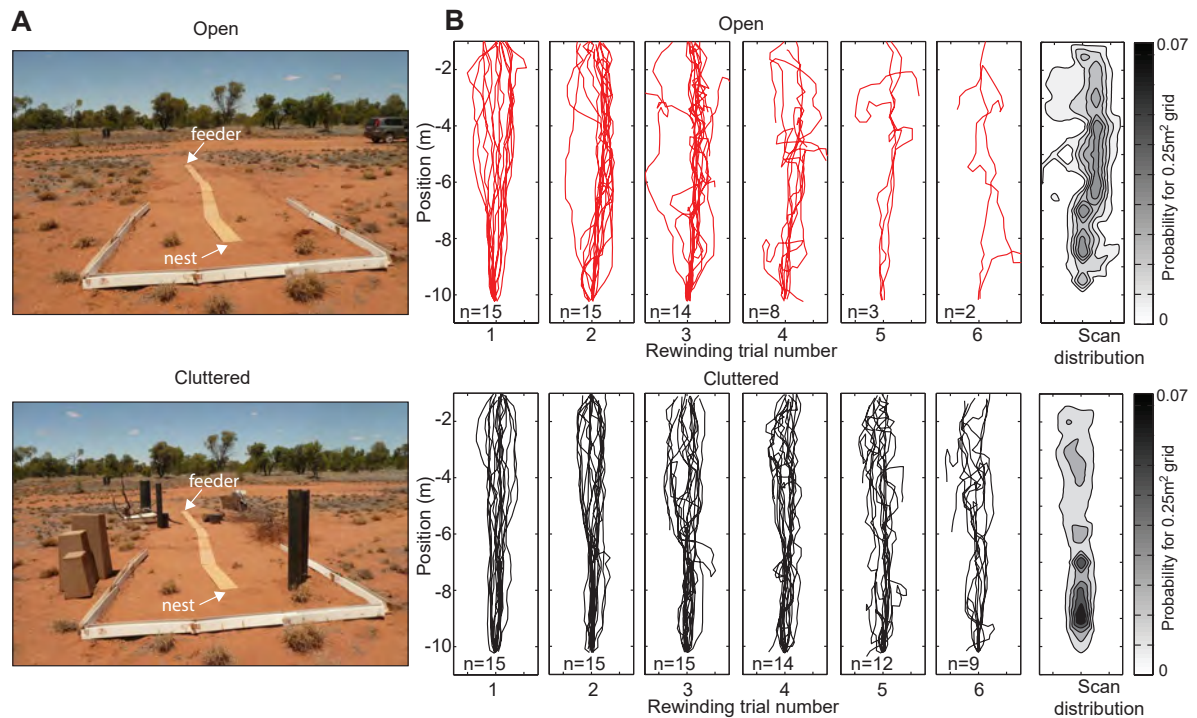


Figure SI4. Set up and paths from Condition 4: comparing performance in an open vs. cluttered landscape. **A.** Photos of the landscapes of the open condition (top) and the cluttered condition (bottom) of Nest 2. **B.** Paths (left 6 columns) and heat maps of scans (right column) of rewinding runs of ants in the open (top) and cluttered (bottom) conditions. In the heat map, the darker colours show a higher Scan rate. Quantitative analyses of these data are given in Fig. 3.