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## Rapid Aversive and Memory Trace Learning during Route Navigation in Desert Ants

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1 **Rapid aversive and memory trace learning**

2 **during route navigation in desert ants**

3

4 **Short title:** Aversive learning during route navigation

5

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24 **Summary**

25 The ability of bees and ants to learn long visually guided routes in complex environments is  
26 perhaps one of the most spectacular pieces of evidence for the impressive power of their  
27 small brains. While flying bees can visit flowers in an optimised sequence over kilometres,  
28 walking solitary foraging ants can precisely recapitulate routes of up to a hundred metres in  
29 complex environments [1]. It is clear that route following depends largely on learnt visual  
30 information and we have a good idea how visual memories can guide individuals along them  
31 [2–6], as well as how this is implemented in the insect brain [7,8]. However, little is known  
32 about the mechanisms that control route learning and development. Here we show that ants  
33 (*Melophorus bagoti* and *Cataglyphis fortis*) navigating in their natural environments can  
34 actively learn a route detour to avoid a pit-trap. This adaptive flexibility depends on a  
35 mechanism of aversive learning based on memory traces of recently encountered stimuli,  
36 reflecting the laboratory paradigm of trace conditioning. The views experienced *before* falling  
37 into the trap become associated with the ensuing negative outcome and thus trigger salutary  
38 turns on the subsequent trip. This drives the ants to orient away from the goal direction and  
39 avoid the trap. If the pit-trap is avoided, the novel views experienced during the detour  
40 become positively reinforced and the new route crystallises. We discuss how such an interplay  
41 between appetitive and aversive memories might be implemented in insect neural circuitry.

42

## 43 RESULTS AND DISCUSSION

44 *Ants can reshape their route to circumvent a trap.*

45 We first let Australian solitarily foraging ants *Melophorus bagoti* shuttle back and forth  
46 between their nest and a feeder full of cookie crumbs located 5 m away. For the outbound  
47 trip, the ants had to walk through a long and narrow channel suspended 15 cm above the  
48 ground that connected the nest directly to the feeder. For the way back to the nest, ants  
49 loaded with a cookie crumb were free to navigate on the desert ground. After a day of  
50 shuttling back and forth, all marked ants had established a fairly direct homing route to the  
51 nest (Figure 1Ai). We then opened a pit-trap, previously buried inconspicuously into the  
52 desert floor, creating a 2 m long, 10 cm wide gap perpendicular to the nest-to-feeder route.  
53 During their first homing trial with the trap, all trained ants ran as usual along the first part of  
54 the route and suddenly dropped into the trap. The trap was 10 cm wide and 10 cm deep (7 ×  
55 7 cm for *Cataglyphis fortis*) so that ants could see only the sky. The trap had slippery walls to  
56 prevent the ants from escaping and contained small twigs, which desert ants naturally tend  
57 to avoid as they impede walking. The trap offered a single possible exit formed by a 20 cm  
58 wide board (5 cm for *C. fortis*) leading from the base of the trap to the second part of the  
59 homeward route. The time the ants were trapped in the trap varied from one to tens of  
60 minutes, but once out, all individuals showed no apparent problem in returning directly to  
61 their nest (Figure 1Aii). We let ants shuttle back and forth with the trap open and recorded  
62 their paths again after 24h. After such training, several ants (4 out of 14, 29%) displayed a  
63 new route that circumvented the trap (Figure 1Aiii green trajectories). The tendency to detour  
64 the trap on the right side may due to two factors: 1- the nest relocated its entrance slightly  
65 on the right (as observed in [9]), so that the trap is not actually perpendicular to the feeder-  
66 to-nest beeline, making detours slightly shorter to the right than to the left; 2-a high cluttered  
67 region stands on the left of the setup, and desert ant species tend to be repelled by region  
68 presenting a high skyline [10,11]. We replicated these experiments at a larger scale (8 m route  
69 and 4 m wide trap) with North African desert ants from Tunisia (*Cataglyphis fortis*) and  
70 obtained similar results (13 out of 47, 28% avoided the trap after 24h) (Figure 1Bi,ii,iii). Note  
71 that black objects were added around the experimental setup to visually enrich the barren *C.*  
72 *fortis* environment (see STAR METHODS).

73 Why some ants did not learn to circumvent the trap may be due to different reasons. A good  
74 proportion of those ants in both species did show modification of their routes by learning to  
75 avoid the trap using alternative strategies such as jumping directly onto the exit stick (see red  
76 paths in SI1), or simply learning a quick route through the trap by systematically falling in at  
77 the same spot and quickly reaching the exit stick with very little search (see Ant 7 in SI1).  
78 Finally, some ants simply did not learn, perhaps because they performed too few training  
79 trials within the 24h period. Our personal observations were that the specifics of how ants hit  
80 the pit-trap influenced their ultimate strategy. Some ants fell by chance near the exit bridge  
81 and so spent less time in the trap itself. These ants were more likely to develop a strategy that  
82 depended on hitting the trap, but also knowing how to get out quickly. Future experiments  
83 could investigate the possibility that a longer time of being trapped yields stronger aversive  
84 memories. In any case, a simple categorisation of whether the ants circumvented the trap or  
85 not is sufficient to show that such an effect is unlikely to happen by chance  
86 (1st\_trial\_with\_trap vs. 24h\_with\_trap:  $N_{(\text{circumvented})} / N_{(\text{all ants})} : 0/30$  vs.  $17/61$ , Fisher's Exact  
87 test  $p < 0.001$ ), and that there was no apparent difference in detour success between species  
88 (24h\_with\_trap *M. bagoti* vs. *C. fortis*:  $N_{(\text{circumvented})} / N_{(\text{all ants})} : 4/14$  vs.  $13/47$ , Fisher's Exact  
89 test  $p = 1$ ).

90

91 *New routes are based on learnt terrestrial cues.*

92 Desert ants are well known to follow habitual routes guided by learnt terrestrial cues  
93 although they also have access to their Path Integration (PI) system at all times [12,13], a  
94 navigational strategy that is particularly pronounced in *C. fortis* [14]. We carried out several  
95 manipulations to ask whether learnt terrestrial cues, rather than PI, were controlling the new  
96 routes of our foragers.

97 Ants captured just before entering their nest and then re-released at the feeder are called  
98 zero vector (ZV) ants because their PI state is zero at the onset of homing, and thus no longer  
99 provides correct homeward information. Such ZV ants that had circumvented the trap during  
100 their previous (full vector, FV) run were equally successful in their subsequent ZV run (Figure  
101 1Biv) whereas ants that had fallen into the trap as FV ants still did so as ZV ants (previous\_FV  
102 circumvented vs. previous\_FV fell:  $N_{(\text{circumvented})} / N_{(\text{all ants})} : 12/12$  vs.  $2/13$ , Fisher exact test

103 p<0.001), showing that guidance along the newly learnt route does not require PI.  
104 Interestingly, ZV ants displayed turns before the trap even if they had failed as FV, showing  
105 that the learning process itself has to do with views rather than the ant's PI state. Note also  
106 that 2 out of 13 ants fell as FV but avoided the trap as ZV, which further supports the  
107 hypothesis of visual route learning. We know that the directional dictates of PI and learnt  
108 visual guidance are integrated, even when pointing in different directions [15–18]. Thus, in  
109 FV ants, the PI vector points directly to the nest and thus may bias the path towards the trap.  
110 Therefore, the paths of ZV ants are more representative of the route as guided by terrestrial  
111 cues. Nevertheless, the extent to which PI information may be associated with aversive  
112 experiences should be more fully studied.

113 We further tested whether ants that had learnt a new route to circumvent the trap would still  
114 follow it, if the trap was removed again. Five out of the six individuals tested displayed the  
115 usual detour even though the trap had been removed (Figure 1Aiv; No\_trap\_initially vs.  
116 trap\_removed\_again:  $N_{(\text{circumvented})} / N_{(\text{all ants})} : 1/20$  vs.  $5/6$ , Fisher exact test  $p < 0.001$ ). This  
117 confirms that the detour does not depend on perceiving the trap, but on route memories.

118 Finally, we recorded a cohort of ants that had started their foraging life while the trap was  
119 already in place. We did not control how many trials each ant produced but within a period  
120 of 24h we observed that several individuals learnt routes that circumvented the trap (Figure  
121 1Av). The proportion of ants that circumvented the trap was similar between these ants and  
122 ants that had some route experience before the trap was set in place (24h\_with\_trap\_naive  
123 vs. 24h\_with\_trap:  $N_{(\text{circumvented})} / N_{(\text{all ants})} : 5/15$  vs.  $4/14$ , Fisher exact test  $p=1$ ). This shows that  
124 a route that circumvents a hidden trap will develop naturally, whether the trap is novel or has  
125 been there for all of an ant's foraging career.

126 The use of chemical trails, scent marks or other social information would be unlikely in these  
127 highly visual solitary foraging ants, however we can further definitively rule them out, by  
128 observing the typical idiosyncrasies of ants' individual routes [19–21]: even though trials were  
129 interleaved between individuals and spanning over different days, routes are remarkably  
130 similar across subsequent trials of a same individual (Figure 2A, Figure S1) but they vary  
131 substantially across individuals (Figure 1, Figure S1). We can be confident that ants were not  
132 using social information, but private information based on learnt terrestrial cues. The nature

133 of these learnt terrestrial cues is not crucial for our purpose here, but based on past evidence  
134 with desert ants, which run at high speed (>50cm/s) with the head and antenna lifted  
135 upwards, we can be confident that it is mostly based on visual cues [9,19,22], typically the  
136 recognition of familiar panoramic views [3,23,24]. To ease the reading, we will now refer to  
137 these learnt terrestrial cues as ‘familiar views’.

138

139 *How do ants reshape their routes? Evidence for aversive and memory trace learning.*

140 To shed light on the processes that lead from an established route to a new route that  
141 circumvents the trap, we tracked all successive trials of individually marked *M. bagoti* ants  
142 from the first time they encountered the trap onwards. In addition to their paths, we recorded  
143 the locations where ants stopped and scanned their surroundings. Scanning is a typical  
144 behaviour in desert ant navigators (and particularly obvious in *M. bagoti*): the ant suddenly  
145 stops and rotates on the spot, pausing in different directions successively [25]. Scans are  
146 triggered when an ant experiences a decrease in visual familiarity [25], when multiple  
147 directional cues are put into conflict [26,27], when running a route has not resulted in success  
148 [4,27], or simply when naïve ants exit their nest for the first time [28–30]. In other words, the  
149 occurrence of scanning reflects a directional uncertainty in an ant’s navigational system [25].

150 As described above, on the first run with the trap in place, ants rush along their direct  
151 homeward route and fall into the trap. In subsequent trials, routes of most ants changed and  
152 some eventually circumvented the trap. We supposed two potential mechanisms. First, it  
153 could be that the negative event of falling into the trap triggers learning oriented behavioural  
154 routines that occur immediately after the negative event. This was not the case. When ants  
155 emerged from the trap, they rushed towards their nest as usual, and displayed neither more  
156 scanning or meandering than before the trap was set (Second part of the route: *Trial before*  
157 *trap vs. Trial with first trap fall*: mode = 0 scan/ant in both cases, glme #Scan:  $t=0.359$   
158  $p=0.721$ ; glme meander  $z=0.027$   $p=0.978$ ; Figures 2Bi and 2Bii, see also Figures 1Aii and Bii)).

159 Second, ants could keep memory traces of the scenes experienced immediately before falling  
160 into the trap, and change the valence of these recent memories given the current negative  
161 experience of being trapped. In our paradigm, this would predict that ant behaviour will be

162 affected on the run subsequent to falling in the trap when in the area immediately before the  
163 trap. Indeed, this is what we observed. Ants displayed a clear increase in scanning behaviours  
164 in the region before the trap (mode and median = 2 scans/ant, Figure 2Biii), significantly more  
165 than they had on their previous run (mode and median = 0 scan/ants, Figure 2Bii) before  
166 falling into the trap for the first time (First part of the route: *Trial with first trap fall vs. Trial*  
167 *following first trap-fall*: glme #Scan:  $t=4.186$   $p<0.001$ ). Similarly, their paths showed  
168 significantly more meandering as they approached the trap for the second time compared to  
169 their previous run (First part of the route: *Trial with first trap fall vs. Trial following first trap-*  
170 *fall*: glme meander:  $z=3.006$   $p=0.003$ ).

171 Mechanistically, learning must be based on a memory trace because the US (i.e., being in the  
172 trap) is experienced after the CS (i.e., the view of the surrounding scenery), and crucially, with  
173 no time overlap: for being in the trap (US) prevents entirely the view of the scenery (CS), and  
174 the trap is invisible from outside. Being exposed to the CS before the US with no time overlap  
175 between both stimuli reflects the laboratory paradigm of 'trace conditioning' [31]. How long  
176 is the delay between CS and US cannot be determined in our natural situation, although the  
177 apparent increase of scanning behaviours up to 2 metres before the trap (Figures 2A, 2C)  
178 suggests that the memory trace of the views may be kept for at least a couple of seconds. The  
179 resulting aversive memory formed, however, lasts across days to trigger salutary avoidance  
180 and scanning behaviours (Figure 2A, 'day2' grey arrow).

181 Aversive trace learning also provides an explanation for past results: ants repeatedly captured  
182 at the same location and asked to repeat their visually guided route display turns and  
183 avoidance behaviours before the capture point after a couple of trials only [27]. Being  
184 captured likely provides a negative US and the views preceding the capture point become  
185 aversive. This happens even though the ants were never allowed to reset their Path Integrator  
186 by being put back to the nest [32], showing that such long term aversive memories can be  
187 formed rapidly and without the need of the PI system.

188

189 *Neural implications.*



190 We now have a good idea of the neural underpinnings of learning in insects from studies of  
191 the Mushroom Bodies (MB) [33–37], which are assumed to be the seat of the route visual  
192 memories [8,38,39] (Figure 3). Each view experienced can be represented by a specific pattern  
193 of activation of Kenyon Cells (KC) in the MB [7], and KCs project onto multiple output neurons  
194 (MBONs) conveying different valences [40,41]. By modulating the synaptic weights between  
195 the active KCs and specific MBONs, views can be thus be associated with positive or negative  
196 valences, presumably inciting forward movements [7,8] or turns respectively (see also [42]).

197 To accommodate these principles of MB function our results simply require that 1- a trace (or  
198 tag) must remain in the KCs neurons (or their output synapses) for at least a few seconds after  
199 they have fired; and 2- A negative event (such as being trapped) must be able to change the  
200 valence associated with the tagged neurons, so that familiar views perceived before falling  
201 into the trap become less positive or aversive (Figure 3).

202 How and where exactly the tagging may happen remains unclear as several types of  
203 modulatory neurons project to these mushroom body compartments [43,44]. Finally, if it is  
204 now clear that being trapped or caught can constitute a negative reinforcement, what  
205 constitutes positive reinforcement during route learning is still unclear: it could be reaching  
206 the nest or running down the PI accumulated home vector [7]. Such positive reinforcement  
207 might also involve the ‘tagged neurons’, so that not only the current but also the previously  
208 experienced views that led to a positive outcome become associated to a positive valence. In  
209 any case, it is clear that route reshaping here results from an interplay between avoidance  
210 behaviour triggered by memory trace learning and appetitive learning based on positive  
211 reinforcement (Figure 3).

## 212 **CONCLUSION**

213 We have demonstrated here how memory trace learning is adaptively applied to navigation  
214 in natural habitats, where a negative experience labels specific locations in space that precede  
215 the aversive event. Behaviourally, this allows an ant to solve a navigational problem by  
216 efficiently reshaping its route through the world. Such route plasticity thus results from an  
217 interesting interplay between aversive and appetitive visual memories, and between  
218 avoidance learning (a form of negative reinforcement) and positive reinforcement, which  
219 matches well our current understanding of insect learning circuits.

220

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229

230 **Author contributions**

231 Conceptualisation: A.W.; Methodology: A.W., C.B., S.S., P.G.; Formal analysis: A.W.  
232 Investigation: A.W., C.B., S.S.; Writing - original draft: A.W., P.G.; Writing - review & editing:  
233 A.W., C.B., S.S., K.C.; P.G.; Funding: A.W., C.B., K.C., P.G.

234

235 **Declaration of interests**

236 The authors declare no competing interests.

237 **Main-text figure legends**

238 **Figure 1. Negative experience shapes ants' routes.** **A.** Australian desert ants (*Melophorus*  
239 *bagoti*) or **B** North African desert ants (*Cataglyphis fortis*) were followed for a series of  
240 homeward routes from a permanent feeder (F), with a pit-trap in place (solid line) or covered  
241 over (dashed line). Two small dashes across the trap line indicate the escape board. Green  
242 paths from ants that circumvent the pit-trap and blue paths from ants that fall into the trap.  
243 (i) Control routes of ants between feeder and nest, without trap (A, n=20; B, n=16). (ii) The  
244 first route after the installation of the pit-trap (A, n=15; B, n=15). (iii) Paths after the pit-trap  
245 has been in place for 24 hours (A, n=14; B, n=47). (iv) and (v) show different conditions for the  
246 two species. (Aiv) paths of *M. bagoti* ants that had previously learnt to circumvent the trap,  
247 tested with the trap covered (n=6). (Av) the paths of ants that had begun their foraging life  
248 (i.e., since naïve stage) with the trap in place and had 24 hours of foraging experience (n=15).  
249 (Biv, v) paths of *C. fortis* ants tested as ZV ants (without useful path integration information,  
250 as the path integrator no longer points towards the nest, but towards the feeder) that had  
251 previously succeeded (Biv, n=12) or failed (Bv, n=13) to circumvent the trap as full-vector ants.  
252 'n' refers to the number of ants tested.

253 **Figure 2. Negative experience modifies the memory of the views experienced before the**  
254 **trap.** Individually marked ants of *Melophorus bagoti* were tracked for a sequence of runs  
255 before and after the activation of the pit-trap. **A.** Sample sequence from a single ant.  
256 Locations where the ant stops and scans the world are marked with a circle. As elsewhere in  
257 the paper, paths are coloured coded: blue for ant paths that fall into the trap and green paths  
258 that avoid the trap. See Figure S1 for more individual examples. **B.** For each ant the number  
259 of scans was recorded before (bottom, blue) and after (top, red) the trap across three focal  
260 trials. (i) Trial before the trap was activated; (ii) Trial with first trap-fall; (iii) Trial following first  
261 trap-fall. From this we calculated the probability of an ant scanning ( $N_{(\text{ants scan}>0)}/N_{(\text{all ants})}$ ), and  
262 the median number of scans, for each trial and route segment. Before the trap: (i) N=31  
263 proba=0.45 Median=0 (ii) N=25 proba=0.40 Median=0 (iii) N=25 proba=0.80 Median=2. Star  
264 indicate a significant increase in scan number (see text for detail). After the trap: (i) N=31  
265 proba=0.35 Median=0 (ii) N=25 proba=0.40 Median=0 (iii) N=25 proba=0.32 Median=0. **C.** For  
266 the same three focal trials, the location of scans is shown relative to the Feeder (F, (0,0)), Nest

267 (N, (0,5)) and Pit Trap (Black line,  $y=2.5$ ). Darker areas represent higher scan numbers. Upward  
268 arrows in left panels indicate route direction.

269 **Figure 3. Overview of the appetitive and aversive learning experiences.** **A.** illustrations of  
270 the concept. Across a sequence of journeys for a typical ant we show the regions of operation  
271 for aversive (red) and appetitive (green) learning. The aversive region is first formed by  
272 memory trace learning on the trials where the ant encounters the trap. Over time a detour  
273 develops, and the new route is maintained by appetitive processes. **B.** Picture of a *Cataglyphis*  
274 *fortis* brain's right hemisphere showing the optic lobes (OL) and Mushroom-Bodies (ML)  
275 (modified from [45]) **C.** Mushroom-Bodies neural architecture derived from the insect  
276 literature [8,46]. Visual information is sparsely projected via visual Projection Neurons (PNs)  
277 to the Kenyon Cells (KCs). KC activity thus represents views that can be associated with the  
278 Mushroom Body output neurons (MBONs) mediating appetitive or aversive valences. Such  
279 associations result from the modulation of KC-to-MBON synapses; the modulation is  
280 generated by the co-activation of KCs and dopaminergic neurons (DANs) relaying the valence  
281 of the current situation. The resulting balance between aversive and appetitive MBON  
282 activities can then control steering. **D.** The current study suggests memory trace learning as a  
283 mechanism to explain the reshaping of routes. First, a trace of the KC activity must be kept  
284 for a few seconds (top panel). Second, the co-activation of a dopaminergic neuron modulates  
285 the KC-to-MBON synapses of these recently activated KCs (bottom panel). Note that  
286 modulation consists of a synaptic depression, which explains why DANs of a given valence  
287 modulate MBONs of the opposite valence. Thus an aversive situation, such as being trapped,  
288 will be mediated by a DAN decreasing the connection strength between the recently activated  
289 KCs and the appetitive MBON. These KCs will no longer activate the appetitive MBON, but still  
290 activate the aversive MBON. In other words, the view experienced before the trap will  
291 henceforth trigger an aversive response.

292

293 **STAR Methods**

294

295 **LEAD CONTACT AND MATERIALS AVAILABILITY**

296 Further information and requests for resources should be directed to and will be fulfilled by  
297 the Lead Contact, Antoine Wystrach ([antoine.wystrach@univ-tlse3.fr](mailto:antoine.wystrach@univ-tlse3.fr)). This study did not  
298 generate new unique reagents.

299

## 300 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

301 Two species were tested in this study: the Australian red honey ant, *Melophorus bagoti* and  
302 the Saharan desert ant *Cataglyphis fortis*. Both species are highly thermophilic ants (Wehner,  
303 1987; Christian & Morton, 1992) that forage solitarily on dead insects and plant materials  
304 (Muser et al., 2005). Experiments with *M. bagoti* were achieved in January-March 2014, ~10  
305 km south of Alice Springs, Australia, on the grounds of the Centre for Appropriate Technology,  
306 in a semi-arid desert habitat characterised by red soil, grass tussocks, bushes, and trees of  
307 Acacia and Eucalyptus species. Field experiments with *C. fortis* were performed in June-July  
308 2015 in a flat salt pan (34.954897 N, 10.410396 E) near the village of Menzel Chaker, Tunisia.  
309 By using two species of desert ants that belong to different phylogenetic tribes, we hoped to  
310 investigate the generality of this route re-learning process.

311

## 312 **METHOD DETAILS**

### 313 *Experimental set-ups*

314 The experimental set-ups for the two species were similar, with a larger scale for *C. fortis* to  
315 reflect their typically longer range of foraging (up to hundreds of meters for *C. fortis* and up  
316 to 70 m in *M. bagoti*, personal observation) [14]. Measurements below are given for *M.*  
317 *bagoti*, followed by *C. fortis* in brackets. Experiments were undertaken with a nest located in  
318 an area cleared of grass but surrounded by bushes and trees (or artificially added large black  
319 cylinders for *C. fortis*) providing rich visual information for navigation.

320 In both experimental set-ups, ants moved between their nest and a feeder full of cookie  
321 crumbs 5 m (8 m for *C. fortis*) away. The ants' nest was covered with an overturned bucket  
322 that had the bottom removed, whose opening at ground level was connected to a straight  
323 outbound channel (5 m long, 10 × 10 cm for *M. bagoti*; 8 m long, 7 × 7 cm for *C. fortis*) elevated  
324 15 cm above the ground, which was always in place and thus belonged to the scenery. This

325 outbound channel suddenly ended above the feeder, into which ants would drop. The feeder  
326 was a small plastic container sunk into the ground providing biscuit crumbs *ad libitum*. To  
327 return to the nest, the ants climbed out of the feeder using a small ramp and walked on the  
328 desert ground back to the nest. Removing the feeder ramp at critical times allowed us to  
329 control which ants ran their homeward journey and when. Halfway along their homing route,  
330 a plastic channel was buried inconspicuously into the desert floor, creating a 2 m long, 10 cm  
331 wide (4 m long 7 cm wide for *C. fortis*) trap perpendicular to the nest-to-feeder route. The  
332 trap was buried entirely so as to remain invisible to the ants. The trap had smooth walls and  
333 was filled with twigs to hinder ant movement. Ants could leave the trap by using a single exit  
334 formed of a stick bridge 20 cm wide (5 cm for *C. fortis*), connecting the bottom of the trap to  
335 the second part of the route. A grid of lines (mesh width: 1 m) was set up by winding strings  
336 around pegs (or by painting on the ground with *C. fortis*) and the ants' homing paths before  
337 and after introducing the trap were recorded on squared paper. During initial training the trap  
338 was covered by a thin board, with desert sand scattered on top, so that the ants could shuttle  
339 unimpeded.

340

#### 341 *Experimental protocols*

342 For both species, ants that arrived at the feeder were marked with a dot of day-specific  
343 enamel paint to ensure that ants were experienced before the trap was set. Only ants with at  
344 least 24 hours experience were recorded. Once the trap was set, the ants' first homing paths  
345 after trap introduction were recorded as well as their paths 24 hours later.

346 With *C. fortis* a group of ants was recorded twice. Here, the ants performed their homing  
347 route and just before they entered the nest they were taken and released again at the feeder  
348 as zero-vector ants, to test whether PI could provide an alternative explanation to views. *C.*  
349 *fortis* is well suited for this control as this species is known to strongly rely on PI (compared  
350 to *M. bagoti*) [14].

351 With *M. bagoti*, an additional treatment was enacted. Successful ants that circumvented the  
352 trap were marked and, once they return to the feeder again, tested with the trap covered  
353 again (as in the initial training).

354 Another condition was tested with naïve *M. bagoti* ants. The trap was set in place and all ants  
355 were marked for 5 consecutive days. After this period, all unpainted ants reaching the feeder  
356 were considered 'naïve' and were painted with a specific colour. Naïve ants were free to  
357 forage for 24 hours before being recorded.

358 Finally, some *M. bagoti* ants were marked with individual colour codes in order to obtain a  
359 record of the evolution of individual routes. In this treatment, we recorded both the path and  
360 the occurrence of the clear cut scanning behaviours typically observed in this species [25].

361

## 362 **QUANTIFICATION AND STATISTICAL ANALYSIS**

363

364 Paths were digitised using the software Graphclick. Meander was calculated as the mean  
365 angular deviation in direction between successive 30 cm chunks of the ants' paths. For the  
366 '*Avoid vs. fell comparison*' we used Fisher's Exact Test to look for differences between groups  
367 in the ratio of ants that circumvented or fell into the trap. For the '*Scan number and meander*'  
368 comparisons ants were followed individually across successive trials. We compared *scan*  
369 *number* and *meander* across three situations: (i) Trial before trap; (ii) Trial with first trap-fall;  
370 (iii) Trial following first trap-fall for two sections of the route, before the trap and after the  
371 trap, separately. To analyse the number of scan per ants, we used a generalised linear mixed  
372 effects model with ants as a random effect for count data. Given that the dispersion  
373 parameter (null deviance / df = 2.67 for 'first part of the route' and 2.08 for 'second part of  
374 the route') is above 2, we used a quasiPoisson distribution rather than a Poisson distribution  
375 (glmmPQL from MASS library in R). For meander values, we scaled the data between 0  
376 (Meander = 0deg) and 1 (Meander = 180deg) and used a generalised linear mixed effects  
377 model for proportional data with ants as a random effect (family="beta", link="probit" ;  
378 glmmTMB library in R).

379 **DATA AND CODE AVAILABILITY**

380 All data are available at:

381 [https://github.com/awystrac/Rapid-aversive-and-memory-trace-](https://github.com/awystrac/Rapid-aversive-and-memory-trace-learning_Current_Biol_2020/commits?author=awystrac)  
382 [learning\\_Current\\_Biol\\_2020/commits?author=awystrac](https://github.com/awystrac/Rapid-aversive-and-memory-trace-learning_Current_Biol_2020/commits?author=awystrac)

383

384

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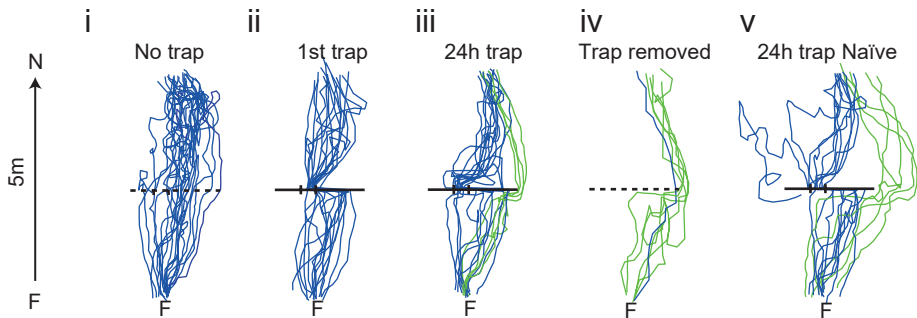
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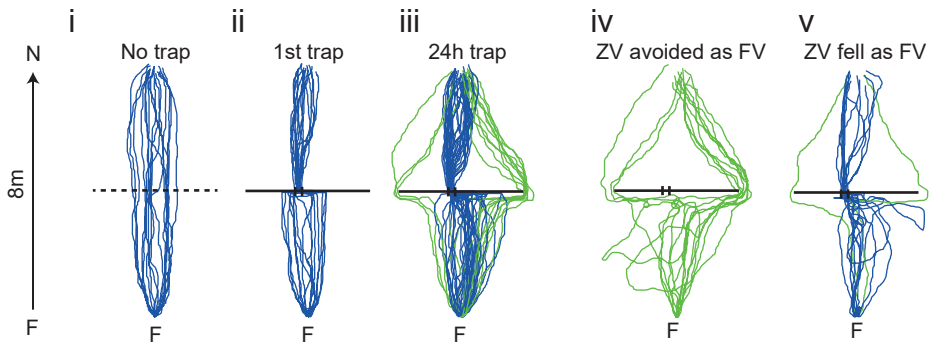
## KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Experimental Models: Organisms/Strains</b>		
<i>Melophorus bagoti</i>	Field site near Alice Springs (Australia)	N/A
<i>Cataglyphis fortis</i>	Field site near Menzel Chaker (Tunisia)	N/A

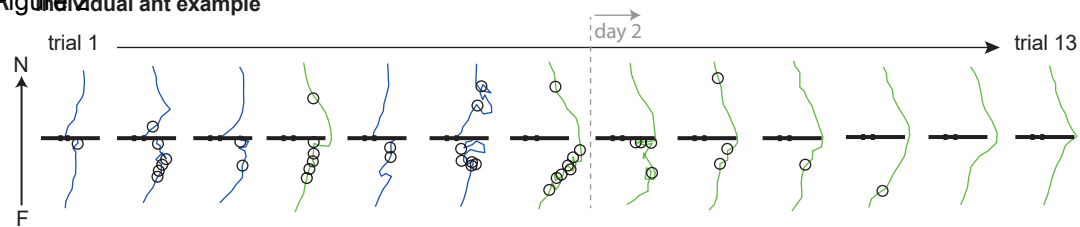
**Figure 1**  
*Metophrus bagoti*



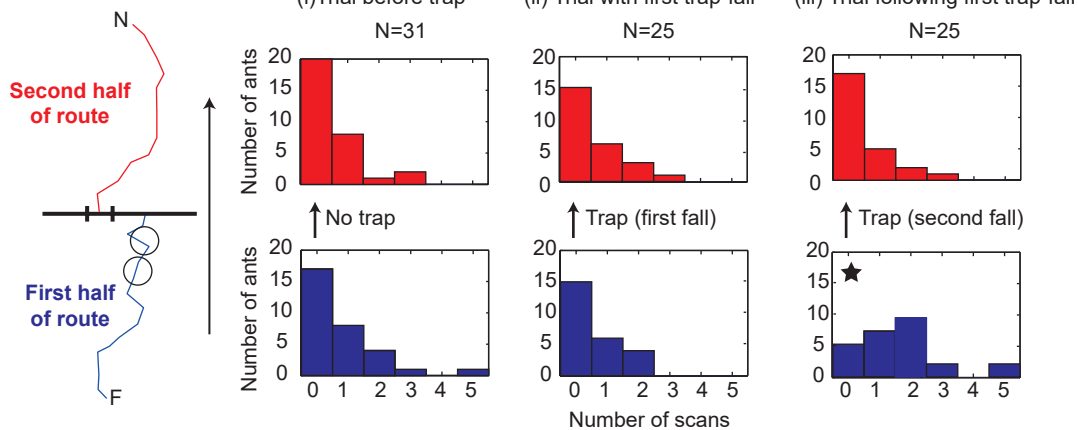
**B** *Cataglyphis fortis*



# Figure 2 dual ant example



## B Frequency of scans



## C Spatial distribution of scans

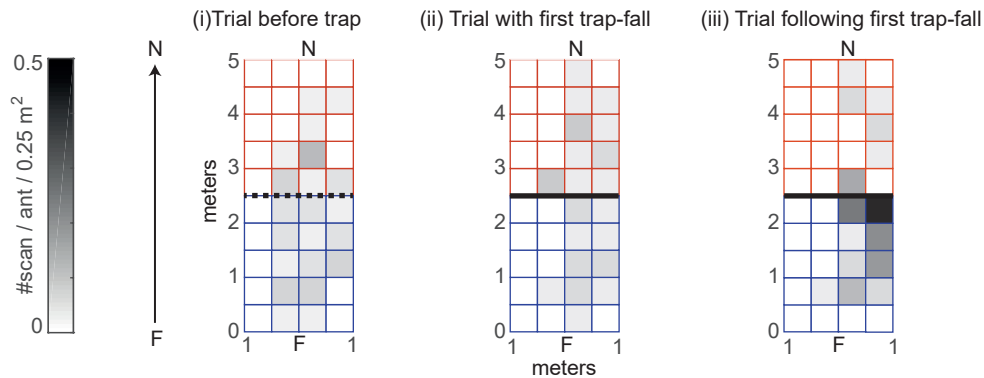


Figure 3  
A

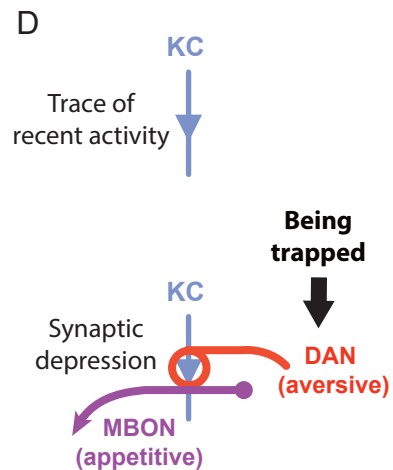
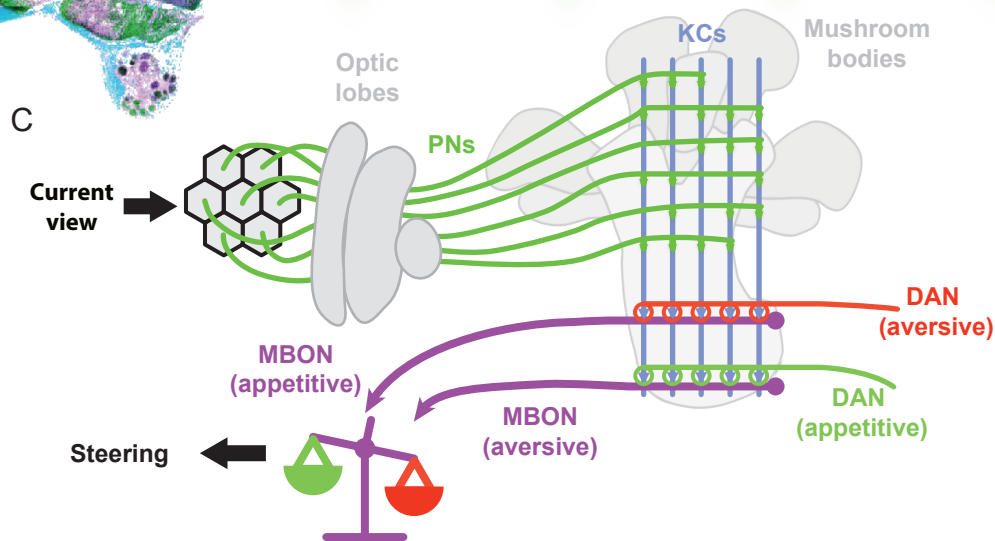
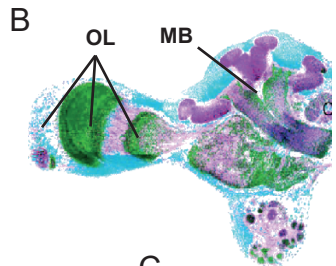
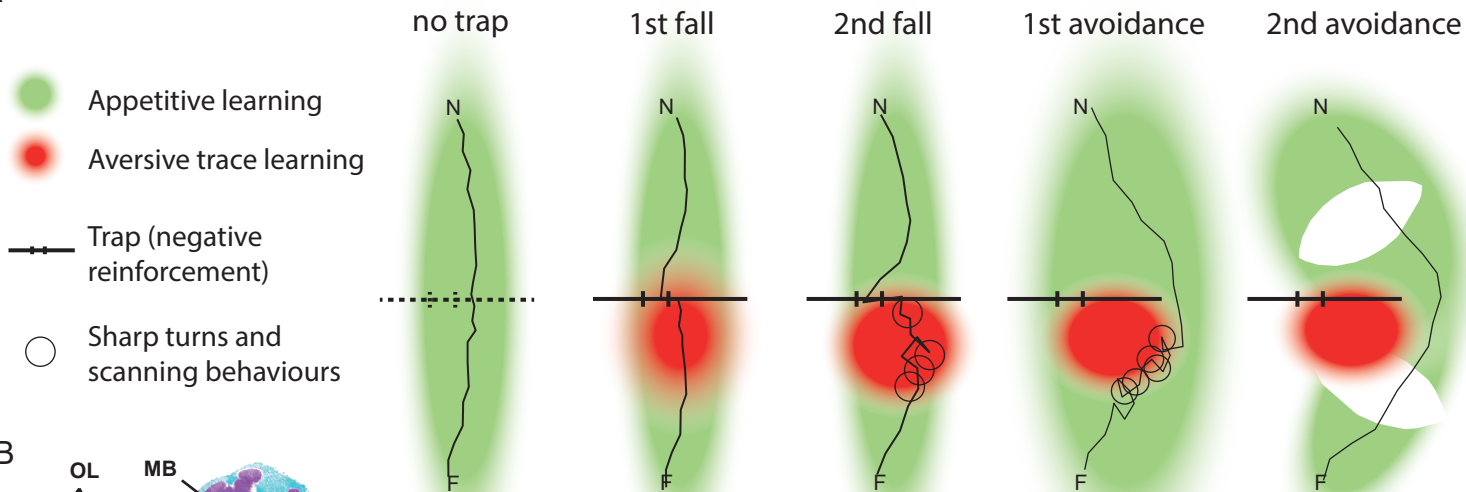
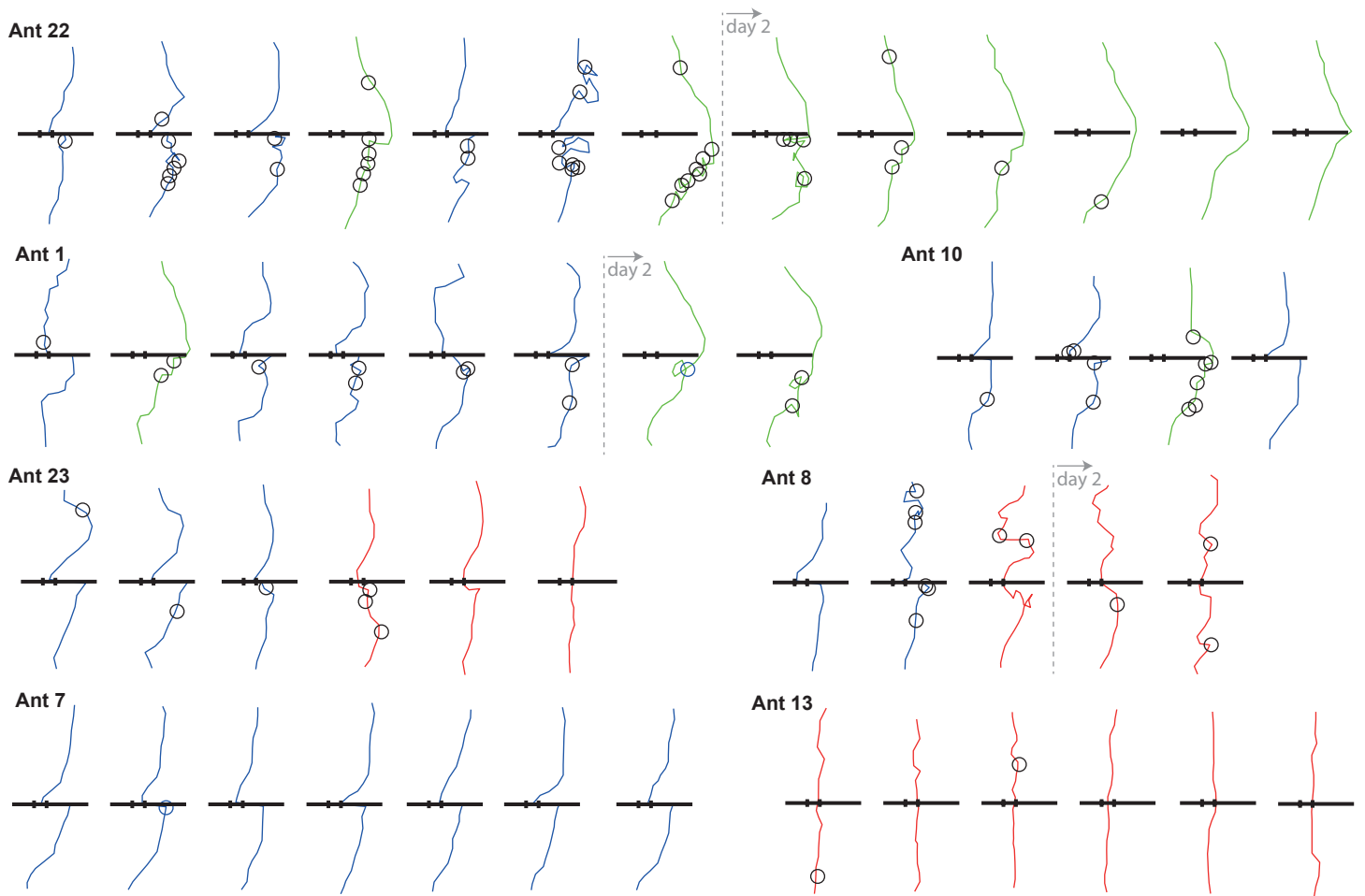


Figure S1



**Figure S1. Route shape and scanning ontogeny for individual ants. Related to Figure 2.**

Successive routes of *Melophorus bagoti* individuals from the first run incorporating the trap onwards. Scan locations are marked with a circle and routes are colour coded as in Figure 1 and 2 with the addition of paths marked in red for ants that learnt to use the stick bridge efficiently.