Detour learning ability and the effect of novel sensory cues on learning in Australian bull ants, *Myrmecia midas*

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Subject Category: Neuroscience and Cognition

Running Head: Learning & Cognitive ability of bull ant

Keywords: Detour Learning, Successful Foragers, Path Straightness, Motor Routine

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ABSTRACT

Many animals navigate in a structurally complex environment which requires them to detour around physical barriers that they encounter. While many studies in animal cognition suggest that they are able to adeptly avoid obstacles, it is unclear whether a new route is learned to navigate around these barriers and, if so, what sensory information may be used to do so. We investigated detour learning ability in the Australian bull ant, *Myrmecia midas*, which primarily uses visual landmarks to navigate. We first placed a barrier on the ants' natural path of their foraging tree. Initially, 46% of foragers were unsuccessful in detouring the obstacle. In subsequent trips, the ants became more successful and established a new route. We observed up to eight successful foraging trips detouring around the barrier. When we subsequently changed the position of the barrier, made a new gap in the middle of the obstacle, or removed the barrier altogether, ants mostly maintained their learned motor routine, detouring with a similar path as before, suggesting that foragers were not relying on barrier cues and therefore learned a new route around the obstacle. In additional trials, when foragers encountered new olfactory or tactile cues, or the visual environment was blocked, their navigation was profoundly disrupted. These results suggest that changing sensory information, even in modalities that foragers do not usually need for navigation, drastically affects the foragers' ability to successful navigate.

KEY WORDS: Detour learning, Successful foragers, Path straightness, Motor routine
_____

INTRODUCTION

In detour learning, a significant paradigm in animal cognition, a direct route is blocked, and a new route must be taken to reach the goal. In a natural environment, animals face various situations where obstacles block their regular shortest path to reach a destination, and in most cases, they can negotiate these obstacles (Zucca et al., 2005; Kabadayi et al., 2018). Several ecological and contextual factors might affect the efficiency of animal detour learning, such as the nature of the barrier, goal distances, detour experiences, reward visibility, and motivation (Kabadayi et al., 2018). To meet these challenges, animals may integrate both external and internal cues that help to redirect their movement (Giurfa, 2015; Kabadayi et al., 2018) but it is unknown what cues are used for establishing a new route.

Two forms of detour learning have been identified: spatial learning, characterized by gradual learning, and spatial reasoning, characterized by quick learning. Spatial learning occurs if an individual develops a tendency to select the shortest route over multiple trips. In contrast, spatial reasoning occurs if an individual selects the shortest path to a destination spontaneously on its first trip based on memory and visual inspection (Kabadayi et al., 2018). Detour abilities have been studied in a wide range of taxa including vertebrates such as apes (Kohler, 1925), rats and mice (Juszczak et al., 2016), dogs (Pongracz et al., 2001; Siniscalchi et al., 2013) goats (Naworth et al., 2016; Langbein et al., 2018), horses (Baragli et al., 2011; Baragli et al. 2017), marsupials (Wynne and Leguet, 2004), fish (Bisazza et al., 1997; Sovrano et al., 2018; Lucon-Xiccato et al., 2019), birds (Vallortigara et al., 1999; Zucca et al., 2005) and invertebrates such as spiders (Tarsitano and Jackson, 1997; Aguilar-Argüello et

61   al., 2019), and bees (Kevan, 2015; Ong et al., 2017). In most detour experiments, animals
62   have been tested repeatedly to examine their learning abilities (Kabadayi et al., 2018).
63   Several studies have shown gradual improvements in detouring over multiple trials (Parker et
64   al., 2005; Smith and Litchfield, 2010; Boogert et al., 2011). For example, horses reach their
65   goal faster over trials when they encounter an obstacle (Baragli et al., 2011). On the very first
66   trip, some horses were unsuccessful in detouring the barrier but from the second trip they
67   improved their success. Similarly, dingos in a detour learning experiment improved their
68   performance significantly over four trials (Smith and Litchfield, 2010). Another detour
69   learning experiment was done on mice, which exhibited a preference for one or the other side
70   of the barrier and optimized the direction of movement based on their previous experiences,
71   showing gradual learning over trials (Juszczak et al., 2016). Another study investigated the
72   ability to progress around a barrier to a desired food source in four quokkas, *Setonix*
73   *brachyurus*, in their natural environment (Wynne and Leguet, 2004). Among them, three
74   quokkas preferred to detour either right or left around the obstacle, showing spatial learning,
75   while the fourth showed no behavioural laterality but exhibited spatial reasoning.
76
77   Ants are highly efficient navigators in complex natural environments (Freas and Cheng,
78   2019; Lihoreau and Schwarz, 2020; Buehlmann et al., 2020). There have been few studies of
79   detour ability in ants. A detour strategy was observed in the crazy ant, *Paratrechina*
80   *longicornis* in obstacle navigation during cooperative transport (McCreery et al., 2016).
81   These ants proved effective at implementing a flexible or stochastic strategy that works for
82   both simple and complex barriers. Another detour experiment on *Cataglyphis fortis* ants
83   showed that when a barrier was set between the nest and releasing point, homing ants, tested
84   just once, usually walked towards the barrier, and when coming close to the barrier, deviated
85   around one of the sides and moved forward to the goal (Schmidt et al., 1992). Desert ants
86   (*Melophorus bagoti* and *Cataglyphis fortis*) can also learn a route detour around a pit trap
87   (Wystrach et al., 2020).
88
89   The Australian nocturnal bull ant, *Myrmecia midas*, is an excellent navigator that forages on
90   eucalyptus trees by using primarily visual information (Freas et al., 2017a; Freas et al., 2018;
91   Freas and Cheng, 2019; Islam et al., 2020). Nest entrances of these bull ants are usually
92   located close to a eucalyptus tree, called their nest-tree (Freas et al., 2018). Ants forage
93   individually in the tree canopy of either their nest tree or other nearby trees for insect prey
94   and sap. There is no evidence of trail pheromone for recruitment or any other recruitment
95   interaction among the foragers. Ants from each nest typically travel to a foraging tree in
96   evening twilight and back to their nest before morning twilight (Freas et al., 2017a; Freas et
97   al., 2018).
98
99   This study investigated the ability of *M. midas* to detour around a barrier during the
100   ascending navigation on their foraging tree, with a focus on four questions. First, would the
101   ants successfully move around the detour on each trial? Second, would the ants show spatial
102   learning or spatial reasoning? Third, how would the bull ants modify their route when the
103   barrier is removed partially or completely? Fourth, how would ants behave when
104   encountering visual and non-visual sensory changes along their new route? We hypothesized

105 that ants would learn to overcome the barrier gradually with repeated trials, as seen in
106 previous detour learning studies (e.g., Parker et al., 2005; Smith and Litchfield, 2010;
107 Boogert et al., 2011). Given their reliance on visual cues for navigation, we predict that the
108 ants would follow their newly learned route when encountering any changes of barrier
109 position or any olfactory or tactile changes on their foraging route, but that they would
110 navigate less successfully when visual cues around the tree were blocked.

111

MATERIAL AND METHODS

Animal and experimental site

114 The experimental site was located at Macquarie University campus, Sydney, Australia
115 (33°4611´´ S,151°06´40´´ E). Nocturnal bull ants, *Myrmecia midas*, typically build their nest
116 close to a Eucalyptus tree. We selected two nests 200 m apart which were located close to a
117 smooth-skinned eucalyptus tree, *E. virminalis*. In Experiment-1, we conducted experiments
118 on both Nest-A and Nest-B during 2018–2019 and on only Nest-A during 2019–2020. In
119 Experiment-2, we conducted experiments on Nest-A during 2019–2020. A tree is usually
120 located less than 30 cm from nest entrances of *M. midas* colonies (Freas et al., 2017a; Freas
121 et al., 2017b). This tree is termed the "nest-tree." Ants typically emerge from the nest
122 entrance, walk a few centimetres on the ground, and then many foragers climb vertically up
123 the nest-tree. We call this behaviour "ascending navigation," which is perpendicular to the
124 direction of navigation on the ground. To record the path of individual foragers during their
125 ascending foraging trips, a grid was drawn from the bottom of the tree to 1.5 m high on the
126 tree trunks of the nest-trees of both Nest-A and Nest-B. Each grid consisted of 10-cm squares
127 (Fig. 1) that covered the diameter of the tree trunk with code numbers on the grid to identify
128 locations on the grid. The circumference of the Nest-A and Nest-B nest-tree trunks were 200
129 cm and 160 cm, respectively.

130

Experimental procedure

132 **Experiment 1:** In the Control Condition, we observed individually marked ants between 7:00
133 p.m. and 9:30 p.m. for seven consecutive nights to record the frequency and paths of natural,
134 unobstructed foraging routes on the nest-tree. On the very first night, we captured the ants
135 from the nest-tree trunk by using plastic tubes and put them into an icebox with ice for 3–5
136 minutes to cool. Then foragers were painted with a unique colour code on the dorsal side of
137 their body with Citadel Paint (Games Workshop, UK). For detour learning tests, we chose
138 those marked ants that ascended to forage on the nest-tree at least three times out of seven
139 consecutive nights (104 foragers out of 135, 18 ants from Nest-A 2018–2019, 14 ants from
140 Nest-B and 72 ants Nest-A 2019–2020). In the Learning Condition, a piece of white art paper
141 (15 cm in diameter and 0.25 mm thick) was placed at 1 m from the bottom of the tree trunk
142 which covered 50% of the perimeter of the nest-tree trunk, obstructing their natural foraging
143 corridor on the nest side (Fig. 1). We tested the selected 104 foragers in up to eight trials each
144 with a detour barrier over 21 consecutive nights immediately after the Control Condition for
145 each nest.

146

147 **Experiment 2:** After the Control and Learning Conditions on Nest-A in 2019–2020, we
148 tested foragers one time in each of six different conditions in order: Extension Condition (we
149 moved the barrier from 1 m to 1.5 m from the bottom of the tree trunk, n=38), Gap in Middle
150 Condition (we made a 20-cm gap in the middle of the barrier, n= 40), No Barrier Condition
151 (we took off the entire barrier, n=36), Tactile Condition [we added a cream-coloured cloth
152 (100 cm × 120 cm) which covered between 0.5 m and 1.5 m height on the front side of the
153 tree trunk, n=38], Olfactory Condition (we sprayed perfume on two different places of the
154 tree trunk at 50 and 80 cm height which made two parallel lines and covered 50% of nest
155 side, n=25), and Visual Condition (we installed a 2-m wide and 1.25-m tall black screen on
156 both the right and left sides symmetrically on the ground and 50 cm from the edge of the tree
157 trunk on both sides, n=36). The barrier was not removed in the Tactile, Olfactory and Visual
158 Conditions. After testing in each Condition, we allowed the foragers 10 days of relearning the
159 route with the barrier (Table S1, Fig. S1). During relearning, we kept the barrier at 1 m from
160 the bottom of the tree trunk as in the Learning Condition. We used the individually marked
161 foragers observed in the Control and Learning Conditions for all of the additional Conditions.
162 Not all ants were tested in each condition because some ants did not forage every night of the
163 experimental conditions. Full details of methods are provided in the supplementary materials.
164

167 **Fig. 1**. The experimental set up for the detour learning experiments on Australian nocturnal
168 bull ants, *Myrmecia midas*, on their nest-tree: A. The tree trunk of a nest-tree (Nest-A) with a
169 grid from the base of the tree (0 m) to 1.5 m. A white cardboard barrier was placed

170 perpendicular to the tree trunk at 1 m from the base of the tree trunk which covered 180
171 degrees at the nest-side. B. A close view of the barrier that the foragers were confronted
172 with. C. A uniquely colour-coded nocturnal bull ant *Myrmecia midas* ascending towards the
173 top of the tree during detour learning. The photo was taken when the worker ant started at the
174 bottom of the foraging tree in the evening twilight.
175

Statistical Analysis

177 Graph-Click (www. arizona-software.ch/graph-click) was used to digitize the path of
178 individual foragers. By using a custom-written MATLAB (MATLAB 2019b) program, we
179 plotted the paths of the foragers and calculated the path straightness of individual ants in both
180 the Control Condition and the Learning Condition in the two different nest-trees separately.
181 We determined the path straightness for the path from the bottom of the tree to one-meter
182 height as the ratio of the straight-line distance to the sum of all segments of the path
183 (Palavalli-Nettimi et al., 2018) transformed so that 0.5 is the maximum value. For the Control
184 Condition, the straight-line distance for individual foragers was ~1 m, but in the Learning
185 Condition, the straight-line distances were 112 cm and 108 cm (from the bottom of the tree
186 trunk to edge of the barrier) in Nest-Tree A and Nest-Tree B, respectively. For all of the
187 other conditions except the Extension Condition the straight-line distance was also 112 cm
188 (straightness measured up to 1 m). For the Extension Condition this distance was 158 cm
189 (straightness measured up to 1.5 m). We considered ants as successful on a trip if they passed
190 the barrier located at 1 m from the bottom of the tree trunk.
191

192 For the Learning Condition, we conducted repeated-measures ANOVAs in SPSS to compare
193 the mean path straightness across the 3rd Control run in the Control Condition and 5
194 successful foraging trips (1st, 2nd, 3rd, 5th and 8th) of the Learning Condition in Nest-A 2018–
195 2019, Nest-A 2019–2020 and Nest B. We determined whether there was a significant
196 difference in path straightness of each successful learning trip in the Learning Condition
197 compared to the Control Condition by using a Bonferroni correction. The foraging duration
198 was timed when individual ants started from the bottom (0 cm) of the nest tree until they
199 reached 1.5 m from the bottom in both the Control Condition and the Learning Condition.
200 We conducted a one-way repeated-measures ANOVA with a Bonferroni correction to
201 compare durations of the 3rd trip of the Control Condition and 5 different successful foraging
202 trips (1st, 2nd, 3rd, 5th and 8th) during the Learning Condition in Nest-A 2018–2019, Nest-A
203 2019–2020 and Nest B. All of the foragers detoured either on the left or right side of the
204 barrier during their learning trips. We reflected the paths of the ants going to the right so that
205 all ants, conceptually, headed to the left side of the barrier. This practice allowed data to be
206 pooled to examine how the foragers adjusted their upward path with the barrier over trips. To
207 compare changes in paths over foraging trips (3rd control trip, 1st, 2nd, 3rd 5th and 8th
208 successful foraging trips), the mean positions on the *x*-axis (horizontal position) of the ants on
209 the tree trunk at 0 cm, 10 cm, 20 cm, 30 cm, 40 cm, 50 cm, 60 cm, 70 cm, 80 cm and 90 cm,
210 we used a two-way repeated-measures ANOVA with height and trip as factors. For all
211 repeated measure ANOVA, while the p-value represents whether the difference between

212 conditions is statistically significant or not, partial eta squared (ηp^2) illustrates about the
213 magnitude of this difference, or effect size. A value of $\eta p^2 \geq 0.14$ is considered a large effect.
214
215 In Experiment-2, to compare path straightness and duration, paired-samples t-tests were
216 conducted to compare the differences between last learning trips or relearning trips and test-
217 condition trips (for instance, Middle Gap tests vs. immediately preceding relearning trip). We
218 report effect sizes (Cohen's d) along with statistical significance levels. Data were analyzed
219 using SPSS Statistics (Version 26), MATLAB (2019b) and R-Studio (Version
220 1.2.1335/2019).
221

RESULTS

222

A. Experiment-1: Detour Learning

223

Successful versus unsuccessful foraging trips

224

225 Pooling results from both Nest-trees A and B in both experiments, first foraging trips with a
226 barrier were challenging for individual foraging ants, with 46% unsuccessful (Fig. 2). The
227 ants improved steadily over trips, with almost all successful by the 5th trip.
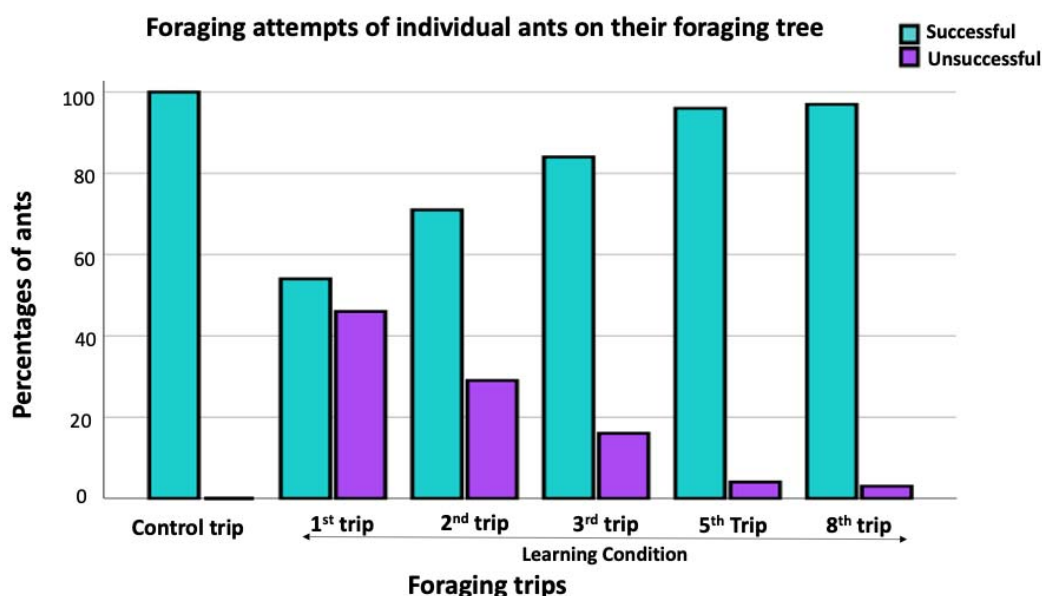

228
229 **Fig. 2.** The percentages of successful and unsuccessful foraging ants (N=104, Nest-Tree A,
230 2018–2019=18, Nest-Tree B=14, Nest-Tree A, 2019–2020=72) in the 1st, 2nd, 3rd, 5th and
231 8th attempts of foraging trips.
232

Position of foragers on the tree trunk on successful foraging trips

233

234 In the Control Condition, foragers' position was close to 0 on the *x*-axis from 0 cm to 90 cm
235 of the tree trunk (data not shown). Over trips in the Learning Condition, foragers' average
236 heading position became farther from 0 on the x-axis, which indicates ants learning to detour.

237 A two-way repeated-measures ANOVA examined the effects of trips and height on the *y*-axis
238 on the dependent variable of *x*-axis position. In Nest-Tree A 2018–2019, there was a
239 statistically significant interaction between trips and *y*-axis height [F (36,600) =6.273, p <
240 .05, ηp^2 = 0.273]. There were also significant main effects of trips [F (4,600) =149.03, p <
241 .05, ηp^2 = 0.499] and height on the *y*-axis [F (9,600) =11.495, p < .05, ηp^2 = 0.407]. In Nest-
242 Tree A 2019–2020, a statistically significant interaction was also observed between trips and
243 position on the *y*-axis [F (36,3599) =9.85, p<.05, ηp^2 = 0.191]. Significant main effects were
244 also found for trips [F (4,3599) =400.78, p < .05, ηp^2 = 0.308] and position on the *y*-axis [F
245 (9,3599) =117.71, p < .05, ηp^2 = 0.227]. Like Nest-A, there was a statistically significant
246 interaction between trips and *y*-axis position of ants in Nest-Tree B [F (4,320) =5.743, p <
247 .05, ηp^2 = 0.392] and also significant main effects of trips [F (4,320) =135.28, p < .05, ηp^2 =
248 0.628] and height on the tree [F (9,320) =13.09, p < .05, ηp^2 = 0.594]. In all experiments, the
249 position of foragers on the tree trunk was not significantly different (p > 0.05) between the
250 Control Condition and the 1st successful foraging trip. However, the 2nd, 3rd, 5th and 8th
251 successful foraging trips were significantly different (p < 0.05) compared to the Control
252 Condition. Importantly, in the Learning Condition, there were no significant differences of
253 ants' mean position on the *x*-axis of the tree trunk between the 5th and the 8th successful
254 foraging trips (p > 0.05). The head positions of foragers on the *x*-axis were not significantly
255 different across trips at 0 cm and 10 cm height, but from 20 to 90 cm height, foragers' *x*-axis
256 positions were significantly different (p < 0.05) across trips, suggesting that detouring started
257 between 10 cm and 20 cm height on the tree trunk (Fig. 3).
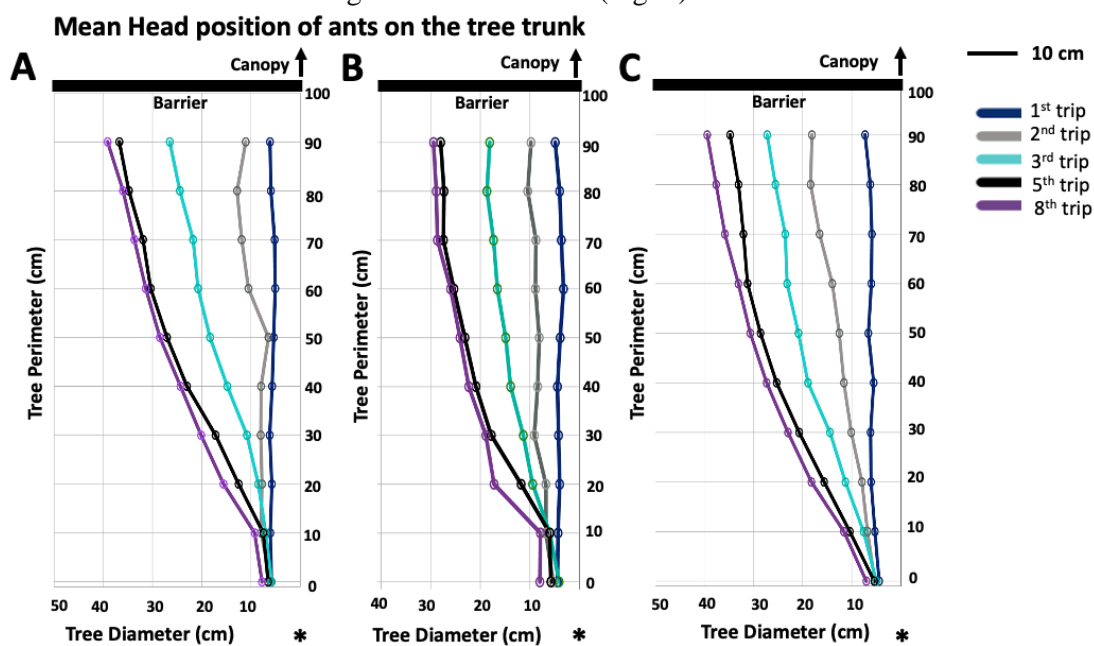
258
259

260 **Fig. 3.** Interaction between trip number in the Learning Condition and height on the tree
261 trunk in the *x*-axis position of ants on the nest-tree trunk in Nest-tree A 2018–2019 (A), Nest-
262 tree B (B) and Nest-tree A 2019-2020 (C). Nest-tree A trunk was 200 cm in circumference
263 where Nest-tree B was 160 cm in circumference and the barrier covered 50% on the front
264 side at 1 m from the bottom of the tree trunk (see details in Experimental Procedure). The

265   figure represents one half of the front (nest) side of the tree trunk. The *x*-axis represents the
266   horizontal position on the tree trunk and the *y*-axis represents the height on the tree trunk. We
267   reflected the paths of the ants going to the right so that all ants are, conceptually, headed to
268   the left side of the barrier. The coloured dotted lines illustrate the mean of *x*-axis positions of
269   ants on the tree trunk in different successful learning trips. The black line indicates the
270   position of the barrier on the tree trunk at 1 m. Ant paths are shown from 0 cm to 90 cm of
271   the tree trunk. The arrow indicates the canopy direction and the '*' indicates the nest
272   position.
273

274   The mean data in figure 3 hide notable individual differences in detour learning. Foragers
275   showed 3 types of detour learning: A. Gradual learning, a forager that learned the detour path
276   gradually (figure S2A); B. Quick learning, a forager that adjusted after 2 trips (figure S2B);
277   C. Barrier-avoiding learning, a forager that followed the foraging corridor up to the barrier
278   and then detoured to one side (Fig. S2C).
279

280   **Path Straightness**

281   The path straightness of foragers in detour learning was compared to their performance in the
282   control condition. Foragers ascended directly to the canopy on the tree-trunk in the Control
283   Condition, thus having a path straightness near the maximum value of 0.5 (figure 4). Path
284   straightness dropped sharply in the 1st successful foraging trip in the Learning Condition, and
285   then improved over trips. In the 5th and 8th successful foraging trips foragers performed
286   straighter paths like those in the Control Condition. A one-way repeated-measures ANOVA
287   showed a statistically significant difference between the Control Condition and successful
288   foraging trips in the Learning Condition of foragers in all experiments: Nest-Tree A 2018–
289   2019 ($F(5,75) = 151.05$, $p < 0.001$, $\eta\rho^2 = 0.988$), Nest-Tree B ($F(5,40) = 38.56$, $p < 0.001$,
290     $\eta\rho^2 = 0.903$) and Nest-tree A 2019–2020 ($F(5,355) = 334.06$, $p < 0.001$, $\eta\rho^2 = 0.825$). Post
291   hoc comparisons using Bonferroni correction showed that the first two successful trips in the
292   Learning Condition were significantly less straight when compared with the Control
293   condition in all experiments, while the third trip was significantly less straight in the 2018-19
294   field season (Fig. 4). The 5th and 8th trips did not differ significantly from the Control
295   Condition in either colony or season.
296
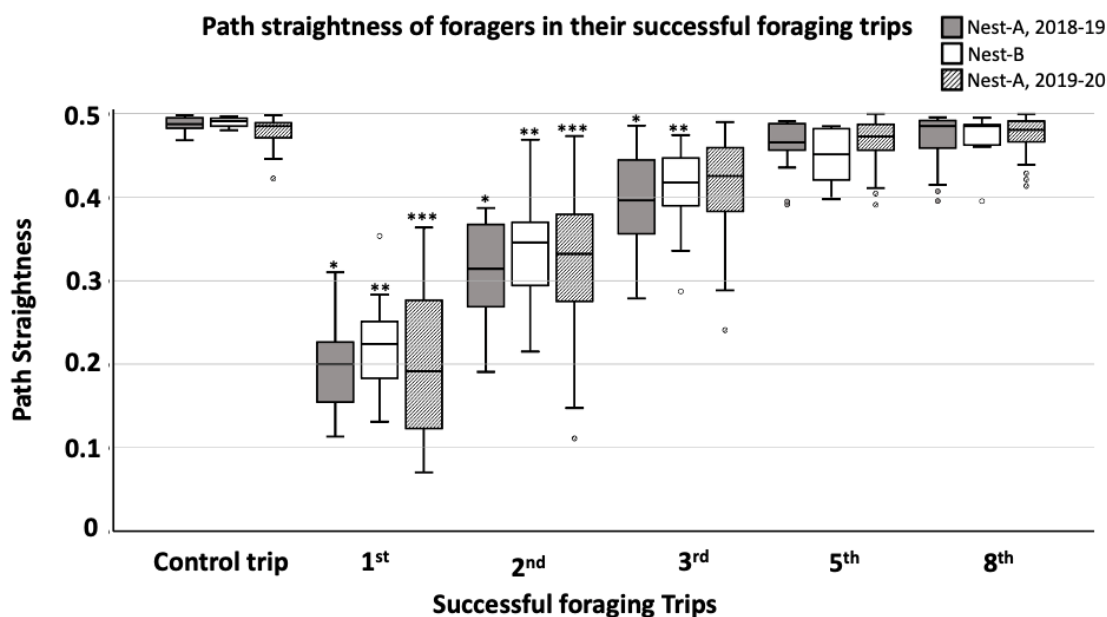
Fig. 4. Path straightness of *Myrmecia midas* during their Control and Learning Conditions. The '*' indicates that the path straightness of the 1ˢᵗ, 2ⁿᵈ and 3ʳᵈ successful foraging trips were significantly different from the Control Condition in Nest-A 2018–2019 and the '**' indicates a significant difference in path straightness compared to the Control Condition in Nest-B. Similarly, '***' represents the significant differences of path straightness in their 1ˢᵗ, 2ⁿᵈ and 3ʳᵈ learning trips compared to the Control Condition in Nest-A 2019–2020. The box plots indicate medians (solid black line), box margins (25th and 75th percentiles) and whiskers (5th and 95th percentiles) in this and all following figures.

Duration of foraging trips

In the 1ˢᵗ successful foraging trip ants took 2–3 fold more time compared to the Control Condition (figure 5). Over successful learning trials foragers took significantly less time to pass the barrier compared to the Control Condition in all experiments: Nest-tree A 2018–2019 ($F_{(5,75)} = 84.02$, $p < 0.001$, $\eta p^2 = 0.851$), Nest-Tree B ($F_{(5,40)} = 34.012$, $p < 0.001$, $\eta p^2 = 0.812$), Nest-tree A 2019–2020 ($F_{(5,355)} = 154.65$, $p < 0.001$, $\eta \rho^2 = 0.685$). Post hoc comparisons using Bonferroni correction indicated significant differences from the Control Condition in the first two trips in the Learning Condition in all experiments, while the third trip differed significantly from the Control Condition in two of the three experiments (Fig. 5). The 5ᵗʰ and 8ᵗʰ trips did not differ significantly from the Control Condition in any experiment.
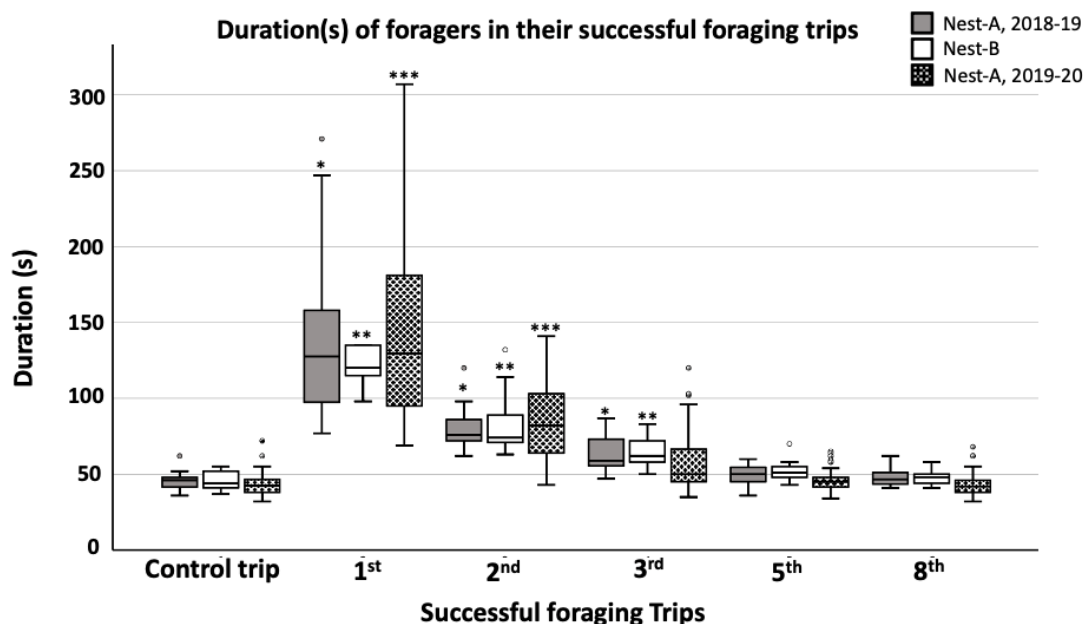
Fig. 5. The duration (up to 1.5 m height) on the ants' successful foraging trips in both Nest-A and Nest-B. The '*' indicates that the duration of 1st, 2nd and 3rd successful foraging trips were significantly different from the Control Condition in Nest-A 2018–2019 and the '**' indicated the significant differences in duration compared to the Control Condition in Nest-B. Similarly, '***' represents the significant differences of duration in their 1st and 2nd learning trips compared to the Control Condition in Nest-A 2019–2020.

Laterality sequence in detour learning

Foragers performed with individual side preferences during their successful learning trips. Only two out of 104 foragers modified their foraging direction and detoured both to the right and to the left sides. In both colonies and seasons, similar numbers detoured on the left and right sides. A binomial test showed the proportions of right and left detours do not differ significantly from the chance of expectation of 50:50 in any group (Table S2).
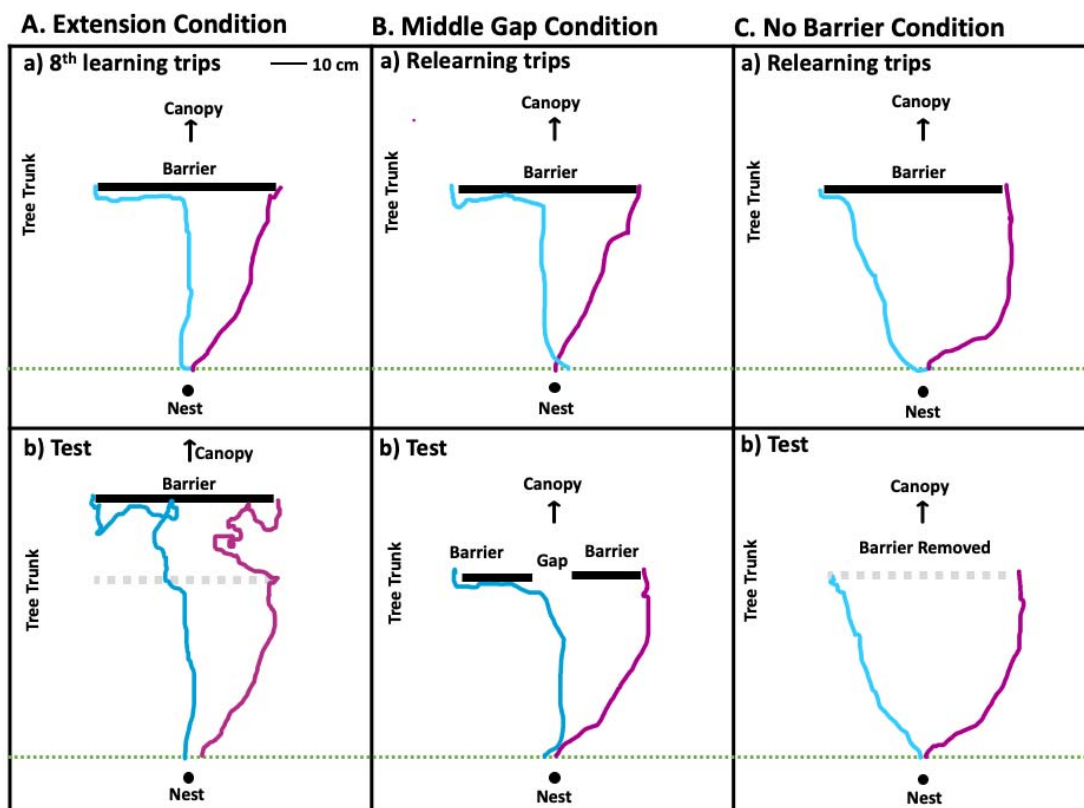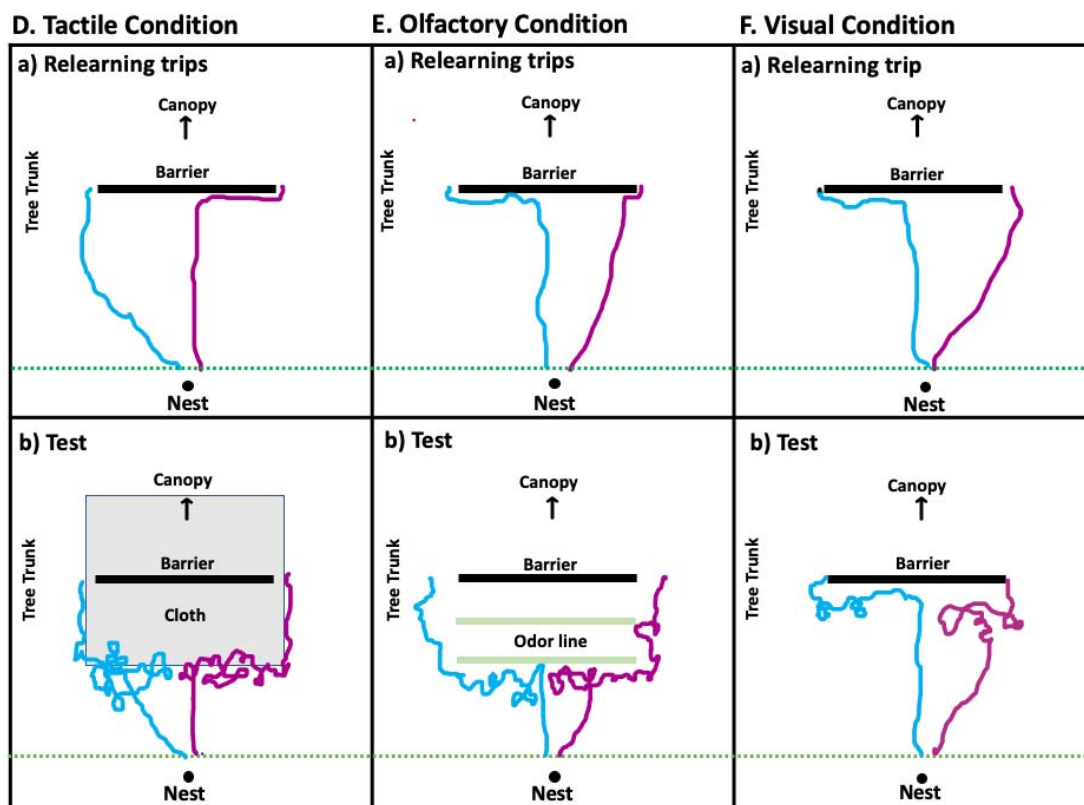
B. Experiment-2: Effect of Changes on Learning

Effects of barrier position on detour learning

Changing the barrier position (Extension Condition), adding a gap to the barrier (Middle Gap Condition), and removing the barrier (No Barrier Condition) did not greatly affect the paths of the ants. The ants took a similar path as in the previous experiment (Fig. 6A-C). Even with no barrier at all, the ants headed to the position of the edge of the barrier. Although path straightness (Fig. 7) and trip duration (Fig. 8) in the first test (Extension Condition) differed from the previous learning trip (path straightness, paired t test, $t(35) = 5.838$, $p < 0.001$; duration, paired t test, $t(35) = -3.468$, $p = 0.001$), these differences disappeared in the subsequent two tests, including the test with the drastic change of not having any barrier at all.

A. Extension Condition

a) 8th learning trips ── 10 cm

Canopy ↑
Barrier
Tree Trunk
Nest

b) Test ↑Canopy
Barrier
Tree Trunk
Nest

B. Middle Gap Condition

a) Relearning trips

Canopy ↑
Barrier
Tree Trunk
Nest

b) Test
Canopy ↑
Barrier   Gap   Barrier
Tree Trunk
Nest

C. No Barrier Condition

a) Relearning trips

Canopy ↑
Barrier
Tree Trunk
Nest

b) Test
Canopy ↑
Barrier Removed
Tree Trunk
Nest

345

D. Tactile Condition

a) Relearning trips

Canopy ↑
Barrier
Tree Trunk
Nest

b) Test
Canopy ↑
Barrier
Cloth
Tree Trunk
Nest

E. Olfactory Condition

a) Relearning trips

Canopy ↑
Barrier
Tree Trunk
Nest

b) Test
Canopy ↑
Barrier
Odor line
Tree Trunk
Nest

F. Visual Condition

a) Relearning trip

Canopy ↑
Barrier
Tree Trunk
Nest

b) Test
Canopy ↑
Barrier
Tree Trunk
Nest

346

347   **Fig. 6.** Exemplary paths of foragers in 6 different conditions with manipulations: (A)
348   Extension Condition, (B) Middle Gap Condition, (C) No Barrier Condition, (D) Tactile
349   Condition, (E) Olfactory Condition, and (F) Visual Condition. In each condition, (a)
350   represents foragers' habitual path after 8th learning trips or relearning trips in the presence of
351   barrier at 1m whereas (b) represents the ant's response to the particular Changes.
352

Effect of tactile changes on learning

354   When foragers encountered the tactile changes on their foraging tree trunk, they mostly
355   avoided the added cloth and moved around it (Fig. 6D). The ants stopped, scanned, and
356   searched for an alternative way to reach the tree canopy (figure S3). As a result, paths were
357   less straight (paired t test, $t(39) = 12.56$, $p < 0.001$) and of longer duration (paired t test, t
358    $(39) = -3.468$, $p < 0.001$) compared to the last relearning trip before the Tactile Condition
359   (Fig. 7 & Fig. 8).
360

Effect of olfactory changes on learning

362   In the Olfactory Condition, individual foragers similarly avoided the new odour and
363   attempted to move around it (Fig. 6E). Foragers stopped just before the first odour line,
364   meandered around the odour line, and then ascended the tree. As a result, trips were less
365   straight (paired t test, $t(25) = 9.05$, $p < 0.001$) and took more time (paired t test, $t(25) = -$
366   7.146, $p < 0.001$) compared to the relearning trips (Fig. 7 and 8).
367

Effect of visual changes on learning

369   Foragers were also affected in the Visual Condition. Paths appeared similar to the relearning
370   trials until the ants approached the cloths on the sides of the tree (figure 6F). Hesitancy,
371   meandering, and scanning then set in (Fig. 7 & Fig. 8). As a result, foragers were less straight
372   (paired t test, $t(35) = 4.306$, $p < 0.001$) and took more time (paired t test, $t(35) = -4.83$, $p =$
373   0.001) compared to the relearning trips. After passing halfway from the bottom of the tree
374   trunk to the barrier, foragers observed the visual changes. Most of the foragers stopped,
375   scanned, and then moved around the barrier to reach the canopy (Fig. 6 and Fig. S3).
376
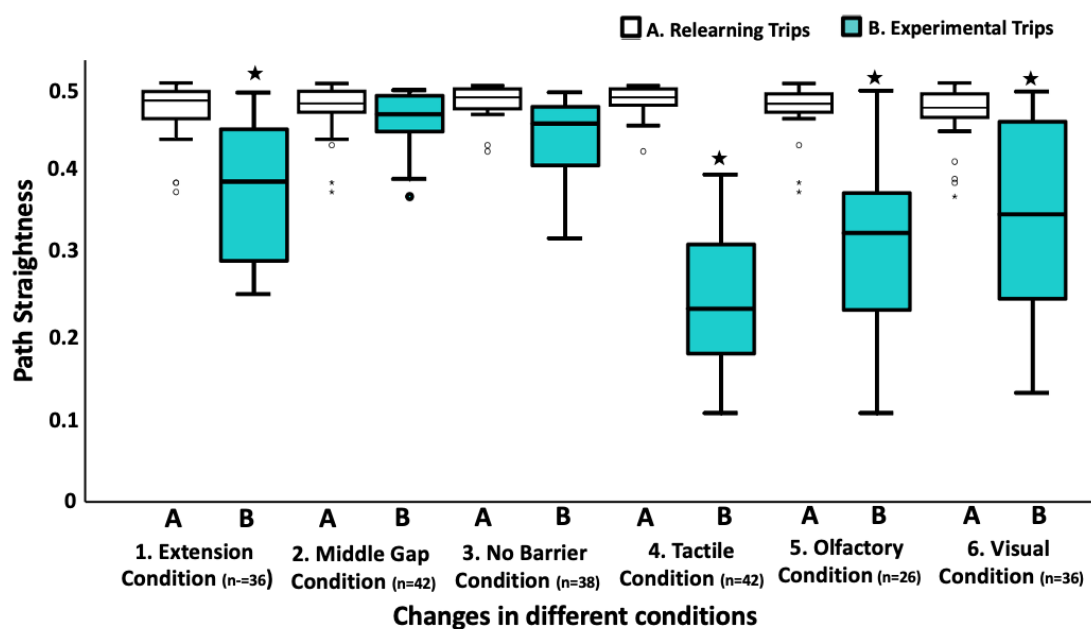
Fig. 7. The path straightness of the nocturnal bull ants in different experimental conditions. The '*' indicates that the path straightness of a condition was significantly different from the relearning trips of the same condition.
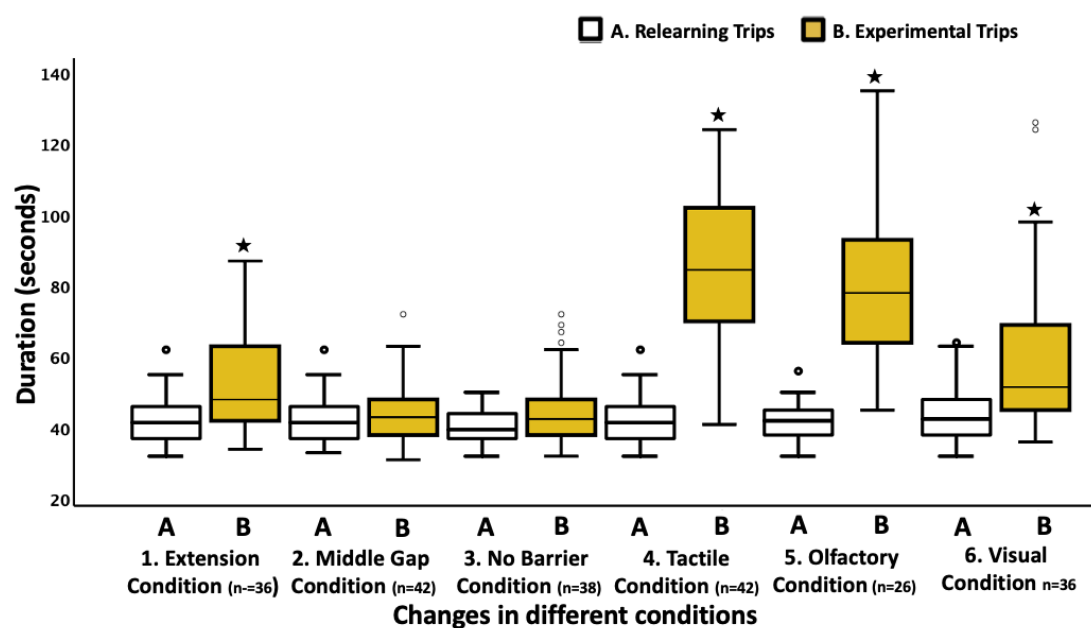
Fig. 8. The time taken to reach 1.5 m above the nest entrance of the nocturnal bull ants in different experimental conditions. The '*' indicates that the duration of a condition was significantly different compared to the relearning trips of the same condition.

DISCUSSION

Our findings demonstrate both how nocturnal bull ants learn to avoid obstacles in their environment and how they adapt their learned motor routine to novel sensory stimuli. When

389   the foragers confronted the barrier on their foraging corridor for the first time, almost half of
390   them failed to overcome the barrier. In subsequent trips, the foragers became more
391   successful. In subsequent detour trials, foragers became faster to pass the barrier and paths
392   became straighter. Some foragers modified their foraging paths quickly, even within two
393   trials, while other ants changed the paths more gradually over trips. When the position of the
394   barrier was changed or removed altogether, foragers predominantly showed their learned
395   motor routine, detouring with a similar path as before. We also found that when foragers
396   detected the tactile or olfactory changes, they immediately showed neophobic behaviour,
397   scanning and avoiding those novel cues. Visual changes on their learned route also adversely
398   affected their navigation and motor routine.

400   The tested ants improved from 54% success on their first detour trial to 98% success on their
401   8th. Studies on other animals have shown a similar progression in increased success rates in
402   multiple trials of detouring around a barrier on their way to a goal. In horses (*Equus*
403   *caballus*), domestic dogs (*Canis familiaris),* domestic chicks, mice and dingoes, more than
404   50% individuals were successful in their first attempt and the success rate improved over
405   trials (Pongrácz et al., 2001; Pongrácz et al., 2005; Baragli et al., 2011, Juszczak et al., 2016).
406   Many studies found improved detouring over trials (Pongrácz et al., 2001; Wallis et al. 2001;
407   Parker et al., 2005; Smith and Litchfield, 2010; Boogert et al., 2011; Anderson et al., 2016).
408   In our detour learning experiments, foragers became faster to pass the barrier and walked
409   straighter paths over several trials. Most of them veered left or right on the trunk well before
410   the barrier. Several studies have found that animals become faster in reaching the destination
411   around a barrier over several trials (Spigel, 1964; Vallortigara et al., 1988; Lockman et al.,
412   2001; Parker et al., 2005; Smith and Litchfield, 2010; Baragli et al., 2011; Juszczak et al.,
413   2016).

415   Our experiments found that ants maintained their newly learned route when the position of
416   the barrier was changed, or the barrier was removed altogether. The ants started at a similar
417   position at the bottom of the tree before and after the barrier was put in place, although with
418   the barrier, most ants headed to a different position on the tree, one edge of the barrier.
419   Retaining the learned new route suggests that foragers were not solely using cues of the
420   barrier to navigate around it. They could have used a combination of a local vector [67] from
421   the bottom of the tree to the edge of the barrier and using surrounding visual cues for route
422   guidance. Two desert ants, *Melophorus bagoti* and *Cataglyphis fortis*, also maintain their
423   newly learned route, which many ants learned after they encountered a pit trap on the direct
424   route home (Collett et al., 1998). The trap was not visible until the ants were right at the edge,
425   so that on the first run with the trap, homing ants fell into the trap. Ants that learned to detour
426   around the trap persisted in their newly learned routes even after the pit was removed.
427   Reinforcement learning was posited to play a key role in maintaining such learned routes
428   (Collett et al., 1998) and reinforcement learning likely played a role in maintaining the new
429   routes of the bull ants in this study as well.

431   When the nocturnal bull ants encountered olfactory and tactile changes on the tree trunk, the
432   navigation of the experienced foragers was profoundly affected. Ants avoided the changed

433   terrain. We interpret such behaviours tentatively as neophobia, an interpretation that requires
434   further study. Novel objects are commonly used to assess neophobia, generally by placing an
435   object near a familiar food source. Fear of novel environments (Mettke-Hofmann et al., 2009;
436   Cohen et al., 2015; Elvidge et al., 2016) and fear of novel objects (Greenberg, 1988; Mettke-
437   Hofmann et al., 2002; Moretti et al., 2015; Brown et al., 2016) produce a conflict between the
438   motivation to feed and the motivation to avoid the object. Trained desert ants, *Cataglyphis*
439   *fortis,* use odours from a food source or at the nest in navigation and searching and use the
440   olfactory cues as route guidance in the absence of other navigational information (Wolf and
441   Wehner, 2000; Buehlmann et al. 2015). Tactile learning experiments on *C. fortis* showed that
442   the ants use tactile cues of the ground structure (Seidl and Wehner, 2006). With a novel or
443   altered ground mark, however, these ants exhibited avoidance responses, likewise suggesting
444   neophobia.

446   As discussed, the tested ants were affected adversely when a part of the surrounding visual
447   scene was blocked. Changes of visual panorama may affect the navigation of ants, sometimes
448   severely, although their performance recovers within a few days or over several trials
449   (Graham and Cheng, 2009; Wystrach et al., 2011; Julle-Daniere et al., 2014; Narendra and
450   Ramirez-esquivel, 2017; Freas et al., 2018a; Freas and Cheng, 2019; Islam et al., 2020). Even
451   small visual changes such as the removal of three trees in a forest of trees may adversely
452   affect bull ants (Narendra and Ramirez-esquivel, 2017). The ants did not require the
453   surrounding visual cues to the sides of the tree as they still managed to negotiate the barrier
454   with those cues blocked, albeit with more meandering and scanning. Meandering and
455   scanning increase when ants encounter visual changes (Narendra and Ramirez-esquivel,
456   2017; Islam et al., 2020) and are interpreted as behaviours for learning the changed cues.
457   Thus, we do not interpret the ants' reactions as neophobia in this case.

459   Taken together, our results suggest that individual foragers learn a new route around a barrier
460   on the tree, gradually in most cases, which is indicative of spatial learning. Most of the ants
461   did not rely on barrier-based cues but rather used a local vector and probably surrounding
462   visual cues to detour. Our findings also suggest a large initial neophobic response to tactile
463   and olfactory changes on the route.

References

Anderson, A. V. J. and Kelly, D. C. D. M. (2016). Inhibition in Clark' s nutcrackers (Nucifraga columbiana): results of a detour-reaching test. *Anim. Cogn.* **19**, 661–665

Aguilar-Argüello, S., Gerhard, D. and Nelson, X. J. (2019). Risk assessment and the use of novel shortcuts in spatial detouring tasks in jumping spiders. *Behav. Ecol.* 1–11.

Baragli, P., Vitale, V., Sighieri, C., Lanata, A., Palagi, E. and Reddon, A. R. (2017). Consistency and flexibility in solving spatial tasks: Different horses show different cognitive styles. *Sci. Rep.* **7**, 1–12.

Baragli, P., Vitale, V., Paoletti, E., Sighieri, C. and Reddon, A. R. (2011). Detour behaviour in horses (Equus caballus). 227–234.

Bisazza, A., Pignatti, R. and Vallortigara, G. (1997). Laterality in detour behaviour: interspecific variation in poeciliid fish. 1273–1281.

Boogert, N. J., Anderson, R. C., Peters, S., Searcy, W. A. and Nowicki, S. (2011). Song repertoire size in male song sparrows correlates with detour reaching, but not with other cognitive measures. *Anim. Behav.* **81**, 1209–1216.

Brown, M. J. and Jones, D. N. (2016). Cautious Crows: Neophobia in Torresian Crows (Corvus orru) Compared with Three Other Corvoids in Suburban Australia. *Ethology* **122**, 726–733.

Buehlmann, C., Graham, P., Hansson, B. S. and Knaden, M. (2015). Desert ants use olfactory scenes for navigation. *Anim. Behav.* **106**, 99–105.

Buehlmann, C., Mangan, M. and Graham, P. (2020). Multimodal interactions in insect navigation. *Anim. Cogn*. **23,** 1129-1141

Cohen, S., Benjamini, Y. and Golani, I. (2015). Coping with Space Neophobia in Drosophila melanogaster: The Asymmetric Dynamics of Crossing a Doorway to the Untrodden. *PLoS One* **10**, 1–14.

Collett, M., Collett, T. S., Bisch, S. and Wehner, R. (1998). Local and global vectors in desert ant navigation. *Nature* **394**, 269–272.

Elvidge, C. K., Chuard, P. J. C. and Bbrown, G. E. (2016). Local predation risk shapes spatial and foraging neophobia patterns in Trinidadian guppies. *Curr. Zool.* **62**, 457–462.

Freas, C. A., Narendra, A. and Cheng, K. (2017a). Compass cues used by a nocturnal bull ant, *Myrmecia midas*. *J. Exp. Biol.* jeb.152967.

Freas, C. A., Narendra, A., Lemesle, C. and Cheng, K. (2017b). Polarized light use in the nocturnal bull ant, Myrmecia midas. *R. Soc. Open Sci.* **4**.

Freas, C. A. and Cheng, K. (2018). Landmark learning, cue conflict, and outbound view sequence in navigating desert ants. *J. Exp. Psychol. Anim. Learn. Cogn.* **44**, 409–421.

Freas, C. A., Wystrach, A., Narendra, A. and Cheng, K. (2018). The view from the trees: Nocturnal bull ants, Myrmecia midas, use the surrounding panorama while descending from trees. *Front. Psychol.* **9**, 1–15.

Freas, C. A. and Cheng, K. (2019). Panorama similarity and navigational knowledge in the nocturnal bull ant Myrmecia midas. *J. Exp. Biol.* **222**.

Graham, P. and Cheng, K. (2009). Ants use the panoramic skyline as a visual cue during navigation. *Curr. Biol.* **19**, R935–R937.

Greenberg, R. (1998). The role of neophobia and neophilia in the development of innovative behaviour of birds. *Book* **Chapter 8**.

Giurfa, M. (2015). Learning and cognition in insects. *Wiley Interdiscip. Rev. Cogn. Sci.* **6**,383–395.

Islam, M., Freas, C. A. and Cheng, K. (2020). Effect of large visual changes on the navigation of the nocturnal bull ant, Myrmecia midas. *Anim. Cogn.* **23,** 1071-1080

Julle-Daniere, E., Schultheiss, P., Wystrach, A., Schwarz, S., Nooten, S. S., Bibost, A. L. and Cheng, K. (2014). Visual matching in the orientation of desert ants (Melophorus bagoti): The effect of changing skyline height. *Ethology* **120**, 783–792.

Juszczak, G. R. and Miller, M. (2016). Detour behavior of mice trained with transparent, semitransparent and opaque barriers. *PLoS One* **11**, 1–23.

Kabadayi, C., Bobrowicz, K. and Osvath, M. (2018). The detour paradigm in animal cognition. *Anim. Cogn.* **21**, 21–35.

Kevan, H. B. M. G. J. M. P. G. (2015). Complex operant learning by worker bumblebees (Bombus impatiens): detour behaviour and use of colours as discriminative stimuli. *Insectes Soc.* **62**, 365–377.

Kohler, W. 1925 The mentality of apes Translated from the second revised edition by Ella Winter, BSc London: Kegan Paul, Trench. Trubner & Co, Ltd.

Langbein, J. (2018). Motor self-regulation in goats (Capra aegagrus hircus) in a detour-reaching task.

Lihoreau, M. and Schwarz, S. (2020). Insect Navigation. 1–8.

Lockman, J. J. and Adams, C. D. (2001). Going around transparent and grid-like barriers: Detour ability as a perception-action skill. *Dev. Sci.* **4**, 463–471.

Lucon-Xiccato, T., Gatto, E. and Bisazza, A. (2019). Male and female guppies differ in problem-solving abilities. *Curr. Zool.* 1–8.

545 **McCreery, H. F., Dix, Z. A., Breed, M. D. and Nagpal, R.** (2016). Collective strategy for
546   obstacle navigation during cooperative transport by ants. *J. Exp. Biol.* **219**, 3366–3375.

547 **Mettke-hofmann, C., Winkler, H. and Leisler, B.** (2002). The Significance of Ecological
548   Factors for Exploration and Neophobia in Parrots. **272**.

549 **Mettke-Hofmann, C., Lorentzen, S., Schlicht, E., Schneider, J. and Werner, F.** (2009).
550   Spatial neophilia and spatial neophobia in resident and migratory warblers (Sylvia).
551   *Ethology* **115**, 482–492.

552 **Moretti, L., Hentrup, M., Kotrschal, K. and Range, F.** (2015). The influence of
553   relationships on neophobia and exploration in wolves and dogs. *Anim. Behav.* **107**, 159–
554   173.

555 **Narendra, A. and Ramirez-esquivel, F.** (2017). Subtle changes in the landmark panorama
556   disrupt visual navigation in a nocturnal bull ant. *Philos. Trans. R. Soc. B Biol. Sci.* **372**,
557   20160068.

558 **Nawroth, C., Baciadonna, L. and Mcelligott, A. G.** (2016). Goats learn socially from
559   humans in a spatial problem-solving task. *Anim. Behav.* **121**, 123–129.

560 **Ong, M., Bulmer, M., Groening, J. and Srinivasan, M. V.** (2017). Obstacle traversal and
561   route choice in flying honeybees: Evidence for individual handedness. *PLoS One* **12**.

562 **Palavalli-Nettimi, R. and Narendra, A.** (2018). Miniaturisation decreases visual
563   navigational competence in ants. *J. Exp. Biol.* **221**, jeb177238.

564 **Parker, K. J., Buckmaster, C. L., Justus, K. R., Schatzberg, A. F. and Lyons, D. M.**
565   (2005). Mild early life stress enhances prefrontal-dependent response inhibition in
566   monkeys. *Biol. Psychiatry* **57**, 848–855.

567 **Pongrácz, P., Miklósi, Á., Kubinyi, E., Gurobi, K., Topál, J. and Csányi, V.** (2001).
568   Social learning in dogs: The effect of a human demonstrator on the performance of dogs
569   in a detour task. *Anim. Behav.* **62**, 1109–1117.

570 **Pongrácz, P., Miklósi, Á., Vida, V. and Csányi, V.** (2005). The pet dogs ability for learning
571   from a human demonstrator in a detour task is independent from the breed and age.
572   *Appl. Anim. Behav. Sci.* **90**, 309–323.

573 **Tarsitano, M. S. and Jackson, R. R.** (1997). Araneophagic jumping spiders discriminate
574   between detour routes that do and do not lead to prey. 257–266.

575 **Santer, R. D. and Hebets, E. A.** (2009). Tactile learning by a whip spider, Phrynus
576   marginemaculatus C. L. Koch (Arachnida, Amblypygi). 393–399.

577 **Schmidt, I., Collett, T. S., Dillier, F. X. and Wehner, R.** (1992). How desert ants cope with
578   enforced detours on their way home. *J. Comp. Physiol. A* **171**, 285–288.

579 **Seidl, T. and Wehner, R.** (2006). Visual and tactile learning of ground structures in desert
580   ants. **4**, 3336–3344.

581 **Siniscalchi, M., Pergola, G. and Quaranta, A.** (2013). Detour behaviour in attack-trained
582   dogs: Left-turners perform better than right-turners. *Laterality* **18**, 282–293.

583 **Smith, B. P. and Litchfield, C. A.** (2010). How well do dingoes, Canis dingo, perform on
584   the detour task? *Anim. Behav.* **80**, 155–162.

585 **Sovrano, V. A., Baratti, G. and Potrich, D.** (2018). A detour task in four species of fishes.
586   *Front. Psychol.* **9**, 1–7.

587 **Spigel, I. M.** (1964). Learning, retention, and disruption of detour behavior in the turtle. *J.*
588   *Comp. Physiol. Psychol.* **57**, 108–112.

589 **Vallortigara, G., Regolin, L. and Pagni, P.** (1999). Detour behaviour, imprinting and visual
590   lateralization in the domestic chick. 307–320.

591 **Wallis, J. D., Dias, R., Robbins, T. W. and Roberts, A. C.** (2001). Dissociable
592   contributions of the orbitofrontal and lateral prefrontal cortex of the marmoset to
593   performance on a detour reaching task. *Eur. J. Neurosci.* **13**, 1797–1808.

594 **Wolf H, Wehner R.** 2000 Pinpointing food sources: Olfactory and anemotactic orientation in
595   desert ants, Cataglyphis fortis. *J. Exp. Biol.* **203**, 857–868

596 **Wynne, C. D. L. and Leguet, B.** (2004). Detour behavior in the Quokka (Setonix
597   brachyurus). **67**, 281–286.

598 **Wystrach A, Beugnon G, Cheng K.** 2011 Landmarks or panoramas: what do navigating
599   ants attend to for guidance? *Front. Zool.* **8**, 21

600 **Wystrach, A., Buehlmann, C., Schwarz, S., Cheng, K. and Graham, P.** (2020). Rapid
601   Aversive and Memory Trace Learning during Route Navigation in Desert Ants. *Curr.*
602   *Biol.* **30**, 1927-1933.e2.

603 **Zucca, P., Antonelli, F. and Vallortigara, G.** (2005). Detour behaviour in three species of
604   birds: quails (Coturnix sp .), herring gulls (Larus cachinnans) and canaries (Serinus
605   canaria). *Anim. Cogn.* **8**, 122–128.