

1 **Aversive View Memory and Navigational Risk Sensitivity in the Desert Ant,**
2 *Cataglyphis Velox*

3
4 Cody A Freas¹, Antoine Wystrach², Sebastian Schwarz², Marcia L Spetch¹

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6 ¹Department of Psychology, University of Alberta, Edmonton, Alberta, Canada

7 ²Research Center on Animal Cognition (CRCA), Center of Integrative Biology (CBI), CNRS -
8 University of Toulouse; CNRS, UPS, 31067 Toulouse, France

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11
12
13
14 Address for correspondence:

15 Cody A Freas

16 Department of Psychology

17 University of Alberta

18 Edmonton, Alberta T6G 2R3 Canada

19 Email: freascody@gmail.com

31 **Abstract**

32 Many ants establish foraging routes through learning views of the visual panorama. Route
33 models have focused primarily on attractive view use, which experienced foragers orient towards
34 to return to known sites. However, aversive views have recently been uncovered as a key
35 component of route learning. Here, *Cataglyphis velox* rapidly learned aversive views, when
36 associated with a negative outcome, a period of captivity in brush, triggering an increase in
37 hesitation behavior. These memories were based on the accumulation of experiences over
38 multiple trips with each new experience regulating forager's hesitancy. Foragers were also
39 sensitive to captivity time differences, suggesting they possess some mechanism to quantify
40 duration. Finally, we analyzed foragers' perception of risky (i.e. variable) versus stable aversive
41 outcomes by associating two sites along the route with distinct captivity schedules, a fixed or
42 variable duration, with the same mean across training. Foragers exhibited significantly less
43 hesitation to the risky outcome compared to the fixed, indicating they perceived risky outcomes
44 as less severe. Results align with a logarithmic relationship between captivity duration and
45 hesitations, suggesting that aversive stimulus perception is a logarithm of its actual value. We
46 conclude by characterizing how these behaviors can be executed within the mushroom bodies'
47 neural circuitry.

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65 **Introduction**

66 The navigational abilities of solitarily foraging ants can be attributed to a toolkit comprised of
67 multiple strategies (Wehner et al. 1996; Collett et al. 2013; Cheng et al. 2014; Freas and
68 Schultheiss 2018). The most well studied components of this toolkit are the path integration (PI)
69 system and learned visual cues of the panorama. The PI system is informed via the celestial
70 compass and a step-counter, creating a vector that continuously updates an estimate of the nest
71 location during the foraging trip (Wehner 2003, 2008). PI can be especially useful for foragers
72 when their environment lacks prominent terrestrial cues (Wehner 2020) or during a naïve
73 forager's first few foraging trips as they learn the surrounding terrestrial cues and form foraging
74 routes (Kohler and Wehner 2005; Muller and Wehner 2010; Mangan and Webb 2012; Schwarz
75 et al. 2017).

76 *View learning*

77 When available, many ant species rapidly learn visual landmark information to navigate.
78 Rather than attend to individual landmarks, foragers learn panoramic views around goal
79 locations and along their foraging routes (Graham and Cheng 2009; Wystrach et al. 2011a).
80 View learning first occurs around the nest before the onset of foraging, during learning walks
81 (Wehner et al. 2004; Zeil and Fleischmann 2019) Foragers also acquire views en-route as they
82 move away from known locations (Graham and Cheng 2009; Wystrach et al. 2011a; Schultheiss
83 et al. 2016; Freas et al. 2018), and can rapidly learn the panorama at a new site, often after only
84 one previous experience (Freas and Cheng 2018; Freas and Spetch 2019). Foragers retain long
85 term-memories of these panoramas (Narendra et al. 2007) and, while navigating, compare these
86 memories to their current view to recover their goal direction (Zeil 2012).

87 *Aversive views*

88 View memories and their importance in route following have been well modeled (Zeil et
89 al. 2003; Wystrach et al. 2011b; Baddeley et al. 2012; Kodzhabashev and Mangan 2015; Möller
90 2012), yet these models rely principally on the forager orienting towards attractive views via
91 view comparison. Recent work has expanded this modelling to include the use of learned views
92 that are repellant and cause foragers to turn away from views not associated with the current
93 goal, resulting in orientation away from incorrect directions (Le Möel and Wystrach 2020;
94 Murray et al. 2020). The interaction between these learned attractive and repellant views permits
95 navigators to compare a single current view to their view memories to quickly decide whether to
96 move toward or turn away from a given direction (Le Möel and Wystrach 2020, Murray et al.
97 2020). It has been suggested that learned views can become repellant depending on their
98 orientation relative to the nest (Jayatikala et al. 2017; Murray et al. 2020), on the foraging
99 motivational context (Schwarz et al. 2020) or when these views are associated with aversive
100 outcomes (Wystrach et al. 2020). Previously attractive views can also become aversive when
101 they are associated with negative outcomes. In both *Cataglyphis* and *Melophorus* desert ants,
102 when a pit trap was added along a forager's homeward route, resulting in foragers falling into
103 brush, ants quickly memorized the views experienced just before this negative experience as

104 repulsive. Eventually, after a few experiences falling into this pit, foragers formed new routes
105 detouring around it. The interplay of aversive and attractive views appears to facilitate the
106 formation of these detours. As foragers attempt to avoid aversive views, novel views that pilot
107 around these areas become positively reinforced, leading to the development of new routes
108 detouring around obstacles and areas with difficult terrain (Wystrach et al. 2020). Vegetation can
109 often be hard for desert ants to move through effectively, especially when carrying food,
110 resulting in increases to both expenditure of effort and the delay to return to the nest. After only a
111 few trips experiencing the pit, foragers began to hesitate near the pit's edge, increasing their
112 hesitancy to pass through the area, evidenced through increases in both scanning behavior and
113 path meander (Wystrach et al. 2020). Scanning behavior consists of a forager stopping forward
114 movement and rotating their body on the spot. This behavior is associated with instances of
115 increased navigational uncertainty: when the familiarity of the panorama decreases, the PI and
116 panorama enter into conflict, or when the current route's panorama is associated with failure
117 (Wystrach et al. 2014, 2019). Thus, the incidence of scanning is a good behavioral proxy to
118 assess the ant's uncertainty and in this study we used it to quantify the strength of the aversion
119 associated with a given location (Schwarz. et al. 2020).

120 *Risk perception*

121 While navigating, foraging animals must make decisions assessing risky or safe options both in
122 the resources they collect and in their foraging routes. Based on predictions within evolutionary
123 theory, individuals were conventionally thought to make foraging choices that were strictly
124 optimal, maximizing their net energy gains to increase fitness (MacArthur and Pianka 1966;
125 Krebs 1986). However, humans and other animals sometimes behave in seemingly non-optimal
126 or irrational ways with regards to their perception and preference for risk (Kahneman and
127 Tversky 1979; Kacelnik and Bateson 1996). For example, when risk is generated by variability
128 in amount, animals are often risk-averse or risk neutral, whereas when risk is generated by
129 variability in delay, animals are typically risk-prone (e.g., Kacelnik and Bateson 1996; Kacelnik
130 and Abreu 1998). Such irrational preferences are believed to flow from animals' perception of
131 the world, where true stimulus strength has a logarithmic relationship with the animal's
132 perception (Fechner 1860; Bruce and Johnson 1996; Kacelnik and Abreu 1998; Stevens and
133 Marks 2017). Based on this principle, animals' choices between risky (variable) and fixed
134 outcomes should be predicted not by the mean value of these options but instead by their
135 geometric means.

136 Risk perception and preference have been studied across a range of animals (for review
137 see: Kacelnik and Bateson 1996; Kacelnik and El Mouden 2013). Much of the risk preference
138 research in Hymenoptera has focused on foragers' preference for risk solely in regards to the
139 amount or quality of a given reward (Kacelnik and Bateson 1996; Hübner and Czaczkes 2017;
140 De Agrò et al. 2021). In honeybees and bumble bees, a variety of outcomes have been reported
141 with foragers showing evidence of no preference, risk avoidance and risk seeking foraging
142 choices based on factors such as colony resource levels (Waddington et al. 1981; Cartar 1991;

143 Perez and Waddington 1996; Fülöp and Menzel 2000). In ants, risk perception and sensitivity
144 have been explored on the colony level, focusing on how collective decision-making influences
145 choice in the assessment of potential nesting site quality and in food reward quality. Rock ants
146 (*Temnothorax albipennis*) were shown to exhibit risk seeking behavioral choices when making
147 collective choices between nests (Burns et al. 2016). The collective decision-making of the
148 colony has also been shown to result in the avoidance of certain irrational choice behaviors
149 observed in individual ants, including reducing the time to choose between potential nest sites
150 (Sasaki et al. 2018, 2019). Recently, De Agrò and colleagues (2021) showed that ant foragers’
151 perceptions of food reward quality are based on the two reward options’ logarithmic values.
152 Individuals were shown to be risk adverse when choosing between food rewards with the same
153 mean values, preferring the fixed option, however this preference disappeared when ants were
154 presented two logarithmically balanced alternatives (De Agrò et al. 2021).

155 In the current study, we characterized foragers’ learning and memories of aversive views
156 when these views are associated with aversive, high effort outcomes, i.e. being kept within a
157 brush-filled phial for set time periods. Forcing foragers into brush simulates areas along the
158 homeward route that contain dense clutter, compelling the forager to struggle through in order to
159 reach the nest with its food piece, increasing both their time and energy expenditure. Foragers’
160 behaviors were recorded using a trial-by-trial approach to describe navigational learning during
161 natural tasks (Freas et al. 2019). We first studied the dynamics of view learning, as well as
162 retention across non-reinforced trials. Second, we explored foragers’ perception of captivity
163 duration by training foragers to associate sites along the route with two distinct fixed time
164 periods within the brush (15s vs. 300s). Finally, we characterized foragers’ perception of risk
165 when sites were associated with ‘Fixed’ or ‘Risky’ outcomes with the same mean duration across
166 training (~150s). Here, the ‘Fixed’ outcome was associated with a constant period within the
167 brush (150s) while the ‘Risky’ outcome was associated with a variable time period where
168 foragers had a 50/50 chance on each trip of being held within the brush for either 1s or 300s. We
169 found that *C. velox* foragers rapidly learn to associate the (previously positive) homeward route
170 views with aversive outcomes, with as few as two prior experiences. These aversive view
171 memories persist over multiple trips after the outcome is removed. Foragers were able to
172 perceive differences in outcome severity, learning more rapidly and exhibiting more overall
173 hesitations to views associated with more severe outcomes (300s) compared to less severe
174 outcomes (15s). Finally, we show that foragers show significantly less apprehension to travel
175 through sites associated with risky aversive outcomes compared to a fixed outcome with the
176 same mean. The observed forager hesitation responses at these sites are in line with the
177 perception of stimulus strength associated with their geometric averages.

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182 **Methods**

183 *Study site and species*

184 Testing was conducted in June and July 2019 on a single *C. velox* nest located at an established
185 field site ~6 km south of Seville, Spain (37°19'51"N, 5°59'23"W). *C. velox* inhabit visually
186 cluttered semi-arid environments, densely covered in grass tussocks, scattered bushes and with
187 distant stands of trees and man-made structures. While navigating, these foragers rely heavily on
188 these visual cues to return to the nest and known food sites, creating stable routes between
189 locations (Mangan and Webb 2012; Wystrach et al. 2015; Schwarz et al. 2017).

190 *Testing arena*

191 A plastic square-shaped feeder (15cm × 15cm × 8cm) was sunk into the ground 12m from the
192 nest entrance and was continuously stocked with crushed cookie pieces (Royal DanskTM). The
193 smooth walls of the feeder prevented foragers that dropped in from exiting without being lifted
194 out by the researcher. All vegetation in a 2m wide band from the nest to the feeder and in a 1m
195 radius around both sites was removed using an edge trimmer. To entice foragers to collect food
196 only from the feeder, an arena was erected using a 10cm high smooth plastic barrier, enclosing
197 the nest and feeder site and restricting the nest to forage only within the arena. This arena was
198 1.5m in width and extended in a 75cm radius semi-circle around both feeder and nest (Fig. 1a).
199 Two collection sites along the feeder-nest route were designated at 8m and 6m (Site 1 and Site 2
200 respectively) from the nest. To record inbound forager behaviour leading up to each site, two
201 grids consisting of a 2 × 2 of 50cm squares were erected using string and metal pegs extending
202 from each collection site 1m towards the feeder (ending at 9m for Site 1 and 7m for Site 2; Fig.
203 1a,b). Two sets of barriers were erected at 45deg angles creating a ~20cm gap at the edge of the
204 grid to funnel foragers toward the centre of the arena's width (Fig. 1a,b). To create two distinct
205 panoramic scenes, at Site 1 the first set of erected barriers were 10cm high plastic walls identical
206 to the walls of the arena, while the second set of barriers leading to Site 2 were 1.2m high (Fig.
207 1c). Additionally, to increase the panorama differences between sites, we placed a number of
208 shorter 15–25cm visual landmarks consisting of stones and bricks around Site 2 (See Fig. 1c).
209 This arena set-up was used for all three experiments.

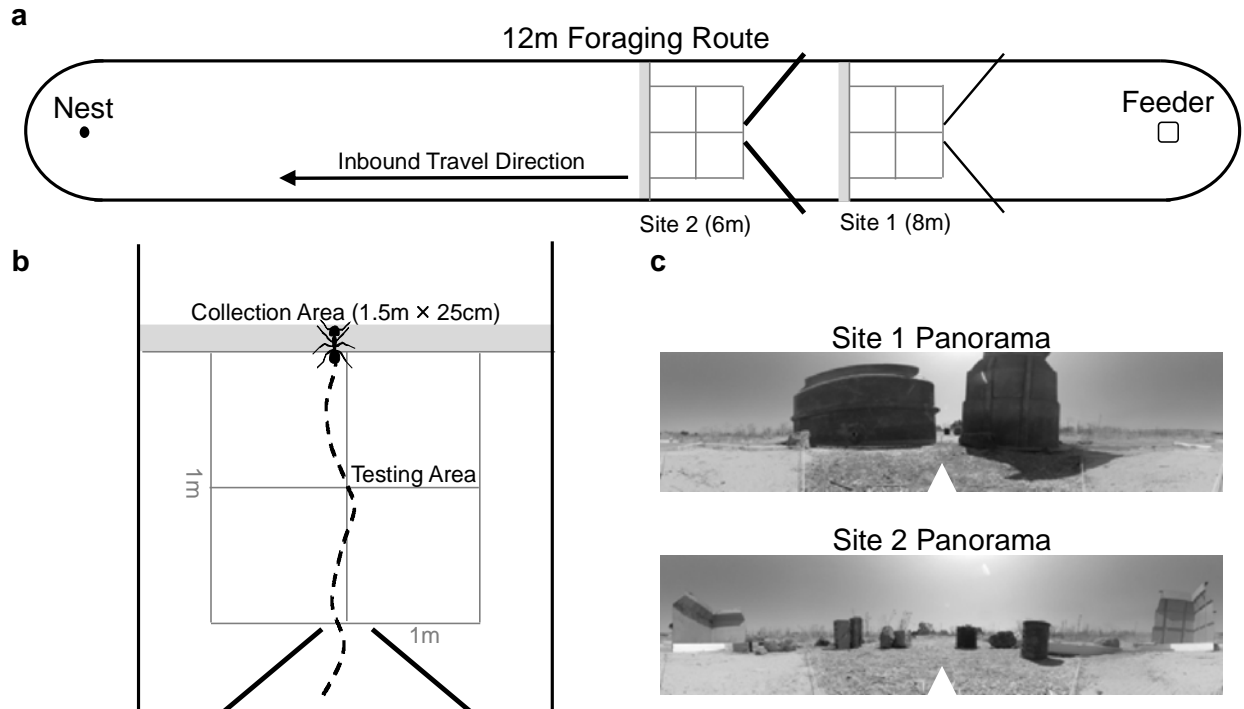
210 Upon the completion of the foraging arena construction, we allowed the nest two days to
211 discover the feeder and begin consistently foraging. For these two days, any foragers reaching
212 the feeder were allowed to enter and exit via a wooden ramp. At the onset of training this ramp
213 was removed. As foraging began on the third day, it was expected foragers used in the
214 experiments had some level of knowledge of the route before the onset of training and had
215 learned the positive association between the views of the route and successful foraging trips.
216 While the exact level of experience with the route prior to training may have individually varied,
217 forager experience of the route during training was strictly controlled. When a researcher was not
218 present to conduct training/testing, all foragers were restricted to a 20cm area around the nest
219 using a plastic cylinder (~20cm in height).

220 *Procedure*

221 *Aversive learning tests*

222 We initially tested foragers' learning of aversive view memories by collecting inbound foragers
223 as they reached Site 2 (Fig. 1a, Fig. 2). When approaching a view that has been associated with a
224 negative outcome, foragers have been shown to hesitate leading up to the site, exhibiting bouts of
225 scanning behaviour as well as attempting to avoid these sites via detours (Wystrach et al. 2020).
226 In the current study, we collected two types of hesitation behaviour, scans and stops. Scans were
227 defined as the ceasing of forager movement that was accompanied by the forager clearly turning
228 on the spot, rotating in place. In contrast, stops were cataloged as the ceasing of forward
229 movement with no accompanying rotation.

230 During training, each forager ($n = 16$) was exposed to ten consecutive training trips,
231 followed by five tests (Fig. 2). At the onset of training, foragers were allowed to travel freely
232 from the nest to the feeder. Once in the feeder, foragers were individually marked using acrylic
233 paint (Tamiya™), returned to the feeder and allowed to collect a cookie piece. After collecting
234 their food piece, foragers were lifted out of the feeder by hand and allowed to travel through the
235 arena to the nest. As foragers neared the grid at Site 2, their paths were recorded using an HD
236 camera at 60 fps with a 3840×2160 pixels image size (GoPro™) positioned 1.2m above the grid
237 facing down. Recording started just before the forager entered the grid area and ceased at
238 collection. As the forager exited the grid (6m from the nest entrance), they were exposed to an
239 aversive outcome in which they were held captive in a brush-filled phial. Specifically, foragers
240 were collected within a 25cm area (grey area, Fig. 1a,b) using an opaque plastic 5cm diameter
241 vial, which was filled with ~10cm of loosely packed grass brush from the surrounding
242 vegetation, and held within this brush for a period of 300s. During this holding period, the vial
243 was covered and placed in a semi-shaded area to prevent overheating. Additionally, the lid of
244 this vial rested lightly upon the top of the brush, preventing foragers from standing on top of the
245 brush during their hold period. After 300s, the vial was placed at the center of Site 2's collection
246 area (grey area, Fig. 1b) and tilted $\sim 75^\circ$ with the opening facing the nest to allow the forager to
247 climb out of the brush and back onto the foraging route and resume navigating. This procedure
248 was repeated for training Trips 2–10.



249
250 **Fig. 1.** Diagram of the experimental set-up in all conditions. (a) Foragers were allowed to travel freely on the
251 outbound trip to the feeder and collect food. After their release from the feeder, foragers travelled back to the nest
252 through the testing areas at Site 1 and Site 2 and were collected based on the condition. (b) During collection
253 conditions, foragers were collected within a 25cm (grey) area after passing 8m (Site 1) or 6m (Site 2) from the nest
254 to allow the collector to remain as far back as possible before collection. After the allotted hold period, the collection
255 phial was placed at the centre of the collection area and foragers were allowed to climb out and resume their
256 homeward trip. The testing areas were arranged with blocking walls to both funnel foragers to the centre of the arena
257 as they reached each testing area as well as to create distinct visual panoramas at each site. (c) Panoramic 360°
258 photos of the surrounding visual cues at each collection site. In each photo, the arrow denotes the nest direction.

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260 On the forager's eleventh trip to the feeder (Test 1), individuals were released from the
261 feeder and allowed to travel the full inbound route to the nest without being collected and held
262 (i.e. in the absence of the aversive outcome). As in training, foragers were recorded at Site 2
263 beginning just before they entered the grid. As there was no collection during testing trips,
264 recording ceased once foragers reached 25cm past the grid at the end of the collection area. All
265 of these foragers reached the nest and freely entered. On their next foraging trip, foragers were
266 tested twice. These inbound foragers were recorded and allowed to pass through Site 2 (Test 2),
267 identically to the previous test, until they reached the nest entrance. As foragers reached within
268 20cm of the nest entrance (with their path integrator now near zero, termed Zero Vector, ZV)
269 each forager was collected using an empty vial and returned to the foraging route 10m from the
270 nest (Fig. 1a). These foragers were allowed to resume their nest-ward journey and were recorded
271 while passing through Site 2 identically to previous tests (Test 3 ZV) without the corresponding
272 vector state present during training at these sites. Returning foragers collected at the nest are
273 described as zero-vector as their path integrator (PI) no longer provides directional information

274 to the nest, however note that the PI system is constantly in use and foragers in this test are still
 275 accumulating PI information. Importantly the foragers' PI states during zero vector testing do not
 276 align with their PI states at the sites while training. During foragers' next two foraging trips to
 277 the feeder their homeward journeys at Site 2 were recorded (Test 4 and Test 5) identically to Test
 278 1. After Test 5 all testing on the individual ceased and foragers were collected at the nest,
 279 marked as tested and then released.

Aversive View Learning and Retention Tests

Training Phase		Testing Phase	
Trip 1-10 16 Foragers - 300s	Test 1 & 2 No collection	Test 3 - Zero Vector No collection	Test 4 & 5 No collection

Hold Duration Tests

Training Phase	Testing Phase
Trip 1-10 14 Foragers - 300s Site 1/ 15s Site 2 14 Foragers - 15s Site 1/ 300s Site 2	Test 1 - Zero Vector No collection at Site 1 & 2

Risky Sensitivity Tests

Training Phase	Testing Phase
Trip 1-20 8 Foragers - 300s or 1s Site 1/ 150s Site 2 7 Foragers - 150s Site 1/ 300s or 1s Site 2	Test 1 - Zero Vector No collection at Site 1 & 2

280

281 **Fig. 2.** Timeline of training and testing procedures in all conditions.

282

283 *Hold duration tests*

284 Next, we characterized whether foragers perceived differences in severity of aversive outcomes
 285 and responded differently to the associated views. Here, foragers (n = 14) were exposed to ten
 286 consecutive training trips where Site 1 was associated with a hold period of 300s while Site 2
 287 was associated with a hold period of 15s (Fig. 2). A mirrored condition (Site 1 – 15s, Site 2 –
 288 300s), was conducted on a second set of foragers (n = 14). Foragers were individually marked at
 289 the feeder and then released once they collected a food piece. During training (Trips 1–10), as
 290 foragers neared the grid at Site 1, they were recorded using the HD camera beginning just before
 291 the forager entered the grid area. As the forager exited the grid (8m from the nest entrance),
 292 recording ceased and they were collected within a 25cm area past the grid (grey area, Fig. 1a,b).
 293 Foragers were collected and held individually within the brush filled vial for 300s or 15 s
 294 (depending on condition) and then released back at the center of Site 1, using the procedure
 295 described in the previous experiment. After release, foragers were allowed to travel to Site 2
 296 where they were again recorded within the grid at this site, then foragers were collected and held
 297 within the brush-filled vial upon exiting the grid (grey area, Fig. 1b). At Site 2, foragers were
 298 held for the other hold time before being released and then allowed to travel back to the nest with

299 their food piece. On Trip 10, after release from Site 2, foragers were collected for testing as they
300 reached the nest. These foragers were collected with no remaining vector (< 20cm from the nest)
301 using an empty vial and immediately released along the route 10m from the nest (Fig. 2).
302 Released foragers were allowed to return to the nest and were recorded while passing through the
303 grid at both Site 1 and Site 2 without the corresponding vector state present during training at
304 these sites. After testing, foragers were collected, marked as completed and released at the nest.

305 *Risk sensitivity tests*

306 In the final group of tests, we characterized foragers' perceptions of fixed and risky aversive
307 outcomes over 20 foraging trips. Here, for one set of foragers (n = 8), Site 1 was associated with
308 a fixed aversive outcome, being held in brush for a period of 150s on every training trip, while
309 Site 2 was associated with a variable outcome, with a 50/50 chance of being held for a longer
310 (300s) or shorter (1s) period. Given the short period within the brush during the 1s hold time,
311 special care was taken to confirm that this hold period did not start until foragers came in contact
312 with the brush. A mirrored condition was conducted on a second group of foragers (n = 7) with
313 these hold periods switched (Site 1 – 50/50 chance of a 300s or 1s hold period; Site 2 – 150s hold
314 period). Foragers were individually marked as they reached the feeder and then allowed to
315 collect food and return towards the nest. At Site 1, foragers were recorded as they entered the
316 grid then collected and held identically to previous conditions. After the designated holding
317 period, foragers were released and allowed to travel to Site 2 where they were recorded as they
318 entered the grid and then collected and held. This training occurred for 20 trips. After the Site 2
319 release on Trip 20, foragers were tested by being collected with a zero-vector state as they
320 reached the nest, released at 10m from the nest and their return trip through Site 1 and Site 2 was
321 recorded without collection.

322 *Data digitization and analysis*

323 Videos were digitized using GraphClick (Arizona Software). Paths were digitized by marking
324 the ant's mesosoma at 200ms intervals beginning when the forager entered the grid and ceasing
325 once foragers were collected during training or when they reached 25cm past the grid edge
326 during testing. Aversive view learning and memory were assessed by recording the number of
327 hesitations exhibited by the forager leading up to collection. Two types of hesitation behavior
328 were observed during testing, scans and stops and these were confirmed during video playback.
329 We classified a 'stop' as a ceasing of forward movement with the ant remaining stationary until
330 forward movement resumed. In contrast, 'scans' were classified as the ceasing of forward
331 movement that is accompanied by the ant pirouetting, or rotating in place before resuming
332 forward movement. Both of these behaviors were collected by the experimenter during the
333 experiment and both positioning and behavior type was confirmed using video analysis. The
334 quantity and position of both behaviors were recorded along the forager's digitized paths. For
335 statistical analysis, scan and stop behaviors were combined to create a total hesitation count for
336 each training and test trip.

337 In the Aversive Learning tests, we compared hesitation numbers (Stops + Scans) across
338 training/testing trips using a General Linear Model (GLM) for count data (Poisson loglinear)
339 with Individual ants as a random effect. In the Hold Duration and Risk Perception tests, where
340 foragers were collected for distinct hold periods at both Site 1 and Site 2, both *Site Number* and
341 *Hold Condition* were analyzed as fixed effects. Post hoc pairwise comparisons of forager
342 hesitations during the baseline during Trip 1 and after training/testing were conducted using p
343 values corrected with the Bonferroni method. Within individual comparisons between hold
344 regimes in the *Hold Condition* (15s v. 300s) and the *Risk Sensitivity* (Fixed vs. Risky) tests were
345 compared using Wilcoxon Signed Rank Tests with p-values corrected with the Bonferroni
346 method.

347 To further characterize the change in hesitation numbers at the Risky site, we calculated
348 the change in hesitation (current hesitations minus hesitations on previous trip) number based on
349 the outcome of the previous trip (held for 1s or 300s). For each individual forager, the mean
350 hesitation change (excluding Trip 1) after a 300s hold time was compared to the mean hesitation
351 change after a 1s hold time using a Wilcoxon Signed Rank Test.

352 For between test comparisons we chose to focus on forager hesitation numbers during
353 training Trip 10 as, up to this point, the training schedule for each individual forager was
354 consistent across all testing. Across testing, we compared the fixed and risky site hesitation
355 numbers to those of the 15s site and 300s hold conditions using Mann-Whitney U tests. Finally,
356 we further analyzed hesitations by calculating the predicted hesitation numbers along a
357 logarithmic curve calibrated by the observed hesitations in the 15s, 150s (Fixed condition), and
358 300s conditions on Trip 10. We then compared the observed hesitations during Trip 10 of the
359 Risky condition ($\mu = 3.33$) to the predicted hesitations based on the conditions arithmetic
360 (150.5s) and geometric (17.32s) hold times using one sample T-tests.

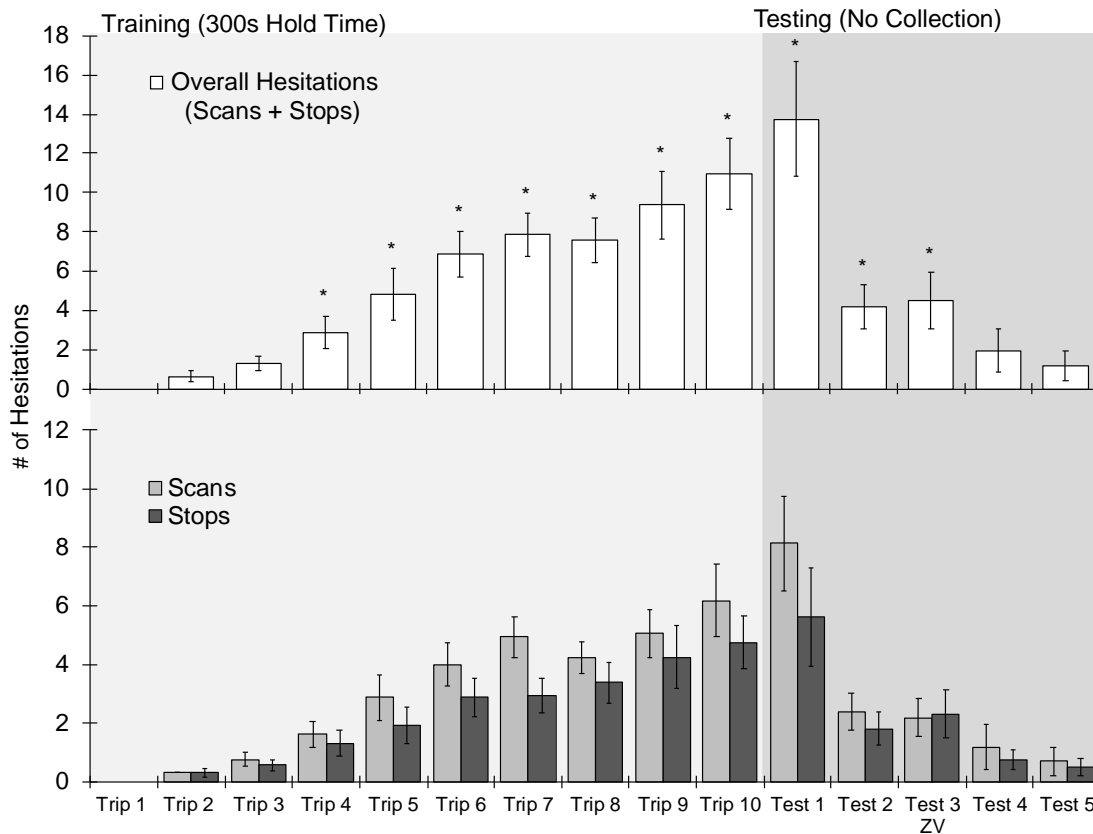
361

362 **Results**

363 *Aversive learning tests*

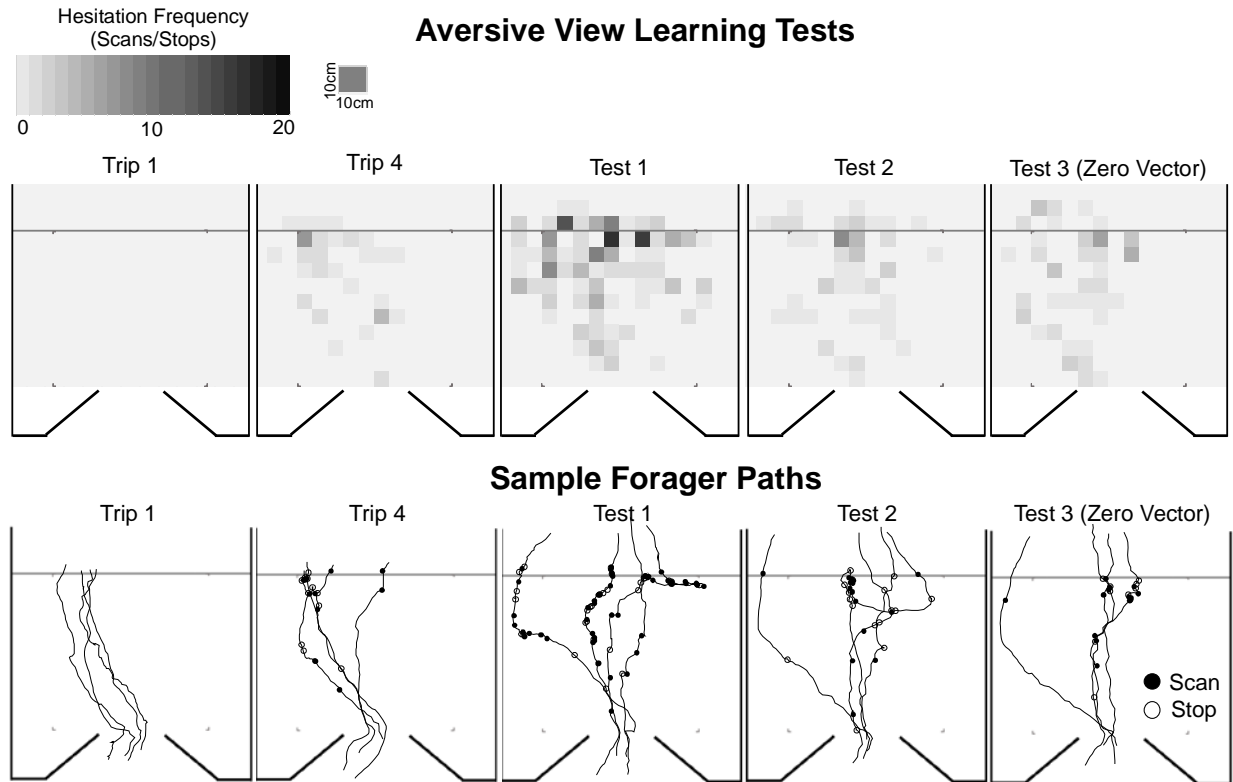
364 Foragers traveling through Site 2 at the onset of training (Trip 1) showed no signs of
365 hesitation leading up to the collection site ($\mu \pm S.E. = 0.0 \pm 0.0$; Fig. 3, 4) and this was used as
366 the baseline for hesitation comparisons during training and testing. *Trip Number* had a significant
367 effect on foragers' hesitation numbers ($Z = 3.84$; $p < 0.001$). Post hoc comparisons showed that
368 hesitations did not significantly increase from the baseline on training trips 2–4 ($p > 0.05$; Fig. 3,
369 4). Beginning on Trip 5 ($T = 3.42$, $p = 0.01$) and continuing through the rest of training (Trip 7–
370 10), hesitations were significantly higher than foragers' baseline hesitation counts ($p < 0.001$).
371 During testing (Test 1–5), post hoc comparisons revealed that hesitation numbers were
372 significantly above baseline (Trip 1) during Test 1 ($T = 6.39$; $p < 0.001$), Test 2 ($T = 3.19$; $p =$
373 0.032), and Test 3 ZV ($T = 3.26$; $p = 0.016$; Fig. 3, 4). Beginning on Test 4 and continuing
374 during Test 5, hesitation counts returned to baseline and were not significantly different from

375 Trip 1 ($p > 0.05$). There was no significant difference between hesitation numbers during the last
 376 training trip, Trip 10 and Test 1 ($T = 0.24$; $p = 1.00$) as well as between Test 2 and Test 3 ZV (p
 377 $= 0.87$). Results suggest foragers showed significant signs of aversive view learning after three
 378 prior experiences and these hesitations increased throughout training, peaking at Test 1 ($\mu \pm$
 379 $S.E. = 13.8 \pm 2.9$). Evidence of memory retention persisted for two trips after the aversive
 380 outcome was removed (Test 2 and Test 3 ZV) before hesitations returned to baseline (Test 4 and
 381 Test 5; Fig. 3). Finally, increased hesitations in zero vector ants confirm that the memories are
 382 associated, at least partially, with the views and not the forager's vector state.



383

384 **Fig. 3.** Forager hesitations during training and testing in the Aversive Learning tests. Foragers were collected at Site
 385 2 during training (Trips 1–10), for 300s and were then allowed to return to the foraging route. During testing (Test
 386 1–5), foragers were allowed to travel through Site 2 and return to the nest. (a) Mean hesitations across training and
 387 testing, consisting of scans and stops combined $\pm SE$. ‘*’ denotes training or test trips where forager hesitations are
 388 significantly above the Trip 1 baseline ($p < 0.05$). (b) Mean scans and stops across training and testing $\pm SE$.



389

390 **Fig. 4.** Heat maps of forager hesitation locations and sample paths in the Aversive Learning Tests. Heat maps of
391 forager hesitations and five foragers' sample paths during Trip 1, Trip 4, Test 1, Test 2 and Test 3. Closed black
392 circles denote locations of forager Scans while open circles denote Stop locations. For heat maps of hesitations for
393 all training and test trips in the Aversive Learning tests, see SFig. 1.

394

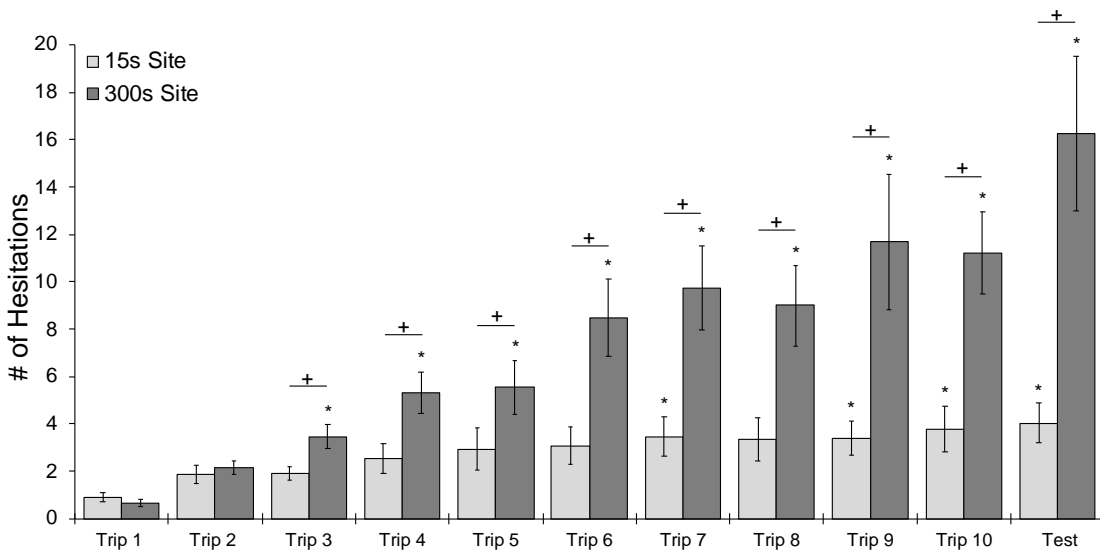
395 *Hold duration tests*

396 Foragers at the onset of training (Trip 1) exhibited few pre-training hesitations leading up to both
397 collection sites (15s Trip 1, $\mu \pm S.E. = 0.89 \pm 0.21$; 300s Trip 1, $\mu \pm S.E. = 0.64 \pm 0.14$; Fig. 5, 6)
398 and these were used as the baselines for comparisons. Both *Trip Number* and *Hold Condition* had
399 a significant effect on hesitations (*Trip Number*, $Z = 6.21$; $p < 0.001$; *Hold Condition*, $Z = -3.47$;
400 $p < 0.001$) and there was a significant interaction between *Trip Number* and *Hold Condition* ($Z =$
401 -4.98 ; $p < 0.001$). There was no significant effect of *Site Number* (condition mirroring) on
402 forager hesitation numbers ($Z = 0.34$; $p = 0.73$) and *Site Number* showed no significant
403 interaction with *Hold Condition* ($Z = 1.05$; $p = 0.30$) or *Trip Number* ($Z = 0.85$; $p = 0.40$).

404 At the 15s hold associated site, post hoc comparisons showed that hesitations did not
405 significantly increase from the baseline on training Trips 2–6 ($p > 0.05$). Beginning on training
406 Trip 7 ($T = 3.11$; $p = 0.02$) hesitations were significantly higher than foragers' baseline hesitation
407 counts, yet this significance increase disappeared during Trip 8 ($T = 2.84$, $p = 0.06$) before re-
408 emerging for the final two training trips (Trip 9, $T = 3.02$; $p = 0.04$; Trip 10, $T = 3.88$; $p = 0.01$)
409 as well as the zero vector Test ($T = 3.69$; $p < 0.001$). In contrast at the 300s hold site, post hoc

410 comparisons showed that foragers learned the association after only two exposures, with
411 hesitations significantly above Trip 1's baseline beginning on Trip 3 ($T = 2.38$; $p < 0.001$). This
412 significantly higher number of hesitations persisted through the rest of training on Trips 4–10 (p
413 < 0.001) as well as the zero vector Test ($p < 0.001$). Comparisons between the final training trip,
414 Trip 10, and the zero vector Test showed no significant difference in hesitations at either the 15s
415 site ($T = 0.19$; $p = 1.00$) or 300s site ($T = 1.14$; $p = 1.00$).

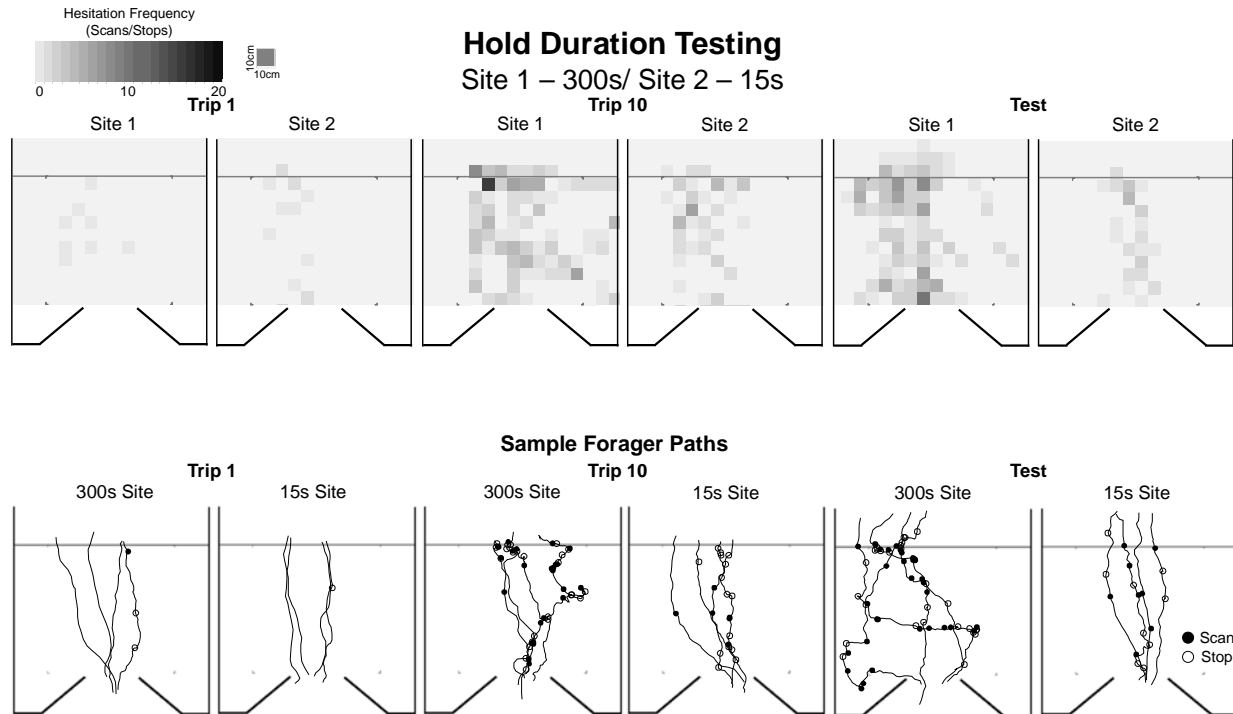
416 Comparisons of forager hesitation numbers between the 15s and 300s sites showed that
417 before training (Trip 1) foragers showed no significant differences between Site 1 and Site 2
418 (Wilcoxon signed-rank, $Z = 43.5$, $p = 1.00$). Foragers also showed no significant increase in their
419 hesitations at the 300s site during Trip 2 ($p = 0.23$). Beginning on Trip 3 ($p = 0.002$), and
420 continuing for the rest of training (Trips 4–10), foragers exhibited significantly higher hesitations
421 at the 300s hold site compared to the 15s hold site ($p < 0.005$) and this difference persisted
422 during the zero vector Test ($p < 0.001$). Foragers were able to perceive differences in outcome
423 severity between two hold times, as foragers learned the association at the 300s hold site faster
424 than the 15s site (Trip 3 vs. Trip 7) and showed higher hesitation counts associated with the more
425 severe outcome associated site.



426

427 **Fig. 5.** Forager hesitations during training Trips 1–10 and the zero vector test during Hold Duration tests. During
428 training, foragers were collected at both Site 1 and Site 2 and held for either 15s or 300s before being released back
429 at the site (hold periods for each site were randomly assigned at the onset of training for each individual). After
430 training Trip 10, foragers were collected at the nest and tested as a 'zero vector' forager. During the test, foragers
431 were allowed to travel through Site 1 and Site 2 without collection. Each '*' denotes training or test trips where
432 foragers' hesitation numbers were significantly above the Trip 1 baseline ($p < 0.05$). Each '+' denotes trips in which
433 the forager showed significantly higher hesitation numbers leading up to the 300s collection site compared to the 15s
434 site ($p < 0.05$).

435



436

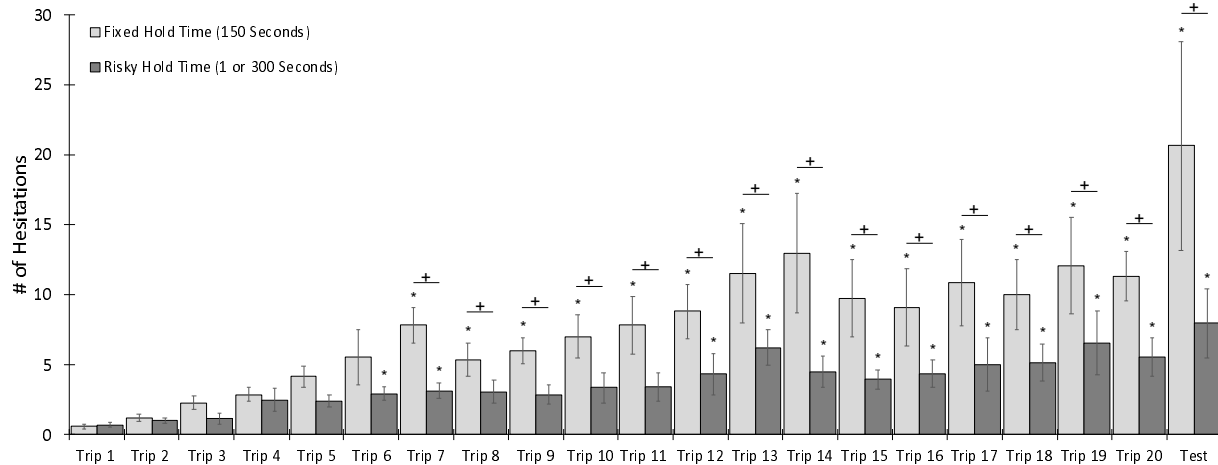
437 **Fig. 6.** Heat maps of forager hesitation locations and sample paths in the Hold Duration testing. Closed black circles
438 denote locations of forager Scans while open circles denote Stop locations. For heat maps of hesitations for all
439 training and test trips in the Hold Duration tests, see SFig. 2,3.

440

441 *Risk sensitivity tests*

442 As in previous conditions, foragers at the onset of training (Trip 1) in both the Fixed and Risky
443 conditions exhibited few hesitations leading up to the collection sites (Fixed Trip 1, $\mu \pm S.E. =$
444 0.60 ± 0.16 ; Risky Trip 1, $\mu \pm S.E. = 0.69 \pm 0.18$; Fig. 7) and this was used as the baseline for
445 future comparisons. Both *Trip Number* and *Hold Condition* (classified as Fixed or Risky) had a
446 significant effect on hesitations (*Trip Number*, $Z = 6.41$; $p < 0.001$; *Hold Condition*, $Z = 3.54$; p
447 < 0.001 ; Fig. 7) and there was no significant interaction between *Trip Number* and *Hold*
448 *Condition* ($Z = 1.1$; $p = 0.24$). There was no significant effect of *Site Number* (condition
449 mirroring) on forager hesitation numbers ($Z = 0.47$; $p = 0.64$) and *Site Number* showed no
450 significant interaction with *Hold Condition* ($Z = 0.34$; $p = 0.73$) or *Trip Number* ($Z = 0.91$; $p =$
451 0.36).

452 At the Fixed site, post hoc comparisons showed that hesitations did not significantly
453 increase from the baseline on training Trips 2–6 ($p > 0.05$; Fig. 7). On Trip 7, hesitations were
454 significantly higher than foragers' baseline hesitation counts ($p < 0.001$) and this significant
455 hesitation increase persisted throughout the rest of training on Trips 8–20 ($p < 0.05$; Fig. 7)
456 During the zero vector Test, forager hesitations were also significantly above baseline ($p <$
457 0.001).



458 **Fig. 7.** Forager hesitations during training Trips 1–20 and one zero vector test in the Risk sensitivity tests. During
459 training, foragers were collected at both Site 1 and Site 2 and held within a brush filled container for either a fixed or
460 risky period. At the fixed site, foragers were held for 150s, while at the risky site foragers had a 50/50 chance of
461 being held for 1s or 300s. After the hold period, foragers were allowed to climb out of the container and return with
462 their food to the nest. After training Trip 20, foragers were collected at the nest and tested as ‘zero vector’ foragers,
463 by placing them back onto the route at 10m from the nest. During the test, released foragers were allowed to travel
464 through Site 1 and Site 2 without collection. Each ‘*’ denotes training or test trips where forager hesitation numbers
465 were significantly above the Trip 1 baseline ($p < 0.05$). Each ‘+’ denotes trips in which the forager showed
466 significantly higher hesitation numbers leading up to the fixed (150s) collection site compared to the risky (1s/300s)
467 site ($p < 0.05$). For heat maps of hesitations for all training and test trips in the Risky Perception tests, see SFig. 4,5.

468

469 At the Risky site, post hoc comparisons showed that hesitations did not significantly
470 increase from the baseline on training Trips 2–5 ($p > 0.05$; Fig. 7). Hesitations were significantly
471 above baseline during training Trip 6 ($p = 0.02$) and Trip 7 ($p = 0.02$), but not during Trip 8 and
472 Trip 9 ($p > 0.05$). Beginning on Trip 10, hesitations were again significantly above baseline ($p =$
473 0.02) and this difference persisted through the rest of training ($p < 0.05$). During the Test, ZV
474 forager hesitations were also significantly above baseline ($p = 0.009$; Fig. 7).

475 Comparisons of forager hesitations between fixed and risky hold schedules showed that,
476 before training (Trip 1), foragers showed no significant differences between Site 1 and Site 2
477 (Wilcoxon signed-rank; $Z = 10.50$; $p = 1.00$). During training, foragers also showed no
478 significant difference in their hesitations between sites during Trip 2 (Wilcoxon signed-rank; $Z =$
479 32.5 ; $p = 1.00$) and this persisted through Trip 6 (Wilcoxon signed-rank; $Z = 11.02$; $p = 0.20$).
480 Beginning on Trip 7 (Wilcoxon signed-rank; $Z = 5.01$; $p = 0.004$) and continuing through the
481 rest of training (Trips 8–20), foragers exhibited significantly higher hesitations at the Fixed hold
482 site compared to the Risky hold site ($p < 0.05$) and this difference was also present during the
483 zero vector Test ($p < 0.001$).

484 During training at the Risky site, changes in hesitation numbers suggest that the effect of
485 training was continuously regulating hesitation behavior up and down based upon differences in
486 the expected outcome and forager’s experience on each trip (Fig. 8a). After experiencing the

487 highly aversive 300s outcome, hesitations are regulated upward (mean hesitation change $\pm S.E.$ =
488 $+1.13 \pm 0.44$) while experiencing the less aversive 1s outcome resulted in hesitations being
489 regulated downward (mean hesitation change $\pm S.E.$ = -0.46 ± 0.28) and these changes based on
490 the last experience were significant (Wilcoxon signed-rank; $Z = 18.00$; $p = 0.02$).

491 *Between Condition Comparisons*

492 During Trip 10, forager hesitations in the two 300s conditions (the Aversive Learning tests and
493 Hold Duration tests) showed no significant differences (Mann-Whitney U; $U = 210.5$; $p = 0.37$)
494 and these data were combined for future comparisons. Hesitation numbers during the Fixed 150s
495 condition were significantly lower than the 300s conditions (Mann-Whitney U; $U = 196.5$; $p =$
496 0.048) and significantly higher than the 15s condition (Mann-Whitney U; $U = 196.5$; $p = 0.002$).
497 In contrast, forager hesitations in the Risky condition, (mean hold time = 150.5s) were not
498 significantly higher from the hesitation numbers of foragers in the 15s condition of the Hold
499 Duration tests (Mann-Whitney U; $U = 196.5$; $p = 0.741$) despite the order of magnitude
500 difference in mean hold time (150.5s vs. 15s; Fig. 8b).

501 Finally, observed hesitations during Trip 10 of the Risky condition ($\mu = 3.33$) were
502 compared along the logarithmic curve of predicted hesitation numbers ($y = 2.2076 \times \ln(x) -$
503 2.544 ; $R = 0.9293$) at both its arithmetic (150.5s) and geometric (17.32s) mean hold times. At
504 Trip 10, observed hesitations in the Risky condition were not significantly different from the
505 predicted hesitation number (3.75) based upon the geometric mean hold time of 17.32s (One
506 sample T-test; $T = 0.377$; $p = 0.712$; Fig. 8b). In contrast, these observed hesitations significantly
507 differed from the predicted hesitation number (8.53) based upon the arithmetic mean hold time
508 of 150.5s (One sample T-test; $T = 4.67$; $p < 0.001$).

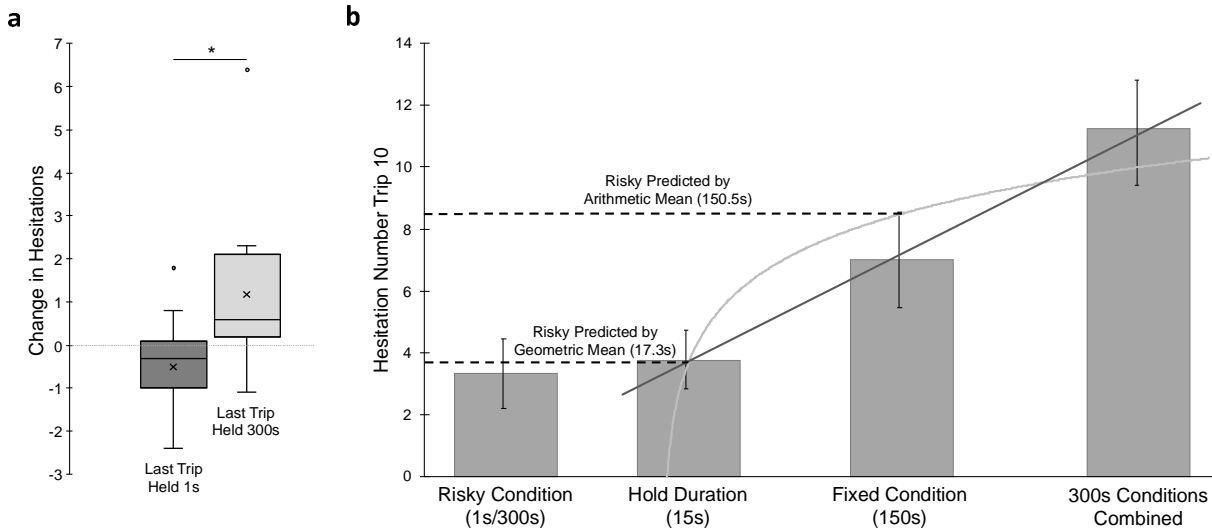
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515 **Fig. 8.** (a) Mean Change in hesitation numbers in the Risky condition across training based on the outcome of the
516 previous trip (Held 300s or 1s). Mean change across training was calculated per individual. Box plots shows the
517 median hesitation change (middle line), mean hesitation change (\boxtimes) and 25th and 75th percentile (box) while the
518 whiskers extend to min and max values (excluding outliers). Outliers were defined as values 150% of the IQR
519 beyond 25th and 75th percentile and represented as individual points. * denotes a significant difference between
520 conditions ($p < 0.05$). (b) Predicted hesitations at training Trip 10 along a logarithmic curve (grey line) calibrated by
521 hesitation numbers in the Hold Duration (15s), Fixed condition (150s), and the combined 300s conditions (mean
522 hesitations \pm S.E.). The Risky condition's mean number of hesitations on Trip 10 is plotted on the left, with the
523 predicted value of either the condition's geometric mean hold time of 17.3s or its arithmetic mean hold time of
524 150.5s. The observed hesitations during Trip 10 of the Risky condition ($\mu \pm S.E. = 3.3 \pm 1.1$) fell well below the
525 predicted hesitation number given the arithmetic mean hold time (3.33 observed vs. 8.5 predicted) yet the
526 logarithmic curve of predicted hesitations (3.8) falls within the standard error of the observed hesitations at the
527 condition's geometric mean. The observed lower hesitations during risky training aligns with the principle that the
528 forager's perception, and resulting hesitation behavior, of the aversive outcome has a logarithmic relationship with
529 stimulus strength ($y = 2.1824 \times \ln(x) - 2.4646$).

530

531 Discussion

532 In all tests, foragers traveling through the grid leading to their first collection exhibited either no
533 or minimal hesitation behaviours. Hesitations, including scanning behaviours, typically occur
534 when there are increases in navigational uncertainty due to inexperience, cue conflicts or
535 decreases in view familiarity (Wystrach et al. 2014). Given the high likelihood that foragers had
536 multiple experiences of the route during previous successful foraging trips to the feeder, it was
537 expected that hesitation numbers during Trip 1 would be low. When foragers were trained at
538 either site along the homeward route with a captivity period in the brush filled container, these
539 individuals learned an association between the views preceding the collection site and the
540 outcome, showing a significant increase in hesitation numbers when encountering these views on
541 the following trips. Foragers retained these view-based associations even after the outcome was
542 removed for multiple trips. Trained foragers continued to hesitate above their baseline on the

543 next three trips where they were allowed to pass through without collection, suggesting the
544 behavioral response was not based solely on the previous foraging trip. Foragers tested without a
545 corresponding vector state showed no change in hesitation numbers, meaning the association was
546 tied primarily to the view memory and not an association between the vector state and outcome.

547 *Aversive view learning*

548 Models of visual navigation currently rely solely on the positive valence, or attractiveness, of
549 familiar views which inhibit search behaviour (turning) and induce forward movement
550 (Wystrach et al. 2011b; Baddeley et al. 2012; Ardin et al. 2016; Kodzhabashev and Mangan
551 2015). These positive valence memories involve reinforcement learning of the associated
552 inbound views that lead to the nest, likely reinforced upon the forager's arrival, though the exact
553 reinforcer remains unknown. Yet, recent work has demonstrated that views can also be
554 associated with negative outcomes leading these views to develop a negative valence, or
555 aversiveness, which inhibits forward movement and induces hesitations, turns and scanning
556 behaviour (Wystrach et al. 2020). Such behaviours increase the likelihood that foragers may
557 avoid the negative outcome experienced on the old route and return to the nest quickly along a
558 new route, leading to these new views developing a positive valence and the formation of detours
559 (Le Möel and Wystrach 2020, Murray et al. 2020; Wystrach et al. 2020).

560 In the current study, just as in Wystrach et al. (2020), our results show rapid acquisition
561 of aversive view memories at specific spatial locations associated with negative outcomes,
562 resulting in increased hesitations leading up to these sites. Views that previously had a positive
563 association, formed during the initial formation of the homeward route, subsequently become
564 negative when associated with the experience of struggling within the brush. Unlike previous
565 work, here foragers were unable to form new positively reinforced routes detouring around these
566 negative outcomes as all available homeward routes resulted in collection. Once the negative
567 outcome was removed, foragers were shown to take two exposures to the route to re-learn its
568 positive association, reinforced through re-entering the nest (during Test 1 and Test 3 ZV), and
569 reduce hesitations to baseline. As Test 2 did not result in the forager successfully entering the
570 nest (due to collection for Test 3 ZV testing), it is likely the observed lack of a decrease in
571 hesitations between Test 2 and Test 3 (Fig. 3) was influenced by the missing reinforcement of re-
572 entering to the nest, rather than reaching a zero-vector state or experiencing the nest panorama.
573 This result hints that positive reinforcement of the route views upon a successful foraging trip
574 may trigger only once the forager enters the nest.

575 *Outcome severity and risk perception*

576 In associating views with negative outcomes, foragers are able to distinguish between levels of
577 severity of outcome, which was evident both in the acquisition rates and overall hesitation
578 responses. Foragers in the Hold Duration tests rapidly learned the association at the 300s site,
579 showing increased hesitation behaviour after only two previous exposures. In contrast, these
580 same foragers required six exposures to show increased hesitation behavior at the 15s site.
581 Overall hesitations also differed by outcome severity, with foragers exhibiting significantly more

582 hesitations associated with the 300s site ($\mu = 11.7$) than the 15s site ($\mu = 3.8$) during the final
583 training trip (Trip 10). In contrast, foragers trained in the Risk Perception tests at the fixed 150s
584 site, exhibited a hesitation response mid-point between these two extremes ($\mu = 7.3$). These
585 differences suggest foragers were able to perceive differences in time spent struggling within the
586 brush and recalled this outcome severity on subsequent foraging trips, leading to distinct levels
587 of aversion behavior expressed at each site.

588 Risk variance did not affect how quickly foragers learned the negative association, but
589 had a significant effect on the degree of hesitation that developed. Over the final five training
590 trips, foragers exhibited twice the number of hesitations at sites that resulted in a fixed negative
591 outcome (150s; $\mu = 10.7$) compared to sites leading to risky outcomes (1s or 300s; $\mu = 5.3$)
592 suggesting foragers perceived the risky outcome as less severe than the fixed outcome despite
593 these schedules having the same mean hold time over the course of training (~150s). This
594 increased hesitation number at the fixed site versus the risky site persisted during the test ($\mu =$
595 20.6 and 7.9 respectively). Furthermore, hesitation number at the risky site was shown to
596 increase or decrease based upon the outcome experienced on the forager's previous trip. When
597 foragers experienced a severe 300s hold time on the preceding trip, hesitations increased ($\mu =$
598 1.16) while when the less aversive 1s hold time was experienced on the preceding trip,
599 hesitations decreased ($\mu = -0.51$). These changes indicate that forager's hesitancy to pass
600 through the site is being continuously regulated up or down with each new experience of the site.
601 During each training trip, the hesitation behavior expressed represents the forager's level of
602 aversion to the expected outcome while each new experience regulates this expectation.

603 Additionally, the hesitation data presented here shows that foragers' perception of risky
604 aversive outcomes was not optimal in terms of the true value of the outcome. Many studies of
605 preference variance in reward quality/quantity show a general risk aversion tied to the animal's
606 perception of rewards balanced only by mean (Kacelnik and Bateson 1996). This has been
607 demonstrated in ants, as De Agrò et al. (2021) showed that ant foragers were risk averse when
608 reward options were balance by mean value. Yet, fixed option preference disappeared when the
609 two reward options were altered to be geometrically balanced (De Agrò et al. 2021). This finding
610 makes sense if animals perceive reward value on a logarithmic scale. For positive outcomes,
611 when the geometric average of a risky reward option falls below the fixed reward option, animals
612 should perceive the fixed option as preferable. Our research suggests that this relationship also
613 fits with the study of variability of negative outcomes. When the geometric average of a negative
614 outcome falls below the fixed outcome, animals should perceive this risky outcome as less
615 severe and become risk seeking, which aligns with our results. In the Risk Sensitivity tests, the
616 two outcomes were only balanced by true value (150s vs. 150.5s), while the geometrical average
617 of the hold duration of the Risky site ($\sqrt{1 \times 300} = 17.32$) was lower than the Fixed site
618 ($\sqrt[3]{150} = 150$). This corresponded with an overall lower level of hesitations at the risky
619 outcome site compared to the fixed outcome site. While we did not test purely geometrically
620 balanced Risky vs. Fixed outcomes, the perceived stimulus strength of the Risky site based on

621 the geometrical average would make it very similar to that of the 15s outcome in the Hold
622 Duration tests (17.32s vs. 15s). Interestingly, the hesitation levels during training (Trip 10)
623 between the 15s outcome ($\mu = 3.8$) and the Risky outcome ($\mu = 3.3$) were not significantly
624 different from one another despite the order of magnitude difference in actual mean hold
625 duration (150.5s vs. 15s; Fig. 8b). Additionally, we calculated a logarithmic curve of expected
626 hesitations based on those observed during the 15s, 150s and 300s conditions on Trip 10 (Fig.
627 8b). When hesitations observed during Trip 10 at the Risky site were compared with this curve,
628 observed and predicted hesitations did not significantly differ at the geometric mean (17.32s) but
629 did significantly differ from the predicted hesitations at the Risky condition's arithmetical mean
630 (150.5s), demonstrating that the geometric mean hypothesis should be favored over the
631 arithmetic mean. This provides further evidence that there is a logarithmic relationship between
632 captivity duration and forager's response, suggesting foragers are perceiving the outcome
633 severity logarithmically rather than its true value.

634 Stimulus strength perception is typically confirmed by animal's choices of varying
635 rewards and is used to explain why animals are typically risk averse to variable rewards (see
636 Scalar Utility Theory, Kacelnik and El Mouden 2013). The current results indicate that such
637 factors also predict risk seeking behavior to negative outcomes. Here, the foragers faced with
638 two identical mean hold times perceived the variable outcome as less severe, and equal to a hold
639 time almost an order of magnitude lower than its true value (17.3s vs. 150.5s) and thus respond
640 less negatively to the associated views, on par with hesitations to a fixed 15s hold time (Fig. 8b).
641 In our Risk Perception testing, the constant site always results in a 150s hold while the risky site
642 may result in a 1s or 300s hold time. Along a logarithmic curve, this 150s hold time would be
643 perceived by the forager as 150 times worse than the 1s hold time while 300s is only two times
644 worse than 150s. If these outcomes were balanced by geometric mean rather than true mean, for
645 example altering the hold times to a constant 15s hold time and a risky schedule of a 50% chance
646 of either 1s or 225s ($\sqrt{1 \times 225} = 15$), we would expect the forager's perception of the aversive
647 outcomes to be equal and result in identical hesitation numbers. While the current findings
648 clearly point to the logarithmic relationship between the outcome's severity and the forager's
649 aversive response, future work could help further untangle the ant's perception of aversive
650 outcomes, effort and risk by testing truly geometrically balanced fixed and variable outcomes.

651 *Underlying neural mechanisms*

652 Specific view memories must be stored through some change within the brain (memory trace)
653 along with their current corresponding positive or negative valence, based on previous
654 experiences along the foraging route. Each memory trace must also be able to be altered between
655 attractive and aversive valences and result in changes of forager behaviour when these views are
656 next experienced.

657 The use of learnt route memory in ants involves the Mushroom bodies, MB (Büehlmann
658 et al. 2020; Khamil et al. 2021), and its known neural circuitry can explain the storage and recall
659 of visual as well as olfactory memories (Heisenberg 2003; Ardin et al. 2016; Webb and

660 Wystrach 2016; Wystrach et al. 2020). Visual information enters the MB via projection neurons
661 from the optic lobes (Habenstein et al. 2020). An individual view can be represented neurally
662 within the MB through activation patterns of Kenyon Cells (KC), which project onto a number of
663 motor output neurons (MBON). Each MBON conveys an attractive or aversive valence, and
664 changes in synaptic strength between KCs and MBONs by activation of dopaminergic neuron
665 mediating negative or positive experiences, modulates the association between a stimulus and its
666 outputted valence (Cohn et al. 2015; Aso and Rubin 2016). Here, changes within these synaptic
667 compartments mediate the view's current overall valence by weighting the attractive and
668 aversive valences of the forager's experiences during previous trips to control the forager's
669 steering behavior. More specifically, the aversive outcome of being captured must result in
670 dopaminergic neurons decreasing the connection strength between the recently encountered view
671 specific pattern of Kenyon cells and attractive valence MBONs (Wystrach et al. 2020). This
672 triggers the hesitation behaviours observed leading up to the collection site after training foragers
673 with the aversive outcome.

674 The current study findings support our current understanding of the memory dynamics
675 within the circuitry of the MBs, mostly stemming from olfactory memory work in other insects.
676 First, the aversive response increased across repeated trial until reaching a plateau (Fig. 7), as
677 observed in drosophila olfactory conditioning (Beck et al. 2000). Second, while aversively
678 trained foragers were highly hesitant to travel through these sites, no forager refused to cross the
679 line despite the aversive association, even when they lacked a corresponding vector (Fig. 4; Fig.
680 5), suggesting that both attractive and aversive valence memory traces are simultaneously at
681 play. Thus, it is likely some underlying attractive valence associated with these views persists in
682 different MBONs, despite the acquired association with the aversive outcome, as demonstrated
683 in fly's MB (Boto and Ramaswami 2021). Third, the changes in hesitation number in the risky
684 condition indicate that forager behaviour is being continuously regulated based by each new
685 trip's experienced outcome (Fig. 8a). Thus, current experience continuously regulates the
686 forager's expected outcome at the site, as observed in the fly's MB (Cohn et al. 2020). However,
687 the persistence of hesitations after the aversive outcome was removed show that valence persist,
688 and is thus the result from an accumulation of experiences over multiple previous trips, not the
689 last experience alone. Finally, the reduction in hesitation following a 1s hold time in the risky
690 condition (Fig. 7) shows that this experience led to a net gain in positive valence, even though
691 the ant has been captured. This supports the idea that learning in the MB follows a prediction-
692 error rule (Bennett et al. 2021). Learning is dependent on the discrepancy between the current
693 experience and the expected one (Rescorla and Wagner 1972). In other words, being captured for
694 1s at a site where one has been previously captured 300s mediates a positive reinforcement,
695 leading to a decrease in aversion. Overall, these results indicate that various dopaminergic
696 neurons are continuously modulating connection strength of various aversive and attractive
697 valence MBONs based on the difference between the expected outcome and the experienced
698 outcome on that trip.

699 *Conclusions*

700 We found that *C. velox* foragers rapidly learn to associate views with aversive outcomes,
701 showing increased hesitations at these sites, in some cases after only two previous experiences.
702 Such memories are not solely based on the forager's most recent trip, as individuals continued to
703 showed increased hesitation at these sites after the aversive outcome was removed, suggesting
704 these aversive memories persist over multiple trips. Foragers were also able to perceive
705 differences in outcome severity, learning more rapidly and exhibiting more hesitations at a site
706 associated with a severe outcome (300s) when compared to a less severe outcome (15s).
707 Additionally, we show that the foragers show significantly less apprehension to travel through a
708 site associated with a risky aversive outcome compared to a fixed outcome with the same mean
709 and that forager hesitation responses at these sites across experiments was in line with the
710 logarithmic relationship between stimulus strength and perception. Finally, our findings fit
711 within the current modeling of view-based route learning and memory in the mushroom bodies
712 of the insect brain. The behavioral dynamics observed here align well with the complex and
713 parallel memory dynamics of the MB as studied in the context of olfaction in flies. In closing, a
714 final intriguing question remains. Namely, the foragers' response to different hold durations
715 suggests that they can somehow quantify or estimate their duration of capture, yet the
716 mechanism by which this duration estimate is accomplished currently remains unknown.

717

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721 Sciences and Engineering Research Council of Canada Discovery grants (#04133 and #2020-
722 03933).

723

724 **Competing Interests**

725 The authors declare no competing or financial interests.

726

727 **Ethical standards**

728 There are no ethical requirements for working with insects in Spain. Manipulations were non-
729 invasive and all individuals were returned to the nest after testing.

730

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