Report

Current Biology

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

Highlights

- Navigating ants learn to reshape their routes to avoid falling into an invisible trap
- The visual memories experienced a few seconds *before* being trapped become aversive
- On subsequent trips, these aversive memories trigger salutary turns prior to the trap
- The new views experienced along safe detours are consolidated as part of a new route

Authors

Antoine Wystrach,
Cornelia Buehlmann,
Sebastian Schwarz, Ken Cheng,
Paul Graham

Correspondence

antoine.wystrach@univ-tlse3.fr

In Brief

Wystrach et al. show that navigating ants can rapidly reshape a familiar foraging route to avoid regions associated with negative experiences. The route views perceived shortly before a negative event become retrospectively associated with the ensuing outcome, and on the subsequent trip this new association is used to trigger salutary turns.

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

Antoine Wystrach,[1,4,*] Cornelia Buehlmann,[2] Sebastian Schwarz,[1] Ken Cheng,[3] and Paul Graham[2]
[1]Centre de Recherches sur la Cognition Animale, CNRS, Université Paul Sabatier, Toulouse, F-31062 Cedex 09, France
[2]School of Life Sciences, University of Sussex, John Maynard Smith Building, Falmer, Brighton, Sussex BN1 9QG, UK
[3]Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia
[4]Lead Contact
*Correspondence: antoine.wystrach@univ-tlse3.fr
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SUMMARY

The ability of bees and ants to learn long visually guided routes in complex environments is perhaps one of the most spectacular pieces of evidence for the impressive power of their small brains. Whereas flying bees can visit flowers in an optimized sequence over kilometers, walking solitary foraging ants can precisely recapitulate routes of up to 100 m in complex environments [1]. It is clear that route following depends largely on learned visual information and we have a good idea of how visual memories can guide individuals along them [2–6], as well as how this is implemented in the insect brain [7, 8]. However, little is known about the mechanisms that control route learning and development. Here we show that ants (*Melophorus bagoti* and *Cataglyphis fortis*) navigating in their natural environments can actively learn a route detour to avoid a pit trap. This adaptive flexibility depends on a mechanism of aversive learning based on memory traces of recently encountered stimuli, reflecting the laboratory paradigm of trace conditioning. The views experienced *before* falling into the trap become associated with the ensuing negative outcome and thus trigger salutary turns on the subsequent trip. This drives the ants to orient away from the goal direction and avoid the trap. If the pit trap is avoided, the novel views experienced during the detour become positively reinforced and the new route crystallizes. We discuss how such an interplay between appetitive and aversive memories might be implemented in insect neural circuitry.

RESULTS AND DISCUSSION

Ants Can Reshape Their Route to Circumvent a Trap

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

Why some ants did not learn to circumvent the trap may be due to different reasons. A good proportion of those ants in both species did show modification of their routes by learning to avoid the trap using alternative strategies such as jumping directly onto the exit stick (see the red paths in Figure S1) or simply learning a quick route through the trap by systematically falling in at the same spot and quickly reaching the exit stick with very little search (see ant 7 in Figure S1). Finally, some ants simply did not learn, perhaps because they performed too few training trials within the 24-h period. Our personal observations were that the specifics of

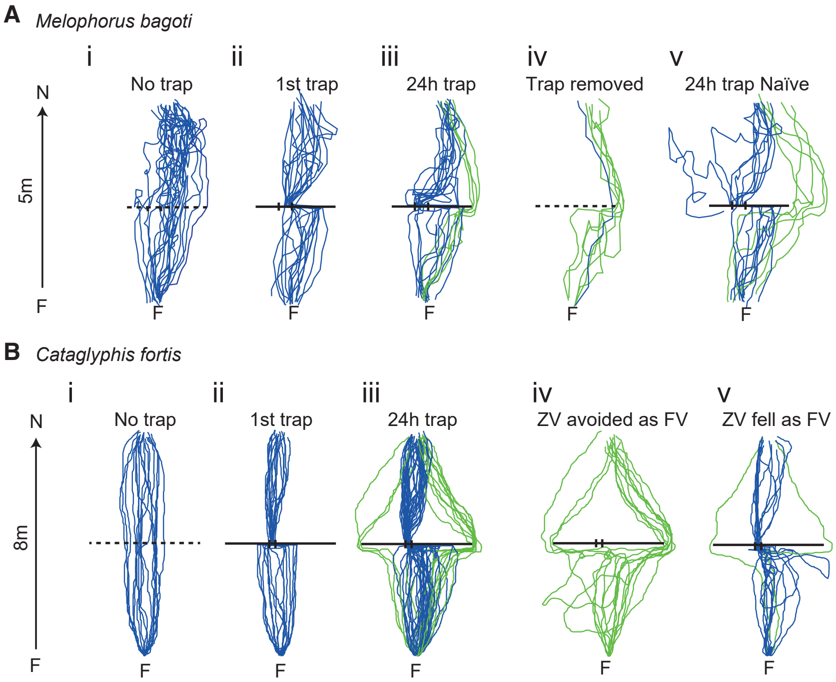

Figure 1. Negative Experience Shapes Ants' Routes

(A and B) Australian desert ants (*Melophorus bagoti*) (A) or North African desert ants (*Cataglyphis fortis*) (B) were followed for a series of homeward routes from a permanent feeder (F) to their nest, with a pit trap in place (solid line) or covered over (dashed line). Two small dashes across the trap line indicate the escape board. Green paths are from ants that circumvent the pit trap and blue paths are from ants that fall into the trap (or would have fallen if the trap was there). N, nest.

(i) Control routes of ants between feeder and nest, without a trap (A, n = 20; B, n = 16).

(ii) The first route after the installation of the pit trap (A, n = 15; B, n = 15).

(iii) Paths after the pit trap has been in place for 24 h (A, n = 14; B, n = 47).

(A, iv) Paths of *M. bagoti* ants that had previously learned to circumvent the trap, tested with the trap covered (n = 6).

(A, v) Paths of ants that had begun their foraging life (i.e., since naive stage) with the trap in place and had 24 h of foraging experience (n = 15).

(B, iv and v) Paths of *C. fortis* ants tested as ZV ants (without useful PI information, as the path integrator no longer points toward the nest but toward the feeder) that had previously succeeded (iv, n = 12) or failed (v, n = 13) to circumvent the trap as full-vector ants.

"n" refers to the number of individual ants tested.

how ants hit the pit trap influenced their ultimate strategy. Some ants fell by chance near the exit bridge and so spent less time in the trap itself. These ants were more likely to develop a strategy that depended on hitting the trap but also knowing how to get out quickly. Future experiments could investigate the possibility that a longer time of being trapped yields stronger aversive memories. In any case, a simple categorization of whether the ants circumvented the trap or not is sufficient to show that such an effect is unlikely to happen by chance (1st_trial_with_trap versus 24 h_with_trap: $N_{(\text{circumvented})}/N_{(\text{all ants})}$: 0/30 versus 17/61, Fisher's exact test, $p < 0.001$), and that there was no apparent difference in detour success between species (24 h_with_trap *M. bagoti* versus *C. fortis*: $N_{(\text{circumvented})}/N_{(\text{all ants})}$: 4/14 versus 13/47, Fisher's exact test, p = 1).

New Routes Are Based on Learned Terrestrial Cues

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

Ants captured just before entering their nest and then re-released at the feeder are called zero-vector (ZV) ants because their PI state is zero at the onset of homing, and thus no longer provides correct homeward information. Such ZV ants that had circumvented the trap during their previous (full-vector; FV) run were equally successful in their subsequent ZV run (Figure 1B, iv), whereas ants that had fallen into the trap as FV ants still did so as ZV ants (previous_FV circumvented versus previous_FV fell: $N_{(\text{circumvented})}/N_{(\text{all ants})}$: 12/12 versus 2/13, Fisher exact test, $p < 0.001$), showing that guidance along the newly learned route does not require PI. Interestingly, ZV ants displayed turns before the trap even if they had failed as FV, showing that the learning process itself has to do with views rather than the ant's PI state. Note also that 2 out of 13 ants fell as FV but avoided the trap as ZV, which further supports the hypothesis of visual route learning. We know that the directional dictates of PI and learned visual guidance are integrated, even when pointing in different directions [15–18]. Thus, in FV ants, the PI vector points directly to the nest and thus may bias the path toward the trap. Therefore, the paths of ZV ants are more representative of the route as guided by terrestrial cues. Nevertheless, the extent to which PI information may be associated with aversive experiences should be more fully studied.

We further tested whether ants that had learned a new route to circumvent the trap would still follow it, if the trap was removed again. Five out of the six individuals tested displayed the usual detour even though the trap had been removed (Figure 1A, iv; no_trap_initially versus trap_removed_again: $N_{(\text{circumvented})}/N_{(\text{all ants})}$: 1/20 versus 5/6, Fisher exact test, $p < 0.001$). This confirms that the detour does not depend on perceiving the trap but on route memories.

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

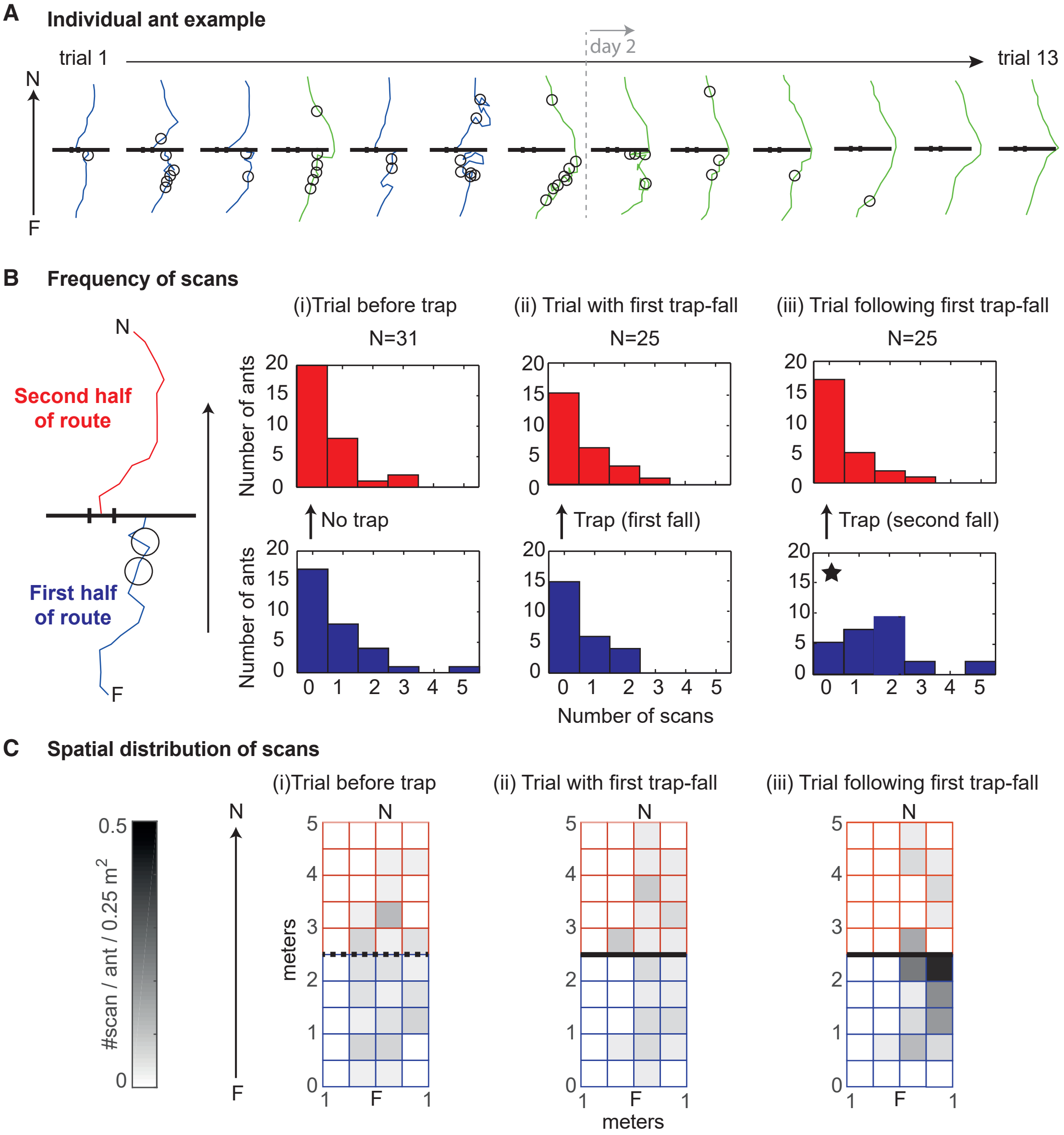

Figure 2. Negative Experience Modifies the Memory of the Views Experienced before the Trap

Individually marked ants of *M. bagoti* were tracked for a sequence of runs before and after the activation of the pit trap.

(A) Sample sequence from a single ant. Locations where the ant stops and scans the world are marked with a circle. As elsewhere in the paper, paths are colored coded: blue for ant paths that fall into the trap and green for paths that avoid the trap. See Figure S1 for more individual examples.

(B) For each ant, the number of scans was recorded before (bottom, blue) and after (top, red) the trap across three focal trials.

(i) Trial before the trap was activated.

(ii) Trial with the first trap fall.

(iii) Trial following the first trap fall.

From this, we calculated the probability of an ant scanning ($n_{(\text{ants scan} > 0)}/n_{(\text{all ants})}$), and the median number of scans, for each trial and route segment. Before the trap: (i) n= 31, probability (proba) = 0.45, median = 0; (ii) n = 25, proba = 0.40, median = 0; and (iii) n = 25, proba = 0.80, median = 2. After the trap: (i) n = 31, proba = 0.35, median = 0; (ii) n= 25, proba = 0.40, median = 0; and (iii) n = 25, proba = 0.32, median = 0. The star indicates a significant increase in scan number (see text for details).

(C) For the same three focal trials, the location of scans is shown relative to the feeder (0,0), nest (0,5), and pit trap (black line, y = 2.5). Darker areas represent higher scan numbers. The upward arrows on the left panels indicate route direction.

The use of chemical trails, scent marks, or other social information would be unlikely in these highly visual solitary foraging ants; however, we can further definitively rule them out, by observing the typical idiosyncrasies of ants' individual routes [19–21]: even though trials were interleaved between individuals and spanned different days, routes are remarkably similar across subsequent trials of a same individual (Figures 2A and S1) but vary substantially across individuals (Figures 1 and S1).

We can be confident that ants were not using social information but private information based on learned terrestrial cues. The nature of these learned terrestrial cues is not crucial for our purpose here, but based on past evidence with desert ants, which run at high speed (>50 cm/s) with the head and antennae lifted upward, we can be confident that it is mostly based on visual cues [9, 19, 22], typically the recognition of familiar panoramic views [3, 23, 24]. To ease the reading, we will refer to these learned terrestrial cues as "familiar views."

How Do Ants Reshape Their Routes? Evidence for Aversive and Memory Trace Learning

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

As described above, on the first run with the trap in place, ants rushed along their direct homeward route and fell into the trap. In subsequent trials, routes of most ants changed and some eventually circumvented the trap. We supposed two potential mechanisms. First, it could be that the negative event of falling into the trap triggers learning-oriented behavioral routines that occur immediately after the negative event. This was not the case. When ants emerged from the trap, they rushed toward their nest as usual, and displayed neither more scanning or meandering than before the trap was set (second part of the route: *trial before trap* versus *trial with first trap fall*: mode, 0 scans/ant in both cases; generalized linear mixed model [glmm] #scan: $t = 0.359$, $p = 0.721$; glmm meander, $z = 0.027$, $p = 0.978$; Figure 2B, i and ii; see also Figures 1A, ii and 1B, ii).

Second, ants could keep memory traces of the scenes experienced immediately before falling into the trap, and change the valence of these recent memories given the current negative experience of being trapped. In our paradigm, this would predict that ant behavior will be affected on the run subsequent to falling in the trap when in the area immediately before the trap. Indeed, this is what we observed. Ants displayed a clear increase in scanning behaviors in the region before the trap (mode and median = 2 scans/ant; Figure 2B, iii), significantly more than they had on their previous run (mode and median = 0 scans/ant; Figure 2B, ii) before falling into the trap for the first time (first part of the route: *trial with first trap fall versus trial following first trap fall*: glmm #scan: $t = 4.186$, $p < 0.001$). Similarly, their paths showed significantly more meandering as they approached the trap for the second time compared to their previous run (first part of the route: *trial with first trap fall versus trial following first trap fall*: glmm meander: $z = 3.006$, $p = 0.003$).

Mechanistically, learning must be based on a memory trace because the US (i.e., being in the trap) is experienced after the conditioned stimulus (CS) (i.e., the view of the surrounding scenery) and, crucially, with no time overlap, for being in the trap (unconditioned stimulus; US) prevents entirely the view of the scenery (CS) and the trap is invisible from outside. Being exposed to the CS before the US with no time overlap between both stimuli reflects the laboratory paradigm of "trace conditioning" [31]. How long is the delay between CS and US cannot be determined in our natural situation, although the apparent increase of scanning behaviors up to 2 m before the trap (Figures 2A and 2C) suggests that the memory trace of the views may be kept for at least a couple of seconds. The resulting aversive memory formed, however, lasts across days to trigger salutary avoidance and scanning behaviors (Figure 2A, day 2, gray arrow).

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

Neural Implications

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

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

How and where exactly the tagging may happen remain unclear as several types of modulatory neurons project to these MB compartments [45, 46]. Finally, if it is now clear that being trapped or caught can constitute a negative reinforcement to escape, what constitutes positive reinforcement during route learning is still unclear: it could be reaching the nest or running down the PI-accumulated home vector [7]. Such positive reinforcement might also involve the tagged neurons,' so that not only the current but also the previously experienced views that led to the positive outcome become associated with a positive valence. In any case, it is clear that route reshaping here results from an interplay between avoidance behavior triggered by memory trace learning and appetitive learning based on positive reinforcement (Figure 3).

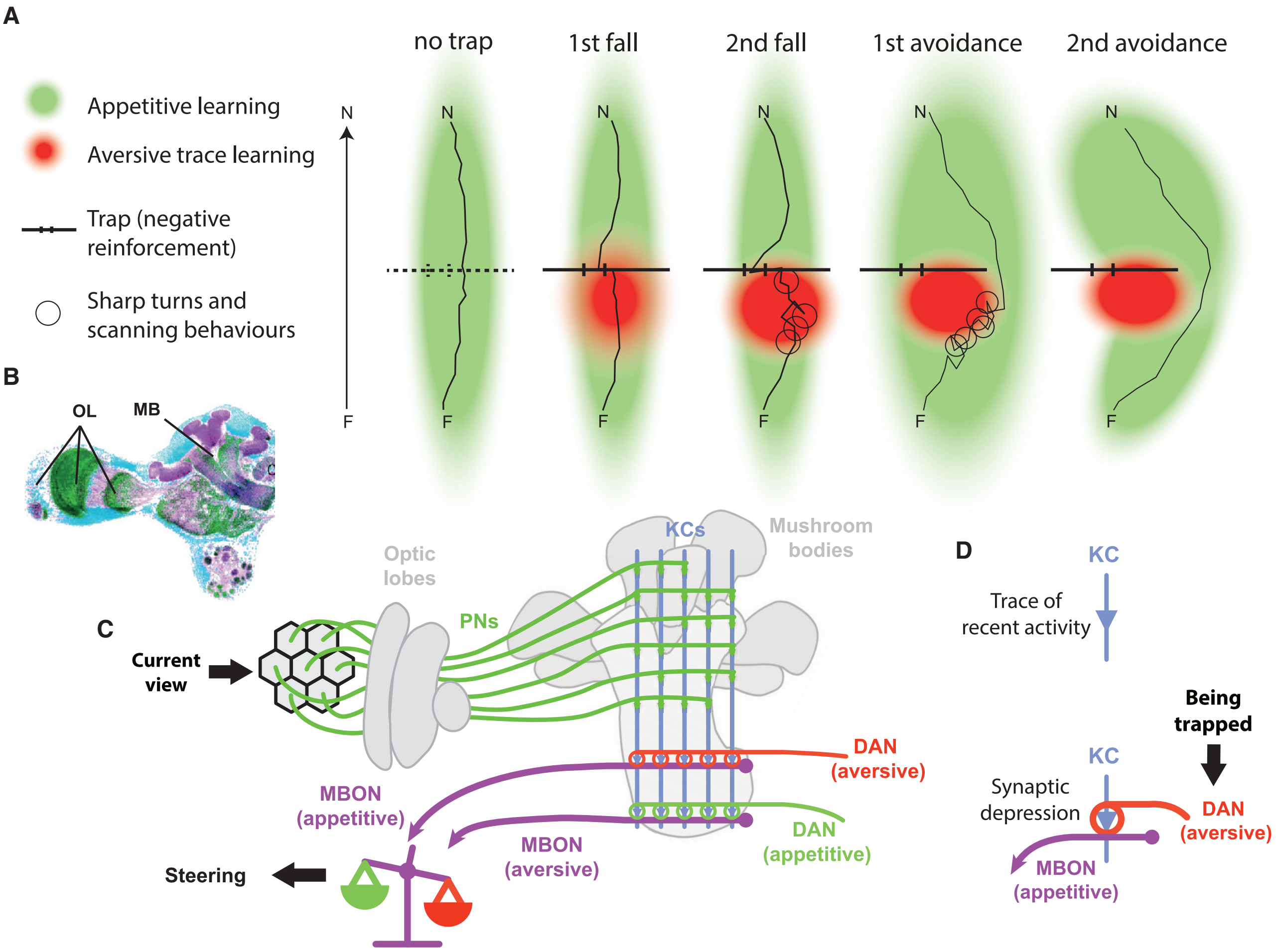

Figure 3. Overview of the Appetitive and Aversive Learning Experiences That Lead to Route Reshaping

(A) Illustrations of the concept. Across a sequence of journeys for a typical ant, we show the regions of operation for aversive (red) and appetitive (green) visual learning. The aversive region is first formed by trace learning on the trials where the ant encounters the trap. Over time a detour develops, and the new route is maintained by appetitive processes. The upward arrow indicates route direction.

(B) Picture of a *Cataglyphis fortis* brain's right hemisphere showing the optic lobes (OLs) and mushroom bodies (MBs) (modified from [43]).

(C) MB neural architecture derived from the insect literature [8, 44]. Visual information is sparsely projected via visual projection neurons (PNs) to the Kenyon cells (KCs). KCs' activity thus represents views that can be associated with the MB output neurons (MBONs) mediating appetitive or aversive valences. Such associations result from the modulation of KC-to-MBON synapses; the modulation is generated by the co-activation of KCs and dopaminergic neurons (DANs) relaying the valence of the current situation. The resulting balance between aversive and appetitive MBONs' activities can then control steering through repulsion/attraction.

(D) The current study suggests memory trace learning as a mechanism to explain the reshaping of routes. First, a trace (or tag) of the KC activity must be kept for a few seconds (top panel). Second, the activation of a dopaminergic neuron (DAN) modulates the KC-to-MBON synapses of these recently activated 'tagged' KCs (bottom panel), thus modulating the valence of the views experienced a few second before. Note that modulation consists of a synaptic depression, which explains why DANs of a given valence modulate MBONs of the opposite valence. Thus, an aversive situation, such as being trapped, will be mediated by a DAN decreasing the connection strength between the recently activated KCs and the appetitive MBON. These KCs will no longer activate the appetitive MBON but still activate the aversive MBON. In other words, the view experienced before the trap will henceforth trigger an aversive response.

Conclusions

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

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- LEAD CONTACT AND MATERIALS AVAILABLITY
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - ○ Experimental set-ups
  - ○ Experimental protocols

- QUANTIFICATION AND STATISTICAL ANALYSIS
- DATA AND CODE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2020.02.082.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.W.; Methodology, A.W., C.B., S.S., and P.G.; Formal Analysis, A.W.; Investigation, A.W., C.B., and S.S.; Writing – Original Draft, A.W. and P.G.; Writing – Review & Editing, A.W., C.B., S.S., K.C., and P.G.; Funding, A.W., C.B., K.C., and P.G.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

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

LEAD CONTACT AND MATERIALS AVAILABLITY

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

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Two species were tested in this study: the Australian red honey ant, *Melophorus bagoti* and the Saharan desert ant *Cataglyphis fortis.* Both species are highly thermophilic ants ([47, 48]) that forage solitarily on dead insects and plant materials ([49]). Experiments with *M. bagoti* were achieved in January-March 2014, ~10 km south of Alice Springs, Australia, on the grounds of the Centre for Appropriate Technology, in a semi-arid desert habitat characterized by red soil, grass tussocks, bushes, and trees of Acacia and Eucalyptus species. Field experiments with *C. fortis* were performed in June-July 2015 in a flat salt pan (34.954897 N, 10.410396 E) near the village of Menzel Chaker, Tunisia. By using two species of desert ants that belong to different phylogenetic tribes, we hoped to investigate the generality of this route re-learning process.

METHOD DETAILS

Experimental set-ups

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

In both experimental set-ups, ants moved between their nest and a feeder full of cookie crumbs 5 m (8 m for *C. fortis*) away. The ants' nest was covered with an overturned bucket that had the bottom removed, whose opening at ground level was connected to a straight outbound channel (5 m long, 10 × 10 cm for *M. bagoti*; 8 m long, 7 × 7 cm for *C. fortis*) elevated 15 cm above the ground, which was always in place and thus belonged to the scenery. This outbound channel suddenly ended above the feeder, into which ants would drop. The feeder was a small plastic container sunk into the ground providing biscuit crumbs *ad libitum*. To return to the nest, the ants climbed out of the feeder using a small ramp and walked on the desert ground back to the nest. Removing the feeder ramp at critical times allowed us to control which ants ran their homeward journey and when. Halfway along their homing route, a plastic channel was buried inconspicuously into the desert floor, creating a 2-m-long, 10-cm-wide (4 m long 7 cm wide for *C. fortis*) trap perpendicular to the nest-to-feeder route. The trap was buried entirely so as to remain invisible to the ants. The trap had smooth walls and was filled with twigs to hinder ant movement. Ants could leave the trap by using a single exit formed of a stick bridge 20 cm wide (5 cm for *C. fortis*), connecting the bottom of the trap to the second part of the route. A grid of lines (mesh width: 1 m) was set up by winding strings around pegs (or by painting on the ground with *C. fortis*) and the ants' homing paths before and after introducing the trap were recorded on squared paper. During initial training the trap was covered by a thin board, with desert sand scattered on top, so that the ants could shuttle unimpeded.

Experimental protocols

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

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

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

Another condition was tested with naive *M. bagoti* ants. The trap was set in place and all ants were marked for 5 consecutive days. After this period, all unpainted ants reaching the feeder were considered "naive" and were painted with a specific color. Naive ants were free to forage for 24 hours before being recorded.

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

QUANTIFICATION AND STATISTICAL ANALYSIS

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

DATA AND CODE AVAILABILITY

All data are available at: https://github.com/awystrac/Rapid-aversive-and-memory-trace-learning_Current_Biol_2020/commits?author=awystrac