Cold-induced anesthesia impairs path integration memory in dung beetles

Highlights

- *Scarabaeus galenus* stores the directional component of its home vector for 60 min
- The distance component of the home vector memory declines gradually
- Cold-induced anesthesia impairs distance memory before directional memory
- An anesthesia-sensitive process is essential to support homing by path integration

In brief

Path integration is a navigational mechanism used by animals to keep track of their position with respect to a geographical reference point. Using the homing dung beetle *Scarabaeus galenus*, Yilmaz et al. demonstrate that a biological process that can be disrupted by cold-induced anesthesia is required for the maintenance of home vector memories.

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Cold-induced anesthesia impairs path integration memory in dung beetles

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SUMMARY

Path integration is a general mechanism used by many animals to maintain an updated record of their position in relation to a set reference point.1–11 To do this, they continually integrate direction and distance information into a memorized home vector. What remains unclear is how this vector is stored, maintained, and utilized for successful navigation. A recent computational model based on the neuronal circuitry of the insect central complex suggests that home vector memories are encoded across a set of putative memory neurons and maintained through ongoing recurrent neural activity.12 To better understand the nature of the home vector memory and experimentally assess underlying mechanisms for maintaining it, we performed a series of experiments on the path integrating dung beetle Scarabaeus galenus.13 We found that, while the directional component of the home vector was maintained for up to 1 h, the distance component of the vector memory decreased gradually over time. Using cold-induced anesthesia, we disrupted the neural activity of beetles that had stored a home vector of known length and direction. This treatment diminished both components of the home vector memory, but by different amounts—the homing beetles lost their distance memory before their directional memory. Together, these findings present new insights into the functional properties of home vector memories and provide the first empirical evidence that a biological process that can be disrupted by cold-induced anesthesia is essential to support homing by path integration.

RESULTS AND DISCUSSION

Homing beetles store the directional component of their home vectors for up to 60 min

Today, more than 50 years after the first behavioral evidence that insects use path integration,14 we still have a limited understanding of how information about direction and distance is integrated in the brain to encode the home vector and how this vector is maintained and utilized for successful navigation. To unravel the functional and mechanistic characteristics of the vector memories of the homing beetle Scarabaeus galenus,13 we trained the beetles to forage over different distances (1.25 or 2.55 m) and immobilized them for periods up to 120 min before releasing them in a test area in their natural environment (Figure S1). It is important to note that S. galenus is known to ignore visual landmarks and rely primarily, if not exclusively, on path integration for homing.15 After moving some distance along a straight path, each homing beetle made a sharp turn. This distinct turning point defines where the beetle expects to find its burrow.13 Beetles trained to a feeder placed 1.25 m away from their burrow and immobilized for 2 min in small opaque tubes moved straight in the direction of their fictive burrows, i.e., the relative location of their burrow if it had been moved with them (Figures 1Ai and 1Bi), and expected to find it 0.90 ± 0.33 m (mean ± SD, n = 11) from their point of release. This clearly confirmed that beetles could maintain and follow the direction and distance components of their home vector even after being caught at the feeder, immobilized, isolated in the dark, and transported to a new location (Figures 1Ai and 1Bi; Table 1).

Beetles with an expected home vector of 1.25 m lost the directional component of this vector within 1 h (Figures 1Aii–1Aiv and 1Bii–1Biv; Table 1), while beetles with a longer home vector (2.55 m) still moved in the direction of their burrows upon release, even after a full hour of immobilization (Figures 2Ai, 2Aii, 2Bi, and 2Bii; Table 1). This increase in directional certainty with increasing foraging distance is consistent with previous findings from other path integrating insects15 and will naturally serve to guide the insect back to the safety of its home when foraging farther away. Nevertheless, this period is rather short compared to, for example, that of ants, which can retain their directional memory for at least 24 h.16 In addition, the accuracy in estimating the angular component of the vector memory degraded much more rapidly in the beetles (exponential time constant t = 55.5 min) (Figure 3A) than it does in ants (t = 4.5 days).16 However, this difference should be treated with caution because the training distance (12 m), as well as the training period (2 days) of the ants,16 was much longer than the distances over which the beetles in this study were tested. Nonetheless, these
differences in foraging distances reflect the natural foraging behavior of the two model animals—while the beetles forage over a median distance of only 1.5 m,13 ants are known to forage for distances of several hundreds of meters. In addition, feeding on fresh dung that quickly dries under the hot African sun, *S. galenus* might not benefit from maintaining a defined compass direction between its burrow and the food source for an extended period of time.

**Distance component of the home vector memory declines gradually**

Unlike the directional component, the distance component of the vector memory in beetles trained to forage over a distance of 1.25 m showed a clear, gradual decline as immobilization time increased—from 1.19 ± 0.19 m after 0 min (data from Dacke et al.13) to 0.90 ± 0.33 m after 2 min (n = 11), 0.74 ± 0.31 m after 10 min (n = 10), 0.65 ± 0.26 m after 30 min (n = 13), 0.5 ± 0.32 m after 60 min (n = 16) (Figure 2C), and 0.49 ± 0.46 m after 120 min (mean ± SD, n = 11). Interestingly, beetles trained over a longer distance (2.55 m) initiated their search behaviors after covering only 40% and 30% of their home vectors (i.e., 1.01 ± 0.56 m [n = 13] and 0.74 ± 0.36 m [mean ± SD, n = 11]) after 10 and 60 min immobilization, respectively (Figures 2Ai, 2Aii, and 2C; Table 1). An increase in accumulated errors with increasing path length9 or number of steps as recently shown in ants and ball-rolling dung beetles17,18 could possibly increase the positional uncertainty, resulting in the observed transition to a systematic search proportionally earlier when trained over longer distances.

The gradual loss of distance memory compared to a more sudden loss of directional memory fits well with the predictions of a recently proposed computational model of the insect central complex.12 In this model, the home vector is encoded as a sinusoidal activity pattern distributed across a set of putative memory neurons (PFN in *Drosophila*, CPU4 cells in beetles) in the fan-shaped body (upper division of the central body in beetles) of the central complex. The phase of the sinusoidal wave represents the home vector direction, while the amplitude (encoded via firing rate) is proportional to the vector length. The amplitude of the sinusoid that represents the vector length can be expected to decline when approaching home or over time. Directional information, on the other hand, would still be discernible for as long

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**Figure 1. Trajectories and angular positions of the turning points of beetles with an expected vector length of 1.25 m**

(A) Trajectories in shades of orange to black represent the paths traveled by different beetles upon their release in the test area after 2, 10, 30, or 60 min (left to right) immobilization in the dark.

(B) Circular graphs represent the position of the turning points in relation to the normalized homeward direction (0°), gray lines indicate the mean angles, and the associated sectors represent the 95% confidence interval of the data.

(Ai–Aiii and Bi–Biii) After up to 30 min immobilization the beetles still ran in the direction of their fictive burrow but were randomly oriented when released in the test area after 60 min immobilization.

(Aiv and Biv) Adjusting the data (white data points in Biv) according to the 4°/10° azimuthal change of solar position over the 60 min of immobilization did not affect the results. Orange stars indicate the expected positions of the fictive burrows, orange lines the expected vector length of 1.25 m.
as there is sufficient distance information retained in the memory. Similar circuits that also rely on recurrent connectivity for maintained headings have been suggested for Drosophila. Given that the beetles in our experiments could retain the directional component of their home vector for up to an hour, labile synaptic modulations (or some other biological mechanism) that store vector information in a more stable state are also likely to be involved in vector memory formation.

From the first part of the study, we can conclude that both the directional and the distance components of the home vector of S. galenus are stored for periods of time limited to less than 2 h. In a natural context, memory dynamics might show specific adaptations depending on the species-specific foraging requirements. Maintaining vector-based information for longer periods of time might not be vital for S. galenus, which forages over short distances and does not occupy a permanent burrow. The fitness cost of not finding home for these beetles is, therefore, likely to be much less than for other navigators such as ants or honeybees that would not survive without the support systems of their colonies.

**Cold-induced anesthesia significantly affects the beetle’s vector memory**

In the second part of the experiments, we tested the effect of cold-induced anesthesia on the vector memories of the beetles. This was done by placing immobilized beetles (with an expected home vector of 2.55 m) on ice for 10 or 60 min (body temperature lowered to 10.3°C ± 1.8°C and 1.5°C ± 0.7°C [mean ± SD, n = 10], respectively) (Figure S2A). This type of cold treatment can be expected to halt or at least drastically modulate various neural and cellular mechanisms that contribute to memory formation. A gradual reduction in neural temperature has, for example, been shown to result in reduced firing rates and speed of synaptic transmission. When released under the African sun in the same test area as above, the cold-anesthetized beetles started to move away from their point of release after 4.5 ± 2.4 min or 5.5 ± 2.1 min (mean ± SD, n = 14, n = 10) after immobilization on ice for 10 and 60 min, respectively, with no obvious disruption to their normal locomotory behaviors.

**Beetles lost their distance memory before their directional memory after cold-induced anesthesia**

Beetles kept on ice for 10 min and cooled down to an internal temperature of 10°C moved straight in the direction of the fictive burrows (Figure 2Aii). The angular positions of the turning points of these beetles were clustered in the direction of their fictive burrows (Figure 2Bii; Table 1) with no significant difference between the angular distribution of their turning points compared to the turning points of the beetles trained to a feeder at the same distance and immobilized for the same amount of time at ambient temperatures (p = 0.16, Mardia Watson Wheeler test) (Figure 2B). In contrast to the stability of the angular component of the vector, the cold anesthetized beetles initiated their search behavior already at a radial distance of 0.43 ± 0.33 m (n = 14) from their point of release. This is significantly shorter than the 1.01 ± 0.56 m (n = 13, mean ± SD) recorded for the beetles immobilized for 10 min at ambient temperatures (p = 0.003, t test) (Figure 2C) and clearly shows that cold-induced anesthesia had a stronger effect on the distance component of the vector memory than on the angular component. When cooled down to an internal temperature of 1.5°C over a period of 60 min, the released beetles were no longer oriented to a specific (fictive burrow) direction (Figure 2Av; Table 1) and initiated their search at a distance of 0.32 ± 0.20 m (mean ± SD, n = 10) from their point of release (Figure 2C). Both the angular distribution and the radial distance traveled differed significantly from that of beetles immobilized for 60 min at ambient temperatures (p < 0.001, Mardia Watson Wheeler test; vector length, p = 0.004, Mann-Whitney rank-sum test, n = 11) (Figures 2Bii and 2Biv). This demonstrates that cold-induced anesthesia significantly affects the directional and distance components of the beetle’s vector memory, but by different amounts (Figures 3A and 3B).

Although our results do not reveal how or where the home vector is encoded, they clearly demonstrate that a biological mechanism that can be disrupted by cold-induced anesthesia is required to support homing by path integration. Again, the earlier loss of distance memory compared to directional memory fits well with the predictions of the recently proposed computational

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**Table 1. Summary data**

| Foraging distance (m) | Foraging runs | Treatment during immobilization | Duration of immobilization (min) | Vector length (m) | Angular difference from homeward direction (degrees) | V test/* | Rayleigh test | Sample size (n) |
|----------------------|--------------|---------------------------------|----------------------------------|------------------|------------------------------------------------------|--------|---------------|----------------|
| 1.25                 | 7            | ambient temperature             | 2                                | 0.90 ± 0.33      | 1.3 ± 4.3                                            | p < 0.001 | 11            |
| 1.25                 | 7            | ambient temperature             | 10                               | 0.74 ± 0.31      | 349.7 ± 17.1                                         | p = 0.001 | 10            |
| 1.25                 | 7            | ambient temperature             | 30                               | 0.65 ± 0.26      | 15.2 ± 17.8                                          | p = 0.001 | 13            |
| 1.25                 | 7            | ambient temperature             | 60                               | 0.50 ± 0.32      | [blank]                                              | [blank]  | 16            |
| 1.25                 | 7            | ambient temperature             | 120                              | 0.49 ± 0.46      | [blank]                                              | [blank]  | 11            |
| 2.55                 | 7            | ambient temperature             | 10                               | 1.01 ± 0.56      | 9.8 ± 7.6                                            | p < 0.001 | 13            |
| 2.55                 | 7            | ambient temperature             | 60                               | 0.74 ± 0.36      | 351.2 ± 15.3                                         | p < 0.001 | 11            |
| 2.55                 | 7            | cold-induced anesthesia         | 60                               | 0.32 ± 0.20      | [blank]                                              | [blank]  | 10            |
| 1.25                 | 2            | cold-induced anesthesia (retrained) | 10                       | 0.98 ± 0.44      | 343.2 ± 10.4                                         | p < 0.001 | 11            |

Beetles trained to forage over distances of 1.25 or 2.55 m were immobilized in the dark for up to 120 min at ambient temperature or on ice. Vector length defines the radial distance traveled from the point of release in the test area until the turning point, i.e., where the beetle expects to find its burrow. Angular difference from homeward direction is calculated as the angular difference between the turning point and the homeward direction.
Figure 2. Trajectories and angular positions of turning points of beetles with an expected vector length of 2.55 m and traveled vector lengths of beetles with expected vector lengths of 1.25 or 2.55 m

(A and B) Trajectories in shades of green to black represent the paths traveled by different beetles upon their release in the test area after 10 (Ai) or 60 min (Aiii) immobilization in the dark. Trajectories in shades of blue to black represent the paths traveled by different beetles upon their release in the test area after 10 (Aii) or 60 min (Aiv) isolation in the dark, on ice. Circular graphs represent the position of the turning points in relation to the normalized homeward direction (0°/C14), gray lines indicate the mean angles, and the associated sectors represent the 95% confidence interval of the data. After 10 (Ai and Bi) or 60 min (Aiii and Biii) immobilization in the dark or 10 min immobilization in the dark, on ice (Aii and Bii), the beetles still ran in the direction of their fictive burrow but were randomly oriented when released in the test area after 60 min immobilization in the dark, on ice (Aiv and Biv).

(legend continued on next page)
model for the insect central complex. The large spread in vector lengths after 10 min on ice (reaching a body temperature of 1.0°C over 60 min) seems to wipe out the memory entirely (Figures 2 and 3). In other words, as cold-induced anesthesia could possibly cause the firing rates across the memory cells to become uniform, the beetle could no longer navigate in its homeward directions. It is important to note that persistent neuronal activity based on recurrent connectivity is most likely not the only mechanism involved in successful homing by path integration. Short-term synaptic modification, where the strength of individual neural connections rapidly changes to encode new information, is also a plausible mechanism behind the formation and maintenance of a range of different memories.

Even if short-term cold-induced anesthesia is not expected to permanently damage or affect neuronal structure, the possibility still exists that the observed loss of vector memories in the cold-anesthetized group was caused by permanent neuronal damage. To control for this, we reintroduced another group of beetles (trained to forage over 2.55 m) straight back to their burrows after they had been kept on ice for 10 min. These beetles were re-trained to a feeder 1.25 m away in a novel direction and transferred to the test area only after their second visit to the feeder. The cooled-retrained beetles moved straight in the direction of their fictive burrows (V test: p < 0.001) and started their characteristic searches after 0.98 ± 0.44 m (mean ± SD, n = 11) (Figures S2B and S2C). We found no significant difference between this group of cooled-retrained beetles and non-cooled beetles transferred to the test area after 2 min immobilization in either the direction (p = 0.20, Mardia Watson Wheeler test) or the distance (p = 0.76, t test) component of their indicated vectors (Figures S2Bi, S2Bii, and S2C). Our experiments with the re-trained group of beetles clearly showed that the effect of the 10 min cold treatment did not damage the path-integrating circuits (Figures S2B and S2C). In addition, our findings suggest that the vector memory of the beetles is not significantly affected by repeated foraging experience (2 versus 7 foraging trips). This is in line with earlier findings in ants and bees where distance estimates are predominantly based on the animal's most recent outbound trip and do not vary as a function of the number of training trials. A path integration mechanism that is accurate enough from the very beginning might not only protect animals from prolonged exposure to predators, but may also help them not to overuse their memory capacity by comparing or averaging different foraging trips. The effect of repeated foraging trips on homing precision will be more closely addressed in a future study of these beetles.

Overall, our findings provide the first empirical data showing that a biological mechanism that can be disrupted by cold-induced anesthesia is required for maintaining home vector memories. However, as it remains unclear exactly what these mechanisms are, there is still much to be resolved. An interesting future direction would be to understand if, or how, vector memories obtained via short- or long-term synaptic modification are integrated into the neuronal circuit of the (beetle) central complex. Comparative analysis of the structure and function of the central complex circuits of homing beetles and other homing insects that integrate visual information into their path integration system over longer distances can be expected to provide exciting and remarkable findings in this direction and aid our rapidly increasing understanding of the neuronal system behind insect navigation.

**STAR METHODS**

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

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2021.10.067.

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AUTHOR CONTRIBUTIONS

A.Y., M.D., and E.B. designed research; A.Y., M.D., E.B., and M.B. performed experiments; Y.G. designed the software for data analysis; and A.Y. analyzed data. A.Y. and M.D. wrote the manuscript. A.Y., M.D., and E.B. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE                        | SOURCE            | IDENTIFIER                           |
|-------------------------------------------|-------------------|--------------------------------------|
| Deposited data                            |                   | GitHub repository: https://github.com/yakir12/MemoryExperiments |
| Raw video tracks of the beetles obtained during the tests | This study        |                                     |
| Experimental models: Organisms/strains    |                   |                                     |
| Diurnal *Scarabaeus galenus*              | Wild caught       | NCBI Taxonomy ID: 205312             |
| Software and algorithms                   |                   |                                     |
| MATLAB                                    | Mathworks         | Version 2019a                        |
| Julia                                     | Open source       | Version 1.6                          |
| Other                                     |                   |                                     |
| Handycam                                  | Sony, Japan       | HDR-HC5E                             |
| Wide angle conversion lens                | RAYNOX, Japan     | 0.66x                                |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for methods and materials may be directed to and will be fulfilled by the lead contact, Ayse Yilmaz (ayse.yilmaz-heusinger@biol.lu.se)

Materials availability
This study did not generate new unique reagents.

Data and code availability
All statistical analyses and behavioral data are available at https://github.com/yakir12/MemoryExperiments

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethical statement
All applicable international, national and/or institutional guidelines for the care and use of animals were followed. Animal care was in accordance with national/institutional guidelines (EU Directive 2010/63/EU; South African National Standard for The Care and Use of Animals for Scientific Purposes).

Animal Collection and Husbandary
Experiments were performed with the homing dung beetle *Scarabaeus galenus* in its natural habitat at Bergsig Lodge in Bela Bela, Limpopo, South Africa (27.95°E, 24.78°S) in November 2019 and February 2020 (Figures S1A–S1E). Beetles were collected while foraging on antelope middens, kept in plastic bins filled with soil (30 cm x 22 cm x 22 cm) and fed everyday *ad libitum* with fresh antelope and cow dung in preparation for the experiments.

METHOD DETAILS

Effect of time on Path integration (PI) memory
To evaluate if and for how long beetles can maintain the direction and distance components of their home vector, beetles marked individually on their thorax with tipp-ex were trained to forage to a feeder in a designated training area (Figures S1A and S1C), according to a set protocol. The training area was a small dirt road lined with small trees and bushes (Figures S1A and S1C). In regular intervals between 06.30 am and 09.00 am on mornings with clear skies, a total of 15-20 beetles were placed on Petri-dish feeders filled with fresh antelope dung (3-5 beetles per feeder). The beetles soon left the Petri-dishes and about half of them started to dig a burrow within the training area (the others walked or flew away). Once a beetle emerged from its finished burrow, a feeder was placed at a distance of 1.25 m (adapted from Dacke et al.13) or 2.55 m (double the distance including the radius of the Petri-dish feeder) from the burrow and in a similar orientation to the original feeder location. As the beetle started to forage by running back and forth...
between the feeder and its burrow, the homeward direction from the feeder was recorded. As the pellet-carrying beetle attempted to leave the feeder after its 7th visit (establishing that it was navigating accurately\textsuperscript{13}) it was picked up and carefully wrapped with Parafilm M Wrapping Film (Fisher Scientific, #S37440) around its thorax and abdomen. The wrapping allowed for some air exchange in these ground dwelling insects that tolerate low levels of \textit{O}.\textsubscript{2}\textsuperscript{42} The parafilm kept the beetles in a stable position and prevented them from moving their legs, and thereby limiting systematic errors from accumulating in their PI vectors.\textsuperscript{15} Immobilized beetles were individually placed inside opaque tubes (diameter: 2.5 cm, height: 5 cm) and put away for 2, 10, 30, 60 and 120 min in the shade (holding time was randomly assigned but restricted to 10 or 60 min in beetles trained to forage over a distance of 2.55 m). After the isolation, the beetles were unwrapped and individually released at a test area, a small dirt road lined by small trees and bushes 30 m away (ca. 30 s-60 s transition) and oriented in a different direction from the first road (Figures S1A and S1B). Here, the paths traveled by the beetles were recorded from above using a Sony HDR-HC5E Handycam camera fitted with a 0.66x wide angle lens and mounted on a tripod.

**Effect of cold-induced anesthesia on PI memory**

To evaluate how neuronal cooling interferes with stored vector information, we also trained a second group of beetles to forage from a feeder placed 2.55 m from their respective burrows. After making their 7\textsuperscript{th} visit to the feeder, the beetles were wrapped in parafilm and black duct tape (to keep the beetles dry and provide sufficient darkness) and carefully placed inside an ice-filled cooling box (The Coleman Company) for 10 min or 60 min. The cold-anesthetized beetles were subsequently released in the test area, where they soon recovered and walked away from their release point. Again, the paths traveled by the beetles were recorded until a clear turning point was observed (see below).

To evaluate any long-term effects of the cold-induced anesthesia on navigation precision, some beetles (n = 19), were returned directly to their burrows after being cold-anesthetized for 10 min (cooled-retrained). The beetles (11 out of 19) that continued to forage in our training area on the same or the following day, after this treatment, were re-trained to a new feeder placed 1.25 m away, in a compass direction that differed by at least 90° to the one they had been foraging at before the cold-induced anesthesia. Due to the many experimental challenges imposed by this training/cooling/training protocol, these re-trained beetles were picked up as they departed from the feeder already after only their second visit (to avoid losing any experimental animals in this challenging part of the study), and transferred directly to the test area where their paths were recorded as described above. To quantify any long-term effects of the cold-induced anesthesia on navigation precision, we compared the results of this cooled and retrained group of beetles (Figure S2) with the results from the beetles that were immobilized for only 2 min before they were caught and tested. As the two groups of beetles were not allowed to visit the feeder the same number of times (2 versus 7), this analysis also allowed us to briefly evaluate the effect of repeated foraging experience on vector memories.

**Measurements of internal body temperature**

The internal body temperature of 20 beetles wrapped in parafilm and black duct tape was measured over a period of 60 min by inserting a temperature probe (Type K connected to Tenmars Thermometer TM-82N) inside the last abdominal segment (the pygidium). The measurements were carried out simultaneously on two beetles of similar body size (thorax width, 15 mm - 17.5 mm), one placed in the ice-filled cooling box as described above and one on top of the box as a control for any probe-induced changes in temperature.

**Data Analysis**

The filmed beetle trajectories were tracked using a custom-made software (dung track) integrated in MATLAB (Mathworks) and converted to real world distance coordinates as described in Dacke et al.\textsuperscript{13} The tracks traveled by the beetles were then further visualized and analyzed in Julia.\textsuperscript{16} A parametric spline with a factor of 500 and an order of two were used to smooth the coordinates of the beetle’s trajectory. During this step, the erratic movements of the beetle as well as minor errors from the manual tracking of the beetles were minimized. For ease of comparison and illustration, the relative position of the fictive burrows in relation to the feeder was rotated into the same frame of reference for all runs of all beetles (directly up in Figures 1 and 2).

The **vector length** for each beetle was calculated as the radial (shortest) distance from the release point (that is, where the beetle was placed on the ground) to the point along its track where the beetle changed its direction of travel by a minimum of 60° and initiated a searching behavior. This turning point indicates the expected position of the burrow.\textsuperscript{13} Vector length and the angular position of the turning point in relation to the beetles’ homeward direction were used as a measure of navigational performance. To further define the accuracy in the beetles’ estimate of direction, we also calculated the mean vector angle (\(\mu\)) and the mean vector length (\(r\)), for each experimental group. From these two measures, we could then calculate the **length of the home vector component**\textsuperscript{16}, that is, the vector length in the homeward direction. To define the accuracy also in the distance component of the home vector, the vector lengths were divided by the expected length of the vector (1.25 m or 2.55 m).

The direction and distance component of the beetle’s home vector was analyzed using Oriana 4.0 (Kovach Computing Services, Anglesey, UK) and Sigmaplot (Systat Software, San Jose, CA, USA), respectively. Rayleigh tests were used to test for the uniformity of the circular distribution of turning points, while V-tests were used to determine whether the angular distributions of turning points – if significantly different from random – were centered on the fictive burrow at 0°. Mardia-Watson Wheeler tests were used to compare the angular distributions of the turning points of the beetles tested under different conditions. One sample t tests, Mann-Whitney rank
sum tests or one-way ANOVAs (depending on the normality test results and number of test groups) were used to compare vector lengths between conditions. A post hoc Holm-Sidak test was used for pairwise comparisons. As a measure for how rapidly the distance and directional components of the home-vector of the beetle decayed, an exponential decay function \( y = ae^{-\lambda x} \) was fitted to the accuracy data (Sigmaplot, Systat Software, San Jose, CA, USA). From this, the exponential time constant (\( \tau, t \)) was calculated \( \left( \frac{1}{\lambda} \right) \).