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Compensation to visual impairments and behavioral plasticity in navigating ants

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Desert ants are known to rely heavily on vision while venturing for food and returning to the nest. During these foraging trips, ants memorize and recognize their visual surroundings, which enables them to recapitulate individually learned routes in a fast and effective manner. The compound eyes are crucial for such visual navigation; however, it remains unclear how information from both eyes are integrated and how ants cope with visual impairment. Here, we manipulated the ants' visual system by covering one of the two compound eyes and analyzed their ability to recognize familiar views. Monocular ants showed an immediate disruption of their ability to recapitulate their familiar route. However, they were able to compensate for this nonnatural impairment in a few hours by engaging in an extensive route-relearning ontogeny, composed of more learning walks than what naïve ants typically do. This relearning process with one eye forms novel memories, without erasing the previous memories acquired with two eyes. Additionally, ants having learned a route with one eye only are unable to recognize it with two eyes, even though more information is available. Together, this shows that visual memories are encoded and recalled in an egocentric and fundamentally binocular way, where the visual input as a whole must be matched to enable recognition. We show how this kind of visual processing fits with their neural circuitry.

desert ants | route-following | monocular | compound eyes; compensation

Significance

We reveal a surprising rigidity in the way ants encode the visual world. Scenes learned with two eyes can only be recognized with two eyes and scenes learned with one eye can only be recognized with one eye (the same eye), showing that ants store egocentric inputs rather than a spatial model of the world. However, this rigidity is compensated by a remarkable behavioral flexibility. Upon covering (or uncovering) one eye, ants—which can no longer recognize their familiar surroundings—do not remain dysfunctional for long. They engage in a step-by-step learning process to store the novel visual inputs in a parallel memory and resume normal foraging activity in a matter of hours.

Self-organized living beings and engineered machines are both able to fulfill their tasks reliably. However, living organisms show flexibility in the way they achieve their functions and can often compensate for unexpected impairments, whereas machines cannot (1). The aptitude for compensation has been well studied in humans with regard to impairments such as cognitive pathologies, aging, or brain damage (2, 3), and is evident after morphological impairments, such as when one manages to achieve with one hand what one used to do with two. These forms of compensations require time and likely involve neural rewiring, so-called structural or network plasticity (4, 5).

In insects, which are often assumed to be less versatile than vertebrates (6, 7), the ability to compensate for impairments is usually studied as an instantaneous response and viewed as the product of the evolved natural redundancy and robustness of these systems, rather than the result of neural plasticity through a life time. For instance, the change in gait following a single or double leg amputation may be interpreted as a robust and "spontaneous" response of the neural machinery governing leg coordination [e.g., in ants (8), cockroaches (9), stick-insects (10), and other arthropods like crabs (11)]. Robustness through redundancy in insects is also well appreciated in the context of navigation. For instance, representation of directions is based on the integration of a vast array of sensory cues such as visual terrestrial cues (12–15), multiple celestial cues (16–20), olfactory cues (21, 22), wind cues (23, 24), magnetic cues (25, 26), and also self-motion cues (27). Hence, depriving a navigating insect from one modality—or all modalities but one—does not necessarily disrupt their ability to orient. Unilateral suppression of one eye input, however, has a direct impact on the navigational performance of ants (28, 29). Monocular ants may still show evidence of recognition of learned terrestrial cues but their navigational behavior is drastically affected (28, 29). For instance, monocular desert ants that have learned a landmark array with one eye only are incapable of recognizing it if the eye cap has been swapped to the other eye, suggesting that there is no interocular transfer of visual terrestrial cues (28). Here again, these studies focused on the insects' response that immediately follows the manipulation. But whether some plastic, compensatory mechanisms are at play, and given time, can enable a recovery of a functional behavior remains unknown.

Here, we investigated this question by conducting various eye-capping manipulations on visually navigating desert ants (*Cataglyphis velox*). Both, their immediate response as well as the potential compensatory effects emerging after a longer period of time were observed and

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analyzed. The results show that any visual manipulations via eye-capping caused immediate, substantial impairments that disrupted the ant's ability to walk straight, and to home. However, a few hours upon the visual impairment, ants recovered a functional navigation behavior, indicating the existence of a profound plasticity and behavioral flexibility. We explored the mechanisms underlying this plasticity and, as a corollary, gained insight into the way visual information is stored in their brain.

Results and Discussion

One-Eye-Capping Disrupts Learned Route-Following. Iberian desert ants (*C. velox*) were individually marked and let free to navigate back and forth along an 8.0 m long route between their nest and a feeder (Fig. 1*A*). The surrounding natural landscape provided plenty of visual information and the route floor was covered with a 1.2 m wide wooden board ensuring an even substrate for the navigating ants. Once experienced to the route (after at least 10 foraging trips), ants were captured at the feeder one by one for the eye-cap treatment: either their left or right compound eye was covered with opaque paint. The eye-capped ant was then provided with a food item, released near the feeder and her homing path was recorded. For illustrative purposes the paths of right eye–capped ants were mirrored and pooled with the paths of left eye–capped ants in Figs. 1–5. Both right and left eye–capped paths can be seen in *SI Appendix*, Fig. S1. Regardless of the side of the eye cap, ants failed to navigate back to their nest. Their initial direction showed a bias toward the side opposite to

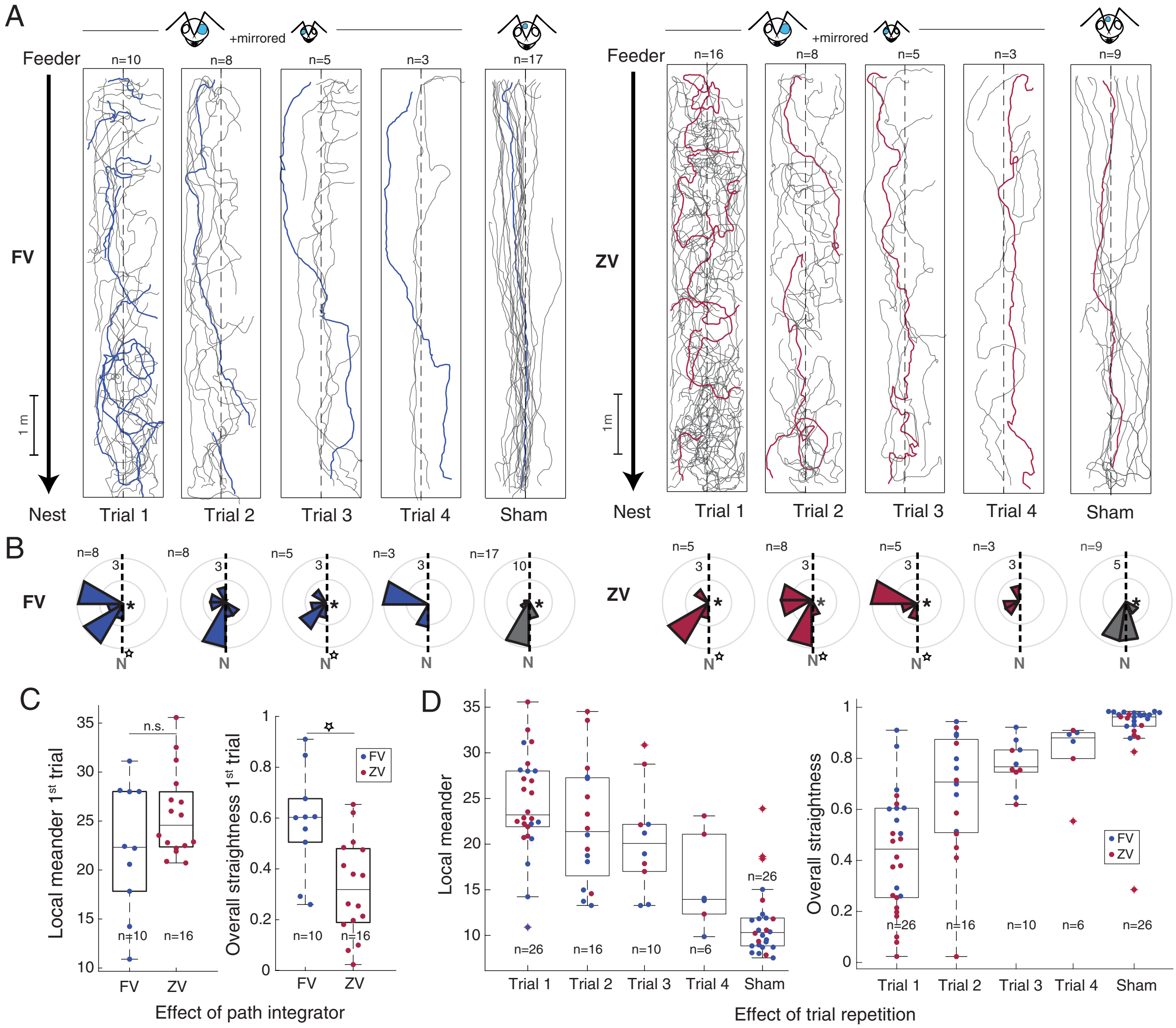

Fig. 1. Route recovery of eye-capped ants over trials. (*A*) Homing paths of ants on a familiar route after covering one eye, from trial 1 to 4. Ants with the left or right (mirrored path) eye covered are pooled (see *SI Appendix*, Fig. S1 to see left and right eye–capped ants separately). Sham groups correspond to ants with uncovered eyes that received a dot of paint somewhere else on the head. Ants were released at the feeder (*Top*) and homed toward the nest (*Bottom*), either with path integration (PI) information [full vector (FV), *Left* panel] or without [zero vector (ZV), *Right* panel]. All ants had previously learned the route with both eyes. One example path is highlighted in color for each condition. Interruptions of the trajectories indicate that the ant ran off the route board and was thus captured and replaced on it. Black arrows indicate the travel direction of the homing route. Dashed lines indicate the route beeline. (*B*) Initial headings at the onset of the homing route (same conditions as in *A* above, with left or right (mirrored path) eye covered ants pooled together). Circular histograms depict headings distribution after 0.2 m of travel. Dashed lines indicate the route beeline with the nest at the bottom, the left side correspond to the open eye direction. Numbers at the outer rims indicate the scale in term of number of ants per sector of 30°. Significant differences from random distributions are indicated with a star (Rayleigh test) at the center of the diagrams. Significant differences away from the nest direction (N) is indicated with an open star (S-test; see *Material and Methods* and *SI Appendix*, Table S1 for statistical details). Side biases appear in both FV and ZV ants but not in Sham controls. (*C*) Local meander and overall straightness of FV and ZV ants during their first homing trial with an eye-cap. Higher overall straightness and lower local meander in FV ants indicate a beneficial effect of PI on route following. (*D*) Local meander and overall straightness of eye-capped ant paths across their first four trials. Increase of overall straightness and decrease of local meander across trials indicate a rapid recovery of route following toward sham group performance.

the eye cover (Fig. 1*B*) as previously reported in Tunisian desert ants (28). All eye-capped ants (10 out of 10) veered sidewise and fell off their usual route corridor, defined by the wood plank on the floor (Fig. 1*A*). To enable them to reach home, ants falling off the wood boards were systematically captured and rereleased back onto the route beeline (Fig. 1*A*, dashed line) where path recording resumed. Eye-capped ants veered off the boards 3.42 times on average before reaching their nest surrounding, where recording was stopped (Fig. 1*A* and *SI Appendix*, Fig. S1). The difficulty to head home was obvious along the complete homing path (Fig. 1*A*). By comparison, Sham ants, which received a paint mark on the head but untouched compound eyes and ocelli, showed clearly oriented (Fig. 1*B*) and much straighter paths (Fig. 1*A* and *SI Appendix*, Fig. S1) and almost never left their route corridor (1 out of 17 ants fell one time, so a probability of 0.06 time/ants on average). Thus, covering one compound eye drastically affects navigation in homing ants.

The compound eyes of ants extract information from both, celestial compass cues—which are key for PI—and terrestrial cues—which are key for learned views during route-following (30). To test whether the behavioral defect observed in the eye-capped ant is due to a disruption of the PI system or the use of learned views, the experiment was repeated by using this time so-called ZV ants. While FV ants are captured at the feeder, ZV ants were captured on their way home just before entering their nest, then received an eye-cap and were released with their food item right near the feeder. The PI vector of a ZV ant does no longer point toward the nest; hence the ant can solely rely on learned terrestrial cues for homing (31). As for FV ants, covering one eye of ZV ants strongly disrupted their ability to navigate home (Fig. 1*A*). Their initial directions were also biased toward the open eye side (Fig. 1*B*) and all of them (16 out of 16) repeatedly ran off their usual route corridor (4.43 times/ant on average) to the contrary of ZV sham ants (0.33 times/ant on average). Their paths showed significantly less overall straightness (Anova: $F_{1,24} = 10.7$, $P = 0.003$) but only marginally more local meander (Anova: $F_{1,24} = 2.42$, $P = 0.133$; Fig. 1*C*) than eye-capped FV ants. Consequently, eye capping strongly impairs the use of learned terrestrial cues and the directional input provided by the PI is helping only slightly the FV eye-capped ants to maintain straighter paths. This is in line with previous evidence that information based on celestial but not terrestrial compass cues undergoes interocular transfer (28).

Eye-Capped Ants Spontaneously Recover Their Route-Following Behavior. Surprisingly, within a few hours, some of the tested eye-capped ants reoccurred at the feeder again, which provided the opportunity to record their subsequent homebound trips. With increasing homing trials, eye-capped ants gradually recovered their navigational efficiency (Fig. 1). To ensure this recovery was not simply due to an increased reliance on PI, eye-capped ants reaching their nest were captured as ZV ants and released again at the feeder for a second run home. Whether as FV- or ZV ants, their initial direction upon release still tended to be biased toward the open eye side (Fig. 1*B*; see *SI Appendix*, Table S1 for circular statistics). However, both FV- and ZV paths showed progressively less local meander (Anova: $F_{0.1,49.4} = 25.170$, $P < 0.001$) and more overall straightness (Anova: $F_{1.1,51.3} = 32.52$, $P < 0.001$; Fig. 1*C*). During the fourth homing trial the path overall straightness of eye-capped ants resembled the one from the sham ants (Anova: $F_{1,30} = 2.68$, $P = 0.112$), and even though they still showed slightly more local meandering (Anova: $F_{1,30} = 5.39$, $P = 0.027$; Fig. 1*C*), most eye-capped foragers (5 out of 6) managed to home while no-longer exiting their route corridor. Within a relatively short time period, eye-capped ants can thus recover the ability to follow their familiar route again, using terrestrial cues. These insects are therefore able to compensate impressively fast for the strong impairment caused by losing the visual input of one eye, showing a remarkable plasticity in their navigational capacities.

Eye-Capped Ants Show a Persistent Lateralized Sensory-Motor Defect. The fact that the initial direction of both FV- and ZV ants upon eye-covering was biased toward the open eye side as compared to the correct route direction (Fig. 1*B*) provides two insights. First, because ZV ants did not head randomly (or backtracked) like they usually do on visually unfamiliar terrain (32), freshly eye-capped ants must still be able to derive some information from their visual route memory. Second, because the side of the directional bias depends purely on the side of the capped eye (Fig. 2 *A* and *C*), a lateralized sensory-motor defect

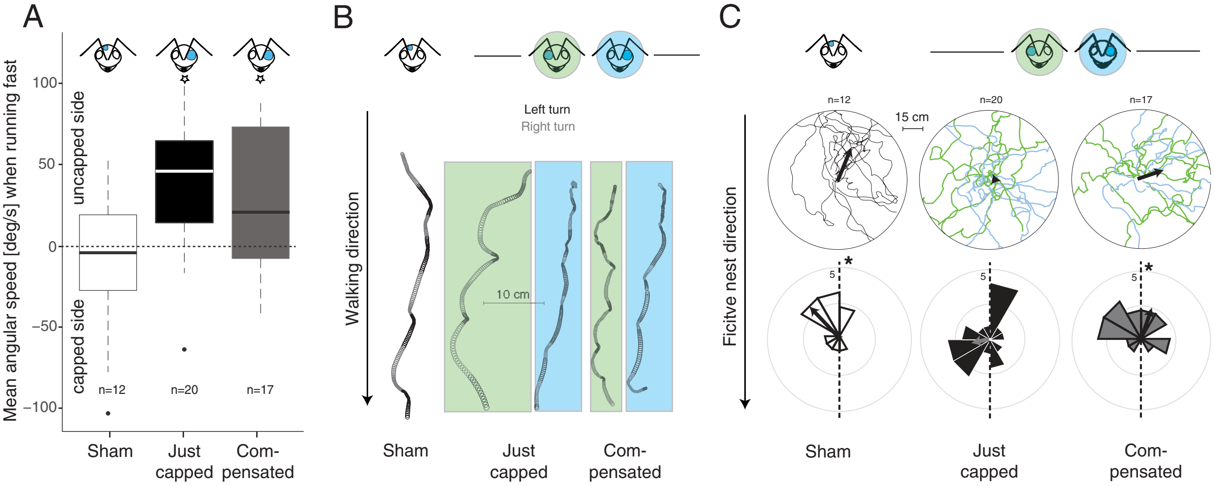

Fig. 2. Effect of eye capping and experience on course control when released in unfamiliar surroundings without PI homing vector (ZV ants). Ants were released on unfamiliar terrain either just after receiving an eye cap (Just capped); after having learned to recapitulate a route with one eye cap (Compensated) or ants receiving a sham painting on the head (Sham). (*A*) Course control in eye-capped ants shows a lateralized bias along their oscillatory cycle that can be highlighted by a tendency to turn toward the uncapped eye side when walking forward. Stars indicate significant difference to a balanced average angular velocity (0 deg/s), one-sided Wilcoxon test (see text for details). (*B*) Example paths of ants with (Just capped, Compensated) and without (Sham) the characteristic course-control deficit caused by eye-capping. (*C*) Tracked paths of Sham, Just capped, and Compensated ants (*Upper* panels) and initial headings at 30 cm (*Lower* panels) upon release in unfamiliar terrain. Black arrows depict the mean vectors of the heading distributions. Significant orientation opposite to the nest compass direction (i.e. so-called backtracking) is indicated with a black star (V-tests: Sham $P = 0.047$; Just capped $P = 0.390$; Compensated $P = 0.013$).

caused by losing one-eye input—notably, a unilateral loss of visual motion (33)—may well impact course control as observed in the lab with this ant species (34).

To investigate this lateralized sensory-motor defect independently of the expression of the recognition of visual memories, freshly eye-capped ants were released in unfamiliar surroundings and their behavior was analyzed based on video recordings. The typical oscillatory movements of ants (35) were altered. Eye-capped ants tended to regularly alternate between brief bursts of speed when turning toward the open eye side and pauses by rotating on the spot toward the covered eye side (Fig. 2*C*). This can be simply quantified by assessing whether the turning direction is biased on one side when the ants forward speed is above the individual's average. While sham ants with both eyes open showed no bias (Sham ants: one-sided Wilcoxon test: $V = 32$, $P = 0.715$, Fig. 2*A*) freshly one-eye capped ant turned more toward the open eye when walking fast (Just-capped ants: one-sided Wilcoxon test: $V = 188$, $P = 0.001$; Fig. 2*A*).

To test whether the observed recovery of route-following behavior is due to a compensation over time of such a lateralized sensory-motor defect, we tested whether the bias persisted in ants after they had recovered their route-following behavior with one eye. Eye-capped ants that had recovered their route still displayed the lateralized defect on unfamiliar terrain (Compensated ants: one-sided Wilcoxon test: $V = 113$, $P = 0.044$, Fig. 2), showing that their route recovery is due to a process that is different from overcoming such a sensori-motor defect. The persistence of this sensori-motor defect may explain, however, while one-eye ants having recovered their route showed more local meandering than sham ants (Fig. 1).

Eye-Capped Ants Compensate by Reengaging in a New Route Ontogeny. We next investigated whether the recovery of route-following behavior in eye-capped ants is based on the ability to eventually recall previous binocular memories, or alternatively, based on the formation of novel, monocular route memories. To do so, we covered the left or right eye of a new cohort of experienced, individually marked ants, and released them back to their nest. Their behavior was recorded once they emerged outdoors again. Upon leaving their nest entrance, these freshly eye-capped ants displayed tight, meandrous paths around their nest entrance (Fig. 3) reminiscent of so-called learning walks (LWs) as observed in naïve ants (36–39).

These convoluted paths and pirouettes enable ants to expose their gaze in multiple directions for visual learning (39). LWs are here likely a consequence of perceiving an unfamiliar scenery when leaving the nest. Indeed, experienced ants with two eyes may also display a few zigzags and pirouettes upon departure if an alteration of the visual surrounding has occurred around the nest; but in general, they rapidly scoot along their familiar outbound route

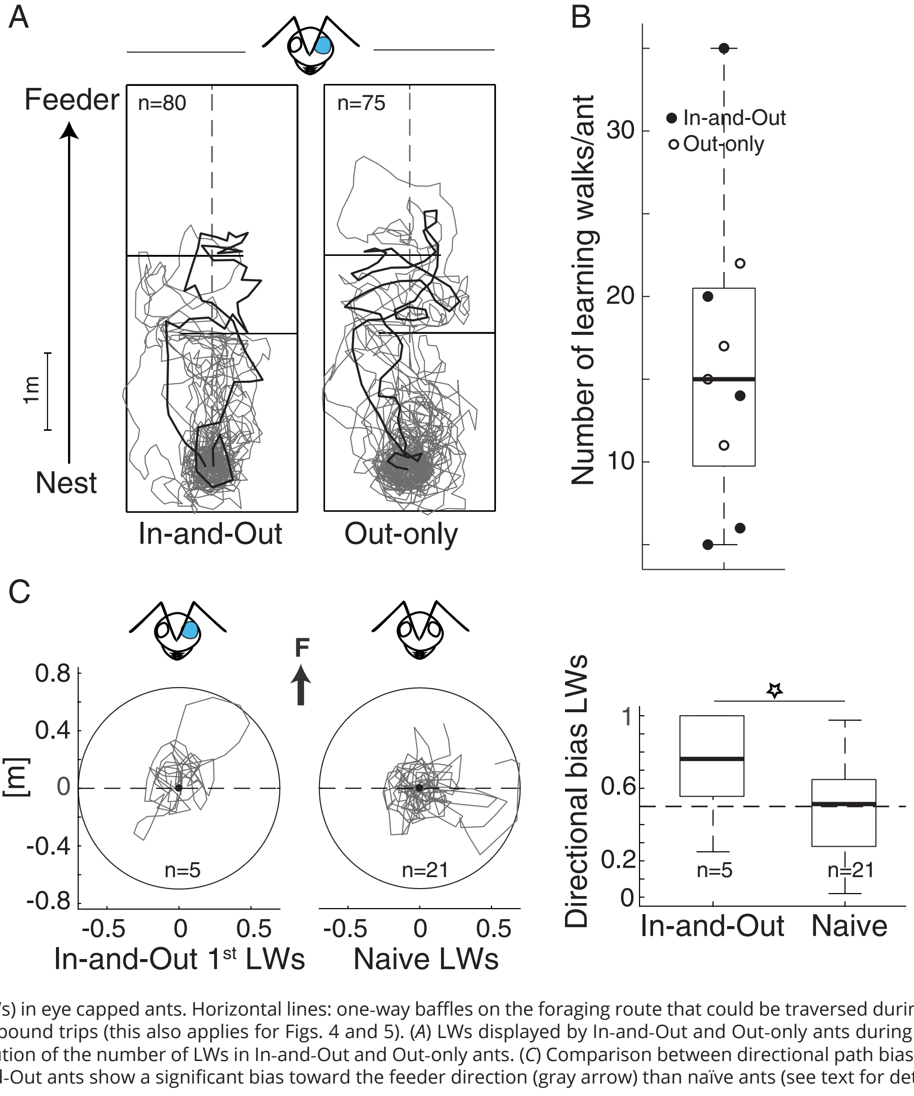

Fig. 3. Learning walks (LWs) in eye capped ants. Horizontal lines: one-way baffles on the foraging route that could be traversed during outbound trips but had to be negotiated during inbound trips (this also applies for Figs. 4 and 5). (*A*) LWs displayed by In-and-Out and Out-only ants during training. Black bold lines: example paths. (*B*) Distribution of the number of LWs in In-and-Out and Out-only ants. (*C*) Comparison between directional path bias of first LWs in In-and-Out ants and naïve ants. In-and-Out ants show a significant bias toward the feeder direction (gray arrow) than naïve ants (see text for details).

again (38, 39). Here, the eye-capped ants remained at first very close to the nest and reentered their colony often, like naïve ants do (Fig. 3*C*) (28, 36). They displayed on average more than 15 (up to 35 in one individual) subsequent learning trips before reaching the familiar feeder located 5.0 m away (Fig. 3 *A* and *B*), which is more than the one to six LWs usually observed in this (40) or other related species (36). This supports the idea that the scenery appeared strongly unfamiliar to these eye-capped ants, and perhaps that visual information for route following is longer to acquire with one eye input. Interestingly and contrary to naïve ants, LWs of freshly eye-capped ants were biased toward the feeder direction (Fig. 3*A*); this was true from the first LWs onward (Fig. 3*C*, One-tailed *T* test: $P = 0.019$) indicating that previous memory of the feeder direction persisted despite the eye-cover. Whether this directional memory was due to remnant memories of terrestrial cues learned with both eyes, or the expression of a stored food-ward celestial compass vector (41, 42) could not be disentangled here.

The subsequent out- and inbound (i.e., homing) trips of these eye-cap ants were also recorded, which, as expected, showed gradual improvements (*SI Appendix*, Figs. S3 and S4). After eight successful trips between the nest and feeder, all the recorded eye-capped ants (In-and-Out) had fully recovered their ability to run between the nest and the feeder without colliding into baffles (Fig. 4*A*). Tested as ZV ants, these individuals could home equally well, (Fig. 4*A*) showing as previously, that they could use learned terrestrial cues.

To ensure that this recovery was actually due to performing LWs, and not simply due to the time passed while navigating outdoors, the experiment was replicated by using two additional cohorts of freshly eye-capped ants that had previous experience (with both eyes) of the route. In one group (In-only), eye-capped ants were systematically captured upon exiting the nest and released at the feeder for homing, preventing them to display LWs around the nest and outbound trips to the feeder. In the other group (Out-only), eye-capped ants were free to display LWs but upon reaching the feeder, these ants were systematically captured and released inside their nest entrance, preventing them to perform their homing runs (inbound trips). This latter group of ants (Out-only) displayed similar LWs than the previous condition (Fig. 3), and after eight successful outbound trials up to the feeder, foragers were able to home quite well, albeit not as successful as ants that had experienced both out- and inbound trips (Fig. 4 and *SI Appendix*, Fig. S3). This shows that inbound trips are helpful but not crucial for route recovery. Contrastingly, ants deprived of LWs (In-only) showed no improvement in their homing ability despite spending a long time navigating outdoor (Fig. 4). On the contrary, they showed a decrease in homing performance across trials (*SI Appendix*, Fig. S3), suggesting again that freshly eye-capped ants have strongly impaired but remnant binocular visual memories of the route, but that subsequent experience outdoor does not enable to reaccess them, or form new functional ones. Note that In-only ants, as being transferred from the nest to the feeder, could not rely upon PI. After eight attempts of homing without LWs, these ants searched at length around the release point (feeder) and only one individual eventually managed to home (Fig. 4). It was rather difficult to conduct this condition, as In-only ants mostly failed to reach their nest during training. After 10 min of search and an unsuccessful inbound trip the foragers had to be put back manually to the nest. Unfortunately, many of those individuals stopped their foraging activity and hence could not be tested anymore.

In sum, performing LWs and outbound trips is crucial for eye-capped ants to recover their route. This echoes what is observed in naïve ants with untouched eyes, for whom LWs and outbound trips are key for learning an inbound route, while inbound experience may help but seems to be insufficient on its own (43–47). Together, this suggests that eye-capped ants cannot recognize properly the route learned with both eyes and thus perceive at first the world as quite unfamiliar. This unfamiliarity triggers numerous, dense LWs upon leaving their nest, which enables them to steadily form novel memories of the terrestrial cues and eventually relearn the route monocularly. Therefore, ants with previous extensive foraging experience, and after a nonnatural treatment preventing them to walk straight and recognize the familiar surroundings, retain the flexibility to reengage in a complete ontogeny of route learning.

Ants Learn Binocular Visual Memories. Previous behavioral work in ants and bees has shown that visual memories learned with one eye cannot be retrieved using the other eye, suggesting that these insects form two separated visual memories for each eye, with an

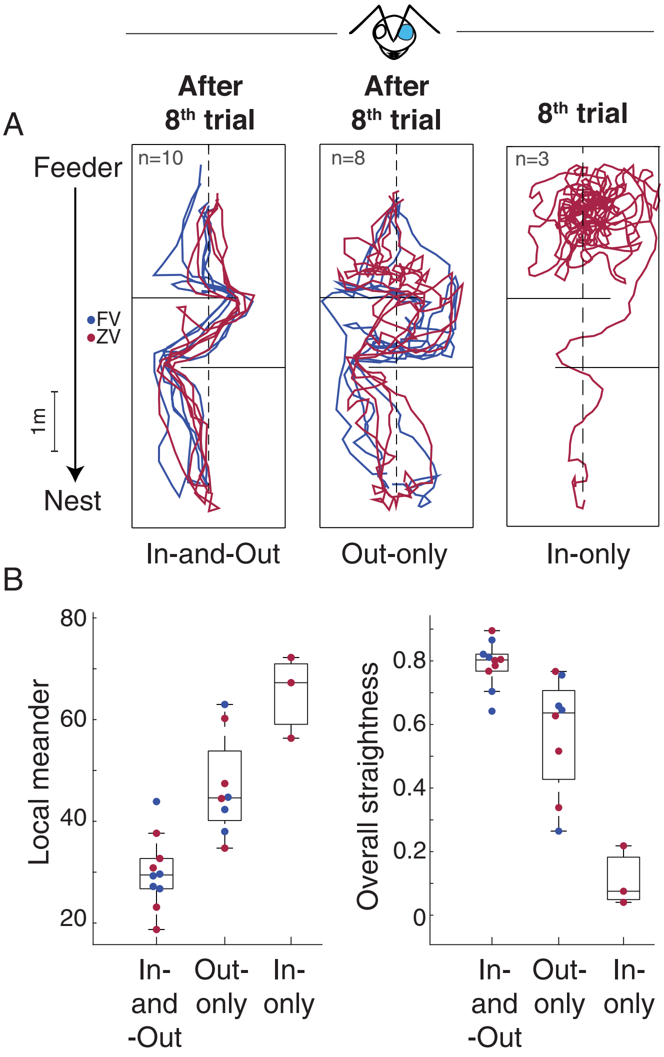

Fig. 4. Paths and path characteristics of eye capped ants after compensation with inbound and outbound trips (In-and-Out), no inbound trip (Out-only), and no outbound trip (In-only). (*A*) Paths of homing ants after 8 training trials. All In-and-Out and Out-only ants were able to home whereas In-only ants struggled and only one individual succeeded (see *SI Appendix*, Fig. S3 for trajectories of all trials). (*B*) Local meander and overall straightness of homing paths after eight training trials. In-and-Out ants displayed paths with the lowest local meander (Anova: In-and-Out vs. Out-only: $F_{0.29,0.07} = 4.122$, $P = 0.001$; In-and-Out vs. In-only: $F_{0.63,0.11} = 5.981$, $P < 0.001$) and highest level of overall straightness (Anova: In-and-Out vs. Out-only: $F_{-0.02,0.06} = -3.436$, $P = 0.003$, In-and-Out vs. In-only: $F_{-0.69,0.09} = -7.327$, $P < 0.001$), followed by Out-only and In-Only ants.

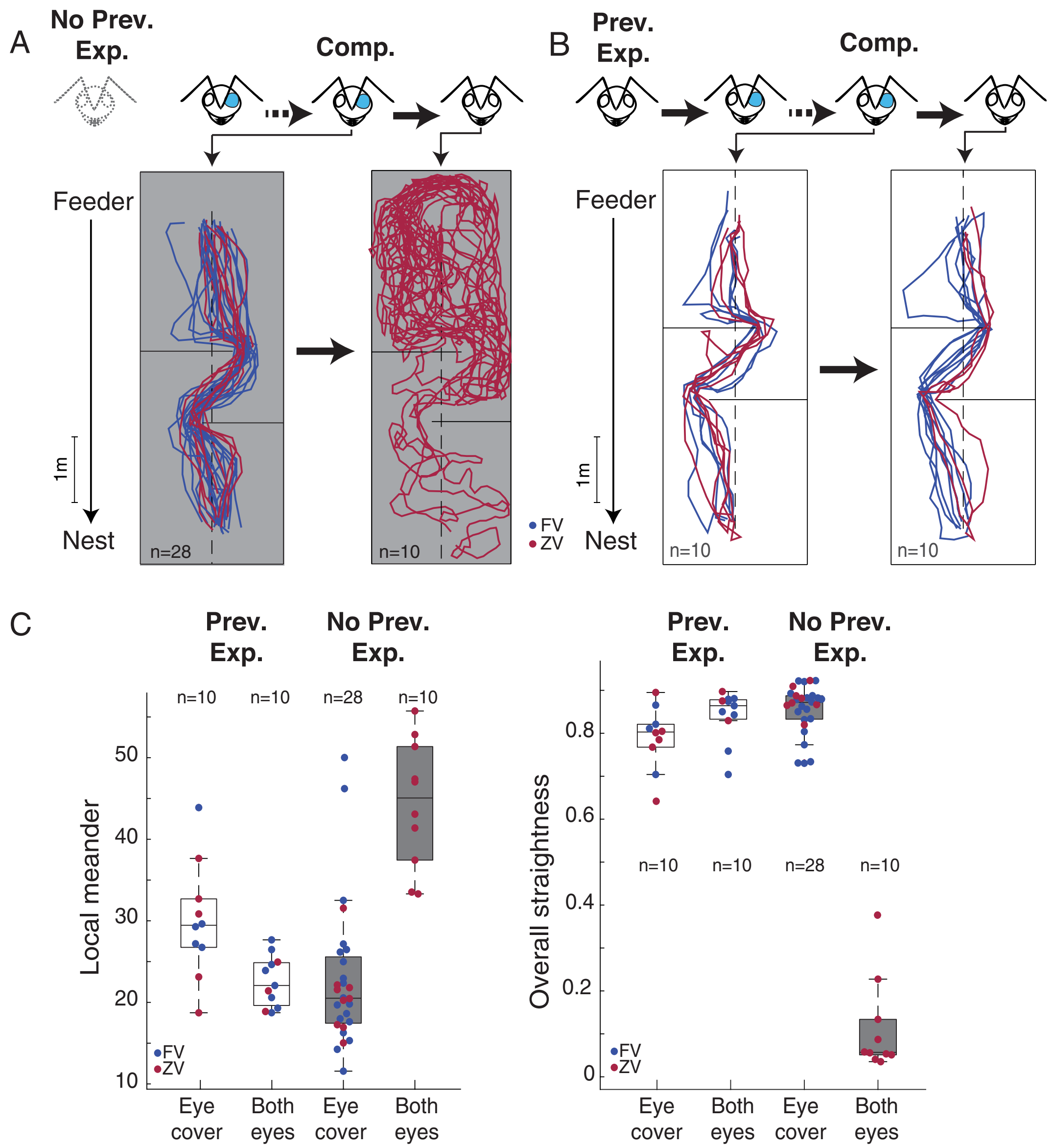

Fig. 5. Route learned with one eye cannot be recognized with two eyes. (*A*) Ants having learned the route with one eye covered, and without previous experience of the route with uncovered eyes (no prev. exp.). After compensation (comp.) and reaching the feeder, homing paths are displayed for ants with the eye cap still in place (*Left* panel) and with uncapped eyes (*Right* panel). Ants without previous experience of the route struggle to find back to the nest once the eye cap is removed. Only 2 ants out of 10 eventually found their nest. (*B*) Ants having learned the route with both eyes, hence with previous experience of the route before eye capping (prev. exp.), and then having relearned the route with one eye cap (comp.). After compensation and upon reaching the feeder, homing paths are displayed for ants with the eye cap still in place (*Left* panel) and the eye cap removed (*Right* panel). Ants with previous binocular experience of the route have no difficulties to recapitulate their route with two eyes again. (*C*) Path characteristics of ants with and without previous experience of the route before eye capping. Ant with previous experience show paths with low local meander and high level of overall straightness after eye cap was removed, whereas ants without previous experience of the route show paths with high local meander and low level of overall straightness with both eyes uncovered (Anova local meander: $F_{-0.13,0.05} = -2.487$, $P = 0.018$, Anova overall straightness: $F_{0.07,0.02} = 3.281$, $P = 0.002$).

absence of interocular transfer between these visual memories (28, 48). The current study revealed that visual memories acquired with both eyes cannot be retrieved with one eye. Rather than two separated visual memories for each eye, one possible explanation is that visual memories are fundamentally binocular, that is, their recall implies the correct and simultaneous combination of both left and right visual input. Indeed, neurobiological studies show that each eye sends bilateral visual projections to the Kenyon cells (KCs) in both the left and right hemispheres of the mushroom bodies (MBs) (49), where visual memories for route-following are formed (50–52). What's more, visual projection to the KCs are pseudorandom; therefore, it seems quite likely that individual KCs, whether in the left or right hemisphere, receives input from both eyes, and thus must receive the correct bilateral input to be activated (Fig. 6). If this hypothesis is correct, it predicts, that memories acquired with one eye could not be retrieved with both eyes.

To test this prediction, a novel cohort of ants from a new nest was eye-capped either on the left or the right eye. Crucially, the training to the foraging route and the transformation of the visual scenery by clearing the floor and altering the natural bushes and other terrestrial cues around the route was done afterward. The set-up was similar to the former experiment: a 5.0 m long route containing two baffles in the middle and one feeder at the end. These ants had binocular memories of the previous natural surroundings but experienced the foraging route and its novel surroundings only with an eye-cap (i.e., monocular).

After a few hours, the eye-capped ants were familiarized with the new surroundings, discovered the feeder, and learned to home successfully along the novel route (Fig. 5*A*). When captured at the nest

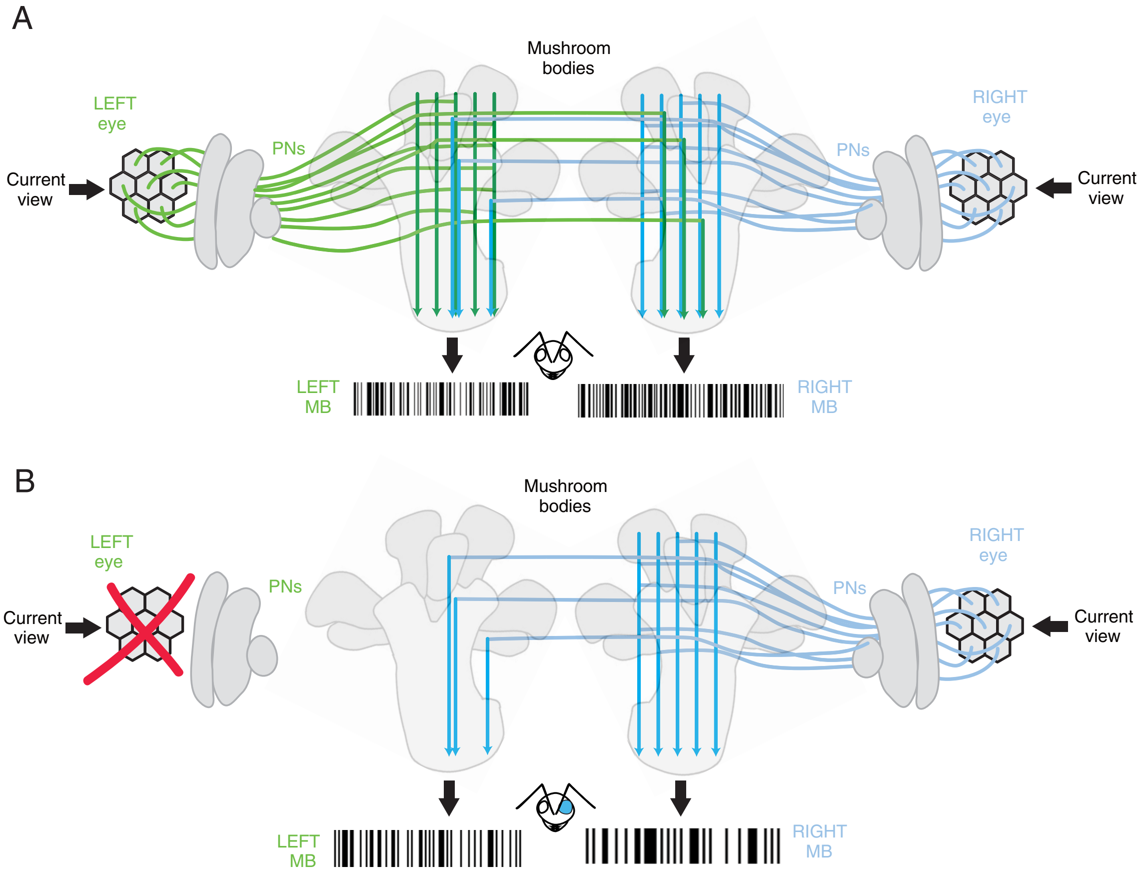

Fig. 6. Simplified model depicting a possible neurobiological implementation. (*A*) Both compound eyes send projection neurons (PNs) to KCs in both ipsi- and contralateral MBs. KCs receiving inputs from both eyes respond to coincidental inputs of both eyes simultaneously (symbolized by the barcodes), perceived along a familiar route. (*B*) If one eye is covered (or uncovered), the same scene viewed from the exact same viewpoint will now produce a different KCs activation pattern in both MBs, preventing the recognition of previously stored route memories. Note that some KCs may receive input from one eye only and may therefore provide a partial familiarity of the scene across manipulations.

and released again as ZV ants near the feeder, these now experienced eye-capped foragers had no difficulties to recapitulate the route, confirming that they had formed (monocular) visual memories of the terrestrial cues (Fig. 5 *A*, *Left* panel). However, when uncapped, that is, when exposed for the first time to this particular route with two eyes, the ants struggled immensely to home (Fig. 5 *A*, *Right* panel). All uncapped ants searched predominantly in the upper section of their foraging route incapable of negotiating the baffles, only 2 out of 10 eventually managed to reach the nest entrance (Fig. 5 *A*, *Right* panel). Even when some (5 out of 10) of these ants were rereleased beyond the baffles, closer to the nest, they were not able to home successfully and wandered around seemingly without purposeful orientation. Thus, ants with two eyes were unable to recognize the route learned with one eye. In other words, adding the visual input of the second eye prevents the access to the visual memories acquired with one eye only. Therefore, ants do not form distinct memories for each eye, but interpret the egocentric visual input to both eyes as a fundamentally binocular impression.

In terms of neural implementation, this supports the idea that many KCs in the MB receive input from both eyes simultaneously (Fig. 6). It remains likely however that some KCs receive input from one eye only, which could explain why some freshly eye-capped ants, or freshly uncapped ants, even though strongly impaired, could still derive a rough estimation of the nest direction, as if they recognized the scene only partially or sporadically. The variation in homing performance observed across individuals may be explained, at least in part, by the more or less fortunate random connectivity in their MB.

Eye-Cap Ants Do Not Forget Past Binocular Memories. To control whether the visual impairment observed with freshly uncapped ants (Fig. 5 *A*, *Right* panel) was indeed due to an inability to access visual memories sorted with one eye and not just an inherent consequence of the recent recovery of bilateral visual input, the previous experiment was rerun with a cohort of ants that, this time, had previous two-eyed experience of the route. These ants were eye-caped, let free to relearn the route with one eye, then "uncapped" and tested. Contrary to the previous cohort of ants with no previous experience of the route, these foragers did not struggle whatsoever to home toward the nest with uncapped eyes, even when tested as ZV ant (Fig. 5*B*). This shows that uncapping the ants bears no inherent issue and thus confirms that memories acquired with one eye are no longer retrieved with two eyes (Fig. 5*A*). In addition, it shows that the latter cohort of ants had not forgotten their former visual memories of the route acquired with two eyes. Learning the route anew with one eye does not override the memories of the previous two-eye memories.

In neurobiological terms, memories acquired with one eye vs. two-eyes are likely to recruit different set of KCs in both hemispheres, with perhaps a certain amount of overlap between them (Fig. 6). This can be viewed as learning and remembering two "mostly" different routes, which desert ants can also do (53). Indeed, ants possess hundreds of thousands of KCs (49) and models of the MBs show that this offers memory space for recognizing a large amount of visual sceneries (54, 55); enough to remember views around the nest, along multiple routes or as shown here, along the same route but with monocular and binocular inputs.

Conclusion

The response of navigating ants to visual impairments shows a surprising mix between rigidity and flexibility. Rigidity in the sense that scene recognition is fundamentally egocentric and dependent of the binocular input, and any alteration in the visual field has dramatic consequences and prevent the ants from walking in a straight line, recognize the (no-longer) familiar scene, or home. Flexibility in the sense that these dysfunctional ants manage to compensate the deficit caused by such a nonnatural alteration by engaging an extensive route relearning process, in a similar way, albeit longer than what naïve ants do at the onset of their foraging ontogeny. Within hours, the altered ants resume a fully functional foraging behavior and the newly acquired route memories do not override previous ones acquired with a different visual field. Investigating the plastic neural mechanisms underlying these feats will form a great agenda for future research.

Material and Methods

Experiments took place during June and July 2017–2019 on a plain open field with grassy vegetation close to the harbor in the metropolitan area of Seville, South of Spain. Three different nests of the Iberian desert ant *C. velox* were used for training and testing. Workers exhibit behaviors typical for solitary foraging ants that venture out of the nest to find food without the help of pheromone trails (56). Instead their navigational guidance is primarily based on visual input derived from celestial and terrestrial sensory cues (57).

General Experimental Set-Up and Protocol. All set-ups shared a similar basic design, which is described in the following while specific differences were appropriately mentioned above. Ants were trained to follow a route from the nest to a feeder that provided food ad libitum in form of a variety of buttery, sweet biscuit crumbs. Nests were enclosed with thin white plastic planks, a smooth material, too slippery for the tarsi of the ants and hence preventing them from foraging elsewhere. A square plastic bowl was sunk into the ground and served as feeder. The walls of the feeder were covered with fluon to prevent ants from climbing out. During training, ants dropped into the feeder and could return to the nest via a small wooden ramp that led the ants out of the feeder onto the foraging route. Training continued until the ants familiarized with the foraging route and scuttled fast and straight forward between the nest and the feeder at least five times. For tests, ants were either caught at the feeder or close to the nest entrance. Ants caught at the feeder have both the familiar visual scenery and the homing vector of their path integrator as scaffold for homing: hence FV ants. Ants caught close to the nest ran off their homing vector and can solely rely on the familiar visual scenery during homing: hence ZV ants. All tested ants were subjected to an eye-cap procedure, which was noninvasive and reversible. For that foragers were manually caught and the first two pair of legs including one of the antenna were carefully fixed between two fingertips. Thus, the head of the ant was immobile and one of the compound eyes could be covered with a drop of opaque enamel paint (Tamiya). The tip of a thin pin acted as brush and painted ants were subsequently checked for an even and complete cover of the targeted eye with the help of a 10× magnifying glass. Afterward ants were transferred into a small plastic vial and tested as soon as the foragers held on to a crumb.

Trajectory Recording and analyses. Paths of tested ants were recorded with pen and paper and the help of a square grid (0.5 × 0.5 m) made of string and tent pegs mounted on the ground. Paths were digitized with GraphClick (Arizona Software) and analyzed with Matlab (Mathworks, Matick, MA). "Local meander" and "Overall straightness" were computed by first segmenting the path into consecutive segment of 30 cm length. Local meander corresponds to the average absolute angle between successive segment. Overall straightness corresponds to the length of the circular average vector (r) of the distribution of all segment directions (independently of their order). Statistics on local meander and overall straightness were achieved with R studio V. 1.0.136. We performed a mixed Anova testing for the continuous effects of trial number (1 to 4) and categorical effect of PI state (FV or ZV) as well as their interaction. Since the interactions were never significant ($Ps > 0.18$), we ran additive models. Ant individuality across trials was systematically informed as a random effect. Circular statistics (58) on the heading directions were calculated with Matlab (circular statistic toolbox) for each condition separately. A Rayleigh test rejects the uniformity of the distribution. A S-test rejects a theoretical direction (here the nest direction in Fig. 1) as an acceptable mean of the distribution, and a V-test rejects the uniformity, given a theoretical direction where the population is expected to head (here, backtracking opposite to the nest compass direction in Fig. 2). The *T* test in Fig. 3*C* was also calculated with Matlab.

Paths in the unfamiliar environment (Sham, Just capped, and Compensated ants) were recorded with a Panasonic Lumix camera (DMC FZ200) fixed on a tripod, digitized via a novel video tracker system of the Risse group at the University of Münster (59, 60) and analyzed with R studio. Left eye–capped ants (LEC) and right eye–capped ants (REC) were pooled by mirroring paths of REC ants, so that for both groups, left on the trajectory correspond to the side of the covered eye. Differences in their tendency to turn left or right were determined by comparing the angular velocity via a calculation of Dtheta and forward velocity. The X and Y values of the paths were smoothed with a Savitzky–Golay filter of order 3 and filter length of 41 frames, followed by a double smoothing of the Dtheta signal by moving the average of window length by three. Finally, all pauses and events longer than 1 s were removed. A one-sided Wilcoxon test was used to calculate the significance of each pooled group against random choice.

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

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