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Importance of magnetic information for neuronal plasticity in desert ants

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Many animal species rely on the Earth's magnetic field during navigation, but where in the brain magnetic information is processed is still unknown. To unravel this, we manipulated the natural magnetic field at the nest entrance of *Cataglyphis* desert ants and investigated how this affects relevant brain regions during early compass calibration. We found that manipulating the Earth's magnetic field has profound effects on neuronal plasticity in two sensory integration centers. Magnetic field manipulations interfere with a typical look-back behavior during learning walks of naive ants. Most importantly, structural analyses in the ants' neuronal compass (central complex) and memory centers (mushroom bodies) demonstrate that magnetic information affects neuronal plasticity during early visual learning. This suggests that magnetic information does not only serve as a compass cue for navigation but also as a global reference system crucial for spatial memory formation. We propose a neural circuit for integration of magnetic information into visual guidance networks in the ant brain. Taken together, our results provide an insight into the neural substrate for magnetic navigation in insects.

celestial compass | compass calibration | memory formation | learning walks | solar ephemeris

The Earth's magnetic field [also geomagnetic field (GMF)] is a crucial cue for spatial orientation in many animal species. It guides birds (1), fish (2), and turtles (3), but also invertebrates like moths (4) and butterflies (5) on their long-distance migrations. The GMF does not only provide compass information but also serves as a geo-stable cue to calibrate other navigational systems (6–9). However, how magnetic cues are encoded in an animal's nervous system has remained enigmatic. While magnetic orientation has traditionally and most intensively been studied in vertebrates, their complex nervous system and behavioral repertoire may complicate access to relevant neuronal circuits involved in the magnetic sense. Due to their tractable and relatively small nervous systems, insects provide a unique opportunity to shed light on the neuronal basis for magnetic orientation (10).

Cataglyphis desert ants provide a favorable experimental model for studying the integration of magnetic information into neuronal circuits due to clear behavioral readouts and well-studied neuronal pathways underlying spatial orientation behaviors (10). Before interior workers become outdoor foragers, the ants perform the so-called learning walks (11) in the vicinity of their nest to calibrate their celestial compass and learn the landmark panorama used for subsequent navigation during foraging. During these learning walks, the ants repeatedly look back to their nest entrance using path integration (11). To do so, the GMF serves as their primary compass cue (12). In addition, the GMF is proposed to serve as a reference system for calibration of the ants' celestial compass (7, 13, 14) and to memorize the panoramic scenery in relation to the position of the nest entrance. The learning processes associated with this sensory experience of directional panoramic information and celestial compass cues are reflected at the neuronal level by the expression of structural changes in neurons, particularly their synaptic connections, in visual circuits in the ants' brains (structural neuronal plasticity) (15, 16). In contrast to passive light exposure, sensory experience of celestial compass cues during learning-walk behavior results in an increase of synaptic connections in high-order brain centers associated with sensory integration and memory formation (mushroom bodies) and with path integration and motor control (central complex) (17–20). Structural neuronal plasticity in these brain centers depends on the sun's rotating sky-polarization pattern, which can be substituted by an artificial polarization pattern that changes over the day (16). However, to calibrate an internal representation of the sun's movement across the sky over the day (solar ephemeris), ants and bees need a geo-stable reference system (7, 21, 22). Such a reference can be provided by the panoramic scenery, as has been shown during calibration in honeybees (22–24), or the GMF, as has been shown in birds (6, 8, 9). Here, we investigated the

Significance

The Earth's magnetic field is an essential navigational cue for many animal species. However, where in the brain magnetic information is processed is still little understood. In this paper, we analyzed structural neuronal plasticity in the brain of *Cataglyphis* desert ants following sky-compass calibration under permanently manipulated magnetic field conditions. Our results demonstrate that information from the Earth's magnetic field is integrated into the ants' internal compass (central complex) and into the learning and memory centers (mushroom bodies). Together with our behavioral analyses, the results show that the ants use magnetic information both as a navigational compass and as a reference system for visual compass calibration.

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influence of magnetic field manipulations on structural neuronal plasticity (referred to as neuroplasticity in the following) in *Cataglyphis nodus* ants freely behaving in their natural habitat (Fig. 1).

Our results show that magnetic field information differentially affects neuroplasticity of visual circuits in the central complex and mushroom bodies. This suggests that magnetic information is processed in these regions of the insect brain and that the GMF provides ants not only with a global compass during learning walks but also with a geo-stable reference system for calibration as demonstrated by neuroplastic change. The results show that magnetic field information plays a crucial role during a critical early learning phase. Based on our present results and previous studies, we discuss a model for neuronal integration of magnetic information into the ants' sky-compass and visual guidance networks.

Results and Discussion

Influence of Permanently Manipulated GMF on Learning Walk Behavior. Under natural conditions, *C. nodus* ants walk in small loops around their nest entrance during their transition from interior worker to outdoor forager (Fig. 1*A*). These so-called learning walks are repeatedly interrupted by pirouettes during which ants turn about their body axes [pirouettes (Fig. 1, *Inset*)]. During these turns, the ants gaze back at their nest entrance [natural control, N (Fig. 1*A*)] (12 and behavioral observation). A chaotic, unreliable magnetic field that varies in both direction and field strength around the nest entrance (Disarrayed, Dis) prevents the ants from looking back to the nest entrance (Fig. 1*B*) (12 and behavioral observation). To assess the influence of manipulated magnetic fields on neuroplastic changes related to learning-walk behavior, we combined and adapted neuroethological assays developed by Grob et al. (15) and magnetic field manipulation techniques established by Fleischmann et al. (12) (for more details, see *Materials ans Methods*). Naive ants that had not yet started learning walks outside the nest were allowed to perform learning walks around their nest entrance on 3 consecutive days. During this period, we presented the ants with a magnetic field that was manipulated throughout the day using a 3D-Helmholtz coil system which was connected to the ants' natural nest (Fig. 1*C*). We first presented the ants with magnetic information with the horizontal component of the GMF eliminated [horizontal elimination (HE)]. Under these conditions, the magnetic field vector is still present, but at a weaker field strength and points straight downward, providing no directional information to the ants in the horizontal plane (cardinal direction). During this GMF manipulation, the ants were no longer able to direct their gazes back to their nest entrance. Their gazes were randomly distributed relative to the nest entrance (Fig. 2*A*; HE: Rayleigh test: Z_0 = 1.355, n = 15, P = 0.262, μ = 199.9°, r = 0.301).

This is well in line with the behavior observed during acute elimination of the horizontal component of the magnetic field

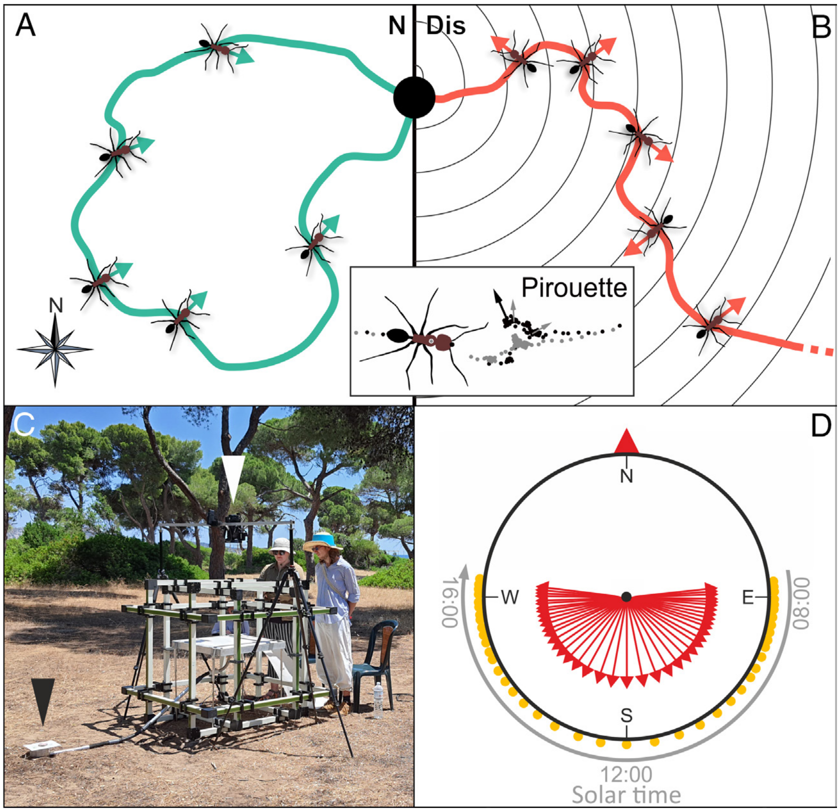

Fig. 1. Magnetic field manipulation in the natural habitat of *C. nodus*. (*A*) Schematic depiction of a learning walk under natural conditions around the nest entrance (black dot). Ants repeatedly stop their forward movement to perform pirouettes (*Inset*, head position marked as black dots, thorax position marked as gray dots). The ants stop their rotation, with the longest stops directed toward the nest entrance [black arrows (gray arrows are shorter stops)]. (*B*) Schematic of a learning walk when an electromagnetic spiral (black circles) disarrays the magnetic field around the nest entrance (Dis). Ants are no longer able to direct their gazes back to the nest entrance. (*C*) Experimental setup in the Schinias National Park, Greece. A 3D-Helmholtz coil system was installed next to the natural nest entrance of a *C. nodus* colony (black arrowhead). The nest entrance was connected via a tube to an experimental platform in the center of the Helmholtz coils. Learning walks were recorded using a camcorder (white arrowhead) installed above the experimental platform. Two observers made sure that only experienced foragers were allowed to leave the experimental platform and recorded the learning walks (for more details, see *Materials and Methods*). (*D*) Under natural conditions, the magnetic field vector points to magnetic north throughout the day (red arrowhead). The sun's azimuth position changes over the day in a way that at solar noon the sun is in the South (yellow dots). During the M + S condition, the magnetic field vector (red arrows) was rotated stepwise every 10 s to follow the sun's course over the day (solar azimuth).

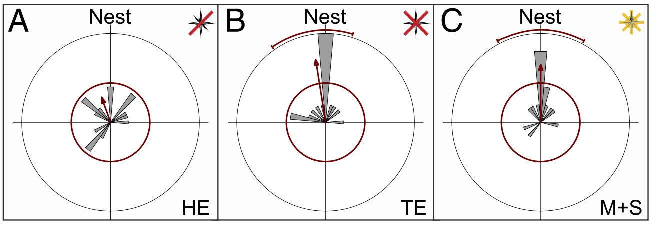

Fig. 2. Gaze directions during learning walks under permanently manipulated magnetic conditions. Look-back behavior under (*A*) elimination of the horizontal component of the GMF (HE, n = 15), (*B*) total elimination of the magnetic field (TE, n = 15), and (*C*) experimental condition when the magnetic vector followed the solar azimuth over the course of the day (M + S, n = 15). Data show the gaze directions during the longest stopping phase of pirouettes relative to the experimental nest entrance (Nest) in the platform. Data are shown in gray: The bins comprise 10°. The circular axis has a maximal scale of five ticks, i.e., the outer black circle comprises five ants. Statistics are shown in red: The arrow indicates the r-vector, which is significantly directed if it exceeds the circle indicating the significance level of the Rayleigh uniformity test (α = 0.05). If data are directed, the arc indicates the 95% CI. For statistical details, see text.

during pirouettes (12) and confirms that the ants use the GMF as a compass during look-back behavior.

We then asked whether the ants might use other directional cues when magnetic information is completely absent. To test this, we eliminated the complete GMF, i.e., both the horizontal and vertical component of the GMF [total elimination (TE)]. Surprisingly, during complete elimination of the magnetic field, ants were still able to gaze back to their nest entrance (Fig. 2*B*; TE: Rayleigh test: $Z_0 = 7.694$, n = 15, $P < 0.001$, μ = 189.1°, r = 0.716, 95% CI: CI95 = 162.9°/215.3°). This suggests that when the magnetic field is permanently manipulated throughout the day, total elimination presents the ants with a different challenge compared to only horizontal elimination of the GMF. In contrast to all other magnetic manipulations (Dis, HE, M + S), total elimination leaves the ants with no magnetic information whatsoever. This extreme situation might trigger the ants to switch to a backup compass for path integration to guide their look-back behavior, which they would not use as a main compass if any magnetic information was available. Such compass information might come from more unreliable directional cues. *Cataglyphis* employs a wide range of navigational cues, including a wind compass (25), visual panoramic and landmark cues (14, 26), olfactory (27, 28), vibratory (29), gravitational (30, 31), and egocentric cues (32). While such directional information is used extensively during foraging where the ants cover vast distances at high walking speeds (33), they have clear disadvantages during learning walks. Some of these cues are not yet calibrated or learned, or do not provide a geo-stable cue (7).

Since the ants' main compass during foraging is a celestial compass, we next tested, whether a magnetic field vector at GMF strength that followed the sun's azimuthal position and related sky-polarization cues (magnetic vector follows solar azimuth (M + S) Fig. 1*D*) may influence the look-back behavior of the ants. The ants were still able to gaze back to their nest when the magnetic field direction followed the course of the sun throughout the day (Fig. 2*C*; M + S: Rayleigh test: $Z_0 = 6.351$, n = 15, $P < 0.001$, μ = 180.0°, r = 0.651, CI95 = 152.1°/207.9°). Learning walks are very short trips around the ants' nest entrance lasting only for several seconds to a few minutes (11). The solar ephemeris and, therefore, the magnetic vector during M + S changes with a mean speed of 15°/h (21). Thus, under M + S the direction of the magnetic vector remained relatively stable for the duration of a learning walk. While the magnetic vector might be stable during a learning walk, its direction does not point to magnetic north (Fig. 1*D*). Our results show that the ants' look-back behavior does not depend on the magnetic field's natural direction (M + S). Since ants cannot inherently know in which relation the Earth's magnetic field is to the outside world, they cannot have an expectation in which direction geomagnetic north is when they leave their nest for the first time to perform learning walks (7). Additionally, "north" is not an important information for the path integrator to be able to work (12). Artificial magnetic fields, therefore, can provide the necessary compass for the look-back behavior.

We also tested whether the magnetic manipulations had an influence on the duration of the longest stopping phase during pirouettes, which under natural magnetic conditions is directed toward the nest entrance (11, 12, 15, 16). The duration of the longest stopping phase might serve as an indicator for the ants' certainty in looking back to the nest entrance. In magnetic conditions under which the ants did not to gaze back at their nest entrance (HE), the longest stopping phases during pirouettes were slightly shorter compared to conditions where they looked back accurately. This difference was significant between HE and TE, but not between HE and M + S (*SI Appendix*, Fig. S1; Kruskal–Wallis test: χ = 9.53, P = 0.0085, n = 45, $n_{TE} = 15$, $n_{HE} = 15$, $n_{M+S} = 15$). This might indicate that the ants recognize that they do not look in their nestward direction. However, the duration of stopping phases of desert ant learning walks can be significantly influenced by temperature (34). Future experiments controlling for the ambient temperature during the experiments will reveal if the stopping phase duration is a reliable readout for look-back certainty. Taken together, our results show that permanent magnetic manipulations interfere with the orientation capabilities of novices suggesting that the GMF is used as a compass cue during learning walks. We next tested whether the same manipulations influence learning-related structural neuroplasticity in visual integration centers triggered by learning walks.

Importance of GMF Information for Compass Calibration. Since the learning walk behavior is accompanied by substantial structural neuronal plasticity in visual integration centers in the ant brain (15, 35, 36), we next asked how magnetic information influences this neuroplasticity. We used the very same ants that had performed learning walks while magnetic cues were manipulated to examine how the treatment influenced the size of central brain areas and numbers of synaptic complexes within them (neuroplasticity). Ants that had not yet performed any learning walks (interior workers) were used as negative control (DD). Previous experiments revealed that both the mushroom bodies and central complex (Fig. 3) increase in volume and numbers of synaptic complexes following the experience of a rotating sky-polarization pattern during active performance of learning walks (16).

We therefore hypothesized that the GMF provides the geo-stable reference system for calibrating the rotation of skylight cues (7). If this is true, permanent alteration of the magnetic field information during learning walks should have a significant impact on neuroplastic changes in visual integration centers. Compared to DD conditions, the volume of the central complex significantly increased to the same extent as under natural conditions (N), both when the geomagnetic field was fully (TE) or partially eliminated (HE) and when the magnetic field around the nest entrance was chaotic (Dis) during 3 d of learning walks (Fig. 4*A*). Only when the magnetic vector followed the solar azimuth over the day (M + S), the volume of the central complex did not increase compared to DD conditions (Fig. 4*A*; Kruskal–Wallis test: relative central complex volume: χ = 30.02, n = 58, P = 0.000015, $n_{DD} = 10$, $n_{HE} = 8$, $n_{TE} = 10$, $n_{Dis} = 10$, $n_{M+S} = 10$, $n_N = 10$). Future studies should

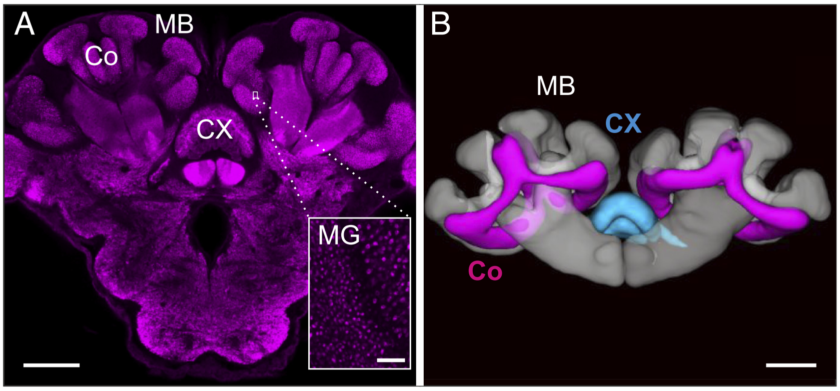

Fig. 3. Neuroanatomical analyses in the brain of *C. nodus*. (*A*) The brain of an ant that participated in the field experiments is labeled with anti-synapsin antibodies (magenta). The central complex (CX) and mushroom body (MB) collar (Co) [microglomeruli (MG) *Inset*] were analyzed. (Scale bar in *Inset*: 10 µm.) (*B*) Three-dimensional reconstruction of MB (gray) cut open to highlight the structure of the Co (magenta). The central complex is shown in blue. (Scale bars: 100 µm.)

test whether the lack of neuronal changes leads to a decrease in the navigational success of the ants.

Interestingly, our data suggest that the magnetic field vector does not necessarily need to point to geomagnetic north or provide any directional information [e.g., when the horizontal component of the magnetic field is eliminated (HE)] to trigger neuronal plasticity in the central complex. Only when the magnetic vector followed the position of the sun and, thus, the angle between celestial and magnetic directional information always remained constant over the day (M + S) the volume of the central complex remained unchanged compared to DD and did not reach the volume of N. Under natural magnetic conditions, a polarization pattern that changes over the day is necessary to trigger neuroplasticity (15, 16). To determine this naturally occurring change in sky-compass cues, the ants need a geo-stable reference system, the magnetic field (7). Our results indicate that differential changes between magnetic and polarization information over time ($\alpha + t_n$) indeed play a crucial role for triggering neuroplastic change in the ants' neuronal compass circuits (Fig. 5).

Only in cases when celestial compass information changes relative to a stable reference system over time ($\alpha + t_n$), central-complex circuits undergo plastic changes (calibration) (Figs.4*A* and 5). Interestingly, under permanent total elimination of the GMF (TE), the ants were able to compensate for the complete absence of magnetic information. Under these conditions, ants were able to gaze back to their nest entrance (Fig. 2*B*) and the volume of their central complex increased (Fig. 4*A*). This indicates that ants may have switched to backup compass systems, like panoramic information ($\beta + t_n$) (Fig. 5).

While the manipulation of celestial compass cues over the course of a day—a changing polarization pattern—triggers neuronal plasticity in both the mushroom body and central complex (15, 16), manipulating magnetic cues affects plasticity in the two brain regions differently. In the visual input regions of the mushroom body calyx (collar), the overall volume increased only when the ants were allowed to perceive the natural magnetic field during learning walks (Fig. 4*B*, Kruskal–Wallis test: relative Co volume: χ = 16.63, n = 56, *P* = 0.0053, n_{DD} = 10, n_{TE} = 10, n_{HE} = 6, n_{Dis} = 10, n_{M+S} = 10, n_N = 10) and the number of synaptic complexes (Fig. 4*C*, Kruskal–Wallis test: relative number MGs: χ = 14.24, n = 56, *P* = 0.0142, n_{DD} = 10, n_{TE} = 10, n_{HE} = 6, n_{Dis} = 10, n_{M+S} = 10, n_N = 10). We conclude that magnetic information seems to be necessary for gating visual circuit plasticity in the mushroom bodies associated with learning-walk behavior. This mechanism could allow ants to learn and store calibrated and most relevant visual snapshots around the nest for later homing guided by visual cues.

A modeling study has shown that mushroom body microcircuits have the theoretical capacity to store the necessary view memories (20) and, therefore, might use a different evaluation of magnetic information than the central complex. Neuroplasticity in the visual collar was triggered only under natural GMF conditions and was absent following all magnetic field manipulations during learning walks [(HE, TE, Dis, and M + S); Fig. 4 *B* and *C*]. The GMF can already be perceived in the dark nest, and it was shown that Hymenoptera are able to use magnetic cues for nest building and sense the GMF in darkness (41). Ants, thus, might already be familiar with the properties of the magnetic field. In contrast, celestial and panoramic cues are unpredictable for naive ants and must be initially calibrated during first learning walks (7). The mushroom bodies are crucial for spatial orientation behaviors based on learned visual cues (18). We suggest that for neuroplasticity related to the storage of long-term view memories (snapshots) in the mushroom bodies appropriate magnetic compass information is necessary. Under natural conditions, the angular relationship between visual and magnetic parameters should not change over time (β) (Fig. 5) and only to an insignificant extent when an ant is moving through space. Our results show that if this relationship is disturbed (HE, Dis, M + S) or when the magnetic field is absent (TE), neuroplastic changes are absent in the mushroom body collar (Fig. 4*A*). This entanglement of magnetic and panoramic directional cues is backed by studies in honeybees. During calibration of the solar ephemeris, honeybees do not use the magnetic field as a geo-stable reference but rather rely on familiar panoramic scenery (44). Similarly, experienced *Cataglyphis* foragers no longer use magnetic information as their main compass during re-learning (45). Overall, this suggests a close interplay between the magnetic and celestial compass and panoramic information. We propose that appropriate magnetic information is necessary for triggering long-term consolidation of view memories (Fig. 5). Long-term consolidation of view memories should be triggered only in cases when ants path integrate correctly and gaze toward the nest entrance, which is associated with an increase of volume and synaptic complexes in the mushroom body collar. In *Drosophila,* it was shown that olfactory long-term memory consolidation is triggered via a long-range serotonergic neuron mediating stage-dependent activation of dopaminergic neurons in olfactory circuits of the mushroom body (46). A similar gating mechanism might mediate consolidation of visual memories in *Cataglyphis*. View memories are used for panoramic view matches, which could be communicated to

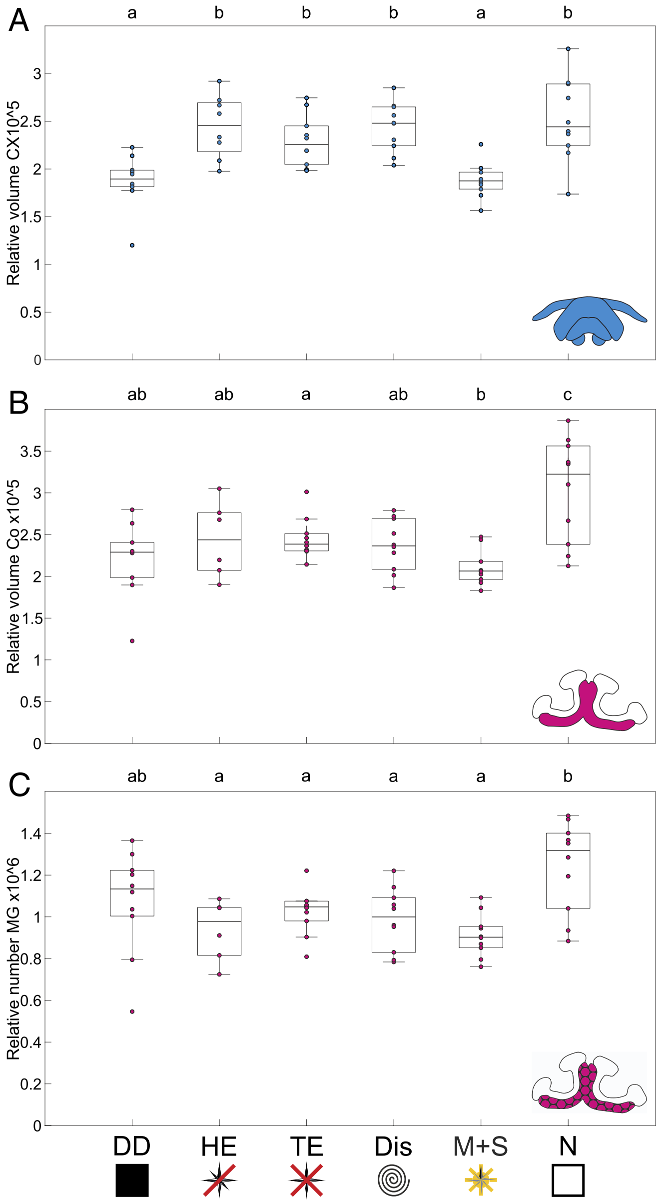

Fig. 4. Influence of different magnetic conditions during learning walks on neuronal plasticity. The size-corrected volume of the (*A*) central complex (CX) and (*B*) the mushroom body collar (Co) is shown. (*C*) The size-corrected total number of synaptic complexes (MG) in the mushroom body collar is shown. The central line of each boxplot depicts the median of the data. The upper and lower limits of the boxes show the 25th and 75th percentiles, while whiskers extend to extreme data points without outliers. Outliers are values that are more than 1.5 times the interquartile range from the edge of the box. All data points are plotted as colored dots. Significant differences between the groups are marked with lowercase letters [Kruskal–Wallis test ($\alpha = 0.05$) and post hoc Mann–Whitney *U* test with Benjamini–Hochberg correction (false discovery rate of $\alpha = 0.05$)]. For statistical details, see text. Experimental groups: interior worker (DD), 3 d of learning walks under a magnetic field with the horizontal component of the geomagnetic field eliminated (HE), under total elimination of the magnetic field (TE), under a chaotic magnetic field (Dis), under a magnetic vector following the solar azimuth (M + S), or under natural magnetic conditions (N).

the central complex via mushroom body output neurons (MBON) synaptically connected to the fan-shaped body (Fig. 5) (42). Future studies in *Cataglyphis* should further test the basis of visual plasticity by rotating the panorama along with the magnetic field vector and by investigating modulatory systems potentially underlying a gating mechanism for visual memory consolidation.

Integration of GMF Information into the Sky-Compass Network. How is magnetic information integrated into the sky-compass network? It has been proposed that the antennae, particularly the Johnston's organ, might be involved in magnetoreception in ants (40, 47). A substantial proportion of Johnston's organ afferent nerve fibers carry information from sensory receptors on the antenna to the posterior slope of the ant brain (40). The posterior slope is considered a multimodal integration center (48) interconnected with the sky-compass network (49) and visual information, including panoramic information from the optic lobes (37). It can also be considered a premotor center as polarization-sensitive descending neurons with dendrites in the posterior slope have been described in locusts (50) and at least one of these neurons' codes for an unpolarized light spot in an azimuth-dependent way. This could encode the position of the sun on its daily course (51). These neurons also show daytime-dependent shifts in polarization-angle preference (50) suggesting a key role of the posterior slope in time-compensated sky-compass navigation (52). Interestingly, afferent nerve fibers from the Johnston's organ in the posterior slope terminate in close proximity to afferent nerve fibers from polarization-sensitive ocelli in *C. nodus* (40), which renders this neuropil a potential substrate for integration of celestial information with other compass cues like the magnetic compass and panoramic information. In *Drosophila* the posterior slope is connected to the central complex and neurons connecting both neuropils were suggested to be involved in neuroplasticity in the central complex (42). Based on our present results, we propose a model in which the directional information from the magnetic field is integrated with celestial and panoramic directional information in the posterior slope and forwarded to the central complex (Fig. 5). Taken together, our results show that magnetic information serves both as a compass cue for orientation and as a geo-stable reference for neuroplasticity associated with memory formation during learning walks. This requires integration of magnetic information in both the central complex and the mushroom bodies.

Materials and Methods

Experimental Model and Subject Details. Ants of the species *C. nodus* (Brullé 1832) were studied in their natural habitat in the pine forest of Schinias National Park, Marathon, Greece, in the summers of 2021 and 2022. The GMF strength at the experimental site (38°08′N 24°01′E) was B = (46.1 ± 0.2) µT; horizontal component H = (26.6 ± 0.2) µT [https://www.ngdc.noaa.gov/geomag-web/; and monitored by a MEDA FVM400 vector magnetometer (Macintyre Electronic Design Associates, Inc.)]. All ants outside the nest were marked with car paint for 3 consecutive days before the experiments started (Motip Lackstift Acryl, MOTIP DUPLI GmbH). This made sure that only unmarked novices that had not performed learning walks were included in the experiments (53).

Experimental Procedure. For the dark control (DD), interior workers that had not yet performed learning walks were collected from an excavated colony. All foragers of the colony had been marked beforehand. The ants were kept in a dark box during excavation. Interior workers of an unspecified age (unmarked ants) were kept in darkness for 3 d before being decapitated and prepared for neuroanatomical staining directly after the excavation.

Naive ants (novices) performed learning walks in a restricted area around their natural nest entrance (60 cm × 60 cm). Only experienced foragers (marked ants) were allowed to leave the fenced area. Novices performed learning walks on 3 consecutive days. On the third day, ants reaching the rim of the restricted area were caught and placed in a dark box until the next day and then prepared for neuroanatomical staining. For the natural control (N), novices performed learning walks under natural magnetic conditions. All learning-walk groups were able to see the natural sky during learning walks. Above the experimental platform, a

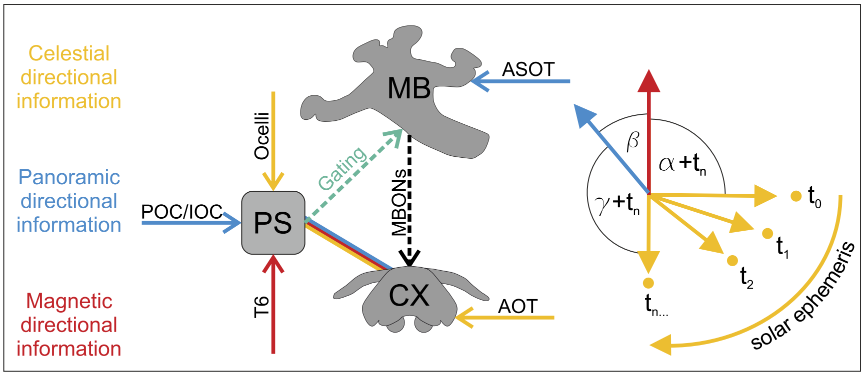

Fig. 5. Model for integration of magnetic information into the compass network. The posterior slope (PS) is a multisensory integration center in the insect brain. It receives input from the polarization-sensitive ocelli (37–39), panoramic information from the compound eyes via the posterior and inferior optic commissures (POC/IOC) (37), and additional information from the ants' antennae via the Johnston's organ afferent nerve fiber tract 6 (T6) (40). The Johnston's organ detects wind compass information and, potentially, magnetic compass information (41). The multisensory input renders the PS a suitable neuropil to integrate the different types of directional information for calibration. While the angle between panoramic and magnetic directional information remains constant throughout the day (β), the relative angle between magnetic compass and celestial compass ($\alpha + t_n$) and the angle between panoramic and celestial compass information ($\gamma + t_n$) change over the day due to celestial rotation (t_n). These relationships might be integrated in the PS. The PS is connected to the central complex (CX) (42), the internal compass in the insect brain (19). Here, directional information, e.g., celestial compass information from the anterior optic tract (AOT), can be processed with heading information. Depending on the relationships between the different directional inputs, neuronal plasticity occurs in the central complex and the mushroom bodies (MB)—which are both needed for learning-dependent spatial orientation (18, 43). In the mushroom bodies, visual memory formation, e.g., based on panoramic snapshots transferred via the anterior superior optic tract (ASOT), might be gated based on PS output. During subsequent navigation, panoramic view matches can be communicated to the central complex fan-shaped body via mushroom body output neurons (MBON).

4K-camcorder (HC-X1000, Panasonic Corporation) recorded learning walks of novices at 50 fps. Every time an unmarked ant left the nest entrance, the observer sitting next to the experimental setup started recording of the 4K-camcorder using the Panasonic Image App (Version 10.9.2, Panasonic Corporation) on a Cat S60 smartphone (Caterpillar or a Xperia Z1, Sony). High-resolution recordings were stopped when the ant returned to the nest or touched the rim of the experimental platform (60 cm × 60 cm). Since all naive ants were unmarked and we could not remove the ones that were already recorded, as we also use them for the neuroanatomical part of the study (i.e., let them perform learning walks for 3 d), we cannot be completely certain that all learning walks recorded are performed by different ants. However, the radius (distance and length) of learning walks expands quite rapidly over time. Only early learning walks can be recorded undisturbed on the experimental platform, while later (more expanded) learning walks become unsuitable for video analyses. Therefore, we are quite confident that the majority of learning walk pirouettes analyzed are performed by different ants.

Magnetic Manipulations. Experimental groups performed learning walks under permanently altered magnetic conditions. To disarray the magnetic field, a flat coil (diameter 70 cm; separation distance between the wire windings: 5 cm; diameter of copper wire: 0.5 mm) was installed around the nest entrance and covered with sand. The electromagnetic spiral was powered by a customized DC (Direct Current) power supply custom built by the Biocenter's electronic workshop (12). To achieve a magnetic field slightly stronger than the Earth's magnetic field close to the ground the current was set to 3 A. The magnetic field was almost eliminated at the northern end of the flat coil (H = 0.6 μT, α = 118.4°), and roughly doubled in strength in the south (H = 62.4 μT, α = −3.9°). Thus, the altered magnetic field provided different directional information at any point on the spiral. Therefore, it was not suitable as a reliable reference system for the ants' look back to the nest entrance behavior during learning walks.

To precisely control the magnetic field around the nest entrance, a Helmholtz coil system was used (Fig. 1*C*; HHS 3D 5213-50–3D Helmholtz-Coil, Schwarzbeck Mess-Elektronik). The experimental procedure with the Helmholtz coil system was based on the method established by Fleischmann et al. (12). Since homogeneity of the magnetic field is highest in the center of the coil system, ants had to perform their learning walks on a platform (60 cm × 60 cm). The area was restricted either by a plastic fence or a water-filled moat. The natural nest entrance was covered with a cylindrical box (nest cover) with a tunnel (diameter: 3 cm) in a way that the ants could still leave the nest but had to use an artificial nest entrance. During the experiment, the tunnel of the nest cover was connected to the experimental table via a flexible tube (diameter: 3 cm). Ants left the tube through a hole (diameter: 3 cm) in the center of the elevated platform (Fig. 1*C*). Foragers (marked ants) could leave the platform by walking on a fabric ramp. The Helmholtz coil system had to be installed every morning and removed every evening. To make sure, novices would not leave the nest under natural magnetic field conditions, the nest entrance was closed as soon as the Helmholtz coil system was switched off. Before opening the nest entrance in the morning, we measured the natural geomagnetic field and the experimental magnetic field by the Helmholtz coil with a magnetometer. The Helmholtz coil system was powered by a lithium battery (YETI 3000 LITHIUM, Goal Zero) and the current was controlled by a R&S®HMP4000 power supply (Rohde & Schwarz GmbH & Co KG). To eliminate the horizontal component (HE) of the geomagnetic field, the magnetic field of the horizontal coil pair of the Helmholtz coil system was set at the exact strength but directed in the other direction. This resulted in elimination of the horizontal component (H = 0.4 μT, B = 37.5 μT), leaving the ants with a magnetic vector pointing straight down to the Earth's surface. To eliminate the full magnetic vector (TE), both the horizontal component and the vertical component of the geomagnetic field were offset by the magnetic field of the Helmholtz coil system (H = 0.05 μT, B = 0.1 μT). To provide the ants with a magnetic vector that followed the course of the sun and the associated changing polarization pattern, the magnetic vector of the Helmholtz coil system was adjusted every 10 s to point in the direction of the solar azimuth (Fig. 1*D*; M + S). This adjustment was controlled by custom written software run on an outdoor notebook.

Neuroanatomical Procedures. To analyze neuroplastic changes following learning walks under altered magnetic fields, the ants' brains were dissected and double labeled in the field laboratory in Greece using a primary antibody against synapsin (SYNORF1, kindly provided by E. Buchner, University of Würzburg, Germany) and CF633 Phalloidin (00046, Biotium Inc.) following established protocols by Habenstein et al. (37).

Ants were anesthetized on ice, decapitated, and the brains were immediately dissected under cooled ringer solution (127 mM NaCl, 7 mM KCl, 1.5 mM $CaCl_2$, 0.8 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 4.8 mM TES, and 3.2 mM trehalose, pH 7.0). The brains were transferred to a 24-well plate and fixed in 4% formaldehyde in phosphate-buffered saline (PBS) in a refrigerator overnight. Brains were rinsed in PBS (three times 10 min each step). The brains were rinsed once with 2% Triton-X 100 in PBS for 10 min and twice with 0.5% Triton-X 100 in PBS for 10 min. To block unspecific binding sites, the brains were incubated for 1 h at room temperature (approximately 27 °C) on a shaker in a 0.5% Triton-X 100 solution in PBS with 2% of Normal Goat Serum (NGS, Jackson ImmunoResearch Laboratories). Subsequently, they were incubated on a shaker in primary antibody to synapsin from mouse 1:50 in PBS with 0.5% Triton-X 100 and 2% NGS for 5 d in a fridge. The brains were then rinsed three times in PBS for 20 min and subsequently incubated in a secondary anti-mouse antibody from goat coupled to AlexaFluor 568 (A12380, Molecular Probes) dye (1:250), and CF633 Phalloidin (2.5 μL Phalloidin from Methanol stock solution in 500 μL PBS) in PBS with 0.5% Triton-X 100 and 1% NGS for 3 d on a shaker in the refrigerator. After incubation, the brains were rinsed in PBS four times (20 min each) and postfixed in 4% Formaldehyde in PBS overnight in the refrigerator. They were washed four times in PBS for 20 min each and then dehydrated in an ascending ethanol series (30%, 50% ,70%, 90%, and 95% for 3 to 4 min each and two times in 100% ethanol for 5 min). The brains were cleared in methyl salicylate (4,529.1, Carl Roth GmbH & Co. Kg) and finally transported from the field lab to the University of Würzburg for confocal laser scanning microscopy and further analyses.

Video Analysis. 4K-videos were converted into image stacks using the Free Video to JPG Converter (v. 5.0.99 build 823, DVDVideoSoft, DIGITAL WAVE LTD.). Pirouettes [full or partial tight turns about the ant's body axis (11)] were analyzed manually using the MATLAB (2015a, MathWorks) application DIGILITE (Jan Hemmi and Robert Parker, The Australian National University, Canberra, Australia). The positions of the thorax and mandibles were marked in each frame. In addition, the nest entrance was marked. Using these coordinates, the gaze directions of the ants could be determined relative to the nest. The direction of the nest was defined as 180°. Stopping phases during pirouettes were defined as in previous studies (minimal duration: 100 ms) (11, 15). The lengths of the longest stopping phases were measured and compared between the different conditions. The longest stopping phase of each pirouette was used to compare gaze directions between experimental groups. Fifteen pirouettes from different learning walks were analyzed for each experimental group in the Helmholtz coil setup (TE, HE, M&S).

Neuroanatomical Analyses. Brains were digitized using a confocal laser scanning microscope (Leica TCS SP8, Leica Microsystems GmbH) at a step size of 5 μm. An APO 20×/0.7 IMM water immersion objective with 0.75 digital zoom was used for whole-brain scans. Closeups of the central complex were scanned with 1.6 zoom, and 2.0 zoom was used for the MB calyx. A PL APO 63×/1.2 W objective with 2.0 digital zoom was used for high-resolution scans in the collar of the mushroom-body calyx.

The neuropils and their subunits are easily distinguishable in anti-synapsin labeled whole mount brains (Fig. 3*A*). The volumes of the subunits of the central complex and the mushroom body (Fig. 3*B*) were analyzed using the 3D-reconstruction software TrakEM2 (54) plugin for ImageJ 1.52n (Wayne Rasband, NIH, USA).

The numbers of microglomeruli in the mushroom body collar (MG, Fig. 3 *A*, *Inset*) were quantified with Amira software (Amira-Avizo Software 2019.1, Thermo Fisher Scientific Inc.) following the study by Groh et al. (55). Anti-synapsin and phalloidin labeled synaptic boutons were counted in three defined volumes (1,000 μm^3 each) by a person blind with respect to the experimental treatments. MG densities were calculated by averaging the analyzed volumes of the collar as numbers of MG per 1,000 μm^3. The total numbers of MG per collar were estimated by extrapolation of the average MG numbers per 1,000 μm^3 to the total volume of the collar. This represents a good approximation, as microglomeruli densities are homogeneous throughout the collar of *C. nodus* neuropils (15).

Statistics. Circular statistics were performed using Oriana 4.02 (Kovach Computing Services). Gaze directions during the longest stopping phases of pirouettes were grouped into 10°-bins. To test whether data were randomly distributed or directed, the Rayleigh test was used ($\alpha = 0.05$). If data were directed, the 95% CIs (CI95) were determined to check whether the expected direction (nest entrance defined as 180°) was within these limits. The lengths of the longest stopping phase of each pirouette were compared between the groups using a Kruskal–Wallis test ($\alpha = 0.05$) and a post hoc Mann–Whitney *U* test with Benjamini–Hochberg correction (56) (false discovery rate $\alpha = 0.05$).

Since the body sizes of *C. nodus* individuals and, therefore, brain volumes differ, the size of the neuropils was corrected for the ants' sizes (which has been shown to correlate with the ants' total brain size), by dividing the volume by the thorax length (16, 57). The neuroanatomical differences between the groups were compared using a Kruskal–Wallis test ($\alpha = 0.05$) and a post hoc Mann–Whitney *U* test with Benjamini–Hochberg correction (56) (false discovery rate $\alpha = 0.05$). All descriptive statistical analyses were done with MATLAB (2022b, The MathWorks Inc.).

Nomenclature. For the nomenclature of neuropils in the ant brain, we refer to the study by Habenstein et al. (37) (see also https://www.insectbraindb.org for 3D data of the *Cataglyphis* brain). For the terminology related to spatial orientation and navigation, we refer to the study by Grob et al. (58).

Data, Materials, and Software Availability. Raw data of confocal image stacks of whole brains and high-resolution videos of learning walks are available on the servers of the University of Oldenburg (DARE) at the following link: https://doi.org/10.57782/GMFGFO (59). All other data are included in the manuscript and/or supporting information (*SI Appendix*, Table S1).

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