

Review Article

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Learning to navigate – how desert ants calibrate their compass systems

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Abstract: Navigating through the environment is a challenging task that animals cope with on a daily basis. Many animal species have impressive capabilities to navigate in complex or even harsh environments. *Cataglyphis* desert ants are a famous example. These ants use a remarkable navigational repertoire to find their way home after far-reaching foraging trips. How do naïve ants calibrate their visual navigational systems? The ants perform stereotyped sequences of learning walks before switching from tasks inside the darkness of their nest, to foraging under bright sunlight. Here, naïve ants align nest-directed views using the earth's magnetic field as a compass reference. Neuronal plasticity was mapped in two visual pathways to higher brain centers during this transition. Both their first exposure to light, and the performance of learning walks lead to distinct changes in synaptic circuits along both visual pathways, reflecting calibration and memory formation in the ants' visual compass systems.

Keywords: central complex; learning and memory; mushroom bodies; magnetosensation; plasticity

Introduction

Finding our way around can be a demanding task. No matter whether we aim to reach the correct gate, a restaurant arrangement, or our favorite nightspot, to efficiently navigate, it is essential to know where we are, and where

we want to go. Many animal species must find their way back to their nest, migrate to a specific destination (sometimes thousands of kilometers), or make their ways in a featureless open ocean. The mammalian brain has a neuronal positioning system for these tasks. Place cells in the hippocampus form an internal map of space, and grid cells in the entorhinal cortex are arranged in a hexagonal pattern providing an internal coordinate system. This discovery is a major breakthrough in research on the neuronal mechanisms of spatial orientation and was awarded with the Nobel Prize in physiology or medicine in 2014 to John O'Keefe, May-Britt Moser and Edvard Moser.

How do ants, with their relatively tiny brains, cope with navigational challenges? Thermophilic desert ants of the genus *Cataglyphis* are famous experimental models for insect navigation. Like other social insects, *Cataglyphis* ants perform cooperative brood care in a common nest, and therefore, are central-place foragers that leave their nest for far-ranging foraging excursions, needing later to return to their nest entrance, an inconspicuous hole in the ground. In contrast to many ant species that employ trail-pheromones to exploit profitable food sources, *Cataglyphis* ants are solitary foragers, relying heavily on a mostly visual guided navigation (Wehner, 2008). While foraging for dead insects during the hottest time of day to avoid predators and competition, the soil temperature may reach a life-threatening 70°C. In the North African desert, ant *Cataglyphis fortis* foraging trips may exceed 1,500 m, with maximum distances exceeding 350 m away from their nest origin. This is equivalent to several thousand times the ants' own body lengths (Buehlmann et al., 2014; Huber and Knaden, 2015; Knaden and Graham, 2016; Ronacher, 2008). Therefore, under high selection pressure, *Cataglyphis* ants evolved to become expert navigators. Many years of behavioral analyses have revealed remarkable navigational capabilities of the foraging *Cataglyphis* ants (reviewed in Ronacher, 2008; Wehner, 2009; Wehner and Rössler, 2013; Rössler, 2019). Investigations on the early ontogeny of these navigational systems have only recently begun.

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Navigation in *Cataglyphis* ants

Desert ants use a powerful navigational tool termed *path integration* when returning to their nest using the shortest way possible – a straight line – even in featureless or unfamiliar terrains. By integrating directional compass and distance information, the ants obtain a vector which leads them home. This vector points towards the nest entrance, with exact direction and path length. Path integration is especially handy in extreme desert habitats, e.g. for *C. fortis* inhabiting featureless salt pans in North Africa. *Cataglyphis* ants use a celestial compass as their main source of directional information. Both the sun position and associated skylight polarization pattern function as global compass cues, enabling the ants to keep track of all walked angles during their random foraging runs (Wehner, 2008). For distance estimation, the ants utilize a step integrating mechanism and optic flow perception (Pfeffer and Wittlinger, 2016; Wittlinger et al., 2006). Since path integration mechanisms are prone to cumulative error, desert ants also use panoramic sceneries as local guidance cues for homing, whenever available. These local visual cues, however, differ among nest sites and may change over time. The ants must therefore learn them before embarking on their first foraging trips (Fig. 1).

However, a skylight-based compass presents additional challenges. For example, both the position of the sun and the associated sky-polarization pattern change throughout the day. Therefore, the internal skylight compass must compensate for this movement, especially during extended foraging trips, or upon subsequent visits to a profitable food site. This is especially true around solar noon, when the sun's horizontal position (azimuth) changes most rapidly. This problem is even more complex considering that the sun's daily path (solar ephemeris) depends on the season and geographical position. An animal, therefore, cannot inherently predict the solar ephemeris. Hence, a celestial compass must be calibrated before embarking upon far-ranging foraging journeys (Grob et al., 2017; Wehner and Lanfranconi, 1981; Wehner and Müller, 1993).

In the following, we discuss recent progress in understanding how *Cataglyphis* ants initiate their navigational systems as they switch from performing tasks under darkness of the nest, to becoming a forager navigating over long distances under bright sunshine (Fig. 1) (Rössler, 2019). In *Cataglyphis*, this behavioral transition is rather sudden, making these ants ideal experimental models for the underlying neuronal plasticity. The foraging success of naïve ants (novices) depends critically on a calibrated celestial compass, and knowledge of the nest-related panoramic scenery. We therefore focus on a conspicuous,

early learning behavior and the related plasticity in visual neuronal circuits of the ants' brains. To understand how ant novices acquire navigational knowledge, it was essential to study their behavior in the context of their natural and ecologically relevant habitat. These studies were combined with quantitative analyses of structural synaptic plasticity in visual circuits of two higher integration centers in the brain.

The choreography of learning walks

Cataglyphis workers undergo an age-related polyethism. The ants spend their first four weeks underground inside a dark nest to perform nest-related tasks. Their first day of adult life starts as young callows with a still pale cuticle and no particular task yet. The ants then proceed into a more or less motionless state to serve the colony as living food-stores (interior I). Subsequently, they perform brood care, nest building, or waste management during the interior II stage (Schmid-Hempel and Schmid-Hempel, 1984) (Fig. 1). After about 4 weeks, the ants leave the dark nest to become foragers. During their first short trips close to the nest entrance, novices never bring back any food items. Instead, the ants perform stereotyped sequences of learning walks by meandering in small loops around the nest entrance (Fleischmann et al., 2016, 2017; Stieb et al., 2012; Wehner et al., 2004). Subsequent learning walks explore different sectors around the nest entrance. With increasing experience, learning-walks increase in distance leading the ants farther away from the nest entrance until they eventually start foraging (Fleischmann et al., 2016, 2017). The ants perform this remarkable behavior for 2–3 days (Fleischmann et al., 2017; Stieb et al., 2012; Wehner et al., 2004). This correlates highly with the time needed to induce structural neuronal changes in high-order sensory integration centers, following first sensory exposure or formation of stable long-term memories after associative learning (Filibene et al., 2015; Hourcade et al., 2010; Schmitt et al., 2016; Scholl et al., 2015; Stieb et al., 2010, 2012).

High-resolution video analyses revealed important details in the choreography of learning walks (Fig. 2). The ants repeatedly stop their forward movement to perform rotational body turns. We identified two distinct types of turns – voltes and pirouettes. During voltes, the ants leave their forward path to walk in a small 360°-circle before moving on in the former path direction (Fleischmann et al., 2017). Interestingly, dung beetles perform 360°-rotations on top of their dung ball to take snapshots of skylight cues for alignment of their celestial compass system (el Jundi

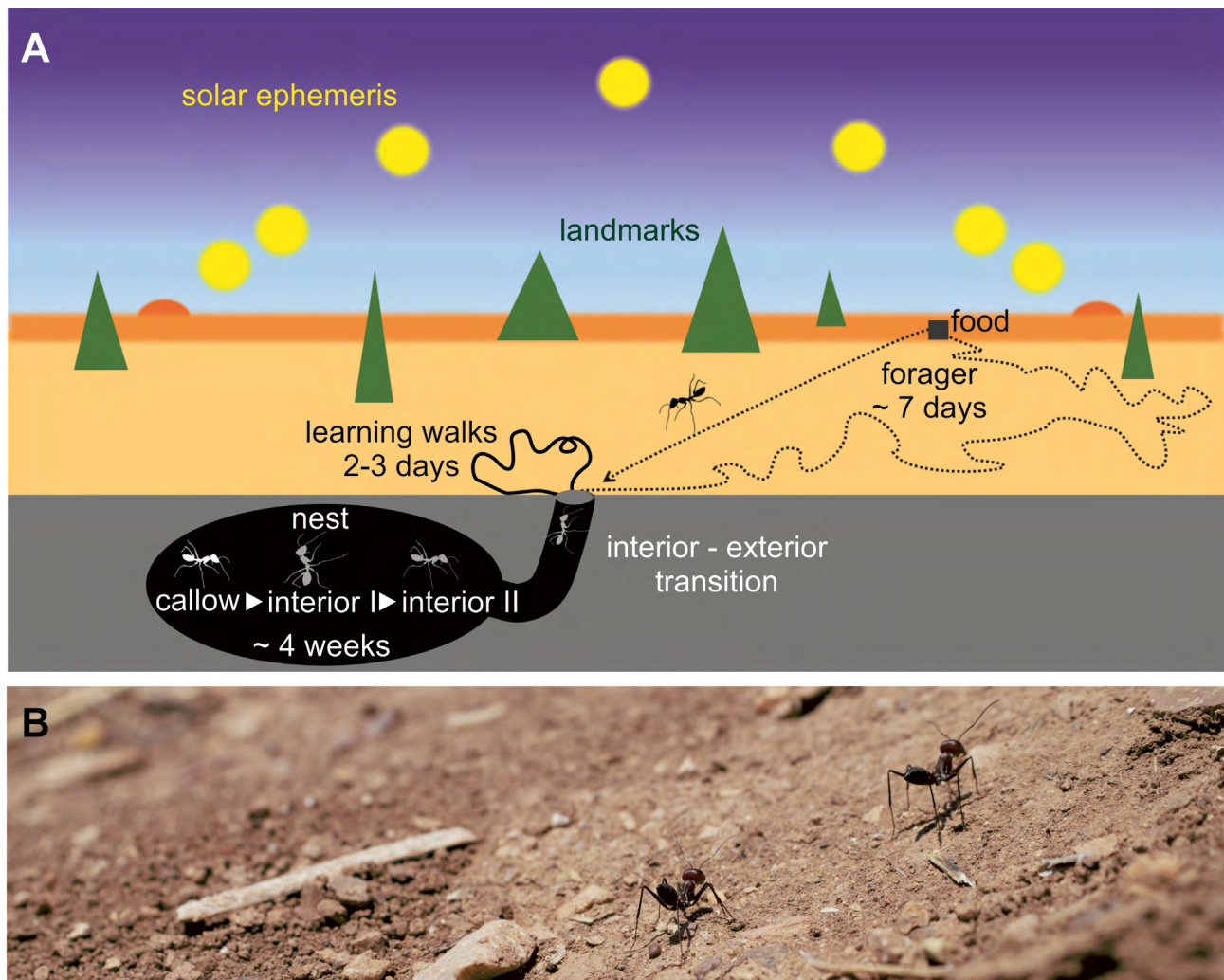


Fig. 1: Individual life history of *Cataglyphis* desert ants. **A.** The ants spend about 4 weeks under darkness of the nest performing interior tasks as callow, interior I, and interior II. They then move on to perform learning walks close to the nest entrance for 2–3 days. Finally, the ants start foraging, using path integration and guidance by panoramic cues for about 7 days, until they die. The daily course of the sun (solar ephemeris) is depicted as snapshots of different horizontal (azimuthal) positions across the sky, together with visual panorama cues (in green) used for navigation. Further details are in the text. Modified from Rössler (2019). **B.** Two *Cataglyphis nodus* ants set out to forage in their natural habitat in Greece.

et al., 2016). Similarly, desert ants may systematically calibrate their celestial compass system over the day during voltes (Fleischmann et al., 2017). During pirouettes the ants perform tight turns about their own body axes. These pirouettes can be either full (360°) or partial turns ($<180^\circ$) (Fleischmann et al., 2017; Grob et al., 2017; Wehner et al., 2004). The ants interrupt their rotational movements multiple times making brief stops (>100 ms). Most interestingly, the gaze direction during the longest stops is precisely directed towards the nest entrance. As the ants cannot see their nest entrance from most positions during learning walks, they must use path integration to align their body axes in the direction of the home vector. Comparison of closely related species living in different habitats, revealed

that only *Cataglyphis* species living in cluttered habitats with a prominent panoramic scenery perform pirouettes with nest-directed views (*Cataglyphis nodus*, *Cataglyphis aenescens*: Fleischmann et al., 2017; *Ocymyrmex robustior*: Müller and Wehner, 2010; *Cataglyphis bicolor*: Wehner et al., 2004; for a review see Rössler 2019). *C. fortis* inhabiting featureless salt pans exclusively perform voltes with rare stops that are not nest-directed (Fleischmann et al., 2017). This suggests that the look-back behavior in pirouettes relates to learning the nest-related panoramic scenery. Snapshot memories from different positions around their nest, allow the ants to determine the nest location from within the panoramic scenery (Cartwright and Collett, 1983; Graham et al., 2010; Zeil, 2012). *Cataglyphis* need at

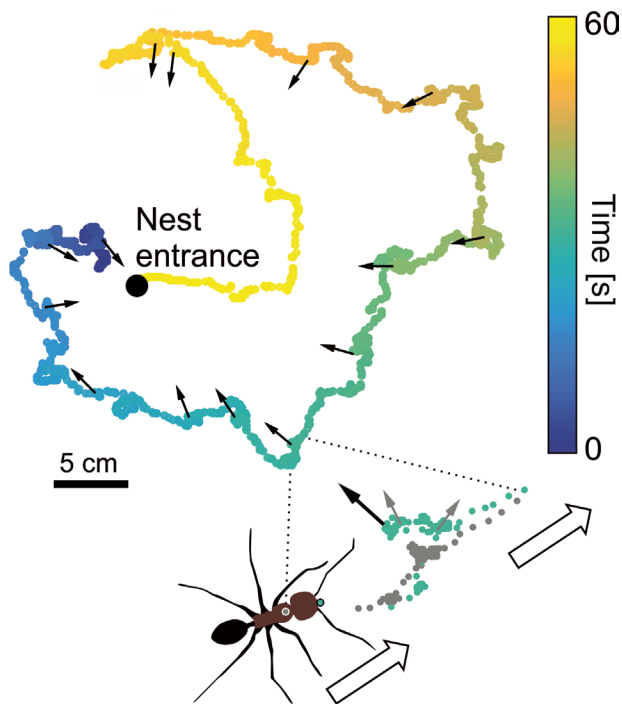


Fig. 2: The choreography of learning walks. During learning walks, desert ants circle around their nest entrance (black dot) in small loops. They repeatedly stop their forward movement to perform pirouettes during which they look back (black arrows) to the nest entrance. Time is color coded. **Inset lower right: Detailed tracking of a pirouette.** During pirouettes, the ants perform a tight turn about their own vertical body axes. The ants stop briefly (> 100 ms) several times while oriented in different directions (arrows). The longest of these stopping phases (black arrow) is precisely directed towards the nest entrance. The tracking positions of the mandibles (green) and the thorax (gray) during a pirouette are indicated. Modified from Fleischmann et al. (2017).

least two days to perform several learning walks in order to use panoramic cues for navigation, as demonstrated in displacement experiments (Fleischmann et al., 2016, 2018b). Furthermore, the ants need enough space during learning walks (at least about 0.5 m distance from the nest entrance), to memorize snapshots from different positions (Fleischmann et al. 2018b). Only after *Cataglyphis* novices had enough time and space to perform learning walks, were they able to use nest-related panoramic cues as a sufficient navigational tool as foragers later on.

The earth's magnetic field is used as initial compass reference

To calibrate their celestial compass and to learn the panoramic scenery surrounding their nest entrance, the ants

need an earthbound reference system. Ideally, such a system is both stable over time and accessible during the complete learning walk sequence. Many previous studies have shown that the celestial compass is the main directional information for path integration in foraging ants. Different filter settings to manipulate the skylight above the nest entrance allowed researchers to ask whether naïve ants also use their celestial compass for the alignment of nest-directed views during learning walks. Surprisingly, even with the sun and skylight polarization pattern blocked, novices still performed nest-directed views that clearly did not rely on the celestial compass (Grob et al., 2017).

The question then arose whether these ants might use the earth's magnetic field as a compass cue during learning walks. Results were unambiguous. After disarray of the earth's magnetic field by a flat coil and elimination of the horizontal component of the earth's magnetic field by a Helmholtz coil setup, the ants' views were no longer directed (Fleischmann et al., 2018a). With systematic rotation of the horizontal magnetic field component using Helmholtz coils, the ants gazed towards a fictive nest entrance rotated by the same angle as the magnetic field (Fig. 3; for more details see Fleischmann et al. 2018a). These results clearly demonstrate that the ants use the geomagnetic field as a necessary and sufficient compass cue for path integration during learning walks. This means that compass information is integrated into the path integration system, adding a new compass to *Cataglyphis*' navigational toolkit. The earth's magnetic field not only provides the initial compass system for navigation in novices, it also represents a geostable compass reference, for learning the visual panorama and for calibrating the celestial compass.

Despite increasing behavioral evidence for a role of the geomagnetic field as a cue for orientation or navigation in various animal species, the behaviorally-relevant sensory mechanisms and especially the neuronal processing of geomagnetic information still remains elusive (Lohmann, 2018; Mouritsen 2018; Nordmann et al., 2017). Due to the clear role of the magnetic sense in path integration during learning walks, *Cataglyphis* ants are a highly promising new experimental model for future studies on insect magnetosensation. Here, the geomagnetic compass is the only compass naïve ants rely on during their learning walks, whereas the use of a magnetic sense for directional orientation in other insect species was less apparent or only in combination with other cues (Dreyer et al., 2018; Wajnberg et al., 2010). The short spatial range of learning walks enables high-resolution video analyses of nest-directed views, providing quantifiable behavioral readout

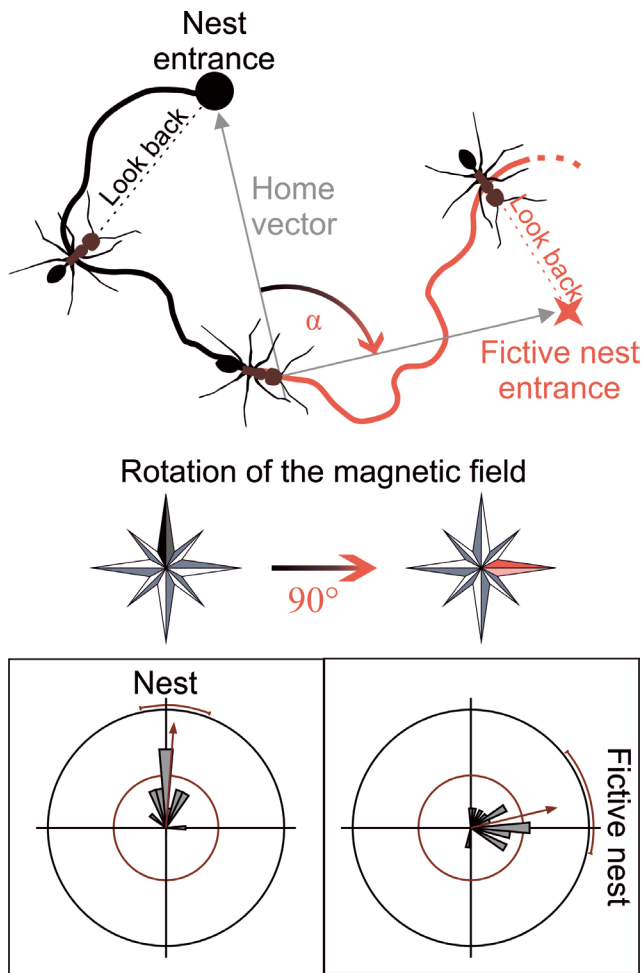


Fig. 3: The earth's magnetic field is a compass cue. **Upper:** During naïve learning walks *Cataglyphis nodus* relies on the geomagnetic field for directional information during path integration. When a naïve ant leaves its nest to perform a learning walk (black line), it looks back to the nest entrance during pirouettes. However, when the horizontal component of the earth's magnetic field is experimentally rotated using a Helmholtz-coil setup (horizontal rotation angle depicted by the compass), the home vector of the ant is rotated likewise, and the ants look back to a fictive nest entrance during the following pirouette. **Lower:** After rotation of the horizontal component of the magnetic field, mean gaze directions during the longest stopping phases in pirouettes become directed towards a fictive nest entrance rotated by the same angle (90°). Inner circles of the circular plots indicate Rayleigh's critical value ($\alpha = 0.05$) (further details in Fleischmann et al. 2018a). These results demonstrate that the ants integrate directional information from the earth's magnetic field into their path integration system. Modified from Fleischmann et al. (2018a).

for path integration during navigation. Future experimental manipulations of magnetosensation in freely behaving ants will be combined with studies aiming to understand the sensory mechanisms and neuronal integration of magnetic compass information.

Two visual pathways for navigation

How is visual navigation information processed in the ants' brains? One hypothesis is that the alignment of views during rotational turns serves to acquire visual information, by using the earth's magnetic field as a geostable compass reference. This may serve both the calibration of celestial information and the acquisition of panoramic snapshot memories (Fleischmann et al., 2017, 2018a; Grob et al., 2017). How are global compass cues and local panoramic image information processed in the ant's brain? Earlier behavioral studies had shown that the ants memorize local panoramic information for up to their lifetimes, whereas global skylight compass and path integration information showed a much faster memory decay, which suggests two channels of visual information transfer (Ziegler and Wehner, 1997).

Physiological studies in the locust and bees, together with recent modeling approaches, suggest that the central complex (CX) integrates sky-compass information, whereas mushroom-body (MB) circuits have the capacity for memorizing image information (Ardin et al., 2016; Hoinville and Wehner, 2018; Homberg et al., 2011; Stone et al., 2017; Webb and Wystrach, 2016). The CX and MB visual pathways in *Cataglyphis* were characterized using neuronal tracing combined with immunolabeling of synaptic proteins and ultrastructural investigations, which allowed the characterization of differences in the synaptic architecture of both circuits (Fig. 4) (Grob et al., 2017; Schmitt et al., 2016). The CX pathway starts from a polarization-sensitive upper region of the eye, the dorsal rim area, and proceeds with neuronal projections to the optic ganglia, and via the anterior optic tract, to the anterior optic tubercle, and from there, to the lateral complex and the lower division of the CX. In contrast, the MB pathway is characterized by direct neuronal connection from projection neurons in the medulla via the anterior superior optic tract to the MB calyces.

Interestingly, both visual pathways comprise exceedingly large synaptic complexes at the input to the two integration centers – the CX and the MBs (Schmitt et al., 2016; Stieb et al., 2010) (Fig. 4 insets). The CX pathways in each hemisphere converge on about 100 synaptic complexes in the lateral complex. The large (>5 μm diameter), cup-shaped presynaptic sites contain ~100 active zones with contacts to many profiles of a relatively small number of postsynaptic tangential neurons that relay information to the lower division of the CX central body. These convergent synaptic circuits might guarantee a reliable and precise transmission of binocular sky-compass information (Schmitt et al., 2016). In the MB pathway, projection

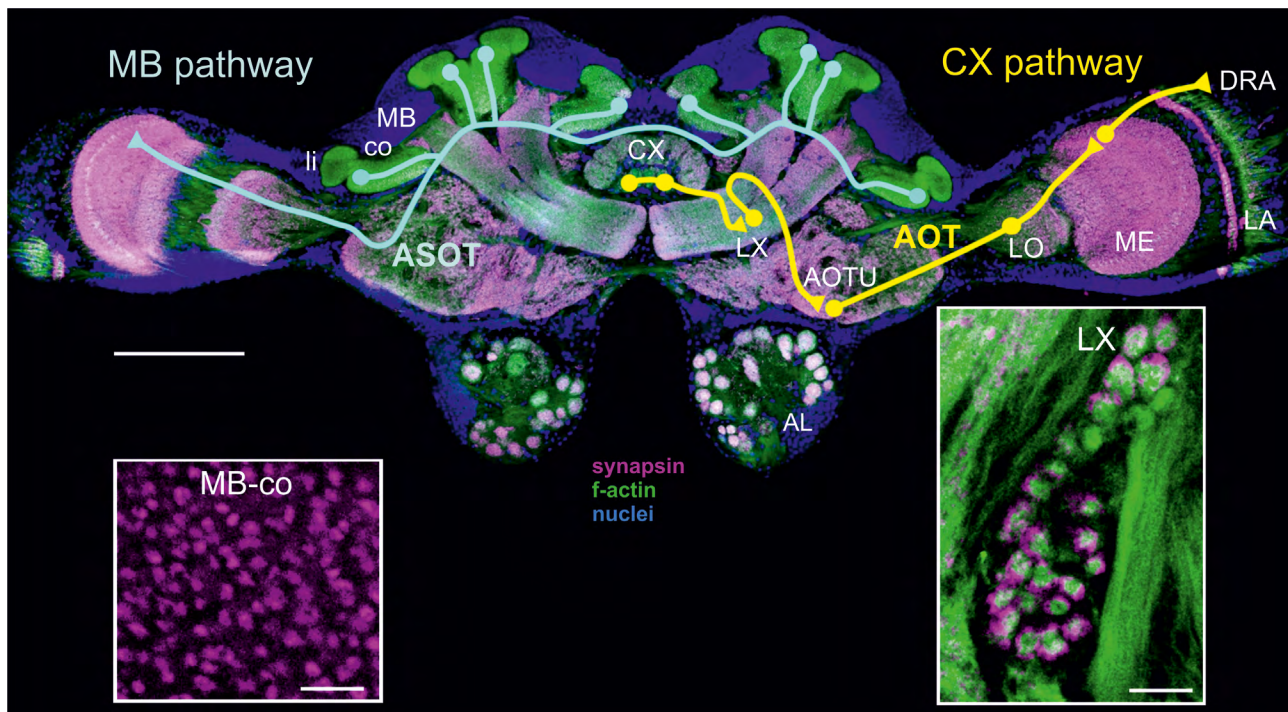


Fig. 4: Two visual pathways for navigation in the *Cataglyphis* brain. The visual pathway to the central complex (CX or sky-compass pathway) is depicted in the right brain hemisphere of a *C. fortis* brain, and the visual pathway to the mushroom bodies (MB pathway) is highlighted in the left hemisphere. The brain is labeled with an antibody to the presynaptic protein synapsin (magenta), staining of f-actin in dendritic and axonal profiles by phalloidin (green), and detection of cell nuclei with Hoechst 3458 (blue). Scale bar = 200 µm. Further abbreviations: AL antennal lobe, AOT anterior optic tract, AOTU anterior optic tubercle, ASOT anterior superior optic tract, co collar, CX central complex, DRA dorsal rim area, LA lamina, li lip, LO lobula, LX lateral complex, ME medulla. The two lower images show high magnifications of large synaptic complexes at the input to the MB calyx collar (MB-co) and the lateral complex (LX). Scale bars = 10 µm. The brain image is modified from Stieb et al. (2012). The pathways are combined from results by Schmitt et al. (2016) and Grob et al. (2017). High magnification whole-mount images of synaptic complexes provided by Kornelia Gröbel.

neurons from the medulla project directly to the MBs to form many synaptic boutons in visual input regions (collar) of the medial and lateral MB calyces in both brain hemispheres (Fig. 4). Individual boutons contain ~50 active zones with divergent connections to many profiles of dendritic spines from a large number of Kenyon cells, the intrinsic MB neurons (Grob et al., 2012; Stieb et al., 2010). Synaptic boutons in the MB-calyx collar are estimated to number up to 400,000 (Grob et al., 2017).

The different circuit architecture in the CX and MB pathway is suggestive for differences in the type of information processing (Fig. 6). The direct connection of projection neurons from the medulla to a large number of parallel MB synaptic circuits promoting memory formation is suggestive for the potential storage of complex image information (Ardin et al., 2016). By contrast, the CX pathway comprises a high level of pre-processing in the optic neuropils and converges on a relatively small number of synaptic complexes in the lateral complex that relay the information via tangential neurons to the CX.

The CX pathway is highly conserved across insect species studied so far, which are as diverse as locusts, bees, ants, butterflies and dung beetles (Heinze, 2014). It was named sky-polarization pathway since light-polarization information is detected by a group of specialized photoreceptors in the dorsal rim area of the eye and mediated to the CX. Physiological studies in locusts, bees, butterflies and dung beetles suggest that skylight-compass information is systematically mapped in both the lower unit of the central body, a major input region of the CX, and the protocerebral bridge, the predominant output region of the CX (Heinze, 2014; Heinze and Homberg, 2007; Homberg et al., 2011) (Fig. 6). Live calcium imaging studies in *Drosophila* show that this results in an internal representation of the insect's heading (Seelig and Jayaraman, 2015). Recent results in bees show that information about speed (processed as optic flow) is transmitted to the noduli of the CX, suggesting that one important function of the CX circuitry (among other functions in spatial orientation), is to compute path-integration information (Stone et al., 2017).

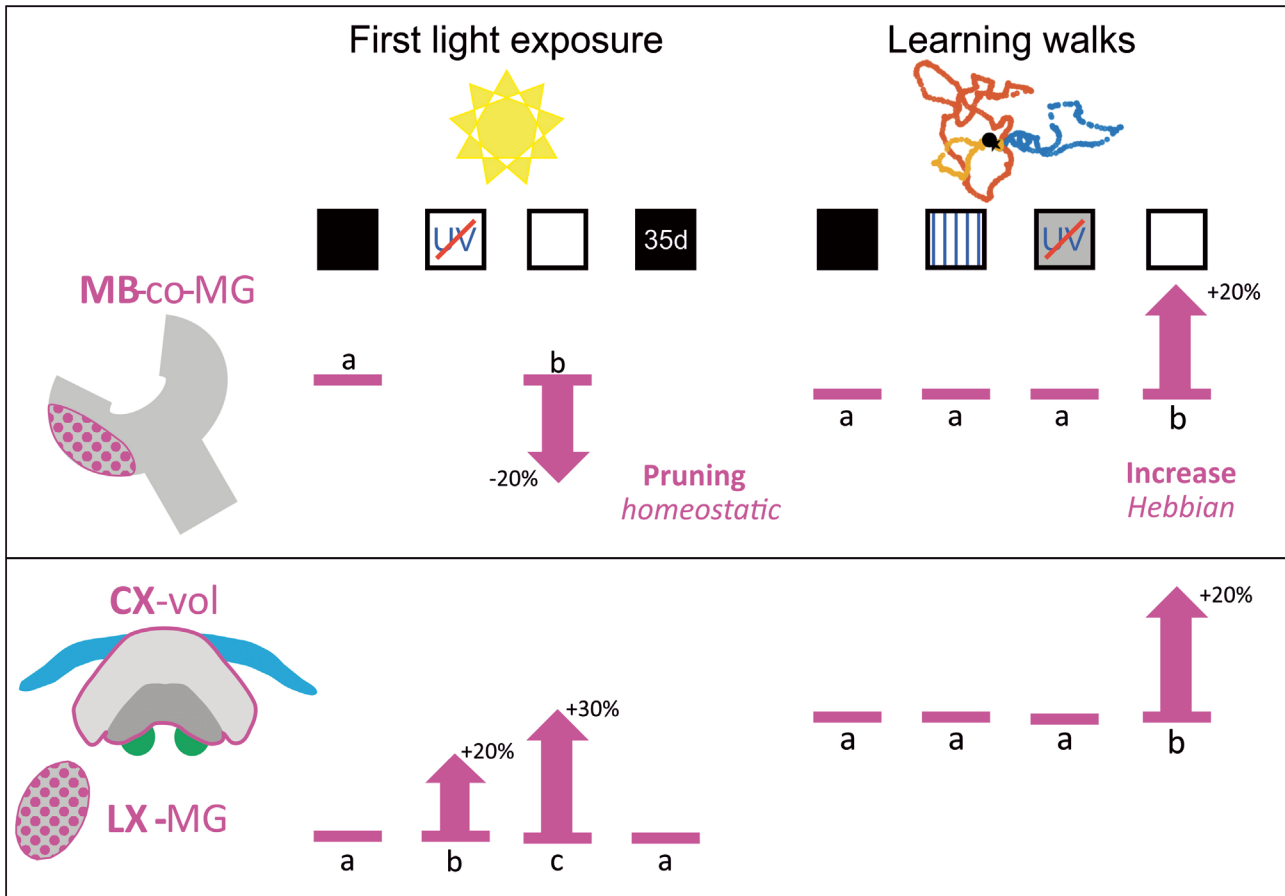


Fig. 5: Neuroplasticity after first light exposure and learning walks. The upper panel summarizes results from quantifications of structural synaptic plasticity in the mushroom body collar (MB-co) after first light exposure and following learning walks. The lower panel shows results on volume changes in the central complex (CX) and numbers of synaptic complexes in the lateral complex (LX). Differences in light conditions are depicted in each case. The percentages represent averages. Statistical significances are indicated by letters (see text for further details on quantitative data). Symbols for first light exposure, from left: same aged without light exposure, UV blocked, full light spectrum, 35 day-old dark reared. Symbols for learning walks, from left: without learning walks (dark), linear polarizer, UV and sun position blocked, full spectrum. Increase or decrease are depicted by arrows including average percentages. Data combined from (Grob et al., 2017; Schmitt et al., 2016; Stieb et al., 2010, 2012).

Microcircuits in the MBs were shown to perform multisensory integration, learning, and associative memory formation (e.g. Menzel, 2014; Oswald and Waddell 2015; Gerber et al., 2004). The associative circuits provide a suitable neuronal substrate for storage of multiple visual panoramic snapshots (Ardin et al., 2015). Interestingly, the visual pathway to the MBs is elaborated in insects with enhanced spatial orientation skills and respective visual ecologies as was shown for beetles (Farris and Roberts, 2005), parasitoid and social Hymenoptera (Farris and Schulmeister, 2011), ants (Grob et al., 2017; Groh et al., 2014; Gronenberg, 2001; Yilmaz et al., 2016) and butterflies (Kinoshita et al., 2012). In *Drosophila*, only a small number of visual projections to the MB calyx were found (Vogt et al., 2016).

To recapitulate, analyses of visual neuronal circuits revealed two elaborate pathways in *Cataglyphis* – the CX

pathway with the capacity for transferring global sky-compass information, and the MB pathway for transferring and storing local image information. Interestingly, in both visual pathways, the last synaptic relay station (in the lateral complex and mushroom body calyx) is mediated by synaptic complexes comprising large presynaptic boutons that form spheroidal (microglomerular) structures, together with multiple postsynaptic dendritic processes (Fig. 4, insets). The large size of these microglomeruli facilitates quantitative confocal imaging analyses for investigating the role of learning walks in triggering structural synaptic plasticity in both visual circuits.

Learning walks trigger structural synaptic plasticity in the CX and MB pathway

The interior-exterior transition leads to structural synaptic plasticity in both visual pathways. These effects were quantified using 3D reconstruction of confocal microscopy images, volume rendering and semi-automated computer guided quantification (Rössler et al. 2017). The changes can be classified into plasticity after first light exposure and learning related plasticity (Fig. 5).

Plasticity after first light exposure: In the CX pathway, the number of synaptic complexes in the lateral complex significantly increases in foragers compared to interior workers (Schmitt et al., 2016) (Fig. 5). The increase was, on average, 30 % (from an average of 95 to 126 synaptic complexes) following first exposure to light. This effect depended on spectral composition and was reduced to, on average, 20 % (~116) synaptic complexes when the UV part of the spectrum was blocked. UV is important in mediating the sky-polarization pattern in photoreceptors of the dorsal rim area. Conversely, in the MB pathway, first light exposure leads to a significant reduction (on average 20 %) in the density of microglomeruli in the visual MB-calyx collar (for details see Stieb et al., 2010, 2012). In both the CX and MB pathway, the densities of synaptic complexes in age-matched dark reared ants remained unchanged, showing that this plasticity is independent of age and triggered by light. Although plasticity in the CX and MB pathways after first light exposure point in opposite directions, we conclude that they represent homeostatic structural plasticity, adjusting the circuits to drastically changing light conditions. This most likely starts before the onset of learning walks, when the ants briefly exit their nest to perform digging activities during the interior II stage (Fleischmann et al., 2017). The increase in lateral complex synaptic complexes, in contrast to pruning in MB microglomeruli, can be assigned to different functional properties of the input neurons: Whereas the majority of tangential neurons projecting to the CX are GABAergic, projection neurons providing input to the MBs are excitatory (cholinergic) (Schmitt et al., 2016).

Plasticity after learning walks: Skylight manipulations during learning walks revealed that ants expressed a volume increase in the CX (on average 20 %), when novices perceived a naturally changing sky-polarization pattern during three days of learning walks (Fig. 5) (Grob et al., 2017). The volume increase was absent under a linear polarization pattern or blocked UV transmission. This suggests a role of neuronal plasticity in the CX pathway in calibrating

the celestial compass system. Our hypothesis is that the geomagnetic compass reference is used for both calibrating path integration input via the sky-compass pathway to the CX, and aligning nest-directed panoramic snapshots transferred to the MBs (Fig. 6). It remains unclear, how and whether sky-compass input is linked to the endogenous clock for proper time compensation, which represents another interesting topic for future research.

Visual compartments of the MB calyces showed an increase in density and number (on average 20 %) of microglomeruli after three days of learning walks, but only when novices had perceived a naturally changing sky-polarization pattern (for details see Grob et al., 2017) (Fig. 5). Plasticity was absent when the ants perceived a static polarization pattern or had no UV input. This is very different from the decrease (pruning) of microglomeruli after passive light exposure under natural skylight conditions (Stieb et al., 2010, 2012). Our results from olfactory learning in honeybees and leaf-cutting ants have shown that associative learning and the formation of stable long-term memory, similarly triggered an increase in microglomeruli, but in olfactory subregions of the MB (Falibene et al., 2015; Hourcade et al., 2010). We therefore conclude that the increase of microglomeruli in visual MB after learning walks is suggestive for the formation of visual long-term memory. This suggests that the increase in microglomeruli in visual MB compartments after learning walks, represents learning-related (Hebbian) structural plasticity associated with formation of stable visual long-term memory.

Investigations in other insects, especially *Drosophila*, and modeling work have shown that the CX is also involved in simple landmark learning, spatial memory, landmark orientation and motion control (Fiore et al., 2017; Neuser et al., 2008; Martin et al., 2015; Seelig and Jayaraman, 2015). However results from *Cataglyphis* ants suggest that storage of more complex panoramic scenes requires input to MBs with elaborate numbers of parallel microcircuits that are suitable for visual long-term memory formation. What could be the associative component for nest-directed snapshot memories? The intrinsic association of the nest with nest-directed views (home vector), might alone serve as an internal reward, which was also termed ‘genetically encoded anticipatory learning’ (Collett and Zeil 2018). Whether and how visual snapshot matches experienced by a homing ant are relayed from the MB output to the CX or to premotor areas, remains another area of future research (Fig. 6). Anatomical data from *Drosophila* suggests potential connections from the MB output via interneurons in the superior protocerebrum to the CX, which still needs to be investigated in *Cataglyphis* (Strausfeld and Hirth, 2013) (Fig. 6).

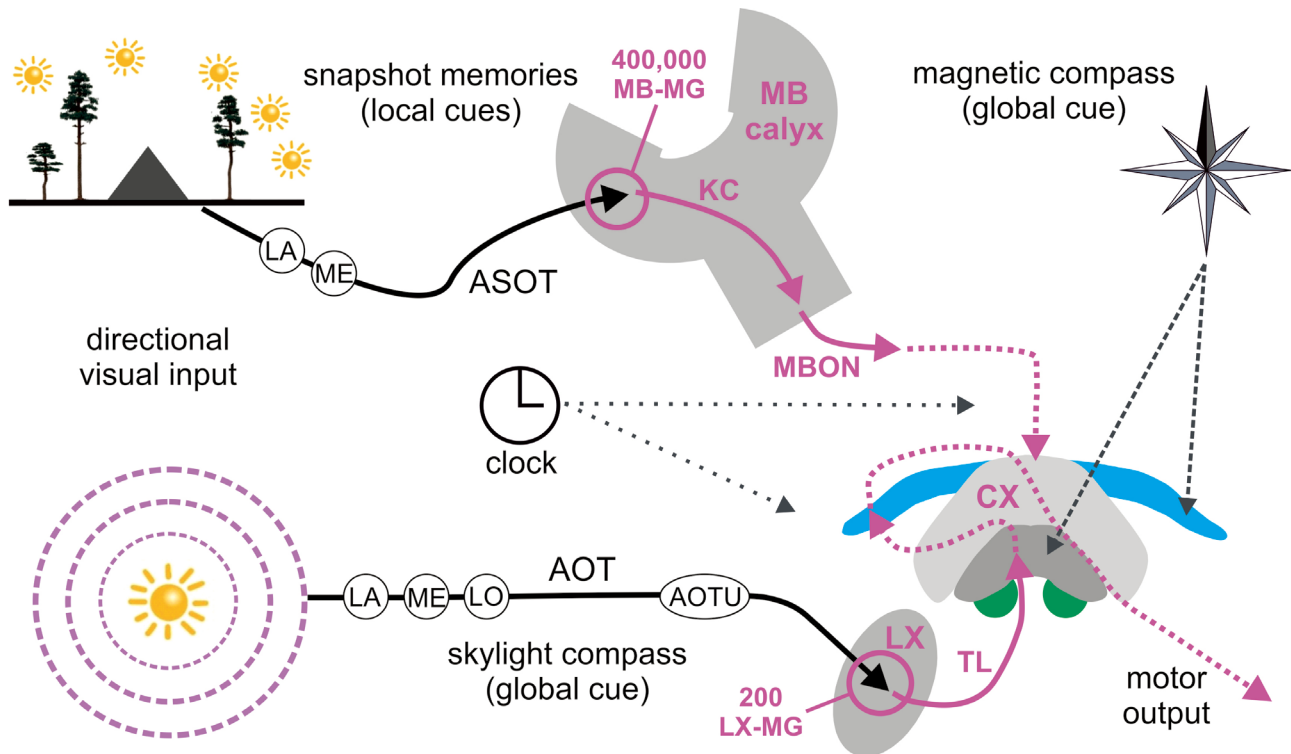


Fig. 6: Model for processing of directional navigational information. The sites of structural synaptic neuroplasticity in visual pathways after first light exposure and following learning walks are labeled in magenta. The left side depicts visual input from the panoramic scenery and skylight-compass cues. Sky-compass information (global cues) is processed via the anterior optic tract (AOT) to the lateral (LX) and central complex (CX), whereas panoramic information (local cues) is processed via the anterior superior optic tract (ASOT) to the mushroom bodies (MB). The input and output connections, together with different numbers of plastic synaptic complexes (microglomeruli, MG) at the input of the MB and LX, are indicated (magenta). The sensory pathways for geomagnetic information, the input of the endogenous clock for time-compensation, a putative connection from the MB output to the CX, and connections to the motor output are still hypothetical and depicted as dashed lines. Further abbreviations: AOTU anterior optic tubercle, KC Kenyon cell, LA lamina, LO lobula, MBON mushroom body output neuron, ME medulla, TL tangential neuron.

Outlook

Learning walks in *Cataglyphis* ants provide an excellent experimental model to study the interaction between behavior, environment, and the brain in the context of navigation. *Cataglyphis* desert ants initiate their visual navigational systems during this early learning phase, by using the geomagnetic field as a geostable compass reference and directional cue for path integration. The learning behavior and the related plasticity in visual pathways opens a wide range of follow-up questions. Where is the magnetosensor located, how does it work, and how is the information integrated with visual information in the path integration circuitry of the CX? Presently, neither the sensor, nor the central pathway for magnetosensation are known. The ants might even use a magnetic compass during underground navigation before leaving the nest. Why do foragers switch from using a magnetic compass during learning walks to using a celestial compass? Expe-

rienced foragers, while apparently ignoring the magnetic compass during foraging runs, perform re-learning walks when confronted with new landmarks around their nest, or when they discover new feeding grounds. In both cases, the ants perform rotational body turns resembling those in naïve learning walks. Do foragers switch back to using the geomagnetic field for navigation under these situations? As the multisensory information must be integrated in the ants' brains during learning walks, future studies will investigate how visual and magnetic compass cues converge in the path integration circuits of the CX and become potentially linked with information from the endogenous clock for time compensation. The remarkable neuronal and behavioral plasticity during the interior-exterior transition of *Cataglyphis* ants, is a highly promising experimental model to investigate the behavioral and neuronal mechanisms underlying the ontogeny of an advanced navigational system housed in a comparatively small brain.

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