Neuroplasticity in desert ants (Hymenoptera: Formicidae) – importance for the ontogeny of navigation

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Abstract

Ants express a remarkable behavioral plasticity ranging far beyond rigid stimulus-response relationships. This review highlights the role of adult neuronal plasticity (neuroplasticity) in ant brains related to behavioral changes at the transition from tasks inside the nest to outdoor foraging. The focus is on desert ants of the genus *Cataglyphis* Foerster, 1850 – a famous experimental model for the study of visually based long-distance navigation. In contrast to ant species using trail-pheromone communication or recruitment strategies to exploit profitable food sources, thermophilic *Cataglyphis* ants are individual scavengers searching for dispersed food items on long excursions. For directional information during foraging, the ants strongly rely on a skylight compass and visual guidance by panoramic scenes. The marked adult transition in the individual life history of the ants offers ideal experimental access to study the behavioral and neuronal plasticity underlying the ontogeny of successful navigation. For the major part of their lives, the ants perform tasks in the dark underground nest interior before switching to largely visually based navigation under bright sunlight. Recent studies demonstrate that learning walks – a stereotyped behavioral routine the ants perform prior to first foraging – represent a crucial element in the ontogeny of successful navigation. Analyses of visual neuronal pathways to the central complex and mushroom bodies – two prominent sensory integration centers in the insect brain – revealed that first light exposure and visual experience during learning walks lead to distinct structural re-organizations of synaptic circuits in both brain centers reflecting initial calibrations and memory processes in the ants' visual compass systems.

Key words: Review, ant brain, transition, behavioral plasticity, orientation, memory, learning walk, vision, earth's magnetic field, central complex, mushroom body, central-place forager.

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Introduction

Successful spatial orientation is crucial for most animal species including humans. For ants, navigation is of paramount importance. To perform collective brood care in a common nest, ants are central-place foragers and find back to their nest after long foraging excursions. Efficient homing strategies, therefore, represented an important behavioral trait in the evolution of insect sociality (Hölldobler & Wilson 1990, Ronacher 2008). In contrast to many ant species employing route following strategies via trail-pheromone communication or nest-mate recruitment to exploit profitable food sources (Hölldobler 1999), thermophilic *Cataglyphis* Foerster, 1850 ants are individual scavengers of dispersed food items that heavily rely on visually guided navigation (Wehner 2009, Wehner & Rössler 2013, Boulay & al. 2017).

Compared with most vertebrates, ants possess miniature brains containing less than a million neurons (Gronenberg 2008, Ronacher 2008, Wehner 2009, Wehner & Rössler 2013). However, their individual behavioral plasticity, communication skills, navigational abilities, and cognitive capabilities are remarkable and, in some cases, not fundamentally different from those in higher vertebrates with much bigger brains (Leadbeater & Chittka 2007, Chittka & Niven 2009). The small size of ant brains poses certain limits, as for example shown for the packing density of synaptic complexes in learning and memory centers (mushroom bodies, MBs) in the brain of the highly polymorphic leaf cutting ant *Atta vollenweideri* Forel, 1893 (Groh & al. 2014). Here, the density of synaptic complexes in the MBs is independent of body size and similar in small and large workers. This causes a limit for caste-specific miniaturization of the MBs. Consequently, the number of synaptic microcircuits involved in sensory integration, learning, and memory formation is smaller

in small workers, despite the relative expansion of MB volumes. However, despite obvious limitations in brain volumes, the individual behavioral repertoires of ants are remarkably diverse and flexible, as expressed by the ability to form multiple olfactory long-term memories (LTMs) that can last for an ant's individual life span. Leaf-cutting ants, for example, form aversive olfactory LTMs for plants unsuitable for fungus cultivation (Herz & al. 2008, Saverscheck & Roces 2011, Falibene & al. 2015). In a similar line, desert ants may store multiple visual memories for recognition of complex panoramic scenes or routes for navigation and form distinct categories of olfactory memories for multiple nest- and food-related odorants (Knaden & Graham 2016, Huber & Knaden 2018). This may shape the behavior of ants over long periods to up to their entire life span, which may be in the range of several months.

Desert ants of the genus *Cataglyphis* possess impressive long-distance navigational capabilities. The behavioral routines and navigational strategies during foraging and homing were discussed in previous reviews (e.g., Ronacher 2008, Wehner 2009, Wehner & Rössler 2013, Knaden & Graham 2016, Freas & Schultheiss 2018). Gronenberg (2008) and Gronenberg & Riveros (2009) compared the general neuroanatomy of ant brains and discuss their evolution in the context of the social brain and ecological brain hypotheses. Kleineidam & Rössler (2009) highlight olfactory adaptations in ant nervous systems, and Kamhi & Traniello (2013) and Kamhi & al. (2017) review the roles of neuromodulators. The focus of this present review is on the striking plasticity of ant behavior and underlying changes of neuronal circuits (neuroplasticity) in the brain during a major transition from tasks inside the nest to outdoor foraging. The emphasis is on adult plasticity – developmental plasticity of the ant brain such as the expression of caste- or sex-specific differences during postembryonic metamorphosis is reported elsewhere, for example for *Camponotus floridanus* (Buckley, 1866) by Zube & Rössler (2008). Non-associative and associative adult plasticity are powerful attributes of nervous systems to adjust to changing external conditions and to memorize stimulus associations. Ants of the genus *Cataglyphis* are favorable experimental models to study plasticity underlying the ontogeny of successful navigation. At the interior-exterior transition, adult *Cataglyphis* experience major changes in sensory input and perform a conspicuous sequence of learning routines that are crucial to become a successful forager. Hence, this review reports recent progress in understanding the behavioral and neuronal ontogeny of navigation.

Navigation in desert ants

Navigation is among the most challenging behavioral tasks for many animal species. The discovery of hippocampal place neurons and cortical (entorhinal) grid neurons as a neuronal substrate for spatial orientation in mammalians were milestones in research on the neuronal basis of spatial orientation (O'Keefe & Burgess 1996, Hafting & al. 2005, Fyhn & al. 2007, Moser & al. 2008). How do ti-ny-brained ants solve similarly complex navigational tasks when finding back to their nest after searching for food over long distances in largely unpredictable and – in some cases – extremely harsh environments? With exception of ants following established chemical pheromone trails, navigation to a specific destination requires advanced spatial orientation skills that enable the constant acquisition of information about the direction and distance of a goal at any point of travel in space (Ronacher 2008). However, even in species with trail pheromone recruitment, scouts need to be able to navigate as well as solitary foraging ants do. Furthermore, Harrison & al. (1989) have demonstrated that also experienced trail-recruiting ants may ignore the pheromone trail and rely on navigation.

The distantly related desert ants of the genera *Cataglyphis*, *Ocymyrmex* Emery, 1886 and *Melophorus* Lubbock, 1883 living in ecologically equivalently hot and arid habitats in Africa, Southern Europe and Australia excluding the use of pheromone trails revealed important insights into ant visual navigation strategies. These ants solve the challenge to find back to their inconspicuous nest entrance - a small hole in the ground - after returning from often long and complex foraging trips (Wehner 2009, Freas & Schultheiss 2018). Over the past years, several reviews have comprehensively reported on the behavioral routines for navigation in foraging desert ants (e.g., Ronacher 2008, Wehner 2009, Knaden & Graham 2016, Freas & Schultheiss 2018) and emphasize the importance of visually-based navigational strategies (Wehner & al. 2014, Zeil 2012). The following section, therefore, only briefly summarizes major findings and subsequently focusses on the behavioral and neuronal ontogeny of visually based navigation.

In the North African desert ant *Cataglyphis fortis* (Forel, 1902) the total lengths of foraging trips may exceed up to c. 1500 m with maximal foraging distances of more than c. 350 m away from the nest entrance. This is equivalent to several thousand times of the ants' own body lengths (Ronacher 2008, Buehlmann & al. 2014, Huber & Knaden 2015, Knaden & Graham 2016). The remarkable long-distance navigational capabilities of *Catagylphis* foragers primarily depend on a process termed path integration (Ronacher 2008, Wehner 2009). In *Cataglyphis* path integration employs a skylight compass using the sky polarization pattern, sun position and chromatic cues of the sky to integrate the directions traveled and a stride (or step) counting mechanism for measuring distances of path segments (Wohlgemuth & al. 2001, Wehner & Müller 2006, Wittlinger & al. 2006, Ronacher 2008, Lebhardt & Ronacher 2014, 2015, Wehner & al. 2016). The ants extrapolate a home vector from the directional and distance information guiding them back to their nest entrance along an almost straight line. As these processes are prone to cumulative errors over long distances (Knaden & Wehner 2005, 2006), the ants combine path integration with visual guidance by panoramic features, whenever these cues are available, and the ants rely on panoramic scenes or the panoramic skyline as visual cues

during navigation (e.g., Lent & al. 2009, Wehner 2009, Huber & Knaden 2015, Buehlmann & al. 2016).

During their final approach to the nest entrance, the ants also use upwind orientation to olfactory cues emanating from the nest, especially CO_2 and make use of environmental odor landmarks to pinpoint the nest entrance (Bregy & al. 2008, Steck & al. 2011, Buehlmann & al. 2012). In the past years, studies on ant navigation increasingly addressed the interplay between path integration, visual panorama guidance systems, and other cues like wind direction or olfactory landmarks (e.g., Kohler & Wehner 2005, Collett & al. 2013, Narendra & al. 2013, Wystrach & al. 2015, Wehner & al. 2016, Huber & Knaden 2017). Experimental combinations of different sensory cues, especially the presentation of cue conflicts revealed that the ants steer intermediate courses during homing indicating that the different navigational routines always run in parallel (Reid & al. 2011, Narendra & al. 2013, Legge & al. 2014, Wystrach & al. 2015). An extended model, recently proposed by Hoinville & Wehner (2018), suggests that parallel processing of multiple navigational cues (resulting in individual vectors) functions without a central integrator. A decentralized neuronal model architecture with simultaneous integration of global guidance cues (global vectors) like the sky-polarization pattern and local guidance cues like the panoramic scenery (local vectors) proved sufficient for modeling the behavior of the ants under natural and manipulated conditions.

The recent advances in behavioral analyses and models based on the behavioral studies provide a very fruitful theoretical and experimental framework in the search for neuronal correlates of navigation in the ants' brains. Whereas a large body of research on desert ant navigation – as briefly outlined above – focused on behavioral analyses of navigational skills in experienced foragers, the identity of neuronal circuits for visually based navigation and their adjustments and memory performance during the behavioral ontogeny of navigation are much less understood (Zeil 2012, Wehner & Rössler 2013, Webb & Wystrach 2016, Freas & Schultheiss 2018). The individual life history of relatively short-lived ants of the genus *Cataglyphis* offers unique experimental access to address these questions as the ants undergo a robust behavioral transition from performing tasks inside the dark nest to outside solitary foraging over long distances in bright sunlight (Wehner & Rössler 2013). This marked transition predicts high levels of plasticity in the underlying neuronal substrates and offers a unique experimental model for interdisciplinary research aimed to link the behavioral ontogeny of navigation with the underlying neuroplasticity.

The individual life history and behavioral ontogeny of *Cataglyphis*

For the major part of their lives, *Cataglyphis* ants stay underground in their dark nest interior and go through distinct behavioral-stage transitions before they switch to far-ranging outdoor foraging trips (Schmid-Hempel & Schmid-Hempel 1984, Stieb & al. 2010, 2012) (Fig. 1). How do the ants' sensory systems and brain centers adjust at this marked transition, and how do the ants acquire all the necessary navigational information before heading out on first foraging trips? The stride (or step) integrating systems for distance estimation can operate largely independent from experience (Wittlinger & al. 2006, Ronacher 2008). In contrast, the directional navigational systems, such as the sky-compass and panoramic guidance routines, need to be calibrated and require learning and memory processes before the onset of foraging (Zeil 2012, Fleischmann & al. 2016, 2018a). They operate with largely variable sensory input such as the seasonally changing course of the sun (solar ephemeris) and unpredictable positions of visual panoramic sceneries with relation to the sky-compass (Towne 2008, Towne & Moscrip 2008, Wehner & Rössler 2013; Fig. 1). Visual systems, therefore, need to adjust to the drastically changing sensory input at the interior-exterior transition, which predicts processes of homeostatic neuroplasticity (sensu Fox & Stryker 2017). In addition, we hypothesize that visual learning and memory formation is associated with learning-related (Hebbian, associative) neuroplasticity in visual integration centers in the ants' brains.

Cataglyphis workers undergo the following age-related ethocaste stages: callows (pale, freshly emerged workers c. 24h old), interior I (motionless food-storage stage indicated by swollen gasters), and interior II (performing brood care and digging behavior). Finally, foragers actively search for food during long-distance foraging trips outside the nest after a period of c. 4 weeks inside the nest (Schmid-Hempel & Schmid-Hempel 1984, Stieb & al. 2010, 2012; Wehner & Rössler 2013, Schmitt & al. 2016) (Fig. 1A). The first activities outside the nest are digging walks performed by interior II ants (Fleischmann & al. 2017). To transport material out of the nest, the ants briefly exit the nest entrance for distances of only a few centimeters. They walk along an almost straight line to deposit soil particles a few centimeters from the nest entrance. After the ants dropped their load, they perform a (180°) U-turn to immediately return to and disappear in the nest entrance. This behavior does not require any advanced orientation skills as digging walks are short and follow a very simple rule for returning to the nest entrance in a hairpin-like fashion. Most importantly, digging walks represent the first activities in the ants' lives when they experience full exposure to sunlight.

Prior to first foraging trips – before they start to actively collect food items – naïve *Cataglyphis* ants (novices) perform elaborate sequences of learning walks (previously also termed orientation or exploration walks) over 2 - 3 consecutive days (Nicholson & al. 1999, Wehner & al. 2004, Stieb & al. 2012, Fleischmann & al. 2016, 2017, Grob & al. 2017, Collett & Zeil 2018, Jayatilaka & al. 2018). Learning walks are short excursions during which the ants slowly meander around the nest entrance without collecting food or performing digging activities (Fig. 2). Given an average total foraging period in *Cataglyphis* of
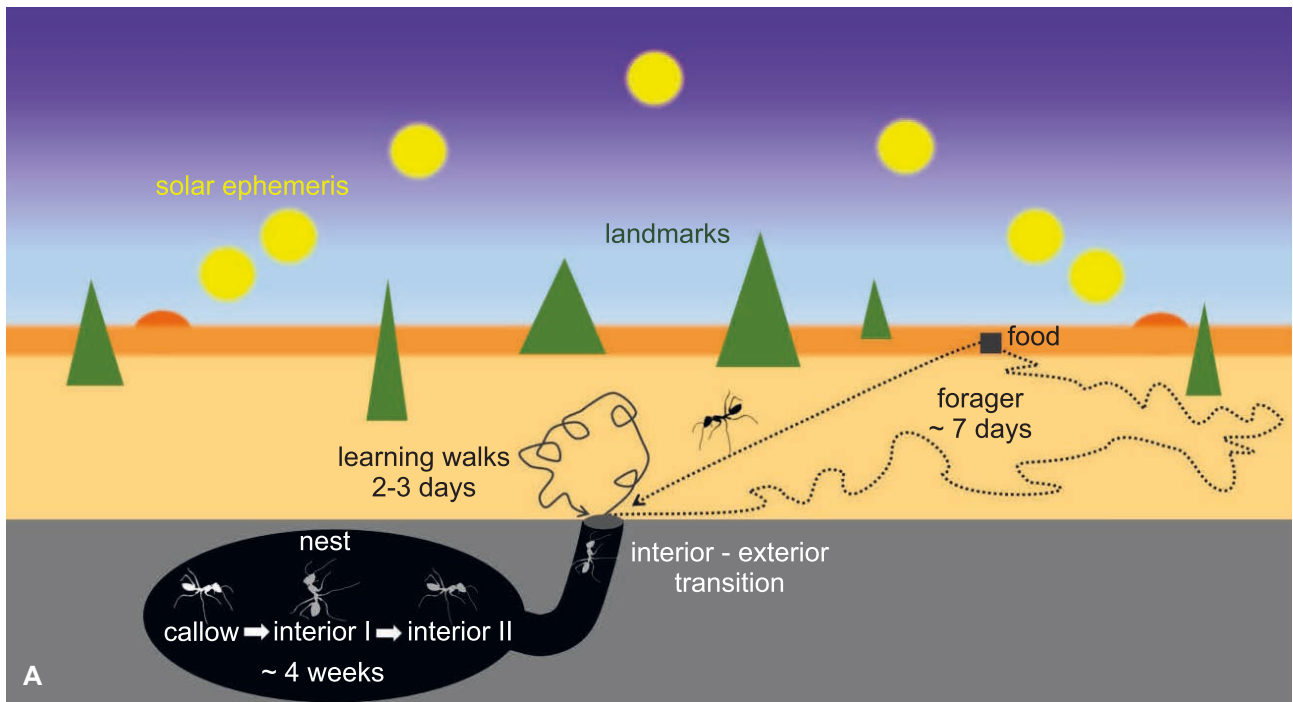
Fig. 1: Individual life history and natural habitats of *Cataglyphis* ants. (A) The ants spend c. 4 weeks in the dark nest performing interior tasks (callow, interior I, II) before they move on to perform learning walks close to the nest entrance for 2 - 3 days and finally to foraging using path integration and guidance by the panoramic scenery for c. 7 days until the ants die. The daily course of the sun (solar ephemeris) depicted as accumulated snapshots of different horizontal (azimuthal) positions across the sky together with visual panorama elements (in green) used for navigation. Further details in the text. (B) Natural habitat of *Cataglyphis fortis* and experimental test field in Tunisia (Menzel Chaker, Tunisia 34° 57' N, 10° 24' E). (C) Natural habitat of *Cataglyphis nodus* and experimental test field in Southern Greece. Photograph in (C) by Pauline Fleischmann (Schinias National Park near Marathon, Greece 38° 08' N, 24° 02' E).

only c. 7 days (Schmid-Hempel & Schmid-Hempel 1984), the investment of 2 to 3 days into learning walks emphasizes the high importance of this behavior in acquiring navigational information prior to first foraging (see below). Once the ants have completed their learning walks, they head out along a straight course on foraging trips of increasing lengths and durations, and the ants often develop a preference for distinct foraging sectors (Wehner & al. 2004). Interestingly, experienced foragers resume (re-) learning walks upon the occurrence of unfamiliar

visual scenery around the nest entrance suggesting that learning walks serve the calibration (and re-calibration) of skylight cues with information about the panoramic scenery (Müller & Wehner 2010).

The importance of learning walks for the acquisition of navigational information

For mainly visually oriented ants, the surrounding habitat contains important variables that cannot be encoded genetically and must be acquired prior to first foraging

excursions (Collett & Zeil 2018). This includes the location of panoramic visual information and the seasonally changing course of the sun (Fig. 1A). Learning walks comprise a stereotyped behavioral routine and represent a crucial element in the behavioral ontogeny of *Cataglyphis* and other ant species (Wehner & al. 2004, Stieb & al. 2012, Fleischmann & al. 2016, 2017; Grob & al. 2017, Jayatilaka & al. 2018). Novices walk in arcs or loops of increasing radius around the nest entrance. Although first learning walks in *Cataglyphis* have an average duration of only c. 14 s, some walks may extend up to 1 min (Fig. 2). Walking speed during learning walks is rather slow compared to the strikingly fast speed of the ants during foraging runs (Wehner & Wehner 1990, Fleischmann & al. 2017, Buehlmann & al. 2018). Subsequent learning walks systematically cover different sectors of increasing size (up to a beeline distance of c. 1 m) around the nest entrance exploring all compass directions (Muser & al. 2005, Fleischmann & al. 2016, 2017, Jayatilaka & al. 2018). Learning walks in *Cataglyphis* and other ant genera exhibit striking parallels, even with orientation flights in bees and wasps (Collett & Zeil 2018). In all cases, the short excursions contain behavioral routines that serve the acquisition of knowledge about the position of the nest in its surroundings and with relation to celestial compass cues (e.g., Zeil 1993, Zeil & al. 1996, 2014, Wehner & al. 2004, Graham & al. 2010, Fleischmann & al. 2016, 2017, 2018a, Grob & al. 2017, Jayatilaka & al. 2018, Collett & Zeil 2018). Experimental manipulations of artificial visual sceneries and tests of the ants at various stages of learning walk activities suggest that the ants gradually learn information about nest-related visual guidance cues during the performance of consecutive learning walks (Fleischmann & al. 2016). In addition to the temporal requirement for a period of at least two days, the ants need enough space for the performance of learning walks. By using experimental barriers formed by a (seawater-filled) moat in the natural habitat to limit learning-walk space, Fleischmann & al. (2018a) showed that insufficient learning-walk space limits the range over which acquired views provide navigational information. Subsequent displacement in different compass directions demonstrate that the ants need several square meters for proper performance of learning walks (Fleischmann & al. 2018a). Obviously, the space covered during learning walks (or the distance from which views across the nest are acquired) determines the range over which such learnt views provide guidance (see also Narendra & al. 2013, Stürzl & al. 2015). Only under spatially unrestricted conditions, the ants were homing successfully after displacement using panoramic guidance cues. Thus, the behavior analyses clearly demonstrate that the undisturbed performance of learning walks including enough time and space represents a crucial element in the ontogeny of successful navigation in *Cataglyphis* and most probably in other ants as well.

Learning walks in different *Cataglyphis* species comprise similarities, but also species-specific differences. Comparative behavior analyses of *Cataglyphis* species
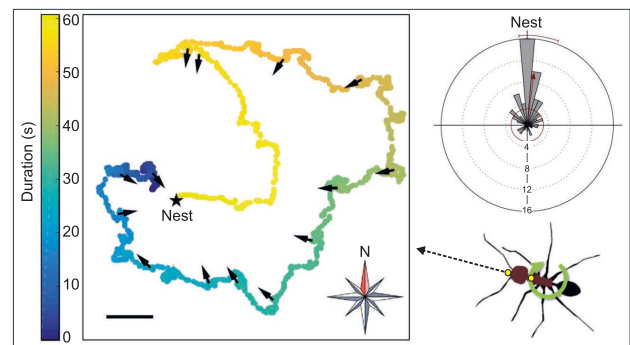

Fig. 2: Learning walks in *Cataglyphis nodus*. (Left) Path and time course of an individual learning walk around the nest entrance (black star). Time is color coded, and indicated on the left, the compass (red) is pointing north (N). Pirouettes, characteristic body rotations during which the ants stop to look back to the nest entrance indicated by arrows depicting the view direction during the longest stopping phases. Scale bar = 5 cm. (Upper right) Mean gaze directions during the longest stopping phases in pirouettes are not significantly different from the nest direction (the inner circle indicates Rayleigh's critical value α = 0.05; further details in Fleischmann & al. 2017). (Lower right) Schematic drawing showing *C. nodus* performing a pirouette with a nest-directed view during a stopping phase. The green circular arrow shows the center of rotation during a pirouette. The alignments of views during pirouettes were measured and quantified by tracking the tip of the mandibles and the position of the thorax (yellow spots). Further details in the text. Images and graphs modified from Fleischmann & al. (2017).

living in differently structured habitats (Fig. 1B and C) revealed that the ants perform learning walks with a similar overall structure and a duration of 2-3 consecutive days. However, different species showed distinct differences in body-rotation elements within learning walks (Fleischmann & al. 2017). *Cataglyphis fortis* inhabits North African salt flats (e.g., Menzel Chaker, Tunisia 34° 57' N, 10° 24' E; Fig. 1B), whereas *Cataglyphis nodus* (Brullé, 1833) and *Cataglyphis aenescens* (Nylander, 1849) build nests in open spaces within pine forests in Southern Greece (e.g., Schinias National Park near Marathon, Greece 38° 08' N, 24° 02' E; Fig. 1C) (see Stieb & al. 2011, Knaden & al. 2012 for phylogenetic relationships). Please note that in some previous publications the name *Cataglyphis nodus* was rendered as *Cataglyphis noda*, to make the species epithet correspond with the feminine gender of the genus. But the Latin word nodus is a masculine noun, which should stand unchanged, in apposition to the name of the genus, in accordance with Articles 11.9.1.2 and 34.2.1 of the International Code of Zoological Nomenclature of 1999 (B. Bolton, pers. comm.). In contrast to the featureless saltpan environment surrounding the nest entrances of *C. fortis*, a prominent visual panorama of tall pine trees and bushes surrounds the nest entrances of *C. nodus* and *C. aenescens*. High-resolution video analyses allowed to dissect characteristic rotational elements

performed during learning walks (FLEISCHMANN & al. 2017). The ants repeatedly interrupt their walks to perform accurate body turns during which the ants briefly stop for more than c. 100 ms when they precisely align their body axes to gaze towards the nest entrance (Fig. 2). Wasps perform similar turns with views directed back towards the nest entrance during their orientation flights (STÜRZL & al. 2016). Body rotations in *Cataglyphis nodus* comprise voltes (small walked circles) and pirouettes (full 360° or partial rotations about the vertical body axis). Interestingly, *C. fortis* performs only voltes, whereas *C. nodus* and *C. aenescens* display both types of turns. Stops, most frequently, occur during pirouettes, when naïve ants precisely gaze back to their nest entrance, even though it is invisible for them from the different positions around the nest entrance (FLEISCHMANN & al. 2017) (Fig. 2). Similar nest-directed views and body turns had been observed in *Cataglyphis bicolor* (FABRICIUS, 1793) (see WEHNER & al. 2004), *Ocymyrmex robustior* STITZ, 1923 (see WEHNER & MÜLLER 2010) and *Myrmecia croslandi* TAYLOR, 1991 (see JAYATILAKA & al. 2018). The results from different studies may indicate that ant species living in more cluttered environments take more panoramic snapshots while performing distinct body rotations during learning walks (FLEISCHMANN & al. 2017). The fact that another desert ant species (*Ocymyrmex robustior*) only stops once or twice during a learning walk – and that *C. fortis* only performs voltes with extremely rare stops – suggests a possible correlation between the richness of the visual scenery and the number of stopping phases during learning walks (MÜLLER & WEHNER 2010, GRAHAM & al. 2010, FLEISCHMANN & al. 2017, COLLETT & ZEIL 2018). These studies and other previous reports support the view that systematically spaced (and timed) turns back to the nest entrance serve the alignment of panoramic features and the sky-compass system to take nest-directed snapshots of the panorama surrounding the nest entrance. Results from CARTWRIGHT & COLLETT (1983) suggested that snapshot matching is used for later pinpointing a place. Experiments by WEHNER & al. (1996) addressed whether this egocentric information is coupled with geocentric compass information. The repeated nest-directed panoramic snapshots are most probably essential to learn and memorize panoramic features associated with the nest direction from different viewing angles. With these snapshot memories, the ants subsequently may identify panoramic view matches while homing from different compass directions (GRAHAM & al. 2010, MÜLLER & WEHNER 2010, BADDELEY & al. 2012, NARENDRA & al. 2013), and the nest-related familiar views serve as local vectors sensu COLLETT & al. (1998). As during naïve learning walks the ants are not able to see the nest entrance from their different positions, and visual panoramic guidance cues are still unfamiliar, the performance of nest-directed views during naïve learning walks must rely on path-integration information (GRAHAM & al. 2010, MÜLLER & WEHNER 2010, FLEISCHMANN & al. 2016, 2017, 2018a).

The earth's magnetic field is a compass cue during learning walks

What provides the directional reference system for performing nest-directed views during naïve learning walks? FLEISCHMANN & al. (2017, 2018b) performed high-resolution video analyses of the stopping phases during pirouettes in *Cataglyphis nodus* as they provide an accurate behavioral readout of the ants' path integrator. This allowed asking whether an ant knows the correct homing direction (home vector) from different positions around the nest entrance under specific experimental conditions or manipulations. Surprisingly, after blocking all relevant directional skylight cues, the analyses of nest-directed views revealed that naïve ants do not rely on their skylight compass for path integration during learning walks (GROB & al. 2017). This contrasts with the firmly established role of the skylight-compass during path integration in foragers as known from many studies before and outlined above. After blocking UV light, polarization information, and obscuring the sun position, the ants still looked back to the nest entrance from different positions around the nest. This clearly suggests that novices use a different compass reference for path integration during naïve learning walks (GROB & al. 2017).

Surprisingly, a follow up study in the ants' natural environment unambiguously demonstrated that the ants use the earth's magnetic field as compass cue and directional reference for path integration during naïve learning walks (FLEISCHMANN & al. 2018b). This finding is even more exciting as it represents the first evidence for an insect using the earth's magnetic field as the necessary and sufficient compass cue for performing path integration. Earlier studies had shown that *Cataglyphis* foragers use artificial high-intensity magnetic field disturbances as landmarks during homing (BUEHLMANN & al. 2012) or that foraging ants, under certain conditions, respond to manipulations of the geomagnetic field (JANDER & JANDER 1998, BANKS & SRYGLEY 2003, RIVEROS & SRYGLEY 2008; reviewed in WAJNBERG & al. 2010). Using a Helmholtz-coil setup in the natural habitat, FLEISCHMANN & al. (2018b) demonstrate that after artificial elimination of the horizontal component of the earth's magnetic field, naïve *Cataglpyhis nodus* were no longer able to gaze back to the nest entrance while performing their learning walks. Moreover, experimental rotation of the horizontal component of the magnetic field systematically changed the direction of the ants' nest-directed views in a predictable way. The ants then gazed towards a fictive nest entrance rotated by the same angle as the magnetic field (FLEISCHMANN & al. 2018b). This suggests that the geomagnetic field serves as the earthbound directional reference system to align nest-directed views for initial calibration of the visual compass systems. To be able to use directional information from the earth's magnetic field for the alignment of visual input, the ants need to integrate magnetic information into their neuronal path integrator. The underlying magnetoreceptive mechanism
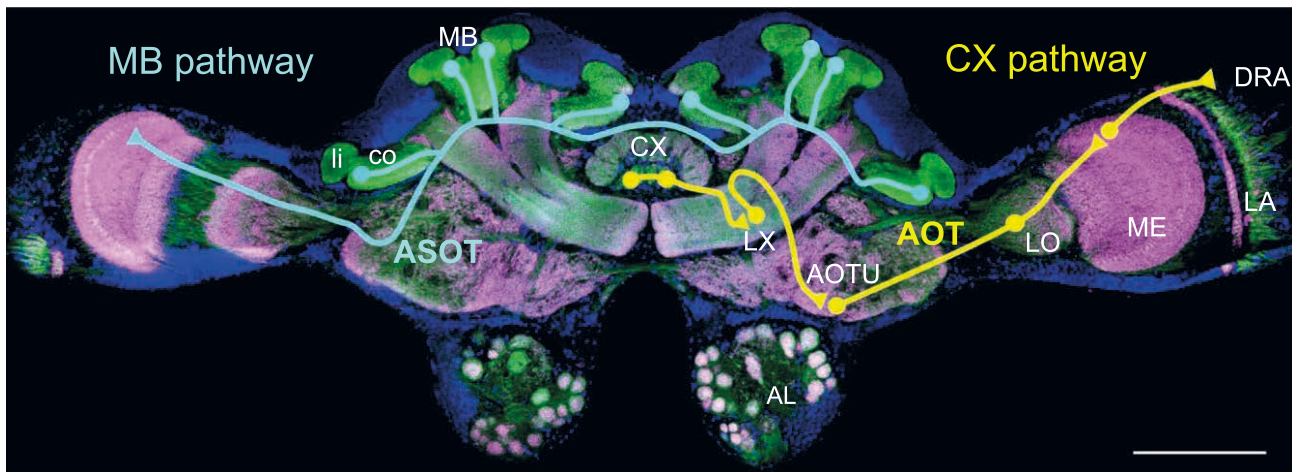
Fig. 3: Two major visual pathways in the *Cataglyphis* brain. The visual pathway to the central complex (CX pathway, or sky-compass pathway) depicted in the right brain hemisphere of a *C. fortis* brain, the visual pathway to the mushroom body (MB pathway) shown on the left side. Brain labeled with an antibody to synapsin (magenta), f-actin staining by phallodin (green) and detection of cell nuclei by 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 brain image is from STIEB & al. (2012), and pathways combined from results by SCHMITT & al. (2016) and GROB & al. (2017).

and sensory pathways in the brain are still unknown and will be subject to future studies.

The striking role a magnetic compass has during the early life stages in *Cataglyphis* triggers several questions. Do the ants use the geomagnetic compass to calibrate the skylight-compass system to the seasonally changing daily course of the sun (solar ephemeris; WEHNER & MÜLLER 1993)? An earthbound reference system is required for time-compensation of a skylight compass. Previous experiments by TOWNE (2008) and TOWNE & MOSCRIP (2008) suggest that experienced honeybees use the landscape for re-calibration. Second, why do the ants switch from a geomagnetic compass during learning walks to a skylight compass during foraging? One possibility is that the underlying magnetosensory mechanism is not accurate and/or fast enough for path integration at high foraging speed over long distances. Another possibility is that the ants use the magnetic field as directional information in their underground dark nest to then replace it by salient directional cues, such as the celestial compass and panoramic scenery. Finally, is the switch to visual compass cues a complete switch, or do experienced foragers still use their magnetic compass system under certain circumstances? Re-learning walks of experienced foragers may be helpful in elucidating these questions. In any case, the magnetic compass in *Cataglyphis* is highly promising in revealing further exciting results on the sophisticated navigational performance of the ants.

After having introduced the crucial role of learning walks for the ontogeny of successful visually based navigation, the next chapters focus on the visual pathways in *Cataglyphis* brains and their neuroplasticity following first light exposure and the performance of learning walks.

Visual circuits that transfer navigational information to sensory integration centers

To investigate plasticity in neuronal circuits that process navigational information, recent work in *Cataglyphis* focused on two visual pathways projecting to two sensory integration centers in the ants' brains. Early behavioral studies on the interocular transfer of visual input using unilateral eye closure experiments in *C. fortis* already predicted two channels for visual information transfer – one for global directional information (e.g., skylight polarization) and one for local visual memories of the panoramic scenery (WEHNER & MÜLLER 1985). More recent modeling studies by CRUSE & WEHNER (2011) and HOINVILLE & WEHNER (2018) claimed that the central complex (CX) and the mushroom bodies (MBs) might represent the neuronal substrates for integration of path integration and panoramic-scenery related information in *Cataglyphis*, respectively (see also WEBB & WYSTRACH 2016, STONE & al. 2017). Additional support for two separate integration systems came from experiments showing that the time-course of memory decay is faster for vector-based (path integration) compared to navigational information based on the panoramic scenery (ZIEGLER & WEHNER 1997, NARENDRA & al. 2007). Whereas the ants memorize local visual information for up to their lifetimes, global vector information from the skylight-compass and path integration system show a much faster memory decay in the range of several hours.

We anatomically mapped two major visual pathways in the brain of *Cataglyphis fortis* and *C. nodus* fulfilling the criteria for two separate visual information streams to two high-order integration centers in the ants' brains (Fig. 3). This was achieved by focal iontophoretic injection of flu-
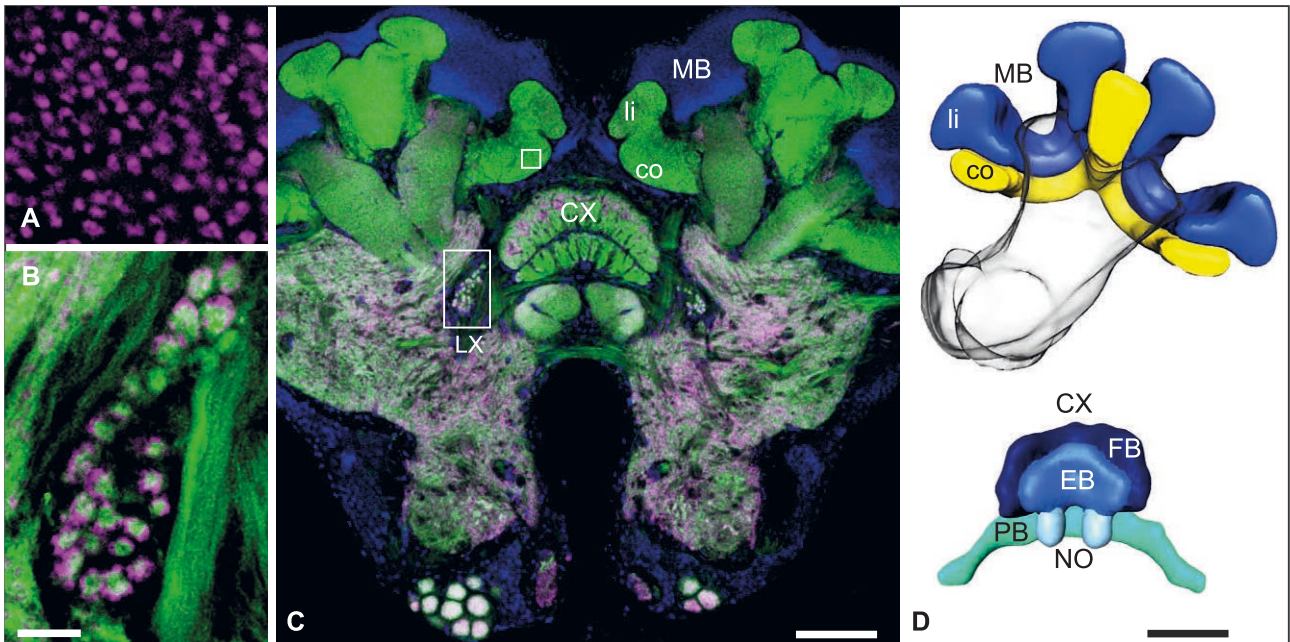
Fig. 4: Quantitative analyses of structural synaptic plasticity in visual integration centers in the lateral complex (LX) and mushroom body (MB) of *Cataglyphis* brains. (A) Anti-synapsin immunolabeled distinct synaptic complexes in the MB (visual) collar. The position in the brain indicated by the square in the MB-calyx collar in (C). (B) Anti-synapsin and f-actin phalloidin co-labeled synaptic complexes in the bulb of the lateral complex (LX). The position in the brain indicated by the rectangle in the LX in (C). The density and numbers of synaptic complexes in the MB calyx (A) and lateral complex (B) are quantified using computer guided analyses. Scale bar in (B), also valid for (A) = 10 μm. (C) Brain of *Cataglyphis fortis* labeled with an antibody to synapsin (magenta), f-actin stained with phallodin (green) and cell nuclei labeled with Hoechst 3458 (blue). Scale bar = 100 μm. (D) 3D reconstruction and surface rendering of the individual components of the mushroom body (upper, frontal view) and central complex (lower, ventral view) for volume analyses in the brain of *Cataglyphis nodus*. Scale bar = 100 μm. Further abbreviations: co collar, EB ellipsoid body, FB fan-shaped body, NO noduli, PB protocerebral bridge, li lip. Combined from Stieb & al. 2012 (C) and Grob & al. (2017) (D). Whole mount images in (A) and (B) provided by Kornelia Grübel.

orescent dyes into projection neurons of the optic ganglia (lamina, medulla and lobula) and subsequent tracing of the neuronal connections into the central brain using confocal microscopy imaging techniques and computer-guided 3D reconstructions (Schmitt & al. 2016, Grob & al. 2017). Fluorescent tracings combined with immunolabeling of synaptic proteins to map major brain neuropils revealed two prominent visual pathways extending from the optic lobes to the CX and to visual subregions of the MB calyces. Whereas the CX pathways from both hemispheres converge in the ellipsoid body of the CX, the MB pathway has bilateral projections from the optic lobes (mainly from the medulla) to the two MB calyces in the ipsi- and contralateral brain hemispheres (Fig. 3).

The anterior sky-compass pathway to the CX origins from photoreceptor neurons of ommatidia in the dorsal rim area of the compound eye and associated structures in the first optic ganglion, the dorsal lamina (Schmitt & al. 2016, Grob & al. 2017 (Fig. 3). The dorsal rim area comprises c. 100 UV-sensitive ommatidia. These are c. 5% of the ommatidia in the compound eye of *Cataglyphis* functioning as UV-sensitive polarization sensors (Labhart & Meyer 1999). Neuronal projections from the optic ganglia (medulla and lobula) join the anterior optic tract

(AOT) to the anterior optic tubercle (AOTU), a distinct neuropil located above the antennal lobe. From there, projections of AOTU neurons terminate in large synaptic complexes forming a single bulb within the lateral complex (LX) (brain nomenclature after Ito & al. 2014). The large LX synaptic complexes (also termed giant synapses or microglomerular synaptic complexes; Schmitt & al. 2016, Held & al. 2016) connect to tangential neurons terminating in the lower division of the central body (also termed ellipsoid body). This pathway appears highly conserved across a wide range of insect species as it exhibits high similarities with the sky-compass pathways previously found in the locust, honeybee, bumblebee, dung beetle, monarch butterfly and fly. Combined physiological and anatomical studies, most extensively done in the locust, but also in bees, the fly and in dung beetles, indicate a highly conserved scheme for processing of celestial information along this pathway (locust: Homberg & al. 2011; bees: Pfeiffer & Kinoshita 2012, Zeller & al. 2015, Held & al. 2016, Stone & al. 2017, Heinze & al. 2018; fly: Seelig & Jayaraman 2013; butterfly: Heinze & al. 2013; dung beetle: Immonen & al. 2017, el Jundi & al. 2018). In addition to polarized skylight information, this pathway transfers chromatic cues and information about
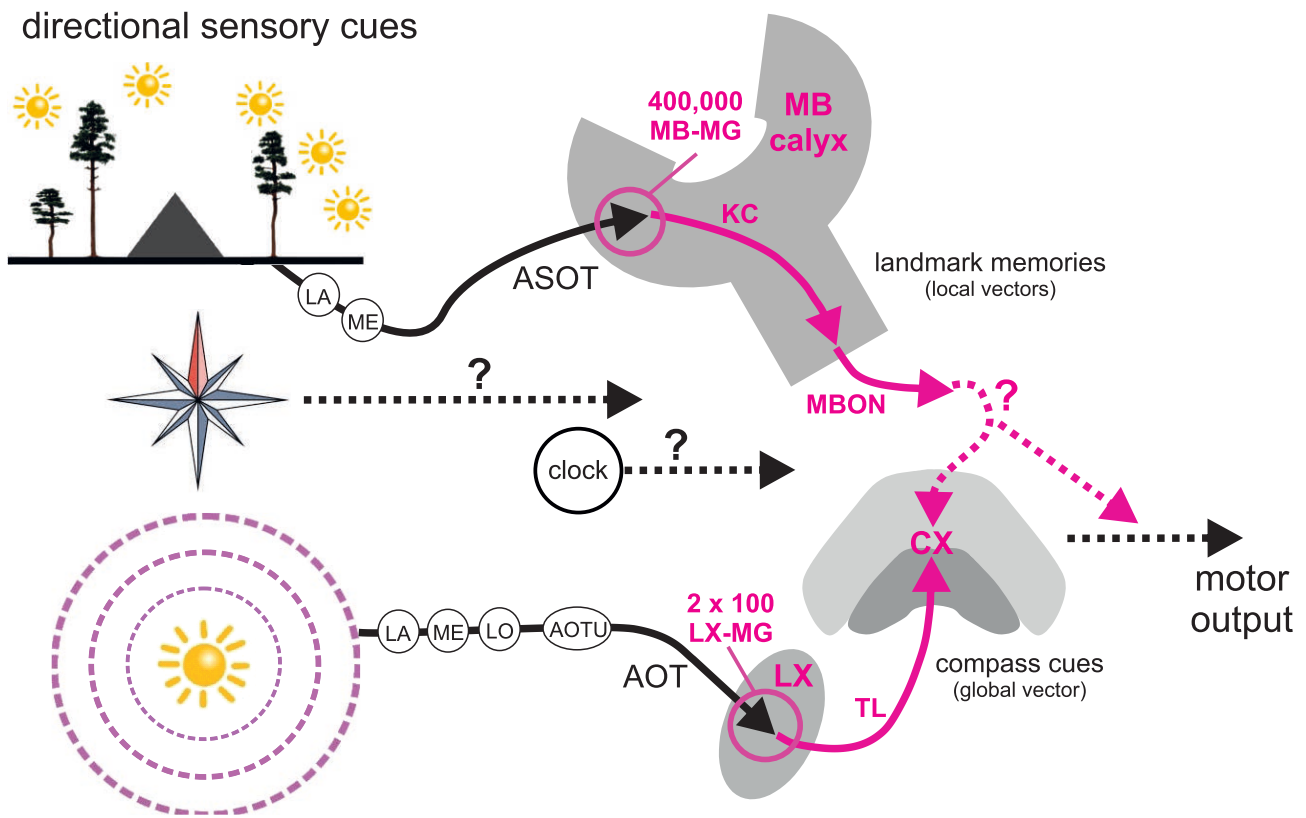
Fig. 5: Model for processing of navigational information and sites of structural synaptic neuroplasticity in visual pathways after first sensory exposure and following learning walks. The left side depicts sensory input from the panoramic scenery, the geomagnetic field and the sky polarization pattern with the position of the sun. Directional sky-compass information (compass cues: global vector) is processed via the anterior optic tract (AOT) to the lateral (LX) and central complex (CX), whereas snapshots from panoramic information (panoramic memories, local vectors) are processed via the anterior superior optic tract (ASOT) to the mushroom bodies (MB). The large difference in the numbers of plastic synaptic complexes (microglomeruli, MG) at the input of the MB and LX indicated in magenta. The sensory pathways for geomagnetic information, the input of the endogenous clock for time-compensation, the connection from the MB output to the CX, and connections to the motor output are still hypothetical and depicted as dashed lines. Regions of structural (synaptic) neuroplasticity and potentially affected downstream connections highlighted in magenta (see text for details). Further abbreviations: AOTU anterior optic tubercle, KC Kenyon cell, LA lamina, LO lobula, MBON mushroom body output neuron, ME medulla, TL tangential neuron.

the position of the sun (PFEIFFER & al. 2005, PFEIFFER & HOMBERG 2007, EL JUNDI & al. 2011). Very similar to the situation described in locusts (TRÄGER & al. 2008), in the *Cataglyphis* brain the last synaptic relay station before entering the CX is mediated by a relatively small number of c. 100 LX large synaptic complexes forming a single bulb (SCHMITT & al. 2016). Here, combined confocal imaging and ultrastructural studies found very large cup-shaped individual presynapses connected to numerous fine postsynaptic processes of (most likely inhibitory) tangential neurons that finally terminate in the lower division of the central body (Figs. 3 & 4).

The visual pathway to the MBs in *Cataglyphis* is mainly formed by axons of projection neurons in the medulla joining the anterior superior optic tract (ASOT) and extending axons to the medial and lateral MB calyces of both brain hemispheres (GROB & al. 2017) (Fig. 3). Visual projections via the ASOT to the MBs previously were shown in various other species of ants (GRONENBERG 1999, GRONENBERG &

HÖLLDOBLER 1999, EHMER & GRONENBERG 2004, YILMAZ & al. 2016). A large comparative survey of MB-calyx volumes within the order Hymenoptera by FARRIS & SCHULMEISTER (2011) suggests that the expansion of visual supply to the MBs via the ASOT is specific to the higher Hymenoptera. The authors argue that visual projections to the MB calyx in advanced Hymenoptera promote the ability to store complex visual memories, which represented an important requirement for elaborate spatial orientation in parasitoid Hymenoptera. The repeated return to a burrow, for example in parasitoid wasps, is required for provisioning larvae with prey and needs advanced visual navigation skills involving visual input to the MBs. Evolution of central-place foraging in eusocial Hymenoptera potentially was based on the co-option of this trait. Only a minority of insect species studied so far from other orders like Coleoptera and Lepidoptera possess comparably elaborate visual projections from the optic lobes to the MB calyces (FARRIS & ROBERTS 2005, KINOSH-

ITA & al. 2015). This is most likely associated with specific foraging ecologies. However, in most non-hymenopteran insect species investigated so far including *Drosophila melanogaster* MEIGEN, 1830, visual projections to the MB calyces are minor or absent (VOGT & al. 2016). It will be interesting for future studies to find out how visual input to the MB calyces correlates with the specific visual ecology in more diverse insect taxa. A general correlation with the behavioral importance of vision appears rather obvious within the ants. Compared with the situation in *Cataglyphis* (see GROB & al. 2017), visual input to the MBs is substantially smaller in preferentially olfactory guided ants (e.g., GRONENBERG & RIVEROS 2009, GROH & al. 2014, YILMAZ & al. 2016). However, also in species that recruit by trails, experiments by HARRISON & al. (1989) showed that scouts were able to navigate individually.

Based on high-resolution confocal imaging data (STIEB & al. 2010, SCHMITT & al. 2016, GROB & al. (2017), the number of visual input synapses (microglomeruli, MG) to the collars of the MB calyces in *Cataglyphis* are estimated to number to up to c. 400,000 MG (Fig. 4 & 5). This large number of synaptic input channels contrasts with the only about 100 synaptic complexes at the input to the CX (Fig. 5). In the MB-calyx visual projection neurons from the optic lobes synapse on an extremely large population of Kenyon cells (KCs) - the intrinsic neurons of the MBs involved in learning and memory formation (MENZEL 2001, SZYSZKA & al. 2005, GROH & al. 2012). In the honeybee, the total number of KCs are estimated to number about 360,000 and comprise c. 45% of all brain neurons (STRAUSFELD 2002, RÖSSLER & GROH 2012). In *Camponotus rufipes* (FABRICIUS, 1775), a comparably large number of c. 130,000 KCs was reported (EHMER & GRONENBERG 2004). Within the MB lobes, modulatory input (dopamine, octopamine) mediates information about reward or punishment during associative learning (e.g., GIURFA 2007, GERBER & al. 2004). In the honeybee, c. 50 GABAergic neurons provide feedback from the MB-lobe output to the MB-calyx input. Functional studies suggest a role of this inhibitory feedback in gain control and regulation of spatial KC activation (FROESE & al. 2014). From the KCs information converges on a much smaller set of MB output neurons (c. 400 in the honeybee). The high level of convergence at the MB output and physiological recordings in the honeybee indicate that MB output neurons extract behaviorally relevant categories from multimodal (olfactory and visual) sensory information processed in the KCs (STRUBE-BLOSS & RÖSSLER 2018). The activity of KCs changes following learning and memory processes as shown by calcium imaging at the KC dendritic input in the MB calyx (SZYSZKA & al. 2005, 2008). The multi-modal (olfactory and visual) integrative function of MB output neurons together with learning and memory-driven changes in their responses are highly interesting attributes asking for more combined behavioral and physiological studies of MB output neurons in the future (STRUBE-BLOSS & al. 2011, 2012, STRUBE-BLOSS & RÖSSLER 2018).

The difference in the synaptic architecture strongly suggests fundamental differences in the quality of visual information processing along the two pathways (Fig. 5). The large number of microglomerular synaptic microcircuits in the MB pathway likely provides an ideal neuronal substrate for the storage of information about rather fine-grained snapshot (image) memories. Behavioral evidence indicates that these panoramic views are retinotopic (COLLETT & ZEIL 2018) meaning that they contain panoramic components that might serve as local cues in spatial orientation (local vector). This view of the potential role of the MBs is not only supported by the large number of parallel synaptic microcircuits, but also by physiological data, theoretical and modeling approaches (SZYSZKA & al. 2008, ARDIN & al. 2016, WEBB & WYSTRACH 2016, PENG & CHITTKA 2017, HOINVILLE & WEHNER 2018, HAENNICKE & al. 2018). For the CX pathway, physiological and circuit analyses in various insect species strongly suggest that this pathway transfers directional information from skylight cues used to determine heading (recently reviewed by HEINZE 2017, HEINZE & al. 2018). The final transfer of global directional (compass) information to the CX obviously requires a much smaller number of input channels. Whereas the MB pathway from the medulla comprises only one synaptic relay before entering the MB calyx, the CX pathway involves at least 4 - 5 synaptic relay stations indicating that input to the CX has a higher level of preprocessing compared to the input to the MB calyx (Figs. 3 and 5) (also indicated by electrophysiological studies from e.g., HOMBERG & al. 2011, STONE & al. 2017). Any direct neuronal connections between the MB and CX are unknown so far. However, indirect pathways via the superior protocerebrum might mediate such interactions between both integration centers (STRAUSFELD 2012, STRAUSFELD & HIRTH 2013). Alternatively, output from the MBs (mediating local vectors) might as well directly modulate global vector information from the CX pathway in downstream premotor pathways (Fig. 5). Along this line, the potential role of other, smaller visual projections from the optic lobes into different parts of the protocerebrum still await future investigation.

First exposure to skylight triggers structural synaptic plasticity in visual circuits

How does the visual sensory system cope with the drastic changes in sensory input once the ants transition from the dark nest interior to foraging in bright sunlight? As structure and function closely interconnect in the nervous system, structural plasticity of neuronal circuits is a powerful tool to adjust the function of neuronal circuits. Therefore, identification and quantification of structural neuroplasticity enables the detection of sites undergoing functional adaptations in response to changes in the quality or intensity of sensory input (GROH & al. 2004, RÖSSLER & GROH 2012, FAHRBACH & VAN NEST 2016, RÖSSLER & al. 2017).

To address whether the two visual neuronal pathways respond with plastic changes to first exposure to

light, Stieb & al. (2010, 2012) and Schmitt & al. (2016) screened ants at different ages and behavioral stages for structural changes in visual circuits to the CX and MB and compared them with control ants and brain regions processing other sensory information. In the CX pathway, experienced foragers showed an increase in the number of LX synaptic complexes of on average c. 15% compared to callows. To find out whether this effect results from first sensory exposure, the ants were precociously stimulated with light and compared with age-matched dark-kept cohorts. This revealed that first exposure to skylight triggers a substantial increase (up to 30%) in the number of LX synaptic complexes (Schmitt & al. 2016, Figs. 4 & 5). Age matched ants kept in darkness did not show any increase in LX synaptic complexes, even when they were past the typical foraging age. This suggests that sensory exposure and not an age-related program triggers this plasticity. Interestingly, the increase in the number of LX synaptic complexes critically depends on the spectral composition of light. The increase was significantly lower when the UV part of the spectrum was blocked compared to the numbers of LX synaptic complexes after stimulation with an intensity-matched full light spectrum.

Conversely, in the MB pathway first light exposure leads to a decrease (pruning) of synaptic complexes and a volume increase in the (visual) MB-calyx collar. This effect occurs in both *Cataglyphis fortis* and the honeybee (Stieb & al. 2010, 2012, Scholl & al. 2014, Muenz & al. 2015, Rössler & al. 2017). In a similar line, Kühn-Bühlmann & Wehner (2006) had previously shown an increase in the MB volume of experienced (aged) foragers compared to dark reared ants in age-controlled *C. bicolor.* In the honeybee, a similar MB expansion occurs in foragers compared to nurses (Muenz & al. 2015). Similarly, in the olfactory system of leaf-cutting ants non-associative exposure to multiple plant odors leads to pruning of synaptic complexes in the (olfactory) MB-calyx lip (Falibene & al. 2015). Modality specific pruning of MB-calyx synaptic complexes is associated with a massive outgrowth of KC dendrites and a resulting overall increase in the volume of the MB-calyx subdivisions. Quantitative ultrastructural analyses in the honeybee using serial electron microscopy followed by 3D reconstructions demonstrated that pruning of synaptic boutons and increase in KC dendritic branching goes along with a c. 33% increase in the number of postsynaptic contacts per presynaptic bouton indicating a substantial increase in the synaptic divergence at the level of individual projection-neuron boutons (Groh & al. 2012). This suggests a massive re-organization of visual and olfactory MB microcircuits in response to changing sensory input at the interior-forager transition. Similarly, experiments in *Cataglyphis* using electron microscopy techniques and confocal imaging of postsynaptic structures suggest axonal pruning and an increase in pre- to postsynaptic divergence (Seid & Wehner 2009, Stieb & al. 2010). Under natural conditions, these changes in MB synaptic complexes occurred at the interior II stage, which is the time when the ants start to perform digging walks

and leave the nest for the first time (Stieb & al. 2010). It is most likely during the digging walks when the ants get their first full exposure to sunlight.

The studies on neuroplasticity in response to first light exposure demonstrate that in *Cataglyphis* both the CX and MB visual circuits express synaptic reorganization at the interior-forager transition. Interestingly, structural synaptic changes at the entrance to the CX and MB go in opposite directions. Whereas activity dependent pruning is a rather common phenomenon in sensory systems following sensory exposure (Fox & Stryker 2017), the increase of LX synaptic complexes is less common and indicates fundamental differences in functional properties within the two visual circuits. It is important to note that in both cases artificial light exposure triggered changes as early as in young callows, and MB synaptic complexes remained plastic in foragers artificially kept in darkness for up to six months suggesting a high degree of plasticity (Stieb & al. 2010, 2012, Schmitt & al. 2016). Interestingly, in the honeybee first light exposure also triggers an increase of juvenile hormone (JH) levels showing that, in addition to the effects on neuronal circuits, the hormonal system is affected by first light exposure (Scholl & al. 2014). However, experimental increase of JH levels did not affect synaptic plasticity showing that both effects of light exposure are independent from each other. The effects on synaptic plasticity in *Cataglyphis* are only partially reversible (Stieb & al. 2012). Whereas honeybee foragers re-introduced in the dark nest expressed an almost complete reversal in the density of MB synaptic complexes (Scholl & al. 2014), *Cataglyphis* showed only a mild reduction of immunoreactivity to the presynaptic protein synapsin (Stieb & al. 2012). The reason for this might be that honeybees are more likely to revert to interior tasks depending on colony status compared to relatively short-lived *Cataglyphis* foragers. In addition to changes in synaptic circuits, investigations in *Camponotus rufipes* revealed both age-related and light-dependent plasticity in opsin gene expression in photoreceptor neurons of the compound eyes demonstrating that adjustments in visual pathways during the interior-exterior transition may even occur at peripheral levels (Yilmaz & al. 2016). As in all experiments outlined above light exposure was passive and not accompanied by any form of associative learning, structural synaptic plasticity after first light exposure can most probably be assigned to homeostatic plasticity sensu Fox & Stryker (2017) reflecting adjustments of neuronal circuits to the drastically increasing light intensities and changing spectral composition of light experienced at the interior-exterior transition.

Learning walks lead to learning-related (Hebbian-like) neuroplasticity

Do learning walks trigger learning-related (Hebbian-like) neuroplasticity in visual circuits to the CX and MB? To investigate the influence of skylight cues during learning walks, Grob & al. (2017) manipulated light perception using UV and polarization filters mounted above the nest

entrance while naïve ants performed their learning walks. After different cohorts of naïve ants had performed learning walks for three days under different manipulated and natural conditions, the authors quantified neuroplastic changes in the CX and MB pathways and compared them with control ants that had not performed learning walks. Three days of learning walks under normal skylight conditions in the ants' natural habitat led to structural plasticity in both visual pathways (Grob & al. 2017). Most interestingly, however, in both neuronal pathways plasticity was absent when the ants did not experience the full spectrum of a UV mediated daily change in the natural sky polarization pattern. Only learning walks performed under a naturally changing sky polarization pattern led to an increase in the density (and number) of synaptic complexes in the visually innervated MB-calyx collar and to a volume increase in the CX. This suggests that structural synaptic plasticity after learning walks under natural skylight conditions is learning-related (Hebbian-like). It is interesting that learning-walks also induced a volume increase in the CX indicating that visual learning and memory formation cause long-term changes in the CX neuronal circuitry. Along this line, a recent study in the honeybee shows that transient silencing of the MB or CX with local anesthesia had differential effects on a specific color learning task supporting that both integration centers are involved in different aspects of visual learning (Plath & al. 2017). The results in *Cataglyphis* further indicate that experience of a UV-mediated dynamic sky polarization pattern may be essential for the ants to learn and memorize nest-related panoramic snapshots during learning walks. The study by Fleischmann & al. (2018b) demonstrates that the earth's magnetic field is an essential compass cue underlying path integration and turn back and look behavior during naïve learning walks. Combined with the effects on neuroplasticity demonstrated by Grob & al. (2017) the results strongly suggest that the ants may calibrate their visual neuronal compass systems only when they have access to both a naturally changing skylight-polarization pattern and the earth's magnetic field as an earthbound reference system.

To elaborate a little deeper on learning-related (Hebbian-like) structural neuroplasticity expressed in MB microcircuits during learning walks, I shall briefly recapitulate results on the formation of long-term memory (LTM) and LTM-related structural changes previously found in olfactory neuronal circuits of the MBs in the honeybee and leaf-cutting ants (Hourcade & al. 2010, Falibene & al. 2015). The insect MBs are neuronal substrates for associative learning and memory processes (Strausfeld & al. 1998, Menzel 1999, Gerber & al. 2004, Davis 2005, Giurfa 2007). First evidence that the MBs are required for place memory based on information from the visual scenery came from bilateral lesions of the MBs in cockroaches (Mizunami & al. 1998). Moreover, split-brain preparations, also in cockroaches, demonstrate that spatial-learning associated changes in the MBs occurred only within the trained brain hemisphere, not within the naïve one

(Lent & al. 2007). *Cataglyphis* spends at least two days for the performance of learning walks. This period correlates well with the minimum time span required for the formation of stable LTM (for honeybees: e.g., Menzel 2001; for ants: e.g., Falibene & al. 2015). Most importantly, the formation of stable LTM leads to modality-specific structural changes in synaptic microcircuits in MB-calyx subregions as shown after both appetitive olfactory learning in the honeybee and aversive olfactory learning in leaf-cutting ants (Hourcade & al. 2010, Falibene & al. 2015). In both cases, formation of stable olfactory LTM led to an increase in the density and number of synaptic complexes in the olfactory subregions (lip) of the MB calyx. This is in line with Hebbian structural synaptic plasticity found in the vertebrate brain leading to an increase of synaptic contacts following a phase of high neuronal activity (Fauth & Tetzlaff 2016). This means that structural reorganization of synaptic microcircuits in the MB calyx relates to functional consequences of a long-term memory trace and a learned behavioral response to an appetitive or aversive conditioned olfactory stimulus. In contrast to the increase of synaptic complexes following learning and LTM formation, pure sensory exposure induced a reduction (pruning) of MB synaptic complexes. These differential effects were found in both the visual (collar) and the olfactory (lip) of the MB calyx (Stieb & al. 2010, Scholl & al. 2014, Falibene & al. 2015). The formation of associative LTM induces new synaptic complexes in the MB calyx (Hourcade & al. 2010, Falibene & al. 2015). This clearly distinguishes learning related (Hebbian-like) structural neuroplasticity (new synapses) following associative learning and LTM formation from effects of pure sensory exposure and the resulting homeostatic plasticity (pruning of synapses).

Similar to the effects in the MB-calyx lip after olfactory LTM formation in leaf cutting ants (Falibene & al. 2015), the density and number of synaptic complexes in the MB-calyx collar of *Cataglyphis nodus* increased after the performance of learning walks under natural skylight conditions (Grob & al. 2017). This strongly suggests that the underlying plasticity is learning-related (Hebbian-like) most likely structural Hebbian plasticity (Fauth & Tetzlaff 2016) leading to new (associative) synaptic connections across different projection neurons boutons and KC dendrites. The exceptionally high numbers of parallel microcircuits and the associated intrinsic MB neurons (Kenyon cells) provide a rich neuronal substrate for such a structural synaptic plasticity and potentially allows the formation of multiple LTMs (Rössler & Groh 2012, Falibene & al. 2015, Grob & al. 2017). Along this line, recent modeling work and physiological data in the honeybee suggest that synaptic plasticity at MB-calyx input synapses is crucial for specific forms of memory formation (Peng & Chittka 2017, Szyszska & al. 2008, Haennicke & al. 2018) and the memory capacity of MB-calyx microcircuits is very high (Ardin & al. 2016). It will be interesting to see in future work whether (and potentially how) LX synaptic complexes change after learning walks.

We hypothesize that repeated experience of nest-directed panoramic snapshots during learning walks triggers the formation of nest-associated visual long-term memories in MB synaptic microcircuits. What could be the associative component for the formation of snapshot LTMs? The intrinsic association of the nest with nest-directed views alone might serve as an internal reward during the look-back behavior and snapshot learning, which was termed "genetically encoded anticipatory learning" by COLLETT & ZEIL (2018). In a similar line, ARDIN & al. (2016) already suggested that alignment with the home vector might serve as an "internal reward" (see also JAYATILAKA & al. 2018). This is an attractive hypothesis, but it remains speculative and is difficult to prove at this point. How do snapshot memories formed in the MB guide a homing ant? If we assume that panoramic snapshots in *Cataglpyhis* are memorized only when the ants briefly stop during pirouettes and gaze towards the nest entrance by using the earth's magnetic field as a compass reference for path integration, this results in a collection of nest-directed panoramic snapshot memories pointing towards the nest from different positions around the nest. A homing forager can then match the experienced scenery with its stored nest-directed visual snapshot memories (GRAHAM & al. 2010, NARENDRA & al. 2013, STÜRZL & al. 2015). These matches may function as local nest-directed guidance cues for a homing forager (HOINVILLE & WEHNER 2018). In that case, the snapshot memories do not require any directional or positional information attached to them as the ants simply proceed forward when they recall them (see BADDELEY & al. 2012). Is memory retrieval fast enough to guide returning foragers via such a mechanism? STRUBE-BLOSS & al. (2012) quantified the neuronal processing properties of MB circuits by measuring responses from MB output neurons in the honeybee. The results show that information is processed surprisingly fast in MB synaptic microcircuits and even outpaces processing in primary sensory centers. As the responses of MB output neurons undergo learning-dependent changes and integrate input from different (olfactory and visual) sensory modalities to categorize sensory input (STRUBE-BLOSS & al. 2011, STRUBE-BLOSS & RÖSSLER 2018), the resulting output can potentially provide feedback to primary sensory centers while they are still processing incoming signals. The fast processing properties of MB microcircuits together with the learning-dependent plasticity of MB output neurons and their stimulus categorization properties provide an ideal neuronal substrate for fast behavioral decisions.

In addition to learning-related changes in MB microcircuits, learning-walks induce an increase in the CX volume suggesting that neuronal circuits in the CX also reorganize during the formation of visual snapshot learning and LTM formation (GROB & al. 2017). This receives support from recent results suggesting a role of the CX in visual learning (PLATH & al. 2017). Structural plasticity in the CX during learning walks might be related to polarization (sky) compass calibration. Combined with the results from functional studies in the locust, bees, butterflies and dung beetles (HOMBERG & al. 2011, PFEIFFER & KINOSHITA 2012, HEINZE & al. 2013, HELD & al. 2016, ZELLER & al. 2015, IMMONEN & al. 2017, STONE & al. 2017), the results strongly suggest that plasticity in the CX pathway adjusts (calibrates) input from global (skylight) compass cues based on sensory-exposure and experience during learning walks. In the future, we need more manipulation experiments combined with functional and structural studies to scrutinize the role of neuroplasticity in LX and CX visual circuits for navigation. Live calcium imaging experiments in *Drosophila melanogaster* suggest that compass information is integrated and mediated by ring neurons (equivalent to tangential neurons) to the CX ellipsoid body encoding general heading of the insect (SEELIG & JAYARAMAN 2013, 2015). In the CX directional visual input further computes with information from other sensory systems, for example proprioreceptive systems measuring body turns and walking speed to direct movement (reviewed in HEINZE 2017, HEINZE & al. 2018). In addition, massive modulatory input relays to the CX (especially fan-shaped body) reporting the internal status and motivation. The behavioral studies combined with the results on plasticity in the CX strongly suggest that learning walks are an important behavioral program needed for the individual adjustment of directional sensory input from skylight cues (Fig. 5). Time-compensation of skylight cues to the daily course of the sun most probably requires modulatory interactions with the endogenous clock at the level of the CX or MB, which is another area for future research (Fig. 5).

Future studies on structural synaptic plasticity in sensory integration centers can benefit from new developments of high-resolution microscopic tools for synaptic-circuit analyses, for example correlative microscopy combining super resolution molecular imaging techniques and ultrastructural tools (array tomography; MARKERT & al. 2017). For further exploration of molecular aspects, research in ants can benefit from ongoing studies and the development of genetic tools in *Drosophila* together with emerging new techniques for genetic manipulation techniques in other insects (SUGIE & al. 2018). Furthermore, combination with functional approaches such as live-calcium imaging (ZUBE & al. 2008), expression of immediate early genes (SOMMERLANDT & al. 2017, 2018) and electrophysiological approaches, as already employed in honeybees (STRUBE-BLOSS & RÖSSLER 2018), are very promising. The small size of ant brains and the lack of genetically encoded sensors, however, require additional adjustments and methodological developments for physiological techniques.

To conclude this section on learning-related neuroplasticity, very much like in the vertebrate hippocampus and cortex (FOX & STRYKER 2017), the formation of stable associative LTM leads to structural reorganization of synaptic microcircuits in the ants' brains. Altogether, the results demonstrate that ant brains express high levels of neuroplasticity allowing them to adapt to new situations and to form complex and multiple stable LTMs for spatial orientation.

What initiates the interior-exterior transition in the first place?

Obviously, the results on the behavioral and neuronal plasticity outlined above trigger many follow-up questions and studies. However, there also is a hen and egg problem still to solve. The studies on neuroplasticity in visual pathways suggest that exogenous factors like first light exposure experienced during digging walks and visual memory formation during learning walks are important elements in the ontogeny of navigational performance as they induce substantial changes and adaptations in visual circuits and behavior (Stieb & al. 2010, Fleischmann & al. 2016, 2018a, Schmitt & al. 2016, Grob & al. 2017). However, the internal factors that cause the ants to leave the nest in the first place, to expose themselves to light for the first time and to perform learning walks, remain largely unknown. Although the focus of this review is on plasticity in neuronal circuits of the ant brain, I shall briefly outline recent progress in investigating the potential role of neuromodulators, especially neuropeptides in initiating diverse behaviors associated with behavioral transitions.

Many previous studies in social Hymenoptera focussed on changes in the hormonal system, especially juvenile hormone (JH), in the context of behavioral transitions and correlated these changes with the onset of foraging (e.g., Bloch & al. 2009, Shpigler & al. 2014, Dolezal & al. 2012). However, direct effects of JH (and the co-regulated egg-yolk protein vitellogenin, Vg) on foraging related neuroplasticity and behavior, in most cases, remained unclear. For example, RNAi-mediated knockdown of Vg and the resulting increase of JH did not affect synaptic maturation in the mushroom bodies (MBs) of the honeybee (Scholl & al. 2014). On the other hand, first exposure to light triggers structural synaptic plasticity in the MBs and, at the same time, an increase in JH level, changes in gene expression, and modulation of epigenetic processes (Scholl & al. 2014, Muenz & al. 2015, Becker & al. 2016). A promising future approach here will be to identify genes that respond to light exposure and might play a role in mediating structural synaptic plasticity (Becker & al. 2016).

Regarding the ontogeny of navigation, one key question is how the distinct changes in diverse behavioral programs at the interior-exterior transition are initiated. Roles of biogenic amines (e.g., serotonine, dopamine, octopamine) in modulating phototaxis and general behavioral thresholds associated with the nurse-forager transition were shown in the honeybee (e.g., Scheiner & Erber 2009, Scheiner & al. 2014), and their potential role as modulators of social behavior and the organization of division of labor in ants were recently reviewed by Kamhi & Traniello (2013) and Kamhi & al. (2017). However, it remained unclear how a very limited number of rather broadly acting modulators controls the diverse behavioral programs and physiological changes associated with different behavioral transitions. Recent work increasingly draws the attention to neuropeptides, a very promising class of most diverse modulators of behavior and physiol-ogy acting as both neuromodulators and neurohormones in insects (Schoofs & al. 2017). Investigations on the potential role of neuropeptides in triggering age- and stage-related behavioral transitions in social Hymenoptera started only recently.

A study by Brockmann & al. (2009) in the honeybee *Apis mellifera* Linnaeus, 1758 suggests potential roles of neuropeptides in regulating social behavior. Until recently, however, very little information was available on neuropeptides in ants. A neuropeptidomic screen based on mass-spectrometric methods in *Camponotus floridanus* revealed c. 40 neuropeptides localized in various parts of the nervous system (Schmitt & al. 2015). Using a targeted approach in *Cataglyphis fortis* combining mass spectrometry and immunohistochemistry, Schmitt & al. (2017) identified neuropeptidergic neurons of the allatotropin (AT), allatostatin A (AstA) and tachykinin (TK) families in the CX, the protocerebrum, and in primary sensory neuropils like the antennal and optic lobes. Interestingly, TK-immunoreactivity (IR) in *C. fortis* expressed age-related changes in the CX (Schmitt & al. 2017), and correlative analyses of neuropeptides of the allatostatin (Ast), short neuropeptide F (sNPF), and TK families indicate a potential role in orchestrating behavioral transitions in the honeybee (e.g., Brockmann & al. 2009, Prataveira & al. 2014, Han & al. 2015). Furthermore, studies in leaf-cutting ants show that TK is involved in modulating caste-specific changes in behavior (Howe & al. 2016). Most recently, a comprehensive analysis by Gaspocic & al. (2017) revealed stage-specific changes in Corazonin (Crz) expression in brains of the ponerine ant, *Harpegnathos saltator* Jerdon, 1851 showing high brain levels in workers compared to low levels in pseudo queens (gamergates). Brain injections of synthetic Crz promoted hunting behavior, a typical behavior of foragers, and, as a long-term effect, reduced reproductive behavior and vitellogenin expression typical for pseudo queens.

The recent approaches on neuropeptides are promising and indicate that age-related internal programs in neuropeptide expression have high potential in initiating specific behavioral routines during the various stages of the interior-exterior transition. These behavioral routines in turn trigger sensory exposure and learning-related changes in the neuronal circuitry. Future combinations of experiments on neuroplasticity and sensory physiology will help to address the complex interplay of internal programs and external (sensory) stimuli in causing appropriate adaptive changes in brain structure, function and behavior. The navigational skills and remarkable behavioral plasticity in *Cataglyphis* desert ants are very promising to tackle these future scientific endeavours aiming at understanding fundamental neuronal principles underlying the behavioral plasticity during the ontogeny of navigational performances in these charismatic ants.

References

ARDIN, P., PENG, F., MANGAN, M., LAGOGIANNIS, K. & WEBB, B. 2016: Using an insect mushroom body circuit to encode route memory in complex natural environments. – Public Library of Science Computational Biology 12: art. e1004683.

BADDELEY, B., GRAHAM, P., HUSBANDS, P. & PHILIPPIDES, A. 2012: A model of ant route navigation driven by scene familiarity. – Public Library of Science Computational Biology 8: art. e1002336.

BANKS, A.N. & SRYGLEY, R.B. 2003: Orientation by magnetic field in leaf-cutter ants, *Atta colombica* (Hymenoptera: Formicidae). – Ethology 109: 835-846.

BECKER, N., KUCHARSKI, R., RÖSSLER, W. & MALESZKA, R. 2016: Age-dependent transcriptional and epigenetic responses to light exposure in the honey bee brain. – FEBS Open Biology 6: 622-639.

BLOCH, G., WHEELER, D.E. & ROBINSON, G.E. 2009: Endocrine influences on the organization of insect societies. In: PFAFF, D.W., ARNOLD, A.P., ETGEN, A.M., FAHRBACH, S.E. & RUBIN, R.T. (Eds.): Hormones, brain and behavior. – Academic Press, San Diego, CA, pp. 1027-1068.

BOULAY, R., ARON, S., CERDA, X., DOUMS, C., GRAHAM, P., HEFETZ, A. & MONNIN, T. 2017: Social Life in Arid Environments: The case study of *Cataglyphis* ants. – Annual Review of Entomology 62: 305-321.

BREGY, P., SOMMER, S. & WEHNER, R. 2008: Nest-mark orientation *versus* vector navigation in desert ants. – The Journal of Experimental Biology 211: 1868-1873.

BROCKMANN, A., ANNANGUDI, S.P., RICHMOND, T.A., AMENT, S.A., XIE, F., SOUTHEY, B.R., RODRIGUEZ-ZAS, S.R., ROBINSON, G.E. & SWEEDLER, J.V. 2009: Quantitative peptidomics reveal brain peptide signatures of behavior. – Proceedings of the National Academy of Sciences of the United States of America 106: 2383-2388.

BUEHLMANN, C., FERNANDES, A.S.D. & GRAHAM, P. 2018: The interaction of path integration and terrestrial visual cues in navigating desert ants: what can we learn from path characteristics? – Journal of Experimental Biology 221: art. jeb167304.

BUEHLMANN, C., GRAHAM, P., HANSSON, B.S. & KNADEN, M. 2014: Desert ants locate food by combining high sensitivity to food odors with extensive crosswind runs. – Current Biology 24: 960-964.

BUEHLMANN C., HANSSON, B.S. & KNADEN, M. 2012: Path integration controls nest-plume following in desert ants. – Current Biology 22: 645-649.

BUEHLMANN, C., WOODGATE, J.L. & COLLETT, T.S. 2016: On the encoding of panoramic visual scenes in navigating wood ants. – Current Biology 26: 2022-2027.

CARTWRIGHT, B.A. & COLLETT, T.S. 1983: Landmark learning in bees. – Journal of Comparative Physiology 151: 521-543.

CHITTKA, L. & NIVEN, J. 2009: Are bigger brains better? – Current Biology 19: R995-R1008.

COLLETT, M., CHITTKA, L. & COLLETT, T.S. 2013: Spatial memory in insect navigation. – Current Biology 23: R789-R800.

COLLETT, M., COLLETT, T.S., BISCH, S. & WEHNER, R. 1998: Local and global vectors in desert ant navigation. – Nature 394: 269-272.

COLLETT, T.S. & ZEIL, J. 2018: Insect learning flights and walks. – Current Biology 28: R984-R988.

CRUSE, H. & WEHNER, R. 2011: No need for a cognitive map: decentralized memory for insect navigation. – Public Library of Science Computational Biology 7: art. e1002009.

DAVIS, R.L. 2005: Olfactory memory formation in *Drosophila*: From molecular to systems neuroscience. – Annual Review in Neuroscience 28: 275-302.

DOLEZAL, A.G., BRENT, C.S., HÖLLDOBLER, B. & AMDAM, G.V. 2012: Worker division of labor and endocrine physiology are associated in the harvester ant, *Pogonomyrmex californicus*. – Journal of Experimental Biology 215: 454-460.

EHMER, B. & GRONENBERG, W. 2004: Mushroom body volumes and visual interneurons in ants: comparison between sexes and castes. – Journal of Comparative Neurology 469: 198-213.

EL JUNDI, B., PFEIFFER, K. & HOMBERG, U. 2011: A distinct layer of the medulla integrates sky compass signals in the brain of an insect. – Public Library of Science One 6: art. e27855.

EL JUNDI, B., WARRANT, E., PFEIFFER, K. & DACKE, M. 2018: Neuroarchitecture of the dung beetle central complex. – Journal of Comparative Neurology 526: 2612-2630.

FAHRBACH, S. & VAN NEST, B.N. 2016: Synapsin-based approaches to brain plasticity in adult social insects. – Current Opinion in Insect Science 18: 27-34.

FALIBENE, A., ROCES, F. & RÖSSLER, W. 2015: Long-term avoidance memory formation is associated with a transient increase in mushroom body synaptic complexes in leaf-cutting ants. – Frontiers in Behavioral Neuroscience 9: art. 84.

FARRIS, S.M. & ROBERTS, N.S. 2005: Coevolution of generalist feeding ecologies and gyrencephalic mushroom bodies in insects. – Proceedings of the National Academy of Sciences of the United States of America 102: 17394-17399.

Farris, S.M. & Schulmeister, S. 2011: Parasitoidism, not sociality, is associated with the evolution of elaborate mushroom bodies in the brains of hymenopteran insects. – Proceedings of the Royal Society B-Biological Sciences 278: 940-951.

Fauth, M. & Tetzlaff, C. 2016: Opposing effects of neuronal activity on structural plasticity. – Frontiers in Neuroanatomy 10: art. 75.

Fox, K. & Stryker, M. 2017: Integrating Hebbian and homeostatic plasticity: introduction. – Philosophical Transaction of the Royal Society B-Biological Sciences 372: art. 20160413.

Fleischmann, P.N., Christian, M., Müller, V.L., Rössler, W. & Wehner, R. 2016: Learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. – Journal of Experimental Biology 219: 3137-3145.

Fleischmann, P.N., Grob, R., Müller, V.L., Wehner, R. & Rössler, W. 2018b: The geomagnetic field is a compass cue in *Cataglyphis* ant navigation. – Current Biology 28: 1440-1444.

Fleischmann, P.N., Grob, R., Wehner, R. & Rössler, W. 2017: Species-specific differences in the fine structure of learning-walk elements in *Cataglyphis* ants. – Journal of Experimental Biology 220: 2426-2435.

Fleischmann, P.N., Rössler, W. & Wehner, R. 2018a: Early foraging life: spatial and temporal aspects of landmark learning in the ant *Cataglyphis noda*. – Journal of Comparative Physiology A 209: 579-592.

Freas, C.A. & Schultheiss, P. 2018: How to navigate in different environments and situations: lessons from ants. – Frontiers in Psychology 9: art. 841.

Froese, A., Szyszka, P. & Menzel, R. 2014: Effect of GABAergic inhibition on odorant concentration coding in mushroom body intrinsic neurons of the honeybee. – Journal of Comparative Physiology A 200: 183-195.

Fyhn, M., Hafting, T., Treves, A., Moser, M.-B. & Moser, E.I. 2007: Hippocampal remapping and grid realignment in entorhinal cortex. – Nature 446: 190-194.

Gaspocic, J., Shields, E.J., Glastad, K.M., Lin, Y., Penick, C.A., Yan, H., Mikheyev, A.S., Linksvayer, T.A., Garcia, B.A., Berger, S.L., Liebig, J., Reinberg, D. & Bonasio, R. 2017: The neuropeptide corazonin controls social behavior and caste identity in ants. – Cell 170: 748-759.

Gerber, B., Tanimoto, R. & Heisenberg, M. 2004: An engram found? Evaluating the evidence from fruit flies. – Current Opinion in Neurobiology 14: 737-744.

Giurfa, M. 2007: Behavioral and neural analysis of associative learning in the honeybee: A taste from the magic well. – Journal of Comparative Physiology A 193: 801-824.

Graham, P., Philippides, A. & Baddeley, B. 2010: Animal cognition: multimodal interactions in ant learning. – Current Biology 20: R639-R640.

Grob, R., Fleischmann, P.N., Grübel, K., Wehner, R. & Rössler, W. 2017: The role of celestial compass information in *Cataglyphis* ants during learning walks and for neuroplasticity in the central complex and mushroom bodies. – Frontiers in Behavioral Neuroscience 11: art. 226.

Groh, C., Kelber, C., Grübel, K. & Rössler, W. 2014: Density of mushroom-body synaptic complexes limits intraspecies brain miniaturization in highly polymorphic leaf-cutting ants. – Proceedings of the Royal Society B-Biological Sciences 281: art. 20140432.

Groh, C., Lu, Z., Meinertzhagen, I.A. & Rössler, W. 2012: Age-related plasticity in the synaptic ultrastructure of neurons in the mushroom body calyx of the adult honeybee *Apis mellifera*. – Journal of Comparative Neurology 520: 3509-3527.

Groh, C., Tautz, J. & Rössler, W. 2004: Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. – Proceedings of the National Academy of Sciences of the United States of America 101: 4268-4273.

Gronenberg, W. 1999: Modality-specific segregation of input to ant mushroom bodies. – Brain Behavior & Evolution 54: 85-95.

Gronenberg, W. 2008: Structure and function of ant (Hymenoptera: Formicidae) brains: strength in numbers. – Myrmecological News 11: 25-36.

Gronenberg, W. & Hölldobler, B. 1999: Morphologic representation of visual and antennal information in the ant brain. – Journal of Comparative Neurology 412: 229-240.

Gronenberg, W. & Riveros, A.J. 2009: Social brains and behavior – past and present. In: Gadau, J. & Fewell, J. (Eds.): Organization of insect societies – from genomes to socio-complexity. – Harvard University Press, Cambridge, MA, pp. 377-401.

Haennicke, J., Yamagata, N., Zwaka, H., Nawrot, M. & Menzel, R. 2018: Neural correlates of odor learning in the presynaptic microglomerular circuitry in the honeybee mushroom body calyx. – eNeuro 11: art. 5(3).

Hafting, T., Fyhn, M., Molden, S., Moser, M.-B. & Moser, E.I. 2005: Microstructure of a spatial map in the entorhinal cortex. – Nature 436: 801-806.

Han, B., Fang, Y., Feng, M., Hu, H., Qi, Y., Huo, X., Meng, L., Wu, B. & Li, J. 2015: Quantitative neuropeptidome analysis reveals neuropeptides are correlated with social behavior regulation of the honeybee workers. – Journal of Proteome Research 14: 4382-4393.

Harrison, J.F., Fewell, J.H., Stiller, T.M. & Breed, M. 1989: Effects of experience on use of orientation cues in the giant tropical ant. – Animal Behaviour 37: 869-871.

Heinze, S. 2017: Unraveling the neural basis of insect navigation. – Current Opinion in Insect Science 24: 58-67.

Heinze, S., Florman, J., Asokaraj, S., El Jundi, B. & Reppert, S. 2013: Anatomical basis of sun compass navigation II: the neuronal composition of the central complex of the monarch butterfly. – Journal of Comparative Neurology 521: 267-298.

Heinze, S., Narendra, A. & Cheung, A. 2018: Principles of insect path integration. – Current Biology 28: R1043-R1058.

Held, M., Berz, A., Hensgen, R., Muenz, T.S., Scholl, C., Rössler, W., Homberg, U. & Pfeiffer, K. 2016: Microglomerular synaptic complexes in the sky-compass network of the honeybee connect parallel pathways from the anterior optic tubercle to the central complex. – Frontiers in Behavioral Neuroscience 10: art. 186.

Herz, H., Hölldobler, B. & Roces, F. 2008: Delayed rejection in a leaf-cutting ant after foraging on plants unsuitable for the symbiotic fungus. – Behavioral Ecology 19: 575-582.

Hoinville, T. & Wehner, R. 2018: Optimal multiguidance integration in insect navigation. – Proceedings of the National Academy of Sciences of the United States of America 115: 2824-2829.

Hölldobler, B. 1999: Multimodal signals in ant communication. – Journal of Comparative Physiology A 185: 129-141.

Hölldobler, B. & Wilson, E.O. 1990: The ants. – Belknap Press, Cambridge, MA, 732 pp.

Homberg, U., Heinze, S., Pfeiffer, K., Kinoshita, M. & El Jundi, B. 2011: Central neural coding of sky polarization in insects. – Philosophical Transactions of the Royal Society B-Biological Sciences 366: 680-687.

Hourcade, B., Muenz, T.S., Sandoz, J.C., Rössler, W. & Devaud, J.M. 2010: Long-term memory leads to synaptic

reorganization in the mushroom bodies: a memory trace in the insect brain? – Journal of Neuroscience 30: 6461-6465.

Howe, J., Schiott, M. & Boomsma, J.J. 2016: Tachykinin expression levels correlate with caste-specific aggression in workers of the leaf-cutting ant *Acromyrmex echinatior*. – Frontiers in Ecology and Evolution 4: art. 55.

Huber, R. & Knaden, M. 2015: Egocentric and geocentric navigation during extremely long foraging paths of desert ants. – Journal of Comparative Physiology A 201: 609-616.

Huber, R. & Knaden, M. 2017: Homing ants get confused when nest cues are also route cues. – Current Biology 27: 3706-3710.

Huber, R. & Knaden, M. 2018: Desert ants possess distinct memories for food and nest odors. – Proceedings of the National Academy of Sciences of the United States of America 115: 10470-10474.

Immonen, E.V., Dacke, M., Heinze, S. & El Jundi, B. 2017: Anatomical organization of the brain of a diurnal and a nocturnal dung beetle. – Journal of Comparative Neurology 525: 1879-1908.

Ito, K., Shinomiy, K., Ito, M., Armstrong, D., Boyan, G., Hartenstein, V., Harzsch, S., Heisenberg, M., Homberg, U., Jenett, A., Keshishian, H., Restifo, L., Rössler, W., Simpson, J., Strausfeld, N.J., Strauss, R. & Vosshall, L.B. Insect Brain Name Working Group. 2014: A systematic nomenclature for the insect brain. – Neuron 81: 755-765.

Jander, R. & Jander, U. 1998: The light and magnetic compass of the weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae). – Ethology 104: 743-758.

Jayatilaka, P., Murray, T., Narendra, A. & Zeil, J. 2018: The choreography of learning walks in the Australian jack jumper ant *Myrmecia croslandi*. – Journal of Experimental Biology; doi:10.1242/jeb.185306.

Kamhi, J.F., Arganda, S., Moreau, C.S. & Traniello, J.F.A. 2017: Origins of aminergic regulation of behavior in complex insect social systems. – Frontiers in Systems Neuroscience 11: 74.

Kamhi, J.F. & Traniello, J.F.A. 2013: Biogenic amines and collective organization in a superorganism: neuromodulation of social behavior in ants. – Brain Behavior & Evolution 82: 220-236.

Kinoshita, M., Shimohigasshi, M., Tominaga, Y., Arikawa, K. & Homberg, U. 2015: Topographically distinct visual and olfactory inputs to the mushroom body in the Swallowtail butterfly, *Papilio xuthus*. – Journal of Comparative Neurology 523: 162-182.

Kleineidam, C. & Rössler, W. 2009: Adaptations of the olfactory system of social Hymenoptera. In: Gadau, J. & Fewell, J. (Eds.): Organization of insect societies – from genomes to socio-complexity. – Harvard University Press, Cambridge, MA, pp. 195-219.

Knaden, M. & Graham, P. 2016: The sensory ecology of ant navigation: From natural environments to neural mechanisms. – Annual Review of Entomology 61: 63-76.

Knaden, M., Tinaut, A., Stökl, J., Cerdá, X. & Wehner, R. 2012: Molecular phylogeny of the desert ant genus *Cataglyphis* (Hymenoptera: Formicidae). – Myrmecological News 16: 123-132.

Knaden, M. & Wehner, R. 2005: Nest mark orientation in desert ants *Cataglyphis*: what does it do to the path integrator? – Animal Behaviour 70: 1349-1354.

Knaden, M. & Wehner, R. 2006: Ant navigation: resetting the path integrator. – Journal of Experimental Biology 209: 26-31.

Kohler, M. & Wehner, R. 2005: Idiosyncratic route-based memories in desert ants, *Melophorus bagoti*: how do they

interact with path-integration vectors? – Neurobiology of Learning and Memory 83: 1-12.

Kühn-Bühlmann, S. & Wehner, R. 2006: Age-dependent and task-related volume changes in the mushroom bodies of visually guided desert ants, *Cataglyphis bicolor*. – Journal of Neurobiology 66: 511-521.

Labhart, T. & Meyer, E.P. 1999: Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. – Microscopy Research & Techniques 47: 368-379.

Leadbeater, E. & Chittka, L. 2007: Social learning in insects – from miniature brains to consensus building. – Current Biology 17: R703-713.

Lebhardt, F. & Ronacher, B. 2014: Interactions of the polarization and the sun compass in path integration of desert ants. – Journal of Comparative Physiology A 200: 711-720.

Lebhardt, F. & Ronacher, B. 2015: Transfer of directional information between the polarization compass and the sun compass in desert ants. – Journal of Comparative Physiology A 201: 599-608.

Legge, E.L.G., Wystrach, A., Spetch L.M.L. & Cheng, K. 2014: Combining sky and earth: desert ants (*Melophorus bagoti*) show weighted integration of celestial and terrestrial cues. – Journal of Experimental Biology 217: 4159-4166.

Lent, D.D., Graham, P. & Collett, T.S., 2009: Visual scene perception in navigating wood ants. – Current Biology 23: 684-690.

Lent, D.D., Pinter, M. & Strausfeld, N.J. 2007: Learning with half a brain. – Developmental Neurobiology 67: 740-751.

Markert, S.M., Bauer, V., Muenz, T.S., Jones, N., Helmprobst, F., Britz, S., Sauer, M., Rössler, W., Engstler, M. & Stigloher, C. 2017: 3D subcellular localization with super-resolution array tomography on ultrathin sections of various species. – Methods in Cell Biology 149: 21-47.

Menzel, R. 1999: Memory dynamics in the honeybee. – Journal of Comparative Physiology A 185: 323-340.

Menzel, R. 2001: Searching for the memory trace in a mini-brain, the honeybee. – Learning & Memory 8: 53-62.

Mizunami, M., Weibrecht, J.M. & Strausfeld, N.J. 1998: Mushroom bodies of the cockroach: Their participation in place memory. – Journal of Comparative Neurology 402: 520-537.

Moser, E.I., Kropff, E. & Moser, M.-B. 2008: Place cells, grid cells, and the brain's spatial representation system. – Annual Review of Neuroscience 31: 69-89.

Muenz, T.S., Groh, C., Maisonnasse, A., Le Conte, Y., Plettner, E. & Rössler, W. 2015: Synaptic plasticity in the mushroom-body calyx during adult maturation in the honeybee and possible pheromonal influences. – Developmental Neurobiology 75: 1368-1384.

Müller, M. & Wehner, R. 2010: Path integration provides a scaffold for landmark learning in desert ants. – Current Biology 20: 1368-1371.

Muser, B., Sommer, S., Wolf, H. & Wehner, R. 2005: Foraging ecology of the thermophilic Australian desert ant, *Melophorus bagoti*. – Australian Journal of Zoology 53: 301-311.

Narendra, A., Gourmaud, S. & Zeil, J. 2013: Mapping the navigational knowledge of individually foraging ants, *Myrmecia croslandi*. – Proceedings of the Royal Society B-Biological Sciences 280: art. 20130683.

Narendra, A., Si, A., Sulinowski, D. & Cheng., K. 2007: Learning, retention and coding of nest-associated visual cues by the Australian desert ant, *Melophorus bagoti*. – Behavioral Ecology and Sociobiology 61: 1543-1553.

Nicholson, D.J., Judd, S.P., Cartwright, B.A. & Collett, T.S. 1999: Learning walks and landmark guidance in wood ants (*Formica rufa*). – The Journal of Experimental Biology 202: 1831-1838.

O'keefe, J. & Burgess, N. 1996: Geometric determinants of the place fields of hippocampal neurons. – Nature 381: 425-428.

Peng, F. & Chittka, L. 2017: A simple computational model of the bee mushroom body can explain seemingly complex forms of olfactory learning and memory. – Current Biology 27: 224-230.

Pfeiffer, K. & Homberg, U. 2007: Coding of azimuthal directions via time-compensated combination of celestial compass cues. – Current Biology 17: 960-965.

Pfeiffer, K. & Kinoshita, M. 2012: Segregation of visual inputs from different regions of the compound eye in two parallel pathways through the anterior optic tubercle of the bumblebee (*Bombus ignitus*). – Journal of Comparative Neurology 520: 212-229.

Pfeiffer, K., Kinoshita, M. & Homberg, U. 2005: Polarization-sensitive and light-sensitive neurons in two parallel pathways passing through the anterior optic tubercle in the locust brain. – Journal of Neurophysiology 94: 3903-3915.

Plath, J.A., Entler, B.V., Kirkerud, N.H., Schlegel, U., Galizia, C.G. & Barron, A.B. 2017: Different roles for honey bee mushroom bodies and central complex in visual learning of colored lights in an aversive conditioning assay. – Frontiers in Behavioral Neuroscience 11: art. 98.

Prataveira, M., Ribeiro Da Silva Menegasso, A., Caviqui-oli Garcia, A.M., Simo Es Dos Santos, D., Gomes, P.C., Malaspina, O., Palma, M.S. 2014: MALDI imaging analysis of neuropeptides in the africanized honeybee (*Apis mellifera*) brain: effect of ontogeny. – Journal of Proteome Research 13: 3054-3064.

Reid, S.F., Narendra, A., Hemmi, J. & Zeil, J. 2011: Polarised skylight and the landmark panorama provide night-active bull ants with compass information during route following. – Journal of Experimental Biology 214: 363-370.

Riveros, A.J. & Srygley, R.B. 2008: Do leafcutter ants, *Atta colombica*, orient their path-integrated home vector with a magnetic compass? – Animal Behavior 75: 1273-1281.

Ronacher, B. 2008: Path integration as the basic navigation mechanism of the desert ant *Cataglyphis fortis* (Forel, 1902) (Hymenoptera: Formicidae). – Myrmecological News 11: 53-62.

Rössler, W. & Groh, C. 2012: Plasticity of synaptic microcircuits in the mushroom-body calyx of the honeybee. In: Eisenhardt, D., Giurfa, M. & Galizia, C.G. (Eds.): Honeybee neurobiology and behavior – a tribute to Randolf Menzel. – Springer Verlag, Heidelberg, New York, NY, pp 141-153.

Rössler, W., Spaethe, J. & Groh, C. 2017: Pitfalls of using confocal-microscopy based automated quantification of synaptic complexes in honeybee mushroom bodies (response to Peng and Yang 2016). – Scientific Reports 7: art. 9786.

Saverscheck, N. & Roces, F. 2011: Foraging leaf cutter ants: olfactory memory underlies delayed avoidance of plants unsuitable for the symbiotic fungus. – Animal Behavior 82: 453-458.

Scheiner, R. & Erber, J. 2009: Sensory thresholds, learning and the division of foraging labor in the honey bee. In: Gadau, J. & Fewell, J. (Eds.): Organization of insect societies – from genomes to socio-complexity. – Harvard University Press, Cambridge, MA, pp. 335-356.

Scheiner, R., Toteva, A., Reim, T., Søvik, E. & Barron, A.B. 2014: Differences in the phototaxis of pollen and nectar foraging honey bees are related to their octopamine brain titers. – Frontiers in Physiology 5: art. 116.

Schmid-Hempel, P. & Schmid-Hempel, R. 1984: Life duration and turnover of foragers in the ant *Cataglyphis bicolor* (Hymenoptera. Formicidae). – Insectes Sociaux 31: 345-360.

Schmitt, F., Stieb, S.M., Wehner, R. & Rössler, W. 2016: Experience-related reorganization of giant synapses in the lateral complex: potential role in plasticity of the sky-compass pathway in the desert ant *Cataglyphis fortis*. – Developmental Neurobiology 76: 390-404.

Schmitt, F., Vanselow, J.T., Schlosser, A., Kahnt, J., Rössler, W. & Wegener, C. 2015: Neuropeptidomics of the carpenter ant *Camponotus floridanus*. – Journal of Proteome Research 14: 1504-1514.

Schmitt, F., Vanselow, J.T., Schlosser, A., Wegener, C. & Rössler, W. 2017: Neuropeptides in the desert ant *Cataglyphis fortis*: mass spectrometric analysis, localization, and age-related changes. – Journal of Comparative Neurology 525: 901-918.

Scholl, C., Wang, Y., Krischke, M., Mueller, M.J., Amdam, G. & Rössler, W. 2014: Light exposure leads to reorganization of microglomeruli in the mushroom bodies and influences juvenile hormone levels in the honeybee. – Developmental Neurobiology 74: 1141-1153.

Schoofs, L., De Loof, A. & Van Hiel, M.B. 2017: Neuropeptides as regulators of behavior in insects. – Annual Review of Entomology 62: 35-52.

Seelig, J.D. & Jayaraman, V. 2013: Feature detection and orientation tuning in the *Drosophila* central complex. – Nature 503: 262-266.

Seelig, J.D. & Jayaraman, V. 2015: Neural dynamics for landmark orientation and angular path integration. – Nature 521: 186-191.

Seid, M.A. & Wehner, R. 2009: Delayed axonal pruning in the ant brain: a study of developmental trajectories. – Developmental Neurobiology 69: 350-364.

Shpigler, H., Amsalem, E., Huang, Z.Y., Cohen, M., Siegel, A.J., Hefetz, A. & Bloch, G. 2014: Gonadotropic and physiological function of juvenile hormone in bumblebee (*Bombus terrestris*) workers. – Public Library of Science One 9: art. e100650.

Sommerlandt, F., Brockmann, A., Rössler, W. & Spaethe, J. 2018: Immediate early genes in social insects: a tool to identify brain regions involved in complex behaviors and molecular processes underlying neuroplasticity. – Cellular and Molecular Life Sciences; doi: 10.1007/s00018-018-2948-z.

Sommerlandt, F.M.J., Rössler, W. & Spaethe, J. 2017: Impact of light and alarm pheromone on immediate early gene expression in the European honeybee, *Apis mellifera*. –Entomological Science 20: 122-126.

Steck, K., Hansson, B.S. & Knaden, M. 2011: Desert ants benefit from combining visual and olfactory landmarks. – Journal of Experimental Biology 214: 1307-1312.

Stieb, S.M., Hellwig, A., Wehner, R. & Rössler, W. 2012: Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. – Developmental Neurobiology 72: 729-742.

Stieb, S.M., Kelber, C., Wehner, R. & Rössler, W. 2011: Antennal-lobe organization in desert ants of the genus *Cataglyphis*. – Brain, Behavior & Evolution 77: 136-146.

Stieb, S.M., Muenz, T.S., Wehner, R. & Rössler, W. 2010: Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. – Developmental Neurobiology 70: 408-423.

Stone, T., Webb, B., Adden, A., Weddig, N.B., Honkanen, A., Templin, R., Wcislo, W., Scimera, L., Warrant, E. & Heinze, S. 2017: An anatomically constrained model for path integration in the bee brain. – Current Biology 20: 3069-3085.

Strausfeld, N.J. 2002: Organization of the honey bee mushroom body: representation of the calyx within the vertical and gamma lobes. – Journal of Comparative Neurology 450: 4-33.

Strausfeld, N.J. 2012: Arthropod brains. Evolution, functional elegance, and historical significance. – Harvard University Press, Cambridge, London, UK, 830 pp.

Strausfeld, N.J., Hansen, L., Li, Y., Gomez, R.S. & Ito, K. 1998: Evolution, discovery, and interpretations of arthropod mushroom bodies. – Learning & Memory 5: 11-37.

Strausfeld, N.J. & Hirth, F. 2013: Deep homology of arthropod central complex and vertebrate basal ganglia. – Science 340: 157-161.

Strube-Bloss, M.F., Herrera-Valdez, M.A. & Smith, B.H. 2012: Ensemble response in mushroom body output neurons of the honey bee outpaces spatiotemporal odor processing two synapses earlier in the antennal lobe. – Public Library of Science One 7: art. e50322.

Strube-Bloss, M.F., Nawrot, M.P. & Menzel, R. 2011: Mushroom body output neurons encode odor-reward association. – Journal of Neuroscience 31: 3129-3140.

Strube-Bloss, M.F. & Rössler, W. 2018: Multimodal integration and stimulus categorization in putative mushroom body output neurons of the honeybee. – Royal Society Open Science 5: art. 171785.

Sugie, A., Marchetti, G. & Tavosanis, G. 2018: Structural aspects of plasticity in the nervous system of *Drosophila*. – Neural Development 13: art. 14.

Stürzl, W., Grixa, I., Mair, E., Narendra, A. & Zeil, J. 2015: Three dimensional models of natural environments and the mapping of navigational information. – Journal of Comparative Physiology A 201: 563-584.

Stürzl, W., Zeil, J., Boeddeker, N. & Hemmi, J. 2016: How wasps acquire and use views for homing. – Current Biology 26: 470-482.

Szyszka, P., Ditzen, M., Galkin, A., Galizia, C.G. & Menzel, R. 2005: Sparsening and temporal sharpening of olfactory representations in the honeybee mushroom bodies. – Journal of Neurophysiology 94: 3303-3313.

Szyszka, P., Galkin, A. & Menzel, R. 2008: Associative and non-associative plasticity in Kenyon cells of the honeybee mushroom body. – Frontiers in Systems Neuroscience 2: art. 3.

Towne, W.F. 2008: Honeybees can learn the relationship between the solar ephemeris and a newly experienced landscape. – Journal of Experimental Biology 211: 3737-3743.

Towne, W.F. & Moscrip, H. 2008: The connection between landscapes and the solar ephemeris in honeybees. – Journal of Experimental Biology 211: 3729-3736.

Träger, U., Wagner, R., Bausenwein, B. & Homberg, U. 2008: A novel type of microglomerular synaptic complex in the polarization vision pathway of the locust brain. – Journal of Comparative Neurology 506: 288-300.

Vogt, K., Aso, Y., Hige, T., Knapek, S., Ichinose, T., Friedrich, A.B., Turner, G.C., Rubin, G.M. & Tanimoto, H. 2016: Direct neural pathways convey distinct visual information to *Drosophila* mushroom bodies. – eLife 5: art. e14009.

Wajnberg, E., Acosta-Avalos, D., Alves, O.C., De Oliveira, J.F., Srygley, R.B. & Esquivel, D.M.S. 2010: Magnetoreception in eusocial insects: an update. – Journal of the Royal Society Interface 7: S207-S225.

Webb, B. & Wystrach, A. 2016: Neuronal mechanisms of insect navigation. – Current Opinion in Insect Science 15: 27-39.

Wehner, R. 2009: The architecture of the desert ant's navigational toolkit (Hymenoptera: Formicidae). – Myrmecological News 12: 85-96.

Wehner, R., Cheng, K. & Cruse, H. 2014: Visual navigation strategies in insects: lessons from desert ants. In: Werner, J.S. & Chalupa, L.M. (Eds.): The new visual neurosciences. – MIT Press, Cambridge, MA, pp. 1153-1163.

Wehner, R., Hoinville, T., Cruse, H. & Cheng, K. 2016: Steering intermediate courses: desert ants combine information from various navigational routines. – Journal of Comparative Physiology A 202: 459-472.

Wehner, R., Meier, C. & Zollikofer, C. 2004: The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor*. – Ecological Entomology 29: 240-250.

Wehner, R., Michel, B. & Antonsen, P. 1996: Visual navigation in insects: coupling of egocentric and geocentric information. – Journal of Experimental Biology 199: 129-140.

Wehner, R. & Müller, M. 1985: Does interocular transfer occur in visual navigation by ants? – Nature 315: 228-229.

Wehner, R. & Müller, M. 1993: How do ants acquire their celestial ephemeris function? – Naturwissenschaften 80: 331-333.

Wehner, R. & Müller, M. 2006: The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. – Proceedings of the National Academy of Sciences of the United States of America 133: 12575-12579.

Wehner, R. & Müller, M. 2010: Path integration provides a scaffold for landmark learning in desert ants. – Current Bioloy 20: 1368-1371.

Wehner, R. & Rössler, W. 2013: Bounded plasticity in the desert ant's navigational toolkit. In: Menzel, R. & Benjamin, P.R. (Eds.): Invertebrate learning and memory. – Academic Press Elsevier, Amsterdam, London, Heidelberg, pp. 514-529.

Wehner, R. & Wehner, S. 1990: Insect navigation: use of maps or Ariadne's thread? – Ethology Ecology and Evolution 2: 27-48.

Wittlinger, M., Wehner, R. & Wolf, H. 2006: The ant odometer: stepping on stilts and stumps. – Science 312: 1965-1967.

Wohlgemuth, S., Ronacher, B. & Wehner, R. 2001: Ant odometry in the third dimension. – Nature 411: 795-798.

Wystrach, A., Mangan, M. & Webb, B. 2015: Optimal cue integration in ants. – Proceedings of the Royal Society B-Biological Sciences 282: art. 20151484.

Yilmaz, A., Lindenberg, A., Albert, S., Grübel, K., Spaethe, J., Rössler, W. & Groh, C. 2016: Age-related and light-induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar-feeding ant *Camponotus rufipes*. – Developmental Neurobiology 76: 1041-1057.

Zeil, J. 1993: Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera) I. Description of flight. – Journal of Comparative Physiology A 172: 189-205.

Zeil, J. 2012: Visual homing: an insect perspective. – Current Opinion in Neurobiology 22: 285-293.

Zeil, J., Kelber, A. & Voss, R. 1996: Structure and function of learning flights in bees and wasps. – Journal of Experimental Biology 199: 245-252.

Zeil, J., Narendra, A. & Stürzl, W. 2014: Looking and homing: how displaced ants decide where to go. – Philosophical Transactions of the Royal Society B-Biological Sciences 369: art. 20130034.

Zeller, M., Held, M., Bender, J., Berz, A., Heinloth, T., Hellfritz, T. & Pfeiffer, K. 2015: Transmedulla neurons in the sky compass network of the honeybee (*Apis mellifera*) are a possible site of circadian input. – Public Library of Science One 10: art. e0143244.

Ziegler, P.E. & Wehner, R. 1997: Time-courses of memory decay in vector-based and landmark-based systems of navigation in desert ants, *Cataglyphis fortis*. – Journal of Comparative Physiology A 181: 13-20.

Zube, C. & Rössler, W. 2008: Caste- and sex-specific adaptations within the olfactory pathway in the brain of the ant *Camponotus floridanus*. – Arthropod Structure & Development 37: 469-479.

Zube, C., Kleineidam, C.J., Kirschner, S., Neef, J. & Rössler, W. 2008: Organization of the olfactory pathway and odor processing in the antennal lobe of the ant *Camponotus floridanus*. – Journal of Comparative Neurology 506: 425-441.