SYNAPTIC PLASTICITY IN VISUAL AND OLFACTORY BRAIN CENTERS OF THE DESERT ANT *CATAGLYPHIS*

SYNAPTISCHE PLASTIZITÄT VISUELLER UND OLFAKTORISCHER GEHIRNZENTREN DER WÜSTENAMEISE *CATAGLYPHIS*

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SUMMARY

Desert ants of the genus *Cataglyphis* have become model systems for the study of insect navigation. An age-related polyethism subdivides their colonies into interior workers and short-lived light-exposed foragers. While foraging in featureless and cluttered terrain over distances up to several hundred meters, the ants are able to precisely return back to their often inconspicuous nest entrance. They accomplish this enormous navigational performance by using a path integration system - including a polarization compass and an odometer - as their main navigational means in addition to landmark-dependent orientation and olfactory cues. *C. fortis*, being the focus of the present thesis, is endemic to the salt flats of western North Africa, which are completely avoided by other *Cataglyphis* species.

The fact that *Cataglyphis* ants undergo a behavioral transition associated with drastically changing sensory demands makes these ants particularly interesting for studying synaptic plasticity in visual and olfactory brain centers. This thesis focuses on plastic changes in the mushroom bodies (MBs) - sensory integration centers supposed to be involved in learning and memory presumably including landmark learning - and in synaptic complexes belonging to the lateral accessory lobe (LAL) known to be a relay station in the polarization processing pathway.

To investigate structural synaptic plasticity in the MBs of *C. fortis*, synaptic complexes (microglomeruli, MG) in the visual (collar) and olfactory (lip) input regions of the MB calyx were immunolabeled and their pre- and postsynaptic profiles were quantified. The results show that a volume increase of the MB calyx during behavioral transition is associated with a decrease of MG number - an effect called pruning - in the collar and, less pronounced, in the lip that goes along with dendritic expansion in MB intrinsic Kenyon cells. Light-exposure of dark-reared ants of different age classes revealed similar effects and dark-reared ants age-matched to foragers had MG numbers comparable to those of interior workers. The results indicate that the enormous structural synaptic plasticity of the MB calyx collar is primarily driven by visual experience rather than by an internal program. Ants aged artificially for up to one year expressed a similar plasticity indicating that the system remains flexible over the entire life-span. To investigate whether light-induced synaptic reorganization is reversible, experienced foragers were transferred back to darkness with the result that their MBs exhibit only some reverse-type characteristics, in particular differences in presynaptic synapsin expression.

To investigate the structure of large synaptic complexes in the LAL of *C. fortis* and to detect potential structural changes, pre- and postsynaptic profiles in interior workers and foragers were immunolabeled and quantified by using confocal imaging and 3D-reconstruction. The results show that these complexes consist of postsynaptic processes located in a central region that is surrounded by a cup-like presynaptic profile. Tracer injections identified input and output tracts of the LAL: projection neurons from the anterior optic tubercle build connections with neurons projecting to the central complex. The behavioral transition is associated with an increase by ~13% of synaptic complexes suggesting that the polarization pathway may undergo some sort of calibration process. The structural features of these synaptic contacts indicate that they may serve a fast and reliable signal transmission in the polarization vision pathway.

Behavioral analyses of *C. fortis* in the field revealed that the ants perform exploration runs including pirouette-like turns very close to the nest entrance for a period of up to two days, before they actually start their foraging activity. During these orientation runs the ants gather visual experience and might associate the nest entrance with specific landmarks or get entrained to other visual information like the polarization pattern, and, concomitantly adapt their neuronal circuitries to the upcoming challenges. Moreover, the pirouettes may serve to stimulate and calibrate the neuronal networks involved in the polarization compass pathway. Video recordings and analyses demonstrate that light experience enhanced the ants' locomotor activity after three days of exposure. The fact that both the light-induced behavioral and neuronal changes in visual brain centers occur in the same time frame suggests that there may be a link between structural synaptic plasticity and the behavioral transition from interior tasks to outdoor foraging.

Desert ants of the genus *Cataglyphis* possess remarkable visual navigation capabilities, but also employ olfactory cues for detecting nest and food sites. Using confocal imaging and 3D-reconstruction, potential adaptations in primary olfactory brain centers were analyzed by comparing the number, size and spatial arrangement of olfactory glomeruli in the antennal lobe of *C. fortis*, *C. albicans*, *C. bicolor*, *C. rubra*, and *C. noda*. Workers of all *Cataglyphis* species have smaller numbers of glomeruli compared to those of more olfactory-guided *Formica* species - a genus closely related to *Cataglyphis* - and to those previously found in other olfactory-guided ant species. *C. fortis* has the lowest number of glomeruli compared to all other species, but possesses a conspicuously enlarged glomerulus that is located close to the antennal nerve entrance. Males of *C. fortis* have a significantly smaller number of glomeruli compared to female workers and queens and a prominent male-specific

macroglomerulus likely to be involved in sex pheromone communication. The behavioral significance of the enlarged glomerulus in female workers remains elusive. The fact that *C. fortis* inhabits microhabitats that are avoided by all other *Cataglyphis* species suggests that extreme ecological conditions may not only have resulted in adaptations of visual capabilities, but also in specializations of the olfactory system.

The present thesis demonstrates that *Cataglyphis* is an excellent candidate for studying the neuronal mechanisms underlying navigational features and for studying neuronal plasticity associated with the ant's lifelong flexibility of individual behavioral repertoires.

ZUSAMMENFASSUNG

Wüstenameisen der Gattung *Cataglyphis* wurden zu Modellsystemen bei der Erforschung der Navigationsmechanismen der Insekten. Ein altersabhängiger Polyethismus trennt deren Kolonien in Innendienst-Arbeiterinnen und kurzlebige lichtausgesetzte Fourageure. Nachdem die Ameisen in strukturlosem oder strukturiertem Gelände bis zu mehrere hundert Meter weite Distanzen zurückgelegt haben, können sie präzise zu ihrer oft unauffälligen Nestöffnung zurückzukehren. Um diese enorme Navigationsleistung zu vollbringen, bedienen sich die Ameisen der sogenannten Pfadintegration, welche die Informationen aus einem Polarisationskompass und einem Entfernungsmesser verrechnet; des Weiteren orientieren sie sich an Landmarken und nutzen olfaktorische Signale. Im Fokus dieser Arbeit steht *C. fortis*, welche in Salzpfannen des westlichen Nordafrikas endemisch ist - einem Gebiet, welches vollständig von anderen *Cataglyphis* Arten gemieden wird.

Die Tatsache, dass *Cataglyphis* eine hohe Verhaltensflexibilität aufweist, welche mit sich drastisch ändernden sensorischen Anforderungen verbunden ist, macht diese Ameisen zu besonders interessanten Studienobjekten bei der Erforschung synaptischer Plastizität visueller und olfaktorischer Gehirnzentren. Diese Arbeit fokussiert auf plastische Änderungen in den Pilzkörpern (PK) - sensorischen Integrationszentren, die mutmaßlich an Lern- und Erinnerungsprozessen, und auch vermutlich am Prozess des Landmarkenlernens beteiligt sind - und auf plastische Änderungen in den synaptischen Komplexen des Lateralen Akzessorischen Lobus (LAL) – einer bekannten Relaisstation in der Polarisations-Leitungsbahn.

Um die strukturelle synaptische Plastizität der PK in *C. fortis* zu quantifizieren, wurden mithilfe immunozytochemischer Färbungen die prä- und postsynaptischen Profile klar ausgeprägter synaptischer Komplexe (Mikroglomeruli, MG) der visuellen Region (Kragen) und der olfaktorischen Region (Lippe) der PK-Kelche visualisiert. Die Ergebnisse legen dar, dass eine Volumenzunahme der PK-Kelche während des Übergangs von Innendiensttieren zu Fourageuren von einer Abnahme der MG-Anzahl im Kragen und, mit einem geringeren Anteil, in der Lippe - dieser Effekt wird als Pruning bezeichnet - und einem gleichzeitigen Auswachsen an Dendriten PK-intrinsischer Kenyonzellen begleitet wird. Im Dunkeln gehaltene Tiere unterschiedlichen Alters zeigen nach Lichtaussetzung den gleichen Effekt und im Dunkel gehaltene, den Fourageuren altersmäßig angepasste Tiere weisen eine vergleichbare MG-Anzahl im Kragen auf wie Innendiensttiere. Diese Ergebnisse deuten darauf hin, dass die immense strukturelle synaptische Plastizität in der Kragenregion der PK-

Kelche hauptsächlich durch visuelle Erfahrungen ausgelöst wird und nicht ausschließlich mit Hilfe eines internen Programms abgespielt wird. Ameisen, welche unter Laborbedingungen bis zu einem Jahr alt wurden, zeigen eine vergleichbare Plastizität. Dies deutet darauf hin, dass das System über die ganze Lebensspanne eines Individuums flexibel bleibt. Erfahrene Fourageure wurden in Dunkelheit zurückgeführt, um zu untersuchen, ob die lichtausgelöste synaptische Umstrukturierung reversibel ist, doch ihre PK zeigen nur einige die Zurückführung widerspiegelnde Plastizitätsausprägungen, besonders eine Änderung der präsynaptischen Synapsinexprimierung.

Mithilfe immunozytochemischer Färbungen, konfokaler Mikroskopie und 3D-Rekonstruktionen wurden die prä- und postsynaptischen Strukturen synaptischer Komplexe des LAL in *C. fortis* analysiert und potentielle strukturelle Änderungen bei Innendiensttieren und Fourageuren quantifiziert. Die Ergebnisse zeigen, dass diese Komplexe aus postsynaptischen, in einer zentralen Region angeordneten Fortsätzen bestehen, welche umringt sind von einem präsynaptischen kelchartigen Profil. Eingehende und ausgehende Trakte wurden durch Farbstoffinjektionen identifiziert: Projektionsneurone des Anterioren Optischen Tuberkels kontaktieren Neurone, welche in den Zentralkomplex ziehen. Der Verhaltensübergang wird von einer Zunahme an synaptischen Komplexen um ~13% begleitet. Dieser Zuwachs suggeriert eine Art Kalibrierungsprozess in diesen potentiell kräftigen synaptischen Kontakten, welche vermutlich eine schnelle und belastbare Signalübertragung in der Polarisationsbahn liefern.

Die Analyse von im Freiland aufgenommener Verhaltenweisen von *C. fortis* enthüllen, dass die Ameisen, bevor sie mit ihrer Fouragiertätigkeit anfangen, bis zu zwei Tage lang in unmittelbarer Nähe des Nestes Entdeckungsläufe unternehmen, welche Pirouetten ähnliche Drehungen beinhalten. Während dieser Entdeckungsläufe sammeln die Ameisen Lichterfahrung und assoziieren möglicherweise den Nesteingang mit spezifischen Landmarken oder werden anderen visuellen Informationen, wie denen des Polarisationsmusters, ausgesetzt und adaptieren begleitend ihre neuronalen Netzwerke an die bevorstehende Herausforderung. Darüber hinaus könnten die Pirouetten einer Stimulation der an der Polarisationsbahn beteiligten neuronalen Netzwerke dienen. Videoanalysen legen dar, dass Lichtaussetzung nach drei Tagen die Bewegungsaktivität der Ameisen heraufsetzt. Die Tatsache, dass die neuronale Umstrukturierung in visuellen Zentren wie auch die Veränderungen im Verhalten im selben Zeitrahmen ablaufen, deutet darauf hin, dass ein Zusammenhang zwischen struktureller synaptischer Plastizität und dem Verhaltensübergang von der Innendienst- zur Fouragierphase bestehen könnte.

Cataglyphis besitzen hervorragende visuelle Navigationsfähigkeiten, doch sie nutzen zudem olfaktorische Signale, um das Nest oder die Futterquelle aufzuspüren. Mithilfe konfokaler Mikroskopie und 3D-Rekonstruktionen wurden potentielle Anpassungen der primären olfaktorischen Gehirnzentren untersucht, indem die Anzahl, Größe und räumliche Anordnung olfaktorischer Glomeruli im Antennallobus von *C. fortis*, *C. albicans*, *C. bicolor*, *C. rubra*, und *C. noda* verglichen wurde. Arbeiterinnen aller *Cataglyphis*-Arten haben eine geringere Glomeruli-Anzahl im Vergleich zu denen der mehr olfaktorisch-orientierten *Formica* Arten - einer Gattung nah verwandt mit *Cataglyphis* - und denen schon bekannter olfaktorisch-orientierter Ameisenarten. *C. fortis* hat die geringste Anzahl an Glomeruli im Vergleich zu allen anderen *Cataglyphis*-Arten und besitzt einen vergrößerten Glomerulus, der nahe dem Eingang des Antennennerves lokalisiert ist. *C. fortis* Männchen besitzen eine signifikant geringere Glomeruli-Anzahl im Vergleich zu Arbeiterinnen und Königinnen und haben einen hervorstechenden Männchen-spezifischen Makroglomerulus, welcher wahrscheinlich an der Pheromon-Kommunikation beteiligt ist. Die Verhaltensrelevanz des vergrößerten Glomerulus der Arbeiterinnen bleibt schwer fassbar. Die Tatsache, dass *C. fortis* Mikrohabitate bewohnt, welche von allen anderen *Cataglyphis* Arten gemieden werden, legt nahe, dass extreme ökologische Bedingungen nicht nur zu Anpassungen der visuellen Fähigkeiten, sondern auch des olfaktorischen Systems geführt haben.

Die vorliegende Arbeit veranschaulicht, dass *Cataglyphis* ein exzellenter Kandidat ist bei der Erforschung neuronaler Mechanismen, welche Navigationsfunktionalitäten zugrundeliegen, und bei der Erforschung neuronaler Plastizität, welche verknüpft ist mit der lebenslangen Flexibilität eines individuellen Verhaltensrepertoires.

INTRODUCTION

GENERAL INTRODUCTION

Social Hymenoptera have evolved a rich behavioral repertoire associated with their highly complex social organization. Interactions among colony members via chemical cues and pheromones are a very important feature in social insects in general, and in ants in particular (Hölldobler and Wilson, 1990; Kleineidam and Rössler, 2009). Ants employ an enormous variety of olfactory communication signals in the context of sexual behavior, nestmate recognition, caste discrimination and/or alarm, recruitment, and trail marking behaviors (Hölldobler and Wilson, 1990). Besides the importance of olfaction, vision also plays a key role in many hymenopteran species. Especially flying bees and wasps or many of the fast-running ants orientate and navigate during their often long-lasting or far-reaching foraging trips by the use of visual information. During navigating and homing, the recognition or recall of visual landmarks or the use of the polarized skylight pattern functioning as a compass serve as visual cues (reviewed in Menzel et al., 1996; Collett and Collett, 2002, 2004; Wehner, 2003; Wehner and Srinivasan, 2003).

The rich behavioral repertoire in social insects requires a highly sophisticated nervous system able to integrate the enormous variety of functions including chemical and visual communication, orientation and navigation skills as well as impressive learning and memory capacities. Moreover, the division of labor in e.g. ants is often characterized by an age-, group-, caste-, or task-related polyethism (for a summary, see Table 8-3 in Hölldobler and Wilson, 1990) with the consequence that the individual behavioral repertoire expresses a life-lasting high flexibility that in turn requires a high plasticity in the underlying neuronal circuitry. In many ant species, workers undergo an age-related polyethism which plays an important role in task allocation and division of labor. Young workers mainly care for brood inside the nest, while older workers forage for food outside the nest. Ants, therefore, provide particularly promising model systems to study the neuronal mechanisms underlying their remarkable behavioral plasticity.

THE DESERT ANT *CATAGLYPHIS*

Desert ants of the genus *Cataglyphis* FOERSTER 1850 – widespread over arid areas of the Old World (Wehner et al., 1983) – have become model systems for the study of insect navigation (Wehner, 2003). An age-related polyethism subdivides their colonies into interior

workers up to an average age of 28 days fulfilling tasks like brood care and food processing and day-active central place foragers with life expectancies of about 6 days possessing impressive navigational skills (shown for *C. bicolor*: Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984).

While foraging for sparsely scattered food items – mostly dead arthropods – in featureless and cluttered terrain over distances up to several hundred meters, the ants are able to precisely and rapidly return back in a straight line to their often inconspicuous nest entrance (Fig. 1). They accomplish this enormous navigational performance by using a path integration system as their main navigational means in addition to landmark-dependent orientation and olfactory cues (reviewed in Wehner et al., 1996; Wehner, 2003, 2009). Even though *Cataglyphis* ants establish quite pronounced individual sector fidelities during their foraging lives (Wehner et al., 2004), i.e. leave the nest and return to it along familiar vectors or landmark-defined routes, they do not use trail pheromones (Schmid-Hempel, 1983; Wehner et al., 1983), as it is the case in many other ant species (Hölldobler and Wilson, 1990).

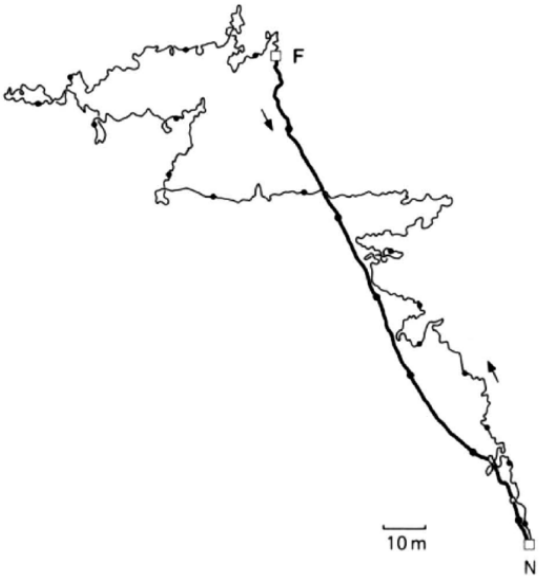

Figure 1. Tortuous outward (foraging) and straight homeward path of the desert ant *Cataglyphis fortis* recorded in a featureless salt pan. F feeding site, N nest. Adapted from Wehner and Wehner (1990).

The path-integrating system (for a review, see Wehner and Srinivasan, 2003) includes a skylight-based visual compass (Wehner and Müller, 2006) and a stride-integrating odometer (Wittlinger et al., 2006). This means that *Cataglyphis* continuously measures all angles steered and all distances covered and integrates them to a home vector pointing from its actual position to the nest entrance. Information about the direction is achieved by skylight information including the azimuthal position of the sun, spectral gradients in the sky (reviewed in Wehner, 1994), but most dominantly the pattern of polarized light in the sky (Wehner et al., 1996; Wehner and Müller 2006). Information about the distance travelled is

mainly gathered by an odometer measuring idiothetic leg movements (Wittlinger et al., 2006; 2007). In contrast to honeybees, the integration of visual flow-field cues ('optic-flow') plays only a minor role in the distance estimation of *Cataglyphis* (Ronacher and Wehner, 1995). However, the mechanism of path integration leads *Cataglyphis* foragers only back to the approximate vicinity of their nest resulting from continuously accumulated errors (Müller and Wehner, 1988; Merkle and Wehner, 2010) that increase with longer-lasting foraging excursion (Merkle et al., 2006). Thus, it is essential for foragers to additionally exhibit navigational backup systems including systematic search behavior (Wehner and Srinivasan, 1981; Müller and Wehner, 1994) and the use of landmarks to define places and routes (Wehner and Räber, 1979; Collett et al., 1992; Bisch- Knaden and Wehner, 2003; Collett and Collett, 2009). The ants link places, e.g. the nest entrance, with visual landmarks and later, when returning to this place, match their stored image with the actual one (reviewed in Wehner et al., 1996; Wehner, 2003). It has been shown that familiar landmark scenes can be matched with local vectors (Collett et al., 1998) and motor commands (Collett et al., 2001; Bisch-Knaden and Wehner, 2001), which enable the ants to navigate from one familiar place to the next. *Cataglyphis* although relies on chemical cues for pinpointing food sources (Wolf and Wehner, 2000), and, in the vicinity of the nest can use olfactory landmarks for homing (Steck et al., 2009; Steck et al., 2010). Furthermore, bimodal sensory input resulting from an interaction of visual and olfactory information enhances the acquisition of landmark information in *Cataglyphis* (Steck et al., 2011).

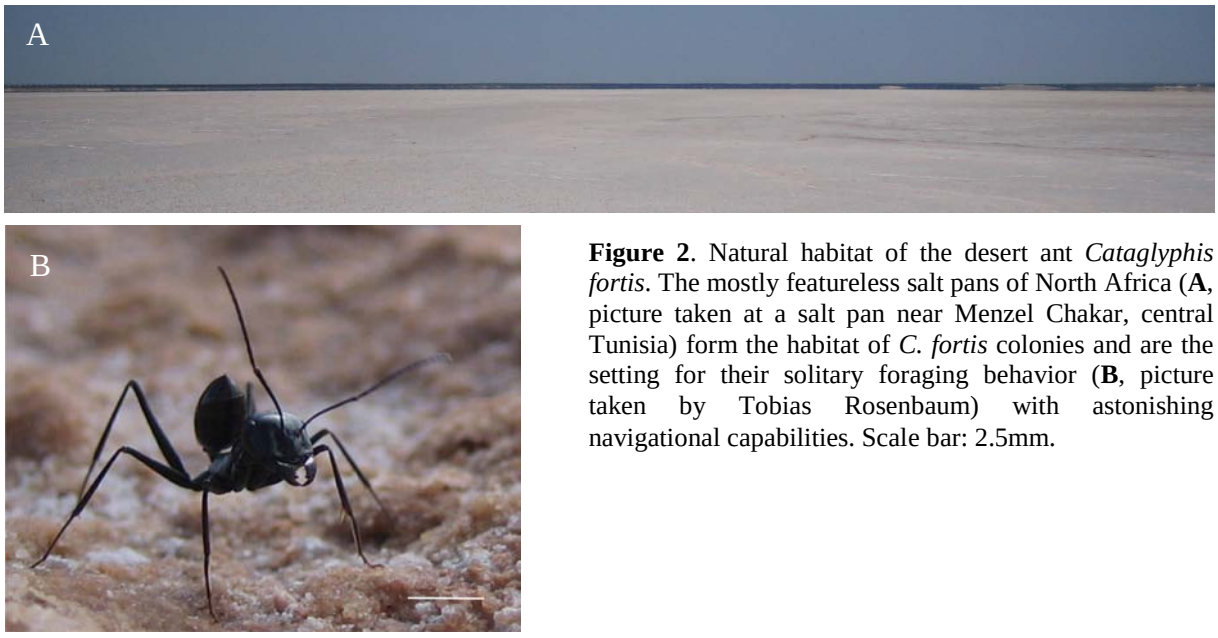

Figure 2. Natural habitat of the desert ant *Cataglyphis fortis*. The mostly featureless salt pans of North Africa (**A**, picture taken at a salt pan near Menzel Chakar, central Tunisia) form the habitat of *C. fortis* colonies and are the setting for their solitary foraging behavior (**B**, picture taken by Tobias Rosenbaum) with astonishing navigational capabilities. Scale bar: 2.5mm.

Cataglyphis fortis, being the focus of the present thesis, is endemic to the salt flats of western North Africa (Fig. 2) (Wehner, 1981a, 1983; Wehner et al., 1994), which are completely avoided by other *Cataglyphis* species inhabiting more steppe-like environments (Wehner, 1983; Dillier and Wehner, 2004). This mostly featureless habitat is perfectly suitable to perform behavioral experiments unraveling insect navigational mechanism and thus, *C. fortis* has become a well-studied model organism. Nevertheless, the neuronal mechanisms underlying both visual and olfactory navigational features have just begun to be explored. The present thesis aims at investigating visual and olfactory brain centers in *C. fortis* (Fig. 3). Moreover, the fact that *Cataglyphis* ants undergo a transition from interior workers to short-lived foragers associated with drastically changing sensory environments and demands makes these ants particularly interesting for studying synaptic plasticity in visual and olfactory brain centers.

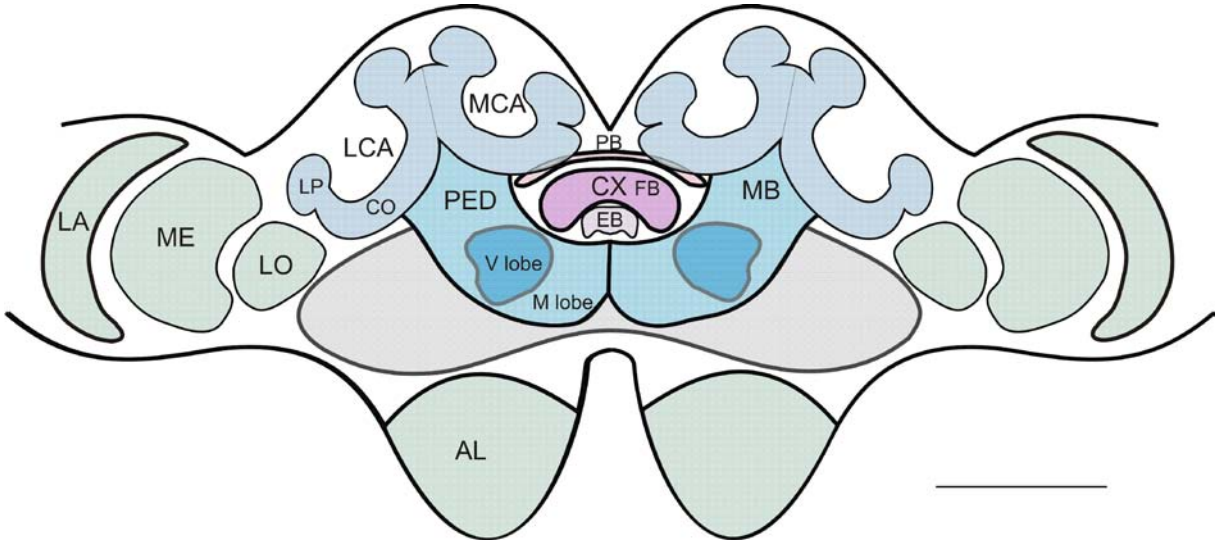

Figure 3. Schematic frontal overview of the *Cataglyphis fortis* brain. Optic lobes with lamina (LA), medulla (ME) and lobula (LO); antennal lobes (AL); mushroom bodies (MB) with medial calyces (MCA) and lateral calyces (LCA); the calyx subdivisions with the olfactory innervated lip (LP) and visual innervated collar (CO; separation between subcompartiments as well as the basal ring (BR) are not displayed); pedunculus (PED); vertical (V) and medial lobes (M lobe); central complex (CX) with fan-shaped (FB) and ellipsoid body (EB); protocerebral bridge (PB); scale bar: 200µm.

THE INSECT VISUAL SYSTEM AND ITS CHARACTERISTICS IN HYMENOPTERA

Visual information in insects is received via visual pigment molecules located in photoreceptor cells of numerous ommatidia (600-1200 in *C. bicolor* (Menzel and Wehner, 1970) and 1180 in a large individual of *C. fortis* (Wenzler, 2010)) building up the compound eye. Color vision in Hymenoptera is rather conservative, mostly with ultraviolet (UV), blue

and green receptors with maximum sensitivities near 340, 430, and 535 nm (Peitsch et al., 1992; for a review see Briscoe and Chittka, 2001). Nevertheless, species differences do exist, e.g. *Cataglyphis* (Labhart, 1986; Mote and Wehner, 1980) as well as all other (for a review, see Briscoe and Chittka, 2001) ants possess only UV (~350 nm) and green-receptors (~510 nm). The primary visual centers in the insect brain, the optic lobes, consist of three neuropils: lamina, medulla and lobula (see Fig. 3) (Bullock and Horridge, 1965). While in some insect groups, e.g. Diptera and Lepidoptera, the lobula is further subdivided in lobula and lobula plate, the hymenopteran lobula is structured more or less uniformly (Wehner, 1976). The topographical arrangement of the ommatidia is retained throughout lamina, medulla and lobula; this organization is called retinotopy. Most of the photoreceptor axons end in the lamina; the remaining ones send their projections to the medulla (for a review, see Sanes and Zipursky, 2010; for *Cataglyphis*, see Meyer, 1979). Medulla projection neurons are either targeting directly in visual centers of the protocerebrum or are interconnected with lobula neurons, which then project to central brain regions (for different visual pathways to the central brain see e.g. Sanes & Zipursky, 2010; Ehmer and Gronenberg, 2002; Homberg et al., 2003; Mota et al.,, 2011). In addition to the compound eyes, most adult insects possess three dorsally located single-lens eyes, the ocelli. Their function is still under consideration and possibly varies between different insects. Ocelli support different behavioral tasks including flight stabilization, navigation and orientation, absolute intensity measurement and neurosecretion (reviewed in Goodman, 1981; Mizunami, 1995; Wehner, 1987). In *Cataglyphis*, the ocelli have been shown to function as an auxiliary celestial compass (Fent and Wehner, 1985; Schwarz et al., 2011).

The present study focuses on two visual pathways, namely the information processing of polarized light and the projection of visual information to the mushroom bodies (MBs), higher sensory integration centers involved in learning and memory. Both pathways are discussed in more detail in the following sections.

THE POLARIZATION PATHWAY

In order to illustrate information processing of polarized light in the insect brain it is relevant to first describe polarized light itself and its abundance in nature. A wave of light is an electromagnetic radiation with an electric field vector (e-vector) oscillating perpendicularly to its direction of propagation. While the e-vector of unpolarized light, like it is directly coming from the sun, may be orientated in any one plane at any one time, linear polarized light constantly oscillates in a single plane. Polarization is generated by the scattering of sunlight

within the atmosphere, and by reflection of shiny, non-metallic, dielectric surfaces like water, particular parts of vegetations or body surfaces like arthropod cuticles or fish scales (for a review see (Wehner and Labhart, 2006). Animals sensitive to polarized light use it for a variety of purposes (reviewed in Horváth and Varju, 2004; Wehner and Labhart, 2006). The present thesis focuses on the use of the celestial polarization pattern in the context of compass navigation used by insect central place foragers such as ants and bees (reviewed in e.g. Collett and Collett, 2000; Wehner and Srinivasan, 2003). The e-vectors are arranged along concentric circles around the sun, and both the polarization pattern and the degree of polarization are strongly linked to the position of the sun (for a review, see e.g. Wehner and Labhart, 2006). Thus, directional information from the sky can be extracted from even a small patch of the blue sky, independently of the visibility of the sun.

Cataglyphis, like many insect species including locusts, crickets, bees, butterflies, beetles as well as other ant species possesses a special region in its compound eyes that is termed dorsal rim area (DRA) composed of ommatidia that are particularly adapted for polarization sensitivity (reviewed in Labhart and Meyer, 1999). In fact, the DRA was first discovered in C bicolor (Herrling, 1976) and shortly thereafter in honeybees (Wehner et al., 1975; for a review, see Wehner, 1982). All insect photoreceptor cells exhibit a high degree of alignment of photopigment in microvillar membranes allowing maximal photon absorption for light with an e-vector oscillating parallel to the microvillar axis and by this are inherently sensitive to polarized light (reviewed in Labhart and Meyer, 1999). However, rhabdomeric twist or misalignment of microvillar orientation along the rhabdomere in the eye regions outside the DRA suppress polarization-sensitivity that otherwise would interfere with the perception of color and brightness (Wehner and Bernard, 1993).

In *Catagylphis* ants, medulla neurons being sensitive to polarized light have been identified, but unfortunately neither physiological details nor morphological reconstruction are available (Labhart, 2000). Information about central neuronal pathways and processing mechanisms of polarization information has mostly been investigated in the larger cricket *Gryllus campestris* and locust *Schistocerca gregaria* (Fig. 4) (reviewed in Labhart and Meyer, 2002; Homberg, 2004; Homberg et al., 2011). From the DRA of the compound eye, polarization-sensitive neurons project to the corresponding area in the lamina and medulla (Blum and Labhart, 2000; Homberg and Paech, 2002). In the cricket, commissural neurons (referred to as POL1 neurons) have bilateral projections with ipsilateral dendritic arborizations in the dorsal medulla and axon projections in the contralateral medulla (Labhart and Petzold, 1993; Petzold, 2001). Dendritic branches of both the ipsilateral and contralateral part of these

neurons also occur in the accessory medulla. According to the locust model, medulla line tangential neurons with ramifications in the dorsal rim of the medulla project through the anterior lobe of the lobula via the anterior lobe tract into the lower unit of the anterior optic tubercle (Homberg et al., 2003; Pfeiffer et al., 2005). From here, heterolateral interneurons (referred to as LoTu1 and TuTu1) interconnect the lower subunits of both brain hemispheres and two types of projection neurons send their axons to certain areas in the lateral accessory lobe - the lateral triangle and median olive (Pfeiffer et al., 2005). Here, incoming axons form synaptic contacts in large microglomerular complexes with tangential neurons of the ellipsoid body of the central complex (CX) (Träger et al., 2008). Polarized-light information is carried to specific layers of the lower division of the CX (Vitzthum et al., 2002). In the fan-shaped body, the ellipsoid body and the protocerebral bridge of the CX, a network of polarization-sensitive neurons has been identified (Vitzthum et al., 2002; Heinze and Homberg, 2007, 2009). A second visual pathway was shown to project polarization information via the accessory medulla and posterior optic tubercle to the CX (el Jundi and Homberg, 2010). Finally, two types of output neurons from the CX network to the lateral accessory lobe and posterior protocerebrum may transfer information to descending thoracic motor circuits (Heinze and Homberg, 2009).

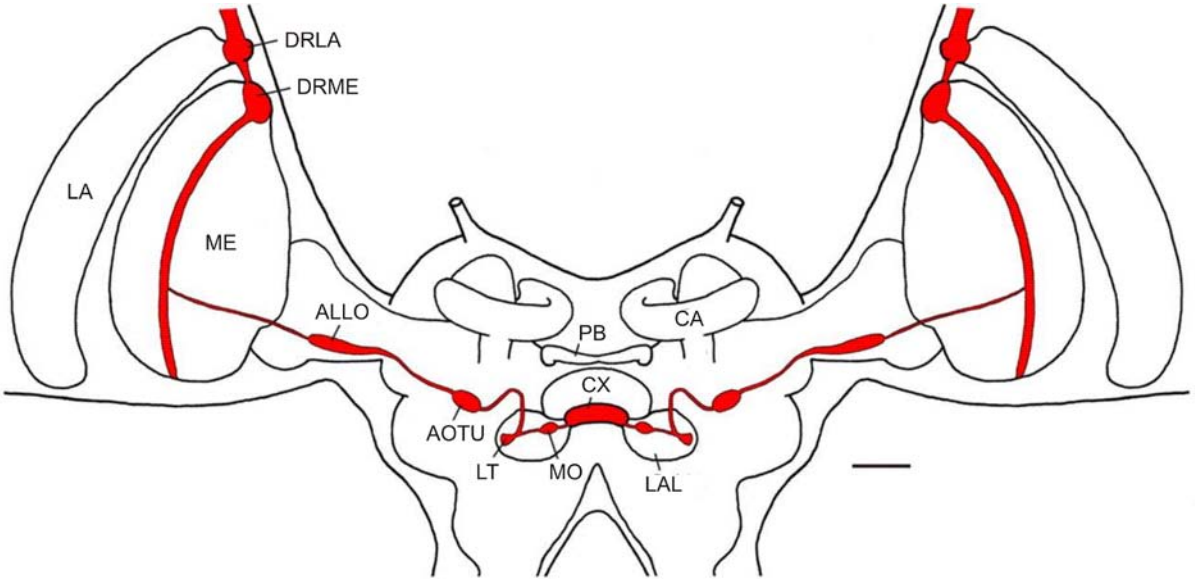

Figure 4. Schematic diagram of the polarization pathway in the locust (*Schistocerca gregaria*) brain. Central processing stages for polarized light include the dorsal rim of the lamina and medulla (DRLA and DRME), the anterior lobe of the lobula (ALLO), the lower unit of the anterior optic tubercle (AOTU), the median olive (MO) and lateral triangle (LT) of the lateral accessory lobe (LAL), and the ellipsoid body of the central complex (CX). Further abbreviations: CA, Calyx; LA, lamina; ME, medulla; PB, protocerebral bridge. Scale bar, 200 mm. Adapted from Homberg et al. (2011).

THE VISUAL PATHWAY TO THE MUSHROOM BODIES

Most insect MBs are assumed to receive indirect rather than direct input from the optic lobes. It has been shown for flies (Strausfeld, 1976) and moths (Pearson, 1971) that no direct afferents end in the MBs. However, visual experience influences the MB organization in flies and thus makes an indirect connection likely (Barth and Heisenberg, 1997). It is only in crickets (Honegger and Schürmann, 1975) and cockroaches (Straussfeld and Li, 1999) that optic lobe terminals have been found in the MBs. In general, MBs have been described as predominantly olfactory neuropils (Strausfeld et al, 1998).

In contrast, in Hymenoptera like ants, bees, wasps and bumblebees, visual input to the MBs is prominent (Mobbs, 1982; Gronenberg, 2001; Ehmer and Gronenberg, 2002, Paulk and Gronenberg, 2008). Both medulla and lobula afferents to the MBs are localized in particular regions: dendritic aborizations in the medulla are not homogeneous and extend from the dorsal to the ventral part; lobula afferents are localized in the dorsal and inner part (Ehmer and Gronenberg, 2002; see Fig. 5). Optic lobe neurons send their axons via the anterior superior optical tract, the anterior inferior tract or the lobula tract to the MBs, whereas medulla and lobula neurons innervate both the ipsi- and contralateral brain hemisphere (Gronenberg, 2001; Strausfeld, 2002; Ehmer and Gronenberg, 2002) (Fig. 6). In bees, recent studies showed that the calyces receive chromatic, temporal, and motion sensitive input from the optic lobes indicating its potential importance for higher visual processing tasks related to visually-guided foraging (Paulk and Gronenberg, 2008).

Since the MBs receive both visual and olfactory input and function as integration and association centers, its detailed innervations is described (see chapter 1.5.) after the insect olfactory system and the MB itself is introduced.

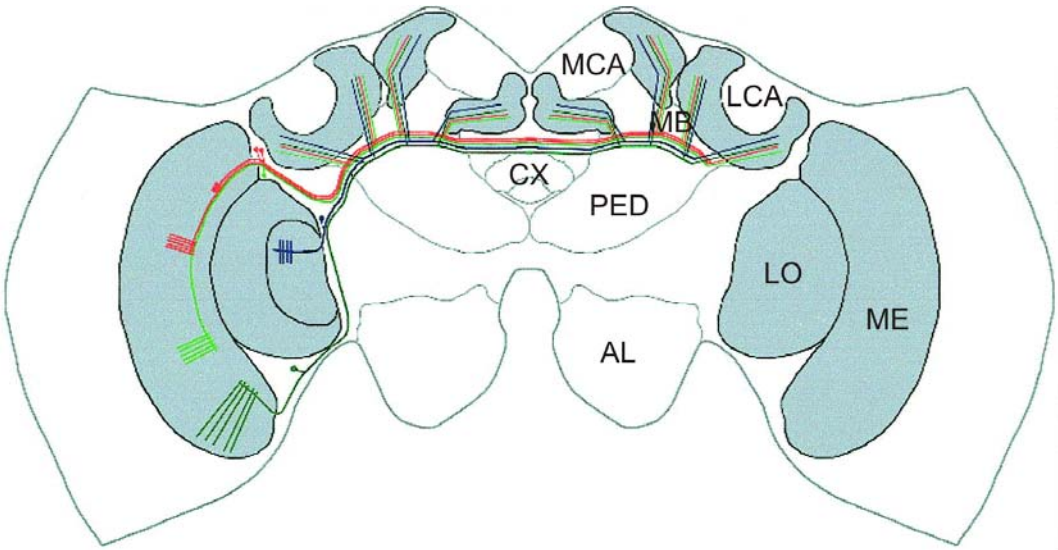

Figure 5. Schematic overview of projections from optic lobes to the mushroom body (MB) calyces in the honeybee brain. Medulla (ME) neurons send their axons via the anterior superior optical tract (red and green) and the anterior inferior tract (dark green), lobula (LO) neurons via the lobula tract (blue) to specific regions in the MB calyces (for detailed description see chapter 1.5). Both medulla and lobula neurons innervate the ipsi- and contralateral brain hemisphere. Further abbreviations: AL, antennal lobe; LCA, mushroom body lateral calyx; MCA, mushroom body medial calyx; PED, pedunculus; CX, central complex. Modified and adapted from Ehmer and Gronenberg (2002).

THE INSECT OLFACTORY SYSTEM AND ITS CHARACTERISTICS IN HYMENOPTERA

In ants, as in most insects, odor information is sensed via olfactory receptor neurons (ORNs) located on the antenna. The antenna not only functions as the insects' nose, it rather is a multimodal sensory organ that serves also for the reception of taste, thermo-, hygro- and mechano-sensory stimuli. All receptor neurons are housed in morphologic distinct types of sensilla, whereas in Hymenoptera one sensillum contains multiple ORNs (for a review, see Keil, 1999). Odor specificity of ORNs is given by its expressed receptor molecules and binding proteins. In nature, odors can be composed of mixtures of many different components; thus, one odor can activate different types of ORNs (Esslen and Kaissling, 1976), and ORNs are often activated by a range of different odors. In contrast to receptors for general odors, receptors specified to the detection of pheromones are often very specific to one single component (for ants, see Dumpert, 1972).

The ORN axons are bundled in the double-stranded antennal nerve (AN) and project to the first olfactory neuropil, the antennal lobe (AL) (see Fig. 3), where they terminate in functional units, the glomeruli (for reviews, see Homberg et al., 1989; Mustaparta, 1990; Hildebrand and Shepherd, 1997; Hansson and Anton, 2000; Kleineidam and Rössler, 2009; Galizia and Rössler, 2010). In moths all ORN axons are sorted into glomerulus specific bundles in a

typical axon sorting zone at the entrance of the antennal nerve (Rössler et al. 1999). As a typical feature in Hymenoptera, the incoming axons of ORNs are sorted into several (1-7) sensory tracts which innervate different clusters of glomeruli (Kirschner et al., 2006; Zube et al., 2008, Kelber et al., 2010). The functional significance of this clustering effect is not yet clear. ORNs expressing the same receptor molecule are assumed to converge onto the same glomerulus (Gao et al., 2000; Vosshall et al., 2000). Consequently, excitation of ORNs by odors results in a spatial neuronal activation pattern of different glomeruli (in honeybees: Joerges et al., 1997; Galizia et al., 1999b; Sachse et al., 1999; in ants: Zube et al., 2008; Kuebler et al., 2010; in *Drosophila*: Fishilevich and Vosshall, 2005). In several insect species, sex-pheromone sensitive ORNs in males of e.g., moths (Anton and Homberg, 1999) or honeybee drones (Arnold et al., 1985; Sandoz, 2006) send their projections to a single or to several enlarged glomeruli, so called macroglomeruli or macroglomerular complexes. In contrast to the sex-pheromone sensitive macroglomerular complex in moths or other insects, in non-sexual individuals of leaf-cutting ants (*Atta* and *Acromyrmex*) an macroglomerulus was found in large workers (Kleineidam et al., 2005; Kelber et al., 2009) and was shown to be involved in the detection and processing of trail pheromone components (Kuebler et al., 2010).

The number of glomeruli varies intra- (caste and sex-specific) and interspecific. While most insects possess glomerular numbers not exceeding 100 (e.g. 43 glomeruli in *Drosophila melanogaster* (Stocker, 1994, Laissue et al., 1999) and 65 in *Manduca sexta* (Huetteroth and Schachtner, 2005)), the number of glomeruli in social Hymenoptera range from 156–166 glomeruli in the honeybee (Arnold et al., 1985; Flanagan and Mercer, 1989; Galizia et al., 1999a; Kirschner et al., 2006) to up to several hundred glomeruli in different ant species (e.g. 257–630 glomeruli in fungus-growing ant speciesss: Kelber et al., 2009; Kuebler et al., 2010; likewise, around 430 glomeruli in carpenter ants: Nishikawa et al., 2008; Zube and Rössler, 2008). The high number of glomeruli might reflect the enormous and diverse olfactory demands existing in social insect colonies.

ORN axons form synaptic contacts with local interneurons - allowing cross-talk between glomeruli and the effect of reformatting olfactory information in the antennal lobe neuronal network (Abel et al., 2001; Flanagan and Mercer, 1989) - and projection neurons (PNs) transfer the olfactory information to higher-order brain centers, the MBs and the lateral horn (Mobbs, 1982; Abel et al., 2001; Kirschner et al., 2006; Zube et al, 2008). Odor information is transferred from PNs to MB intrinsic neurons (Kenyon cells KCs) that in contrast to the PNs

show a very sparse activation pattern (Perez-Orive et al., 2002; Szyszka et al., 2005) requiring synchronized activation by multiple PNs (Lei et al., 2004).

In Hymenoptera, the MBs and the LH are innervated via a dual olfactory pathway (see Fig. 6): olfactory PNs transfer olfactory input via the medial (m) - and lateral (l) – antennal lobe-protocerebral tracts (APTs) to specific regions in the MB calyces (for a detailed description see 5.1) whereas the projections are restricted to the ipsilateral side of the brain (Mobbs, 1982; Abel et al., 2001; Gronenberg, 2001; Kirschner et al., 2006; Zube et al, 2008). The dual olfactory pathway presumably represents an adaptation of the Hymenopteran olfactory system to more sophisticated olfactory processing associated with social life (Kleineidam and Rössler, 2009; Galizia and Rössler, 2010; Kirschner et al., 2006). However, comparative studies among hymenopteran species indicate that a dual olfactory pathway is not restricted to social species but may have evolved in basal Hymenoptera, and the associated advances in olfactory processing may represent a preadaptation for life styles with high demands on olfactory discrimination like parasitoism, central place foraging, and sociality (Rössler and Zube, 2011).

Since the MBs receive both visual and olfactory input and function as integration and association centers, its detailed innervations is described in the next chapter (see chapter 1.5).

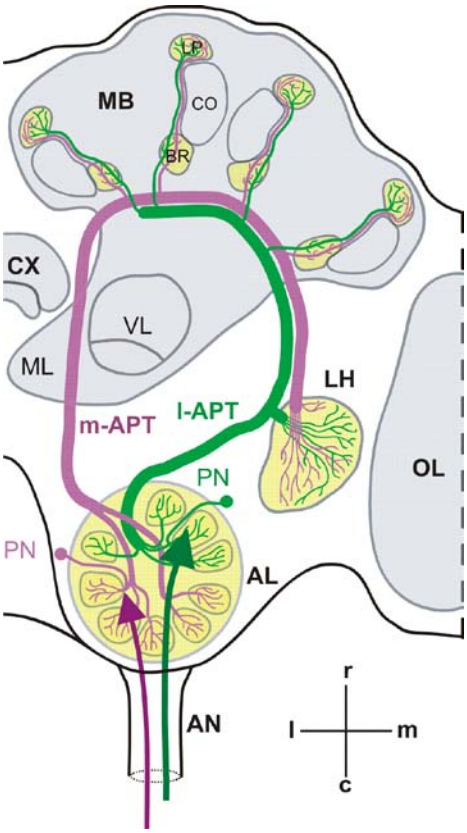

Figure 6. Schematic overview of projections from the antennal lobes (AL) to the mushroom body (MB) calyces in the honeybee brain. The antennal nerve (AN) sensory input tracts innervate the olfactory glomeruli in the (AL. Projection neurons (PNs) receiving input from individual glomeruli in two hemilobes of the AL send their axons via the medial (m-) and lateral (l-) antenno-protocerebral tracts (APTs) to specific regions in the basal ring (BR) and lip (LP) of the MB-calyces and separate domains in the lateral horn (LH; for a detailed description see chapter 5.1). Further abbreviations: c, caudal; CX, central body; CO, collar; l, lateral; m, medial; ML, medial lobe; OL, optic lobe; r, rostral; VL, ventral lobe. Modified and adapted from Kirschner et al. (2006) and Galizia and Rössler 2010.

THE MUSHROOM BODIES
- MULTIMODAL INTEGRATION AND ASSOCIATION CENTERS

The paired MBs function as higher sensory integration and association centers involved in the organization of complex behaviors, especially learning and memory (Heisenberg et al., 1995; Strausfeld et al., 1998; Menzel, 1999; Gerber et al., 2004; Davis, 2005; Giurfa, 2007, Hourcade et al., 2010). In all neopteran insects, the cellular organization of the MB is similar (Strausfeld et al., 1998): each MB consists of single, paired (e.g. in Hymenoptera) or fused cup-shaped calyces, a pedunculus, and a vertical and medial lobe (respectively α- and β-lobe, Mobbs, 1982; Strausfeld et al., 2009). MB calyces, pedunculus and output lobes are formed by MB intrinsic neurons, the Kenyon cells (KCs): while KC dendrites compose, together with axonal terminal arborizations from visual and olfactory PNs, the calyces, their axonal projections run parallel in the lobes (for reviews, see Straussfeld et al, 1998; Fahrbach, 2006). In Hymenoptera different-sized KCs can be subdivided in three populations regarding their location: the inner compact cells (IC), the non-compact cells (NC) and the outer compact cell (OC) (Mobbs, 1982; Farris et al., 1999). Both the densely packed IC and the not so densely packed NC are located inside the calyx cup with the IC forming the innermost center whereas the OC surround the calyx cup. Particularly in ants (Gronenberg et al., 1996; Seid et al., 2005), bees (Groh et al., 2004), and social wasps (Ehmer and Hoy, 2000), the MBs are very prominent structures in relation to other brain regions. In bees and ants, the MB calyces comprise three sensory input regions: the olfactory innervated lip receiving input from the ALs, the visually innervated collar receiving input from the OLs, and the basal ring receiving input from both sensory modalities (Mobbs, 1982; Gronenberg, 2001; Farris and Sinakevitch, 2003). The NC and IC dendrites thereby are segregated into the calyx subregions with NC dendrites forming the lip and collar and IC forming the basal ring, whereas the OCs dendrites diffuse in all regions of the calyx (Strausfeld, 2002; Farris, 2005). Along olfactory and visual input the upper collar and the basal ring receive gustatory and mechanosensory input (Schröter and Menzel, 2003). Both olfactory and visual projection tract input regions are not homogenously distributed in the calycal subcompartments giving strong indication of segregated information processing. Dorsal and ventral medulla PNs terminate in alternating order in five layers within the collar; a sixth layer represents input from the lobula PNs; the remaining lobula neurons terminate in the outer part of the basal ring, and inputs from the medulla are restricted to a small area in the basal ring (see Fig. 5) (Ehmer and Gronenberg, 2002). The m-APT PNs innervate the whole lip with highest innervations in the outer lip cortex and the peripheral part of the basal ring. In contrast, PNs belonging to the l-APT

innervate the central core of both the basal ring and the lip (see Fig. 6) (Kirschner et al., 2006).

The neuropils of the MB calyces consist of distinct synaptic complexes, so called microglomeruli (MG). Each MG comprises a central presynaptic bouton of PNs - originating from either the antennal lobes (AL) or the optic lobes (OL) - and a surrounding shell of numerous postsynaptic profiles, mostly from intrinsic KC dendritic spines (Steiger, 1967; Ganeshina and Menzel, 2001; Gronenberg, 2001; Yasuyama et al., 2002; Frambach et al., 2004; Groh et al., 2004, 2006; Seid and Wehner, 2008; Leiss et al., 2009). Furthermore, the calyx also receives processes from GABAergic terminals of protocerebral feedback-neurons (Bicker et al., 1985; Grünewald, 1999) and other extrinsic neurons considered to have an inhibitory or modulatory function (Schürmann, 1987; Ganeshina and Menzel, 2001). Calycal MG and packing density are highest in Hymenopteran species compared to other neopteran species indicating an increase in synaptic microcircuits, which could enhance the computational capacities of the MBs (Rössler and Zube, 2011).

STRUCTURAL PLASTICITY IN THE MUSHROOM BODIES

The age polyethism typical for workers of e.g. honeybees (Lindauer, 1953; Seeley, 1978) and many ant species (for a summary, see Table 8-3 in Hölldobler and Wilson, 1990) including *Cataglyphis* (Schmid-Hempel, 1983) provides an excellent model to investigate structural modifications of the mature brain. During the adult life, the workers' sensory environment and demands as well as the motor activities change dramatically with the transition from the in-nest/hive phase to the outdoor phase. Whereas interior workers rely extensively on olfactory inputs to fulfill their tasks inside the dark nest/hive, foragers must integrate visual and olfactory information. Moreover, learning and memory of visual and olfactory landmarks get more essential for foragers. The MBs and their role as learning and memory as well as multimodal higher sensory integration centers have become the region of interest in the study of age-and experience-related changes in the neuronal network of the insect brain.

Previous studies have shown that both age and sensory experience can lead to volumetric changes in the MBs. Volume changes in the MB calyces associated with the transition from interior workers to foragers were reported for bees (Withers et al., 1993; Durst et al., 1994; Farris et al., 2001), wasps (O'Donnell et al., 2004), and ants (Gronenberg et al., 1996) including *Cataglyphis* (Kühn-Bühlmann and Wehner, 2006). For example, age-matched honeybees with foraging experiences had larger MB neuropils than the bees without any outdoor-activities (Durst et al., 1994; Farris et al., 2001; Ismail at al., 2006). The same holds

true for *Cataglyphis* ants (Kühn-Bühlmann and Wehner, 2006). The volume changes in the MB calyx may be generated by structural plasticity of neuronal axons, dendrites, and synapses. For example, foraging activity in the honeybee is associated with dendritic outgrowth in MB intrinsic KCs (Farris et al., 2001). It has been demonstrated for crickets, honeybees and flies that f-actin, a highly motile cytoskeletal element, is concentrated in MG of the MB calyces due to its accumulation in KC dendritic spines (Frambach et al., 2004; Groh et al., 2004, 2006; Groh and Rössler, 2008, 2010; Leiss et al., 2009). Consequently, MG could be sites of structural synaptic plasticity and may play important roles in long-term memory formation. In fact, electron microscopic studies in *Cataglyphis* ants showed that the transition from indoor-workers to outdoor foragers involves changes in synaptic complexes in the MBs calyx (Seid and Wehner, 2009). Also, for honeybees it has been shown that the MB microglomerular numbers are affected by the formation of stable olfactory long-term memory (Hourcade et al., 2010). At earlier life stages, i.e., during larval-adult metamorphosis, structural synaptic plasticity in the MB calyx of honeybees was shown to depend on larval feeding and pupal thermoregulation (Groh et al., 2004, 2006), and subsequent adult behavior was affected by these factors (Jones et al., 2005; Becher et al., 2009). In summary, the MB calyx synapses express long-term structural and functional plasticity associated with developmental and adult behavioral plasticity and are consequently a perfect candidate for the study of neuronal plastic changes and adaptations.

THESIS OUTLINE

AIMS AND QUESTIONS

The fact that *Cataglyphis* ants undergo a behavioral transition that is associated with changing sensory environments and demands makes these ants particularly interesting for studying the underlying neuronal mechanisms. The impressive navigational performance during their foraging trips is based on the combination of different, visually as well as olfactory, stimuli and includes learning and memory tasks. Thus, the present thesis aims to study structural synaptic plasticity in visual and olfactory brain centers by addressing the following main questions:

- Does the behavioral transition from interior workers to foragers and the changing visual environment affect the synaptic organization of visual and olfactory brain centers in *C. fortis*? (see 1, 3)
- If yes, are these plastic changes age-related or rather triggered by sensory experience? (see 1)
- Are plastic neuronal changes reversible? (see 2)
- Does visual experience affect the ants' behavior? (see 2)
- How is the information of polarized light projected in the central brain of *Cataglyphis fortis*? (see 3)
- Are olfactory centers specifically adapted in *C. fortis* and are species-specific differences evident in comparison with closely related species? (see 4)

Correspondingly, the thesis focuses in detail on following aspects:

1) The present thesis aims at dissecting age- and experience-related changes in the synaptic organization in the MB calyces of *Cataglyphis fortis*. The sensory environment and behavioral repertoire of these ants change dramatically with the transition from the indoor to the outdoor stage, and visual input becomes extremely important for long-distance foraging. To investigate structural synaptic plasticity associated with the dramatic changes in behavior, pre- and postsynaptic profiles of synaptic complexes in the visual and olfactory input regions of the MB calyx at various stages of interior workers and foragers were immunolabeled and quantified by using confocal microscopy analyses.

This study focused on the following questions. What is the cellular basis of the volume changes found so far in the MB calyces of *Cataglyphis* ants during the transition from interior workers to light-exposed foragers (Kühn-Bühlmann and Wehner, 2006)? Are both visual and olfactory input regions affected in a similar way? Are changes in synaptic organization mainly triggered by an internal age-related program or by external stimuli, in particular by light exposure?

The corresponding results are summarized in Manuscript I (see below).

2) Manuscript I revealed that the natural transition of interior workers to foragers is associated with remarkable structural synaptic plasticity in visual and olfactory input regions within the MBs and that the reorganization of synaptic complexes is triggered by sensory exposure rather than an internal program. This section aims at unraveling underlying neuronal mechanisms of the high and rapid flexibility in the ants' behaviors. Video analyses at the natural nest site and activity recordings after artificial light treatments were used to investigate whether the ants expose themselves to light before onset of foraging and whether this changes the ants' behavior by raising the ants' locomotor activity and inducing premature foraging behavior. To prove whether the synaptic reorganization occurs in a similar time window, pre- and postsynaptic compartments of visual and olfactory microglomeruli were immunolabeled and quantified after different periods of light-exposure. Ants returned back to dark nest conditions were used to investigate whether synaptic reorganization is reversible.

   The corresponding results are summarized in Manuscript II (see below).

3) As *Cataglyphis* ants use a path integration system combining a polarization compass and a proprioceptive odometer, a focus of this thesis is the identification of information processing stages in the insect brain. Polarization-sensitive neurons in the *Cataglyphis* brain have been identified physiologically but not morphologically only in the medulla (Labhart, 2000). Thus, central neural pathways in the *Cataglyphis* brain are not yet resolved. This section of the thesis aims at identifying the polarization-pathway through tracer injections in consecutive relay stations beginning in the periphery. Relay stations for tracer injections were compared to the polarization processing stages already identified for the locust brain (for a review, see Homberg et al., 2011), in order to identify possible similarities between, or deviations from the locust and desert ant central polarization pathways. Furthermore, since the perception and processing of polarized light form the basis of compass navigation as the main feature during long-distance foraging trips and comes into play as soon as an ant

switches from interior to outdoor tasks, it is presumed that visual centers involved in processing of polarization information exhibit a high synaptic plasticity. Of special interest are large synaptic complexes ("giant synapses") in the lateral accessory lobe that have been shown in the locust brain to transfer polarization-sensitive information (Träger et al. 2008). To detect potential structural synaptic plasticity that might be associated with the dramatic changes in behavior, pre- and postsynaptic profiles of the large synaptic complexes in the lateral accessory lobe of naïve callows and experienced foragers were immunolabeled and quantified by using confocal imaging and 3D reconstruction.

The corresponding results are summarized in Manuscript III (see below).

4) Although *Cataglyphis* species are predominantly visually guided navigators that lack a trail pheromone system, behavioral studies have shown that the ants use olfactory cues during various phases of its foraging journeys (Wolf and Wehner, 2000, 2005; Steck et al., 2009, 2010). This part of the thesis focuses on potential adaptations in the primary olfactory centers of *Cataglyphis*. In particular, using confocal imaging and 3D reconstruction, the glomerular organization of the AL of five species of *Cataglyphis* is compared by focusing on the number, size and spatial arrangement of the olfactory glomeruli. The question is addressed whether differences in microhabitats and resulting variances in foraging strategies (e.g., lengths of outbound runs) have led to adaptations in the olfactory system of different *Cataglyphis* species inhabiting different desert environments (steppe-like, sandy, or salt-pan areas). Of special interest is *C. fortis* that is endemic to the salt flats of western North Africa, which are completely avoided by other *Cataglyphis* species (Wehner, 1983). The utilization of such a specialized habitat results in outstanding traits like extremely large foraging ranges (Wehner, 1983) and high nest-site stabilities (Dillier and Wehner, 2004) compared to other *Cataglyphis* species. Furthermore, the number and size of glomeruli in different *C. fortis* castes (workers, queens and males) are compared.

   The corresponding results are summarized in Manuscript IV (see below).

OVERVIEW OF MANUSCRIPTS

Manuscript I

Visual Experience and Age Affect Synaptic Organization in the Mushroom Bodies of the Desert Ant *Cataglyphis fortis*.

Sara Mae Stieb, Thomas Muenz, Rüdiger Wehner, Wolfgang Rössler

Developmental Neurobiology (2010) 70: 408-423

Abstract

Desert ants of the genus *Cataglyphis* undergo an age-related polyethism from interior workers involved in brood care and food processing to short-lived outdoor foragers with remarkable visual navigation capabilities. The quick transition from dark to light suggests that visual centers in the ant's brain express a high degree of plasticity. To investigate structural synaptic plasticity in the mushroom bodies (MBs) - sensory integration centers supposed to be involved in learning and memory - we immunolabeled and quantified pre- and postsynaptic profiles of synaptic complexes (microglomeruli, MG) in the visual (collar) and olfactory (lip) input regions of the MB calyx. The results show that a volume increase of the MB calyx during behavioral transition is associated with a decrease in MG numbers in the collar and, less pronounced, in the lip. Analysis of tubulin-positive profiles indicates that presynaptic pruning of projection neurons and dendritic expansion in intrinsic Kenyon cells are involved. Light-exposure of dark-reared ants of different age classes revealed similar effects. The results indicate that this structural synaptic plasticity in the MB calyx is primarily driven by visual experience rather than by an internal program. This is supported by the fact that dark-reared ants age-matched to foragers had MG numbers comparable to those of interior workers. Ants aged artificially for up to 1 year expressed a similar plasticity. These results sug gest that the high degree of neuronal plasticity in visual input regions of the MB calyx may be an important factor related to behavior transitions associated with division of labor.

Manuscript II

Visual Experience Affects Both Behavioral and Neuronal Aspects in the Individual Life History of the Desert Ant *Cataglyphis fortis*.

Sara Mae Stieb, Anna Hellwig, Rüdiger Wehner, Wolfgang Rössler

Developmental Neurobiology (accepted)

Abstract

The individual life-history of the desert ant *Cataglyphis fortis* is characterized by a fast transition from interior tasks to mainly visually guided foraging. Previous studies revealed a remarkable structural synaptic plasticity in visual and olfactory input regions within the mushroom bodies of the ants' brains - centers involved in learning and memory. Reorganization of synaptic complexes (microglomeruli) was shown to be triggered by sensory exposure rather than an internal program. Using video analyses at the natural nest site and activity recordings after artificial light treatments we investigated whether the ants get exposed to light before onset of foraging and whether this changes the ants' activity levels. We asked whether synaptic reorganization occurs in a similar time window by immunolabeling and quantification of pre- and postsynaptic compartments of visual and olfactory microglomeruli after periods of light-exposure. Ants reverted back to dark nest conditions were used to investigate whether synaptic reorganization is reversible. The behavior analyses revealed that late-interior ants (*diggers)* are exposed to light and perform exploration runs up to two days before they start foraging. This corresponds well with the result that artificial light treatment over more than two to three days significantly increased the ants' locomotor activities. At the neuronal level, visual exposure of more than one day was necessary to trigger reorganization of microglomeruli, and light induced changes were only partly reversible in the dark. We conclude that visual pre-exposure is an important and flexible means to prepare the ants' visual pathway for orientation capabilities essential during foraging.

Manuscript III

Plasticity in Giant Synaptic Complexes of the Lateral Accessory Lobe - a Possible Relay Station in the Polarization Pathway in the Brain of the Desert Ant *Cataglyphis fortis*.

Sara Mae Stieb, Franziska Schmitt, Rüdiger Wehner, Wolfgang Rössler

Abstract

Desert ants of the genus *Cataglyphis* undergo an age-related polyethism from interior workers to short-lived foragers with remarkable visual navigation capabilities. As their main navigational means, solitary foragers use a path integration system including a skylight-based visual compass and a stride-integrating odometer. While this enormous navigational behavior is well described, the central neural pathways in the *Cataglyphis* brain are largely unknown. The quick transition from dark to light suggests that visual centers in the ant's brain express a high degree of plasticity.

To investigate the structure of large synaptic complexes in the lateral accessory lobe – a possible relay station in the polarization pathway – and detect potential structural synaptic plasticity that might be associated with the dramatic changes in behavior, pre- and postsynaptic profiles of these synaptic complexes in naïve callows and experienced foragers were immunolabeled and quantified by using confocal imaging and 3D-reconstruction. Tracer injections were used to identify input and output tracts of the lateral accessory lobe. The results show that these complexes consist of postsynaptic processes located in a central region that is surrounded by a cup-like presynaptic profile. The large presynaptic terminals are formed by neurons of the tubercle-accessory lobe tract that have dendritic ramifications in the anterior optic tubercle. Tangential neurons forming the dendritic complexes in the lateral accessory lobe project to the ellipsoid body of the central complex and are GABAergic. The behavioral transition from interior workers to foraging is associated with an increase in the number of these synaptic complexes by ~13%. An outgrowth of additional synaptic complexes at the transition to foraging suggests some sort of calibration process in these potentially strong synaptic contacts which are likely to deliver fast and reliable signal transmission in the polarization vision pathway.

Manuscript IV

Antennal-Lobe Organization in Desert Ants of the Genus *Cataglyphis*.

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Abstract

Desert ants of the genus *Cataglyphis* possess remarkable visual navigation capabilities. Although *Cataglyphis* species lack a trail pheromone system, *Cataglyphis fortis* employs olfactory cues for detecting nest and food sites. To investigate potential adaptations in primary olfactory centers of the brain of *C. fortis*, we analyzed olfactory glomeruli (odor processing units) in their antennal lobes and compared them to glomeruli in different *Cataglyphis* species. Using confocal imaging and 3D reconstruction, we analyzed the number, size and spatial arrangement of olfactory glomeruli in *C. fortis*, *C. albicans*, *C. bicolor*, *C. rubra*, and *C. noda*. Workers of all *Cataglyphis* species have smaller numbers of glomeruli (198– 249) compared to those previously found in olfactory-guided ants. Analyses in 2 species of *Formica* – a genus closely related to *Cataglyphis* – revealed substantially higher numbers of olfactory glomeruli (c. 370), which is likely to reflect the importance of olfaction in these wood ant species. Comparisons between *Cataglyphis* species revealed 2 special features in *C. fortis*. First, with c. 198 *C. fortis* has the lowest number of glomeruli compared to all other species. Second, a conspicuously enlarged glomerulus is located close to the antennal nerve entrance. Males of *C. fortis* possess a significantly smaller number of glomeruli (c. 150) compared to female workers and queens. A prominent male-specific macroglomerulus likely to be involved in sex pheromone communication occupies a position different from that of the enlarged glomerulus in females. The behavioral significance of the enlarged glomerulus in female workers remains elusive. The fact that *C. fortis* inhabits microhabitats (salt pans) that are avoided by all other *Cataglyphis* species suggests that extreme ecological conditions may not only have resulted in adaptations of visual capabilities, but also in specializations of the olfactory system.

MANUSCRIPT I

Visual Experience and Age Affect Synaptic Organization in the Mushroom Bodies of the Desert Ant *Cataglyphis fortis*

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ABSTRACT: **Desert ants of the genus *Cataglyphis* undergo an age-related polyethism from interior workers involved in brood care and food processing to short-lived outdoor foragers with remarkable visual navigation capabilities. The quick transition from dark to light suggests that visual centers in the ant's brain express a high degree of plasticity. To investigate structural synaptic plasticity in the mushroom bodies (MBs)—sensory integration centers supposed to be involved in learning and memory—we immunolabeled and quantified pre- and postsynaptic profiles of synaptic complexes (microglomeruli, MG) in the visual (collar) and olfactory (lip) input regions of the MB calyx. The results show that a volume increase of the MB calyx during behavioral transition is associated with a decrease in MG numbers in the collar and, less pronounced, in the lip. Analysis of tubulin-positive profiles indicates that presynaptic pruning of projection neurons and dendritic expansion in intrinsic Kenyon cells are involved. Light-exposure of dark-reared ants of different age classes revealed similar effects. The results indicate that this structural synaptic plasticity in the MB calyx is primarily driven by visual experience rather than by an internal program. This is supported by the fact that dark-reared ants age-matched to foragers had MG numbers comparable to those of interior workers. Ants aged artificially for up to 1 year expressed a similar plasticity. These results suggest that the high degree of neuronal plasticity in visual input regions of the MB calyx may be an important factor related to behavior transitions associated with division of labor.** © 2010 Wiley Periodicals, Inc. Develop Neurobiol 70: 408–423, 2010

***Keywords: Cataglyphis*; mushroom bodies; synaptic plasticity; behavioral maturation; microglomeruli**

INTRODUCTION

In many social-insect species behavioral repertoires can change dramatically over an individual's lifespan (Hölldobler and Wilson, 1990). Ant workers, for example, undergo an age-related polyethism which plays an important role in task allocation and division of labor. Young workers mainly care for brood inside the nest, while older workers forage for food outside the nest. Previous neuroanatomical studies have shown that adult behavioral maturation is correlated

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with volume changes in particular brain regions, most prominently the mushroom bodies (MBs), which are higher association centers in the insect brain (e.g., Gronenberg et al., 1996; for *Cataglyphis* see Kühn-Bühlmann and Wehner, 2006). Ants, therefore, provide particularly promising model systems to study the neuronal mechanisms underlying their remarkable behavioral plasticity.

In the North African desert ant *Cataglyphis bicolor* an age-related polyethism divides the colony into interior workers up to an average age of 28 days followed by an outdoor foraging period of about 6 days (mean forager life expectancy 6.1 days: Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984). During this foraging phase, *Cataglyphis* ants are predominantly visually guided navigators (Wehner, 2003, 2009). In addition, olfactory cues are used to pinpoint the nest entrance (Steck et al., 2009) and food sources (Wolf and Wehner, 2005). The fact that in *Cataglyphis* ants the light exposed period of life covers only a small fraction of the entire life cycle makes these ants particularly interesting for studying synaptic plasticity in visual brain centers.

The MBs are supposed to function as higher sensory integration and association centers involved in the organization of complex behaviors, especially in learning and memory (Strausfeld et al., 1998; Menzel, 1999; Gerber et al., 2004; Davis, 2005; Giurfa, 2007). In Hymenoptera, particularly in ants (Gronenberg et al., 1996; Seid et al., 2005), bees (Groh et al., 2004), and social wasps (Ehmer and Hoy, 2000), they are very prominent structures in relation to other brain regions. In bees and ants, the MB calyces comprise three sensory input regions: the olfactory innervated lip, the visually innervated collar, and the basal ring receiving input from both sensory modalities (Mobbs, 1982; Gronenberg, 2001; Farris and Sinakevitch, 2003). In bees, recent studies showed that the collar receives chromatic, temporal, and motion sensitive input from the optic lobes indicating its potential importance for higher visual processing tasks related to visually-guided foraging (Paulk and Gronenberg, 2008).

The neuropils of the MB calyces comprise distinct synaptic complexes, so called microglomeruli (MG). Each MG comprises a central presynaptic bouton of the projection neurons—originating from either the antennal lobes (AL) or the optic lobes (OL)—and a surrounding shell of numerous postsynaptic profiles, mostly from Kenyon-cell (KC) dendritic spines (Steiger, 1967; Ganeshina and Menzel, 2001; Gronenberg, 2001; Yasuyama et al., 2002; Frambach et al., 2004; Groh et al., 2004, 2006; Seid and Wehner, 2008; Leiss et al., 2009). Previous studies have shown that both age and sensory experience can lead to volumetric changes in the MBs. Volume changes in the MB calyces associated with the transition from interior workers to foragers were reported for bees (Withers et al., 1993; Durst et al., 1994; Farris et al., 2001), wasps (O'Donnell et al., 2004), and ants (Gronenberg et al., 1996; Kühn-Bühlmann and Wehner, 2006). For example, age-matched honeybees with foraging experiences had larger MB neuropils than the bees without any outdoor-activities (Durst et al., 1994; Farris et al., 2001; Ismail at al., 2006). The same holds true for *Cataglyphis* ants (Kühn-Bühlmann and Wehner, 2006). The volume changes in the MB-calyx may be generated by structural plasticity of neuronal axons, dendrites, and synapses. For example, foraging activity in the honeybee is associated with dendritic outgrowth in MB intrinsic KCs (Farris et al., 2001), and electron microscopic studies in *Cataglyphis* ants showed that the transition from indoor-workers to outdoor foragers involves changes in synaptic complexes in the MB calyx (Seid and Wehner, 2009). At earlier life stages, i.e., during larval-adult metamorphosis, structural synaptic plasticity in the MB-calyx of honeybees was shown to depend on larval feeding and pupal thermoregulation (Groh et al., 2004, 2006), and subsequent adult behavior was affected by these factors (Jones et al., 2005; Becher et al., 2009).

In the present study we aimed at dissecting age- and experience-related changes in the synaptic organization in the MB calyces of *Cataglyphis fortis*, a predominantly visually guided desert ant (Wehner, 1983). The sensory environment and behavioral repertoire of these ants change dramatically with the transition from the indoor to the outdoor stage, and visual input becomes extremely important for long-distance foraging. To detect and study the potential structural synaptic plasticity that might be associated with the dramatic changes in behavior, we selectively labeled pre- and postsynaptic profiles of synaptic complexes in the MB calyces at various stages of interior workers and foragers. Changes were quantified using confocal-microscopy analysis. In particular, we addressed the following questions:

1. What is the cellular basis of the volume changes found so far in the MB calyces of *Cataglyphis* ants during the transition from interior workers to light-exposed foragers (Kühn-Bühlmann and Wehner, 2006)?

2. Are both visual and olfactory input regions affected in a similar way?
3. Are changes in synaptic organization mainly triggered by an internal age-related program or by external stimuli, in particular by light exposure?

METHODS

Animals

A cohort experiment was performed with a queenright colony of *Cataglyphis fortis* in a salt-pan near Menzel Chaker, Tunisia, in July 2006 (34°58′N, 10°25′E). The complete colony was excavated in the dark under red light illumination to prevent the ants from visible light exposure. Different stages of worker ants were classified into the following groups: foragers were defined as workers collected while actively searching for food outside the nest for at least one full day. A previous study in the closely related species *Cataglyphis bicolor* has shown that workers do not forage before they reach the age of about 28 days (Wehner et al., 1972). All foragers were marked several days prior to the excavation of the colony. Callows were defined as workers that had emerged very recently (within about the last 24 h). They could be identified easily by their still pale cuticle. During the excavation process interior I workers were characterized by their swollen gasters and expanded whitish intersegmental membranes, and the ants sticked motionless to the walls of the nest chambers (Schmid-Hempel and Schmid-Hempel, 1984). The remaining unmarked and hence not yet foraging workers inside the nest belong to a later stage of this life cycle and thus were termed interior II workers.

The different groups of ants were transferred immediately to the Biozentrum of the University of Würzburg. This procedure added another day to the determined age. An adequate number of ants per group was dissected immediately upon arrival and further processed for immunocytochemistry. For experiments involving precocious light exposures (see below) and for having access to 1-day-old callows, freshly emerged *Cataglyphis fortis* ants were collected in 2009 at night from a different colony. Brains of these 2009 ants were dissected immediately and put into fixative solution (see Methods below) before they were transferred to the Würzburg laboratory for further histochemical procedures. The immediate onsite dissection guaranteed that the callows were not older than 24 h. Light exposure experiments and subsequent immunohistochemical treatments of dark-reared ants at different ages were performed in the field with 2009 callows and in the laboratory with 6-month- and 12-month-old dark-reared workers collected at the same location in Tunisia in July 2007. Finally, ants from a fourth colony collected in July 2008 were kept in complete darkness so that ants that had emerged under these dark conditions (and were kept there all the time), could later be compared as a dark-reared control group (termed dark foragers in the following) with age-matched foragers.

Light-Treatment

Light-treatment experiments were either performed in the field near the salt-pan at Menzel Chaker under natural sun light or in the laboratory under artificial light. Freshly emerged callows were separated from the colony and divided into two groups: a control group (10–20 ants) and a light-exposed group (10–20 ants). Both groups of ants were kept in $19 \times 9 \times 6$ cm^3 plastic boxes with the floor covered with gypsum. After 36 h of acclimatization, the light-treatment started. Whereas the control group remained in the dark, the experimental group was exposed to the sun five times a day for 45 min at 9:00, 11:00, 13:00, 15:00, and 17:00 for 4 days in a row simulating an average light exposure during typical foraging trips on four subsequent days (Wehner, 1987). The mean temperature in the experimental boxes during light exposure and between light exposures remained constant at 28.3 ± 2C°. Light exposures took place in a shaded area with mean light intensities of 7.4 W m^{-2} for UVA (280–315 nm), 0.2 W·m^{-2} for UVB (315–400 nm), and 170.9 W m^{-2} for PAR (photosynthetic active radiation 400–700 nm) inside the box (measured with an optometer (Gigahertz-Optik Model X1-2)). The relative humidity remained constant over time at a mean of 56.0% ± 7%. The ants were fed at night with dead cockroaches. Water supply was provided during the entire experiment. Afterward the brains of the control and the light exposed ants were dissected and further processed for immunocytochemistry (see later).

Light-treatment experiments with 6-month- and 12-month-old dark-reared ants were performed in the laboratory using an artificial light source. The experimental procedure (experimental chambers and light exposure time) remained unchanged. The experimental groups were exposed to a mercury arc lamp (125W, Exo-Terra Solar Glo) emitting UV light (with high amounts of UVA and UVB), light in the visible range, and infrared radiation at a distance of 50 cm. The light intensity was 4.3 W m^{-2} for UVA (280–315 nm), 0.05 W m^{-2} for UVB (315–400 nm), and 69.0 W m^{-2} for PAR inside the box. During these light exposures the temperature increased from 25°C up to 32°C, whereas the relative humidity remained constant at 60–70%. Between the light exposure periods the boxes were placed into the dark at a temperature of 25°C.

Immunocytochemistry

To analyze structural plasticity in synaptic complexes in the olfactory lip and visual collar region of the MB calyx, pre- and postsynaptic profiles of individual MG were visualized by double-labeling with an antibody to synapsin and fluorescently-labeled phalloidin binding to f-actin using the method introduced by Groh et al. (2004). In the insect MBs, f-actin is highly concentrated in MG of the MB calyces due to its accumulation in KC dendritic spines, whereas

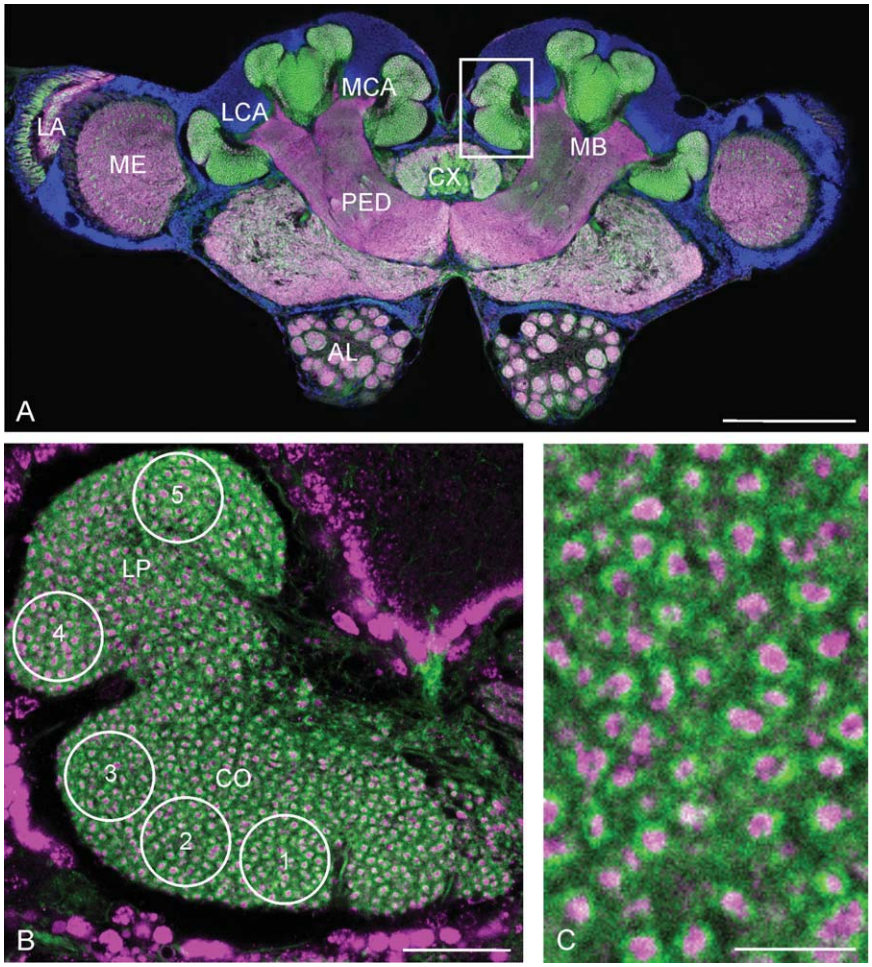

Figure 1 Immunofluorescence stainings of the *Cataglyphis fortis* brain. **A**. Frontal overview of a brain (100-μm section) labeled with anti-synapsin (magenta), phalloidin-labeled f-actin (green), and Hoechst (blue) to highlight cell nuclei. Mushroom body (MB) with medial calyces (MCA), lateral calyces (LCA), and pedunculus (PED); optic lobes with lamina (LA), medulla (ME), and lobula (LO, not displayed in this section); antennal lobes (AL); central complex (CX). For the quantification of structural changes in MBs, the medial branch of the MCA was used (squared box). **B**. Magnification of the medial branch of the MCA with the olfactory innervated lip (LP) and the visually innervated collar (CO). Areas 1–3 represent the region used for quantification of the microglomeruli (MG) number in the CO and Areas 4 and 5 for quantification in the LP. **C**. Magnification of the CO with distinct visible MG. Scale bars: A 200 μm, B 20 μm, C 5 μm.

synapsin is associated with synaptic vesicles aggregated in presynaptic boutons of projection neurons [Figs. 1(C) and 2; see also (Groh et al., 2004)].

The ants were anesthetized with CO_2, decapitated, and the head capsules were fixed in dental-wax-coated dishes. The head capsule was covered with fresh ant-saline solution (127 m*M* NaCl, 7 m*M* KCl, 1.5 m*M* $CaCl_2$, 0.8 m*M* Na_2HPO_4, 0.4 m*M* KH_2PO_4, 4.8 m*M* TES, 3.2 m*M* Trehalose, pH 7.0) and opened by cutting a square window in between the compound eyes. Glands and tracheae were gently removed, and the brains were dissected out and fixed immediately in cold 4% formaldehyde in phosphate-buffered saline (PBS, pH 7.2) overnight at 4°C. The brains were then rinsed in PBS (3×, 10 min). After embedding in 5% low melting point agarose (Agarose II, no. 210-815, Amresco, Solon, OH), brains were carefully adjusted in a frontal plane and sectioned at 100 μm thickness with a vibrating microtome (Leica VT 1000S, Nussloch, Germany). Free-floating agarose sections were preincubated in PBS containing 0.2% Triton X-100 (PBST) and 2% normal goat serum (NGS, 005-000-121, Jackson ImmunoResearch Laboratories) for 1 h at room temperature.

To label neuronal f-actin, sections were incubated in 0.2 U of Alexa Fluor 488 phalloidin (Molecular Probes, A12379) in 500 μl PBST with 2% NGS for 3 days at 4°C (Rössler et al., 2002; Groh et al., 2004). For double-labeling, sections were simultaneously incubated with either a monoclonal antibody to the *Drosophila* synaptic-vesicle-

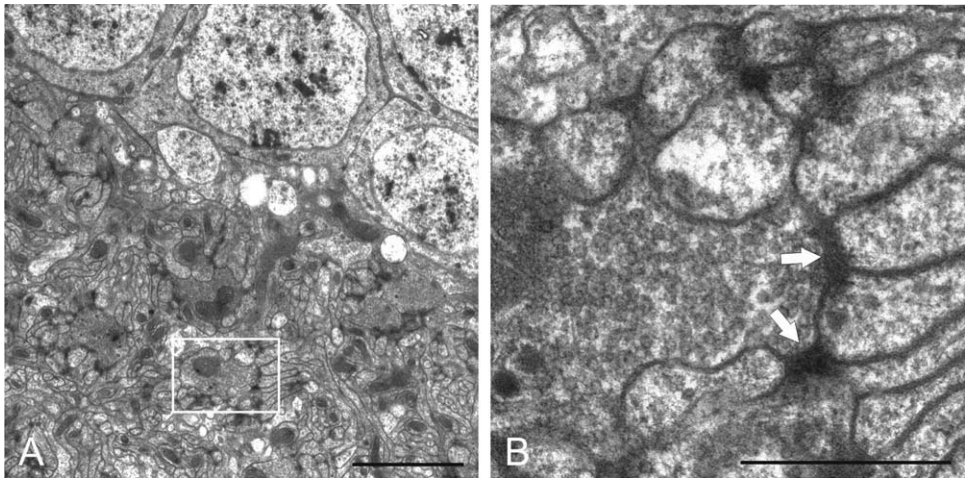

Figure 2 Electron microscopic images of the mushroom body calyx. **A**. Ultrastructural image of the lip region neighbored by Kenyon Cell somata. White frame indicates one individual microglomerulus (MG). **B**. Detail of one MG. Multiple dendrites get in contact with one vesicle packed central presynaptic bouton at the active zones (indicated by arrows). Scale bars: A 2 μm, B 0.5 μm.

associated protein synapsin I (1:50, SYNORF1, kindly provided by E. Buchner, University of Würzburg, Germany) (Klagges et al., 1996) or with mouse anti α-tubulin antibody (1:500, Calbiochem, CP06). After five rinses in PBS, double-labeled preparations were incubated in Alexa Fluor 568-conjugated goat anti-mouse secondary antibody (1:250, Molecular Probes, A11004) in 1% normal goat serum/PBS for 2 h at room temperature to visualize synapsin or tubulin.

To label cell nuclei, sections were incubated for 15 min in 2.5 mg ml^{-1} Hoechst 34580 (1:500; Molecular Probes, H21468) in PBS with 0.2% Triton X-100 at room temperature. Sections were finally washed in at least five changes of PBS, transferred into 60% glycerol/PBS for 30 min, and mounted on slides in 80% glycerol/PBS.

Laser Scanning Confocal Microscopy, Image Processing, and Data Analysis

Preparations were viewed with a laser-scanning confocal microscope (Leica TCS SP2 AOBS; Leica Microsystems, Wetzlar, Germany) equipped with argon/krypton and UV lasers. Excitation wavelengths were 568 nm for synapsin, 488 nm for Alexa Fluor, and 488 and 405 nm for Hoechst 34580. Two different HC PL APO objective lenses were used for image acquisition (20 $\times$ 0.7 NA imm and 63 $\times$ 1.20 NA imm), and in certain cases in combination with a 2–2.5$\times$ digital zoom. In double- or triple-labeled preparations, the different channels were merged with the use of pseudocolors. Images were further processed in ImageJ 1.39u (Wayne Rasband, National Institute of Health, USA) and Corel Draw X3 software (Corel Corporation, Ottawa, ON, Canada).

Quantification of synaptic complexes in the MB calyx was performed at a defined region in the central brain at a plane where the MB calyces and other landmarks such as the lower and upper division of the central complex and the pedunculi and medial lobes of the MBs were clearly identifiable [Fig. 1(A)]. MG profiles were quantified in the olfactory lip and the visual collar of the MB calyx using a modified protocol of the method introduced by Groh et al. (2004) [Fig. 1(B)]. Individual MG were visualized at high magnification using a 63$\times$ objective and a 2.5$\times$ digital zoom. MG profiles were quantified in the inner branch of the medial calyx in both hemispheres (see Results for details). MG numbers were counted and averaged for three circular areas (200 μm^2 per area) in the dense portion of the collar and in some cases extrapolated to the total dense area within which the MG are uniformly distributed (see Fig. 3). The MG numbers in the two calyces of each brain were averaged, and a mean was calculated based on the number of brains analyzed. In the lip region, two circular areas (200 μm^2 per area) were analyzed, and the number of MG in the two calyces of each brain were averaged separately for each area in each individual, and a mean was calculated based on the number of investigated brains. MG were counted blindly, and the criterion used was that a MG contained a magenta-labeled synapsin positive bouton encircled by a green-labeled f-actin-phalloidin halo. Comparison of the size of a microglomerular bouton with electron microscopy data confirmed a range of about 3 μm in diameter (Seid and Wehner, 2008) and assured that MG that fulfilled these criteria were exactly centered in an optical section with the distance between MG profiles closely resembling the density (Figs. 1 and 2).

All statistical analyses were performed with SPSS 15.0 software (SPSS, Chicago, IL). After testing for normal distribution (One-Sample Kolmogorov-Smirnov Procedure, $p < 0.05$) one-way ANOVA was used to compare mean numbers of MG between the different age groups (factorial ANOVA, $p < 0.05$) with subsequent post hoc tests (Tukey's honestly significant difference (HSD)). Independent samples *t*-test were performed to test differences between light-treated and dark-kept ants (*t*-test for Equality of

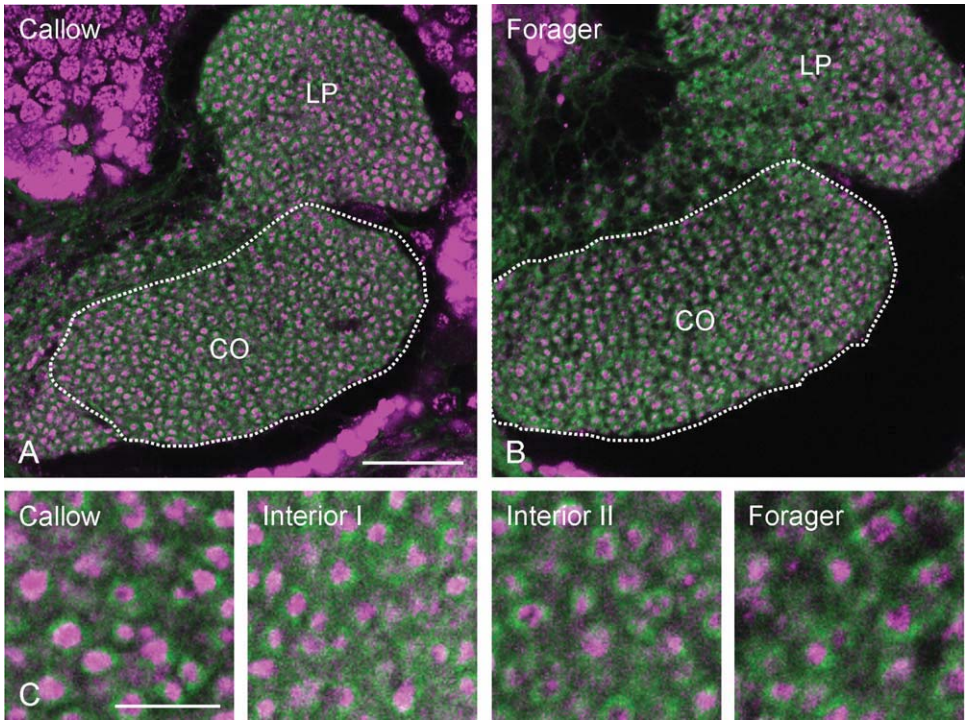

Figure 3 Task-dependent plasticity in the mushroom body (MB) calyx. **A, B**. Medial calyx labeled with anti-synapsin (magenta) and phalloidin-labeled f-actin (green). The MB calyx, both the lip (LP) and collar (CO), increase in size during the transition from callows (A) to foragers (B). Synapsin is localized in the presynaptic endings within the MB neuropil in all age groups whereas it is also found in the Kenyon Cell somata of very young ants (A). Dotted line outlines the dense area of the CO used for the microglomeruli (MG) number extrapolations. **C**. Detailed magnification of the dense collar region. The MB calyx size increase is accompanied by a decrease in the numbers of MG per area, and thus the MG-density (C) in the CO during the transition from interior workers (callows, interior I+II) to foragers. Scale bars: A, B 20 μm, C 5 μm.

Means, $p < 0.05$). Preliminary tests contained independent and paired samples t-tests ($p < 0.05$) as well as GLM (General Line Model) Univariate Procedure ($p < 0.05$).

Electron Microscopy Preparation

Brains were dissected as described above and immediately transferred to cold 2.5% formaldehyde, 2.5% glutaraldehyde buffered with 0.1 *M* cacodylate (pH 7.2–7.4), and 0.04% CaCl overnight at 4°C. The brains were then washed in 0.1 *M* cacodylate and 0.04% $CaCl_2$ for 15 min and post-fixed in 2% aqueous OsO_4 (buffered with cacodylate buffer) for 2 h at room temperature under the fume hood. After fixation the brains were washed in 50% ethanol for a few seconds followed by dehydration in an ascending series of ethanol (50%, 70%, 90%, 95%, and 2× 100%) and two times in propylene oxide for 10 min each step. Brains were incubated in a mixture of Epon with propylene oxide (1:1) over night at room temperature under the fume hood. Next day the brains were put in fresh Epon for 4 h at room temperature and then placed in beem capsules filled with fresh Epon and placed in oven (60°C) for 48 h for polymerization (modified after Takemura et al., 2008).

The embedded brains were taken out of the beem capsules and trimmed to the region of interest (the MBs and the central complex). Semithin slices (0.5–1 μm) were then cut using a microtome (Reichert Jung 2050) with a glass knife, mounted on polylysine laminated glass slides, stained with methylene blue azure II to achieve proper orientation under light microscopy for ultrathin sectioning, and finally washed three times with distilled water. Semithin sections were cut up to a defined plane in the central brain in which the MB calyces and several landmarks were clearly identifiable (see above). After the region was found, ultrathin sections (50 nm) were cut into series using a diamond knife (Diatome, USA) on an ultramicrotome (RMC MT-7000, Boeckeler Instruments, Tuscon, USA) and put on one hole electron microscope grids (Provac GmbH, Oestrich-Winkel, Germany) covered with a thin Pioloform F film (Wacker Chemie, München, Germany). For electron microscopy MG in the lip and collar regions were counterstained with Reynolds lead citrate and visualized using Proscan (Lagerlechfeld, Germany) slow-scan camera attached to the Zeiss (Oberkochen, Germany) EM 10CR with the corresponding software analySIS 3.0 Doku (Soft Imaging System, Münster, Germany).

RESULTS

Age-Related and Task-Dependent Structural Plasticity of the Microglomeruli in the Mushroom-Body Calyx

We investigated changes in the organization of synaptic complexes in the MB calyces during the transition from interior workers (callows, interior I+II) to 1-day-old foragers. While Kühn-Bühlmann and Wehner (2006) described age-dependent and task-related volume increases in all neuropils of the *Cataglyphis* MBs [quite prominent structures in the brain of this genus; Fig. 1(A)], we focused on the cellular basis of these volume changes and on the factors that may underlie these changes in the sensory input regions of the MB calyces.

All following data used for statistical tests were normally distributed (Kolmogorov-Smirnov Procedure, $p < 0.05$). As the inner branches of the medial calyces are most perfectly matched in the sectional plane, we determined the density of the MG in the collar and lip regions of the different age groups by quantification of the MG profiles within five circular areas (three areas in the dense region of the collar; two areas in the lip) in the two inner branches of the medial calyx in both hemispheres. The criteria used for this quantification are described in detail in the Methods section [Fig. 1(A,B)]. To test for potential differences in MG density between the calyces, the inner branches of the medial calyx and the outer branches of the lateral calyx in the left and right hemispheres (collar and lip region) were compared in single individuals. No differences in the MG density in the medial and lateral calyces were found in the collar (GLM Univariate: individual-1: $p = 0.664$; individual-2: $p = 0.262$; individual-3: $p = 492$) and in the lip (GLM Univariate: individual-1: $p = 0.847$; individual-2: $p = 0.665$; individual-3: $p = 125$). A similar observation was previously made in the honeybee (Groh et al., 2004). As in all age groups no significant differences were found between the MG numbers in the three collar areas (GLM Univariate: callow: $p = 0.795$; interior I: $p = 0.956$; interior II: $p = 0.888$; forager: $p = 0.671$), the MG numbers in these three areas of one individual were averaged. Similarly, in nearly all age groups the MG numbers in Areas 4 and 5 (lip region) did not differ significantly (paired *t*-test: callow: $p = 0.825$; interior I: $p < 0.05$; interior II: $p = 0.516$; forager: $p = 0.643$). However, due to the more uneven spatial distribution of MG in between lip Areas 4 and 5, the two subregions were treated separately for subsequent analysis. Furthermore, to exclude any structural differences between the inner branches of the medial calyx in the left and right hemispheres (for both the collar and the lip region), MG numbers in the collar and the lip of both hemispheres were tested per age group. MG numbers in the collar (Areas 1–3 averaged) of the two inner branches of the medial calyces did not differ (paired *t*-test: callow: $p = 0.288$; interior I: $p = 0.652$; interior II: $p = 0.218$; forager: $p = 0.881$), and the same was true for the MG numbers in the lip (Areas 4 and 5 separated) of the two inner branches of the medial calyces [paired *t*-test: callow: $p = 0.821$ (Area 4), 0.247 (Area 5); interior I: $p = 0.638$ (Area 4), 0.233 (Area 5); interior II: $p = 0.528$ (Area 4), 0.799 (Area 5); forager: $p = 0.363$ (Area 4), 0.094 (Area 5)]. Therefore, for subsequent calculations, the two hemispheres were no longer treated separately. As the interior II group comprises ants of moderately different body sizes, we tested the effect of body size. No significant differences in MG numbers were found in the collar and lip regions of ants with head widths larger or smaller than 2 mm [unpaired *t*-test. collar: $p = 0.193$, lip: 0.379 (Area 4), 0.470 (Area 5)]. Finally, to exclude that slight differences in the selection of microscopic focal planes [see Methods section and Fig. 1(A) for the selected plane in the central brain] delivered different results, MG numbers in the brain of one exemplary ant were compared at different focal and sectional planes [single measurements were used; paired *t*-test: different focal planes of one section: collar: $p = 0.184$; lip: $p = 0.205$ (Area 4), 0.070 (Area 5); different sections of one brain: collar: $p = 0.423$; lip: $p = 0.500$ (Area 4), 0.795 (Area 5)]. As a result of these methodological pretests, the MG numbers in the collar region of the three circular areas in the two medial calyces was averaged for each individual ant and were then used to calculate mean values across individuals. In the lip region of the two medial calyces this was done separately for Areas 4 and 5 within and across individuals. In total, data were collected from six callows, six interior I workers, nine interior II workers, and five foragers.

The images in Figure 3 show that MG in the MBs are densely packed in young callows [Fig. 3(A)] as compared with the more loosely distributed MG in the foragers [Fig. 3(B)]. This means that the density of the MG, as well as presumably the number of the MG, decreases with behavioral maturation [Fig. 3(C)]. In detail, in the collar significant differences were found across age-groups [Fig. 4(A); One way ANOVA: $p < 0.01$] especially between callows and foragers [post-hoc-test: $p < 0.05$ (Tukey-HSD)] and callows and interior II workers [post-hoc-test: $p < 0.05$ (Tukey-HSD)]. This holds true for lip Area 4 as well [Fig. 4(B); One way ANOVA: $p < 0.01$;

post-hoc-test: callows-interior II: $p < 0.05$ (Tukey-HSD); interior I-interior II: $p < 0.05$ (Tukey-HSD), but not for lip Area 5 [Fig. 4(C); One way ANOVA: $p = 0.123$], which may be due to the olfactory role of the lip and potentially different olfactory input to different lip subregions (Kirschner et al., 2006; Zube et al., 2008).

To estimate whether the reduction of the MG density in the collar also reflects a decrease in the total number of the MG and not only an expansion of the MG correlated with the volumetric expansion of the MB calyx [Fig. 3(A); Kühn-Bühlmann and Wehner, 2006], MG numbers in the three areas in the collar were extrapolated to the entire area of the collar [region shown in Fig. 3(A,B)]. Indeed, the results indicate a significant reduction of the total MG number in the collar (19.4% reduction in MG number; One way ANOVA: $p < 0.05$). Extrapolations to the entire collar area are in a summary figure at the end.

To exclude that the 1- to 2-day older age of the callows due to the transport of the colony from Tunisia to Würzburg had any effect on the MG number, the data given above were compared with those from freshly emerged callows of another colony. Significant differences were not found in either the collar or the lip [unpaired *t*-test: $n_{(\text{callows} < 24\ \text{h})} = 6$, $n_{(\text{callows} = 2\text{–}3\ \text{days})} = 6$; collar: $p = 0.311$; lip: $p = 0.202$ (Area 4), 0.827 (Area 5)].

Comparison of ants within a natural life span of 1 month with laboratory ants of an unnaturally extended lifespan of 6 months showed that in the latter group the collar area had increased on average by 27% [Fig. 5(A–C)]. This drastic long-term expansion occurred despite the fact that the artificially aged ants were continuously kept in complete darkness and lacked any visual experience. When the MG number was extrapolated to the total area of the dense collar, the size increase of the collar area was accompanied by a significant increase of synaptic complexes [Fig. 5(D); unpaired *t*-test: $p < 0.05$] indicating that new MG had emerged with high age.

In conclusion, during the behavioral development within the natural lifespan of *Cataglyphis* ants, age- and task-related changes occur in the number of MG in the MB calyx, particularly in the visually innervated collar (see Fig. 4). The decrease in MG density,

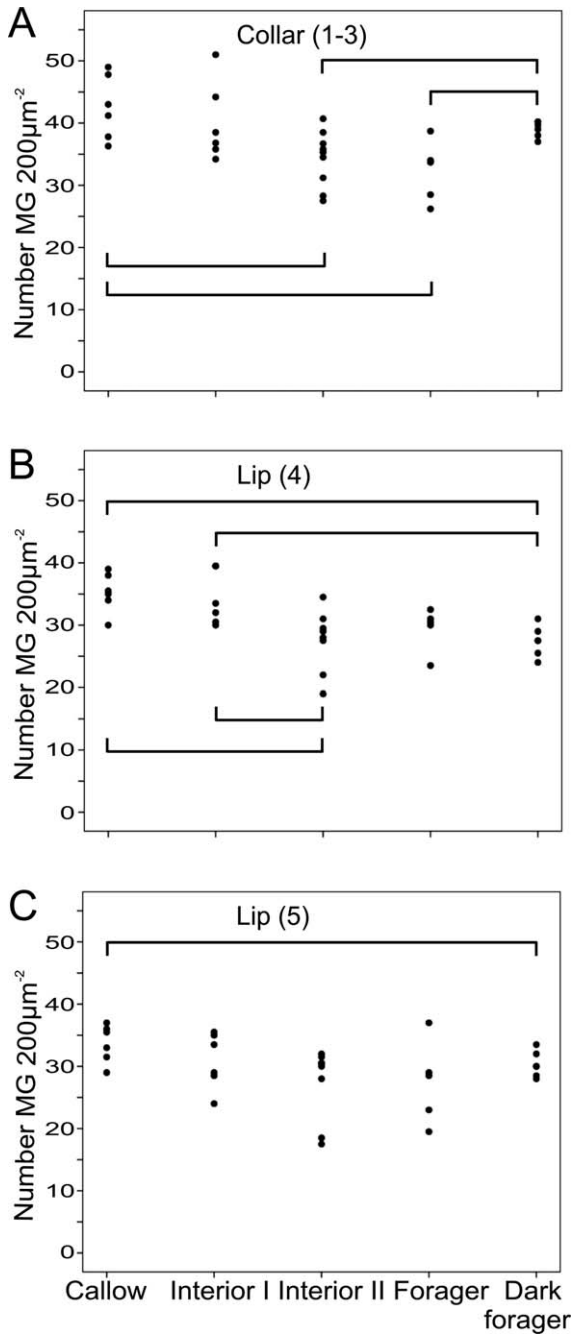

Figure 4 Task-related effect on the microglomeruli (MG) number in the mushroom-body calyx. **A**. Structural plasticity in the collar. In the visually innervated collar (CO) there is a significant reduction in the mean MG number of the three measured 200 μm^2 circular areas (Area 1–3) during the transition from callows to foragers with a significant difference between the age groups callows-interior II workers and callows-foragers. Dark-reared ants (dark foragers) have significantly more MG compared to age-matched foragers but were approximately at the same level as interior I workers. **B, C**. Structural plasticity in the lip (LP). Significant differences in MG numbers are found in the LP in Area 4 (B), but not in Area 5 (C). Dark-foragers showed differences in the MG number compared to callows in both lip areas and compared with interior I workers in Area 4.

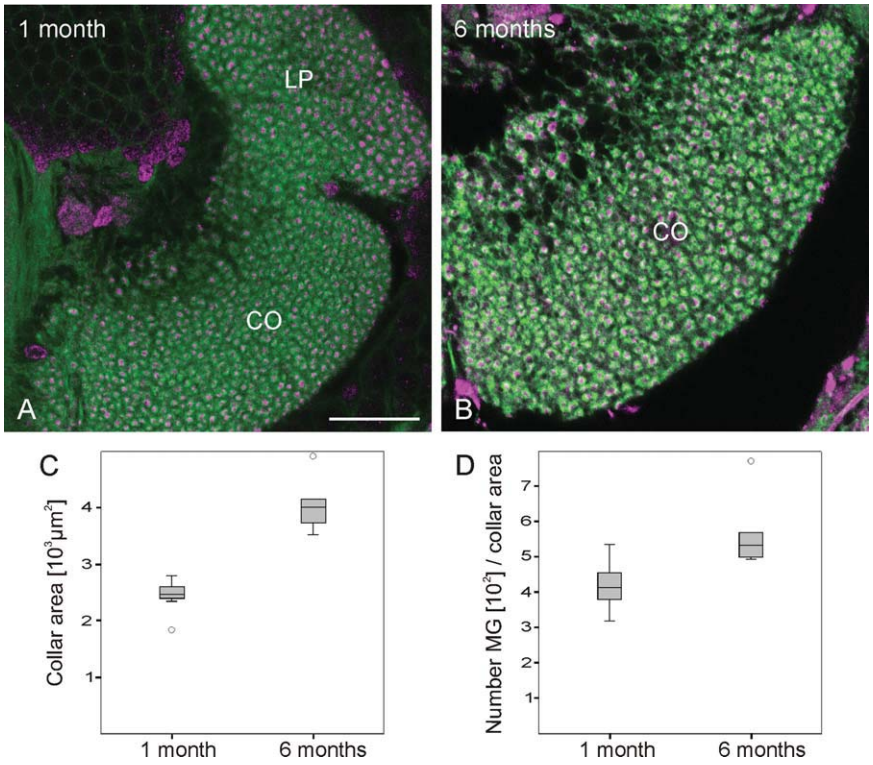

Figure 5 Age-related structural plasticity in the mushroom body (MB) calyx. **A**, **B**. MB calyces stained with anti-synapsin (magenta) and phalloidin-labeled f-actin (green). The comparison of interior workers rose under natural conditions [about 1-month old (A)] and older dark-reared ants [about 6-month old (B)] indicate that there is an enormous volume increase in the collar (CO) as well as in the lip (LP; not shown in B). **C**. Age-related area increase of the dense CO region. The area expansion of the dense CO region is about 27% from 1-month-old ants to 6-month-old ants with a significant difference between the age groups. **D**. Effect of high age on the microglomeruli (MG) number in the dense CO region. Extrapolations of the MG numbers to the whole dense collar area (mean MG numbers) indicate that new MG may emerge. Scale bar: A, B 20 μm; o = outliers.

most likely accompanied by presynaptic pruning, is obviously caused by an increase in dendritic arborizations among the MG profiles (see below). It is accompanied by a slight volumetric expansion of the MB calyces [Fig. 3(A,B)]. In the lip changes in MG density are less pronounced [Fig. 4(B,C)]. In addition, with increasing age (>6 months) new MG may be generated in the MB calyx resulting in a drastic age-related increase of the MB-calyx volume (see Fig. 5).

Structural Plasticity in the Mushroom-Body Calyx Triggered by Light Exposure

To investigate whether changes in MG numbers of the visually innervated collar, as they occur during the transition from interior workers to foragers, are caused by an age-related internal program, or whether light exposure may play a role in triggering these changes, we artificially exposed different cohorts of dark-reared ants to light (see Fig. 6). For example, does premature light exposure trigger a premature reduction in MG numbers of the collar? To answer this question we exposed freshly emerged callows to natural light (see Methods for details). Indeed, this light treatment resulted in a significant reduction of MG numbers in the collar, but not in the lip, when compared with the dark-reared control ants of the same age under the same rearing conditions [Fig. 6(A); unpaired t-test: $n_{(\text{light-exposed ants})} = 8$, $n_{(\text{dark-control ants})} = 8$; collar: $p < 0.001$; lip: $p = 0.607$ (Area 4), $p = 0.237$ (Area 5)]. On the other hand, if light-exposed foragers were compared in their MG number with age-matched foragers that lacked any light exposure (dark foragers; $n = 6$), they exhibited significantly lower MG densities in the collar than the dark foragers [Fig. 4(A): unpaired t-test: dark foragers-foragers: $p < 0.05$]. If the MG numbers of interior workers are included, the statistics read as follows: [Fig. 4(A); dark foragers-callows: $p = 0.138$; dark

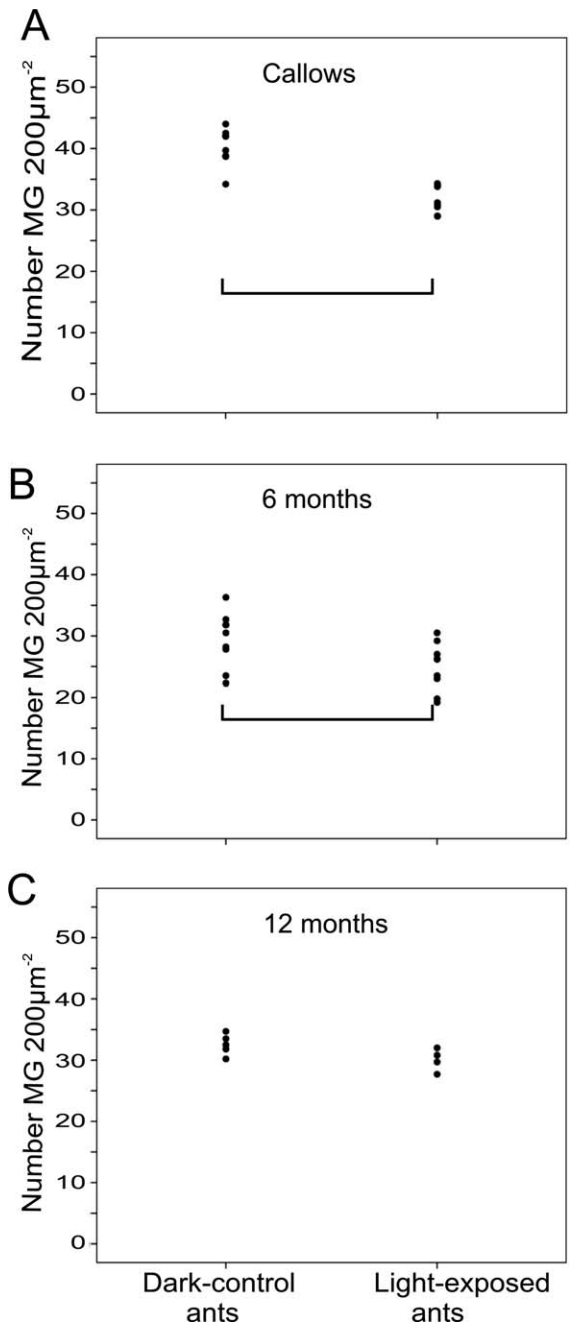

Figure 6 Effect of light on the structural composition in the visually innervated collar (CO) compared to dark-kept ants of different age. **A**. Light-triggered plasticity in the mushroom body (MB) CO of callows. Callows that were exposed to light showed a significant reduction in the microglomeruli (MG) number in comparison to dark-kept animals. **B**. Light-triggered plasticity in the CO of 6-month-old ants. Light exposure had a significant effect on the MG numbers of 6-month-old ants in comparison to the control group of dark reared ants. **C**. Light-triggered plasticity in the CO of 12-month old ants. Even after 1 year in complete darkness, light exposure revealed a tendency to affect MG numbers, albeit not statistically significant.

foragers-interior I: $p = 0.696$; dark foragers-interior II: $p < 0.05$ (unpaired t-test)]. The MG numbers in the lip showed no differences between the dark forager and age-matched foragers [Fig. 4(B,C); unpaired t-test: dark-foragers-callows: $p \leq 0.001$ (Area 4), $p = 0.052$ (Area 5); dark foragers-interior I: $p < 0.05$ (Area 4), $p = 0.780$ (Area 5); dark foragers-interior II: $p = 0.948$ (Area 4), $p = 0.256$ (Area 5); dark foragers-foragers: $p = 0.276$ (Area 4), $p = 0.330$ (Area 5)]. To exclude the possibility that the differences found in the MG numbers of the collar between dark foragers and foragers is caused by the fact that the experimental groups were taken from different colonies, interior I workers of both colonies ($n = 6$ for both groups) were compared. Differences in MG densities were not found in either the collar or the lip [unpaired t-test; collar: $p = 0.614$; lip: $p = 0.070$ (Area 4), 0.488 (Area 5)].

In further asking the question whether light exposure is able to affect structural synaptic plasticity in the MB calyx even in old, dark-reared ants, we exposed 6-month- and 12-month-old, dark-reared ants to light. To our surprise, in the 6-month ants, artificial light exposure (in the laboratory; see Methods) had a still significant effect on the MG numbers in the collar as compared with the control group of dark-reared ants [Fig. 6(B); unpaired t-test: $n_{(\text{light-exposed ants})} = 9$, $n_{(\text{dark-control ants})} = 9$, $p < 0.05$]. In the lip the MG numbers did not change after light treatment [unpaired t-test: $n_{(\text{light-exposed ants})} = 9$, $n_{(\text{dark-control ants})} = 9$, $p = 0.501$ (Area 4), 0.907 (Area 5)]. Even after the ants had been held in complete darkness for 1 year, light exposure still showed an obvious, though not significant trend to reduce MG density in the collar [Fig. 6(C); unpaired t-test: $n_{(\text{light-exposed ants})} = 4$, $n_{(\text{dark-control ants})} = 5$, $p = 0.073$].

In summary, artificial and/or natural light exposure results in a marked reduction of the density of MG in the MB collar. It does so not only in freshly emerged ants but also in really old ants (see Fig. 6). Furthermore, if foragers are artificially prevented from light exposure, the otherwise expected changes do not occur. This supports the role of light as a sensory trigger for structural remodeling of the synaptic organization in the collar region of the MBs. Dark foragers remained at a stage comparable to that of interior I workers which had no light experience [Fig. 4(A)].

Underlying Cellular Mechanisms

The expansion of the MG associated with a reduction of MG density goes hand in hand with an overall

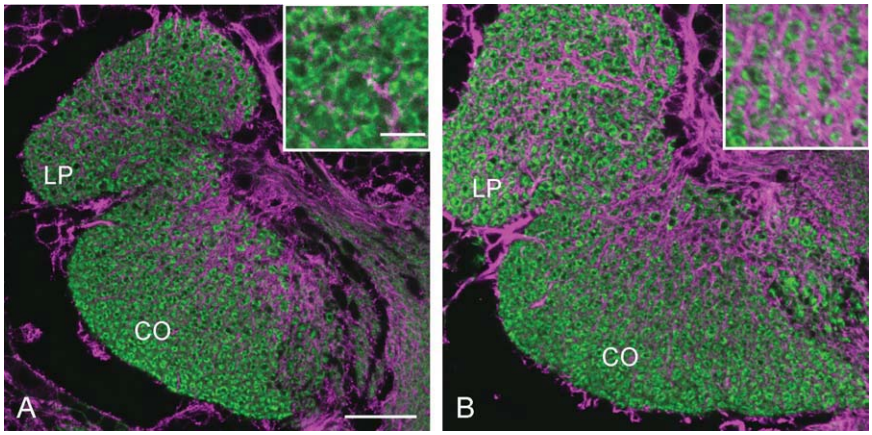

Figure 7 Cellular mechanisms of mushroom-body (MB) volume expansion and changes in microglomeruli (MG) density. **A**, **B**. MB calyx labeled with antitubulin (magenta) and phalloidin-labeled f-actin (green). Tubulin-rich profiles are completely filling out spaces between the dispersing MG during the transition from callows (A) to foragers (B). The magnification of the collar region (squared boxes) indicates that f-actin is highly concentrated in dendritic spines, whereas tubulin-positive profiles are found in the dendritic shafts of presumably Kenyon cells. Scale bars: A, B 20 μm, squared box 5 μm; collar (CO), lip (LP).

volume expansion of the MB calyx. These marked changes immediately raise the question of what the underlying cellular processes might be. To address this question, we combined f-actin-phalloidin labeling with anti-tubulin staining to investigate whether volume expansion may be associated with structural plasticity in main dendritic branches of MB KCs. Whereas f-actin-phalloidin staining predominantly labels KC-dendritic spines (Frambach et al., 2004; Groh et al., 2004), KC dendritic shafts are likely to contain microtubules that are labeled with the anti-tubulin antibody. At a qualitative level the results of this double-labeling procedure suggest that the spaces between the individual MG of callows and foragers are in both cases more or less completely filled with tubulin positive profiles that can be traced into the KC soma layer (Fig. 7). This may reflect an increase in the number of KC dendritic shafts present between the MG profiles of foragers. It further indicates that volume increase and structural remodeling of the MB-calyces during maturation involves pruning of presynaptic projecting neuron boutons and, at the same time, expansion of dendritic arborizations of MB intrinsic neurons (KCs).

DISCUSSION

In *Cataglyphis fortis*, the transition from interior workers to foragers, and thus the reorganization of the ant's behavioral repertoire is accompanied by structural changes in the visual—and to a much smaller extent in the olfactory—regions of the MB calyces. Our results demonstrate in particular that visual experience plays a crucial role in this task-related synaptic plasticity in the collar, but not in the lip of the MB calyx.

The role of insect MBs as brain areas involved in olfactory learning and memory consolidation are well established (Erber et al., 1980; Strausfeld et al., 1998; Heisenberg, 1998, 2003; Menzel, 1999, 2001; Davis, 2005; Menzel and Giurfa, 2006; Gerber et al., 2004; Giurfa, 2007). In visually-guided insects such as honeybees and *Cataglyphis* ants visual learning and memory capabilities are essential during the diurnal outdoor lives of these central place foragers. Visual information from the OLs is projected to the MBs in ants, bees, wasps, and bumblebees (Mobbs, 1982; Gronenberg, 2001; Ehmer and Gronenberg, 2002; Paulk and Gronenberg, 2008). First evidence that the pedunculus and medial lobes of the MBs are required for place memory based on visual landmarks came from bilateral lesions of these MB areas in cockroaches (Mizunami et al., 1998). Moreover, split-brain preparations in cockroaches demonstrated that learning-associated changes in MBs occurred within the trained brain hemisphere, but not within the naïve one (Lent et al., 2007).

Cataglyhis ants rely nearly completely on visual cues when negotiating their way through open or cluttered environments. For pinpointing a goal, be it nesting or feeding site, they mainly rely on visual cues and potentially—in the last stage of nestward orientation—also on olfactory cues (Steck et al., 2009).

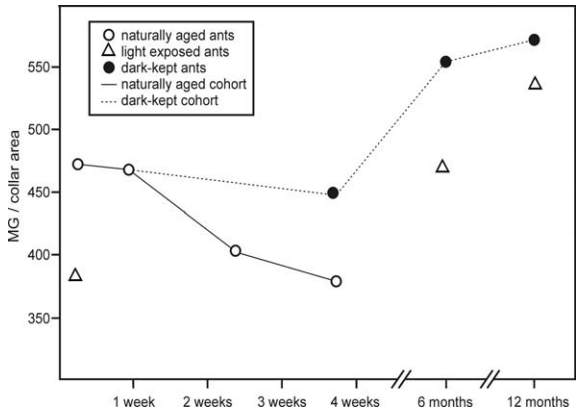

Figure 8 Age-related and light-triggered plasticity in synaptic complexes (microglomeruli, MG) of the visually innervated collar region of the mushroom-body calyces. MG numbers are extrapolated to the dense area of the collar, averaged for each group and plotted versus the ant's age. Naturally aged ants show a decrease in MG density (and in the resulting MG number), most likely initiated by the first light input (Week 2–3) with the transition from interior workers to foragers. In the absence of light input in dark-kept ants the MG density remains unchanged for the first weeks of life, but MG numbers increase at very high age (6 and 12 months). Light exposure of all age groups (1 day, 6 months, and 12 months) results in a decrease in MG density comparable to the decrease in MG density in the course of a natural transition in behavior.

Regarding their visual capabilities, they employ a visual compass system based on skylight cues (Wehner, 1997) and various landmark guidance routines (Wehner et al., 1996; Wehner 2003, 2009), and even can combine information from terrestrial and celestial cues (Åkesson and Wehner, 2002). In addition, early laboratory experiments have shown that the ants spontaneously respond toward landmark changes as the workers undergo the developmental transition from indoor to the outdoor stage (Wehner et al., 1972). Whether the MBs are the places for a landmark memory (see above) remains speculative, but our results suggest that visual experiences have an important influence on the synaptic rewiring in the visual input region of the MB calyces. Furthermore, structural neuronal plasticity occurs at the time when foraging and hence the necessity to acquire and store visual information start.

Age- and Task-Related Structural Plasticity of Microglomeruli in the Mushroom-Body Calyx

Our results in *Cataglyphis fortis* are in line with recent studies that showed volume increases and changes of the synaptic organization in the MB calyx in the congeneric *C. bicolor* and *C. albicans* (Kühn-Bühlmann and Wehner, 2006; Seid and Wehner, 2009) and in the honeybee (Krofczik et al., 2008; Muenz et al., 2008). They further document a significant reduction of MG density in the visually innervated collar while the ants undergo the transition from interior workers to foragers [Figs. 4(A) and 8). The changes in the lip were not significant across age groups, except for a particular subregion, [Fig. 4(B,C)], which may be due to the fact that olfaction (in contrast to vision) already plays a prominent role inside the nest. The net decrease in the density of MG and thus presynaptic boutons in the collar is likely to be caused by axonal pruning in the visual projection neurons and, at the same time, by dendritic expansions of the KCs. A similar conclusion was drawn by Seid and Wehner (2009) in their comparison of indoor ants and foragers. We assume that axonal pruning is a universal process in adapting neuronal circuits during behavioral development and maturation (e.g., Truman and Reiss, 1976; Technau and Heisenberg, 1982; Levine and Truman, 1985; Weeks and Truman, 1986; Lee et al., 1999; Raff et al., 2002; Watts et al., 2003). As structural synaptic plasticity associated with this process is most likely driven by synaptic activity, the effect of synaptic pruning may be an important process in adjusting the MB-calyx microcircuits to the new sensory input occurring during the transition from interior workers to outdoor foragers in social insects. The associated changes in behavior represent an important element regulating the division of labor in insect colonies.

Seid and Wehner (2009) concluded on the basis of their electron-microscopical analyses and volume extrapolations in *C. albicans* that during the transition from indoor to outdoor workers the number of boutons is reduced in the collar and lip region. Their extrapolations showed that the MG number in the lip of young as well as old foragers was significantly smaller than that in the lip of callows. In the collar, however, MG numbers were decreased only in old foragers. Our cohort experiments with *Cataglyphis fortis* confirmed these findings in general, but allow for a more detailed differentiation between the different age groups by confocal-microscopy sampling of synaptic profiles in much wider areas of the MB calyx. This shows that a significant decrease of MG density in the collar already occurs in the interior workers (Figs. 3, 4, and 8) suggesting that pruning effects take place already during the late stages of indoor life and not at the beginning of the foraging phase.

The study of Kühn-Bühlmann and Wehner (2006) in *C. bicolor* revealed age-dependent and task-related enlargements of all compartments of the MBs. On the one hand these authors described a foraging-related volume increase in the collar. On the other hand age-dependent volume increase occurred in the collar (and other MB neuropils) also in dark-reared animals that were older than 150 days (5–6 months). Our results confirmed both these effects in *C. fortis* (for foragers see Fig. 3, for very old dark-reared ants see Figs. 5 and 8). As compared with interior workers, artificially Aged 6-month-old dark-reared ants exhibit significantly larger MB collar regions. Hence, even in the absence of light input or foraging experience the numbers of MG and consequently the total volume of the collar slowly increases with age. Extrapolations of the MG numbers measured in the cross-sectional areas of the collar indicate that new MG may be generated at a slow rate during aging (see Fig. 8). A similar long-term plasticity effect in the MG of the MB-calyx was also found in honeybee queens up to an age of 36 months (Groh et al., 2006). In this case, however, the increase predominantly occurred in the lip region, whereas the collar region decreased in size with age. This indicates that age-dependent programs for structural synaptic plasticity may differ across species, castes and sensory modalities, and in their timing.

Task-related plasticity and long-term structural changes in the MB calyces are likely to be caused by different cellular mechanisms. The size increase of the MB collar in the 6-month-old dark-reared ants is due to an increase in the number of MG, whereas the increase in size that occurs during the transition from the interior workers to the forager stage appears to be accompanied by an axonal pruning process and dendritic growth (see below). Obviously, a steady increase in the number of synaptic complexes is permanently but slowly running in the background as the ants get older. This formation of new MG might prepare for new modulatory events triggered by upcoming sensory inputs and associated with pruning effects within synaptic complexes. Under natural conditions, such modulatory pruning effects may mask the slow-running, long-term changes found in isolation in the dark-reared animals.

Is the task-related volume increase of the MB calyces that occurs during the transition of the interior worker to the forager stage and that is accompanied by an increase in the distance between individual MG (and decrease in the total MG number) really due to an outgrowth of dendritic branches of MB KCs? Our anti-tubulin stainings strongly support that this is the case. The spaces between individual MG were almost completely filled with tubulin-rich profiles, which most likely belong to dendritic branches of KCs (see Fig. 7) as large tubulin-positive tracts can be followed directly into the KC neuronal somata cluster. By this we support the finding of Farris et al. (2001), who showed that dendritic-branching growth in the collar of the honeybee MB increase with age and is promoted by the bees' foraging experience. Previous investigations by Fahrbach et al. (1995) and Gronenberg et al. (1996) had shown that adult neurogenesis is absent in the brains of the honeybee and ants, whereas in the cricket adult neurogenesis was shown to be triggered by sensory input and learning (Scotto-Lomasesse et al., 2002, 2003). We cannot completely exclude neurogenesis in *Cataglyphis*, but axonal pruning and dendritic outgrowth seem to represent important mechanisms of how MB calyx neuropils adjust themselves to changing sensory inputs. In fact, Seid and Wehner (2009) have shown in *C. albicans* that at the level of single boutons a parallel increase of synaptic sites per bouton occurs during the indoor–outdoor transition phase.

Structural Synaptic Plasticity in the Mushroom-Body Calyx is Triggered by Visual Experience

The most important result of this study is the strong influence of sensory stimulation in triggering structural plasticity in the organization of synaptic complexes. Our light exposure experiments clearly revealed that plastic changes in the collar region, but not in the lip, of the MB calyx can be reliably induced with light pulses in dark-reared ants of all age groups (1-day, 6-month, and 12-month ants; see Figs. 6 and 8). This means that synaptic rewiring in the MB calyx is not under the exclusive control of an age-dependent program, but rather regulated to a large extent by sensory input. Unfortunately, a similar test is not possible in the olfactory system because ants completely deprived from olfactory input die within a short period of time, and unilateral removal of antennal sensory input had no effect on the synaptic organization in the lip region indicating that bilateral deprivation is necessary (Kleineidam and Rössler, 2009). The light-trigger effect on the synaptic plasticity in the collar is strengthened by the fact that significant differences in MG densities occurred between natural foragers and age-matched dark foragers (see also Kühn-Bühlmann and Wehner, 2006), and that the latter group did not differ from the interior I and callow groups [see Results section 3; Figs. 4(A) and 8]. These results strongly support the significance of

visual input and are at variance with the hypothesis of an internal program starting during the interior II phase shortly before foraging commences. Similarly, in *Drosophila*, visual stimulation and monocular deprivation affected the volume of the optic lobes (Barth et al., 1997). Likewise, olfactory experience in *Drosophila* and olfactory learning in the honeybee modified the AL structure as well as behavioral responses to odors (Devaud et al., 2003; Hourcade et al., 2009). The visual and odor evoked structural modifications in the *Drosophila* brain are restricted to a critical period of the first days of adult life, whereas in *Cataglyphis fortis* the structural changes in the MBs are not limited to an early stage. It is also in vertebrates that artificial manipulation of the environment could induce neurochemical and neuroanatomical plasticity in the adult brain (Bennett et al., 1964; Floeter and Greenough, 1979; Tieman and Hirsch, 1982).

If light exposure is that decisive, why then do we find such marked effects already in the interior II group (Figs. 3, 4, and 8)? The behavior of workers during their indoor stages might provide a glimpse for answering this question. Whenever one excavates a *Cataglyphis* nest in the field or observes interior workers in laboratory colonies, interior I workers are extremely sluggish and often stick motionless to the walls of the nest chambers, while the interior II workers are actively running around. Interior II workers might also be on their way of becoming diggers carrying sand particles out of the nest and depositing them near the nest entrance, and *diggers* might be on the transition of becoming foragers (Wehner et al., 1983). Hence, interior II workers could potentially appear at the nest entrance every now and then and by this expose themselves to light stimulation. In this context it might be worth mentioning that when we exposed callows to early light treatments, these ants afterwards exhibited higher locomotor activities than the untreated callows. This observation raises the question whether light exposure is able to trigger the transition from interior workers to foragers (see also Wehner et al., 1972). This is certainly a hypothesis worth testing in *Cataglyphis*.

Let us finally conclude by again emphasizing that age-related and task-dependent structural changes in the *Cataglyphis* brain, especially in the visually driven collar of the MBs, are a result of a slow internal program running also in complete darkness, but can be triggered to a large extent and in all age groups by visual stimulation. The structural changes initiated by the visual input mainly consist of a decrease in the density of the MG most likely resulting from an outgrowth of dendritic arborizations of the KCs, and lead to an overall increase in the volumetric size of the collar. This structural plasticity stimulated and exploited in the visual input centers of the MBs of *Cataglyphis* makes these visually guided long-distance insect navigators a particularly promising model organism for future investigations on visually mediated brain plasticity.

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REFERENCES

Åkesson S, Wehner R. 2002. Visual navigation in desert ants *Cataglyphis fortis*: Are snapshots coupled to a celestial system of reference? J Exp Biol 205:1971–1978.

Barth M, Hirsch HVB, Meinertzhagen IA, Heisenberg M. 1997. Experience-dependent developmental plasticity in the optic lobe of *Drosophila melanogaster*. J Neurosci 17:1493–1504.

Becher MA, Scharpenberg H, Moritz RFA. 2009. Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera L.*). J Comp Physiol 195:673–679.

Bennett EL, Diamond MC, Krech D, Rosenzweig MR. 1964. Chemical and anatomical plasticity of brain: Changes in brain through experience, demanded by learning theories, are found in experiments with rats. Science 146:610–619.

Davis RL. 2005. Olfactory memory formation in *Drosophila*: From molecular to systems neuroscience. Ann Rev Neurosci 28:275–302.

Devaud JM, Acebes A, Ramaswami M, Ferrús A. 2003. Structural and functional changes in the olfactory pathway of adult *Drosophila* take place at a critical age. J Neurobiol 56:13–23.

Durst C, Eichmüller S, Menzel R. 1994. Development and experience lead to increased volume of subcompartments of the honeybee mushroom body. Behav Neural Biol 62: 259–263.

Ehmer B, Gronenberg W. 2002. Segregation of visual input to the mushroom bodies in the honeybee (*Apis mellifera*). J Comp Neurol 451:362–373.

Ehmer B, Hoy R. 2000. Mushroom bodies of vespid wasps. J Comp Neurol 416:93–100.

Erber J, Masuhr TH, Menzel R. 1980. Localization of short-term memory in the brain of the bee, *Apis mellifera*. Physiol Entomol 5:343–358.

Fahrbach SE, Strande JL, Robinson GE. 1995. Neurogenesis is absent in the brains of adult honey bees and does

not explain behavioral neuroplasticity. Neurosci Lett 197:145–148.

Farris SM, Robinson GE, Fahrbach SE. 2001. Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. J Neurosci 21:6395–6404.

Farris SM, Sinakevitch I. 2003. Development and evolution of the insect mushroom bodies: Towards the understanding of conserved developmental mechanisms in a higher brain center. Arthropod Struct Dev 32:79–101.

Floeter MK, Greenough WT. 1979. Cerebellar plasticity: Modification of Purkinje cell structure by differential rearing in monkeys. Science 206:227–229.

Frambach I, Rössler W, Winkler M, Schürmann FW. 2004. F-actin at identified synapses in the mushroom body neuropil of the insect brain. J Comp Neurol 475:303–314.

Ganeshina O, Menzel R. 2001. GABA-immunoreactive neurons in the mushroom bodies of the honeybee: An electron microscopic study. J Comp Neurol 437:335–349.

Gerber B, Tanimoto R, Heisenberg M. 2004. An engram found? Evaluating the evidence from fruit flies. Curr Opin Neurobiol 14:737–744.

Giurfa M. 2007. Behavioral and neural analysis of associative learning in the honeybee: A taste from the magic well. J Comp Physiol A 193:801–824.

Groh C, Ahrens D, Rössler W. 2006. Environment- and age-dependent plasticity of synaptic complexes in the mushroom bodies of honeybee queens. Brain Behav Evol 68:1–14.

Groh C, Tautz J, Rössler W. 2004. Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. Proc Natl Acad Sci USA 101:4268–4273.

Gronenberg W. 2001. Subdivisions of hymenopteran mushroom body calyces by their afferent supply. J Comp Neurol 435:474–489.

Gronenberg W, Heeren S, Hölldobler B. 1996. Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. J Exp Biol 199:2011–2019.

Heisenberg M. 1998. What do the mushroom bodies do for the insect brain? An introduction. Learn Mem 5:1–10.

Heisenberg M. 2003. Mushroom body memoir: From maps to models. Nat Rev Neurosci 4:266–275.

Hölldobler B, Wilson, EO. 1990. The Ants. Cambridge, MA: Belknap Press.

Hourcade B, Perisse E, Devaud JM, Sandoz JC. 2009. Long-term memory shapes the primary olfactory center of an insect brain. Learn Mem 16:607–615.

Ismail N, Robinson GE, Fahrbach SE. 2006. Stimulation of muscarinic receptors mimics experience-dependent plasticity in the honey bee brain. Proc Natl Acad Sci USA 103:207–211.

Jones JC, Helliwell P, Beekman M, Maleszka R, Oldroyd BP. 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. J Comp Physiol 191: 1121–1129.

Kirschner S, Kleineidam CJ, Zube C, Rybak J, Grünewald B, Rössler W. 2006. Dual olfactory pathway in the honeybee, *Apis mellifera* J Comp Neurol 499:933–952.

Klagges BRE, Heimbeck G, Godenschwege TA, Hofbauer A, Pflugfelder GO, Reifegerste R, Reisch D, et al. 1996. Invertebrate synapsins: A single gene codes for several isoforms in *Drosophila*. J Neurosci 16:3154–3165.

Kleineidam CJ, Rössler R. 2009. Adaptations of the olfactory system of social Hymenoptera. In: Gadau J, Fewell J, forworded by EO Wilson, editors. Organization of Insect Societies. Cambridge/London: Harvard University Press, pp 195–219.

Krofczik S, Khojasteh U, Hempel de Ibarra N, Menzel R. 2008. Adaptation of microglomerular complexes in the honeybee mushroom body lip to manipulations of behavioral maturation and sensory experience. Dev Neurol 68: 1007–1017.

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

Lee T, Lee A, Luo L. 1999. Development of the *Drosophila* mushroom bodies: Sequential generation of three distinct types of neurons from a neuroblast. Development 126: 4065–4076.

Leiss F, Groh C, Butcher NJ, Meinertzhagen IA, Tavosanis G. 2009. Synaptic organization in the adult *Drosophila* mushroom body calyx. J Comp Neurol 517:808–824.

Lent DD, Pintér M, Strausfeld NJ. 2007. Learning with half a brain. Dev Neurobiol 67:740–751.

Levine RB, Truman JW. 1985. Dendritic reorganization of abdominal motoneurons during metamorphosis of the moth, *Manduca sexta*. J Neurosci 5:2424–2431.

Menzel R. 1999. Memory dynamics in the honeybee. J Comp Physiol 185:323–340.

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

Menzel R, Giurfa M. 2006. Dimensions of cognition in an insect, the honeybee. Behav Cog Neurosci Rev 5:24–40.

Mizunami M, Weibrecht JM, Strausfeld NJ. 1998. Mushroom bodies of the cockroach: Their participation in place memory. J Comp Neurol 402:520–537.

Mobbs PG. 1982. The brain of the honeybee *Apis mellifera*. The connections and spatial organization of the mushroom bodies. Phil Trans R Soc Lond B 298:309–354.

Muenz TS, Oberwallner B, Gehring K, Rössler W. 2008. Plasticity of synaptic complexes in the mushroom-bodies of the honeybee brain depends on age, experience and season. FENS Abstr 4:143.

O'Donnell S, Donlan NA, Jones TA. 2004. Mushroom body structural change is associated with division of labor in eusocial wasp workers (*Polybia aequatorialis*. Hymenoptera: Vespidae) Neurosci Lett 356:159–162.

Paulk AC, Gronenberg W. 2008. Higher order visual input to the mushroom bodies in the bee, *Bombus impatiens*. Arthropod Struct Dev 37:443–458.

Raff MC, Whitmore AV, Finn JT. 2002. Axonal self-destruction and neurodegeneration. Science 296:868–871.

Rössler W, Kuduz J, Schürmann FW, Schild D. 2002. Aggregation of f-actin in olfactory glomeruli: A common feature of glomeruli across phyla. Chem Senses 27:803–810.

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

Scotto-Lomassese S, Strambi C, Aouane A, Strambi A, Cayre M. 2002. Sensory inputs stimulate progenitor cell proliferation in an adult insect brain. Curr Biol 12:1001–1005.

Scotto-Lomassese S, Strambi C, Strambi A, Aouane A, Augier R, Rougon G, Cayre M. 2003. Suppression of adult neurogenesis impairs olfactory learning and memory in an adult insect. J Neurosci 23:9289–9296.

Seid MA, Harris KM, Traniello JFA. 2005. Age-related changes in the number and structure of synapses in the lip region of the mushroom bodies in the ant *Pheidole dentata*. J Comp Neurol 488:269–277.

Seid MA, Wehner R. 2008. Ultrastructure and synaptic differences of the boutons of the projection neurons between the lip and collar regions of the mushroom bodies in the ant, *Cataglyphis albicans*. J Comp Neurol 507:1102–1108.

Seid MA, Wehner R. 2009. Delayed axonal pruning in the ant brain: A study of developmental trajectories. Dev Neurol 69:1–15.

Steck K, Hansson BS, Knaden M. 2009. Smells like home: Desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. Front Zool 6:5.

Steiger U. 1967. Über den Feinbau des Neuropils im Corpus pedunculatum der Waldameise. Z Zellforsch 81: 511–536.

Strausfeld NJ, Hansen L, Li Y, Gomez RS, Ito K. 1998. Evolution, discovery, and interpretations of arthropod mushroom bodies. Learn Mem 5:11–37.

Takemura S, Lu Z, Meinertzhagen IA. 2008. Synaptic circuits of the *Drosophila* optic lobe: The input terminals to the medulla. J Comp Neurol 509:493–513.

Technau G, Heisenberg M. 1982. Neural reorganization during metamorphosis of the corpora pedunculata in *Drosophila melanogaster*. Nature 295:405–407.

Tieman SB, Hirsch HV. 1982. Exposure to lines of only one orientation modifies dendritic morphology of cells in the visual cortex of the cat. J Comp Neurol 211:353–362.

Truman JW, Reiss SE. 1976. Dendritic reorganization of an identified motoneuron during metamorphosis of the tobacco hornworm moth. Science 192:477–479.

Watts RJ, Hoopfer ED, Luo L. 2003. Axon pruning during *Drosophila* metamorphosis: Evidence for local degeneration and requirement of the ubiquitin-proteasome system. Neuron 38:871–885.

Weeks JC, Truman JW. 1986. Steroid control of neuron and muscle development during the metamorphosis of an insect. J Neurobiol 17:249–267.

Wehner R. 1983. Taxonomie, Funktionsmorphologie und Zoogeographie der saharischen Wüstenameise Cataglyphis fortis (Forel 1902) stat nov (Insecta: Hymenoptera: Formicidae) Senckenbergiana Biol 64:89–132.

Wehner R. 1987. Spatial organization of foraging behavior in individually searching desert ants, *Cataglyphis* (Sahara Desert) and *Ocymyrmex* (Namib Desert). In: Pasteels JM, Deneubourg JL, editors. From Individual to Collective Behavior in Social Insects. Basel: Birkhäuser Verlag, pp 15–42.

Wehner R. 1997. The ant's celestial compass system: Spectral and polarization channels. In: Lehrer M, editor. Orientation and Communication in Arthropods. Basel: Birkhäuser Verlag.

Wehner R. 2003. Desert ant navigation: How miniature brains solve complex tasks. J Comp Physiol 189:579–588.

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

Wehner R, Harkness RD, Schmid-Hempel P. 1983. In: Foraging Strategies in Individually Searching Ants *Cataglyphis bicolor* (Hymenoptera: Formicidae). Akad Wiss Lit Mainz Math-Naturwiss KI. Stuttgart: Gustav Fischer Verlag.

Wehner R, Herrling PL, Brunnert A, Klein R. 1972. Periphere Adaptation und zentralnervöse Umstimmung im optischen System von *Cataglyphis bicolor* (Formicidae. Hymenoptera). Rev Suisse Zool 79:197–228.

Wehner R, Michel B, Antonsen P. 1996. Visual navigation in insects: Coupling of egocentric and geocentric information. J Exp Biol 199:129–140.

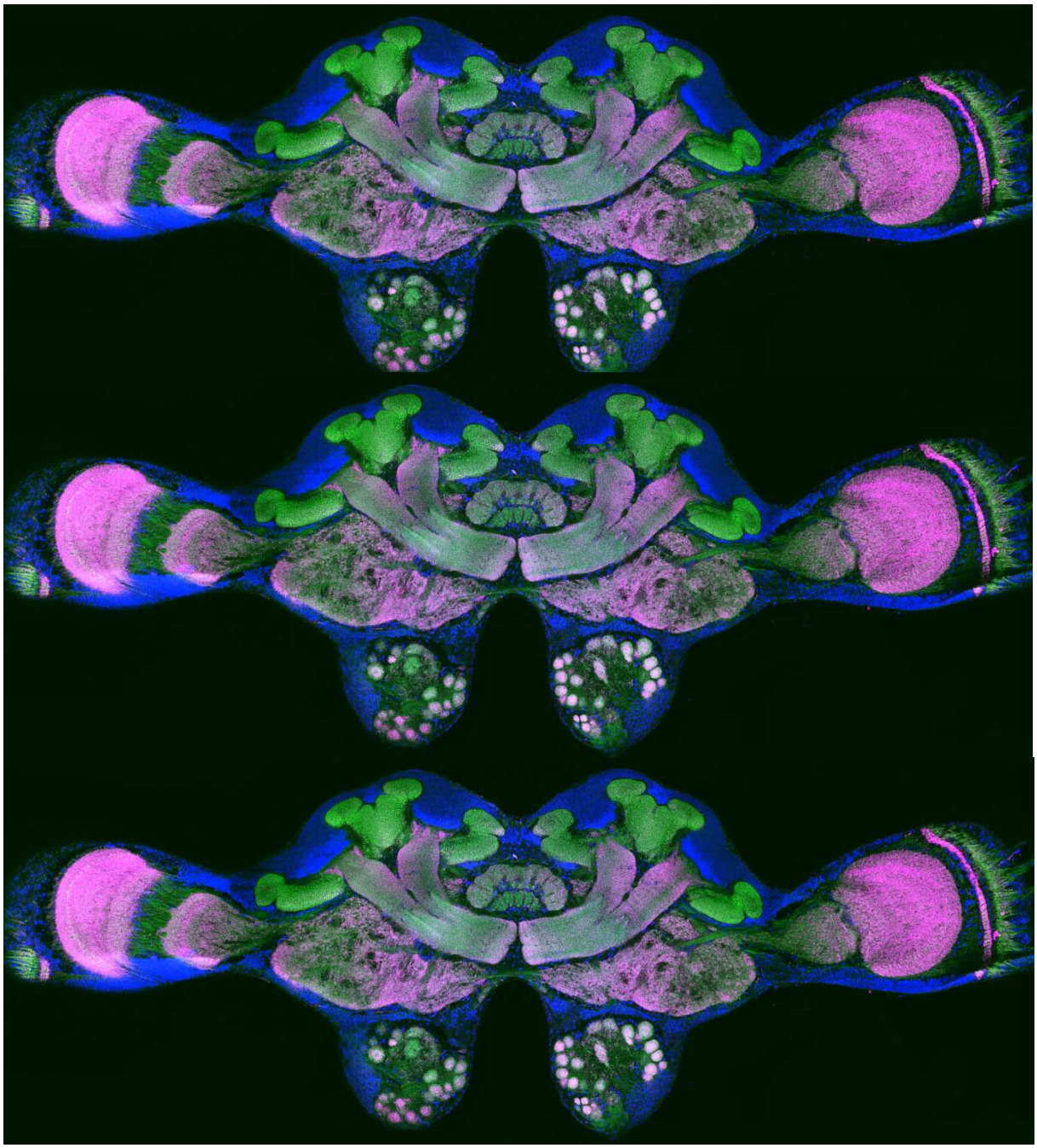
Withers GS, Fahrbach SE, Robinson GE. 1993. Selective neuroanatomical plasticity and division of labour in the honeybee. Nature 364:238–240.

Wolf H, Wehner R. 2005. Desert ants compensate for navigation uncertainty. J Exp Biol 208:4223–4230.

Yasuyama K, Meinertzhagen IA, Schürmann FW. 2002. Synaptic organization of the mushroom body calyx in *Drosophila melanogaster*. J Comp Neurol 445:211–226.

Zube C, Kleineidam CJ, Kirschner S, Neef J, Rössler W. 2008. Organization of the olfactory pathway and odor processing in the antennal lobe of the ant *Camponotus floridanus*. J Comp Neurol 506:425–41.

MANUSCRIPT II



Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*

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The individual life-history of the desert ant *Cataglyphis fortis* is characterized by a fast transition from interior tasks to mainly visually guided foraging. Previous studies revealed a remarkable structural synaptic plasticity in visual and olfactory input regions within the mushroom bodies of the ants' brains - centers involved in learning and memory. Reorganization of synaptic complexes (microglomeruli) was shown to be triggered by sensory exposure rather than an internal program. Using video analyses at the natural nest site and activity recordings after artificial light treatments we investigated whether the ants get exposed to light before onset of foraging and whether this changes the ants' activity levels. We asked whether synaptic reorganization occurs in a similar time window by immunolabeling and quantification of pre- and postsynaptic compartments of visual and olfactory microglomeruli after periods of light-exposure. Ants reverted back to dark nest conditions were used to investigate whether synaptic reorganization is reversible. The behavior analyses revealed that late-interior ants (*diggers)* are exposed to light and perform exploration runs up to two days before they start foraging. This corresponds well with the result that artificial light treatment over more than two to three days significantly increased the ants' locomotor activities. At the neuronal level, visual exposure of more than one day was necessary to trigger reorganization of microglomeruli, and light induced changes were only partly reversible in the dark. We conclude that visual pre-exposure is an important and flexible means to prepare the ants' visual pathway for orientation capabilities essential during foraging.

INTRODUCTION

Division of labor is a fundamental trait in insect societies. In eusocial insects it is often characterized by an age-related polyethism (Hölldobler and Wilson, 1990) expressed in a lifelong high flexibility of individual behavioral repertoires that require plasticity in the underlying neuronal circuitries. The North African desert ant *Cataglyphis* has become a particularly promising model system to study the neuronal basis of behavioral plasticity. The brief transition from interior workers performing tasks like brood care and food processing to short-lived (Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984) predominantly visually guided foragers with astonishing navigational abilities (Wehner, 2003, 2009) has been shown to be associated with volume changes and structural synaptic plasticity in the mushroom bodies (MBs) (Kühn-Bühlmann and Wehner, 2006; Seid and Wehner, 2009; Stieb et al., 2010). Furthermore, visual experience and visual deprivation affect the behavior of interior workers and foragers (Wehner et al., 1972), and light exposure triggers synaptic reorganization in the MBs throughout the natural life span and even after artificially extended life stages of up to one year (Stieb et al., 2010).

The MBs are higher sensory association centers involved in learning and memory (Strausfeld et al., 1998; Menzel, 1999; Gerber et al., 2004; Davis, 2005; Giurfa, 2007; Hourcade et al., 2010). In honeybees and ants, the MB calyces are multimodal and divided into the olfactory innervated lip, the visually innervated collar and the basal ring receiving multimodal sensory input (Mobbs, 1982; Gronenberg, 2001; Farris and Sinakevitch, 2003). Projections from the optic (OL) and antennal (AL) lobes form large presynaptic boutons in the MB calyx neuropil that are surrounded by numerous postsynaptic profiles from Kenyon-cell (KC) spine-like dendritic protrusions (Steiger, 1967; Gronenberg, 2001; Ganeshina and Menzel, 2001; Yusuyama et al., 2002; Frambach et al., 2004; Groh et al., 2004, 2006; Seid and Wehner, 2008; Leiss et al., 2009). These nodular synaptic complexes have been termed microglomeruli (MG).

In *Cataglyphis* ants, behavioral maturation correlates with volume expansion of the MB calyces (Kühn-Bühlmann and Wehner, 2006) that goes along with pruning of MG (Seid and Wehner, 2009; Stieb at al., 2010) and, at the same time, a massive expansion of the KC dendritic network (Stieb et al., 2010) as also reported for honeybees (Farris et al., 2001; Dobrin et al., 2011). Our previous study (Stieb et al., 2010) showed that light exposure is sufficient to mimic the effect caused by natural behavioral maturation in the visual subregion of the MBs.

As *Cataglyphis* foragers have short life expectancies in which they accomplish remarkable visual navigational tasks, there is only a short time window during which the underlying neuronal circuitries and the behavioral performances can adapt to the new environmental conditions. Hence, it is a likely hypothesis that interior workers may gain some exposure to light prior to becoming foragers, possibly promoting an adjustment in their visual system that may be linked to improved performance as foragers. Previous studies indicate that interior workers in a later stage operate as *diggers* and might thus get first visual input prior to the actual foraging period (Wehner et al., 1983). In other ant species it has been shown that foragers are even able to reverse their behavioral sequence and turn from foraging to brood care behavior (*Solenopsis invicta*: Sorensen et al., 1984; *Pheidole dentata*: Calabi and Traniello, 1989). It is also in *Cataglyphis* that preferences for light and dark areas along the skyline can be reversed from the outside-state to the inside-state behavior of the workers (Wehner et al., 1972).

While our previous investigations had documented the high behavioral as well as neuronal plasticity in *Cataglyphis* ants, our present study aims at correlating both aspects, namely the high and rapid flexibility in the ants' behavior and that of its underlying neuronal mechanisms. We do so by raising the following questions.

1. Do advanced (late-stage) interior workers exhibit a particular behavior to get exposed to light before the onset of foraging?
2. Is there a critical time window for light exposure to trigger synaptic plasticity in the MBs?
3. Can precocious light exposure actually induce premature foraging behavior, and is it associated with changes in the ants' locomotor activity?
4. Is synaptic reorganization in the MBs as triggered by light exposure reversible?

METHODS

To detect a potential light-sampling behavior of a late stage of interior ants before they become foragers and to demonstrate general effects of light exposure on the ants' behavior, we performed observations and video analyses at the natural nest site and after artificial light treatment. To investigate light-triggered effects and their potential reversibility on synaptic reorganization, pre- and postsynaptic profiles of microglomerular synapses in the MB calyx were immunolabeled and quantified after the ants were light-exposed and returned to dark (nest) conditions. All experimental treatments are summarized in table 1.

Table 1. Experimental groups and their treatments. * data were included from Stieb et al (2010).

Experiments	Experimental groups (n)	Treatment detail
Light-Treatment	1-d dark callows (7)	callows were kept in darkness for 1 day
	4-d dark callows (7) *	callows were kept in darkness for 4 days
	1-d light callows (9)	callows were exposed to light 5x a day for 1 day
	4-d light callows (8) *	callows were exposed to light 5x a day for 4 days
Activity Recordings	dark callows (32) - in dark - in light	callows were kept in darkness for 5 days and light-exposed on the 5th day (5x); recordings were performed - daily in darkness - on the 5th day in light
	light callows (24) - in dark - in light	callows were exposed to light 5x a day for 5 days; recordings were performed - daily in darkness - daily in light
Light-Deprivation	light foragers (9)	1-d old (light experienced) foragers were exposed to light 5x a day for further 3 days
	reversed foragers (9)	1-d old (light experienced) foragers were transferred in darkness for 3 days

Animals

Experiments were conducted at the Maharès study site in Tunisia in May 2009 and June/July 2010 with workers of different stages belonging to the species *C. fortis*. During the lifetime of the workers the following ethocaste stages occur one after another: callows (freshly emerged workers not more than about 24 hours old), interior I workers (characterized by their motionless state of behavior, their swollen gaster and the expanded whitish intersegmental membranes of the gaster), interior II workers (the remaining inside workers) and foragers (actively searching for food outside the nest; we define the start of the foraging stage by an ant's searching for food for at least one full day).

Three queen-right colonies were excavated in a salt-pan near Menzel Chaker, Tunisia, in May 2009 and June 2010 (34°58'N, 10°25'E) and then kept in complete darkness. This treatment guaranteed that newly emerging callows were raised in complete darkness. For subsequent experiments (light treatment and activity recordings, see Methods below), these callows were separated from the colony under red light illumination (red light LEDs: λ_{max} = c. 600-650 nm) to prevent the ants from visible light exposure as they exhibit two spectral types of photoreceptors maximally sensitive at 510 nm and 350 nm (green and UV type respectively: Mote and Wehner, 1980). To get access to age-marked foragers (the amount of days an ant has been foraging), all foragers of one colony in the salt pan were marked with a spot of paint on the abdomen on three subsequent days. It has been shown that the short-lived foragers

show a steady state turnover rate and do not stay inside the nest for an extended period without taking part in foraging (Schmid-Hempel and Schmid-Hempel, 1984). This procedure guaranteed that on the fourth day all unmarked foragers (ants returning to the nest with food) were foragers with 1 day of foraging experience (1-day foragers). These 1-day foragers were collected in the late afternoon of the fourth day for subsequent experiments (light deprivation, see Methods below). Behavioral recordings (see Methods below) of foragers and ants that were about to become foragers (belonging presumably to the interior II worker stage) were performed at another colony in the salt pan. For this, four food traps sunk into the salt-pan floor were established in five meter distance from the entrance of a selected nest, and ants entering the traps were marked three days in a row. On the fourth day, unmarked ants represented ants leaving the nest for the first time.

Brains of callows and foragers that had undergone the light treatment or deprivation experiments, respectively, were dissected on-site in Tunisia and put into fixative solution (see Methods below) before they were transferred to the Würzburg laboratory for further neurohistochemical procedures.

For behavioral observations outside the natural nest, ants were kept in plastic boxes (19 x 9 x 6 cm^3). They were fed at night with dead cockroaches. Water supply was provided during the entire time. All light-exposure experiments took place under natural sunlight.

Light Treatment

Freshly emerged callows were separated from the colony and divided into two groups: a control group (dark callows; 10-20 ants) and a light-exposed group (light callows; 10-20 ants). After one night of acclimatization, the light treatment started. Whereas the dark callows remained in the dark, the light callows were exposed to the sun 5 times for 45 min each at 9:00, 11:00, 13:00, 15:00 and 17:00 for one day. This treatment simulated an average light-exposure regime that the ants would experience during their outdoor foraging trips (Wehner, 1987). Between the light-exposure periods the boxes were placed into the dark. During the next three days both groups were kept in complete darkness.

The mean temperature in the experimental boxes of both the light-exposed as well as the dark-kept group did not differ between the two groups and remained constant over time at a mean of 28.5 ± 3 C°. Light-exposure took place in a shaded outdoor area with mean light intensities of 3.4 $W \cdot m^{-2}$ for UVA (280-315 nm), 0.1 $W \cdot m^{-2}$ for UVB (315-400 nm) and 238.1 $W \cdot m^{-2}$ for PAR (Photosynthetic Active Radiation 400-700 nm) inside the box (measured with an optometer (Gigahertz-Optik Modell X1-2)). The relative humidity was 52 ± 12 %. After this

procedure the brains of the control and the light-exposed ants were dissected and further processed for immunocytochemistry (see below).

Light Deprivation

1-day foragers were collected from their nest side in the salt-pan and transferred to Maharès where they were divided into two groups. One group was subjected to the light treatment described above (light foragers; 10-20 ants), the other remained in the dark (reversed foragers; 10-20 ants). After one night of acclimatization, the light foragers experienced sunlight three days in a row. Between the light-exposure periods the boxes were placed into the dark. The reversed foragers were under complete darkness during these three days.

The mean temperature and the relative humidity in the experimental boxes of both the light-exposed as well as the dark-kept group did not differ between the two groups and remained constant over time at a mean of 30.5 ± 1 C° and 54.2 ± 8 %. Light-exposure took place in a sunny area with mean light intensities of for 13.9 $W \cdot m^{-2}$ UVA, 0.3 $W \cdot m^{-2}$ for UVB and 666.8 $W \cdot m^{-2}$ for PAR inside the box. Afterwards the brains of the light-exposed and the dark-kept ants were dissected and further processed for immunocytochemistry (see below).

Immunocytochemistry

To analyze structural changes in synaptic organization in the olfactory lip and visual collar region of the MB calyx in the different experimental groups, pre- and postsynaptic profiles of individual MG were visualized by double-labeling with an antibody to synapsin and fluorescently labeled phalloidin binding to f-actin using the method introduced by Groh et al. (2004) (Fig. 1). In the insect MBs, f-actin is highly concentrated in MG of the MB calyces due to its accumulation in KC dendritic spines, whereas synapsin is associated with synaptic vesicles aggregated in presynaptic boutons of projection neurons (see also Groh et al, 2004; Stieb et al., 2010).

The ants were anaesthetized with CO_2, decapitated, and the head capsules were fixed in dental-wax coated dishes. The head capsule was covered with fresh ant-saline solution (127 mM NaCl, 7 mM KCl, 1.5 mM CaCl2, 0.8 mM Na2HPO4, 0.4 mM KH2PO4, 4.8 mM TES, 3.2 mM Trehalose, pH 7.0) and opened by cutting a square window in between the compound eyes. Glands and tracheae were gently removed, and the brains were dissected out and fixed immediately in cold 4 % formaldehyde in phosphate-buffered saline (PBS, pH 7.2) overnight at 4 °C. The brains were then rinsed in PBS (3 x 10 min). After embedding in 5 % low melting point agarose (Agarose II, no. 210–815, Amresco, Solon, OH, USA), brains were carefully adjusted in a frontal plane and sectioned at 100 µm thickness with a vibrating

microtome (Leica VT 1000S, Nussloch, Germany). Free-floating agarose sections were pre-incubated in PBS containing 0.2% Triton X-100 (PBST) and 2 % normal goat serum (NGS, 005-000-121, Jackson ImmunoResearch Laboratories) for 1 hour at room temperature.

To label neuronal f-actin, sections were incubated in 0.2 units of Alexa Fluor 488 phalloidin (Molecular Probes, A12379) in 500 µl PBST with 2 % NGS for three days at 4 °C (Rössler et al., 2002; Groh et al., 2004). For double-labeling, sections were simultaneously incubated with a monoclonal antibody to the *Drosophila* synaptic-vesicle-associated protein synapsin I (1:50, SYNORF1, kindly provided by E. Buchner, University of Würzburg, Germany) (Klagges et al., 1996). In order to visualize synapsin after five rinses in PBS, double-labeled preparations were incubated in Alexa Fluor 568-conjugated goat anti-mouse secondary antibody (1:250, Molecular Probes, A11004) in 1 % NGS/PBS for 2 hours at room temperature.

To label cell nuclei, sections were incubated for 15 min in 2.5 mg/ml of the DNA marker bisbenzimidazole derivative Hoechst 34580 (1:500; Molecular Probes, H21468) in PBST at room temperature. Sections were finally washed in at least five changes of PBS, transferred into 60 % glycerol/PBS for 30 min, and mounted on slides in 80 % glycerol/PBS.

Laser Scanning Confocal Microscopy, Image Processing and Data Analysis

Preparations were viewed with a laser-scanning confocal microscope (Leica TCS SP2 AOBS; Leica Microsystems, Wetzlar, Germany) equipped with an argon/krypton laser. Excitation wavelengths were 568 nm for synapsin, 488 nm for Alexa Fluor 488 and 405 nm for Hoechst 34580. Two different HC PL APO objective lenses were used for image acquisition (20 x 0.7NA imm and 63 x 1.20NA imm), and in certain cases in combination with a 2–2.5x digital zoom. In double- or triple-labeled preparations, the different channels were merged with the use of pseudocolors. Images were further processed with FIJI-win32 (ImageJ 1.44c, Wayne Rasband, NIH, Bethesda, MD), and Corel Draw X3 software (Corel Corporation, Ottawa, ON, Canada).

Quantification of synaptic complexes (microglomeruli, MG) in the MB calyx was performed in a single section per brain at a defined region in the central brain (method according to Stieb et al., 2010). At this sectional plane, all MB calyces and other landmarks such as the ellipsoid and fan-shaped bodies of the central complex and the pedunculi and medial lobes of the MBs were clearly visible (Fig. 1A, B). MG profiles were quantified in the olfactory lip and the visual collar of the MB calyx using a modified protocol of the method introduced by Groh et al. (2004). Individual MG were visualized at high magnification using a 63x objective and a 2.5x digital zoom. MG profiles were quantified in the medial halves of the medial calyx in

both hemispheres as these are most perfectly matched in the sectional plane (Fig. 1B). In a previous study no differences were found in the MG numbers between either the medial and lateral calyces or the right and left brain hemispheres, or between different sectional and focal planes for both the collar and the lip region (see Stieb et al., 2010). In accord with the method applied by Stieb et al. (2010), MG numbers were counted and averaged for three circular areas (Area 1-3; 200 µm² per area) in the dense portion of the collar and in some cases extrapolated to the total dense area within which the MG are uniformly distributed. In the lip region, two circular areas (Area 4 and 5; 200 µm² per area) were analyzed separately for subsequent analyses due to the more uneven spatial distribution of MG in between lip areas. The MG numbers for the collar and the lip in the two calyces of each brain were averaged, and a mean was calculated based on the number of brains analyzed. MG were counted blind to the treatment of the respective specimens, and the criterion used was that a MG contained a magenta-labeled synapsin positive bouton encircled by a green-labeled f-actin-phalloidin halo.

In addition, to quantify differences in the immunoreactivity (IR) of conjugated synapsin in the lip and collar region of foragers that were transferred back to darkness (reversed foragers), a particle analysis was conducted for synapsin fluorescence in this group as well as in a control group of light-adapted, age-matched foragers (light foragers) and in a control group of 1-day foragers (data obtained for this group from Stieb et al., 2010). For this, the total lip and collar area was measured. The intensity of synapsin-fluorescence for this region was analyzed by first creating a black-to-white image, whereas the contrast-threshold for synapsin-IR was set to automatic for each image and by then analyzing particles in the selected area (*Analyze particles* tool (FIJI-win32)). The percental ratio of fluorescence between the lip and collar area was calculated for each MB calyx, whereas within one MB calyx both lip and collar were scanned with the same intensity of fluorescence that in turn compensates for possible differences in fluorescence intensity between preparations. The ratios in fluorescence of lip to collar for each ant were compared between the two groups (light foragers and reversed foragers).

All statistical analyses were performed with SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). After testing for normal distribution (One-Sample Kolmogorov-Smirnov Procedure, $p>0.05$) independent samples t-test were performed to test differences between light-treated and dark-kept ants (t-test for Equality of Means, $p<0.05$).

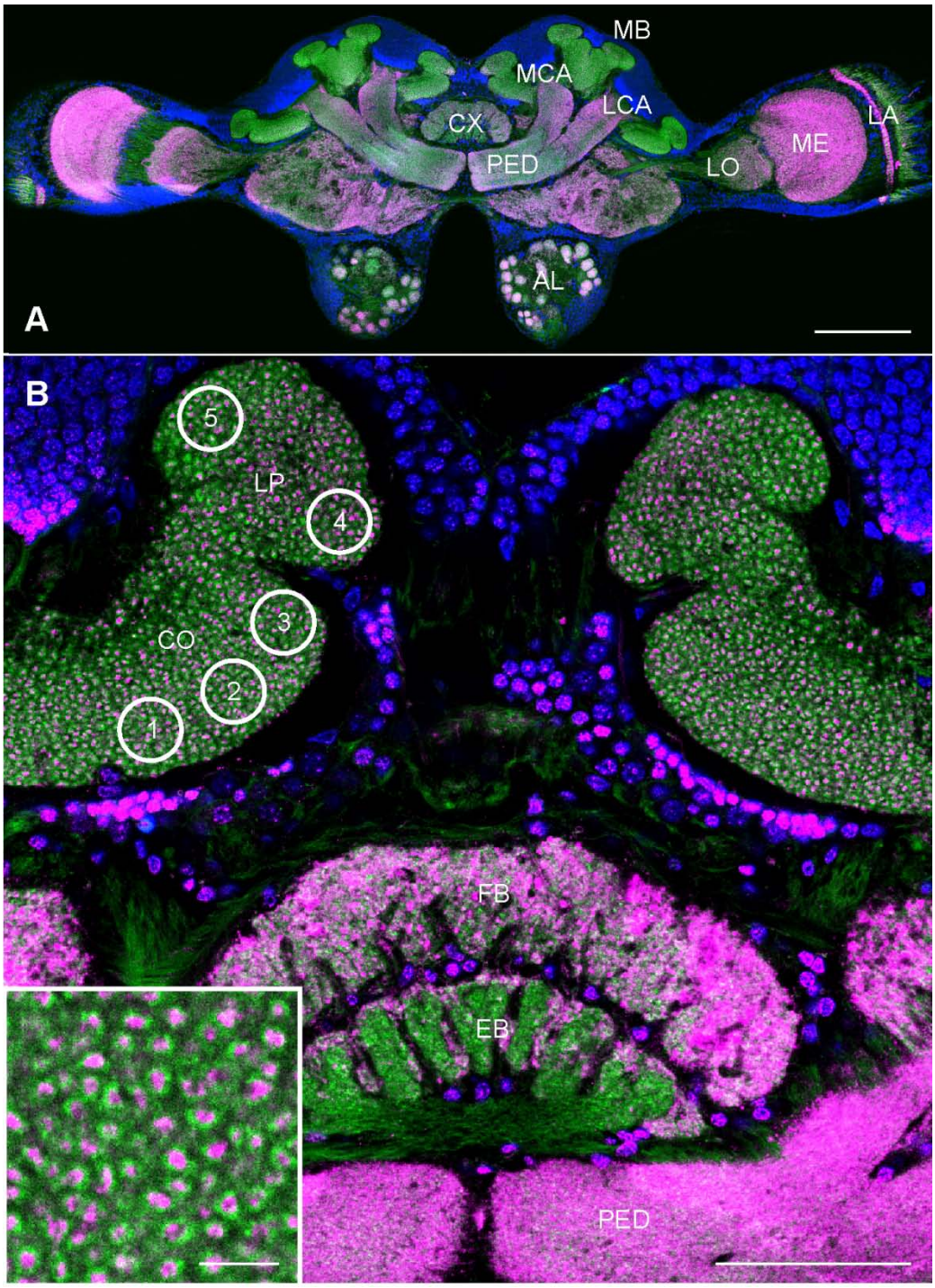

Figure 1. Immunofluorescence stainings of the *Cataglyphis fortis* brain. **A** Frontal overview of a brain (100 µm section) labeled with anti-synapsin (magenta), phalloidin-labeled f-actin (green) and Hoechst (blue) to highlight cell nuclei. Clearly visible are the mushroom bodies (MB) with medial calyces (MCA), lateral calyces (LCA) and pedunculus (PED), optic lobes with lamina (LA), medulla (ME) and lobula (LO), antennal lobes (AL) and the central complex (CX). **B** Magnification of the medial halves of the MCA used for the quantification of structural changes in MBs. Areas 1-3 represent the region used for quantification of the microglomeruli (MG) number in the visually innervated collar (CO) and areas 4 and 5 for quantification in the olfactory innervated lip (LP). Ellipsoid (EB) and fan-shaped body (FB) of the CX. Inset: Magnification of the CO with distinct visible MG. Scale bars: A 200µm, B 50µm, inset 5µm.

Premature Foraging Behavior

Experiments were conducted in 2009 and repeated in 2010. Freshly emerged callows were individually marked, separated from the colony and divided into two groups: a control group (dark callows: 23 ants in 2009 and 20 ants in 2010) and a light-exposed group (light callows: 23 ants in 2009 and 20 ants in 2010). The experiment as described above in the light treatment section started after one night of acclimatization. Whereas the control group remained in the dark, the experimental group was exposed to sunlight for four days. After the treatment both groups of callows were transferred back into the colony, which was kept in an artificial, darkened nest in the field. During the following two weeks all out-coming (foraging and non-foraging) ants were observed.

The mean temperature in the experimental boxes of both the light as well as the dark callows did not differ between the two groups and remained constant over time at a mean of 26.4 ± 2.1 °C in 2009 and 28.8 ± 3.4 °C in 2010. Light-exposure took place in a shaded area with mean light intensities of 9.03 (2009) and 12.77 (2010) $W \cdot m^{-2}$ for UVA, 0.27 (2009) and 0.33 (2010) $W \cdot m^{-2}$ for UVB, and 236.5 (2009) and 612.65 (2010) $W \cdot m^{-2}$ for PAR inside the box. The relative humidity fluctuated around 55-70% (2009, 2010).

Activity Recordings and Data Analysis

Activity of ants was recorded with a camcorder (Panasonic HDC SD300 in HD mode, 1920 x 1080 Pixel, fixed on a tripod with pivoting centre column). All experiments were performed in the field in Maharès under natural sunlight conditions. Freshly emerged callows were separated from the colony and divided into two groups: a control group (dark callows; 32 ants) and a light-exposed group (light callows; 24 ants). Due to the different emergence times of callows, both groups were further subdivided into smaller experimental groups consisting at each time of eight ants of the same age. The experiment as described above in the light treatment section started after one night of acclimatization. Whereas the control groups remained in the dark, the experimental groups were exposed to sunlight for five day.

Video-recordings of 15 min length of the light callows were performed during each light-exposure period and during each subsequent dark period under red light illumination (red light LEDs in approximately 1 meter distance to the experimental boxes) after an acclimatization time of 10 min to the changing light conditions. The age-matched dark callows were recorded for 15 min 5 times a day for four days in the dark under red light illumination and 5 times on the fifth day under sunlight. The latter recording of the dark callows under light-exposure together with the dark recordings of the light callows served as a control to exclude an age

effect as well as the potential direct influence of light (e.g. stress-related escape/running behavior) on the activity.

Due to the very time intensive data acquisition for activity measurements the data recorded on each day were analyzed only for the last light and dark phases. Activity of a single ant was defined by the total amount of time the ant moved within the 15-min recording time. The activity measurement only started when the ant moved for at least one ant-length and stopped as soon as the ant stopped moving. Grooming, trophallaxis, reciprocal antennating, and rotation movements were not included in the activity measurements. The overall activity in the different groups represents the sum of all activity recordings of the individual ants in each group.

The mean temperature in the experimental boxes of both the light as well as the dark callows did not differ between the two groups and remained constant over time at a mean of 28.8 ± 3.4 °C. Light-exposure took place in a shaded area with mean light intensities of 12.77 $W \cdot m^{-2}$ for UVA, 0.33 $W \cdot m^{-2}$ for UVB and 612.65 $W \cdot m^{-2}$ for PAR inside the box. The relative humidity fluctuated around 55-70 %.

All statistical analyses were performed with SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). After testing for normal distribution (One-Sample Kolmogorov-Smirnov Procedure, $p>0.05$; all data are not normally distributed), non-parametric tests were performed to test for differences between light-treated ants during light and dark phases and dark-kept ants for each recording day (Kruskal-Wallis-H-Test; $p<0.05$) and to test for differences between time-varied-light-exposed ants (Mann-Whitney-U-Test; $p<0.05$).

Exploration Runs

To test whether ants started foraging right on the first day of outdoor activity, ants leaving the nest for the first time were observed and recorded with a Panasonic HDC SD300 Camcorder in HD mode. The runs of these ants were recorded for up to three subsequent days, whereas single ants were individually marked to track them for the entire time. In order to measure the distance to the nest of out-coming ants, circles were marked into the sand with a 30, 50 and 70 cm radius centered at the nest entrance.

RESULTS

Effect of Short-Term Light-Exposure on Structural Synaptic Plasticity in the Mushroom Body Calyx

As shown in our previous study, 1-day foragers as well as interior II workers showed a significant reduction of MG numbers in the visual collar region compared to the early stages of interior workers (callows and interior I workers). Furthermore, callows with four days of visual exposure showed a similar decrease in MG numbers as found in foragers (Stieb et al., 2010). The question posed here is whether one day of light-exposure is sufficient to trigger structural changes in MB calyx MG. To answer this question callows were exposed to light for one day (1-d light callows) and compared to an age-matched dark-control group (1-d dark callows). This brief light-exposure of callows resulted in a tendency, albeit not statistically significant, to reduce MG numbers in the collar compared to those in the dark callows (see Fig. 2; unpaired t-test: $n_{(1\text{-d light callows})}=9$, $n_{(1\text{-d dark callows})}=7$, $p=0.255$). In the lip no differences in MG numbers were observed (unpaired t-test: Area 4: $p=0.936$; Area 5: $p=0.555$). To illustrate the course of the MG reduction in the collar region with increasing days of light input, we included the data of callows exposed for four days to light (4-d light callows) from Stieb et al. (2010) showing significant differences across all groups (Fig. 2). The dark callows were not statistically different from the 1-d light callows, but both the dark callows as well as 1-d light callows differed significantly from the 4-d light callows (One way ANOVA: $n_{(1\text{-d dark callows})}=7$, $n_{(1\text{-d light callows})}=9$, $n_{(4\text{-d light callows})}=8$, $p < 0.01$; post-hoc-test: 1-d light callows – 1-d dark callows: $p = 0.382$, 4-d light callows – 1-d dark callows: $p < 0.01$, 4-d light callows – 1-d light callows: $p < 0.01$ (Tukey- HSD)). Age-induced differences in the MG numbers of 1-d and 4-d light callows could be excluded by the fact that both dark callow groups (1- and 4-day old ants) did not differ (unpaired t-test: $n_{(1\text{-d dark callows})}=7$, $n_{(4\text{-d dark callows})}=7$, $p=0.085$). The results show that a light-exposure of one day is not sufficient to induce statistically significant structural changes in MG such as caused by a four day light treatment.

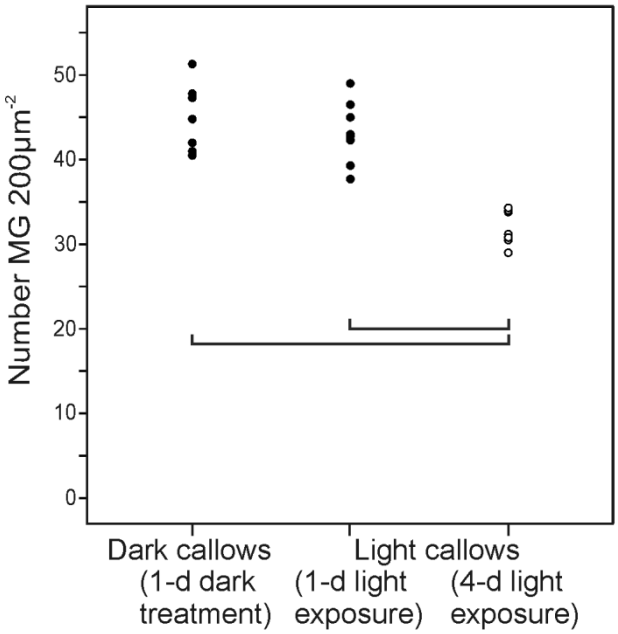

Figure 2. Effect of light exposure over different time periods on structural synaptic reorganization in the visually innervated collar (CO) of the mushroom body calyces in callows. Callows that were exposed to light for one day (light callows: 1-d light exposure) showed a tendency, albeit not statistically significant, to decrease microglomeruli (MG) numbers in comparison to age-matched dark-kept ants (dark callows: 1-d dark treatment). Callows that were exposed to light for four days (light callows: 4-d light exposure) showed a significant reduction in their MG numbers in comparison to both dark callows and the 1-d light-exposed callows. Data obtained from the 4-d light-exposed callows are from Stieb et al. (2010) and are indicated in the graph with open circles.

Reversibility of Structural Synaptic Plasticity in the Mushroom Body Calyx

Next we asked whether the light-induced structural plasticity at the transition from interior workers to foragers (Stieb et al., 2010) is reversible. To answer this question we transferred 1-day light experienced foragers back to darkness for three days (reversed foragers) and compared them to age-matched foragers with natural light experience (light foragers). Our previous study has shown that 1-day foragers do already have a significant decrease in their microglomerular numbers compared to young interior workers (callows and interior I; Stieb et al., 2010). The MG-numbers in the collar of the experimental groups were not significantly different (Fig. 3C: unpaired t-test: $n_{(reversed\ foragers)}=9$, $n_{(light\ foragers)}=9$; collar: $p=0.980$). When the MG numbers were extrapolated to the dense collar area, there were no significant differences in MG numbers between both groups (unpaired t-test: $p=0.342$). In contrast to the collar region, MG numbers of reversed foragers in the MB calyx lip were increased compared to those counted in the control light foragers (Fig. 3C: unpaired t-test: $p<0.05$ (Area 4); $p=0.093$ (Area 5)).

The particle analyses of synapsin-conjugated fluorescence revealed a higher concentration of synapsin conjugated fluorescence in the lip than in the collar region of both the 4-d light foragers (Fig. 3A: around 1.5 times higher) as well as the foragers being transferred back into darkness (Fig. 3B: around 2.3 times higher). Between both groups the ratios of synapsin conjugated fluorescence between the lip and collar differed significantly (Fig. 3D: unpaired t-test: $n_{(reversed\ foragers)}=9$, $n_{(light\ foragers)}=9$; collar: $p<0.05$). The control group of 1-day foragers revealed a similar synapsin concentration as recorded for the 4-d light-foragers (around 1.5 times higher in the lip than in the collar; n=5; data obtained from Stieb et al., 2010).

In summary, the light-induced structural reorganization of the MBs in foragers is affected by a subsequent visual deprivation. In the visual collar, light-induced structural plasticity of MG appeared to be not reversible after three days of visual deprivation back to the state of an interior worker. However, whereas the total number of MG in the collar remained similar for the light foragers and the reversed foragers, the intensity of synapsin was down-regulated in the visually deprived foragers. In the olfactory lip, a significant increase in the MG number occurred in foragers transferred back to darkness compared to those that remained further exposed to light.

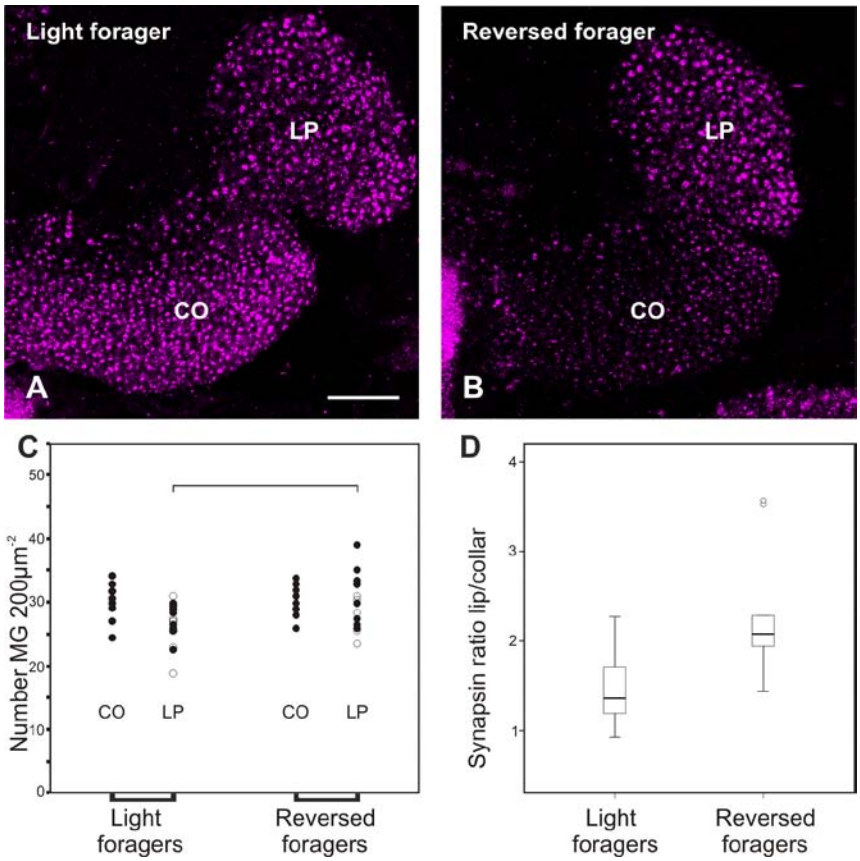

Figure 3. Reversibility of light-induced structural synaptic plasticity in the mushroom body calyces. **A, B** Mushroom body calyces labeled with anti-synapsin (magenta). To compare 4-day foragers with a natural light experience (A: light foragers), age-matched ants with one day of foraging experience subsequently were transferred back to darkness for three additional days (B: reversed foragers) indicate that the intensity of synapsin immunoreactivity (IR) in the collar (CO) is weaker of reversed foragers. In the lip (LP), synapsin IR appears to be equal in both groups. **C** Effect of visual deprivation on microglomeruli (MG) numbers. In the collar, the MG numbers of light and reversed foragers did not differ significantly, even when the MG numbers where extrapolated to the whole dense area of the collar. In the lip, visual deprivation resulted in an increase of MG both in area 4 (filled circles) and area 5 (open circles). **D** Effect of visual deprivation on the intensity of synapsin IR in MG of the lip and collar. Comparison of the ratios of synapsin IR as measured by the ratios in fluorescence intensities between the lip and collar in reversed and light foragers revealed that synapsin fluorescence intensity in the lip is 2.3 times higher as in the collar in the reversed foragers, whereas it is only 1.5 times higher in the light foragers. Both groups differed significantly in their lip-collar ratios of synapsin IR. Scale bar: A, B 20 µm; o=outliers in D (defined as values more than 3 interquartile ranges from the end of a box).

Foragers Receive Light Input Before their Actual Foraging Activity Starts

In total six ants leaving the nest for the first time could be caught, individually marked and released again. These ants were video-recorded on this and the following days. On the first day, all ants performed non-foraging exploration runs that did not go beyond 30 cm from the nest entrance and took a mean time of 26 sec. Typical examples are shown in Figure 4A. In addition, this behavior could also be recorded for some ants that had not been individually marked. Only ants determined and marked as foragers (see Methods) left the nest for more than about 30 cm. On day two, two marked ants ran around the nest entrance at a distance of less than 30 cm and a mean time of 32 sec (Fig. 4B), whereas three marked ants left the nest for a distance of more than 70 cm and actually brought back food to the nest. On day three, all marked ants performed foraging runs by leaving the nest for more than 70 cm (Fig. 4C). During the runs without any foraging behavior and during the first foraging runs, all ants ran in an apparently un-oriented/un-directed manner and showed up to 15 pirouette-like full turns during the exploration runs (arrows in Fig. 4). In contrast, experienced foragers always ran straight out of the nest and performed pirouettes only very rarely (Fig. 4C).

In addition to these first exploration runs, ants could be observed appearing from time to time at the nest entrance to deposit sand material (*diggers*). Due to their very short appearance outside the nest it was not possible to catch them for individual marking.

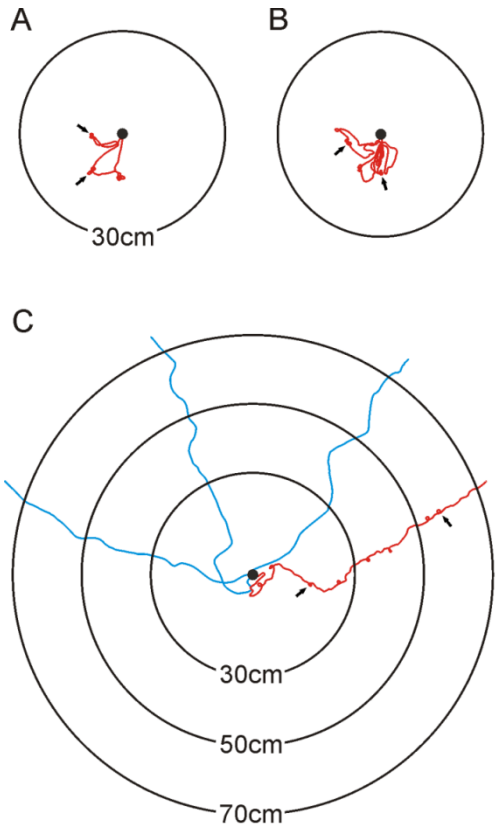

Figure 4. Examples of orientation (A, B) and foraging runs (C) of increasingly experienced ants. **A, B** During orientation runs without foraging activity on the first (A) and second day (B) of light experience ants stay within 30 cm of the nest entrance and perform pirouette-like turns (exemplary indicated by arrows). **C** Foraging runs (day 2 or 3) extend beyond 70 cm from the nest entrance. The first foraging runs (red line) are still circuitous and contain pirouette-like turns (indicated by arrows); experienced ants run straighter and faster (blue lines). All four foraging ants returned with a food item to the nest.

Effect of Precocious Light-Exposure on Premature Foraging Behavior

In order to test whether precocious light-exposure induces premature foraging behavior in contrast to a naturally occurring foraging onset after around 28 days (Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984), newly emerged callows were exposed to daylight for four days. After the treatment, these ants, together with ants of a dark control group, were transferred back into the colony. One light-exposed callow at the age of 17 days was observed performing 25 foraging runs on one day before it died. All other light-exposed callows died without external damages within two weeks.

Precocious Light-Exposure Boosts the Ants' Locomotor Activity

In accord to test whether precocious light-exposure has an effect on the ants' locomotor activity, newly emerged callows were exposed to a sunlight regime (see methods) for five days and their activity was video-recorded in the light (light callows in light) and in the dark (light callows in dark) and compared to a dark-control group (dark callows) for all experimental days. Whereas no difference or slight differences in the ants' activities between the three groups was recorded on the first three days, significant activity differences occurred on day four and five (Kruskal-Wallis-H-Test: day 1: $p=0.172$; day 2: $p=0.018$; day 3: $p=0.053$; day 4: $p<0.001$; day 5: $p<0.001$): the activity of the light callows strongly increased during light-exposure (Fig. 5A). In order to control for age-related effects as well as a direct influence of sunlight on the ants' activity, both groups (light and dark callows) were recorded on the 5^{th} day in dark (5-d light and 5-d dark callows in dark, respectively) and light (5-d light and 5-d dark callows in light, respectively) conditions: whereas no differences in the ants' activity could be recorded in dark phases (Mann-Whitney-U-Test: $p=0.462$), the activity of the same ants during light-exposure was significantly higher in the group of ants that had already 5 days of light experience (light callows) than in ants which had been exposed to light for just one day (Fig. 5B: Mann-Whitney-U-Test: $p<0.05$).

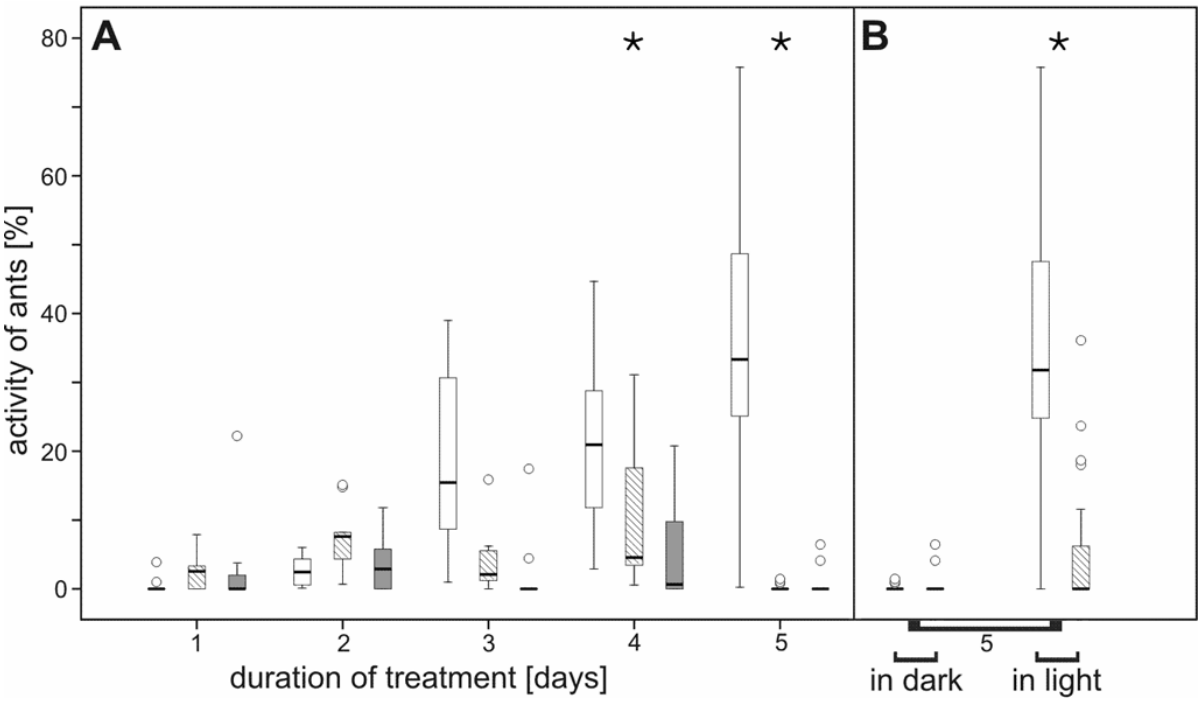

Figure 5. Effect of light on the ants' behavioral activity. **A** Walking activity of light-exposed callows (n=24) during light and dark phases in comparison to a dark-control group (n=32) over five subsequent days. Callows that were exposed to sunlight for five days in a row (white bars) increased their activity during the light phases after three days, compared to the same group of callows under dark conditions (striped bars) and a control group that was completely kept in darkness (grey bars). **B** Effect of the duration of light exposure on the ants' activity. In light conditions, light-exposed callows (white bars) being exposed to light for 5 days showed a significant higher activity on day 5 than age-matched ants that were only exposed to light for 1 day (grey bars). During the dark phases, their activity did not differ. o=outliers (defined as values more than 3 interquartile ranges from the end of a box). Asterisks indicate highly significant differences between groups.

DISCUSSION

Light Exposure Affects Neuronal Networks in the Mushroom Bodies and Behavioral Aspects of Foraging Behavior

The results show that a brief light-exposure over only 1-day is not sufficient to induce structural synaptic plasticity compared with a 4-day light treatment as shown earlier (Stieb et al., 2010). After 1-day of light exposure callows showed a slight tendency of MG reorganization in the visual MB-calyx. Structural reorganization of synaptic complexes in the visual region of the MB calyx was also reported during the natural transition from interior workers to 1-day foragers (Stieb et al., 2010). Furthermore, the previous study has shown that significant synaptic reorganization (MG pruning and KC dendritic expansion) occurs as early as in the interior II worker state. As similar effects can be induced by light-exposure over four consecutive days, in callows as well as in dark-kept ants of different ages (Stieb et al., 2010), we conclude that synaptic reorganization at the transition from interior workers to foragers is

not determined by a strict age-dependent internal program. This raises the question whether advanced (late stage) interior II workers may gain visual experience even before they start foraging.

Whereas interior I workers behave in a very sluggish manner and often stick motionless to the walls inside the nest chambers, interior II workers are more active, run around and may be on their way to become *diggers* carrying sand particles out of the nest to deposit them near the nest entrance and, by this, get exposed to light (Wehner et al., 1983). Our current behavioral observations in the natural habitat confirm these earlier indications and the hypothesis derived from them. Moreover, the recorded trajectories of ants being on their way of becoming foragers demonstrate that the ants perform exploration runs including pirouette-like turns very close to the nest entrance for a period of up to two days, before they actually start their foraging activity (Fig. 4). We therefore conclude that the workers previously referred to as "1-day foragers" had already gained light experience over more than one day, and synaptic reorganization had thus proceeded before these ants actually started their foraging runs. This conclusion is supported by the fact that in a significant proportion of interior II workers the collar exhibited a significant reduction in MG numbers (Stieb et al., 2010). In honeybees, a change in MG numbers is associated with olfactory stable long-term memory three days after associative learning had occurred (Hourcade et al., 2010). In *Cataglyphis*, the same amount of time is necessary to induce sensory-triggered synaptic and behavioral changes. The fact that both the light-induced neuronal changes in visual brain centers and the rise in the ants' locomotor activity occur between one and four days of light-exposure suggests that there is a link between structural synaptic plasticity in the MB calyx and the behavioral transition from interior tasks to outdoor foraging (Fig. 2; 5A). This suggestion in turn raises the question whether precocious light exposure can induce premature foraging behavior. Unfortunately the experiment to test this hypothesis failed because all light exposed ants died within two weeks. A possible reason for this high mortality might be the heat stress to which callows had been exposed. Even foragers die within around six days of foraging (Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984; and under natural conditions predatory stress adds to heat stress). The fact that ants which had not been pre-exposed to light did not perform premature foraging behavior, and that one pre-exposed ant prematurely performed foraging runs might lend support to the hypothesis that light exposure can indeed promote subsequent foraging behavior. In any way, the present study clearly demonstrates an enhancement of the ants' locomotor activity after light pulses. Obviously there is a link between pre-forager light-sampling behavior and an increase in the locomotor activity required for foraging. In

conclusion, in the sequence of behavioral transitions during an individual's life cycle the transition from the interior II worker stage to the *digger* stage directs the ants to the nest entrance where they are exposed to daylight for the first time. Later on the ants perform orientation runs exposing themselves to light for even longer periods. Thence, the system seems to be a self-reinforcing process: once the ants get light input, their locomotor activity is enhanced and their neuronal network is restructured in preparation of their upcoming tasks as foragers. In honeybees, the internal or age-based program forming the basis of the behavioral transition from interior workers to foragers has been shown to be associated with a rise in the circulating levels of juvenile hormone (Robinson et al., 1989; Robinson, 1992; Fahrbach, 1997), which is highest in foragers (Robinson et al., 1989; Huang et al., 1991, 1994; Fahrbach, 1997).

A behavior identical to the orientation runs in *C. fortis* has been reported for *C. bicolor* (Wehner et al., 2004). Both species, *C. bicolor* and *C. fortis*, take short-lasting exploration runs during which the ants move in a number of loops around the nest entrance and show no foraging activity. During these exploration runs as well as during the first foraging runs, ants of either species (Wehner et al., 2004) perform rotation movements (pirouettes) comparable to the 360° rotations at the beginning of long-distance runs in *C. bombycina* (Wehner and Wehner, 1990; Wehner, 1994) and the 360° pirouettes occurring during the spiral-like "learning walks" reported for the Namibian desert ant *Ocymyrmex robustior* when learning new landmarks around the nest entrance (Müller and Wehner, 2010). Furthermore, honeybees (Vollbehr, 1975; Zeil et al., 1996) and wasps (Zeil, 1993; Zeil et al., 1996) show orientation flights during which they perform turning loops to learn the nest location and nest-surrounding landmarks. Honeybees perform a variable number of orientation flights before they become foragers. During these orientation flights they fly increasingly faster, further and straighter (Capaldi et al., 2000). This compares to our observations that the ants performed orientation runs for up to two days, and that the runs increased in distance, straightness and speed in the course of the ants becoming foragers (personal observations). It is likely that in *C. fortis* the orientation runs might also serve as learning runs to memorize the nest entrance and nest surroundings.

In summary, during their "orientation runs" the ants might associate the nest entrance with specific landmarks or get pre-exposed (entrained) to other visual information like the polarization pattern required for path integration, and, concomitantly adapt their neuronal circuitries to the upcoming challenges. A previous study in cockroaches suggests that the MBs are required for place memory based on visual landmarks (Mizunami et al., 1998). The

present study shows that visual exposure raises the ants' locomotor activity and, at the same time, induces synaptic reorganization in visual regions of the MB calyces (Stieb et al., 2010). We therefore hypothesize that associated changes in the neuronal circuitry in the MBs are necessary to improve or prepare the ants' visual orientation capabilities. This needs to be thoroughly tested in future knockdown experiments of certain components that control structural plasticity in the MB-calyx synaptic microcircuits.

Reversibility of Structural Synaptic Plasticity in the Mushroom Body Calyces

Environmental events leading to a reversal of foragers back to the interior worker stage are reported for honeybees (for a review see Robinson, 1992). For *Cataglyphis* it was shown already three decades ago that the stages of interior workers and foragers can be reversed by altering the light conditions to which the ants are exposed (Wehner et al., 1972). In a choice situation presenting dark and white surfaces, interior workers frequently chose the dark silhouettes, whereas foragers oriented towards the white ones. However, if interior workers kept in illumination conditions characteristic for foragers, they reversed their preferences after 24 hours to 40 % and after 72 hours to 100 %. Foragers kept in darkness also reversed their orientation behavior, but much slower: after 24 hours no reversed behavior was observed; after 120 hours some of the ants still oriented towards the white surfaces. These behavioral findings (Wehner et al., 1972) correlate with our present results in so far as synaptic changes occur in the MB collar after a 4-day light-treatment of dark-kept interior workers (Stieb et al., 2010). However, light-induced foragers transferred back to darkness exhibit only some reverse-type characteristics. Whereas in the reversed foragers the expected increase in MG numbers occur in the olfactory lip region, in the visual collar region no changes are observed (Fig. 3C). However, what does occur in the MG of the reversed foragers is a significant decrease in synapsin immunoreactivities (IR; Fig. 3B, D) when compared with age-matched light-exposed foragers. The fact that 1-day foragers (data obtained from Stieb et al., 2010) have similar intensity ratios in synapsin IR as recorded in 4-day light-exposed foragers indicates that not the time of light-exposure but rather the situation of being transferred back to darkness affects the amount of synapsin expression. Reduction of the amount of synapsin in the terminals of the presynaptic projection neurons is likely to affect synaptic transmission and aspects of synaptic strength (Michels et al., 2011). Our staining techniques using f-actin phalloidin labeling of KC dendrites did not indicate major changes in postsynaptic profiles. A previous study in the honeybee has shown that the reversal of honeybee foragers, either due to the onset of winter or following experimental manipulation of the colony structure, does not reduce the MB volume (Fahrbach et al., 2003). This is in accordance with our present findings

and is supported by the results that changes in the KC dendritic network are a major factor in MB calyx volumetric changes (for honeybees Farris et al., 2001; for *Cataglyphis* Stieb et al., 2010). The difference in the olfactory and visual compartments of the MB calyces can most likely be explained by the fact that processing of olfactory information is important for foragers and reversed foragers during both foraging and accomplishing intranidal tasks, whereas visually mediated activities become less important in the dark. This might explain why reversed foragers exhibit a weaker amount of synapsin expression in the visual region of the MB calyx that might be caused by a lack of continued sensory input. We cannot exclude that longer extension of dark treatment of reversed-foragers may eventually affect the dendritic network as well. However, this seems rather unlikely as the MB calyx volume was shown to be not affected, even after longer periods of dark treatment (Fahrbach et al., 2003).

In conclusion our results demonstrate that structural changes in the synaptic organization of the MB calyces that occur during the transition from interior workers to foragers are reversible and hence indicate that the MB circuitries are plastic in both directions. This high degree of neuronal plasticity provides ideal conditions for underpinning the ants' high behavioral plasticity in response to changing environmental conditions and social requirements.

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REFERENCES

Calabi P, Traniello JFA. 1989. Behavioral flexibility in age castes of the ant *Pheidole dentata*. J Insect Behav 2: 663-677.

Capaldi EA, Smith AD, Osborne JL, Fahrbach SE, Farris SM, Reynolds DR, Edwards AN, Martin A, Robinson GE, Poppy GM, Riley JR. 2000. Ontogeny of orientation flight in the honeybee revealed by harmonic radar. Nature 403: 537-540.

Davis RL. 2005. Olfactory memory formation in *Drosophila*: from molecular to systems neuroscience. Annu Rev Neurosci 28: 275-302.

Dobrin SE, Herlihy JD, Robinson GE, Fahrbach SE. 2011. Muscarinic regulation of Kenyon cell dendritic arborizations in adult worker honey bees. Arthropod Struct Dev (in press).

Fahrbach SE. 1997. Regulation of age polyethism in bees and wasps by juvenile hormone. Adv Stud Behav 26: 285–316.

Fahrbach SE, Farris SM, Sullivan JP, Robinson GE. 2003. Limits on volume changes in the mushroom bodies of the honey bee brain. J Neurobiol 57: 141-151.

Farris SM, Robinson GE, Fahrbach SE. 2001. Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. J Neurosci 21: 6395-6404.

Farris SM, Sinakevitch I. 2003. Development and evolution of the insect mushroom bodies: towards the understanding of conserved developmental mechanisms in a higher brain center. Arthropod Struct Dev 32: 79-101.

Frambach I, Rössler W, Winkler M, Schürmann F-W. 2004. F-actin at identified synapses in the mushroom body neuropil of the insect brain. J Comp Neurol 475: 303-314.

Ganeshina O, Menzel R. 2001. GABA-immunoreactive neurons in the mushroom bodies of the honeybee: an electron microscopic study. J Comp Neurol 437: 335-349.

Gerber B, Tanimoto H, Heisenberg M. 2004. An engram found? Evaluating the evidence from fruit flies. Curr Opin Neurobiol 14: 737-744.

Giurfa M. 2007. Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. J Comp Physiol 193: 801-824.

Groh C, Tautz J, Rössler W. 2004. Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. P Natl Acad Sci USA 101: 4268-4273.

Groh C, Ahrens D, Rössler W. 2006. Environment- and age-dependent plasticity of synaptic complexes in the mushroom bodies of honeybee queens. Brain Behav Evolut 68: 1-14.

Gronenberg, W. 2001. Subdivisions of hymenopteran mushroom body calyces by their afferent supply. J Comp Neurol 436: 474-489.

Huang Z-Y, Robinson GE, Borst DW. 1994. Physiological correlates of division of labor among similarly aged honey bees. J Comp Physiol 174: 731-739.

Hölldobler B, Wilson, EO. 1990. The Ants. Cambridge, MA: Belknap Press.

Hourcade B, Muenz TS, 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? J Neurosci 30: 6461-6465.

Huang Z-Y, Robinson GE, Tobe SS, Yagi KJ, Strambi C, Strambi A, Stay B. 1991. Hormonal regulation of behavioural development in the honey bee is based on changes in the rate of juvenile hormone biosynthesis. J Insect Physiol 37: 733-741.

Klagges BRE, Heimbeck G, Godenschwege TA, Hofbauer A, Pflugfelder GO, Reifegerste R, Reisch D, Schaupp M, Buchner S, Buchner E. 1996. Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila*. J Neurosci 16: 3154-3165.

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

Leiss F, Groh C, Butcher NJ, Meinertzhagen IA, Tavosanis G. 2009. Synaptic organization in the adult *Drosophila* mushroom body calyx. J Comp Neurol 517: 808-824.

Menzel R. 1999. Memory dynamics in the honeybee. J Comp Physiol 185: 323-340.

Michels B, Chen Y-C, Saumweber T, Mishra D, Tanimoto H, Schmid B, Engmann O, Gerber B. 2011. Cellular site and molecular mode of synapsin action in associative learning. Learn Memory 185: 332-344.

Mizunami M, Weibrecht JM, Strausfeld NJ. 1998. Mushroom bodies of the cockroach: their participation in place memory. J Comp Neurol 402: 520-537.

Mobbs PG. 1982. The brain of the honeybee *Apis mellifera*. I. The connections and spatial organization of the mushroom bodies. Philos T R Soc Lond 298 :309-354.

Mote MI, Wehner R. 1980. Functional characteristics of photoreceptors in the compound eye and ocellus of the desert ant, *Cataglyphis bicolor*. J Comp Physiol 137: 63-71.

Müller M, Wehner R. 2010. Path integration provides a scaffold for landmark learning in desert ants. Curr Biol 20: 1-4.

Robinson GE. 1992. Regulation of division of labor in insect societies. Annu Rev Entomol 37: 637-665.

Robinson GE, Page RE, Strambi C, Strambi A. 1989. Hormonal and genetic control of behavioral integration in honey bee colonies. Science 246: 109-112.

Rössler W, Kuduz J, Schürmann FW, Schild D. 2002. Aggregation of f-actin in olfactory glomeruli: a common feature of glomeruli across phyla. Chem Senses, 27: 803-810.

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

Seid MA, Wehner R. 2008. Ultrastructure and synaptic differences of the boutons of the projection neurons between the lip and collar regions of the mushroom bodies in the ant, *Cataglyphis albicans.* J Comp Neurol 507: 1102-1108.

Seid MA, Wehner R. 2009. Delayed axonal pruning in the ant brain: a study of developmental trajectories. Dev neurobiol 69: 1-15.

Sorensen AA, Busch TM, Vinson SB. 1984. Behavioral flexibility of temporal subcastes in the fire ant, *Solenopsis invicta*, in response to food. Psyche 91: 319-332

Steiger U. 1967. Über den Feinbau des Neuropils im Corpus pedunculatum der Waldameise. Z Zellforsch 81: 511–536.

Stieb SM, Muenz TS, Wehner R, Rössler W. 2010. Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. Dev Neurobiol 70: 408-423.

Strausfeld NJ, Hansen L, Li Y, Gomez RS, Ito K. 1998. Evolution, discovery, and interpretations of arthropod mushroom bodies. Learn Memory 5: 11-37.

Vollbehr, J. 1975. Zur Orientierung junger Honigbienen bei ihrem ersten Orientierungsflug. Zool Jb Allg Zool Physiol 79: 33–69.

Wehner R. 1987. Spatial organization of foraging behavior in individually searching desert ants, *Cataglyphis* (Sahara Desert) and *Ocymyrmex* (Namib Desert). In: From individual to collective behavior in social insects (eds. Pasteels JM, Deneubourg JL). Basel: Birkhäuser, pp 15-42.

Wehner R. 1994. The polarization-vision project: championing organismic biology. Forts Zool 39: 103-143.

Wehner R. 2003. Desert ant navigation: how miniature brains solve complex tasks. J Comp Physiol 189: 579-588.

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

Wehner R, Wehner S. 1990. Insect navigation: use of maps or Ariadne's thread? Ethol Ecol Evol 2: 27-48.

Wehner R, Herrling PL, Brunnert A, Klein R. 1972. Periphere Adaptation und zentralnervöse Umstimmung im optischen System von *Cataglyphis bicolor* (Formicidae, Hymenoptera). Rev Suisse Zool 79: 197–228.

Wehner R, Harkness RD, Schmid-Hempel P. 1983. In: Foraging strategies in individually searching ants *Cataglyphis bicolor* (Hymenoptera: Formicidae). Akad Wiss Lit Mainz Math-Naturwiss KI. Stuttgart: Gustav Fischer .

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

Yusuyama K, Meinertzhagen IA, Schürmann F-W. 2002. Synaptic organization of the mushroom body calyx in *Drosophila melanogaster*. J Comp Neurol 445: 211-226.

Zeil J. 1993. Orientation flights of solitary wasps (*Cerceris*; Spheeidae; Hymenoptera) II . Similarities between orientation and return flights and the use of motion parallax. J Comp Physiol 172: 207-222.

Zeil J, Kelber A, Voss R. 1996. Structure and function of learning flights in bees and wasps. J Exp Biol 199: 245-252.

MANUSCRIPT III

Plasticity in Giant Synaptic Complexes of the Lateral Accessory Lobe - a Possible Relay Station in the Polarization Pathway in the Brain of the Desert Ant *Cataglyphis fortis*.

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ABSTRACT

Desert ants of the genus *Cataglyphis* undergo an age-related polyethism from interior workers to short-lived foragers with remarkable visual navigation capabilities. As their main navigational means, solitary foragers use a path integration system including a skylight-based visual compass and a stride-integrating odometer. While this enormous navigational behavior is well described, the central neural pathways in the *Cataglyphis* brain are largely unknown. The quick transition from dark to light suggests that visual centers in the ant's brain express a high degree of plasticity.

To investigate the structure of large synaptic complexes in the lateral accessory lobe – a possible relay station in the polarization pathway – and detect potential structural synaptic plasticity that might be associated with the dramatic changes in behavior, pre- and postsynaptic profiles of these synaptic complexes in naïve callows and experienced foragers were immunolabeled and quantified by using confocal imaging and 3D-reconstruction. Tracer injections were used to identify input and output tracts of the lateral accessory lobe. The results show that these complexes consist of postsynaptic processes located in a central region that is surrounded by a cup-like presynaptic profile. The large presynaptic terminals are formed by neurons of the tubercle-accessory lobe tract that have dendritic ramifications in the anterior optic tubercle. Tangential neurons forming the dendritic complexes in the lateral accessory lobe project to the ellipsoid body of the central complex and are GABAergic. The behavioral transition from interior workers to foraging is associated with an increase in the number of these synaptic complexes by ~13%. An outgrowth of additional synaptic complexes at the transition to foraging suggests some sort of calibration process in these potentially strong synaptic contacts which are likely to deliver fast and reliable signal transmission in the polarization vision pathway.

INTRODUCTION

Desert ants of the genus *Cataglyphis* have become model systems for the study of insect navigation (Wehner, 2003). Most striking is the orientational behavior of *C. fortis* that is native to featureless salt pans in North Africa. Solitary, central place foragers cover distances up to more than hundred meters until they find food before they precisely and rapidly return in a straight line back to their often inconspicuous nest entrance. The ants accomplish this enormous navigational performance by using a path integration system as their main navigational means (for a review, see Wehner and Srinivasan, 2003) in addition to landmark-dependent orientation (Wehner and Räber, 1979; Collett et al., 1992; Bisch-Knaden and Wehner, 2003; Collett and Collett, 2009) and olfactory cues (Wolf and Wehner, 2000**;** Steck et al., 2009, 2010). The path integrating system (for a review, see Wehner and Srinivasan, 2003) includes a skylight-based visual compass (Wehner and Müller, 2006) and a stride-integrating odometer (Wittlinger et al., 2006). Information about the direction is achieved by skylight information including the azimuthal position of the sun, spectral gradients in the sky (reviewed in Wehner, 1994), but most dominantly the pattern of polarized light in the sky (Wehner et al., 1996; Wehner and Müller 2006).

Cataglyphis, like many insect species including locusts, crickets, bees, butterflies, beetles as well as other ant species possesses a special region in its compound eyes that is termed dorsal rim area (DRA) composed of ommatidia that are particularly adapted for polarization sensitivity (reviewed in Labhart and Meyer, 1999). In *Catagylphis* ants, medulla neurons being sensitive to polarized light have been identified, but unfortunately neither physiological details nor morphological reconstruction are available (Labhart, 2000). Information about central neuronal pathways and processing mechanisms of polarization information has mostly been investigated in the larger cricket *Gryllus campestris* and locust *Schistocerca gregaria* (reviewed in Labhart and Meyer, 2002; Homberg, 2004; Homberg et al., 2011). In the locust brain, the entire polarization-vision pathway from the DRA to the central complex (CX) has been analyzed (reviewed in Homberg, 2004; Homberg et al., 2011). One relay station within the polarization vision pathway is the lateral accessory lobe (LAL) forming connections between projection neurons of the anterior optic tubercle (AOTU) and tangential neurons of the CX (Pfeiffer et al., 2005). The LAL encompasses extremely large synaptic complexes consisting of postsynaptic processes located in a central region that is surrounded by a cup like presynaptic profile (Träger et al., 2008). The size of these complexes suggests particularly strong synaptic contacts with fast and reliable signal transmission. Similar large

synaptic complexes have also been described in moths (Homberg et al., 1990) and in honeybees (Mota et al., 2011).

Cataglyphis not only is a model system for insect navigation based on predominantly visual cues but also for studying neuronal adult plasticity in visual brain centers, since visual input only becomes especially important as the ants start long-distance foraging trips. An age-related polyethism subdivides *Cataglyphis* colonies into interior workers fulfilling tasks like brood care and food processing and day-active foragers covering only a small fraction of the entire life cycle (*C. bicolor*: Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984). The sensory environment and motor demands of these ants change dramatically within the behavioral transition. Indeed, it has been shown for visual centers possibly involved in landmark learning that the behavioral transition is correlated with volumetric (Kühn-Bühlmann and Wehner, 2006) and structural (Seid and Wehner, 2009; Stieb at al., 2010) synaptic changes that can be triggered by visual experience throughout the natural life-span (Stieb et al., 2010). This demonstrates the enormous plastic capability of the adult neuronal network of insects.

In the present study we identify input and output tracts of the LAL synaptic complexes by ionotophoretic tracer applications in potential relay station of the polarization pathway based on the locust model (reviewed in Homberg, 2004; Homberg et al., 2011). Furthermnore, we analyze the synaptic structure of LAL-neurons in the polarization vision patwhay and their potential structural synaptic plasticity in *C. fortis* that might be associated with the dramatic changes in the ants' behavior during the transition from the indoor to the outdoor stage. To visualize the synaptic structure and detect potential changes, we labeled pre-and postsynaptic profiles of the LAL synaptic complexes of interior workers and foragers. Changes were quantified using confocal microscopy analysis and 3D-reconstructions.

METHODS

Animals

Experiments were conducted at the Maharès study site in Tunisia in June/July 2010 and in the Würzburg laboratory with workers of different stages belonging to the species *C. fortis*. During the lifetime of the workers the following ethocaste stages occur one after another: callows (freshly emerged workers not more than about 24 hours old), interior I workers (characterized by their motionless state of behavior, their swollen gaster and the expanded whitish intersegmental membranes of the gaster), interior II workers (the remaining inside workers) and foragers (actively searching for food outside the nest).

Two queen-right colonies were excavated in a salt-pan near Menzel Chaker, Tunisia, in June 2010 (34°58'N, 10°25'E) and then kept in complete darkness. This treatment guaranteed that newly emerging callows were raised in complete darkness. To get access to age-marked foragers (the amount of days an ant has been foraging), all foragers of one colony in the salt pan were marked during three subsequent days. This procedure guaranteed that on the fourth day all unmarked foragers (ants returning to the nest with food) were foraging for the first day. These newly outcoming foragers were color coded for each subsequent day five days in a row. On the late afternoon of the fifth day, all foragers having 5 days of foraging experience (5-day foragers) were collected for subsequent experiments. Brains of callows and 5-day foragers were dissected immediately and put into fixative solution before they were transferred to the Würzburg laboratory for further histochemical procedures (see Methods below). The immediate onsite dissection guaranteed that callows were not older than 24 h and foragers were dissected right after 5 days of visual experience.

Finally, age-undefined ants from a fourth colony collected in July 2008 and held since then in the Würzburg laboratory were used for ionotophoretic tracer applications (see Methods below).

Iontophoretic Tracer Injection

To trace the sensory tracts of incoming and outgoing neurons of the LAL, iontophoretic dye injections were conducted in *C. fortis* workers (n = 2). The ants were immobilized in a plexiglass holder and fixed with dental wax. The head capsule was opened by cutting a square window in-between the compound eyes, covered with fresh ant saline solution (127 m M NaCl, 7 m M KCl, 1.5 m M CaCl 2, 0.8 m M Na 2 HPO 4, 0.4 m M KH 2 PO 4, 4.8 m M TES, and 3.2 m M trehalose, pH 7.0), and glands and tracheae were gently removed. A stainless silber platform was inserted under the brain and served as a stabilizer and ground electrode. The microelectrodes with a resistance of 10–125 MΩ in the tissue were inserted into the AOTU. Microelectrodes were pulled from glass capillaries (Kwik-Fil™, World Precision-Instruments Inc., Sarasota, USA), containing a micro-filament, with a DMZ Universal puller (Zeitz Instruments GmbH, Germany). The microelectrode tips were filled with N-(2 aminoethyl)biotinamide hydrochloride (Neurobiotin™; A1593 Molecular Probes, Eugene, Oreg., USA) dissolved in 1 M potassium chloride at 5 %. The shank was filled with 0.5 M potassium chloride solution. The tracer was injected iontophoretically into the cell with constant depolarizing current (20-60 nA, 10–15 min, Neurobiotin). Signals were amplified with a Neuroprobe Amplifier 1600 (AM-Systems Inc., Washington, USA), controlled by a harmonic oscillator (SFG-2004, Good Will Instrument Co., Ltd., New Taipei City, Taiwan),

and monitored with an audiomonitor and a digital oscilloscope (Hameg HM 205–3; Hameg, Frankfurt/Main, Germany) for visualization. Following the injection, the animal was placed in a moist chamber for up to 4 hours guaranteeing that the dye is transported along the neuron.

Immunocytochemistry

To trace the projections from and to the LAL, neurobiotin-filled neurons were visualized with fluorochrome-coupled antibodies. The brains were dissected (with the ant being still in the Plexiglas holder) in saline solution followed by fixation in cold 4 % formaldehyde in phosphate-buffered saline (PBS, pH 7.2) for 4-5 days. After being rinsed in PBS (3 x, 10 min), whole brains were pre-incubated in PBS containing 2% Triton X-100 (PBST) for 1 hour. To label neurobiotin, brains were incubated in Strepdavidin Alexa 488 (1:250, MoBiTec, AZS11223) in 500 µl PBST for 1 to 5 days. The brains were then rinsed in PBS (3 x, 10 min), dehydrated in an ascending series of ethanol (50, 70, 80, 90, 95, and 3 x 100%, 10 min each), and finally transferred into methylsalicylic acid (M-2047; Sigma-Aldrich, Steinheim, Germany).

To analyze the structure of synaptic organization in the LAL of callows and 5-day foragers, synaptic complexes were visualized by double-labeling with an antibody to synapsin and fluorescently labeled phalloidin binding to f-actin using the method introduced by Groh et al. (2004) (Fig. 2). The ants were anaesthetized with CO_2, decapitated, and the head capsules were fixed in dental-wax coated dishes. The head capsule was covered with fresh ant-saline solution and opened by cutting a square window in between the compound eyes. Glands and tracheae were gently removed, and the brains were dissected out and fixed immediately in cold 4 % formaldehyde in PBS overnight at 4 °C. Brains were then rinsed in PBS (3 x 10 min). After embedding in 5 % low melting point agarose (Agarose II, no. 210–815, Amresco, Solon, OH, USA), brains were carefully adjusted in a frontal plane and sectioned at 100 µm thickness with a vibrating microtome (Leica VT 1000S, Nussloch, Germany). Free-floating agarose sections were pre-incubated in PBS containing 0.2 % PBST and 2 % normal goat serum (NGS, 005-000-121, Jackson ImmunoResearch Laboratories) for 1 hour at room temperature. To label neuronal f-actin, sections were incubated in 0.2 units of Alexa Fluor 488 phalloidin (Molecular Probes, A12379) in 500 µl PBST with 2 % NGS for three days at 4 °C (Rössler et al., 2002; Groh et al., 2004). For double-labeling, sections were simultaneously incubated with either a monoclonal antibody to the *Drosophila* synaptic-vesicle-associated protein synapsin I (1:50, SYNORF1, kindly provided by E. Buchner, University of Würzburg, Germany) (Klagges et al., 1996) or with a rabbit anti GAD1 (glutamic acid decarboxylase) antibody (1:1000, kindly provided by Rob Jackson, Tufts University, USA). After five rinses

in PBS, double-labeled preparations were incubated in Alexa Fluor 568-conjugated goat anti-mouse respectively anti-rabbit secondary antibody (1:250, Molecular Probes, A11004) in 1 % NGS/PBS for 2 hours at room temperature to visualize general distribution of synapsin or GABA (γ-aminobutyric acid). To label cell nuclei, sections were incubated for 15 min in 2.5 mg/ml Hoechst 34580 (1:500; Molecular Probes, H21468) in PBST at room temperature. Sections were finally washed in at least five changes of PBS, transferred into 60 % glycerol/PBS for 30 min, and mounted on slides in 80 % glycerol/PBS.

Laser Scanning Confocal Microscopy, Image Processing and Data Analysis

Confocal images and image stacks with an optical thickness of 0.5 µm were taken at a resolution of 1,024 x 1,024 pixels from the LAL in whole mount preparations and in sections using a laser-scanning confocal microscope (Leica TCS SP2 AOBS, 20 x 0.7 NA and 63 x 1.20 NA lens; Leica Microsystems AG, Wetzlar, Germany). Excitation wavelengths were 568 nm for synapsin or GAD1, 488 nm for Alexa Fluor 488 and Strepdavidin Alexa 488 and 405 nm for Hoechst 34580. In double- or triple-labeled preparations, the different channels were merged with the use of pseudocolors. Images were further processed with FIJI-win32 (ImageJ 1.44c, Wayne Rasband, NIH, Bethesda, MD), and Corel Draw X3 software (Corel Corporation, Ottawa, ON, Canada). 3D-software (AMIRA 3.1.1; Mercury Computer Systems, Berlin, Germany) was used for reconstruction of the phalloidin-profile of individual synaptic complexes within the LAL and parts of the CX. The total number of all synaptic complexes was assessed by complete reconstructions in 9 callows and 10 foragers.

All statistical analyses were performed with SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Non-parametric tests were performed to test for differences between callows and 5-day foragers (Mann-Whitney-U-Test; $p<0.05$).

RESULTS

Structure of Synaptic Complexes in the Lateral Accessory Lobe

Tracer injections into the AOTU revealed output fibers that form the tubercle-accessory lobe tract (TALT) and target in the medial protocerebrum (Fig. 1A). The TALT runs laterally around the ipsilateral vertical lobe (Fig. 1A-2-4) before entering the LAL from rostral (Fig. 1A-5-7) where the neuronal endings form distinct arborizations patterns (Fig. 1A-7). The similarity of the TALT neurons with honeybee TALT neurons (Mota et al., 2011) both showing no visible varicosities inside the AOTU and both following the same pathway suggests that they are presynaptic in the LAL i.e., that they represent outputs from the AOTU to the LAL. Tracer injections in a cell nuclei cluster near the AOTU (Fig. 1B-7) stained a set of tangential neurons (TL) (Fig. 1B) with distinct knob-like terminals in the LAL (Fig. 1B-2) and wide ramifications in the ellipsoid body (EB) of the CX (Fig. 1B-3-6) indicating output neurons from the LAL to the CX.

The large synaptic complexes in the LAL are very conspicuous in the *Cataglyphis* brain and form one cluster embedded dorsally in a notch of the medial lobe located laterally with respect to the EB of the CX (Fig2. A, B). Immunostaining of the large synaptic complexes showed that several dendritic endings form the central core that is surrounded by a presynaptic profile (Fig. 2B). The high accumulation of f-actin in dendritic spines and of synapsin, being associated with synaptic vesicles, in the presynaptic side of projection neurons correlates with the distribution shown in the insect MBs (see Groh et al, 2004; Stieb et al., 2010). However, in this case the presynapse forms a cup shaped large terminal (see locusts: Träger et al., 2008) and the postsynaptic fibers fork numerous small profiles inside the cup. GABA immunostaining revealed that TL output neurons from the LAL to the EB of the CX are GABA-immunoreactive (ir) in *Cataglyphis* (Fig.2C, D), similar as in the locust (Träger et al., 2008).

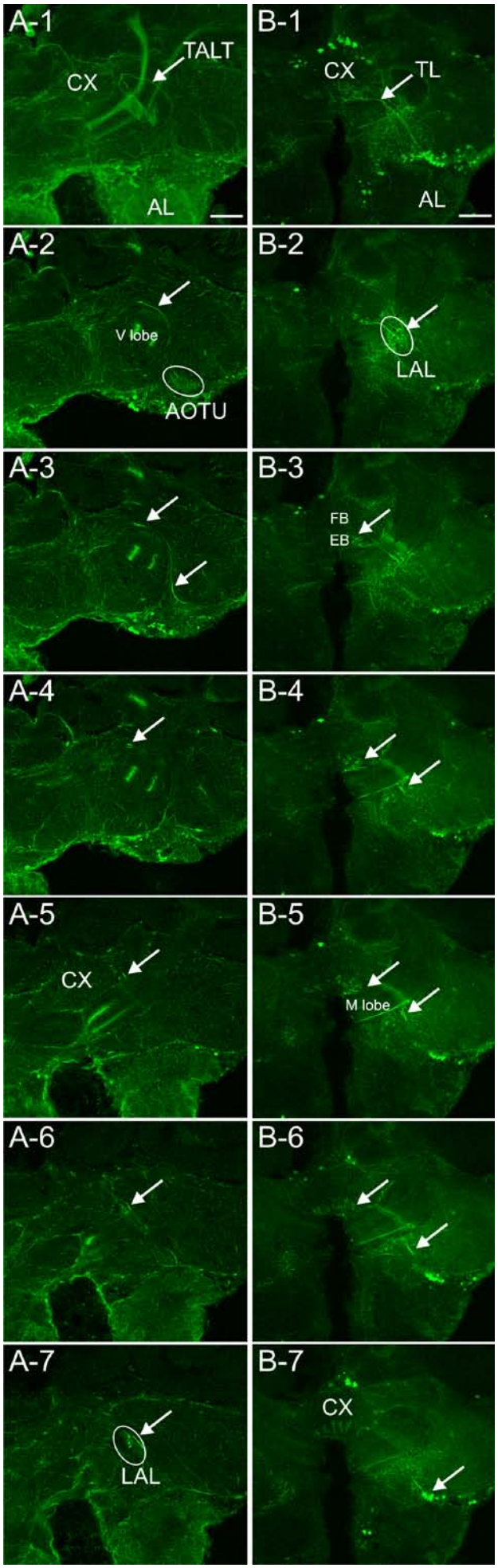

Figure 1. Output and input neurons of large synaptic complexes of the lateral accessory lobe (LAL). **A**. Localized tracer injections in the anterior optic tubercle (AOTU) reveal neurons from the tubercle-accessory lobe tract (TALT). Merged image stacks (A-1: 122 with a step size of 1µm) and single images (arrows in A-2-7) show that the TALT has dendritic ramifications in the AOTU (A-2), and runs laterally around the ipsilateral vertical lobe (V-lobe; A-2-4) before entering the LAL from rostral (A-5-7) where the neuronal endings form distinct presynaptic profiles (A-7). **B**. Localized tracer injections in cell nuclei (B-7) besides the AOTU reveal tangential neurons (TL). Merged image stacks (B-1: 143 with a step size of 1µm) and single images (arrows in B-2-7) show that the TL have distinct postsynaptic knob-like terminals in the LAL (B-2) and widely ramifications in the ellipsoid body (EB) of the central complex (CX; B-3-6). Antennal lobe (AL); fan-shaped body (FB) of the CX; medial lobe (M lobe). Scale bars: 50µm.

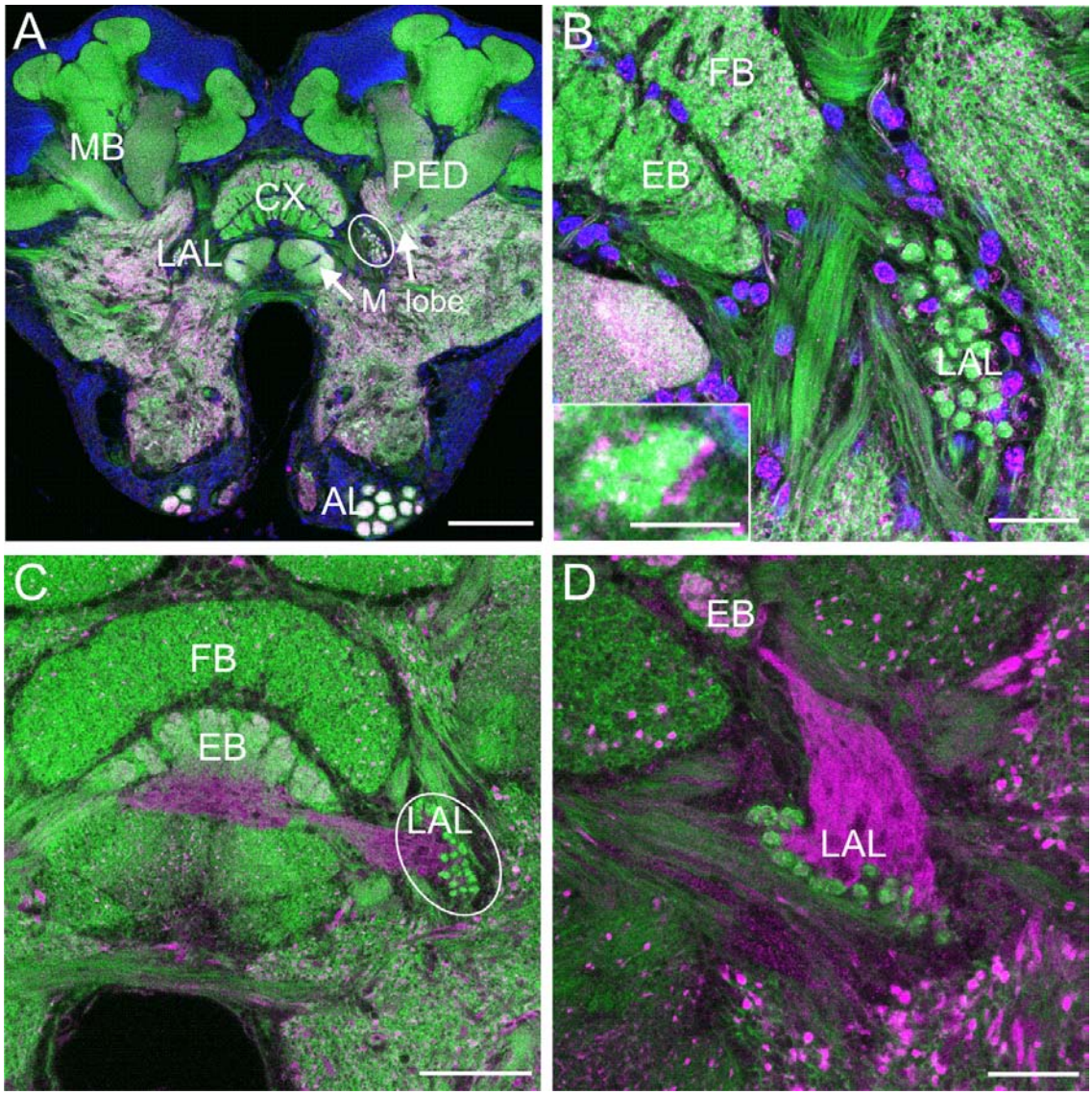

Figure 2. Immunofluorescence stainings of the *Cataglyphis fortis* brain. **A**. Frontal overview of the central brain (100µm section) labeled with anti-synapsin (magenta), phalloidin-labeled f-actin (green), and Hoechst (blue) to highlight cell nuclei. The cluster of large synaptic complexes (encirclement) of the lateral accessory lobe (LAL) is embedded dorsally in a notch of the medial lobe (M lobe; arrows) located laterally in respect to the central complex (CX). Antennal lobe (AL); mushroom body (MB); pedunculus (PED). **B**. Magnification of the LAL large synaptic complexes comprised of postsynaptic profiles forming a central core surrounded by a presynaptic terminal (inset). Ellipsoid body (EB) and fan-shaped body (FB) of the CX. **C**, **D**. Central brain labeled with anti-GAD1 highlighting GABA-immunoreactive (ir) neurons (magenta) and phalloidin-labeled f-actin (green). Tangential neurons giving output from the LAL to the EL of the CX are GABA-ir. Scale bars: A: 200µm; B: 20µm with inlet: 5µm; C: 50µm; D: 20µm.

Age-Related and Task-Dependent Structural Plasticity of Synaptic Complexes in the Lateral Accessory Lobe

We investigated changes in the number of synaptic complexes in the LAL between callows and 5-day foragers. Whereas callows (Fig. 3A) without any light experience had a great variance in their number of synaptic complexes with a mean of 111.3 ± 10.1, in 5-day light-experienced foragers (Fig. 3B) numbers showed only little variation with a mean of 127.4 ± 3.2. This difference between the two age groups with foragers having more synaptic complexes was highly significant (Mann-Whitney-U-Test: $p<0.005$) (Fig. 3C). No specialized region for the outgrowth of new synaptic complexes within their cluster in the LAL could be identified.

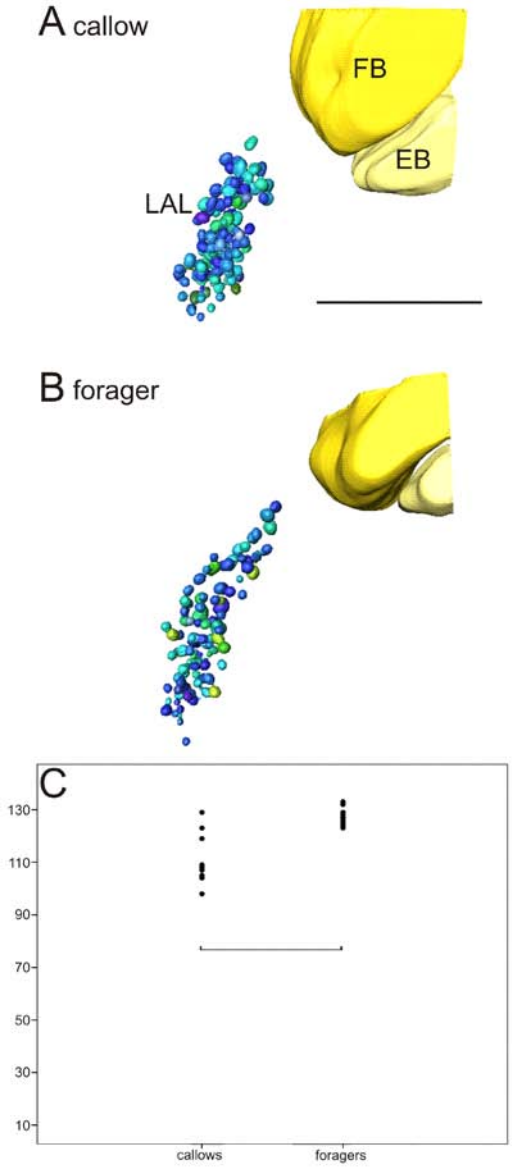

Figure 3. Age/task-related structural plasticity in synaptic complexes located in the lateral accessory lobe (LAL). **A**, **B**. Reconstruction of large synaptic complexes in a callow (A) and in a 5-day forager (B). Reconstructions of the phalloidin-profile highlight the central core of synaptic complexes built up of dendritic spines belonging to neurons of the tubercle-accessory lobe tract. Ellipsoid body (EB) and fan-shaped body (FB) of the central complex. **C**. Age/task-related effect on the number of LAL synaptic complexes. 5-day light-experienced foragers have a significant increase in the number of synaptic complexes compared to dark-kept callows. Scale bar: 50µm.

DISCUSSION

Structure of Synaptic Complexes in the Lateral Accessory Lobe

We have analyzed large synaptic complexes in the LAL of the desert ant *C. fortis*. These complexes consist of postsynaptic processes located in a central region that is surrounded by a cup like presynaptic profile. The presynaptic terminals are formed by neurons of the TALT that have dendritic ramifications in the AOTU. TL neurons forming the dendritic complexes in the LAL project to the EB of the CX and are GABA-ir. The prominent glomerular structures in the LAL have also been reported in other insects where they exhibit similarities in their incoming and outgoing trajectories (locusts: Homberg et al., 2003; Träger at al., 2008; honeybees: Mota et al., 2011). In locusts and honeybees the synaptic complexes are distributed in two separated clusters within the LAL, whereas in *Cataglyphis* only a single cluster was found. Presynaptic terminals belonging to the TALT and providing input to the giant synaptic complexes are identified in the sphinx moth (Homberg et al., 1990), locusts (referred to as TuLAL neurons: Homberg et al., 2003; Pfeiffer et al., 2005) and honeybees (Mota et al., 2011). TL neurons with compact dendritic bushes located laterally of the CX have been reported for locusts (Müller et al., 1997; Vitzthum et al., 2002; Träger at al., 2008), honeybees (Mota et al., 2011), crickets (Sakura and Labhart, 2005) and fruit flies (referred to as R-neurons: Hanesch et al., 1989). As in *Cataglyphis*, these neurons are GABAergic in locusts (Homberg at al., 1999, Träger et al., 2008) and in fruit flies (Hanesch et al., 1989). It has been shown for the locust brain that presynaptic elements from the AOTU end in small numbers of large cup- shaped terminals. These cups enclose many small postsynaptic profiles from TL neurons (Träger et al., 2008). It seems very likely that the configuration of the synaptic complexes in *Cataglyphis* is similar as in locusts but electron microscopic analyses are needed to describe the cellular fine-structure of these large synaptic complexes and to identify single pre- and postsynaptic profiles.

Another type of large synaptic complexes is described for the mushroom bodies (MBs) that are association centers involved in learning and memory (Strausfeld et al., 1998; Menzel, 1999; Gerber et al., 2004; Davis, 2005; Giurfa, 2007; Hourcade et al., 2010). The MB calyx is built up of synaptic complexes (microglomeruli, MG) that have been described for a series of insect species (e.g. in ants: Steiger, 1967; Gronenberg, 2001; Seid and Wehner, 2008; Stieb et al., 2010; Groh and Rössler, 2011; in honeybees: Gronenberg, 2001; Ganeshina and Menzel, 2001; Groh et al., 2004, 2006; Groh and Rössler, 2011; in crickets: Frambach et al., 2004; in fruit flies: Yasuyama et al., 2002, Leiss et al., 2009). Each MG comprises a central

presynaptic bouton of the projection neurons - originating from either the antennal lobes or the optic lobes - and a surrounding shell of numerous postsynaptic profiles, mostly from MB intrinsic Kenyon-cell dendritic spines. In comparison to the *Cataglyphis* MG that is around 1µm in diameter (Stieb et al., 2010), the synaptic complexes in the LAL are giant with around 5µm in diameter (Fig. 2B inset). Furthermore, the synaptic complexes differ in respect to their organization, i.e. the central bouton in an MG is presynaptic while the central region in the LAL complexes is formed by numerous postsynaptic profiles.

In the vertebrate brain, extremely large synapses that share particular features with the LAL synaptic complexes are found in the mammalian auditory brainstem, i. e. in the calyx of Held (Held, 1893; Sätzler et al., 2002; Schneggenburger and Forsythe, 2006) and the endbulb of Held (Ryugo et al., 1996; Schneggenburger and Forsythe, 2006). The giant synapses are formed by a large cup-like presynaptic element that makes multiple synaptic contacts with the cell body of a principal neuron in the medial nucleus of the trapezoid body (MNTB; calyx of Held) and with a bushy cell in the anterior ventral cochlear nucleus (aVCN; endbulb of Held; Sätzler et al., 2002; Schneggenburger and Forsythe, 2006).

The function of the giant synaptic complexes in the LAL of *Cataglyphis* is speculative, but their organization is similar to the ones described in locusts (Pfeiffer et al., 2005; Träger et al., 2008) and honeybees (Mota et al., 2011), dissimilar to any other synaptic complexes reported so far for the insect brain. The remarkable size of these complexes suggests particularly strong synaptic contacts with fast and reliable (scatter free) signal transmission with potentially high temporal precision. In addition, the results of our tracer injections revealing a high number of neurons projecting into EB of the CX (Fig. 1, 2) imply that visual information is carried from the AOTU by TALT neurons and might contribute to neural processing in the CX. This pathway is very similar to the one described for the locust brain that is part of the polarization pathway (reviewed in Homberg, 2004; Homberg et al., 2011). Thus, it is very likely that the giant LAL synaptic complexes in *Cataglyphis* are also a polarization-sensitive relay and information processing center. Tracer injections in corresponding areas in the optic lobes are necessary to confirm the hypothetic pathway from the DRA to the CX (Fig. 4) and physiological studies (in particular electrophysiological recordings) in the different hypothetical relay stations are needed to confirm the polarization sensitivity of these neurons and synapses.

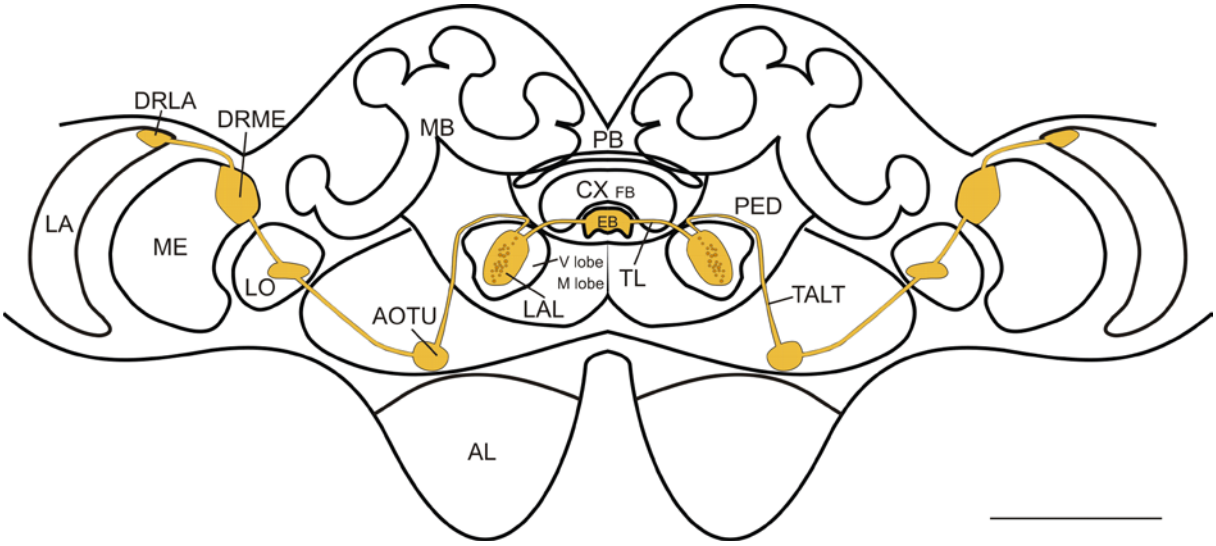

Figure 4. Schematic diagram of the presumed polarization pathway in the *Cataglyphis fortis* brain. Central processing stages for polarized light are the dorsal rim of the lamina (DRLA) and medulla (DRLA). Further presumed stages for polarized light are specific layers in the lobula (LO), the anterior optic tubercle (AOTU), the lateral accessory lobe (LAL) with large synaptic complexes and the ellipsoid body (EB) of the central complex (CX). Further abbreviations: antennal lobe (AL); fanshaped body (FB); lamina (LA); mushroom body (MB); medulla (ME); protocerebral bridge (PB); pedunculus (PED) with medial (M) lobe and vertical (V) lobe; tangential neurons (TL); tubercle-accessory lobe tract (TALT). Scale bar: 200µm.

Age-Related and Task-Dependent Structural Plasticity of Synaptic Complexes in the Lateral Accessory Lobe

Our results clearly demonstrate that the behavioral transition from interior workers to visually guided foragers in *C. fortis* is accompanied by structural changes in synaptic complexes of the LAL. The total number of synaptic complexes was significantly higher in foragers compared to young interior workers (Fig.3). Electron microscopy studies are needed to specify these structural changes on a subcellular level. Also in another brain region, the MBs that are included in visual information processing, synaptic reorganization in the course of becoming a forager has been reported in *C. fortis*. Here, the behavioral maturation correlates with volume expansion of the MB calyces (Kühn-Bühlmann and Wehner, 2006) that goes along with a decrease – an effect called pruning - of MG (Seid and Wehner, 2009; Stieb at al., 2010) in the visual MB region (collar) and, at the same time, a massive expansion of the MB intrinsic dendritic network (Stieb et al., 2010), the Kenyon cells, as was also reported for honeybees (Farris et al., 2001; Dobrin et al., 2011). The present study together with our previous one highlights two different mechanisms of synaptic reorganization in visual brain centers of *C. fortis* during the ant's behavioral transition. While massive dendritic outgrowth and synaptic pruning constitute a mechanism of neuronal adaptation to the new sensory demands and motor tasks in the MB collar, an increase of the total number of synaptic complexes occurs in

the LAL synapses. An explanation of these different neuronal machineries may be provided by the involvement in dissimilar visual information processes of the LAL and the MBs. While the LAL synaptic complexes are most possibly involved in the processing of polarized light information in *Cataglyphis* as described for locusts (Pfeiffer et al., 2005), the MBs are suspected to be involved in landmark-based processing indicated by studies in cockroaches (Mizunami et al., 1998). In foraging and homing, *Cataglyphis* ants use the path integration system as their main navigational means during which steered angles and travelled distances are used to continuously compute a vector pointing to the nest (reviewed in Wehner et al., 1996; Wehner, 2003, 2009). Once the ant has vanished into its nest, the home vector is reset to zero (Knaden and Wehner, 2006), but a copy of the full vector pointing from the previously visited foraging site to the nest is stored in memory. When the ant starts another foraging run, the memorized reference vector is retrieved and used again, reversed in sign, to steer the animal to the previously visited site (for reviews, see Collett et al., 1998; Collett and Collett, 2000; Wehner et al., 2002; Wehner and Srinivasan, 2003). Since the mechanism of path integration leads *Cataglyphis* foragers only back to the approximate vicinity of their nest resulting from continuously accumulated errors (Müller and Wehner, 1988; Merkle and Wehner, 2010), it is essential for foragers to additionally exhibit navigational backup systems including the use of landmarks to define places and routes (Wehner and Räber, 1979; Collett et al., 1992; Bisch- Knaden and Wehner, 2003; Collett and Collett, 2009). The ants link places, e.g. the nest entrance, with visual landmarks like low shrubs, stones or elevations of the ground and later, when returning to this place, match their stored image with the actual one (reviewed in Wehner et al., 1996; Wehner, 2003).While the vector information provided by the ant's path integrator vanishes rapidly in the course of several days, landmark-based information is stored over the entire lifetime of *C. fortis* (Ziegler and Wehner, 1997; *C. bicolor*: Wehner, 1981). Thus, the memory capacity of synaptic complexes in the LAL and MBs may differ in time, structure and amount. Since the extremely large synaptic complexes in the LAL suggest particularly strong synaptic contacts and might therefore deliver fast and very reliable signal transmission in the polarization vision pathway, an outgrowth of additional synaptic complexes during the foraging phase may represent some sort of callibration to the actual light conditions.

In a previous study we could show that *C. fortis* ants perform pre-foraging "orientation runs" during which they might associate the nest entrance with specific landmarks or get pre-exposed (entrained) to other visual information like the polarization pattern, and, concomitantly adapt their neuronal circuitries to the upcoming challenges (see Manuscript II).

For the visual region of the MB circuitry it has been demonstrated that visual experience throughout the natural life-span triggers plastic changes (Stieb et al., 2010). Whether the plasticity in the LAL-synapses is also driven by visual experience rather than an age-based internal programm, is very likely but needs to be proven. The short-lasting exploration runs as well as the very first foraging runs of *C. fortis* are characterized by 360° rotation movements (pirouettes) (see Manuscript II) that are also reported for *C. bicolor* (Wehner et al., 2004) and are comparable to the rotations at the beginning of long-distance runs in *C. bombycina* (Wehner and Wehner, 1990; Wehner, 1994) and the pirouettes occurring during the spiral-like "learning walks" reported for the Namibian desert ant *Ocymyrmex robustior* (Müller and Wehner, 2010). These pirouettes may serve to stimulate and calibrate the neuronal networks involved in the polarization compass pathway. Behavioral studies during the very first exploration runs are needed to identify the possible role of skylight polarization information on the neuronal structure of the LAL synaptic complexes. For this, ants leaving the nest for performing their first orientation runs ought to be deprived of any polarization information by encircleling the nest entrance with an unpolarized shielding and in addition covering the visible portion of the sky with a diffuser preventing any e-vector orientation. Whether or not the ants perform pirouettes during their exploration runs in the absence of polarization information, the outgrowth of additionally synaptic complexes in the LAL should be absent if this process serves as stimulation and calibration mechanism.

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REFERENCES

Bisch-Knaden S, Wehner, R. 2003. Local vectors in desert ants: context-dependent landmark learning during outbound and homebound runs. J Comp Physiol 189: 181–187.

Collett M, Collett TS. 2000. How do insects use path integration for their navigation? Biol cybern 83: 245-259.

Collett M, Collett TS. 2009. The learning and maintenance of local vectors in desert ant navigation. J Exp Biol 212: 895–900.

Collett TS, Dillmann E, Giger A, Wehner R. 1992. Visual landmarks and route following in desert ants. J Comp Physiol 170: 435– 442.

Collett M, Collett TS, Bisch S, Wehner R. 1998. Local and global vectors in desert ant navigation. Nature 450, 269-272.

Davis RL. 2005. Olfactory memory formation in *Drosophila*: From molecular to systems neuroscience. Ann Rev Neurosci 28: 275–302.

Dobrin SE, Herlihy JD, Robinson GE, Fahrbach SE. 2011. Muscarinic regulation of Kenyon cell dendritic arborizations in adult worker honey bees. Arthropod Struct Dev in press.

Farris SM, Robinson GE, Fahrbach SE. 2001. Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. J Neurosci 21:6395–6404.

Frambach I, Rössler W, Winkler M, Schürmann FW. 2004. F-actin at identified synapses in the mushroom body neuropil of the insect brain. J Comp Neurol 475: 303–314.

Ganeshina O, Menzel R. 2001. GABA-immunoreactive neurons in the mushroom bodies of the honeybee: An electron microscopic study. J Comp Neurol 437: 335– 349.

Gerber B, Tanimoto R, Heisenberg M. 2004. An engram found? Evaluating the evidence from fruit flies. Curr Opin Neurobiol 14: 737–744.

Giurfa M. 2007. Behavioral and neural analysis of associative learning in the honeybee: A taste from the magic well. J Comp Physiol 193: 801–824.

Groh C, Rössler W. 2011. Comparison of microglomerular structures in the mushroom body calyx of neopteran insects. Arthropod Struct Dev 40: 358-367.

Groh C, Tautz J, Rössler W. 2004. Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. Proc Natl Acad Sci USA 101: 4268–4273.

Groh C, Ahrens D, Rössler W. 2006. Environment- and age-dependent plasticity of synaptic complexes in the mushroom bodies of honeybee queens. Brain Behav Evolut 68: 1-14.

Gronenberg W. 2001. Subdivisions of hymenopteran mushroom body calyces by their afferent supply. J Comp Neurol 435: 474–489.

Hanesch U, Fischbach KF, Heisenberg M. 1989. Neural architecture of the central complex in *Drosophila melanogaster*. Cell Tissue Res 457: 343– 366.

Held H. 1893. Die zentrale Gehörleitung. Arch Anat Physiol Anat Abt 17: 201-248.

Homberg U. 2004. In search of the sky compass in the insect brain. Die Naturwissenschaften, 91: 199-208.

Homberg U, Kingan TG, Hildebrand JG. 1990. Distribution of FMRFamide-like immunoreactivity in the brain and suboesophageal ganglion of the sphinx moth *Manduca sexta* and colocalization with SCPB-, BPP-, and GABA-like immunoreactivity. Cell Tissue Res 259: 401-19.

Homberg U, Vitzthum H, Müller M, Binkle U. 1999. Immunocytochemistry of GABA in the central complex of the locust *Schistocerca gregaria*: identification of immunoreactive neurons and colocalization with neuropeptides. J Comp Neurol 409: 495–507.

Homberg U, Hofer S, Pfeiffer K, Gebhardt S. 2003. Organization and neural connections of the anterior optic tubercle in the brain of the locust, *Schistocerca gregaria*. J Comp Neurol 462: 415–430.

Homberg U, Heinze S, Pfeiffer K, Kinoshita M, el Jundi B. 2011. Central neural coding of sky polarization in insects. Philos T R Soc B, 366: 680-687.

Hourcade B, Muenz TS, 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? J Neurosci 30: 6461-6465.

Klagges BRE, Heimbeck G, Godenschwege TA, Hofbauer A, Pflugfelder GO, Reifegerste R, Reisch D, Schaupp M, Buchner S, Buchner E. 1996. Invertebrate synapsins: A single gene codes for several isoforms in *Drosophila*. J Neurosci 16:3154–3165.

Knaden M, Wehner R. 2006. Ant navigation: resetting the path integrator. J Exp biol 209: 26-31.

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

Labhart T. 2000. Polarization-sensitive interneurons in the optic lobe of the desert ant *Cataglyphis bicolor*. Naturwissenschaften 87: 133–136.

Labhart T, Meyer EP. 1999. Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. Microsc Res Techniq 47: 368-379.

Labhart T, Meyer EP. 2002. Neural mechanisms in insect navigation: polarization compass and odometer. Curr Opin Neurobiol 12: 707-714.

Leiss F, Groh C, Butcher NJ, Meinertzhagen IA, Tavosanis G. 2009. Synaptic organization in the adult *Drosophila* mushroom body calyx. J Comp Neurol 517: 808–824.

Menzel R. 1999. Memory dynamics in the honeybee. J Comp Physiol 185: 323–340.

Merkle T, Wehner R. 2010. Desert ants use foraging distance to adapt the nest search to the uncertainty of the path integrator. Behav Ecol 21: 349-355.

Mizunami M, Weibrecht JM, Strausfeld NJ. 1998. Mushroom bodies of the cockroach: Their participation in place memory. J Comp Neurol 402: 520–537.

Mota T, Yamagata N, Giurfa M, Gronenberg W, Sandoz J-C. 2011. Neural Organization and visual processing in the anterior optic tubercle of the honeybee brain. J Neurosci 31: 11443-11456.

Müller M, Wehner R. 1988. Path integration in desert ants, *Cataglyphis fortis*. Proc Natl Acad Sci 85: 5287-5290.

Müller M, Wehner R. 2010. Path integration provides a scaffold for landmark learning in desert ants. Curr Biol 20: 1-4.

Müller M, Homberg U, Kühn A. 1997. Neuroarchitecture of the lower division of the central body in the brain of the locust *Schistocerca gregaria*. Cell Tissue Res 288: 159–176.

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. J Neurophysiol 94: 3903– 3915.

Rössler W, Kuduz J, Schürmann FW, Schild D. 2002.Aggregation of f-actin in olfactory glomeruli: A common feature of glomeruli across phyla. Chem Senses 27: 803– 810.

Ryugo DK, Wu MM, Pongstaporn T. 1996. Activity-related features of synapse morphology: a study of endbulbs of Held. J Comp Neurol 365: 141–158.

Sakura M, Labhart T. 2005. Polarization-sensitive neurons in the central complex of the cricket, *Gryllus bimaculatus*. In: Neuroforum 2005 Suppl. (eds. Krieglstein K, Zimmermann H). Heidelberg: Spektrum, p. 154B.

Sätzler K, Söhl LF, Bollmann JH, Borst JGG, Frotscher M, Sakmann B, Lübke JHR. 2002. Three-dimensional reconstruction of a calyx of Held and its postsynaptic principal neuron in the medial nucleus of the trapezoid body. J Neurosci 22: 10567–10579.

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

Schneggenburger R, Forsythe ID. 2006. The calyx of Held. Cell Tissue Res 326: 311–337.

Seid MA, Wehner R. 2008. Ultrastructure and synaptic differences of the boutons of the projection neurons between the lip and collar regions of the mushroom bodies in the ant, *Cataglyphis albicans*. J Comp Neurol 507: 1102–1108.

Seid MA, Wehner R. 2009. Delayed axonal pruning in the ant brain: A study of developmental trajectories. Dev Neurol 69: 1–15.

Steck K, Hansson BS, Knaden M. 2009. Smells like home: Desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. Front Zool 6: 1-8.

Steck K, Knaden M, Hansson BS. 2010. Do desert ants smell the scenery in stereo? Anim Behav 79: 939-945.

Steiger U. 1967. Über den Feinbau des Neuropils im Corpus pedunculatum der Waldameise. Z Zellforsch 81: 511–536.

Stieb SM, Muenz TS, Wehner R, Rössler W. 2010. Visual experience and age affect syn- aptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. Dev Neurobiol 70: 408–423.

Strausfeld NJ, Hansen L, Li Y, Gomez RS, Ito K. 1998. Evolution, discovery, and interpretations of arthropod mushroom bodies. Learn Mem 5: 11–37.

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. J Comp Physiol 506: 288-300.

Vitzthum H, Müller M, Homberg U. 2002. Neurons of the central complex of the locust *Schistocerca gregaria* are sensitive to polarized light. J Neurosci 22: 1114-1125.

Wehner R. 1981. Spatial vision in arthropods. In: Handbook of sensory physiology (ed. Autrum H). Springer, Berlin, pp. 288-616.

Wehner R. 1994. The polarization-vision project: championing organismic biology. Forts Zool 39: 103-143.

Wehner R. 2003. Desert ant navigation: how miniature brains solve complex tasks. J Comp Physiol 189: 579-588.

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

Wehner R, Räber F. 1979. Visual spatial memory in desert ants, *Cataglyphis bicolor* (Hymenoptera: Formicidae). Experientia 35: 1569– 1571.

Wehner R, Wehner S. 1990. Insect navigation: use of maps or Ariadne's thread? Ethol Ecol Evol 2: 27-48.

Wehner R, Srinivasan MV. 2003. Path integration in insects. In: The Neurobiology of Spatial Behaviour (ed. Jeffery KJ). OUP Oxford, pp. 9-30.

Wehner R, Müller M. 2006: The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. Proc Natl Acad Sci USA 103: 12575–12579.

Wehner R, Herrling PL, Brunnert A, Klein R. 1972. Periphere Adaptation und zentralnervöse Umstimmung im optischen System von *Cataglyphis bicolor* (Formicidae, Hymenoptera). Revue Suisse de Zoologie 79: 197-228.

Wehner R, Michel B, Antonsen P. 1996. Visual navigation in insects: coupling of egocentric and geocentric information. J Exp Biol 199: 129-140.

Wehner R, Gallizzi K, Frei C, Vesely M. 2002. Calibration processes in desert ant navigation: vector courses and systematic search. J Comp Physiol 188: 683-693.

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

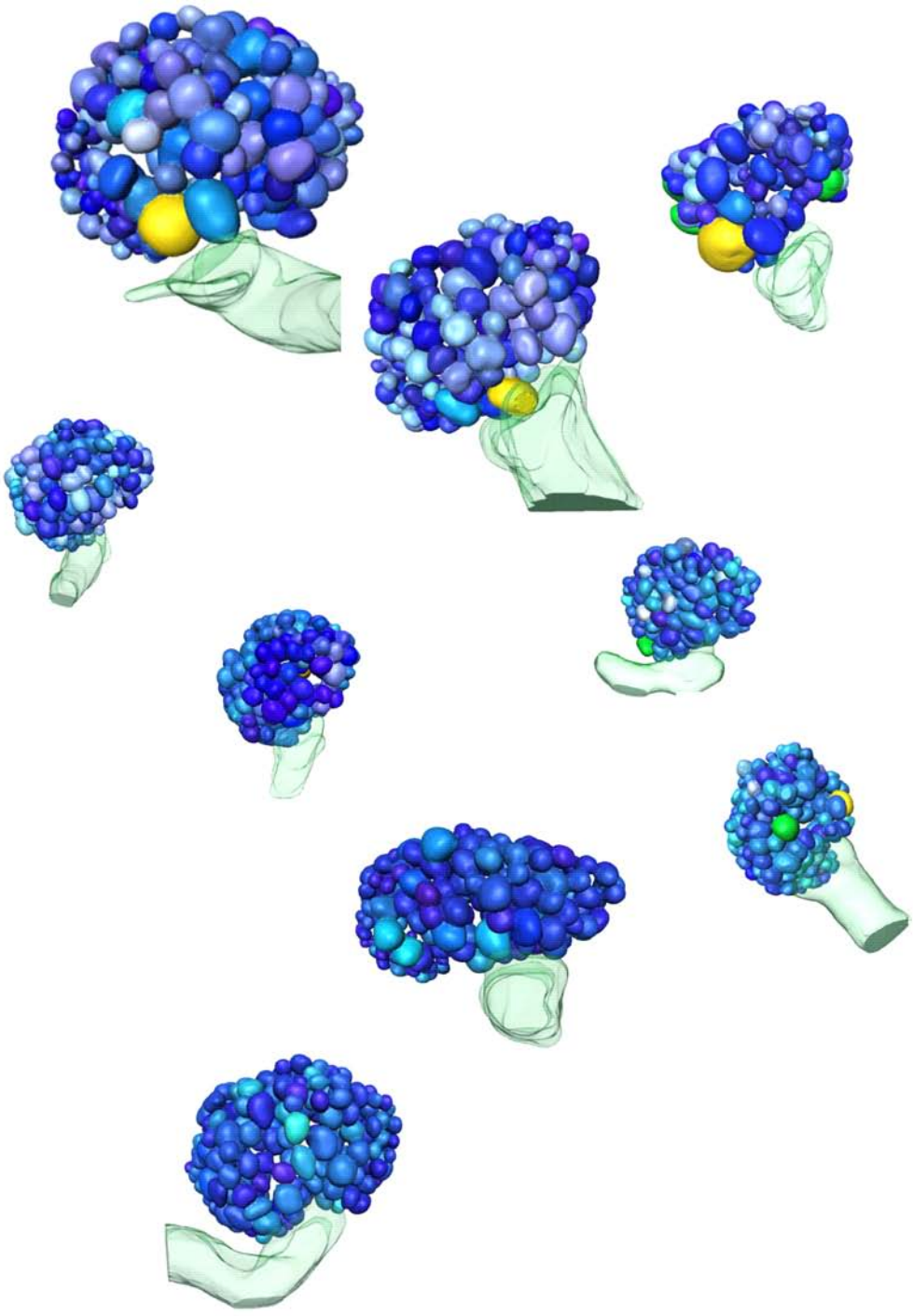
Wittlinger M, Wehner R, Wolf H. 2006. The ant odometer: stepping on stilts and stumps. Science 312: 1965–1967.

Wolf H, Wehner R. 2000. Pinpointing food sources: olfactory and anemotactic orientation in desert ants, *Cataglyphis fortis*. J Exp Biol 203: 857-868.

Yasuyama K, Meinertzhagen IA, Schürmann F-W. 2002. Synaptic organisation of the mushroom body calyx in *Drosophila melanogaster*. J Comp Neurol 445: 211–226.

Ziegler PE, Wehner R. 1997. Time-courses of memory decay in vector-based and landmark-based systems of navigation in desert ants, *Cataglyphis fortis*. J Comp Physiol 81: 13-20.

MANUSCRIPT IV



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Antennal-Lobe Organization in Desert Ants of the Genus *Cataglyphis*

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Abstract
Desert ants of the genus *Cataglyphis* possess remarkable visual navigation capabilities. Although *Cataglyphis* species lack a trail pheromone system, *Cataglyphis fortis* employs olfactory cues for detecting nest and food sites. To investigate potential adaptations in primary olfactory centers of the brain of *C. fortis*, we analyzed olfactory glomeruli (odor processing units) in their antennal lobes and compared them to glomeruli in different *Cataglyphis* species. Using confocal imaging and 3D reconstruction, we analyzed the number, size and spatial arrangement of olfactory glomeruli in *C. fortis*, *C. albicans*, *C. bicolor*, *C. rubra*, and *C. noda*. Workers of all *Cataglyphis* species have smaller numbers of glomeruli (198–249) compared to those previously found in olfactory-guided ants. Analyses in 2 species of *Formica* – a genus closely related to *Cataglyphis* – revealed substantially higher numbers of olfactory glomeruli (c. 370), which is likely to reflect the importance of olfaction in these wood ant species. Comparisons between *Cataglyphis* species revealed 2 special features in *C. fortis*. First, with c. 198 *C. fortis* has the lowest number of glomeruli compared to all other species. Second, a conspicuously enlarged glomerulus is located close to the antennal nerve entrance. Males of *C. fortis* possess a significantly smaller number of glomeruli (c. 150) compared to female workers and queens. A prominent male-specific macroglomerulus likely to be involved in sex pheromone communication occupies a position different from that of the enlarged glomerulus in females. The behavioral significance of the enlarged glomerulus in female workers remains elusive. The fact that *C. fortis* inhabits microhabitats (salt pans) that are avoided by all other *Cataglyphis* species suggests that extreme ecological conditions may not only have resulted in adaptations of visual capabilities, but also in specializations of the olfactory system.

Introduction

Desert ants of the genus *Cataglyphis* have become model systems for the study of animal navigation in general, and visually guided behavior in particular [Wehner, 2003]. While foraging they employ a path-integrating system [for a review, see Wehner and Srinivasan, 2003] including a skylight-based visual compass [Wehner and Müller, 2006] and a stride-integrating odometer [Witt-

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linger et al., 2006] as their main navigational means. In addition, they use landmarks to define places and routes [Wehner and Räber, 1979; Collett et al., 1992; Bisch-Knaden and Wehner, 2003; Collett and Collett, 2009]. Even though they establish quite pronounced individual sector fidelities, i.e. they leave the nest and return to it along familiar vectors or landmark-defined routes, during their foraging lives [Wehner et al., 2004], they do not use trail pheromones [Schmid-Hempel, 1983; Wehner et al., 1983], as it is the case in many other ant species [Hölldobler and Wilson, 1990]. However, they rely on chemical cues for pinpointing food sources [Wolf and Wehner, 2000]. Furthermore, in the vicinity of the nest, they can use olfactory landmarks for homing [Steck et al., 2009, 2010]. Ants, in general, employ a variety of olfactory signals in the context of sexual communication, nestmate recognition and caste discrimination, alarm or recruitment behavior, or trail marking. In fact, chemical communication is the most prominent type of social interactions among ants [Hölldobler and Wilson, 1990; Kleineidam and Rössler, 2009]. Therefore, the question arises whether the olfactory system of the visual navigator *Cataglyphis* is specialized and/or adapted to the particular means of foraging and homing under the extreme ecological conditions of desert environments inhabited by *Cataglyphis* species.

In ants as in all insects, odor information is received via olfactory receptor neurons (ORNs) located in the olfactory sensilla on the antennae. The axons of ORNs project to the first olfactory neuropil, the antennal lobe (AL), and terminate in the functional units of the AL, the glomeruli [for reviews, see Homberg et al., 1989; Mustaparta, 1990; Hildebrand and Shepherd, 1997; Hansson and Anton, 2000; Kleineidam and Rössler, 2009; Galizia and Rössler, 2010]. In social Hymenoptera, the incoming axons of ORNs are sorted into several sensory tracts, which innervate different clusters of glomeruli [Kirschner et al., 2006; Zube et al., 2008; Kelber et al., 2010]. Excitation of ORNs by odors results in a spatial activation pattern of different glomeruli, e.g. in honeybees [Joerges et al., 1997; Galizia et al., 1999b; Sachse et al., 1999], *Drosophila* [Fishilevich and Vosshall, 2005], and in ants [Zube et al., 2008; Kuebler et al., 2010]. In several insect species, sex-pheromone-sensitive ORNs in males of moths [Anton and Homberg, 1999] or honeybee drones [Arnold et al., 1985; Sandoz, 2006], for example, send their projections to a single or to several enlarged glomeruli, the so-called macroglomeruli (MGs) or MG complexes (MGCs). In non-sexual individuals of leaf-cutting ants *(Atta* and *Acromyrmex)*, an MG was found in large workers [Kleineidam et al., 2005; Kelber et al., 2009], where it is involved in the detection and processing of trail pheromone components [Kuebler et al., 2010].

As in *Cataglyphis* behavioral studies have shown that olfactory cues are used during various phases of the ants' foraging journeys [Wolf and Wehner, 2000, 2005; Steck et al., 2009, 2010], we inquired about potential adaptations in the primary olfactory centers of *Cataglyphis*. In particular, we investigated the glomerular organization of the AL of 5 species of *Cataglyphis* by focusing on the number, size, and spatial arrangement of the olfactory glomeruli. We addressed the question of whether differences in microhabitats and resulting variances in foraging strategies (e.g. lengths of outbound runs) have led to adaptations in the olfactory system of different *Cataglyphis* species inhabiting different desert environments (steppe-like, sandy, or salt pan areas). In particular, *C. fortis* is endemic to the salt flats of western North Africa, which are completely avoided by other *Cataglyphis* species [Wehner, 1983]. The utilization of such a specialized habitat results in outstanding traits, e.g extremely large foraging ranges [Wehner, 1987] and high nest-site stabilities [Dillier and Wehner, 2004], compared to other *Cataglyphis* species. In fact, *C. fortis* shows an enlarged glomerulus near the AL entrance that is absent in other *Cataglyphis* species. Hence, the AL structure of *C. fortis* workers is analyzed in more detail. Furthermore, we compare the number and size of glomeruli in different *C. fortis* castes (workers, queens, and males). Finally, we include 2 species of the wood ant *Formica*, which inhabits mesic environments and employs trail pheromone recruitment. Even though ecologically different from *Cataglyphis*, *Formica* is phylogenetically very closely related to *Cataglyphis*, as both genera belong to the same subfamily of ants (Formicinae) and even to the same tribe (Formicini).

Materials and Methods

Animals

In total 5 *Cataglyphis* species and 2 *Formica* species were compared regarding their AL anatomy: *C. fortis* (Forel, 1902), *C. albicans* (Roger, 1859), *C. bicolor* (Fabricius, 1793), *C. rubra* (Forel, 1903), *C. noda* (Brullé, 1832), *F. sanguinea* (Latreille, 1798), and *F. rufibarbis* (Fabricius, 1793). Workers and males/females of *C. fortis* were collected in a salt pan near Menzel Chaker (348580N/108250E), and workers of *C. albicans*, *C. rubra,* and *C. bicolor* were obtained from shrubby habitats around the salt pan as well as near Mahares (343149N/102949E), Tunisia, in July 2007 and July 2010. Workers of *C. noda* came from the southern Peloponnese (370166N/222639E), Greece (collected in June 2007).

All *Cataglyphis* ants were either transferred alive to the Biocenter of the University of Würzburg, or dissection and fixation of the brains were conducted on-site. The phylogenetic relationships between the *Cataglyphis* species examined in the present account are depicted in figure 1.

Workers of *F. rufibarbis* and *F. sanguinea* were collected in the surroundings of the Biocenter of the University of Würzburg (494644N, 95817E) in April 2010.

Neuroanatomical Procedures

For 3D reconstruction of single glomeruli in the AL, all brains were treated as described in the following: the ants were anesthetized with CO_2, decapitated, and the head capsules were fixed in dental-wax-coated dishes. The head capsule was covered with fresh ant saline solution (127 mM NaCl, 7 mM KCl, 1.5 mM $CaCl_2$, 0.8 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 4.8 mM TES, and 3.2 mM trehalose, pH 7.0) and opened by cutting a square window in-between the compound eyes. Glands and tracheae were gently removed, and the brains were dissected and fixed immediately in ice-cold Fix-Mix (2% paraformaldehyde/2% glutaraldehyde) in phosphate-buffered saline (PBS, pH 7.2) and stored for 3–30 days in the fridge. This fixation leads to increased autofluorescence which, compared to formaldehyde fixation only, allowed us to better identify the outlines of glomeruli [Kelber et al., 2009]. The brains were then rinsed in PBS (3×, 10 min), dehydrated in an ascending series of ethanol (50, 70, 80, 90, 95, and 3× 100%, 10 min each), and finally transferred into methylsalicylic acid (M-2047; Sigma-Aldrich, Steinheim, Germany).

To trace the sensory tracts of antennal ORN axons subdividing the AL glomeruli into clusters and later localize these clusters, anterograde stainings of the antennal nerve were conducted in *C. fortis* workers (n = 2). The ants were immobilized in a plexiglass holder, and 1 antenna was cut off at the base of the scapus. A droplet of tetramethylrhodamine dextran with biotin ('micro-ruby', D-7162; Molecular Probes, Eugene, Oreg., USA) dissolved in distilled water was applied in a ring of wax on the cut base and left in place for 1 h. After staining, workers were allowed to move freely for 4 h before the brains were dissected in saline solution followed by fixation with 4% paraformaldehyde in PBS (pH 7.2) and dehydration as described above.

To visualize the synaptic neuropil in individual glomeruli in more detail at higher magnification, preparations were doubly labeled with an antibody to synapsin and f-actin-phalloidin staining (methods according to Rössler et al. [2002]). Brains were dissected, fixed in cold 4% formaldehyde in PBS (pH 7.2) overnight, and sectioned at 100 μm thickness with a vibrating microtome (Leica VT 1000S; Leica, Nussloch, Germany). Afterwards, they were simultaneously incubated in Alexa Fluor 488 phalloidin (A12379; Molecular Probes) and in monoclonal antibody to the *Drosophila* synaptic-vesicle-associated protein synapsin I (1:50, SYNORF1, kindly provided by E. Buchner, University of Würzburg) followed by incubation in Alexa-Fluor-568-conjugated goat anti-mouse secondary antibody (1:250, A11004; Molecular Probes); precise descriptions have been published by Rössler et al. [2002]; Groh et al. [2004], and Stieb et al. [2010].

Laser Scanning Confocal Microscopy, 3D Reconstruction, and Data Analysis

Confocal images and image stacks were taken at a resolution of 1,024 × 1,024 pixels and an optical thickness of 1 μm from the ALs in whole-mount preparations using a laser-scanning confocal microscope (Leica TCS SP2 AOBS, 20 × 0.7 NA and 63 × 1.20 NA lens; Leica Microsystems AG, Wetzlar, Germany). 3D software (AMIRA 3.1.1; Mercury Computer Systems, Berlin, Germany) was used for reconstruction of individual glomeruli within the AL, sensory tracts, and the antennal nerve (AN). The total number and volume of all glomeruli were assessed by complete AL reconstructions in 17 ants in total (*C. fortis*: workers n = 4; males n = 2, and queens n = 2; *C. albicans*: workers n = 3; *C. bicolor*: workers n = 1; *C. noda*: workers n = 1; *C. rubra*: workers n = 1; *F. sanguinea*: workers n = 2, and *F. rufibarbis*: workers n = 1; table 1). Additional *Cataglyphis* specimens were analyzed by visual inspection.

In order to compare the size of glomeruli between different ALs and due to the fact that the number and size of glomeruli in the AL varied considerably between the investigated species, we used a relative measurement of the glomerular volume. Therefore, the median (all data were not normally distributed: Kolmogorov-Smirnov procedure, $p < 0.05$) of all glomerular volumes was calculated for each AL to subsequently compute the ratio of the volume of each glomerulus and the median volume (method modified after Kelber et al. [2009]). This normalized volume describes how many times bigger a single glomerulus is compared to the median size of all glomeruli. Boxplots were used to illustrate the normalized volume values of all glomeruli within 1 AL (fig. 2). Outliers were defined as cases with values that are 1.5–3 box

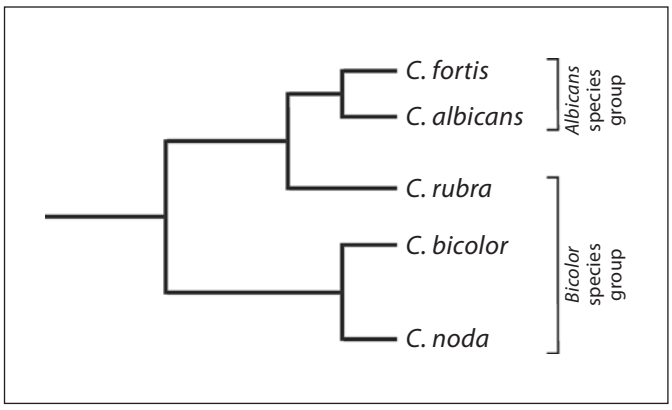

Fig. 1. The cladogram is based on morphometric data (male sexual organs [Agosti, 1990] and worker characteristics [Radchenko, 2001]) as well as molecular phylogentic analyses [Knaden et al., in prep.]. In all these studies, the *bicolor* species group and the *albicans* species group appear as sister taxa. Within the *albicans* species group, *C. fortis* (Forel, 1902) and *C. rubra* (Forel, 1903) have originally been classified as subspecies of *C. albicans*, and were raised to species rank only much later (*C. fortis* [Wehner 1983] and *C. rubra* independently by Collingwood [1985] and Wehner [1986]). While in the steppe-like arid zones of North Africa *C. albicans* and *C. rubra* occur sympatrically [Dillier and Wehner, 2004], *C. fortis* – the species that is most closely related to *C. albicans* [Wehner, 1983] – is endemic to the salt pan areas of Tunisia and Algeria [Wehner et al., 1994].

Table 1. Information on the investigated *Cataglyphis* and *Formica* species, and their AL characteristics determined in this study

Genus (common name)	Species	Country of origin	Habitat	Caste (n)	Glo. n	Vol_{min} μm^3	Vol_{max} μm^3
Desert ant	*C. fortis*	Tunisia	salt pan	w (4)	198 ± 7	424	42,683
				m (2)	150 ± 2	672	90,342
				q (2)	219 ± 3	1,027	21,302
	C. albicans		low-shrub area	w (3)	226 ± 4	586	13,171
	C. bicolor			w (1)	249	503	25,582
	C. rubra			w (1)	249	436	14,915
	C. noda	Greece		w (1)	226	1,542	38,488
Wood ant	*F. rufibarbis*	Germany	grassland	w (1)	373	538	7,608
	F. sanguinea		grassland	w (2)	372 ± 1	403	9,858

Genus (common name)/species = Analyses of the AL structure were performed for the listed desert ant *(Cataglyphis)* and wood ant *(Formica)* species; country of origin and habitat = description of the locality where ants were collected; caste (n) = belonging to the nonsexual (w: worker) or sexual (q: queen; m: male) caste (number of 3D-reconstructed ALs); Glo. = mean ± SD of glomeruli found in the AL; Vol_{min} = volume of the smallest glomerulus; Vol_{max} = volume of largest glomerulus.

lengths, extreme values (with the largest glomerulus termed 'enlarged glomerulus') as cases with values >3 box lengths distant from the upper and lower margins of the box, whereas the length of the box equals 1 interquartile range. All descriptive statistical analyses were performed with SPSS 18.0 software (SPSS, Chicago, Ill., USA).

Results

Number of Glomeruli

In the non-sexual caste of the 5 *Cataglyphis* species tested in this account, the average number of glomeruli in the AL was lowest in *C. fortis* (198 ± 7 glomeruli; table 1). In workers of *C. albicans* and *C. noda*, the 3D reconstructions revealed about 226 glomeruli, while *C. bicolor* and *C. rubra* workers had maximal numbers, namely 249 glomeruli. Compared to workers, males of *C. fortis* had a reduced number of 150 ± 2 glomeruli, whereas *C. fortis* queens had a slightly higher number of 219 ± 3 glomeruli. In comparison to the 2 *Formica* species (about 370 glomeruli), all tested *Cataglyphis* species had a smaller number of glomeruli.

Size of Glomeruli

In workers of all *Cataglyphis* species, the size of the glomeruli ranged from 424 to 42,683 μm^3. The difference between the smallest and the largest glomeruli (table 1) was the highest in *C. fortis* (see numbers given above). With respect to the normalized glomerular volumes, all workers of the *Cataglyphis* and *Formica* species showed several outliers. Extreme values were found in workers of all species with the exception of *C. noda*. Among the *Cataglyphis* workers, *C. fortis* possessed the biggest singular glomerulus (up to 7.2 times bigger) compared with the median size of all glomeruli within 1 AL (fig. 2).

In the sexual castes of *C. fortis*, the size ranges of glomerular volumes were smaller for queens (1,027–21,302 μm^3) and largest for males (672–90,342 μm^3; table 1). Regarding the normalized volumes, *C. fortis* queens exhibited extreme values (besides several outliers) that lay within the range of those of the worker caste (fig. 2). In contrast, the extreme values of *C. fortis* males were up to 21.6 times larger than the median size of all glomeruli (again besides several outliers; fig. 2). These severely enlarged male-specific glomeruli are termed MGs in the following (using the criteria described by Kelber et al. [2009]).

Position of Enlarged Glomeruli

The largest glomerulus within 1 AL (as defined by the criteria mentioned in the Materials and Methods section and termed 'enlarged glomerulus'; fig. 2) could be well localized in the 3D reconstructions (fig. 3). All enlarged glomeruli in workers of *C. rubra* and *C. bicolor* as well as

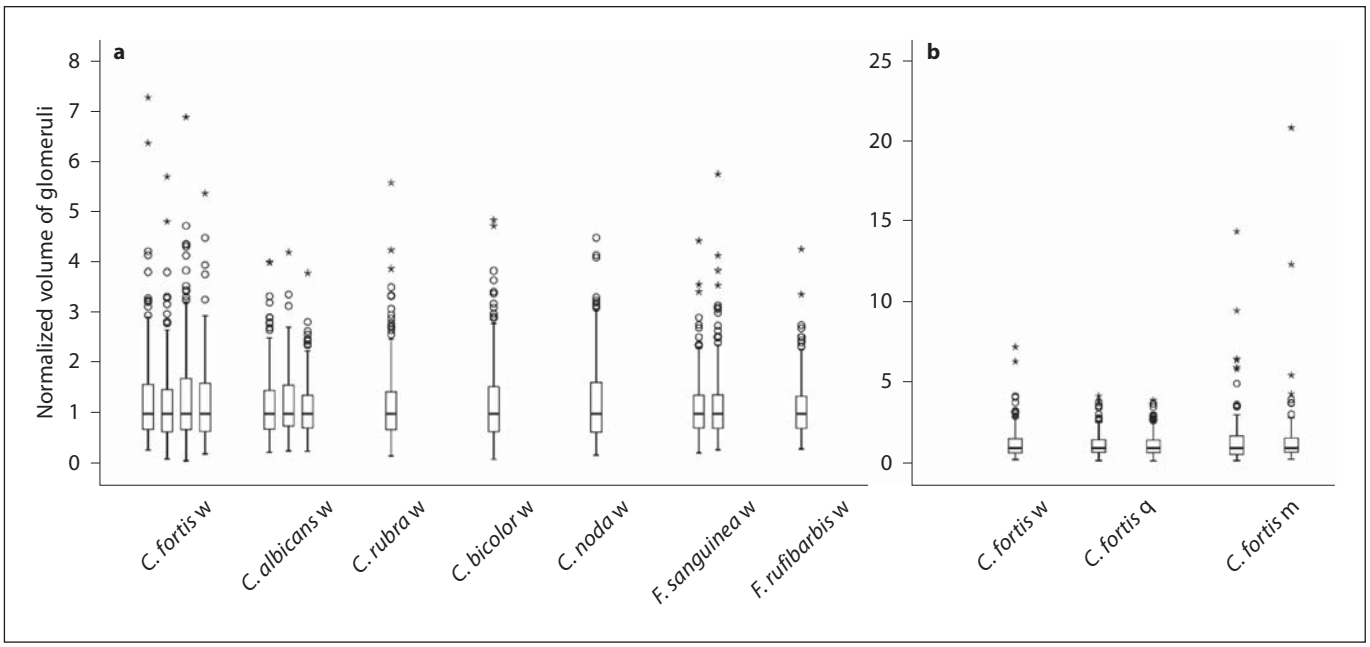

Fig. 2. Descriptive statistics for the identification of enlarged glomeruli in all *Cataglyphis* and *Formica* species. Boxes illustrate the median and variance of all normalized glomerular volumes. Extreme values are displayed as asterisks, outliers as open circles, and corresponding glomeruli are visualized in figure 3. **a** Normalized volumes of glomeruli in different *Cataglyphis* and *Formica* workers (w). In all species, the AL comprises glomeruli identified as outliers and extreme values (with the exception of *C. noda*). **b** Normalized volumes of glomeruli in different *C. fortis* castes: worker (w), queen (q), and male (m). Females and males possess extreme values 4.0–7.2 and 14.4–21.6 times larger, respectively, than the median size of all glomeruli.

the enlarged glomeruli of 2 *C. albicans* workers were located in a group of glomeruli occupying the dorsalmost part of the AL. In the 3rd *C. albicans* worker, the enlarged glomerulus was located near, but not directly at, the entrance of the AN. In all *C. fortis* specimens, the largest glomeruli of the workers were located at the same position close to the entrance of the AN (fig. 3a; enlarged glomerulus; yellow). F-actin phalloidin and anti-synapsin labeling confirmed the existence of a glomerulus containing very large synaptic neuropil located directly adjacent to the AN (fig. 4b). Anterograde mass stainings of ORN axons demonstrated that the enlarged glomerulus at the AN entrance is innervated by a high number of ORN axons (fig. 4b, c). Figure 4c clearly shows that all other glomeruli in the same focal plane are less innervated than the enlarged glomerulus. The remaining glomeruli of *C. fortis* workers with extreme values were located either in the already mentioned dorsalmost part of the AL or more laterally.

In *C. fortis* queens, the largest glomerulus was located in a position corresponding to that of *C. fortis* workers, i.e. close to the AN entrance (fig. 3b). In both *C. fortis* males, the largest glomerulus was found medial to the AN with 1 normal-sized glomerulus in between the AN entrance and the largest glomerulus (fig. 3c). The other glomeruli identified as extreme values of both males and queens were located in different positions in the AL. We would also like to mention 2 glomeruli identified as extreme values which in males are located at the dorsalmost part of the AL, near the dorsal lobe (DL).

In both *Formica* species, all enlarged glomeruli were found in different locations in the AL even within the same species.

Sensory Tracts in C. fortis

Mass labeling of ORN axonal projections allowed the classification of sensory-tract-specific glomerular clusters. The cluster classification was done for 2 *C. fortis* workers: 1 by using 3D reconstruction and 1 by performing careful visual inspection. Figure 4c shows a confocal image with the enlarged glomeruli clearly visible, while figure 4d provides the corresponding 3D reconstruction. In *C. fortis* workers, the ORN axons form 4 distinct sensory tracts (T1–T4) innervating 4 characteristic clusters of glomeruli within the AL. In the following, these glo-

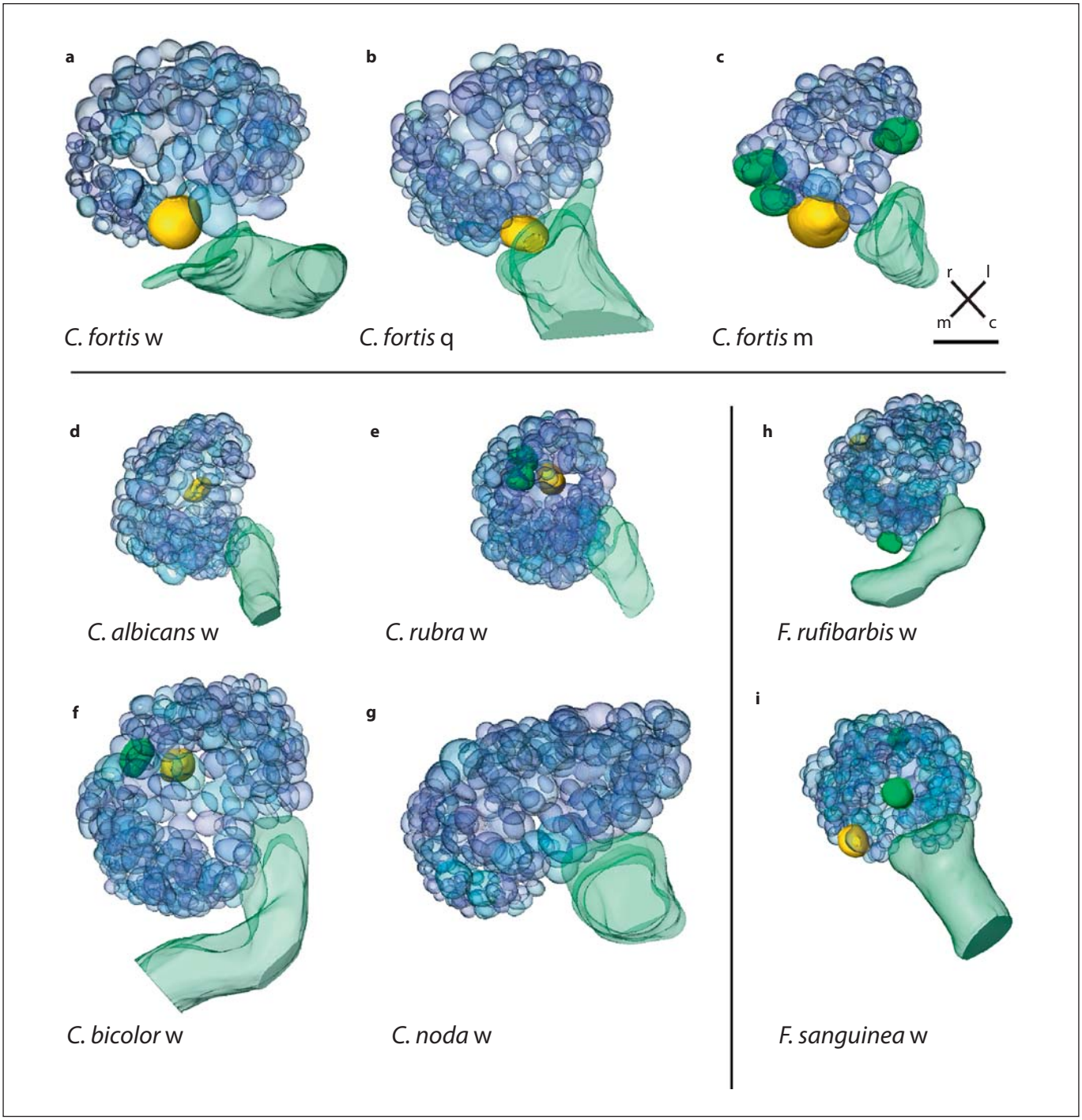

Fig. 3. AL reconstructions in *Cataglyphis* and *Formica* species. In all illustrations, the AN is outlined transparently, the largest glomerulus (if identified as an extreme value; fig. 2) is colored in yellow, further glomeruli classified as extreme values (fig. 2) are colored in green, and the remaining glomeruli, including outliers (fig. 2), in transparent blue. **a–c** 3D reconstructions of AL glomeruli in a *C. fortis* worker (w; **a**), queen (q; **b**) and male (m; **c**). Whereas in females the largest glomerulus is located next to the AN entrance, the MG in males is located medial to the AN with one normal-sized glomerulus in between the AN entrance and itself. **d–g** 3D reconstructions of AL glomeruli in workers of *C. albicans* (**d**), *C. rubra* (**e**), *C. bicolor* (**f**), and *C. noda* (**g**). All enlarged glomeruli are located at the dorsalmost part in the AL. **h**, **i** 3D reconstructions of AL glomeruli in workers of *F. rufibarbis* (**h**) and *F. sanguinea* (**i**). Directions are indicated in the coordinate planes: r = rostral; c = caudal; m = medial; l = lateral. Scale bar: **a–i** 50 μm.

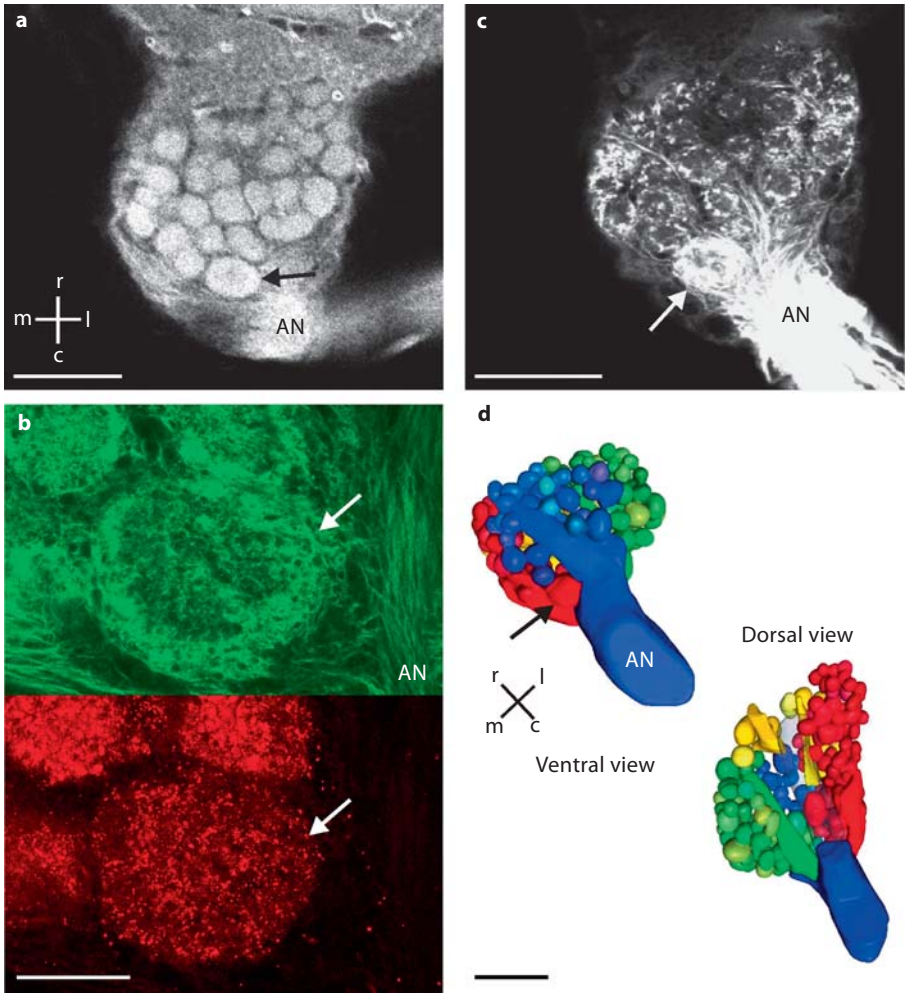

Fig. 4. Anatomy of the AL and the division of the AN into sensory tracts in *C. fortis* workers. **a**, **c** The position of the enlarged glomerulus (indicated with an arrow in **a**–**d**) next to the AN entrance is clearly visible. **a** Single optical section showing the AN and the enlarged glomerulus next to it. **b** Immunolabeling of the AL neuropil. The enlarged glomerulus is clearly defined by its postsynaptic sides visualized by the phalloidin-labeled f-actin (labeled in green) and the presynaptic input stained with an antibody to synapsin (labeled in red). **c** Confocal image of the AL with an anterogradely labeled AN showing the sensory innervation of AL glomeruli. In comparison to all other glomeruli in this sectional plane, the enlarged glomerulus is innervated by a large number of axons from ORNs. Based on these backfill stainings, the sensory tracts and the resulting partitioning in glomerular clusters of tract-associated glomeruli were reconstructed (**d**). **d** Ventral and dorsal views of 3D reconstructions of sensory tracts and associated glomerular clusters. Four sensory tracts innervate 4 glomerular clusters (T1 cluster with 37 glomeruli: blue; T2 cluster with 69 glomeruli: green; T3 cluster with 85 glomeruli: red; T4 cluster with 18 glomeruli: yellow) in the AL. The enlarged glomerulus belongs to the T3 cluster. Dorsal tract: transparent blue. Directions are indicated in the coordinate planes: r = rostral; c = caudal; m = medial; l = lateral. Scale bars = 50 μm (**a**, **c**, **d**) and 20 μm (**b**).

merular subregions are termed T1–T4 clusters (fig. 4d). T1 proceeds at the ventral surface of the AL and innervates 37 glomeruli of the T1 cluster (blue glomeruli in fig. 4d) located on the ventral part of the AL flanked laterally by the T2 (green glomeruli in fig. 4d) and medially by the T3 (red glomeruli in fig. 4d) cluster. The T2 cluster comprises 69 glomeruli spreading along the lateral side of the AL from ventral to dorsal. Similarly, the medial-situated T3 cluster disperses from ventral to dorsal and comprises the highest number of glomeruli (85 glomeruli). Next to the AN, this cluster contains the enlarged glomerulus, whereas the dorsal part of this cluster consists of a high number of small glomeruli. Only 18 glomeruli form the T4 cluster (yellow glomeruli in fig. 4d) located on the dorsalmost AL region near the DL. In contrast to the ORNs of sensory tracts T1–T3, the ORNs of the T4 subpopulation innervate not only the cortical layer but also the entire glomerulus, being similar to findings in the honeybee [Arnold et al., 1985]. One tract, from which the T4 tract is emerging, projects toward the DL.

Discussion

Intraspecific Variations in the Number of Glomeruli in C. fortis

Many insect species exhibit caste- and sex-specific variations in general features of the ALs. The behavioral repertoires of hymenopteran males are in general restricted, which in turn results in a reduced number of glomeruli compared to females (*Apis mellifera* [Arnold et al., 1985; Brockmann and Brückner, 2001; Sandoz, 2006]; *Harpegnathos saltator* [Hoyer et al., 2005]; *Camponotus floridanus* [Zube and Rössler, 2008]; *Camponotus ja-*

ponicus [Nishikawa et al., 2008], and *Atta vollenweideri* [Kuebler et al., 2010]). Analyses of the number of glomeruli in *C. fortis* males confirm these findings. Within the female caste, *C. fortis* queens have a higher number of glomeruli than workers and males. Other studies in ants showed that the number of glomeruli in queens exceeded that in males, but was still lower or comparable to the numbers found in workers (*A. vollenweideri* [Kuebler et al., 2010]; *A. mellifera* [Groh and Rössler, 2008]; *C. japonicus* [Nishikawa et al., 2008], and *C. floridanus* [Zube and Rössler, 2008]). That this ratio is reversed in *C. fortis* might most likely be due to the especially low number of glomeruli in the workers rather than to a relatively larger number of glomeruli in the queens.

Interspecific Variations in the Number of Glomeruli

With a mean of 198 glomeruli, *C. fortis* has by far the lowest glomerular number among all *Cataglyphis* species tested in this account (see table 1: from 198 glomeruli in *C. fortis* to 249 glomeruli in *C. rubra* and *C. bicolor*). Compared to other hymenopteran species (156–166 glomeruli in the honeybee [Arnold et al., 1985; Flanagan and Mercer, 1989; Galizia et al., 1999a; Kirschner et al., 2006] and 186–198 glomeruli in 2 parasitoid wasps [Smid et al., 2003]), ant workers in general have a higher number of glomeruli per AL: the highly olfactory fungus-growing ant species possess 257–630 glomeruli [Kelber et al., 2009; Kuebler et al., 2010]; likewise, the group-recruiting and trail-following carpenter ants [Haak et al., 1996] also have high glomerular numbers (430 glomeruli in *C. japonicus* [Nishikawa et al., 2008] and 434 glomeruli in *C. floridanus* [Zube and Rössler, 2008]). *Formica* species, which have been tested in this account, perform chemical recruitment [Hölldobler and Wilson, 1990] which nicely correlates with high numbers of about 370 glomeruli in the 2 tested species (table 1). In contrast to all the ant species mentioned above, *Cataglyphis* ants forage solitarily and do not use trail pheromones, but rely primarily on visual guidance mechanisms [Wehner, 2003].

The interspecific variations in glomerular numbers indicate that the number of glomeruli appears to be generally correlated with the amount of chemically mediated behaviors. Ant species with a pronounced olfactory-guided behavior like the use of a trail pheromone have a high glomerular number [Kleineidam and Rössler, 2009]. The results in *Cataglyphis* indicate that species relying more on visual cues may possess a reduced glomerular number. This hypothesis is further supported by a small number of glomeruli in the Namibian desert ant (223 glomeruli [Wenzler, pers. commun.]), which in contrast to *Cataglyphis* occasionally exhibits a particular kind of recruitment behavior [Marsh, 1985; Wehner, 1987]. In the same way, *Gigantiops destructor*, which is characterized by huge compound eyes and impressive capabilities of visually detecting moving objects and navigating by visual means [Hölldobler and Wilson, 1990, pp. 259; Gronenberg and Hölldobler, 1999; Beugnon et al., 2001], as well as the mostly visually guided ponerine ant *H. saltator* have low numbers of glomeruli (250 glomeruli in *G. destructor* [Kelber, pers. commun.]; 200 glomeruli in *Formica pratensis* [Goll, 1967], and around 177 glomeruli in *H. saltator* [Hoyer et al., 2005]).

The assumed correlation between a reduced glomerular number in *C. fortis* and the largely visually guided behavior of this species goes along with comparative studies among different ant species showing that *Cataglyphis* has not only relatively large optic lobes, but also possesses an exceedingly large collar – the visual input region of the learning- and memory-associated mushroom bodies [Gronenberg and Hölldobler, 1999; Kühn-Bühlmann and Wehner, 2006].

Clusters of Glomeruli in C. fortis *Workers*

In *C. fortis* workers, the AN is divided into several sensory tracts of which 4 innervate the AL glomeruli. The grouping of glomeruli into a variable number of clusters is a common feature in Hymenoptera and allows for interspecific comparisons and potential phylogenetic inferences. In the honeybee, there are 4 glomerular clusters as in *C. fortis* [Arnold et al. 1985; Flanagan and Mercer, 1989; Galizia et al., 1999a; Kirschner et al., 2006], but 6 and 7 sensory tracts innervate the AL glomeruli in the ants *A. vollenweideri* [Kelber et al., 2010] and *C. floridanus* [Zube et al., 2008] as well as *C. japonicus* [Nakanishi et al., 2010], respectively. The reduced number of glomerular clusters in *C. fortis* compared to other ant species correlates with the small number of glomeruli and might be indicative of the importance of vision rather than olfaction in this species.

The MG in C. fortis *Males and Its Possible Function*

A sexual dimorphism in the AL structure based on sex pheromone communication is reported for a number of insect species [for review, see Rospars, 1988; Galizia and Rössler, 2010]. The occurrence of an MG or an MGC always located near the AN entrance and exclusively receiving input from sex-pheromone-sensitive ORNs has been described for males of several insect species, e.g. moths [Hansson et al., 1991; Anton and Homberg, 1999; Rössler et al., 1999] and honeybees [Arnold et al., 1985; Sandoz, 2006]. The increased size of MGs results from a

high number of terminating ORNs [Vickers and Baker, 1997; Berg et al., 1998]. Similarly, in males of several ant species, enlarged glomeruli have been described (*H. saltator* [Hoyer et al., 2005]; *A. vollenweideri* [Kuebler et al., 2010], and *C. japonicus* [Nakanishi et al., 2010]), but their specific involvement in sex pheromone communication remains to be elucidated.

An MG near the AN entrance was also found in the males of *C. fortis*. The MG location differs from that of the enlarged glomeruli found in the female castes. Due to the fact that this MG was only found in *C. fortis* males, it is likely that sex pheromone detection plays a prominent role for the male during courtship and mating behavior. In some *Cataglyphis* species, it has been shown that the males respond to the sexual 'calling behavior' of potentially pheromone-emitting females (*C. iberica* [Cerdá, 1988]; *C. cursor* [Lenoir et al., 1988], and *C. savignyi* [Wehner and Wehner, unpubl. data]).

Enlarged Glomeruli in the Different Cataglyphis *Species and Their Possible Functions*

With only one exception, all enlarged glomeruli in the workers of *C. rubra*, *C. bicolor* and *C. albicans* are located in a group of glomeruli constituting the dorsalmost part of the AL nearest to the DL. Analyses of the sensory tracts in *C. fortis* revealed that these glomeruli belong to the T4 cluster. Based on the conspicuous position of this dorsalmost cluster of glomeruli and the fact that some of these glomeruli are enlarged in all tested *Cataglyphis* species, we assume that the corresponding glomeruli in all *Cataglyphis* workers belong to the T4 cluster and might be involved in the processing of CO_2-, thermo- or hygroreceptive information. In a corresponding region of the AL, a CO_2-sensitive glomerulus has been described for several moth species [Bogner et al., 1986; Kent et al., 1986; Lee and Altner, 1986; Kent et al., 1999; Guerenstein et al., 2004], and thermo- and hygroreceptive glomeruli were identified in cockroaches, honeybees, and carpenter ants [Nishikawa et al., 1995; Nishino et al., 2003, 2009; Nakanishi et al., 2010].

In *C. fortis* workers, the largest glomerulus is located next to the AN entrance. An MG not involved in the processing of sex pheromone information was discovered for the first time in leaf-cutting ants [Kleineidam et al., 2005]. There the MG is found only in large workers and is located close to the AN. Physiological as well as anatomical studies indicate that this MG is involved in the detection and processing of trail pheromone components [Kleineidam et al., 2005; Kelber et al., 2009, 2010; Kuebler et al., 2010]. These studies clearly demonstrate that the occurrence of enlarged glomeruli or even MGs may vary substantially across ant species and even within the same species, and may correlate with certain behavioral requirements of odor processing. Despite the fact that the enlarged glomerulus of *C. fortis* workers does not fulfill all the criteria of an MG (if we use the criteria used for defining an MG by Kelber et al. [2009]), its size, stable location among tested workers, and its absence in all other tested *Cataglyphis* species indicate a specialized function of this glomerulus. As the backfill stainings clearly show that the enlarged glomerulus receives an increased input from ORN axons (fig. 4c), it is very likely that this glomerulus serves a particular and unique function that may be related to the extreme habitat in this species.

If we now recall that *C. fortis* possesses the AL with the lowest number of glomeruli among all tested *Cataglyphis* species and assume that the number of glomeruli reflects the complexity of olfactory-guided behavior, why is it that of all *Cataglyphis* species just *C. fortis* has an enlarged glomerulus at its AN entrance? Physiological (in particular functional imaging) studies are needed to characterize the odor specificity of the enlarged glomerulus. The fact that the enlarged glomerulus in the AL entrance of *C. fortis* is absent in the AL of the most closely related *C. albicans* (fig. 1) may indeed indicate that this glomerulus represents an adaptation to the special behavioral ecology of *C. fortis,* which among all its congeners takes an outstanding position regarding its preferred habitat, the food-impoverished salt pan flats of the Algerian and Tunisian chotts and sebkhas [Wehner, 1981, 1983; Wehner et al., 1994]. The North African *C. bicolor*, *C. albicans* and *C. rubra* inhabit the nutritionally richer low-shrub semi-deserts surrounding the salt pans [Dillier and Wehner, 2004]. To unravel which of the many differences in the behavioral ecology between *C. fortis* and its congeners [Schmid-Hempel, 1983; Wehner, 1983, 1987; Dillier and Wehner, 2004] are functionally related to the presence of the remarkably enlarged glomerulus in *C. fortis* remains a challenging task to accomplish.

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References

Agosti D (1990): Review and reclassification of *Cataglyphis* (Hymenoptera, Formicidae). J Nat Hist 24:1457–1505.

Anton S, Homberg U (1999): Antennal lobe structure; in Hansson BS (ed): Insect Olfaction. Berlin, Springer, pp 97–124.

Arnold G, Masson C, Budharugsa S (1985): Comparative study of the antennal lobes and their afferent pathway in the worker bee and the drone (*Apis mellifera*). Cell Tissue Res 242:593–605.

Berg BG, Almaas TJ, Bjaalie JG, Mustaparta H (1998): The macroglomerular complex of the antennal lobe in the tobacco budworm moth *Heliothis virescens*: specified subdivision in four compartments according to information about biologically significant compounds. J Comp Physiol A 183:669–682.

Beugnon G, Chagné P, Dejean A (2001): Colony structure and foraging behavior in the tropical formicine ant, *Gigantiops destructor*. Insect Soc 48:347–351.

Bisch-Knaden S, Wehner, R (2003): Local vectors in desert ants: context-dependent landmark learning during outbound and homebound runs. J Comp Physiol A 189:181–187.

Bogner F, Boppré M, Ernst KD, Boeckh J (1986): CO_2 sensitive receptors on labial palps of *Rhodogastria* moths (Lepidoptera: Arctiidae): physiology, fine structure and central projection. J Comp Physiol A 158:741–749.

Brockmann A, Brückner D (2001): Structural differences in the drone olfactory system of two phylogenetically distant *Apis* species, *A. florea* and *A. mellifera*. Naturwissenschaften 88:78–81.

Cerdá X (1988): La carrera nupcial de *Cataglyphis iberica* (Hymeoptera: Formicidae). Congr Iberico Entomol 3:511–514.

Collett M, Collett TS (2009): The learning and maintenance of local vectors in desert ant navigation. J Exp Biol 212:895–900.

Collett TS, Dillmann E, Giger A, Wehner R (1992): Visual landmarks and route following in desert ants. J Comp Physiol A 170:435–442.

Collingwood CA (1985): Hymenoptera: Fam. Formicidae of Saudi Arabia. Fauna Saudi Arabia 7:230–302.

Dillier FX, Wehner R (2004): Spatio-temporal patterns of colony distribution in monodomous and polydomous species of North African desert ants, genus *Cataglyphis*. Insect Soc 51:186–196.

Fishilevich E, Vosshall LB (2005): Genetic and functional subdivision of the *Drosophila* antennal lobe. Curr Biol 15:1548–1553.

Flanagan D, Mercer AR (1989): An atlas and 3-D reconstruction of the antennal lobes in the worker honey bee, *Apis mellifera* L. (Hymenoptera: Apidae). Int J Insect Morphol 18: 145–159.

Galizia CG, McIlwrath SL, Menzel R (1999a): A digital three-dimensional atlas of the honeybee antennal lobe based on optical sections acquired by confocal microscopy. Cell Tissue Res 295:383–394.

Galizia CG, Rössler W (2010): Parallel olfactory systems in insects: anatomy and function. Annu Rev Entomol 55:399–420.

Galizia CG, Sachse S, Rappert A, Menzel R (1999b): The glomerular code for odor representation is species specific in the honeybee *Apis mellifera*. Nat Neurosci 2:473–478.

Goll W (1967): Strukturuntersuchungen am Gehirn von *Formica*. Z Morph Ökol Tiere 59: 143–210.

Groh C, Rössler W (2008): Caste-specific postembryonic development of primary and secondary olfactory centers in the female honeybee brain. Arthropod Struct Dev 37:459–468.

Groh C, Tautz J, Rössler W (2004): Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. Proc Natl Acad Sci USA 101:4268–4273.

Gronenberg W, Hölldobler B (1999): Morphologic representation of visual and antennal information in the ant brain. J Comp Neurol 412:229–240.

Guerenstein PG, Christensen TA, Hildebrand JG (2004): Sensory processing of ambient CO_2 information in the brain of the moth *Manduca sexta*. J Comp Physiol A 190:707–725.

Haak U, Hölldobler B, Bestmann HJ, Kern F (1996): Species-specificity in trail pheromones and Dufour's gland contents of *Camponotus atriceps* and *C. floridanus* (Hymenoptera: Formicidae). Chemoecology 7:85–93.

Hansson BS, Anton S (2000): Function and morphology of the antennal lobe: new developments. Annu Rev Entomol 45:203–231.

Hansson BS, Christensen TA, Hildebrand JG (1991): Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. J Comp Neurol 312:264–278.

Hildebrand JG, Shepherd GM (1997): Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. Annu Rev Neurosci 20:595–631.

Hölldobler B, Wilson EO (1990): The Ants. Cambridge, Belknap Press of Harvard University Press.

Homberg U, Christensen TA, Hildebrand JG (1989): Structure and function of the deutocerebrum in insects. Annu Rev Entomol 34: 477–501.

Hoyer SC, Liebig J, Rössler W (2005): Biogenic amines in the ponerine ant *Harpegnathos saltator*: serotonin and dopamine immunoreactivity in the brain. Arthropod Struct Dev 34:429–440.

Joerges J, Küttner A, Galizia CG, Menzel R (1997): Representation of odours and odour mixtures visualized in the honeybee brain. Nature 387:285–288.

Kelber C, Rössler W, Kleineidam CJ (2010): Phenotypic plasticity in number of glomeruli and sensory innervation of the antennal lobe in leaf-cutting ant workers (*A. vollenweideri*). Dev Neurobiol 70:222–234.

Kelber C, Rössler W, Roces F, Kleineidam CJ (2009): The antennal lobes of fungus-growing ants (Attini): neuroanatomical traits and evolutionary trends. Brain Behav Evol 73: 273–284.

Kent KS, Harrow ID, Quartararo P, Hildebrand JG (1986): An accessory olfactory pathway in Lepidoptera: the labial pit organ and its central projections in *Manduca sexta* and certain other sphinx moths and silk moths. Cell Tissue Res 245:237–245.

Kent KS, Oland LA, Hildebrand JG (1999): Development of the labial pit organ glomerulus in the antennal lobe of the moth *Manduca sexta:* the role of afferent projections in the formation of identifiable olfactory glomeruli. J Neurobiol 40:28–44.

Kirschner S, Kleineidam CJ, Zube C, Rybak J, Grünewald B, Rössler R (2006): Dual olfactory pathway in the honeybee, *Apis mellifera*. J Comp Neurol 499:933–952.

Kleineidam CJ, Obermayer M, Halbich W, Rössler W (2005): A macroglomerulus in the antennal lobe of leaf-cutting ant workers and its possible functional significance. Chem Senses 30:1–10.

Kleineidam CJ, Rössler R (2009): Adaptations in the olfactory system of social Hymenoptera; in Gadau J, Fewell J (eds): Organization of Insect Societies. Cambridge, Harvard University Press, pp 195–219.

Kuebler LS, Kelber C, Kleineidam CJ (2010): Distinct antennal lobe phenotypes in the leaf-cutting ant (*Atta vollenweideri*). J Comp Neurol 518:352–365.

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

Lee JK, Altner H (1986): Primary sensory projections of the labial palp-pit organ of *Pieres rapae* L. (Lepidoptera: Pieridae). Int J Insect Morphol 15:439–448.

Lenoir A, Querard L, Pondicq N, Berton F (1988): Reproduction and dispersal in the ant *Cataglyphis cursor* (Hymenoptera, Formicidae). Psyche 95:21–44.

Marsh AC (1985): Microclimatic factors influencing foraging patterns and success of the thermophilic desert ant, *Ocymyrmex barbiger*. Insect Soc 32:286–296.

Mustaparta H (1990): Chemical information processing in the olfactory system of insects. Physiol Rev 70:199–245.

Nakanishi A, Nishino H, Watanabe H, Yokohari F, Nishikawa M (2010): Sex-specific antennal sensory system in the ant *Camponotus japonicus*: glomerular organizations of antennal lobes. J Comp Neurol 518:2186–2201.

Nishikawa M, Nishino H, Misaka Y, Kubota M, Tsuji E, Satoji Y, Ozaki M, Yokohari F (2008): Sexual dimorphism in the antennal lobe of the ant *Camponotus japonicus*. Zool Sci 25: 195–204.

Nishikawa M, Yokohari F, Ishibashi T (1995): Central projections of the antennal cold receptor neurons and hygroreceptor neurons of the cockroach *Periplaneta americana*. J Comp Neurol 361:165–176.

Nishino H, Nishikawa M, Mizunami M, Yokohari F (2009): Functional and topographic segregation of glomeruli revealed by local staining of antennal sensory neurons in the honeybee *Apis mellifera*. J Comp Neurol 515: 161–180.

Nishino H, Yamashita S, Yamazaki Y, Nishikawa M, Yokohari F, Mizunami M (2003): Projection neurons originating from thermo- and hygrosensory glomeruli in the antennal lobe of the cockroach. J Comp Neurol 455:40–55.

Radchenko AG (2001): The phylogeny and faunagenesis of the genus *Cataglyphis* (Hymenoptera, Formicidae) (in Russian). Ent Obozr 80:885–895.

Rospars JP (1988): Structure and development of the insect antennodeutocerebral system. Int J Insect Morphol 17:243–294.

Rössler W, Kuduz J, Schürmann FW, Schild D (2002): Aggregation of F-actin in olfactory glomeruli: a common feature of glomeruli across phyla. Chem Senses 27:803–810.

Rössler W, Randolph PW, Tolbert LP, Hildebrand JG (1999): Axons of olfactory receptor cells of transsexually grafted antennae induce development of sexually dimorphic glomeruli in *Manduca sexta*. J Neurobiol 38: 521–541.

Sachse S, Rappert A, Galizia CG (1999): The spatial representation of chemical structures in the antennal lobe of honeybees: steps towards the olfactory code. Eur J Neurosci 11: 3970–3982.

Sandoz JC (2006): Odour-evoked responses to queen pheromone components and to plant odours using optical imaging in the antennal lobe of the honey bee drone *Apis mellifera L*. J Exp Biol 209:3587–3598.

Schmid-Hempel P (1983): Foraging Ecology and Colony Structure of Two Sympatric Species of Desert Ants, *Cataglyphis bicolor* and *C. albicans;* PhD Thesis, University of Zurich.

Smid HM, Bleeker MA, van Loon JJ, Vet LE (2003): Three-dimensional organization of the glomeruli in the antennal lobe of the parasitoid wasps *Cotesia glomerata* and *C. rubecula*. Cell Tissue Res 312:237–248.

Steck K, Hansson BS, Knaden M (2009): Smells like home: desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. Front Zool 6:5.

Steck K, Knaden M, Hansson BS (2010): Do desert ants smell the scenery in stereo? An Behav 79:939–945.

Stieb SM, Muenz TS, Wehner R, Rössler W (2010): Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. Dev Neurobiol 70:408–423.

Vickers NJ, Baker TC (1997): Chemical communication in heliothine moths. VII. Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioral antagonist. J Comp Physiol A 180:523–536.

Wehner R (1981): Verhaltensphysiologische, funktionsmorphologische und taxonomische Studien an zwei Arten der *Cataglyphis albicans*-Gruppe (Formicidae, Hymenoptera). Mitt Dtsch Ges Allg Angew Ent 3:214–217.

Wehner R (1983): Taxonomie, Funktionsmorphologie und Zoogeographie der saharischen Wüstenameise *Cataglyphis fortis* (Forel 1902) stat. nov. Senckenbergiana Biol 64:89–132.

Wehner R (1986): Art Charakterisierung von *Cataglyphis diehlii* und *C. rubra*. Jb Akad Wiss Lit Mainz 86:108–111.

Wehner R (1987): Spatial organization of foraging behavior in individually searching desert ants, *Cataglyphis* (Sahara Desert) and *Ocymyrmex* (Namib Desert); in Pasteels JM, Deneubourg JL (eds): From Individual to Collective Behavior in Social Insects. Basel, Birkhäuser, pp 15–42.

Wehner R (2003): Desert ant navigation: how miniature brains solve complex tasks. J Comp Physiol A 189:579–588.

Wehner R, Harkness RD, Schmid-Hempel P (1983): Foraging strategies in individually searching ants, *Cataglyphis bicolor* (Hymenoptera: Formicidae). Akad Wiss Lit Mainz, Abh Math Naturwiss KI. Stuttgart, Gustav Fischer.

Wehner R, Meier C, Zollikofer C (2004): The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor*. Ecol Entomol 29:240–250.

Wehner R, Müller M (2006): The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. Proc Natl Acad Sci USA 103:12575–12579.

Wehner R, Räber F (1979): Visual spatial memory in desert ants, *Cataglyphis bicolor* (Hymenoptera: Formicidae). Experientia 35:1569–1571.

Wehner R, Srinivasan MV (2003): Path integration in insects; in Jeffery KJ (ed): The Neurobiology of Spatial Behaviour. Oxford, Oxford University Press, pp 9–30.

Wehner R, Wehner S, Agosti D (1994): Patterns of biogeographic distribution within the *bicolor* species group of the North African desert ant, *Cataglyphis* Foerster (1850). Senckenbergiana Biol 74:163–191.

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

Wolf H, Wehner R (2000): Pinpointing food sources: olfactory and anemotactic orientation in desert ants, *Cataglyphis fortis*. J Exp Biol 203:857–868.

Wolf H, Wehner R (2005): Desert ants compensate for navigation uncertainty. J Exp Biol 208:4223–4230.

Zube C, Kleineidam CJ, Kirschner S, Neef J, Rössler W (2008): Organization of the olfactory pathway and odor processing in the antennal lobe of the ant *Camponotus floridanus*. J Comp Neurol 506:425–441.

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

DISCUSSION

GENERAL DISCUSSION

The present thesis demonstrates that *Cataglyphis* - the model organism for insect navigation (Wehner, 2003) – is an excellent candidate for studying the neuronal mechanisms underlying navigational features and for studying neuronal plasticity associated with the ant's lifelong flexibility of individual behavioral repertoires.

An age-related polyethism subdivides *Cataglyphis* colonies into interior workers and day-active foragers covering only a small fraction of the entire life cycle (Wehner et al., 1972; Schmid-Hempel and Schmid-Hempel, 1984). During the lifetime of the workers the following ethocaste stages occur one after another: callows (freshly emerged workers not more than about 24 hours old), interior I workers (characterized by their motionless state of behavior, their swollen gaster and the expanded whitish intersegmental membranes of the gaster), interior II workers (the remaining inside workers) and foragers (actively searching for food outside the nest). During the daily foraging trips, the ants use a path integration system combining a polarization compass (Wehner and Müller, 2006) and a proprioceptive odometer (Wittlinger et al, 2006) in addition to landmark-dependent orientation (Bisch-Knaden and Wehner, 2003) and olfactory cues (Wolf and Wehner, 2005, Steck et al., 2009, 2010). The remarkable navigational performance of *Cataglyphis* foragers addresses the question how the underlying neuronal machinery controls for the different homing strategies and how they are integrated. Moreover, the behavioral transition implicates drastically changing sensory environments and demands and visual input becomes especially important not until the ants start long-distance foraging trips. Hence, the adult maturation delivers a fascinating substrate to ask for plasticity effects in visual and olfactory brain centers. The fact that *Cataglyphis* foragers are predominantly visually guided and live under extreme ecological conditions may not only have resulted in adaptations of visual capabilities, but also in specializations of the olfactory system that are also discussed in the following.

PRESUMED CENTRAL PATHWAYS OF POLARIZATION AND LANDMARK INFORMATION

The function of the giant synaptic complexes in the lateral accessory lobe (LAL) of *Cataglyphis* is not yet solved, but their organization (see Manuscript III) is similar to the ones described in locusts (Pfeiffer et al., 2005; Träger et al., 2008) and honeybees (Mota et al., 2011) and represents a very special type of synaptic complexes reported so far for the insect

brain. The remarkable size of these synapses suggests particularly strong synaptic contacts with fast and reliable (scatter free) signal transmission with potentially high temporal precision. In addition, the results of the tracer injections revealing a high number of neurons projecting into ellipsoid body of the central complex (CX) imply that visual information is carried from the anterior optic tubercle and contributes to neural processing in the CX (Manuscript III). This pathway is very similar to the one described for the locust brain that is part of the polarization pathway (reviewed in Homberg, 2004; Homberg et al., 2011). Thus, it is very likely that the giant LAL synaptic complexes in *Cataglyphis* are also a polarization-sensitive relay and information processing center.

The role of insect mushroom bodies (MBs) as brain areas involved in olfactory learning and memory consolidation are well established (Erber et al., 1980; Strausfeld et al., 1998; Heisenberg, 1998, 2003; Menzel, 1999, 2001; Zars et al., 2000; Menzel and Giurfa, 2006). But in visually-guided insects such as honeybees and *Cataglyphis* ants visual learning and memory capabilities are essential during the diurnal outdoor lives of these central place foragers. Visual information from the optic lobes is projected to the MBs in ants, bees, wasps, and bumblebees (Mobbs, 1982; Gronenberg, 2001; Ehmer and Gronenberg, 2002; Paulk and Gronenberg, 2008). First evidence that the pedunculus and medial lobes of the MBs are required for place memory based on visual landmarks came from bilateral lesions of these MB areas in cockroaches (Mizunami et al., 1998). Moreover, split-brain preparations in cockroaches demonstrated that learning-associated changes in MBs occurred within the trained brain hemisphere, but not within the naïve one (Lent et al., 2007).

Cataglyhis ants rely predominantly on visual cues when navigating through open or cluttered environments. Regarding their visual capabilities, they employ a visual compass system based on skylight cues (Wehner, 1997) and various landmark guidance routines (Wehner et al., 1996; Wehner 2003, 2009), and even can combine information from terrestrial and celestial cues (Åkesson and Wehner, 2002). In addition, early laboratory experiments have already shown that the ants spontaneously respond towards landmark changes as the workers undergo the developmental transition from the indoor to the outdoor stage (Wehner et al., 1972).

Whether the MBs are the places for a landmark memory and the LAL synaptic complexes are part of the polarization pathway remains speculative, but this thesis shows that visual exposure raises the ants' locomotor activity and, at the same time, suggests that visual experiences have an important influence on the synaptic rewiring in the visual input region of the MB calyces (see Manuscript I, II, III). Furthermore, structural neuronal plasticity occurs at the time when foraging and hence the necessity to acquire and store visual information starts.

The behavioral studies demonstrate that during the pre-foraging "orientation runs" (see Manuscript II) the ants might associate the nest entrance with specific landmarks or get pre-exposed (entrained) to other visual information like the polarization pattern required for path integration, and, concomitantly adapt their neuronal circuitries to the upcoming challenges. I therefore hypothesize that associated changes in the neuronal circuitry in the MBs and LAL are necessary to improve or prepare the ants' visual orientation capabilities.
For a more detailed discussion see Manuscript I, II, III.

AGE- AND TASK-RELATED BEHAVIORAL AND NEURONAL PLASTICITY

In *Cataglyphis fortis*, the behavioral transition correlates with structural changes in the visual – and to a much smaller extent also in the olfactory - regions of the MB calyces and in the LAL.
Starting with plastic changes in the MBs, the transition from interior workers to foragers is associated with volume expansion of the MB calyces (Kühn-Bühlmann and Wehner, 2006) that goes along with a decrease – an effect called pruning - of microglomeruli (MG) (Manuscript I; Seid and Wehner, 2009) in the visual MB region (collar) and, at the same time, a massive expansion of the MB intrinsic dendritic network (Manuscript I) as also reported for honeybees (Farris et al., 2001; Dobrin et al., 2011). The significant decrease of MG density in the collar already occurs in interior workers. This suggests that dendritic expansion and pruning effects start during the late stages of indoor life and not at the beginning of the foraging phase. Changes in the lip region were not significant across age groups (Manuscript I). Axonal pruning might very likely be a universal process in adapting neuronal circuits during behavioral development and maturation (e.g. Truman and Reiss, 1976; Technau and Heisenberg, 1982; Levine and Truman, 1985; Weeks and Truman, 1986; Lee et al., 1999; Raff et al., 2002; Watts et al., 2003). As structural synaptic plasticity associated with this process is most likely driven by synaptic activity, the effect of dendritic growth and presynaptic pruning may be an important process in adjusting the MB-calyx microcircuits to the new sensory input occurring during the transition from interior workers to outdoor foragers in social insects. The associated changes in behavior represent an important element regulating the division of labor in insect colonies.
The study of Kühn-Bühlmann and Wehner (2006) in *C. bicolor* described a foraging-related volume increase in the collar and also an age-dependent volume increase in the collar (and other MB neuropils) of dark-reared animals that were older than 150 days (5-6 months). The results in Manuscript I confirmed both these effects in *C. fortis* and relate them to subcellular

changes of the synaptic organization. As compared to interior workers, artificially aged 6-month old dark-reared ants exhibit significantly larger volumes of the MB collar regions. Hence, even in the absence of light input or foraging experience the numbers of MG and consequently the total volume of the collar slowly increases with age. Even though the density of the MG decreases during the same time span, extrapolations of MG in the collar indicate that new MG may be generated at a very slow rate during aging. A similar long-term plasticity effect in the MG of the MB-calyx was also found in honeybee queens up to an age of 36 months (Groh et al., 2006). In this case, however, the increase predominantly occurred in the lip region, whereas the collar region decreased in size with age. This indicates that age-dependent programs for structural synaptic plasticity may differ across species, castes and sensory modalities, and they may differ in their timing. Interestingly, in *Cataglyphis* even 1 year old ants responded with plastic changes in the synaptic organization in response to light stimulation indicating that high levels of plasticity are maintained during aging.

Task-related plasticity and long-term structural changes in the MB calyces during aging are likely to be caused by different cellular mechanisms. The size increase of the MB collar in the 6-month old dark-reared ants is due to an increase in the number of MG, whereas the increase in size that occurs during the transition from the interior workers to the forager stage is accompanied by a pruning process and mainly caused by dendritic growth. Obviously, a steady increase in the number of synaptic complexes is permanently but slowly running in the background as the ants get older. This formation of new MG might prepare for new modulatory events triggered by upcoming sensory inputs and associated with pruning effects within synaptic complexes. The expansion of the dendritic network in response to light may prepare the system for novel input and learning-related associations.

The behavioral transition from interior workers to visually guided foragers in *C. fortis* is additionally accompanied by structural changes in synaptic complexes of the LAL. The total number of synaptic complexes was significantly higher in foragers compared to young interior workers (Manuscript III). These results together with the structural changes reported for the MBs highlights two different mechanisms of synaptic reorganization in visual brain centers of *C. fortis* during the ant's behavioral transition. An explanation of these different neuronal machineries may be provided by the involvement in dissimilar visual information processes of the LAL and the MB. While the LAL synaptic complexes are most possibly involved in the processing of polarized light information in *Cataglyphis* as described for locusts (Pfeiffer et al., 2005), the MBs are suspected to be involved in landmark-based processing indicated by studies in cockroaches (Mizunami et al., 1998). In foraging and

homing, *Cataglyphis* ants use the path integration system as their main navigational means (reviewed in Wehner et al., 1996; Wehner, 2003, 2009). Once the ant has vanished into its nest, the home vector is reset to zero (Knaden and Wehner, 2006), but a copy of the full vector pointing from the previously visited foraging site to the nest is stored in memory to be later recalled (for reviews, see Collett et al., 1998; Collett and Collett, 2000; Wehner et al., 2002; Wehner and Srinivasan, 2003). Since the mechanism of path integration is error-prone (Müller and Wehner, 1988; Merkle and Wehner, 2010), it is essential for foragers to additionally exhibit navigational backup systems including the use of landmarks to define places and routes (Wehner and Räber, 1979; Collett et al., 1992; Bisch- Knaden and Wehner, 2003; Collett and Collett, 2009). The ants link places, e.g. the nest entrance, with visual landmarks and later, when returning to this place, match their stored image with the actual one (reviewed in Wehner et al., 1996; Wehner, 2003).While the vector information provided by the ant's path integrator vanishes rapidly in the course of several days, landmark-based information is stored over the entire lifetime of *C. fortis* (Ziegler and Wehner, 1997; *C. bicolor*: Wehner, 1981b). Thus, the memory capacity of synaptic complexes in the LAL and MBs may differ in temporal dynamics and structure. While it is essential for a long-term memory to strengthen and expand synaptic contacts and thus rewiring the retained synaptic complexes as it is the case in the visual region of the MB calyx, an outgrowth of additional synaptic complexes in the LAL during the foraging phase is demandable since their extremely large size suggests particularly strong synaptic contacts and might therefore deliver fast signal transmission in the polarization vision pathway.

For a more detailed discussion see Manuscript I, III.

SENSORY EXPERIENCE TRIGGERS BEHAVIORAL AND NEURONAL PLASTICITY

One of the most important results of this thesis is the strong influence of sensory stimulation in triggering structural plasticity in the organization of synaptic complexes. The light exposure experiments clearly revealed that plastic changes in the collar region of the MBs can be reliably induced with light pulses in dark-reared ants of all age groups (see Manuscript I). This means that synaptic rewiring in the MB calyx is not under the exclusive control of an age-dependent program, but rather regulated to a large extent by sensory input. The light-trigger effect on the synaptic plasticity in the collar is strengthened by the fact that significant differences in MG densities occurred between natural foragers and age-matched dark foragers (see also Kühn-Bühlmann and Wehner, 2006), and that the latter group did not differ from the interior I and callow groups (see Manuscript I). Whether the plasticity in the LAL-synapses is

also driven by visual experience rather than an age-based internal programm, is very likely but needs to be proven.

If light exposure is that decisive, why does the structural reorganization already occur in the interior II group not showing any foraging behavior? Whereas interior I workers behave in a very sluggish manner and often stick motionless to the walls inside the nest chambers, interior II workers are more active, run around and may be on their way to become *diggers* carrying sand particles out of the nest to deposit them near the nest entrance and, by this, get exposed to light (Wehner et al., 1983). The behavioral observations in the natural habitat confirm these earlier indications and the hypothesis derived from them. Moreover, the recorded trajectories of ants being on their way of becoming foragers demonstrate that the ants perform exploration runs including pirouette-like turns very close to the nest entrance for a period of up to two days, before they actually start their foraging activity (see Manuscript II). The fact that both the light-induced neuronal changes in visual brain centers and the rise in the ants' locomotor activity occur between one and four days of light-exposure suggests that there is a link between structural synaptic plasticity in the MB calyx and the behavioral transition from interior tasks to outdoor foraging (see Manuscript II). In conclusion, in the sequence of behavioral transitions during an individual's life cycle the transition from the interior II worker stage to the *digger* stage directs the ants to the nest entrance where they are exposed to daylight for the first time. Later on the ants perform orientation runs exposing themselves to light for even longer periods. Thence, the system seems to be a self-reinforcing process: once the ants get light input, their locomotor activity is enhanced and their neuronal network is restructured in preparation of their upcoming tasks as foragers.

A behavior identical to the orientation runs in *C. fortis* has been reported for *C. bicolor* (Wehner et al., 2004). During these exploration runs as well as during the first foraging runs, ants of either species (Wehner et al., 2004) perform rotation movements (pirouettes) comparable to the 360° rotations at the beginning of long-distance runs in *C. bombycina* (Wehner and Wehner, 1990; Wehner, 1994) and the 360° pirouettes occurring during the spiral-like "learning walks" reported for the Namibian desert ant *Ocymyrmex robustior* when learning new landmarks around the nest entrance (Müller and Wehner, 2010). Furthermore, honeybees (Vollbehr, 1975; Zeil et al., 1996) and wasps (Zeil, 1993; Zeil et al., 1996) show orientation flights during which they perform turning loops to learn the nest location and nest-surrounding landmarks. Honeybees perform a variable number of orientation flights before they become foragers. It is likely that in *C. fortis* the orientation runs might also serve as learning runs to memorize the nest entrance and nest surroundings. Moreover, the pirouettes

may serve to stimulate and calibrate the neuronal networks involved in the polarization compass pathway.

In summary, during their "orientation runs" the ants might associate the nest entrance with specific landmarks or get pre-exposed (entrained) to other visual information like the polarization pattern required for path integration, and, concomitantly adapt their neuronal circuitries to the upcoming challenges.

For a more detailed discussion see Manuscript I, II, III.

REVERSIBILITY OF BEHAVIORAL AND NEURONAL PLASTICITY

Environmental events leading to a reversal of foragers back to the interior worker stage are reported for honeybees (for a review see Robinson, 1992). For *Cataglyphis* it was shown that the stages of interior workers and foragers can be reversed by altering the light conditions to which the ants are exposed (Wehner et al., 1972). On a neuronal basis this thesis reveals that light-induced foragers transferred back to darkness exhibit only some reverse-type characteristics in the MB calyx (Manuscript II). Whereas in the reversed foragers the expected increase in MG numbers occur in the olfactory lip region, in the visual collar region no changes are observed. However, a significant reduction in the amount of synapsin in the terminals of the presynaptic projection neurons likely caused by a lack of continued sensory input is recorded for reversed-foragers when compared with age-matched light-exposed foragers. A previous study in the honeybee has shown that the reversal of honeybee foragers, either due to the onset of winter or following experimental manipulation of the colony structure, does not reduce the MB volume (Fahrbach et al., 2003). This is in accordance with the present findings indicating no major changes in postsynaptic profiles and is supported by the results that changes in the Kenyon cell dendritic network are a major factor in MB calyx volumetric changes (for honeybees: Farris et al, 2001; for *Cataglyphis*: Manuscript I).

In conclusion the results demonstrate that structural changes in the synaptic organization of the MB calyces that occur during the transition from interior workers to foragers are reversible and hence indicate that the MB circuitries are plastic in both directions. This high degree of neuronal plasticity provides ideal conditions for underpinning the ants' high behavioral plasticity in response to changing environmental conditions and social requirements.

For a more detailed discussion see Manuscript II.

SPECIALIZATION OF THE OLFACTORY SYSTEM IN *CATAGLYPHIS*

Among insects, there are many interspecific as well as intraspecific (caste- and sex-specific) variations in general features of the antennal lobes (ALs).

All tested *Cataglyphis* species (*C. fortis*, *C. bicolor*, *C. albicans*, *C. rubra* and *C. noda*) have smaller numbers of glomeruli (198-249; see Manuscript IV) compared to those previously found in olfactory guided ants using trail marking or chemical recruitment (257-630 glomeruli in fungus-growing species: Kelber et al., 2009; Kuebler et al., 2010; ~430 in carpenter ants: Zube and Rössler, 2008; Nishikawa et al., 2008**;** ~370 in *Formica* species: Manuscript IV). The interspecific variations in glomerular numbers indicate that the number of glomeruli appears to be generally correlated with the amount of chemically mediated behaviors (Kleineidam and Rössler, 2009) and by this is in accordance to the reduced glomerular number in *Cataglyphis*. In contrast to all the ant species mentioned above, *Cataglyphis* ants forage solitarily, do not use trail pheromones, but rely primarily on visual guidance mechanisms (Wehner, 2003).

Comparisons between the worker castes of different *Cataglyphis* species revealed that *C. fortis* has with c. 198 the lowest number of glomeruli compared to all other species (226-249) and has a conspicuously enlarged glomerulus located close to the antennal nerve entrance (see Manuscript IV). With only one exception all enlarged glomeruli in the workers of *C. rubra*, *C. bicolor* and *C. albicans* are located in a group of glomeruli constituting the dorsalmost part of the AL nearest to the dorsal lobe and might be involved in the processing of CO_2-, thermo- or hygroreceptive information like it has been shown for several insect species (CO_2-sensitive glomeruli: Lee and Altner, 1986; Bogner et al., 1986; Kent et al., 1986; Kent et al., 1999; Guerenstein et al., 2004; thermo- and hygroreceptive glomeruli: Nishikawa et al., 1995; Nishino et al., 2003; Nishino et al., 2009; Nakanishi et al., 2010). In *C. fortis* workers, the largest glomerulus is located next to the antennal nerve entrance. A macroglomerulus not involved in the processing of sex-pheromone information but most likely in the detection and processing of trail-pheromone components was discovered for the first time in leaf-cutting ants (Kleineidam et al., 2005; Kelber et al., 2009; Kelber et al., 2010; Kuebler et al., 2010). These studies clearly demonstrate that the occurrence of enlarged glomeruli or even macroglomeruli may vary substantially across ant species and even within one species, and may correlate with certain behavioral requirements of odor processing. The size, stable location among tested workers, and its absence in all other tested *Cataglyphis* species, indicate a specialized function of the enlarged glomerulus in *C. fortis*.

By recalling the fact that *C. fortis* possesses the AL with the lowest number of glomeruli among all tested *Cataglyphis* species and assuming that the number of glomeruli reflects the complexity of olfactory-guided behavior, why is it that of all *Cataglyphis* species just *C. fortis* has an enlarged glomerulus at its antennal nerve entrance? The solely presence of this glomerulus may represent an adaptation to the special behavioral ecology of *C. fortis*, which among all its congeners takes an outstanding position regarding its preferred habitat, the food-impoverished salt pan flats (Wehner, 1981a, 1983; Wehner et al., 1994). The North African *C. bicolor*, *C. albicans* and *C. rubra* inhabit the nutritionally richer low-shrub semi-deserts surrounding the salt pans (Dillier and Wehner, 2004). To unravel which of the many differences in the behavioral ecology between *C. fortis* and its congeners (Schmid-Hempel, 1983; Wehner, 1983, 1987; Dillier and Wehner, 2004) are functionally related to the presence of the remarkably enlarged glomerulus in *C. fortis* remains a challenging task to accomplish.
Caste- and sex-specific variations in *C. fortis* (see Manuscript IV) were found in males possessing a significantly smaller number of glomeruli (c. 150) compared to female workers (198) and queens (219) reflecting the in general restricted behavioral repertoires of hymenopteran males (*Apis mellifera*: Arnold et al., 1985; Brockmann and Brückner, 2001; Sandoz, 2006; *Harpegnathos saltator*: Hoyer et al., 2005; *Camponotus floridanus*: Zube and Rössler, 2008; *Camponotus japonicus*: Nishikawa et al., 2008*; Atta vollenweideri*: Kuebler et al., 2010**)**. A prominent male-specific macroglomerulus likely to be involved in sex pheromone communication as described for several insect species (moths: Hansson et al., 1991; Anton and Homberg, 1999; Rössler et al., 1999; honeybees: Arnold et al., 1985; Sandoz, 2006) occupies a position different from that of the enlarged glomerulus in females.
For a more detailed discussion see Manuscript IV.

OUTLOOK

The structural synaptic plasticity stimulated and exploited in the visual input centers of the MBs and the giant synaptic complexes in the LAL of *Cataglyphis* make these visually guided long-distance insect navigators a particularly promising model organism for future investigations on visually mediated brain plasticity.
This thesis outlines that sensory exposure rather than an internal program induces synaptic reorganization in visual regions of the MB calyces. One likely hypothesis is that associated changes in the neuronal circuitry in the MBs are necessary to improve or prepare the ants' visual orientation capabilities. This needs to be thoroughly tested in future knockdown experiments of certain components that control structural plasticity in the MB-calyx synaptic

microcircuits. Furthermore, learning induced long-term memory (as shown for olfactory memory in the honeybee by Hourcade et al. 2010) may also lead to changes in the synaptic organization of visual centers, which need to be explored in the future

For the visual region of the MB circuitry it has been demonstrated that visual experience throughout the natural life-span triggers plastic changes. Whether the plasticity in the LAL-synapses is also driven by visual experience rather than an age-based internal programm, is very likely but needs to be proven. For this, the number of LAL synaptic complexes of dark-reared ants age-matched to naturally experienced foragers have to be analyzed. In order to confirm the polarization sensitivity of the LAL neurons and of all other relay stations belonging to the hypothetic pathway from the dorsal rim area to the CX, further tracer injections in corresponding areas and physiological studies (in particular electrophysiological recordings) in the different relay stations are necessary.

While synaptic pruning and dendritic outgrowth is the mechanism of neuronal adaptation to the new sensory demands and motor tasks in the MB collar, an increase of the total number of synaptic complexes is the mechanism in the LAL. By this, the present thesis highlights two different mechanisms of synaptic reorganization in visual brain centers and emphasizes that the structural machinery for plasticity is context specific and best adapted to the behavioral requirements. The structural changes in synaptic complexes of both the MBs and the LALs need to be specified on a cellular level by electron microscopy studies. Molecular studies need to be performed in order to identify the mechanisms that operate and regulate the structural changes on the pre- and postsynaptic side.

Furthermore, this thesis outlines that visual exposure affects not only the neuronal organization but also raises the ants' locomotor activity. Whether the two are causally linked needs to be proven in knock-down experiments of MB calyx plasticity. In the natural situation, a late stage of interior workers perform pre-foraging "orientation runs" during which the ants might associate the nest entrance with specific landmarks or get pre-exposed (entrained) to other visual information like the polarization pattern required for path integration, and, concomitantly adapt their neuronal circuitries to the upcoming challenges. These runs include pirouettes that may serve to stimulate and calibrate the neuronal networks involved in the polarization compass pathway. Behavioral studies during the very first exploration runs are needed to identify the possible role of skylight polarization information on the neuronal structure of the LAL synaptic complexes. For this, the structure and number of LAL synaptic complexes have to be analyzed in ants that are deprived of polarization information during their orientation runs.

Additionally, this thesis identifies specializations in the olfactory system in the highly visually guided *Cataglyphis* species. In order to characterize the odor specificity of the enlarged glomerulus in *C. fortis*, physiological (in particular functional imaging) studies are needed. The functional significance may uncover whether the enlarged glomerulus is an adaptation on the behavioral ecology that distinguishes *C. fortis* from its congeners. Moreover, functional imaging studies in the sexual castes of *C. fortis* are needed to test the very likely hypothesis that the male-specific macroglomerulus is sex-pheromone sensitive and whether the enlarged glomerulus in queens is homologues to the one found in workers.

The present thesis demonstrates that *Cataglyphis* is an excellent candidate for studying neuronal plasticity associated with the ant's lifelong behavioral flexibility and for studying the neuronal mechanisms underlying navigational features. It remains a challenging task to unravel how the multimodal informations used for navigation - ranging from landmark learning or the use of olfactory signals to the navigation by the polarization compass and the proprioreceptive odometer - are integrated in the nervous system.

ABBREVIATIONS

AL	antennal lobe
ALLO	anterior lobe of the lobula
AN	antennal nerve
AOTU	anterior optic tubercle
BR	basal ring
c	caudal
CO	collar
d	dorsal
DR	dorsal lobe
DRA	dorsal rim area
DRLA	dorsal rim lamina
DRME	dorsal rim medulla
EB	ellipsoid body
e-vector	electric field vector
FB	fan-shaped body
IC	inner compact cells
IR	immunoreactivity
KC	Kenyon cell
l	lateral
LA	lamina
LAL	lateral accessory lobe
l-APT	lateral antennal lobe-protocerebral tract
LCA	lateral calyces
LH	lateral horn
LO	lobula
LP	lip
LT	lateral triangle
m	medial
m-APT	medial antennal lobe-protocerebral tract
MB	mushroom body
MCA	medial calyces

ME	medulla
MG	microglomerulus (with the exception of Manuscript IV:.macroglomerulus)
MGC	macroglomerular complex
M lobe/ML	medial lobe
MO	median olive
NC	non-compact cells
OC	outer compact cells
OL	optic lobe
ORN	olfactory receptor neuron
PB	protocerebral bridge
PED	pedunculus
PN	projection neuron
r	rostral
T	sensory tract
TALT	tubercle-accessory lobe tract
TN	tangential neurons
UV	ultraviolet
V lobe/VL	vertical lobe
CX	central complex

REFERENCES

Abel R, Rybak J, Menzel R. 2001. Structure and response patterns of olfactory interneurons in the honeybee, *Apis mellifera*. J Comp Neurol 437: 363-383.

Agosti D. 1990. Review and reclassification of *Cataglyphis* (Hymenoptera, Formicidae). J Nat Hist 24: 1457-1505.

Åkesson S, Wehner R. 2002. Visual navigation in desert ants *Cataglyphis fortis*: are snapshots coupled to a celestial system of reference? J Exp Biol 205: 1971-1978.

Anton S, Homberg U. 1999. Antennal lobe structure. In: Insect olfaction (ed. Hansson BS). Berlin, Heidelberg: Springer, pp. 97-124.

Arnold G, Masson C, Budharugsa S. 1985. Comparative study of the antennal lobes and their afferent pathway in the worker bee and the drone (*Apis mellifera*). Cell Tissue Res 242: 593-605.

Barth M, Heisenberg M. 1997. Vision affects mushroom bodies and central complex in *Drosophila melanogaster*. Learn Mem 4: 219-229.

Barth M, Hirsch HVB, Meinertzhagen IA, Heisenberg M. 1997. Experience-dependent developmental plasticity in the optic lobe of *Drosophila melanogaster*. J Neurosci 17: 1493-1504.

Becher MA, Scharpenberg H, Moritz RFA. 2009. Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera* L.). J Comp Physiol 195: 673-679.

Bennett EL, Diamond MC, Krech D, Rosenzweig MR. 1964. Chemical and anatomical plasticity of brain: changes in brain through experience, demanded by learning theories, are found in experiments with rats. Science 146: 610-619.

Berg BG, Almaas TJ, Bjaalie JG, Mustaparta H. 1998. The macroglomerular complex of the antennal lobe in the tobacco budworm moth *Heliothis virescens*: specified subdivision in four compartments according to information about biologically significant compounds. J Comp Physiol A 183: 669-682.

Beugnon G, Chagné P, Dejean A. 2001. Colony structure and foraging behavior in the tropical formicine ant, *Gigantiops destructor*. Insectes Sociaux 48: 347-351.

Bicker G, Schafer S, Kingan TG. 1985. Mushroom body feedback interneurons in the honeybee show GABA-like immunoreactivity. Brain Res 360: 394-397.

Bisch-Knaden S, Wehner R. 2001. Egocentric information helps desert ants to navigate around familiar obstacles. J Exp Biol 204: 4177-4184.

Bisch-Knaden S, Wehner, R. 2003. Local vectors in desert ants: context-dependent landmark learning during outbound and homebound runs. J Comp Physiol 189: 181-187.

Blum M, Labhart T. 2000. Photoreceptor visual fields, ommatidial array, and receptor axon projections in the polarisation-sensitive dorsal rim area of the cricket compound eye. J Comp Physiol 186: 119-128.

Bogner F, Boppré M, Ernst KD, Boeckh J. 1986. CO_2 sensitive receptors on labial palps of *Rhodogastria* moths (Lepidoptera: Arctiidae): physiology, fine structure and central projection. J Comp Physiol A 158: 741-749.

Briscoe AD, Chittka L. 2001. The evolution of color vision in insects. An Rev Entomol 46: 471–510.

Brockmann A, Brückner D. 2001. Structural differences in the drone olfactory system of two phylogenetically distant *Apis* species, *A. florea* and *A. mellifera*. Naturwissenschaften 88: 78-81.

Bullock TH, Horridge GA. 1965. Structure and Function in the Nervous System of Invertebrates. San Francisco: Freeman.

Calabi P, Traniello JFA. 1989. Behavioral flexibility in age castes of the ant *Pheidole dentata*. J Insect Behav 2: 663-677.

Capaldi EA, Smith AD, Osborne JL, Fahrbach SE, Farris SM, Reynolds DR, Edwards AN, Martin A, Robinson GE, Poppy GM, Riley JR. 2000. Ontogeny of orientation flight in the honeybee revealed by harmonic radar. Nature 403: 537-540.

Cerdá X. 1988. La carrera nupcial de *Cataglyphis iberica* (Hymeoptera: Formicidae). Congr Iberico Entomol 3: 511-514.

Collett M, Collett TS. 2000. How do insects use path integration for their navigation? Biol cybern 83: 245-259.

Collett TS, Collett M. 2002. Memory use in insect visual navigation. Nat Rev Neurosci 3: 542-552.

Collett TS, Collett M. 2004. How do insects represent familiar terrain? J Physiol 98: 259-264.

Collett M, Collett TS. 2009. The learning and maintenance of local vectors in desert ant navigation. J Exp Biol 212: 895-900.

Collett TS, Dillmann E, Giger A, Wehner R. 1992. Visual landmarks and route following in desert ants. J Comp Physiol 170: 435-442.

Collett M, Collett TS, Bisch S, Wehner R. 1998. Local and global vectors in desert ant navigation. Nature 450, 269-272.

Collett TS, Collett M, Wehner R. 2001. The guidance of desert ants by extended landmarks. J Exp Biol 204: 1635-1639.

Collingwood CA. 1985. Hymenoptera: Fam. Formicidae of Saudi Arabia. Fauna of Saudi Arabia 7: 230-302.

Davis RL. 2005. Olfactory memory formation in *Drosophila*: from molecular to systems neuroscience. Annu Rev Neurosci 28: 275-302.

Devaud JM, Acebes A, Ramaswami M, Ferrús A. 2003. Structural and functional changes in the olfactory pathway of adult *Drosophila* take place at a critical age. J Neurobiol 56: 13-23.

Dillier FX, Wehner R. 2004. Spatio-temporal patterns of colony distribution in monodomous and polydomous species of North African desert ants, genus *Cataglyphis*. Insectes Sociaux 51: 186-196.

Dobrin SE, Herlihy JD, Robinson GE, Fahrbach SE. 2011. Muscarinic regulation of Kenyon cell dendritic arborizations in adult worker honey bees. Arthropod Struct Dev (in press).

Dumpert K. 1972. Receptors for alarm substances on antenna of *Lasius fuliginosus* (Latr) (Hymenoptera, Formicidae). Zeitschrift Vergleichende Physiologie 76: 403–425.

Durst C, Eichmüller S, Menzel R. 1994. Development and experience lead to increased volume of subcompartments of the honeybee mushroom body. Behav Neural Biol 62: 259-263.

Ehmer B, Hoy R. 2000. Mushroom bodies of vespid wasps. J Comp Neurol 416: 93-100.

Ehmer B, Gronenberg W. 2002. Segregation of visual input to the mushroom bodies in the honeybee (*Apis mellifera*). J Comp Neurol 451: 362-373.

Erber J, Masuhr TH, Menzel R. 1980. Localization of short-term memory in the brain of the bee, *Apis mellifera*. Physiol Entomol 5: 343-358.

Esslen J, Kaissling KE. 1976. Number and distribution of sensilla on antennal flagellum of honeybee (*Apis mellifera* L). Zoomorphologie 83: 227-251.

Fahrbach SE. 1997. Regulation of age polyethism in bees and wasps by juvenile hormone. Adv Stud Behav 26: 285–316.

Fahrbach SE. 2006. Structure of the mushroom bodies of the insect brain. Ann Rev Entomol 51: 209-232.

Fahrbach SE, Farris SM, Sullivan JP, Robinson GE. 2003. Limits on volume changes in the mushroom bodies of the honey bee brain. J Neurobiol 57: 141-151.

Farris S. 2005. Evolution of insect mushroom bodies: old clues, new insights. Arthropod Struct Dev 34: 211-234.

Farris SM, Sinakevitch I. 2003. Development and evolution of the insect mushroom bodies: towards the understanding of conserved developmental mechanisms in a higher brain center. Arthropod Struct Dev 32: 79-101.

Farris SM, Robinson GE, Davis RL, Fahrbach SE. 1999. Larval and pupal development of the mushroom bodies in the honey bee, *Apis mellifera*. J Comp Neurol 414: 97-113.

Farris SM, Robinson GE, Fahrbach SE. 2001. Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. J Neurosci 21: 6395-6404.

Fent K, Wehner R. 1985. Oceili: A celestial compass in the desert ant *Cataglyphis*. Science 228: 192–194.

Fishilevich E, Vosshall LB. 2005. Genetic and functional subdivision of the *Drosophila* antennal lobe. Curr Biol 15: 1548-1553.

Flanagan D, Mercer AR. 1989. An atlas and 3-D reconstruction of the antennal lobes in the worker honey bee, *Apis mellifera* L. (Hymenoptera: Apidae). Int J Insect Morphol 18: 145-159.

Floeter MK, Greenough WT. 1979. Cerebellar plasticity: modification of Purkinje cell structure by differential rearing in monkeys. Science 206: 227-229.

Frambach I, Rössler W, Winkler M, Schürmann F-W. 2004. F-actin at identified synapses in the mushroom body neuropil of the insect brain. J Comp Neurol 475: 303-314.

Galizia CG, Rössler W. 2010. Parallel olfactory systems in insects: anatomy and function. Annu Rev Entomol 55: 399-420.

Galizia CG, McIlwrath SL, Menzel R. 1999a. A digital three-dimensional atlas of the honeybee antennal lobe based on optical sections acquired by confocal microscopy. Cell Tissue Res 295: 383-394.

Galizia CG, Sachse S, Rappert A, Menzel R. 1999b. The glomerular code for odor representation is species specific in the honeybee *Apis mellifera*. Nat Neurosci 2: 473-478.

Ganeshina O, Menzel R. 2001. GABA-immunoreactive neurons in the mushroom bodies of the honeybee: an electron microscopic study. J Comp Neurol 437: 335-349.

Gao Q, Yuan B, Chess A. 2000. Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. Nature Neurosci 3: 780-785.

Gerber B, Tanimoto H, Heisenberg M. 2004. An engram found? Evaluating the evidence from fruit flies. Curr Opin Neurobiol 14: 737-744.

Giurfa M. 2007. Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. J Comp Physiol 193: 801-824.

Goll W .1967. Strukturuntersuchungen am Gehirn von *Formica*. Z Morph Ökol Tiere 59: 143-210.

Goodman LJ. 1981. Organisation and physiology of the insect dorsal ocellar system. In: Handbook of Sensory Physiology vol. VII 6B (ed. Autrum H). Berlin: Springer, pp. 471-592.

Groh C, Rössler W. 2008. Caste-specific postembryonic development of primary and secondary olfactory centers in the female honeybee brain. Arthropod Struct Dev 37: 459-468.

Groh C, Rössler W. 2011. Comparison of microglomerular structures in the mushroom body calyx of neopteran insects. Arthropod Struct Dev 40: 358-367.

Groh C, Tautz J, Rössler W. 2004. Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. P Natl Acad Sci USA 101: 4268-4273.

Groh C, Ahrens D, Rössler W. 2006. Environment- and age-dependent plasticity of synaptic complexes in the mushroom bodies of honeybee queens. Brain Behav Evolut 68: 1-14.

Gronenberg, W. 2001. Subdivisions of hymenopteran mushroom body calyces by their afferent supply. J Comp Neurol 436: 474-489.

Gronenberg W, Hölldobler B. 1999. Morphologic representation of visual and antennal information in the ant brain. J Comp Neurol 412: 229-240.

Gronenberg W, Heeren S, Hölldobler B. 1996. Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. J Exp Biol 199: 2011-2019.

Grünewald B. 1999. Morphology of feedback neurons in the mushroom body of the honeybee, *Apis mellifera*. J Comp Neurol 404: 114-126

Guerenstein PG, Christensen TA, Hildebrand JG. 2004. Sensory processing of ambient CO_2 information in the brain of the moth *Manduca sexta*. J Comp Physiol 190: 707-725.

Haak U, Hölldobler B, Bestmann HJ, Kern F. 1996. Species-specificity in trail pheromones and Dufour's gland contents of *Camponotus atriceps* and *C. floridanus* (Hymenoptera: Formicidae). Chemoecology 7: 85-93.

Hanesch U, Fischbach KF, Heisenberg M. 1989. Neural architecture of the central complex in *Drosophila melanogaster*. Cell Tissue Res 457: 343-366.

Hansson BS, Anton S. 2000. Function and morphology of the antennal lobe: new developments. Annu Rev Entomol 45: 203-231.

Hansson BS, Christensen TA, Hildebrand JG. 1991. Functionally distinct subdivisions of the macroglomerular complex in the antenna1 lobe of the male sphinx moth *Manduca sexta*. J Comp Neurol 312: 264-278.

Heinze S, Homberg U. 2007. Maplike representation of celestial E-vector orientations in the brain of an insect. Science 315: 995-997.

Heinze S, Homberg U. 2009. Linking the input to the output: new sets of neurons complement the polarization vision network in the locust central complex. J Neurosci 29: 4911-4921.

Heisenberg M. 1998. What do the mushroom bodies do for the insect brain? An introduction. Learn Mem 5: 1-10.

Heisenberg M. 2003. Mushroom body memoir: From maps to models. Nat Rev Neurosci 4: 266–275.

Heisenberg M, Heusipp M, Wanke C. 1995. Structural plasticity in the *Drosophila* brain. J Neurosci 15: 1951-1960.

Held H. 1893. Die zentrale Gehörleitung. Arch Anat Physiol Anat Abt 17: 201-248.

Herrling PL. 1976. Regional distribution of three ultrastructural retinula types in the retina of *Cataglyphis bicolor* Fabr. (Formicidae, Hymenoptera). Cell Tissue Res 169: 247-66.

Hildebrand JG, Shepherd GM. 1997. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. Annu Rev Neurosci 20: 595-631.

Hölldobler B, Wilson EO. 1990. The Ants. Cambridge, MA: Belknap Press.

Homberg U. 2004. In search of the sky compass in the insect brain. Die Naturwissenschaften 91: 199-208.

Homberg U, Paech A. 2002. Ultrastructure and orientation of ommatidia in the dorsal rim area of the locust compound eye. Arthropod Struct Dev 30: 271-280.

Homberg U, Christensen TA, Hildebrand JG. 1989. Structure and function of the deutocerebrum in insects. Ann Rev Entomol 34: 477-501.

Homberg U, Kingan TG, Hildebrand JG. 1990. Distribution of FMRFamide-like immunoreactivity in the brain and suboesophageal ganglion of the sphinx moth *Manduca sexta* and colocalization with SCPB-, BPP-, and GABA-like immunoreactivity. Cell Tissue Res 259: 401-419.

Homberg U, Vitzthum H, Müller M, Binkle U. 1999. Immunocytochemistry of GABA in the central complex of the locust *Schistocerca gregaria*: identification of immunoreactive neurons and colocalization with neuropeptides. J Comp Neurol 409: 495–507.

Homberg U, Hofer S, Pfeiffer K, Gebhardt S. 2003. Organization and neural connections of the anterior optic tubercle in the brain of the locust, *Schistocerca gregaria*. J Comp Neurol 462: 415–430.

Homberg U, Heinze S, Pfeiffer K, Kinoshita M, el Jundi B. 2011. Central neural coding of sky polarization in insects. Philos T R Soc B 366: 680-687.

Honegger H-W, Schürmann FW. 1975. Cobalt sulphide staining of optic fibres in the brain of the cricket, *Gryllus campestris*. Cell Tissue Res 159: 213–225.

Horváth G, Varju D. 2004. Polarized Light in Animal Vision: Polarization Patterns in Nature. Berlin: Springer.

Hourcade B, Muenz TS, 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? J Neurosci 30: 6461-6465.

Hoyer SC, Liebig J, Rössler W. 2005. Biogenic amines in the ponerine ant *Harpegnathos saltator*: serotonin and dopamine immunoreactivity in the brain. Arthropod Struct Dev 34: 429-440.

Huang Z-Y, Robinson GE, Tobe SS, Yagi KJ, Strambi C, Strambi A, Stay B. 1991. Hormonal regulation of behavioural development in the honey bee is based on changes in the rate of juvenile hormone biosynthesis. J Insect Physiol 37: 733-741.

Huang Z-Y, Robinson GE, Borst DW. 1994. Physiological correlates of division of labor among similarly aged honey bees. J Comp Physiol 174: 731-739.

Huetteroth W, Schachtner J. 2005. Standard three-dimensional glomeruli of the *Manduca sexta* antennal lobe: a tool to study both developmental and adult neuronal plasticity. Cell Tissue Res 319: 513-524.

Ismail N, Robinson GE, Fahrbach, SE. 2006. Stimulation of muscarinic receptors mimics experience-dependent plasticity in the honey bee brain. Proc Natl Acad Sci USA 103: 207-211.

Joerges J, Küttner A, Galizia CG, Menzel R. 1997. Representation of odours and odour mixtures visualized in the honeybee brain. Nature 387: 285-288.

Jones JC, Helliwell P, Beekman M, Maleszka R, Oldroyd BP. 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. J Comp Physiol 191: 1121-1129.

el Jundi B, Homberg U. 2010. Evidence for the possible existence of a second polarization vision pathway in the locust brain. J Insect Physiol 56: 971-979.

Keil T. 1999. Morphology and development of the peripheral olfactory organs. In: Insect Olfaction (ed. Hansson BS). Heidelberg: Springer, pp. 5-48.

Kelber C, Rössler W, Roces F, Kleineidam CJ. 2009. The antennal lobes of fungus-growing ants (Attini): neuroanatomical traits and evolutionary trends. Brain Behav Evol 73: 273-284.

Kelber C, Rössler W, Kleineidam CJ. 2010. Phenotypic plasticity in number of glomeruli and sensory innervation of the antennal lobe in leaf-cutting ant workers (*A. vollenweideri*). Dev Neurobiol 70: 222-234.

Kent KS, Harrow ID, Quartararo P, Hildebrand JG. 1986. An accessory olfactory pathway in Lepidoptera: the labial pit organ and its central projections in *Manduca sexta* and certain other sphinx moths and silk moths. Cell Tissue Res 245: 237-245.

Kent KS, Oland LA, Hildebrand JG. 1999. Development of the labial pit organ glomerulus in the antennal lobe of the moth *Manduca sexta*: the role of afferent projections in the formation of identifiable olfactory glomeruli. J Neurobiol 40: 28-44.

Kirschner S, Kleineidam CJ, Zube C, Rybak J, Grünewald B, Rössler R. 2006. Dual olfactory pathway in the honeybee, *Apis mellifera*. J Comp Neurol 499: 933-952.

Klagges BRE, Heimbeck G, Godenschwege TA, Hofbauer A, Pflugfelder GO, Reifegerste R, Reisch D, Schaupp M, Buchner S, Buchner E. 1996. Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila*. J Neurosci 16: 3154-3165.

Kleineidam CJ, Rössler R. 2009. Adaptations of the olfactory system of social Hymenoptera. In: Organization of Insect Societies. Cambridge/London: Harvard Univ Press, pp. 195-219.

Kleineidam CJ, Obermayer M, Halbich W, Rössler W. 2005. A macroglomerulus in the antennal lobe of leaf-cutting ant workers and its possible functional significance. Chem Senses 30: 1-10.

Knaden M, Wehner R. 2006. Ant navigation: resetting the path integrator. J Exp Biol 209: 26-31.

Krofczik S, Khojasteh U, Hempel de Ibarra N, Menzel R. 2008. Adaptation of microglomerular complexes in the honeybee mushroom body lip to manipulations of behavioral maturation and sensory experience. Dev Neurol 68: 1007-1017.

Kuebler LS, Kelber C, Kleineidam CJ. 2010. Distinct antennal lobe phenotypes in the leaf-cutting ant (*Atta vollenweideri*). J Comp Neurol 518: 352-36.

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

Labhart T. 1986. The electrophysiology of photoreceptors in different eye regions of the desert ant, *Cataglyphis bicolor*. J Comp Physiol 158: 1-7.

Labhart T. 2000. Polarization-sensitive interneurons in the optic lobe of the desert ant *Cataglyphis bicolor*. Naturwissenschaften 87: 133–136.

Labhart T, Petzold J. 1993. Processing of polarized light information in the visual field of crickets. In: Sensory Systems of Arthropods (eds. Wiese K, Gribakin FG, Poppv AV, Renninger G). Basel, Boston, Berlin: Birkhäuser, pp. 158-168.

Labhart T, Meyer EP. 1999. Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. Microsc Res Techniq 47: 368-379.

Labhart T, Meyer EP. 2002. Neural mechanisms in insect navigation: polarization compass and odometer. Curr Opin Neurobiol 12: 707-714.

Laissue PP, Reiter C, Hiesinger PR, Halter S, Fischbach KF, Stocker RF. 1999 Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. J Comp Neurol 405: 543-552.

Lee JK, Altner H. 1986. Primary sensory projections of the labial palp-pit organ of *Pieres rapae* L. (Lepidoptera: Pieridae). Int J Insect Morphol 15: 439-448.

Lee T, Lee A, Luo L. 1999. Development of the *Drosophila* mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Dev 126: 4065-4076.

Lei H, Christensen TA, Hildebrand JG. 2004. Spatial and temporal organization of ensemble representations for different odor classes in the moth antennal lobe. J Neurosci 24: 11108-1119.

Leiss F, Groh C, Butcher NJ, Meinertzhagen IA, Tavosanis G. 2009. Synaptic organization in the adult *Drosophila* mushroom body calyx. J Comp Neurol 517: 808-824.

Lenoir A, Querard L, Pondicq N, Berton F. 1988. Reproduction and dispersal in the ant *Cataglyphis cursor* (Hymenoptera, Formicidae). Psyche 95: 21-44.

Lent DD, Pintér M, Strausfeld NJ. 2007. Learning with half a brain. Dev Neurobiol 67: 740-751.

Levine RB, Truman JW. 1985. Dendritic reorganization of abdominal motoneurons during metamorphosis of the moth, *Manduca sexta*. J Neurosci 5: 2424-2431.

Lindauer M .1953. Division of labour in the honey bee colony. Bee World 34: 63-73, 85-90.

Marsh AC. 1985. Microclimatic factors influencing foraging patterns and success of the thermophilic desert ant, *Ocymyrmex barbiger*. Insectes Sociaux 32: 286-296.

Menzel R. 1999. Memory dynamics in the honeybee. J Comp Physiol 185: 323-340.

Menzel R. 1990. Learning, memory and "cognition" in honey bees. In: Neurobiology of comparative cognition (ed. Kesner RP, Olton DS). Hillsdale, NJ: Erlbaum Inc, pp. 237-292.

Menzel R. 2001. Searching for the memory trace in a mini-brain, the honeybee. Learn Mem 8: 53-62.

Menzel R, Wehner R. 1970. Augenstruktur bei verschieden großen Arbeiterinnen von *Cataglyphis bicolor* Fabr. (Formicidae, Hymenoptera). Z Verg Physiol: 68: 446-449.

Menzel R, Giurfa M. 2006. Dimensions of cognition in an insect, the honeybee. Behav Cog Neurosci Rev 5: 24-40.

Menzel R, Geiger K, Chittka L, Joerges J, Kunze J, Müller U. 1996. The knowledge base of bee navigation. J Comp Exp Biol 199: 141-146.

Merkle T, Wehner R. 2010. Desert ants use foraging distance to adapt the nest search to the uncertainty of the path integrator. Behav Ecol 21: 349-355.

Merkle T, Knaden M, Wehner R. 2006. Uncertainty about nest position influences systematic search strategies in desert ants. J Exp Biol 209*:* 3545-3549.

Meyer EP. 1979. Golgi-EM-study of first and second order neurons in the visual system of *Cataglyphis bicolor* Fabricius (Hymenoptera, Formicidae). Zoomorphologie 92: 115-139.

Michels B, Chen Y-C, Saumweber T, Mishra D, Tanimoto H, Schmid B, Engmann O, Gerber B. 2011. Cellular site and molecular mode of synapsin action in associative learning. Learn Memory 185: 332-344.

Mizunami M. 1995. Functional diversity of neural organization in insect ocellar systems. Vision Res 35: 443-452.

Mizunami M, Weibrecht JM, Strausfeld NJ. 1998. Mushroom bodies of the cockroach: their participation in place memory. J Comp Neurol 402: 520-537.

Mobbs PG. 1982. The brain of the honeybee *Apis mellifera*. I. The connections and spatial organization of the mushroom bodies. Philos T R Soc Lond 298: 309-354.

Mota T, Yamagata N, Giurfa M, Gronenberg W, Sandoz J-C. 2011. Neural Organization and visual processing in the anterior optic tubercle of the honeybee brain. J Neurosci 31: 11443-11456.

Mote MI, Wehner R. 1980. Functional characteristics of photoreceptors in the compound eye and ocellus of the desert ant, *Cataglyphis bicolor*. J Comp Physiol 137: 63-71.

Müller M, Wehner R. 1988. Path integration in desert ants, *Cataglyphis fortis*. Proc Natl Acad Sci 85: 5287-5290.

Müller M, Wehner R. 1994. The hidden spiral: systematic search and path integration in desert ants, *Cataglyphis fortis*. J Comp Physiol 175: 525-530.

Müller M, Wehner R. 2010. Path integration provides a scaffold for landmark learning in desert ants. Curr Biol 20: 1-4.

Müller M, Homberg U, Kühn A. 1997. Neuroarchitecture of the lower division of the central body in the brain of the locust *Schistocerca gregaria*. Cell Tissue Res 288: 159-176.

Muenz TS, Oberwallner B, Gehring K, Rössler W. 2008. Plasticity of synaptic complexes in the mushroom-bodies of the honeybee brain depends on age, experience and season. FENS Abstr., vol.4, 143.

Mustaparta H. 1990. Chemical information processing in the olfactory system of insects. Physiol Rev 70: 199-245.

Nakanishi A, Nishino H, Watanabe H, Yokohari F, Nishikawa M. 2010. Sex-specific antennal sensory system in the ant *Camponotus japonicus*: glomerular organizations of antennal lobes. J Comp Neurol 518: 2186-2201.

Nishikawa M, Yokohari F, Ishibashi T. 1995. Central projections of the antennal cold receptor neurons and hygroreceptor neurons of the cockroach *Periplaneta americana*. J Comp Neurol 361: 165-176.

Nishikawa M, Nishino H, Misaka Y, Kubota M, Tsuji E, Satoji Y, Ozaki M, Yokohari F. 2008. Sexual dimorphism in the antennal lobe of the ant *Camponotus japonicus*. Zool Sci 25: 195-204.

Nishino H, Yamashita S, Yamazaki Y, Nishikawa M, Yokohari F, Mizunami M. 2003. Projection neurons originating from thermo- and hygrosensory glomeruli in the antennal lobe of the cockroach. J Comp Neurol 455: 40-55.

Nishino H, Nishikawa M, Mizunami M, Yokohari F. 2009. Functional and topographic segregation of glomeruli revealed by local staining of antennal sensory neurons in the honeybee *Apis mellifera*. J Comp Neurol 515: 161-180.

O'Donnell S, Donlan NA, Jones TA. 2004. Mushroom body structural change is associated with division of labor in eusocial wasp workers (*Polybia aequatorialis*, Hymenoptera: Vespidae). Neurosci Lett 356: 159-162.

Paulk AC, Gronenberg W. 2008. Higher order visual input to the mushroom bodies in the bee, *Bombus impatiens*. Arthropod Struct Dev 37: 443-458.

Pearson L. 1971. The corpora pedunculata of *Sphinx ligustri* L. and other Lepidoptera: an anatomical study. Philos Trans R Soc Lond B 259: 477-516.

Peitsch D, Fietz A, Hertel H, de Souza J, Ventura DF, Menzel R. 1992. The spectral input systems of hymenopteran insects and their receptor-based colour vision. J Comp Physiol 170: 23-40.

Perez-Orive J, Mazor O, Turner GC, Cassenaer S, Wilson RI, Laurent G. 2002. Oscillations and sparsening of odor representations in the mushroom body. Science 297: 359-365.

Petzold J. 2001. Polarisationsempfindliche Neuronen im Sehsystem der Feldgrille, *Gryllus campestris*: Elektrophysiologie, Anatomie und Modellrechnungen. PhD Thesis, Univ of Zurich.

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. J Neurophysiol 94: 3903-3915.

Radchenko AG. 2001. The phylogeny and faunagenesis of the genus *Cataglyphis* (Hymenoptera, Formicidae). Ent Obozr 80: 885-895 [in Russian].

Raff MC, Whitmore AV, Finn JT. 2002. Axonal self-destruction and neurodegeneration. Science 296: 868-871.

Robinson GE. 1992. Regulation of division of labor in insect societies. Annu Rev Entomol 37: 637-665.

Robinson GE, Page RE, Strambi C, Strambi A. 1989. Hormonal and genetic control of behavioral integration in honey bee colonies. Science 246: 109-112.

Ronacher B, Wehner R. 1995. Desert ants *Cataglyphis* use self-induced optic flow to measure distances travelled. J Comp Physiol 177*:* 21-27.

Rössler W, Zube C. 2011. Dual olfactory pathway in Hymenoptera: evolutionary insights from comparative studies. Arthropod Struct Dev 40: 349-357.

Rössler W, Randolph PW, Tolbert LP, Hildebrand JG. 1999. Axons of olfactory receptor cells of transsexually grafted antennae induce development of sexually dimorphic glomeruli in *Manduca sexta*. J Neurobiol 38: 521-541.

Rössler W, Kuduz J, Schürmann FW, Schild D. 2002. Aggregation of f-actin in olfactory glomeruli: a common feature of glomeruli across phyla. Chem Senses 27: 803-810.

Rospars JP. 1988. Structure and development of the insect antennodeutocerebral system. Int J Insect Morphol 17: 243-294.

Ryugo DK, Wu MM, Pongstaporn T. 1996. Activity-related features of synapse morphology: a study of endbulbs of Held. J Comp Neurol 365: 141-158.

Sachse S, Rappert A, Galizia CG. 1999. The spatial representation of chemical structures in the antennal lobe of honeybees: steps towards the olfactory code. Eur J Neurosci 11: 3970-3982.

Sätzler K, Söhl LF, Bollmann JH, Borst JGG, Frotscher M, Sakmann B, Lübke JHR. 2002. Three-dimensional reconstruction of a calyx of Held and its postsynaptic principal neuron in the medial nucleus of the trapezoid body. J Neurosci 22: 10567-10579.

Sakura M, Labhart T. 2005. Polarization-sensitive neurons in the central complex of the cricket, *Gryllus bimaculatus*. In: Neuroforum 2005 Suppl. (eds. Krieglstein K, Zimmermann H). Heidelberg: Spektrum, p 154B.

Sanes JR, Zipursky SL. 2010. Design principles of insect and vertebrate visual systems. Neuron 66: 15-36.

Sandoz JC. 2006. Odour-evoked responses to queen pheromone components and to plant odours using optical imaging in the antennal lobe of the honey bee drone *Apis mellifera L*. J Exp Biol 209: 3587-3598.

Schmid-Hempel P. 1983. Foraging ecology of colony structure of two sympatric species of desert ants, *Cataglyphis bicolor* and *C. albicans.* PhD Thesis, Univ of Zurich.

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

Schneggenburger R, Forsythe ID. 2006. The calyx of Held. Cell Tissue Res 326: 311-337.

Schürmann FW.1987. The architecture of the mushroom bodies and related neuropils in the insect brain. In: Arthropod brain: its evolution, development, structure, and functions (ed. Gupta AP). New York: John Wiley and Sons, pp. 231-264.

Schwarz S, Albert L, Wystrach A, Cheng K. 2011. Ocelli contribute to the encoding of celestial compass information in the Australian desert ant *Melophorus bagoti*. J Exp Biol 214: 901-906.

Seeley TD. 1978. Life history strategy of the honey bee, *Apis mellifera*. Oecologia 32: 109-118.

Seid MA, Wehner R. 2008. Ultrastructure and synaptic differences of the boutons of the projection neurons between the lip and collar regions of the mushroom bodies in the ant, *Cataglyphis albicans*. J Comp Neurol 507: 1102-1108.

Seid MA, Wehner R. 2009. Delayed axonal pruning in the ant brain: a study of developmental trajectories. Dev neurobiol 69: 1-15.

Seid MA, Harris KM, Traniello JFA. 2005. Age-related changes in the number and structure of synapses in the lip region of the mushroom bodies in the ant *Pheidole dentata*. J Comp Neurol 488: 269-277.

Smid HM, Bleeker MA, van Loon JJ, Vet LE. 2003. Three-dimensional organization of the glomeruli in the antennal lobe of the parasitoid wasps *Cotesia glomerata* and *C. rubecula*. Cell Tissue Res 312: 237-248.

Sorensen AA, Busch TM, Vinson SB. 1984. Behavioral flexibility of temporal subcastes in the fire ant, *Solenopsis invicta*, in response to food. Psyche 91: 319-332.

Steck K, Hansson BS, Knaden M. 2009. Smells like home: Desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. Front Zool 6: 5.

Steck K, Knaden M, Hansson BS. 2010. Do desert ants smell the scenery in stereo? An Behav 79: 939-945.

Steck K, Hansson Bill S, Knaden M. 2011. Desert ants benefit from combining visual and olfactory landmarks. J Exp Biol 214: 1307-1312.

Steiger U. 1967. Über den Feinbau des Neuropils im Corpus pedunculatum der Waldameise. Z Zellforsch 81: 511-536.

Stieb SM, Muenz TS, Wehner R, Rössler W. 2010. Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. Dev neurobiol 70:408-423.

Stocker RF. 1994 The organisation of the chemosensory system in *Drosophila melanogaster*. Cell Tissue Res 275: 3-26.

Strausfeld NJ. 1976. Atlas of an insect brain. Berlin: Springer .

Strausfeld NJ. 2002. Organization of the honey bee mushroom body: representation of the calyx within the vertical and gamma lobes. J Comp Neurol 450: 4-33.

Strausfeld NJ, Li Y. 1999. Representation of the calyces in the medial and vertical lobes of cockroach mushroom bodies. J Comp Neurol 409: 626-646.

Strausfeld NJ, Hansen L, Li Y, Gomez RS, Ito K. 1998. Evolution, discovery, and interpretations of arthropod mushroom bodies. Learn Memory 5: 11-37.

Strausfeld NJ, Sinakevitch I, Brown SM, Farris SM. 2009. Ground plan of the insect mushroom body: functional and evolutionary implications. J Comp Physiol 513: 265-291.

Szyszka P, Ditzen M, Galkin A, Galizia CG, Menzel R. 2005. Sparsening and temporal sharpening of olfactory representations in the honeybee mushroom bodies. J Neurophysiol 94*:* 3303-3313.

Takemura S, Lu Z, Meinertzhagen IA. 2008. Synaptic circuits of the *Drosophila* optic lobe: the input terminals to the medulla. J Comp Neurol 509: 493-513.

Technau G, Heisenberg M. 1982. Neural reorganization during metamorphosis of the corpora pedunculata in *Drosophila melanogaster*. Nature 295: 405-407.

Tieman SB, Hirsch HV. 1982. Exposure to lines of only one orientation modifies dendritic morphology of cells in the visual cortex of the cat. J Comp Neurol 211: 353-362.

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. J Comp Physiol 506: 288-300.

Truman JW, Reiss SE. 1976. Dendritic reorganization of an identified motoneuron during metamorphosis of the tobacco hornworm moth. Science 192: 477-479.

Vickers NJ, Baker TC. 1997. Chemical communication in heliothine moths. VII. Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioral antagonist. J Comp Physiol 180: 523-536.

Vitzthum H, Müller M, Homberg U. 2002. Neurons of the central complex of the locust *Schistocerca gregaria* are sensitive to polarized light. J Neurosci 22: 1114-1125.

Vollbehr J. 1975. Zur Orientierung junger Honigbienen bei ihrem ersten Orientierungsflug. Zool Jb Allg Zool Physiol 79: 33–69.

Vosshall LB, Wong AM, Axel R. 2000. An olfactory sensory map in the fly brain. Cell 102: 147-159.

Watts RJ, Hoopfer ED, Luo L. 2003. Axon pruning during *Drosophila* metamorphosis: Evidence for local degeneration and requirement of the ubiquitin-proteasome system. Neuron 38: 871-885.

Weeks JC, Truman JW. 1986. Steroid control of neuron and muscle development during the metamorphosis of an insect. J Neurobiol 17: 249-267.

Wehner R. 1976. Structure and function of the peripheral visual pathways in Hymenoptera. In: Neural principles in vision (eds. Zettler F, Weiler R). Berlin, Heidelberg, New York: Springer, pp. 280-333.

Wehner R. 1981a. Verhaltensphysiologische, funktionsmorphologische und taxonomische Studien an zwei Arten der *Cataglyphis albicans*-Gruppe (Formicidae, Hymenoptera). Mitt dtsch Ges allg angew Ent 3: 214-217.

Wehner R. 1981b. Spatial vision in arthropods. In: Handbook of sensory physiology (ed. Autrum H). Springer, Berlin, pp. 288-616.

Wehner R. 1982. Himmelsnavigation bei Insekten. Neurophysiologie und Verhalten. Neujahrsbl Naturforsch Ges Zürich 184: 1-132.

Wehner R. 1983. Taxonomie, Funktionsmorphologie und Zoogeographie der saharischen Wüstenameise *Cataglyphis fortis* (Forel 1902) stat. nov. (Insecta: Hymenoptera: Formicidae). Senckenbergiana Biol 64: 89-132.

Wehner R. 1986. Artcharakterisierung von *Cataglyphis diehlii* und *C. rubra*. Jb Akad Wiss Lit Mainz 86: 108-111.

Wehner R. 1987. Spatial organization of foraging behavior in individually searching desert ants, *Cataglyphis* (Sahara Desert) and *Ocymyrmex* (Namib Desert). In: From individual to collective behavior in social insects (eds. Pasteels JM, Deneubourg JL). Basel: Birkhäuser, pp. 15-42.

Wehner R. 1994. The polarization-vision project: championing organismic biology. Forts Zool 39: 103-143.

Wehner R. 1997. The ant's celestial compass system: spectral and polarization channels. In: Orientation and communication in arthropods. Basel: Birkhäuser Verlag.

Wehner R. 2003. Desert ant navigation: how miniature brains solve complex tasks. J Comp Physiol 189:579-588.

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

Wehner R, Räber F. 1979. Visual spatial memory in desert ants, *Cataglyphis bicolor* (Hymenoptera: Formicidae). Experientia 35: 1569-1571.

Wehner R, Srinivasan M. 1981. Searching behaviour of desert ants, genus *Cataglyphis* (Formicidae, Hymenoptera). J Comp Physiol *142:* 315-338.

Wehner R, Wehner S. 1990. Insect navigation: use of maps or Ariadne's thread? Ethol Ecol Evol 2: 27-48.

Wehner R, Bernard GD. 1993. Photoreceptor twist: a solution to the false-color problem. Proc Natl Acad Sci 90: 4132-4235.

Wehner R, Srinivasan MV. 2003. Path integration in insects. In: The Neurobiology of Spatial Behaviour (ed. Jeffery KJ). Oxford University Press, pp. 9-30.

Wehner R, Labhart T. 2006. Polarisation vision. In: Invertebrate Vision (eds. Warrant E, Nilsson D-E). Oxford University Press, pp. 291-248.

Wehner R, Müller M. 2006. The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. Proc Natl Acad Sci 103: 12575-12579.

Wehner R, Herrling PL, Brunnert A, Klein R. 1972. Periphere Adaptation und zentralnervöse Umstimmung im optischen System von *Cataglyphis bicolor* (Formicidae, Hymenoptera). Rev Suisse Zool 79: 197–228.

Wehner R, Bernard GD, Geiger E. 1975. Twisted and non-twisted rhabdoms and their significance for polarization detection in the bee. J Comp Physiol 104: 225-245.

Wehner R, Harkness RD, Schmid-Hempel P. 1983. In: Foraging strategies in individually searching ants *Cataglyphis bicolor* (Hymenoptera: Formicidae). Akad Wiss Lit Mainz Math-Naturwiss KI. Stuttgart: Gustav Fischer.

Wehner R, Wehner S, Agosti D. 1994. Patterns of biogeographic distribution within the *bicolor* species group of the North African desert ant, *Cataglyphis* Foerster (1850). Senckenbergiana Biol 74: 163-191.

Wehner R, Michel B, Antonsen P. 1996. Visual navigation in insects: coupling of egocentric and geocentric information. J Exp Biol 199: 129-140.

Wehner R, Gallizzi K, Frei C, Vesely M. 2002. Calibration processes in desert ant navigation: vector courses and systematic search. J Comp Physiol 188: 683-693.

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

Wenzler N. 2010. A special type of olfactory recruitment in the Namibian desert ant *Ocymyrmex robustior*. Diploma Thesis, Univ of Würzburg.

Withers GS, Fahrbach SE, Robinson GE. 1993. Selective neuroanatomical plasticity and division of labour in the honeybee. Nature 364: 238 - 240.

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

Wittlinger M, Wehner R, Wolf H. 2007. The desert ant odometer: a stride integrator that accounts for stride length and walking speed. J Exp Biol 210: 198-207.

Wolf H, Wehner R. 2000. Pinpointing food sources: olfactory and anemotactic orientation in desert ants, *Cataglyphis fortis*. J Exp Biol 203: 857-868.

Wolf H, Wehner R. 2005. Desert ants compensate for navigation uncertainty. J Exp Biol 208: 4223-4230.

Yusuyama K, Meinertzhagen IA, Schürmann F-W. 2002. Synaptic organization of the mushroom body calyx in *Drosophila melanogaster*. J Comp Neurol 445: 211-226.

Zars T, Fischer M, Schulz R, Heisenberg M. 2000. Localization of a short-term memory in *Drosophila*. Science 288: 672-675.

Zeil J. 1993. Orientation flights of solitary wasps (*Cerceris*; Spheeidae; Hymenoptera) II . Similarities between orientation and return flights and the use of motion parallax. J Comp Physiol 172: 207-222.

Zeil J, Kelber A, Voss R. 1996. Structure and function of learning flights in bees and wasps. J Exp Biol 199: 245-252.

Ziegler PE, Wehner R. 1997. Time-courses of memory decay in vector-based and landmark-based systems of navigation in desert ants, *Cataglyphis fortis*. J Comp Physiol 81: 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 Struct Dev 37: 469-479.

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

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EDUCATION

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10.2006	**University of Würzburg** Diploma in Biology Main subject: Behavioral Physiology & Sociobiology Minor subjects: Neurobiology; Animal Ecology & Tropical Biology
10.2002 – 10.2006	Main study of Biology, **University of Würzburg**
10.2000 – 10.2002	Basic study of Biology, **University of Bremen**
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TEACHING EXPERIENCE & SCIENTIFIC INITIATIVES

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INTERNSHIPS /FIELD STAYS

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10.2005 – 03.2006	**Hawai'i Institute of Marine Science; University of Hawai'I (USA)** Research stay: Localisation- and density-determination of acoustically active snapping shrimps of the genera *Synalpheus* and *Alpheus* and temporal variations in their acoustical activity in Kaneohe Bay (Oahu, Hawai'i)
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LIST OF PUBLICATIONS

PEER-REVIEWED ARTICLES

Stieb SM, Hellwig A, Wehner R, Rössler W. Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis.* Developmental neurobiology: accepted

Stieb SM, Kelber C, Wehner R, Rössler W. 2011. Antennal lobe organization in desert ants of the genus *Cataglyphis.* Brain, Behavior and Evolution 77 (3): 136-146 (DOI 10.1159/000326211)

Stieb SM, Muenz TS, 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 (6): 408-423 (DOI 10.1002/dneu.20785)

Mettke-Hofmann C, Ebert C, Schmidt T, Steiger S, Stieb S. 2005. Personality traits in resident and migratory warbler species. Behaviour 142 (Part 9-10): 1357-1375 (DOI 10-1163/156853905774539427)

CONFERENCE PRESENTATIONS

Stieb SM, Schmitt F, Wehner R, Rössler W (2011) Synaptic plasticity in visual and olfactory brain centers of the desert ant *Cataglyphis fortis.* Insect Homing: Mechanisms and Models, ZiF University Bielefeld, Germany (*poster*)

Stieb SM, Hellwig A, Wehner R, Rössler W (2011) Visual experience affects both behavioral and neuronal aspects of the life history in the desert ant *Cataglyphis fortis.* Gordon Research Conference – Neuroethology: Behavior, Evolution & Neurobiology, Stonehill College Easton, USA (*poster*)

Stieb SM, Kelber C, Wehner R, Rössler W (2011) Organization of the antennal lobe in desert ants of the genus *Cataglyphis.* 9th Göttingen Meeting of the German Neuroscience Society, Göttingen, Germany (*poster*)

Hellwig A, Stieb SM, Wehner R, Rössler W (2011) Synaptic plasticity in visual and olfactory brain centers after unilateral sensory deprivation and light-triggered behavioral effects in *Cataglyphis fortis.* 9th Göttingen Meeting of the German Neuroscience Society, Göttingen, Germany (*poster*)

Wenzler N, Stieb SM, Rössler W, Wehner R (2011) A special type of olfactory recruitment behavior in the Namibian desert ant *Ocymyrmex robustior.* 9th Göttingen Meeting of the German Neuroscience Society, Göttingen, Germany (*poster*)

Stieb SM, Muenz M, Wehner R, Rössler W (2010) Light functions as a trigger for synaptic plasticity in visual brain centers of the ant *Cataglyphis fortis* – but can it also bias the ants' behavior? 9th International Congress of Neuroethology, Salamanca, Spain (*poster)*

Wenzler N, Stieb SM, Rössler W, Wehner R (2010) A special type of olfactory recruitment behavior in the Namibian desert ant *Ocymyrmex robustior*. 103 conference of the DZG, Hamburg, Germany (p*oster*)

Stieb SM, Muenz M, Wehner R, Rössler W (2009) Synaptic plasticity in visual pathways in the brain of the desert ant *Cataglyphis fortis*. Visual Processing in Insects: From Anatomy to Behavior II, Janelia Farm Research Campus, USA *(poster)*

Stieb SM, Muenz M, Wehner R, Rössler W (2009) Synaptic plasticity in visual integration centers in the brain of the desert ant *Cataglyphis fortis*. 8th Göttingen Meeting of the German Neuroscience Society, Göttingen, Germany (*poster*)

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Stieb SM (2008) Synaptic plasticity in visual pathways in the brain of the desert ant *Cataglyphis fortis*. Seminar 'Plasticity and Envelopment of the insect nervous system' Rauischholzhausen, Germany (*talk*)

Lammers MO, Stieb SM, Au WW L, Mooney TA (2006) Temporal, geographic, and density variations in the acoustic activity of snapping shrimp. Fourth Joint Meeting: ASA and ASJ. J. Acoust. Soc. Am., Vol. 120, No. 5 (*poster*)