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

Sara Mae Stieb,[1] Thomas Sebastian Muenz,[1] Rüdiger Wehner,[2] Wolfgang Rössler[1]

[1] Department of Behavioral Physiology and Sociobiology, Biozentrum, University of Würzburg, Germany

[2] Brain Research Institute, University of Zürich, Switzerland

Received 23 December 2009; accepted 26 January 2010

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

This work was performed while Rüdiger Wehner was Humboldt Awardee at the Biozentrum of the University of Würzburg.

Rüdiger Wehner and Wolfgang Rössler contributed equally to this work.

Correspondence to: Prof. Dr. W. Rössler (roessler@biozentrum.uni-wuerzburg.de).

Contract grant sponsor: Deutsche Forschungsgemeinschaft (DFG; University of Würzburg); contract grant number: SFB 554/A8.

Published online 3 February 2010 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/dneu.20785

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

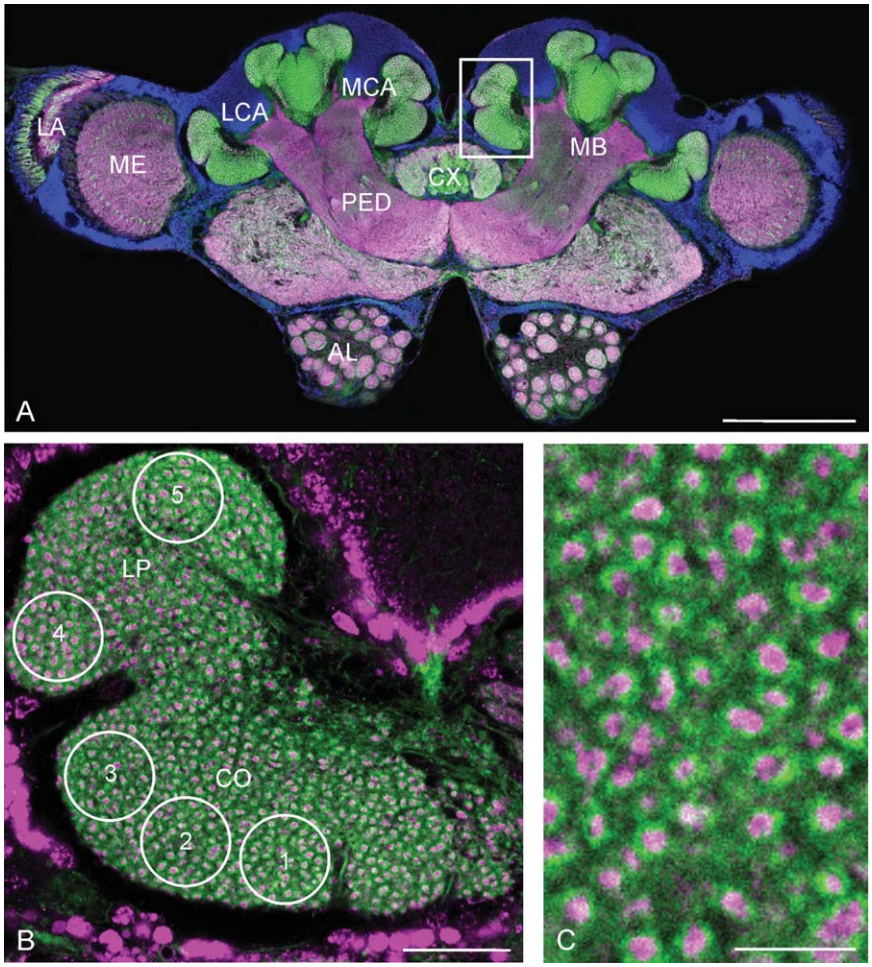

Figure 1 Immunofluorescence stainings of the *Cataglyphis fortis* brain. **A**. Frontal overview of a brain (100-μm section) labeled with anti-synapsin (magenta), phalloidin-labeled f-actin (green), and Hoechst (blue) to highlight cell nuclei. Mushroom body (MB) with medial calyces (MCA), lateral calyces (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-

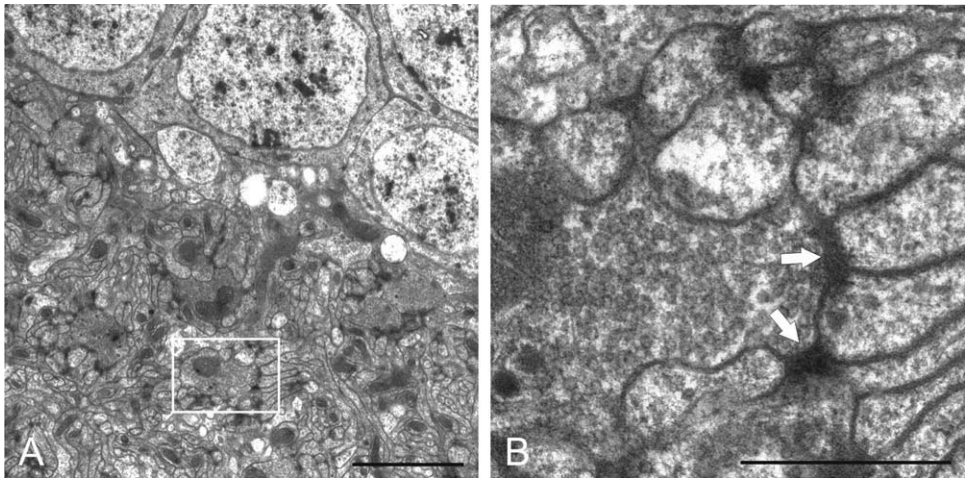

Figure 2 Electron microscopic images of the mushroom body calyx. **A**. Ultrastructural image of the lip region neighbored by Kenyon Cell somata. White frame indicates one individual microglomerulus (MG). **B**. Detail of one MG. Multiple dendrites get in contact with one vesicle packed central presynaptic 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

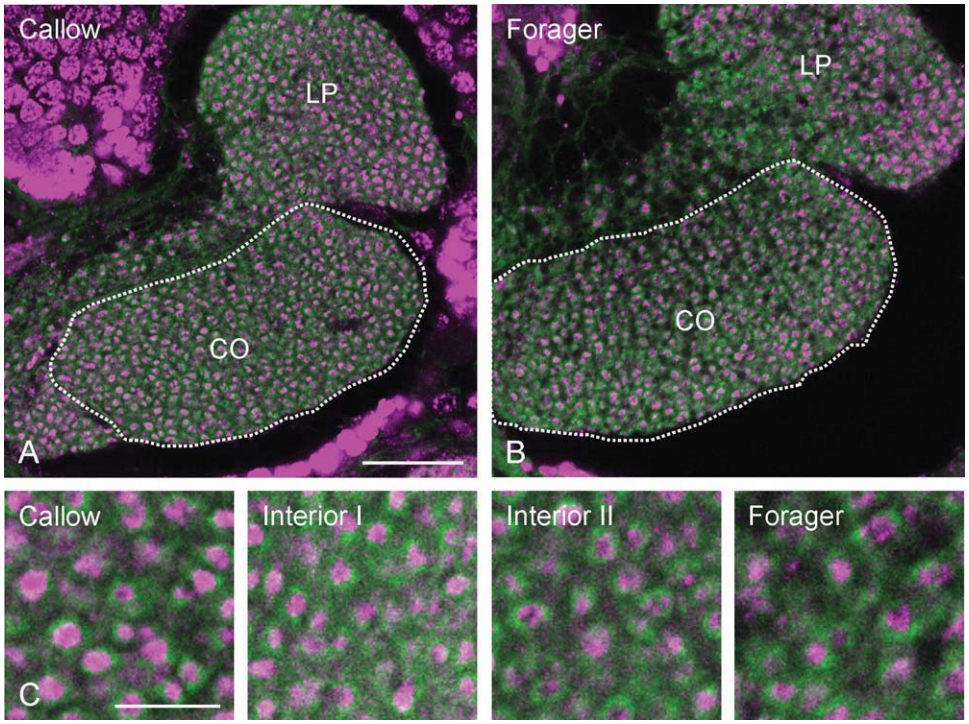

Figure 3 Task-dependent plasticity in the mushroom body (MB) calyx. **A, B**. Medial calyx labeled with anti-synapsin (magenta) and phalloidin-labeled f-actin (green). The MB calyx, both the lip (LP) and collar (CO), increase in size during the transition from callows (A) to foragers (B). Synapsin is 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,

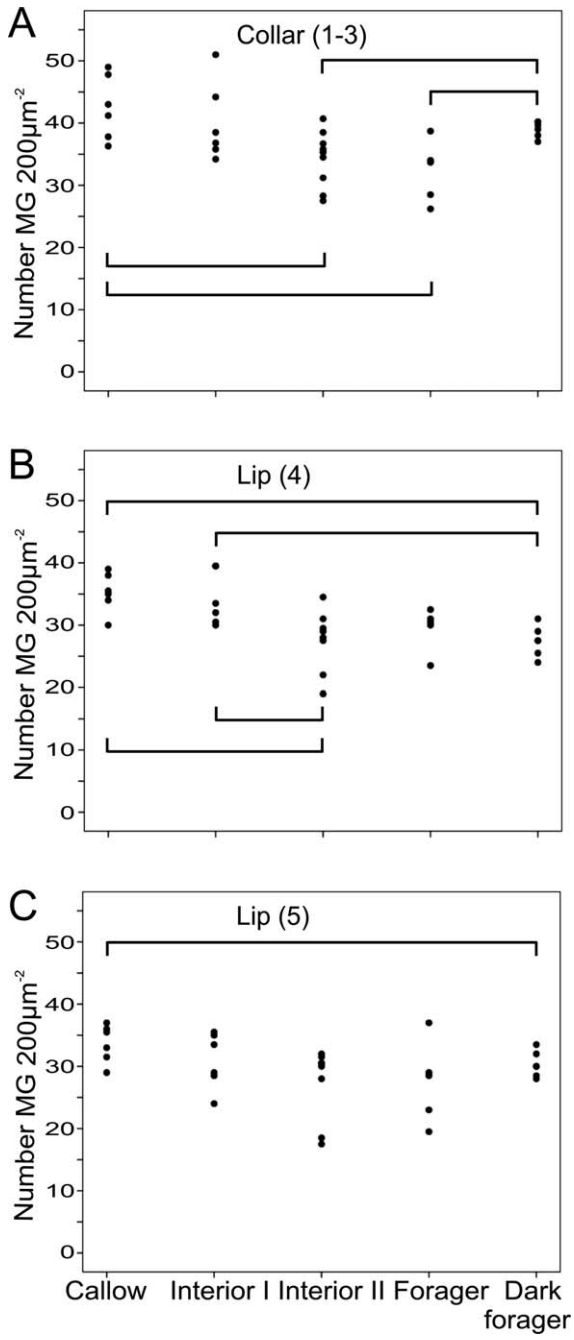

Figure 4 Task-related effect on the microglomeruli (MG) number in the mushroom-body calyx. **A**. Structural plasticity in the collar. In the visually innervated collar (CO) there is a significant reduction in the mean MG number of the three measured 200 μm^2 circular areas (Area 1–3) during the transition 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.

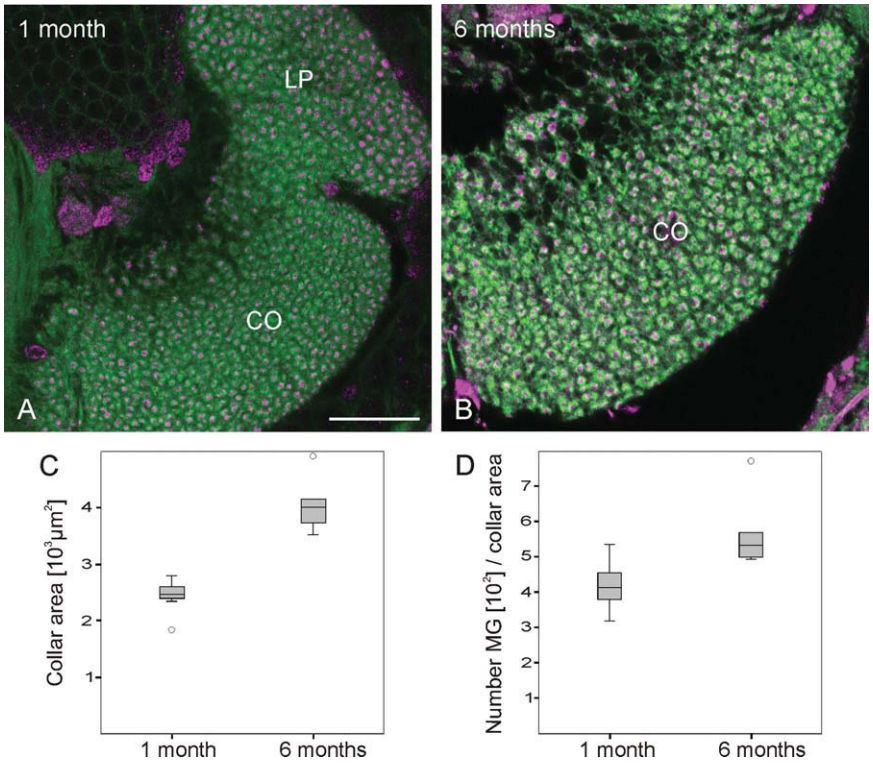

Figure 5 Age-related structural plasticity in the mushroom body (MB) calyx. **A**, **B**. MB calyces stained with anti-synapsin (magenta) and phalloidin-labeled f-actin (green). The comparison of interior workers rose under natural conditions [about 1-month old (A)] and older dark-reared ants [about 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

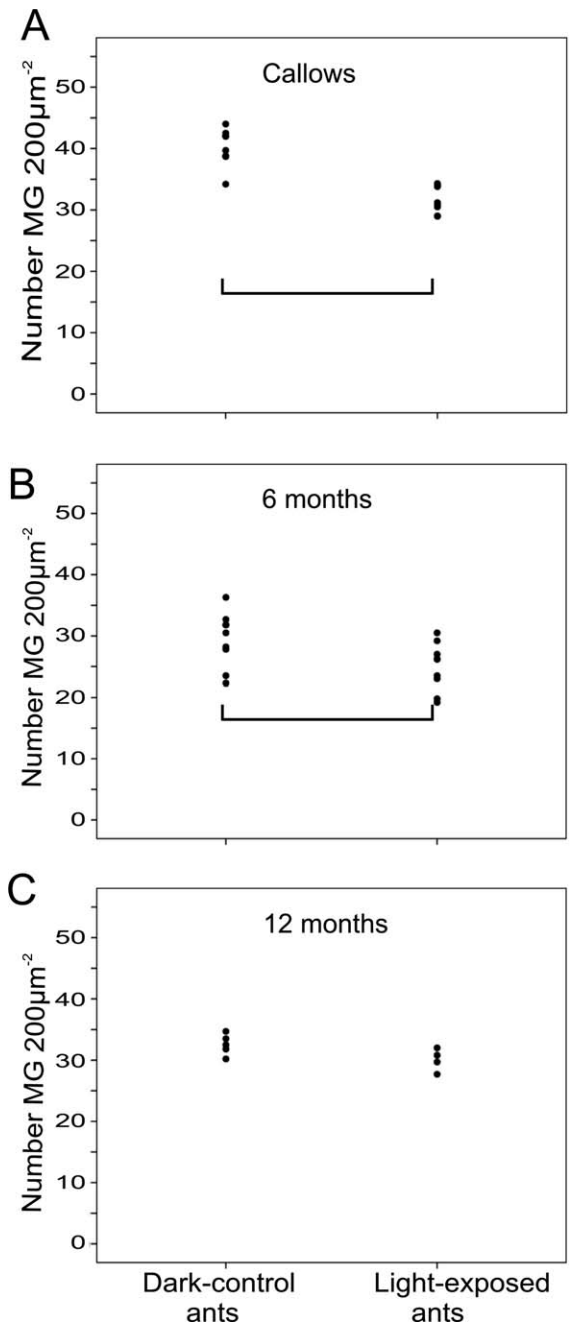

Figure 6 Effect of light on the structural composition in the visually innervated collar (CO) compared to dark-kept ants of different age. **A**. Light-triggered plasticity in the mushroom body (MB) CO of callows. Callows that were exposed to light showed a significant reduction in the microglomeruli (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

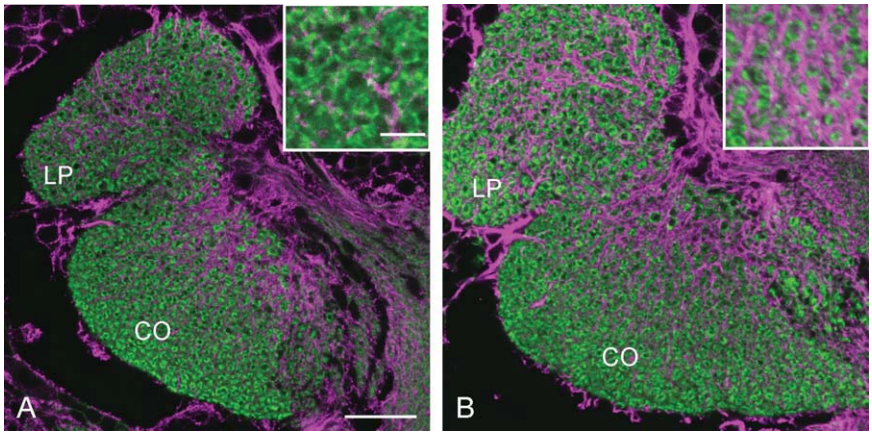

Figure 7 Cellular mechanisms of mushroom-body (MB) volume expansion and changes in microglomeruli (MG) density. **A**, **B**. MB calyx labeled with antitubulin (magenta) and phalloidin-labeled f-actin (green). Tubulin-rich profiles are completely filling out spaces between the dispersing MG during the 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).

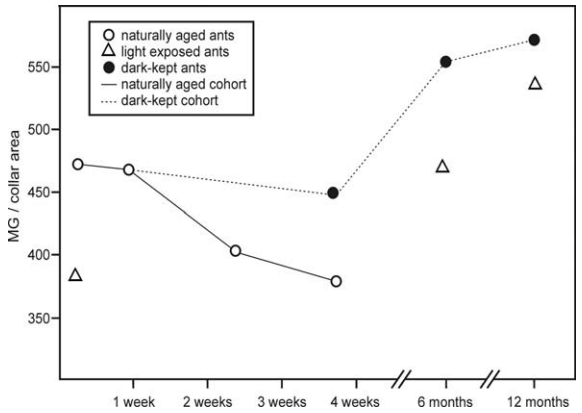

Figure 8 Age-related and light-triggered plasticity in synaptic complexes (microglomeruli, MG) of the visually innervated collar region of the mushroom-body calyces. MG numbers are extrapolated to the dense area of the collar, averaged for each group and plotted versus the ant's age. Naturally aged ants 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.

The authors thank Marieluise Obermayer, Claudia Groh, Christina Zube, Martin Brill, and Cornelia Grübel for their help with immunohistochemistry; Nadine Wenzler, Markus Knaden, Kathrin Steck, and Sebastian Schwarz for providing valuable assistance during the field work in Tunesia; Erich Buchner for the kind gift of anti-synapsin antibodies; and Caroline Müller for lending her optometer. They also thank the Graduate School of Life Sciences, University of Würzburg (SMS and TSM are associated members).

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.