Adaptation of Microglomerular Complexes in the Honeybee Mushroom Body Lip to Manipulations of Behavioral Maturation and Sensory Experience

Sabine Krofczik, Uldus Khojasteh, Natalie Hempel de Ibarra, Randolf Menzel
Institut für Biologie - Neurobiologie, Freie Universität Berlin, Königin-Luise Str. 28-30, 14195 Berlin, Germany

Received 8 October 2007; accepted 2 January 2008

ABSTRACT: Worker honeybees proceed through a sequence of tasks, passing from hive and guard duties to foraging activities. The underlying neuronal changes accompanying and possibly mediating these behavioral transitions are not well understood. We studied changes in the microglomerular organization of the mushroom bodies, a brain region involved in sensory integration, learning, and memory, during adult maturation. We visualized the MB lips’ microglomerular organization by applying double labeling of presynaptic projection neuron boutons and postsynaptic Kenyon cell spines, which form microglomerular complexes. Their number and density, as well as the bouton volume, were measured using 3D-based techniques. Our results show that the number of microglomerular complexes and the bouton volumes increased during maturation, independent of environmental conditions. In contrast, manipulations of behavior and sensory experience caused a decrease in the number of microglomerular complexes, but an increase in bouton volume. This may indicate an outgrowth of synaptic connections within the MB lips during honeybee maturation. Moreover, manipulations of behavioral and sensory experience led to adaptive changes, which indicate that the microglomerular organization of the MB lips is not static and determined by maturation, but rather that their organization is plastic, enabling the brain to retain its synaptic efficacy. © 2008 Wiley Periodicals, Inc. Develop Neurobiol 68: 1007–1017, 2008

Keywords: Apis mellifera; plasticity; microglomerular complexes; projection neurons; Kenyon cells

INTRODUCTION

Age- and experience-dependent neuronal changes are known to accompany naturally occurring behavioral plasticity in vertebrates and invertebrates (Kolb and Whishaw, 1998; Farris et al., 2001). Structural plasticity during experience-dependent maturation of the nervous system is common to many sensory systems (Wilson et al., 2006). Regarding olfactory systems, in rodents it has been shown that olfactory learning influences olfactory sensory neuron architecture (Kerr and Belluscio, 2006). Striking results were obtained for eusocial insects, which provide the opportunity for a socio-neuroethological analysis of neuronal and behavioral maturation. The honeybee Apis mellifera has a discrete system of age-related division of labor (Roesch, 1925). Worker honeybees perform brood rearing (nursing) for the first week of their adult life, engage in other hive maintenance duties when they are 2- to 3-weeks-old, and subsequently carry out foraging tasks (Winston, 1987). This schedule is rather flexible, to meet the colony’s varying needs, which are influenced by demographic and environmental conditions (Seeley, 2002). Such
flexibility makes it possible to experimentally manipu-
late age- and experience-dependent changes in the 
bee brain, particularly in the mushroom bodies (MBs) 
(Withers et al., 1993; Durst et al., 1994; Fahrbach 
et al., 1998; Ismail et al., 2005).

The MBs are multisensory integration centers that 
play a dominant role in odor learning (Heisenberg, 
1998; Menzel and Giurfa, 2001). Their calyces 
receive second-order sensory input with different 
modalities and thus are believed to integrate sensory 
information, possibly leading to context-dependent 
forms of learning (Menzel and Giurfa, 2001). Olfac-
tory input is conveyed to the MB lip region by excita-
tory projection neurons connecting the primary olfac-
tory neuropil, the antennal lobe (AL) with the MB
and transferring sensory information to the dendrites
of the mushroom body-intrinsic neurons, the Kenyon
cells (Mobbs, 1982; Sun et al., 1997; Abel et al., 
2001). In the lip region of the MB calyces projection
neuron boutons and Kenyon cell spines form micro-
glomerular complexes. Each microglomerular complex
contains a presynaptic projection neuron bouton
as its central core, surrounded by a shell of Kenyon
cell dendritic tips (Gronenberg, 2001; Roessler et al., 
2002; Yusuyama et al., 2002; Frambach et al., 2004; 
Groh et al., 2004, 2006). The MBs integrate informa-
tion from different neural circuits and previous studies
revealed that they undergo processes reflecting age-
and experience-related plasticity (Withers et al., 
1993; Durst et al., 1994; Fahrbach et al., 1998; Ismail
et al., 2005). Investigations of the MB neuronal archi-
tecture showed that foraging experience correlates
with the complexity of the Kenyon cell dendritic
branching pattern (Farris et al. 2001). However, the
question whether the microglomerular organization
of the MB lips is influenced by the bee’s age or its
behavior remains elusive. This study addresses this
question by applying a combination of behavioral
manipulations, pre- and postsynaptic labeling and 3D-based quantification and volume measurement
techniques to investigate the MB lips’ microglomeru-
or organization during adult maturation.

Our results show that the microglomerular organiza-
tion of the MB lips changes according to the behavioral
and sensory experience of the bee, which might imply
alterations of the synaptic connectivity between preсин-
aptic PN boutons and postsynaptic KC spines.

MATERIALS AND METHODS

Honeybees

Honeybees (Apis mellifera carnica) were obtained from
colonies kept in our lab according to standard apicultural
Developmental Neurobiology

Experimental Part I: Natural Colonies Under Different Environmental Conditions

The colonies used for this experiment were housed either in
a hive outside—referred to as the “rich” environment—or
in a hive contained within a flight net situated in a glass-
house (the “poor” environment). In the rich environment
the colony contained a naturally mated queen, adult non-
marked bees and about 700 marked bees collected immedi-
ately after eclosion. Foragers were marked with an addi-
tional paint mark to ensure a minimum of 2 days of forag-
ing experience. The poor environment colony contained a
single-inseminated active queen in a small hive with about
1000 adult bees and 300 marked bees collected immedi-
ately after eclosion. Bees foraged at artificial gray odorless
sugar feeders or collected pollen from a dark chamber. Be-
havioral observations were carried out as described above
and bees of dedicated ages (4, 14, 18, and 37 days for the
rich environment bees and 14, 18, and 37 days for the poor
environment) were collected for immunocytochemistry (see
Fig. 1).

Experimental Part II: Manipulations of the Bees’ Social Behaviors and Sensory Experience

The single-cohort colony of about 3000 bees used for this
experiment was housed in an observation hive containing a
single-inseminated queen. Observations of flight and forag-
ing behavior were made as described above. Additional
observations of nursing and overall activity were made via
the glass window of the observation hive. Extended nursing activities performed by the marked bees were observed until Day 14 presumably because new eclosed bees took on further nursing activities. About 1000 bees were marked with numbered bee tags of different colors. Following the procedure described by Withers et al. (1995), plastic discs were glued to the thoraces of an additional 200 bees (hereafter referred to as hive-bound bees). The thickness of the plastic disk prevented hive-bound bees from entering the comb to perform nursing behavior. The hive entrance was restricted such that hive-bound bees could not leave. Foragers were additionally marked with paint. The experiment was undertaken until Day 18, since after this period the survival rate of hive-bound bees decreased progressively. This observation might be explained by the fact that hive-bound bees were detained to perform defecation/orientation flights which might have affected their metabolic activity and positive phototaxic behavior essential for the bees’ natural life span.

At each adult developmental age (5, 10, 14, 18 days) hive-bound, nurse, and forager bees were collected and dissected for immunocytochemistry (see Fig. 1).

**Intracellular Markings of Projection Neurons**

Electrodes (Hilgenberg) were pulled with a Brown-Flaming horizontal puller (P-2000 Sutter Instruments, Novarto, CA), and their tips were filled with 4% tetramethylrhodamin-biotin dextran (Micro-Ruby; Molecular Probes, Eugene, OR) in 0.2 M potassium acetate. The electrodes were inserted into the brain tissue of individual worker bees at the given time-point to label individual identified projection neurons (PN). The PN were then visualized and quantified with immunocytochemistry (see Fig. 2).

![Figure 1](image1.png)

**Figure 1** Experimental design to investigate changes of microglomerular complexes during adult maturation. Bees were either reared in a natural colony (top row) under rich or poor environmental conditions or in a single-cohort colony (bottom row). Bees from the natural colony were collected during the first (4-day-old nurses) and after the second week (14-, 18-, and 37-day-old foragers). Bees from the single-cohort colony were collected during the first (5-day-old hive-bound bees, nurses, and precocious foragers) and after the second week (14-day-old hive-bound bees, nurses, and foragers as well as 18-day-old hive-bound bees and foragers. All samples were used for immunocytochemistry to visualize microglomerular complexes (confocal images) and to apply 3D quantification and volume measurement. All measurements were undertaken on optical slices whose plane was defined by the central body (cb) [Scale bars: 100 μm; inset, 50 μm].

![Figure 2](image2.png)

**Figure 2** Identification, and 3D quantification of microglomerular complexes within the MB lip. (A) Segmentation of the lip neuropil based on anti-synapsin IR. Anti-synapsin IR gray-value image and segmented lip neuropil (hatched purple) superimposed. (B) Segmentation (hatched green) of anti-synapsin IR positive regions. (C) Identification of microglomeruli complexes by alignment with a phalloidin gray-value image. (D) High-resolution image of a single PN bouton and anti-synapsin IR superimposed (yellow). (E) Surface model of the segmented anti-synapsin IR positive region, the PN bouton. (F) Illustration of the six-voxel neighborhood algorithm applied by the connected components module of the software Amira. A single voxel i is compared with its neighboring voxel (iU, iW, iN, iS, iE, iD) based on thresholding. Voxels of the same gray value are assigned to a cluster representing a single microglomerulus. The 3D area of each cluster represents the anti-synapsin IR interpreted as bouton volume and the number of clusters the number of microglomerular complexes. Scale bars: A: 20 μm, B and C: 5 μm, D and E: 0.1 μm.
into the AL for recordings from projection neurons at about 50–180 μm depths. Electrode resistances in the tissue ranged from 140 to 200 MΩ. A silver wire placed into the eye served as the indifferent electrode. To ensure that the recording was performed from a projection neuron Micro-Ruby was injected by using depolarizing pulses of 1–2 Hz, 0.2-s duration and 2–4 nA. Complete filling of the PN required dye injection for 30–45 min. After intracellular filling, the dye was allowed to diffuse for 3 h.

**Immunocytochemistry**

Collected bees were immobilized by cooling. Brains were dissected in bee physiological saline solution (in mmol⁻¹, 136 NaCl, 3 KCl, 10 Na2HPO4, 2 KH2PO4, 105 sucrose, pH 6.7) and fixed in 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS) (overnight at 4°C). Brains were washed three times in PBS, embedded in 7% low-melting agarose (Sigma) and sectioned in a frontal plane (100 μm) with a vibrating microtome (Leica, VT 1000 S). Agarose sections were transferred into marked tetrimitric plates and preincubated in PBS with 0.2% Triton-X100 (PBST) and 2% normal goat serum (NGS; Sigma, St. Louis, MO) for 1 h at room temperature. For visualization of MCs within the calycal lip we used a double labeling technique adapted from Groh et al., 2004. This technique allows simultaneous pre- and postsynaptic labeling in the lip region of the MB calyx by combining anti-synapsin with f-actin labeling. Preparations were incubated simultaneously with a monoclonal antibody against the Drosophila synaptic-vesicle-associated protein synapsin I (Klagges et al., 1996) (1:50; SynOrf1; kindly provided by E. Buchner, University of Wuerzburg, Wuerzburg, Germany) and with 0.2 units of Alexa Fluor 488 phalloidin (Molecular Probes, A-12379) in PBS for 2 days at 4°C. To visualize synapsin, preparations were rinsed five times in PBS and incubated with a Cy5-conjugated goat anti-mouse secondary antibody (Jackson Immunoresearch, West Grove, PA; dilution 1:250 in PBS) overnight at 4°C. For triple labeling of the intracellularly stained PN, neuronal f-actin, and synapsin, the preparations containing marked PNs were fixed and processed as described above. To intensify the intracellular staining of the PN, 1:500 diluted streptavidin-Cy3 (Invitrogen, Kalsruhe, Germany) was added to the primary antiserum.

**Confocal Microscopy**

Vibratome sections were imaged with a confocal laser scanning microscope (Leica TCS SP2) using a Leica HCX PL APO CS 40 × 1.25 oil UV lens objective and a voxel size of 0.1 × 0.1 × 0.5 μm³. For clear visualization of microglomerular complexes a defined zoom factor between 2 and 3 was used for each preparation. The preparation containing the intracellularly recorded and marked projection neuron was imaged with a HC PL APO 20 × 0.70 IMM/CORR UV lens objective with a voxel size of 0.5 × 0.5 × 1 μm³ and a zoom factor of 1.4. Synapsin was visualized by a secondary antibody conjugated to Cy5, which was excited with the 633-nm line of a HeNe laser. F-actin stained with phalloidin was visualized by a conjugated Alexa-488, which was excited using the 488-nm line of an ArKr laser. The projection neuron staining was intensified by using streptavidin-Cy3, which was excited with the 543-nm line of a He-Ne laser.

**3D Quantification and Volume Measurement**

Quantitative neuroanatomical techniques were applied to optical sections at a defined plane in the region of the central body (Fig. 1 right panel) and further analyzed with the Amira 3.1.1 software (Mercury Computer Systems). The 3D quantification and volume measurements were accomplished on images normalized to a voxel size of 0.15 × 0.15 × 1 μm³ and a resolution of 796 × 512 × 11. The confocal image stacks were cropped according to the area of the MB lip region, which was identified using anti-synapsin immunoreactivity (IR) (see Fig. 1 inset). Fully automated threshold-based identification and quantification failed due to the complexity of the structures, and therefore semiautomatic techniques were applied. Because of the high number of preparations and the time-consuming semiautomatic process the analysis was performed on a total thickness of 11 μm. In each preparation lip and bouton volume as well as number and density of microglomerular complexes were measured.

Figure 2 illustrates the stepwise process of the 3D-based volume measurement and quantification technique. To visualize the image stacks in 3D we converted the 8-bit gray scale stacks into another format, the so-called Labelfield tile, which is closely related to a uniform scalar field. This conversion was done by applying a technique referred to as ‘segmentation’. Thereby each voxel was assigned to a material representing either the MB lip or a microglomerular complex [Fig. 2(B,C)]. Each microglomerular complex was identified by segmenting the anti-synapsin IR positive regions in the x, y, and z direction and aligning them with the phalloidin staining [Fig. 2(B,C)]. Segmentation was performed blindly by two independent investigators without knowledge about the animals’ group affiliation. The Labelfield containing the structures of interest was then converted into a 3D scalar field and visualized as a surface model [Fig. 2(E)]. The 3D volumetric measurements and quantification were accomplished by using the ‘connected components’ module of the Amira software. Based on gray-value thresholding this module searches for connected voxel in a 3D image volume meaning that voxel of the same gray value are interpreted as connected and thus belonging to the same region (here to the class of microglomeruli). The segmented anti-synapsin IR positive regions represent the microglomeruli throughout the entire defined MB lip region [Fig. 2(D,E)] show a single PN bouton (red) counterstained with anti-synapsin (green), superimposed yellow. The connected components module operates on a 6-voxel neighborhood screening algorithm, which compares a central voxel...
(i) with its six neighboring voxels (iU, iW, iN, iS, iE, iD) [Fig. 2(F)] by means of their gray-values (for further details see Holden et al., 1995). Figure 2(E,F) illustrate this algorithm by representing each voxel as a transparent cube in a 3D space. The algorithm operates throughout the 3D image stack and assigns each voxel as either lip or microglomerular. Voxel of a microglomerulus [Fig. 2(E) surface model], are assigned to one cluster in the 3D space. The 3D area of each cluster represents the segmented synapsin-IR positive region (here interpreted as the bouton volume) and the number of clusters the number of microglomerular complexes.

**Data Analysis**

Statistical analysis was performed using SPSS 13. The density of microglomerular complexes was calculated on the basis of the 3D lip volume and the number of microglomerular complexes within this volume (figures show density/10 μm³). The mean and standard deviation of number and density of microglomerular complexes and bouton volumes were calculated for same-aged animals. For comparison of animals within one group Kruskal-Wallis non-parametric statistics were performed. Statistical analysis of differently aged animals was performed by applying Mann-Whitney U comparisons. Relationships between the measured parameters we analyzed by applying Pearson correlation. Illustrations were created with Amira 3.1.1, MatLab, Adobe Illustrator CS2, Adobe Photoshop 7.0 and Corel Draw 11.

**RESULTS**

**Projection Neuron Boutons Represent the Microglomerular Complex Core**

The present study focuses on the analysis of the microglomerular organization within the MB lips as related to the animals’ age, behavior and experience. Previous studies using PN mass labeling revealed that PN boutons synapse onto f-actin rich KC dendritic spines (Frambach et al., 2004; Groh et al., 2004). In this present study, we combined intracellular staining of single PNs with immunocytochemistry to visualize the location of single PN boutons and their postsynaptic KC spines. Figure 2(D, E) shows a single marked PN (red) counterstained with the synapsin antibody SynOrf1 (green) (superimposed yellow) and the f-actin marker Alexa-488 phalloidin (blue, appears diffuse due to high resolution of the image, see also Fig. 1 inset). The triple labeling technique of a single PN, anti-synapsin and the f-actin marker phalloidin shows that the antibody SynOrf1 marks presynaptic terminals of olfactory PNs, which are surrounded by KC dendritic spines. More importantly, this technique identifies microglomerular complexes as seen in electron microscopic sections (Ganeshina and Menzel, 2001; Ganeshina et al., 2006).

**Microglomerular Organization of the MB Lips During Adult Maturation**

Previous studies have shown that MB calycal neuropils undergo volumetric expansions during adult honeybee maturation (Withers et al., 1993; Durst et al., 1994; Fahrbach et al., 1998, 2003; Farris et al., 2001; Ismail et al., 2005). The goal of our study was to investigate whether the MB lip’s microglomerular organization undergoes structural changes according to the sensory-guided behaviors during maturation. Therefore we analyzed the number and density of microglomerular complexes as well as the bouton volume in bees at different adult stages. The bees used (see Materials and Methods, Experimental part I) showed an onset of orientation flights between day 5 and 6. Pollen foraging was already observed in a few animals between Days 9 and 11, with an increase around Day 15. Since we did not observe significant changes between nectar and pollen foragers (data not shown), the number and density of microglomerular complexes and bouton volume were pooled into one forager (Fo) group.

Animals performing age-related tasks in a natural environment [rich environment, Fig. 3(A,B)] showed an increase in the number of microglomerular complexes accompanied by an increase in bouton volume. The number of microglomerular complexes and the bouton volume stayed almost constant until the 18th day [Fig. 3(A), white bars, Fig. 3(B)], and then increased significantly (number of microglomerular complexes: Kruskal-Wallis, $\chi^2 = 9.966$, df = 4, $p = 0.041$, Mann-Whitney $U$ $p < 0.05$; bouton volume: Kruskal-Wallis $\chi^2 = 13.217$, df = 4, $p = 0.01$, Mann-Whitney $U$ $p < 0.05$) until the age of Day 37 [Fig. 3(A,B)]. Similar age-dependent effects were observed in the group of animals reared under poor environmental conditions [Fig. 3(C,D)]. The number of microglomerular complexes showed a not significant but slight increase whereas the bouton volume increased significantly most pronounced on Day 37 [Fig. 3(C), white bars; Fig. 4(D); bouton volume: Kruskal-Wallis $\chi^2 = 8.799$, df = 3, $p = 0.032$]. In comparison to the results observed in bees reared under rich environmental conditions, the number of microglomerular complexes increased to a higher degree, whereas the density of microglomerular complexes stayed constant [Fig. 3(A,D), white and gray bars on day 37].
We did not observe statistical differences for the measured parameters comparing same-aged forager bees reared under either rich or poor environmental conditions. Thus either the increase of the number of microglomeruli and bouton volume is independent of the rich versus poor environmental difference or other parameters (e.g. flight activity, internal conditions of the colony) regulate the number of microglomeruli bouton volume.

**Microglomerular Organization During Manipulated Behavioral Maturation and Sensory Experience**

Manipulations of the bees’ olfactory-guided behavior were applied to determine whether maturation of the microglomerular organization of the MB lips is influenced by the bee’s behavior and its olfactory experience. For this purpose bees were reared in a single-cohort hive in which same-aged bees were prevented from nursing and foraging (hive-bound, hb), or allowed to engage in extended nursing (Nu) and precocious foraging (pFO) (see Materials and Methods, Experimental part II). In contrast to bees reared in a natural colony, the changes in the microglomerular organization of the MB lips depend on the behavioral maturation and olfactory experience [Fig. 4(A–F)]. Hive-bound bees show a significant steady decrease in the number [Fig. 4(A), white bars, Kruskal-Wallis $\chi^2 = 10.275, \text{df} = 4, p = 0.036$] and density [Fig. 4(A), gray bars, Kruskal-Wallis $\chi^2 = 11.347, \text{df} = 4, p = 0.023$] of microglomerular complexes until Day 14 (Mann-Whitney U, $p = 0.05$) with a slight increase on Day 18 (Mann-Whitney U, $p = 0.034$). The bouton volume showed a slight continuous increase until Day 18 [Fig. 4(B)] Bees engaged in extended nursing showed a minor decrease in the number [Fig. 4(C), white bars] and density [Fig. 4(C), gray bars] of microglomerular complexes and a significant increase in bouton volume most pronounced on Day 14 [Fig. 4(D), Kruskal-Wallis, $\chi^2 = 10.413$, $\text{df} = 4, p = 0.023$].
Bees engaged in precocious foraging showed a significant decrease in the number [Fig. 4(E) white bars, Kruskal-Wallis, $\chi^2 = 10.880$, $df = 4$, $p = 0.028$] of microglomerular complexes until Day 14 (Mann-Whitney U, $p < 0.05$). The bouton volume showed
a significant increase [Fig. 4(F), Kruskal-Wallis, $\chi^2 = 12.903$, df = 4, $p = 0.012$] until Day 14 followed by a slight decrease (Mann-Whitney U, $p < 0.05$).

Comparing the changes in the number of microglomerular complexes and bouton volume observed in the three groups our results showed that the number of microglomerular complexes is negatively correlated with the bouton volume in hive-bound (Pearson correlation coefficient $r = -0.607$, $p = 0.024$) and extended nurse bees (Pearson correlation coefficient $r = -0.910$, $p < 0.001$) but not in precocious forager bees.

The most striking difference between the three groups of bees (hive-bound, extended-nursing and precocious foraging bees) relates to the different stages of maturation in which changes in the microglomerular organization occurred. In hive-bound bees and precocious forager bees the number and density of microglomerular complexes continuously decreased [Fig. 4(A, E)], whereas in extending nursing bees the significant decrease occurred from Day 10 to 14 [Fig. 4(C)]. Statistically the number and density of microglomerular complexes differs significantly comparing hive-bound and precocious foragers with extended nurses aged 10 days (Mann-Whitney U, $p < 0.04$).

Comparing the results observed in bees reared in a natural colony with those observed in bees from the single-cohort colony, our results show different dependencies between the number or density of microglomerular complexes and the boutons’ volume (see Fig. 5). The single-cohort colony may have been in suboptimal conditions, since a considerable proportion of bees (the hive-bound bees) were constrained to the hive and their survival rate was reduced. These conditions could well have led to an imbalance in the social life within this colony. Nevertheless, in the context of our question it was interesting to ask whether the parameters of the microglomerular complexes change in hive-bound bees in a similar or a different manner, and no attempt was made to trace the factors acting on bees in a single-cohort colony and, in particular, on hive-bound bees.

In bees reared in a natural colony the number and density of microglomerular complexes and the bouton volume increased, whereas in bees reared in a single-cohort colony the number and density of microglomerular complexes decreased and the bouton volume increased. This negative correlation was most pronounced on Day 14 in hive-bound bees and extended nurses, whereas the effect was weaker in precocious foragers. Comparing manipulated with non-manipulated bees on Day 14 our results revealed that foragers from the natural colony—irrespective of the rearing conditions (rich and poor)—exhibit a significantly higher number [Fig. 5(A) white bars, Kruskal-Wallis, $\chi^2 = 9.820$, df = 4, $p = 0.04$] and density [Fig. 5(A) gray bars, Kruskal-Wallis, $\chi^2 = 12.276$, df = 4, $p = 0.015$] of microglomerular complexes and a significantly lower bouton volume [Fig. 5(B), Kruskal-Wallis, $\chi^2 = 16.040$, df = 4, $p = 0.003$].

**DISCUSSION**

This study provides the first 3D-based analysis of the MB lips’ microglomerular organization during adult
behavioral maturation of the honeybee (see Fig. 1). We visualized microglomerular complexes within the mushroom body lips by simultaneously labeling pre- and postsynaptic components (Figs. 1 and 2) following a study by Groh et al. (2004), in which microglomerular complexes in adult worker and queen bees were studied. A 3D-based quantification and volumetric measurement technique allowed us to quantify changes in the microglomerular organization of the MB lip, using light microscopical techniques. We also aimed for a better distinction between effects of environmental experience and those that result from manipulations of behavioral maturation and sensory experience, especially the transition from nursing to foraging behavior.

Previous studies focused on age- and experience-related neuroanatomical changes associated with the mushroom bodies' volume, but not with their microglomerular organization. The fact that age and foraging experience is associated with an increase of mushroom body neuropil (lip, collar, basal ring) volume has been observed in several studies (Withers et al., 1993; Durst et al., 1994; Fahrbach et al., 1998; Ismail et al., 2005). More recently Farris et al. (2001) have shown that foraging experience is associated with an increase in the complexity of the Kenyon cell dendritic branching pattern. A comparison with the microglomerular complexes analyzed in our study is limited since these observations were made on the visual part of the MB, the collar region, and not on the lip region. With respect to the microglomerular organization it was observed that this organization is influenced by brood-temperature in worker bees and queens, respectively (Groh et al., 2004, 2006). Adding to that our study now revealed that the quantitative microglomerular organization is also influenced by age-dependent and behavioral maturation. Our results showed that the number and density of microglomerular complexes and their bouton volumes as measured by anti-synapsin IR increased during maturation (see Fig. 3) and depend on experience and behavior particularly inside the hive. Hive-bound, extended nurses and precocious foragers showed a decrease in the number and density of microglomerular complexes associated with an increase in bouton volume (see Fig. 4). The comparison between non-manipulated and manipulated bees indicates that the number and density of microglomerular complexes—as well as the bouton volume—depend on the bees’ behavior, and not just their age. However such a comparison is complicated by the fact that the colonies in the rich/poor environment experiments (experimental part I) and the one in the experimental part II (age cohorts of behaviorally manipulated animals in an observation hive) were exposed to a range of different factors. Not only that the bees in these two different conditions lived in colonies of different size and environments they were also exposed to different factors which resulted from differences between a normal hive and one of an observation hive with a glass window. These latter differences could have resulted in differences in colony temperature and light exposure. Therefore, the higher number and density of microglomerular complexes found in animals of the rich/poor experiment cannot be traced to any single parameter. However, since we are dealing with a neural structure, the mushroom body lip, which is dedicated to the neural processing of olfactory in formation in the bee brain we can at least conclude that the factors relevant here have an impact on the organization of this structure, most likely with the consequence of altered synaptic transmission. Larger numbers of microglomerular complexes and their higher density combined with smaller volumes might indicate that olfactory processing is more advanced and more detailed, and such a neural substrate appears to be more related to the normal colony life than to the rich/poor conditions of the external environment.

Changes in anti-synapsin IR indicate changes in the volume of the microglomerular complexes which might imply altered synaptic functions at this input sites to the olfactory part of the mushroom body. It is thus tempting to interpret the correlation between number/density and volume of microglomerular complexes as indicating a compensatory mechanism, which increases effective synaptic transmission in the microglomeruli surviving a putative selection process. A similar interpretation has been provided by Seid et al. (2005) who found new synapses to be formed in the lip region of the mushroom body of the ant Pheidole dentate.

Compensatory functional changes triggered by sensory deprivation have been observed in developing mammalian brains. Deprived olfactory bulbs become more sensitive to odor stimulation: mitral cells become more responsive and more glomeruli are activated by odors (Leon, 1998). A recent study by Tyler et al. (2007) revealed that deprivation increased the strength of primary synapses by acting at both presynaptic and postsynaptic sites. Their results argue against a change in the number of olfactory sensory neuron synapses but rather suggest a change in the efficiency of existing synapses. If similar conditions would apply to the system studied here we have to postulate that the larger volume of boutons leads to more efficient synaptic transmission probably because of the larger presynaptic (and possibly also postsynaptic) compartments. On the other
side, if the relationship between pre- and postsynaptic membrane area is kept constant, as it appears to be the case in the first neuropil in flies (Meinertzhagen, 1993) the larger bouton volume would reflect a larger number of presynaptic sites on the grown area of the PN boutons. The observed changes in the number and density of microglomerular complex might also induce a remodeling process (Stepanyants et al., 2002) at their postsynaptic sites, the formation or elimination of KC dendritic spines (postsynaptic sites) or changes in the arborization pattern of KCs and inhibitory feedback neurons from the MB lobes, both of which receive input and serve as presynaptic sites to the PN bouton (Ganeshina and Menzel, 2001). This would imply changes of the microcircuit structure and function around each bouton, not only quantitatively with respect to synaptic weights, but possibly also qualitatively by rewiring the connectivity within each microglomerular complex.

It is well documented that the age- and experience-dependent neural plasticity may be restricted to sensitive periods. For example, sensory deprivation or inadequate stimulation in zebra finches leads to an extension of the sensory period, which is accompanied by a change in spine density (Bischof et al., 2002). We found for the bee that reducing the sensory input at a time when the animals are prepared to change their behavioral tasks leads to the structural changes explained above. In hive-bound bees these structural changes evolved already on Day 10 [Fig. 4(A,B)] whereas in nurse bees at a later stage on Day 14 [Fig. 4C,D)]. The earlier onset of these structural changes in hive-bound bees might be explained by the fact that the brain is more readily prepared for structural plasticity and thus more sensitive to sensory/behavioral impairment at a particular time of its age dependent maturation program.

Age-related programs appear to prepare the animal for particular sensory/motor conditions through hormonal controls and thus coordinate the formation of the neural structures to the expected inputs and/or outputs. Only under conditions of a full- balanced colony life do animals develop normally, and the working of coordinated developmental programs and experience effects can only be uncovered by interfering with normal life conditions. Interfering with the sensory/motor experience of any subgroup of animals within the colony will also have an effect on the normal life history of the whole colony through reciprocal feedback loops up to the extreme case in which a colony loses the balance of social life and deteriorates, as may have been the case in our colony 3. In the domain of olfactory cues, which we studied here, the general effect of such disturbance was the reduction in the number and density of microglomerular complexes, accompanied by an increase of bouton volume. It is tempting to conclude that the microglomerular complexes are the substrate for central nervous olfactory processing that provides the structural modules for both developmental programs as well as experience-dependent plasticity. Further physiological and structural studies need to be brought to the single neuron and the network level, taking advantage of the insect nervous system with its identified neurons and well-described neural nets (Abel et al., 2001; Gronenberg, 2001; Kirschnen et al., 2006). The strength of synaptic transmission between projection neurons and Kenyon cells can be monitored in Ca-imaging recordings (Szyszka et al., 2005), and electron microscopy studies can be combined with single-cell marking and immunolabeling (Ganeshina and Menzel, personal communication). These and other experiments, which are easily carried out in Apis mellifera, will allow us to test the hypothesis that microglomerular organization changes lead to a rewiring of cell-to-cell connections, enabling the brain to adapt its synaptic efficacy.

We thank L. Müßig and D. Drenské for motivated help during experiments, P. Knoll for beekeeping, and B. Brembs, G. Leboulle, N. Stollhoff and the Journal Club for helpful comments regarding this manuscript.

REFERENCES


