Age-Dependent and Task-Related Volume Changes in the Mushroom Bodies of Visually Guided Desert Ants, *Cataglyphis bicolor*

Simone Kühn-Bühlmann, Rüdiger Wehner*

Department of Zoology, Section of Neurobiology, University of Zürich, Winterthurerstrasse 190, Zürich, Switzerland

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ABSTRACT: **Desert ants of the genus *Cataglyphis* are skillful long-distance navigators employing a variety of visual navigational tools such as skylight compasses and landmark guidance mechanisms. However, the time during which this navigational toolkit comes into play is extremely short, as the average lifetime of a *Cataglyphis* forager lasts for only about 6 days. Here we show, by using immunohistochemistry, confocal microscopy, and three-dimensional reconstruction software, that even during this short period of adult life, *Cataglyphis* exhibits a remarkable increase in the size of its mushroom bodies, especially of the visual input region, the collar, if compared to age-matched dark-reared animals. This task-related increase rides on a much smaller age-dependent increase of the size of the mushroom bodies. Due to the variation in body size exhibited by *Cataglyphis* workers we use allometric analyses throughout and show that small animals exhibit considerably larger task-related increases in the sizes of their mushroom bodies than larger animals do. It is as if there were an upper limit of mushroom body size required for accomplishing the ant's navigational tasks.** © 2006 Wiley Periodicals, Inc. J Neurobiol 66: 511–521, 2006

Keywords: **ant brain; mushroom bodies; optic lobes; age effects; task effects**

INTRODUCTION

During the last decades desert ants of the genus *Cataglyphis* have become model systems for the study of insect navigation (e.g., Wehner, 1982, 2003; Collett et al., 1998; Andel and Wehner, 2004). Path integration based on the simultaneous use of a visual skylight compass (Wehner and Srinivasan, 2003) and a proprioceptive odometer (Sommer and Wehner, 2004), and accompanied by the landmark-dependent learning of places and routes (Wehner et al., 1996; Åkesson and Wehner, 2002) constitutes the ant's major navigational toolkit. Finally, food items, i.e. arthropod corpses scattered across the desert floor, are detected and localised by exploiting anemotactic and olfactory cues (Wolf and Wehner, 2000). Different kinds of memory stores seem to be involved in learning path-integration vectors, site-based as well as route-based landmarks (Ziegler and Wehner, 1997; Bisch-Knaden and Wehner, 2003). While performing their long-distance foraging journeys the ants accomplish all these demanding tasks of processing, storing and retrieving various kinds of sensory information during an extremely short period of their lifetime (less than one week at the end of their life).

Like ants in general (Hölldobler and Wilson, 1990), workers of *Cataglyphis bicolor* exhibit an age-dependent polyethism. Having stayed within their subterranean nests as 1-day callows, later as repletes, and finally as interior workers (Wehner et al., 1972) for a total of about 28 days, they become outdoor foragers for another 6 days (mean life expectancy of for-

Correspondence to: Rüdiger Wehner (rwehner@zool.unizh.ch).

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agers: 6.1 days, Schmid-Hempel and Schmid-Hempel, 1984). Hence, the large-sized *Cataglyphis bicolor* is an extremely short-lived species of ant if compared, for instance, with species of other genera, in which the lifetimes of foragers have been measured in the field (e.g. *Formica*: Rosengren, 1971; *Myrmica*: Brian 1972). Here we ask whether in spite of the ant's extremely short foraging lives any kind of age-related and/or task-dependent structural plasticity occurs in the *Cataglyphis* brain. In particular, we inquire about potential volumetric changes of the mushroom bodies (corpora pedunculata) and their constituent parts (calyces with lip and collar, peduncle, vertical and medial lobes) and the two major visual neuropiles (medulla and lobula).

Ever since Dujardin's (1859) classical account have the mushroom bodies been considered to be the highest site of multimodal interactions between afferent and efferent signals, and to be involved in the organisation of complex and plastic behaviours, especially in learning and memory (for reviews see Heisenberg, 1998; Strausfeld, 1973, 2001; Strausfeld et al., 1998; Zars, 2000). For example, in *Drosophila* the mushroom bodies are not only required for olfactory learning, but are also involved in decision making and context generation (DeBelle and Heisenberg, 1994; Liu et al., 1999; Heisenberg, 2003). In fact, learning and memory mutants of *Drosophila* exhibit structural defects in the mushroom bodies (Heisenberg et al., 1985). If compared to the size of the remainder of the brain, the mushroom bodies are largest in social hymenopterans, especially in social wasps (Ehmer and Hoy, 2000) and ants (Gronenberg, 1999).

Over the last decades several studies have shown that honey bees (Withers et al., 1993, 1995; Durst et al., 1994; Fahrbach et al., 1995, 1998; Farris et al., 2001; Fahrbach et al., 2003), carpenter ants (Gronenberg et al., 1996; Ehmer and Gronenberg, 2004) and paper wasps (O'Donnell et al., 2004) exhibit distinct patterns of volume changes during various stages of their adult lives. For example, even in totally light deprived honey bees the volume of the mushroom bodies increases during the first week after the adults have hatched from the pupa (Fahrbach et al., 1998), and this growth continues after the bees have started foraging. Even in age-matched cohorts of bees, the ones with foraging experience have significantly larger mushroom bodies than the ones that lack such experience (Farris et al., 2001). In ants, the most extensive study on neural plasticity during adult life has been performed by Gronenberg et al. (1996). In workers of the wood ant *Camponotus floridanus*, which have never left their nest and have not been engaged in any significant intracolony activity such as nursing, the mushroom body neuropiles double their volumes during the first 6 months of post-pupal life. Brood care and foraging lead to a further increase in the size of the mushroom bodies in this species.

As compared to the forest-living C*amponotus* species, desert ants of the genus *Cataglyphis* are extremely short-lived, especially during their foraging stage. In *Cataglyphis bicolor* this last phase of life lasts for less than a week (only late-summer foragers might overwinter and continue foraging next spring), while *Camponotus* workers may forage over periods of several months (Hölldobler, personal communication). Furthermore, workers of *C. floridanus* do not leave the nest before the age of 10 weeks (Gronenberg et al., 1996), while workers of *Cataglyphis bicolor* do so already after 4 weeks, when they begin to forage immediately (Schmid-Hempel and Schmid-Hempel, 1984; Wehner et al., 2004). Hence we ask whether the mushroom body (and optic lobe) neuropiles change in size even during the short lifespans of *Cataglyphis* workers, especially *Cataglyphis* foragers, which accomplish quite demanding tasks of visual navigation.

METHODS

Ants

The experiments designed to study age-related volumetric changes in brain neuropiles ("age relations") were performed on adult ants obtained from one queenright colony of *Cataglyphis bicolor* excavated at our Maharès field site in Tunisia (34.58°N, 10.50°E). The colony was maintained in complete darkness within an artificial nest consisting of interconnected plaster chambers. The chambers were connected to a foraging arena (0.55×0.55 m^2), within which the ants could forage for food (honey and dead fruit flies, *Drosophila melanogaster*). The temperatures ranged from 29°C to 31°C during daytime, but decreased to 23°C during the night; humidity was about 20%. Freshly eclosed ants (callows, in the following denoted as 1-day ants) were taken out of the chambers, code-marked with dots of paint (Lackstift, Dupli-Color, Germany) as long as they exhibited their pale colour for one and maximally two days, and returned to the colony. The ants were fed, marked, and observed by using a pocket lamp equipped with a red light filter (cut-off filter, 665 nm).

In the field, workers of *C. bicolor* have been shown to start foraging at approximately four weeks after hatching (Schmid-Hempel and Schmid-Hempel 1984). However, as under our laboratory conditions predation pressure and heat or desiccation stress have been virtually absent, the ants have been able to live and forage for much longer times than they are able to do in their natural environment. Hence, in order to examine age-related changes of the mushroom bodies and optic lobes we succeeded in generating and finally testing four different age cohorts: 1-day (n = 6), 30-day (n =

10), 60-day (n = 6), and 150-or-more day (5- to 6-month) old ants. The latter (n = 9) are denoted as (150+)-day ants. All these ants had never been observed (during 20-min observation periods per day) outside the nest in the arena, which anyway was in complete darkness as well.

In order to test for task-dependent changes in neuropile volumes, we compared ants that had been light deprived, i.e. reared in the dark, with those age-matched cohorts of ants that had been observed foraging in their natural habitat at our North African (Maharès) field site. After being captured the latter ants were transported to Zurich alive and dissected there immediately. Two groups of foragers were studied: (i) foragers which had hibernated inside their subterranean colony ("previous-year foragers") and were captured thereafter in the field during the month of May (as the colony, from which these workers were taken, had not yet produced new brood, all foragers appearing in the field must have belonged to previous-year cohorts of ants); (ii) "same-year foragers", which were captured in the month of July long after the *C. bicolor* colonies had started their brood cycles again. Although the exact age of the same-year foragers was unknown, their age was estimated to lie somewhere between 28 and 46 days (Schmid-Hempel and Schmid-Hempel, 1984). The same-year foragers (n = 12) were compared with dark-reared ants which had no foraging experience at all, but belonged to comparable age classes (30-day and 60-day ants, n = 16). The previous-year foragers (n = 14) were compared with age-matched dark-reared (150+)-day ants (n = 9), as their age was supposed to be at least 6 months.

Immunostaining Procedure and Volume Measurements

Ant brains were dissected from the head capsule and fixed in 4% paraformaldehyde overnight at 4°C. Fixed brains were quickly incubated in 0.1% collagenase (Sigma; 1mg/ml 0.1M phosphate buffered saline PBS), washed with PAT (PBS with 1% bovine serum albumine and 3% Triton-X-100, Fluka) (Rein 1998) for 6 hours, and then placed in blocking solution (10% normal goat serum, Jackson Immuno Research) for 1 hour. Synaptic-vesicle-associated protein synapsin I (SYN-ORF1) was used as primary antibody as it is particularly well suited to label neuropile (Klagges et al. 1996). After blocking brains were treated with the mouse-monoclonal primary antibody SYN-ORF1 (1:50) for about 60 hours at room temperature, then washed for 6 hours, and stained with the secondary antibody (fluorochrome CY3.18, Jackson Immuno Research, 1:200 in 10% normal goat serum) for about 36 hours at room temperature. Stained brains were rinsed in PAT, dehydrated in an increasing ethanol series, transferred to 30% methyl salicylate (Fluka) in ethanol, and mounted in pure methyl salicylate.

The wholemount preparations were imaged with a confocal laser scanning microscope (Leica TCS SP2) using a Leica HC PL APO 10x/0.4 dry lens. Optical sections were made at intervals of 2.5 μm and saved as stacks of 150–170 images of a size of 512 × 512 pixels.

For the volumetric analysis of the mushroom bodies and the optic lobes, the neuropile areas of interest were manually traced on each slice using Amira 3.1, a 3D scientific visualisation and data analysis package (Indeed-Visual Concepts, Berlin, Germany). We distinguished three different compartments of the mushroom bodies (lip, collar, and peduncles including vertical and medial lobes, see Fig. 1), and two of the optic lobes, medulla and lobula (for terminology of vertical, formerly alpha, and medial, formerly beta, lobes see Strausfeld 2002). The total volume of the mushroom bodies was calculated by summing up the volumes of its compartments. Like in most ant species (Gronenberg 2001), in *C. bicolor* the basal ring, another region of the mushroom bodies receiving both visual and olfactory input, was not distinguishable from lip and collar. The medulla and lobula were only considered for the statistics when both were intact, and due to damage during processing the lamina could not be reliably measured.

Statistical Analyses

Even though *C. bicolor* is a monomorphic species, the (linear) body sizes can vary by a factor of about two among the individuals of the same colony (Wehner, 1983). The relationship between ant size and mushroom body size was investigated by calculating Pearson's coefficient of product-moment correlation (Sokal and Rohlf, 1995: p 559–566). As larger ants have relatively smaller mushroom bodies (see Gronenberg and Hölldobler, 1999, and our results; for bumblebees see Mares et al., 2005), the effect of age and foraging experience on neuropile volume had to be analysed using allometry and size corrected data. Because the ants used in the present study had to be marked with paint (providing a colour code), their body weights could be estimated only indirectly. Therefore, by using a wedge micrometer (Porter, 1983), we measured head widths (for definition see Wehner, 1983), and transformed these data into body weights by applying a previously determined calibration function that correlated head width with body weight (R = +0.989, n = 58, Fukushi and Wehner, unpublished data).

The allometric relationship (Huxley and Teissier 1936) between body weight (W_{body}) and mushroom body volume (V_{mb}) is

$$V_{mb} = a \cdot W_{body}^{b}. \quad (1)$$

This equation also holds for the allometry of the subcompartments of the mushroom bodies and of the optic lobes. The allometric exponent b and the allometric coefficient a were estimated by fitting the bivariate regression (reduced major axis; Sokal and Rohlf, 1995: p 541–549) to $\log_{10}$-transformed data (using PROC PRINCOMP in SAS 8.2):

$$\log_{10} V_{mb} = \log_{10} a + b \cdot \log_{10} W_{body} \quad (2)$$

The 95% confidence intervals for $\log_{10} a$ and b were calculated following Sokal and Rohlf (1995: p 586–593). The

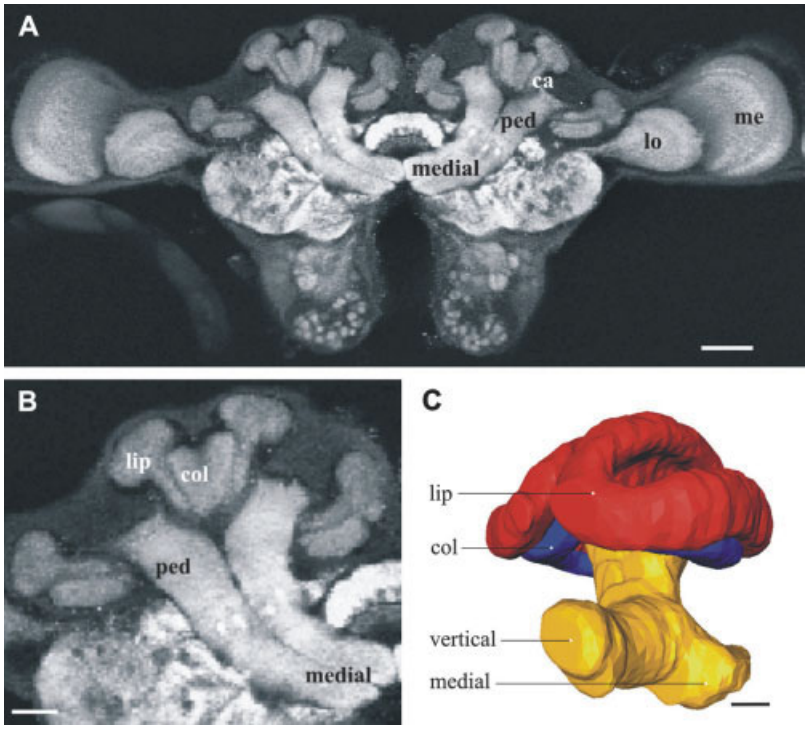

Figure 1 Anatomy of the brain of the desert ant, *C. bicolor*. (A) Frontal view of an immunohistochemically stained brain obtained from confocal microscopy and processed using Amira software (ca: calyx; ped: peduncle; medial lobe; lo: lobula; me: medulla). (B) Frontal section of the mushroom body (col: collar). (C) 3D model of the mushroom body as reconstructed by the Amira software (vertical lobe). Scale bars 100 μm (A), 50 μm (B and C).

estimates for $\log_{10} a$ and the corresponding 95% confidence intervals were then back-transformed to a.

Due to the small sample sizes in some age classes, b and a could not be estimated reliably. Therefore, the age effect was assessed by calculating the difference between the measured volume and the predicted volume:

$$\Delta V = V_{measured} - V_{predicted} \quad (3)$$

$V_{predicted}$ was calculated from equation [1] by pooling all age classes for the estimation of b and a.

In order to identify homogenous groups (i.e. age classes that did not differ statistically from each other) multiple comparisons among pairs of age classes were done on ΔV applying the Tukey-Kramer method with $\alpha = 0.05$. This method is a true "multiple comparison" test, appropriate when all pairwise comparisons are of interest, and it is especially suited for comparisons of groups with unequal sample sizes (SAS OnlineDoc, v. 8, 1999; SAS Institute, Cary, N.C. USA).

The effect of foraging experience was analysed by comparing b and a (equation [1]) between "foragers" and "non-foragers". Differences between groups were considered to be statistically firm if the confidence intervals for b and/or a did not overlap.

RESULTS

Age-Related Effects

In our experimental sample of *C. bicolor* ants, the sizes of the mushroom bodies ranged from 0.012 to 0.033 mm^3. There was a positive correlation between the body weights of the ants and the volumes of their mushroom bodies ($R = +0.68$, $p < 0.0001$, $n = 53$). Therefore we used size-corrected data for unravelling age-dependent and task-related variations in the various substructures of the brain.

Age-related volume changes of the mushroom bodies and optic lobes were examined by comparing four different age classes of ants that had been reared in complete darkness (1-day ants, $n = 6$; 30-day ants, $n = 10$; 60-day ants, $n = 6$; (150+)-day ants, $n = 9$). This comparison revealed a significant age-correlated increase of the volumes of all neuropiles studied (as shown for the mushroom body neuropile in Fig. 2). Five to six months old workers ((150+)-day ants denoted as DARK 150+) had significantly larger mushroom bodies than all other age classes, whereas the 1-day to 60-day cohorts (denoted as DARK 1-60)

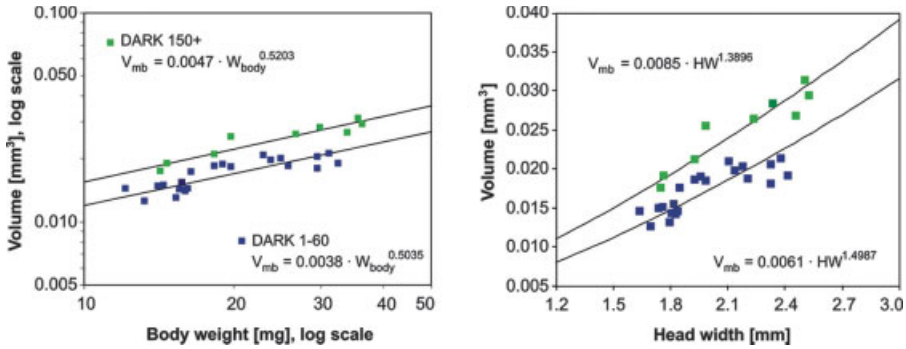

Figure 2 Age-dependent volume changes of the mushroom bodies in dark-reared workers of *C. bicolor* (double-logarithmic plot of neuropile volume on body weight and linear plot of neuropile volume on head width HW). The mushroom body volume of (150+)-day ants (DARK 150+, green squares, n = 9) is significantly larger than that of all other age classes (at $\alpha = 0.05$, Tukey-Kramer method). As the cohorts of the 1-day to 60-day ants could not be separated statistically (see text), they were all pooled for this illustration (1-day, 30-day, and 60-day ants; DARK 1-60, blue squares, n = 22).

could not be separated statistically (at $\alpha = 0.05$, Tukey-Kramer method). The same effect was observed in the subcompartments of the mushroom bodies, i.e. in the lip, the collar, and the peduncle plus lobes. The ratio of the volumes of lip and collar in dark-reared animals (1-day to 60-day cohorts) was 2.85 ± 0.14, and the volume ratios of medulla and lobula was 3.50 ± 0.24 (mean ± s.d., n = 22).

Task-Related Effects

In order to investigate the influence of visual experience or, more generally, diurnal foraging activity on the volumes of the mushroom bodies and the optic lobes, we first compared ants that had been actively foraging in the field ("same-year foragers", denoted as FOR1) with ants that had been kept in complete darkness during their entire lifetimes and had been prevented from foraging ("non-foragers" denoted as DARK 30-60), but were age-matched with the same-year foragers.

The effect of foraging activity on the volumes of the various neuropiles is striking (Fig. 3). The same-year foragers (age 28 to 46 days, see Method) have significantly larger brains and brain subcompartments than the visually deprived non-foragers. This result is clearly documented by the allometric coefficient *a* in Table 1, and applies to the volumes of all neuropiles except for the peduncle plus vertical and medial lobes and the lobula. It is only in these two neuropiles that the 95% confidence intervals for *a* of same-year foragers and non-foragers do overlap. As there are no age effects between dark-reared 30-day and 60-day ants (see above), the observed differences in neuropile volume between non-foraging and foraging workers must be ascribed to the outdoor foraging experience of the latter. Moreover, in 10-mg foragers there is a 58% and 82% increase in the volumes of lip and collar, respectively, if compared with age-matched or even slightly older dark-reared animals. In 30-mg foragers, the corresponding increases are 23% and 29%.

The two results described so far - the age-dependent and the task-related increase of the size of the mushroom body - made us wonder whether the two effects could be combined by generating (150+)-day foragers. Foragers of that age can be obtained only by using ants that had hibernated and had been the first to forage in springtime next year. The results show that the previous-year foragers (denoted as FOR2) indeed had larger brain subcompartments than the age-matched non-foragers (denoted as DARK 150+, Fig. 4). Once more, it was only in the lobula that the 95% confidence intervals for *a* did overlap (Table 2), so that this brain structure did not seem to differ between foragers and non-foragers. Moreover, the comparison of the neuropile volumes of same-year foragers (FOR1) and previous-year foragers (FOR2) revealed an increase in mushroom body size (see *a*-values for FOR1 and FOR2 in Tables 1 and 2). This increase was the larger, the smaller the animal (see Fig. 3 and 4). It appears that in the previous-year foragers an upper limit of about 0.03mm^3 is reached that is not exceeded even by the largest animals (body weight about 50 mg).

Furthermore, as shown by the allometric regression line, the neuropiles of foragers tend to increase

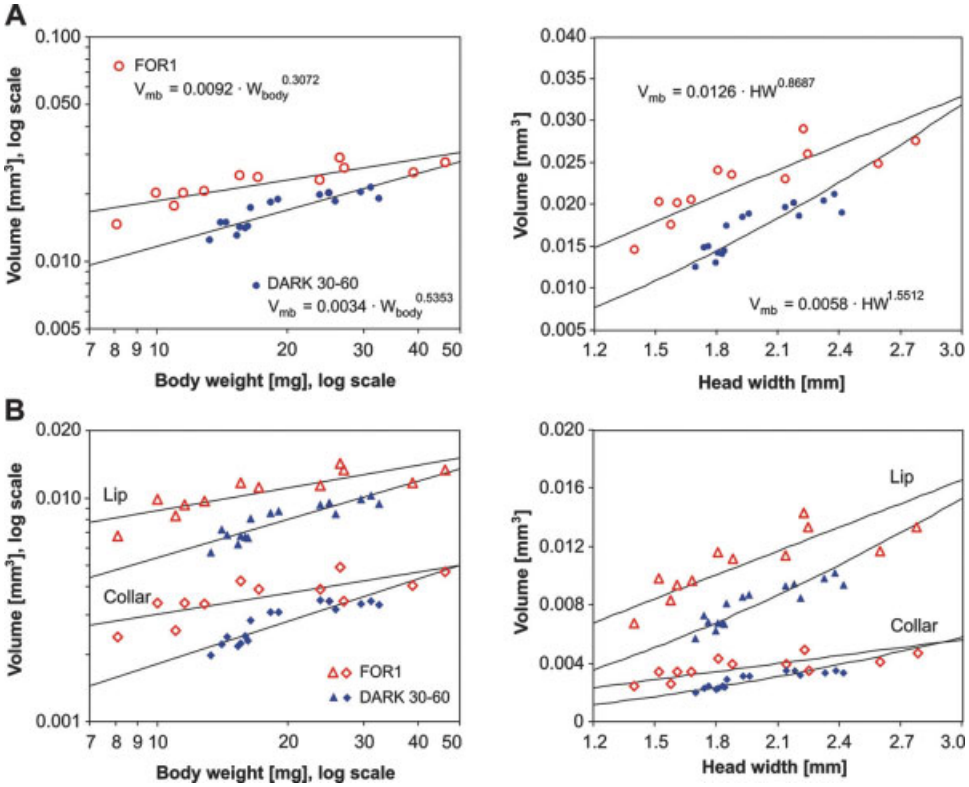

Figure 3 Task-related volume changes of (A) the total mushroom body neuropile and (B) that of its subcompartments lip and collar between same-year foragers (FOR1, open red symbols, n = 12) and 30-day and 60-day non-foragers (DARK 30-60, full blue symbols, n = 16) of *C. bicolor* (double-logarithmic plot of neuropile volume on body weight and linear plot of neuropile volume on head width HW). Note that the ordinate scaling is different in (A) and (B).

proportionally less with body size than the neuropiles of light-deprived workers do: the allometric exponent *b* is larger in the non-foragers than in the foragers, although the 95% confidence intervals do overlap partially (Table 1 and 2). In any way, however, negative brain allometry ($b < 1$) holds for either group.

Table 1 Parameter Estimates of the Allometric Equation $V = a \cdot W_{body}^{b}$ for Same-Year Foragers (FOR1) and Age-Matched Nonforagers (DARK 30-60)

Neuropile	Cohort	Volume (mm^3) ± S.D.	*a* (95% CI)	*b* (95% CI)	R^2	*n*
MB (total)	FOR1	0.0226 ± 0.0041	0.0092 (0.0061, 0.0133)	0.3072 (0.1780, 0.4464)	0.73	12
	DARK 30-60	0.0170 ± 0.0029	0.0034 (0.0019, 0.0056)	0.5353 (0.3682, 0.7294)	0.75	16
Lip	FOR1	0.0109 ± 0.0022	0.0040 (0.0025, 0.0062)	0.3381 (0.1896, 0.5014)	0.71	12
	DARK 30-60	0.0080 ± 0.0014	0.0015 (0.0009, 0.0023)	0.5656 (0.4093, 0.7461)	0.79	16
Collar	FOR1	0.0037 ± 0.0008	0.0015 (0.0008, 0.0024)	0.3132 (0.1343, 0.5124)	0.59	12
	DARK 30-60	0.0028 ± 0.0005	0.0004 (0.0002, 0.0007)	0.6280 (0.4567, 0.8305)	0.79	16
Ped+lobes	FOR1	0.0080 ± 0.0013	0.0037 (0.0026, 0.0050)	0.2676 (0.1614, 0.3799)	0.75	12
	DARK 30-60	0.0062 ± 0.0010	0.0015 (0.0007, 0.0028)	0.4620 (0.2567, 0.7053)	0.59	16
Medulla	FOR1	0.0067 ± 0.0012	0.0026 (0.0017, 0.0039)	0.3307 (0.1874, 0.4873)	0.74	11
	DARK 30-60	0.0059 ± 0.0012	0.0008 (0.0006, 0.0012)	0.6454 (0.5213, 0.7854)	0.89	16
Lobula	FOR1	0.0018 ± 0.0004	0.0006 (0.0005, 0.0008)	0.3496 (0.2677, 0.4360)	0.90	12
	DARK 30-60	0.0016 ± 0.0003	0.0004 (0.0002, 0.0005)	0.5014 (0.3832, 0.6319)	0.86	15

Abbreviations: W_{body}, body weight; MB, mushroom body; Ped, peduncle; S.D., standard deviation; *a*, allometric coefficient; CI, confidence interval; *b*, allometric exponent; R^2, coefficient of determination; *n*, sample size.

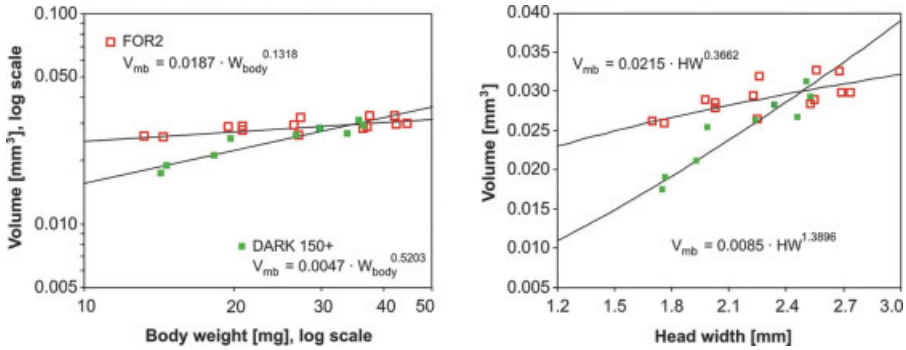

Figure 4 Task-related changes of the mushroom body neuropile volume between previous-year foragers (FOR2, open red squares, n = 14) and (150+)-day non-foragers (DARK 150+, full green squares, n = 9) of *C. bicolor* (double-logarithmic plot of neuropile volume on body weight and linear plot of neuropile volume on head width HW).

DISCUSSION

At the present state of discussions about brain plasticity in social insects, desert ants of the genus *Cataglyphis* are especially well suited for asking a number of basic questions. First, *Cataglyphis* foragers are visually guided, long-distance navigators, which leave their underground colonies for distances of sometimes more than one hundred metres (Wehner and Wehner, 1990), and which by doing so rely heavily on skylight compasses, odometry, path-integration, and the ability to acquire and use landmark-based information about particular sites and routes within their foraging terrain (for reviews see Wehner and Srinivasan, 2003; Wehner, 2003). Second, *Cataglyphis* foragers are extremely short-lived. They exhibit a mean life expectancy of merely 6.1 days (Schmid-Hempel and Schmid-Hempel, 1984). Hence, how and to what extent is this rich repertoire of visual behaviours and memory stores reflected in the relative sizes of the ant's visual neuropiles? Furthermore, do the neuropiles potentially involved in performing these tasks undergo developmental changes in their sizes, even though the navigational task will be accomplished only during a few days?

Let us inquire about the first question by starting with an influential paper (Gronenberg and Hölldobler, 1999), in which the authors have correlated the sizes of the eyes and various brain structures with the basic behavioural repertoires of 14 species of ants ranging from purely olfactory to mainly visually behaving species. For example, Gronenberg and Hölldobler (1999) compared the relative sizes of the olfactory and visual input regions of the mushroom bodies, the lip and the collar, respectively. Whereas in honey bees and social wasps, which are predominantly visually guided hymenopterans, the size of the collar always exceeds that of the lip (plus basal ring) region (Jawlowski, 1959; Howse,

Table 2 Parameter Estimates of the Allometric Equation $V = a \cdot W_{body}^{b}$ for Previous-Year Foragers (FOR2) and Age-Matched Nonforagers (DARK 150+)

Neuropile	Cohort	Volume (mm^3) ± S.D.	*a* (95% CI)	*b* (95% CI)	R^2	*n*
MB (total)	FOR2	0.0291 ± 0.0022	0.0187 (0.0141, 0.0248)	0.1318 (0.0471, 0.2185)	0.49	14
	DARK 150+	0.0249 ± 0.0047	0.0047 (0.0027, 0.0077)	0.5203 (0.3657, 0.6975)	0.89	9
Lip	FOR2	0.0142 ± 0.0013	0.0087 (0.0060, 0.0127)	0.1449 (0.0321, 0.2614)	0.39	14
	DARK 150+	0.0120 ± 0.0023	0.0022 (0.0012, 0.0037)	0.5242 (0.3619, 0.7116)	0.88	9
Collar	FOR2	0.0047 ± 0.0005	0.0022 (0.0014, 0.0034)	0.2246 (0.0997, 0.3566)	0.55	14
	DARK 150+	0.0039 ± 0.0007	0.0007 (0.0005, 0.0011)	0.5217 (0.3900, 0.6698)	0.92	9
Ped+ lobes	FOR2	0.0102 ± 0.0006	0.0080 (0.0059, 0.0107)	0.0729 (−0.0158, 0.1628)	0.21	14
	DARK 150+	0.0091 ± 0.0017	0.0017 (0.0009, 0.0030)	0.5183 (0.3414, 0.7251)	0.86	9
Medulla	FOR2	0.0098 ± 0.0014	0.0054 (0.0024, 0.0112)	0.1778 (−0.0448, 0.4190)	0.21	13
	DARK 150+	0.0076 ± 0.0018	0.0010 (0.0005, 0.0017)	0.6546 (0.4788, 0.8642)	0.92	8
Lobula	FOR2	0.0024 ± 0.0004	0.0010 (0.0005, 0.0021)	0.2566 (0.0474, 0.4891)	0.36	14
	DARK 150+	0.0020 ± 0.0004	0.0003 (0.0002, 0.0006)	0.5729 (0.4057, 0.7682)	0.91	8

For abbreviations see Table 1.

1974; Gronenberg and Heeren, 1997), the opposite is true for ants (Gronenberg and Hölldobler, 1999). However, depending on the relative importance of vision and olfaction in the species examined by Gronenberg and Hölldobler (1999), the size ratio of lip/collar ranged from 105 and 238 in workers of almost (*Mystrium*) or completely (C*erapachays*) blind ants, respectively, to 1.7 in the highly elaborate visual predator *Gigantiops*. The four specimens of *Cataglyphis bicolor*, which the authors included in their survey, ranked highly among all species of ants (lip/collar ratio: 2.97), even though the eyes and optic lobes were much smaller than one would expect for a visual predator such as the afore-mentioned *Gigantiops,* or as *Harpegnathos*. Our data (lip/collar ratio: 2.85, see Results) are in full accord with those of Gronenberg and Hölldobler (1999), even if the workers examined in the present account had been deprived of vision, i.e. had been kept in complete darkness from their time of hatching to the maximum of 60 days. This result means that even in visually inexperienced *Cataglyphis* workers the visual input region of the mushroom bodies is much larger than the size of the eyes and optic lobes would let one assume.

Furthermore, Gronenberg and Hölldobler (1999) hypothesized that complex processing and long-term storage of visual information acquired and used in spatial orientation might not require high spatial resolution and, hence, large eyes and correspondingly large visual lobes as needed by predators that use vision for localising and tracking down their moving prey, i.e. for more immediate high-acuity tasks. This hypothesis is supported by the more recent finding that in *Cataglyphis* food detection does not involve vision at all, but occurs by purely olfactory means (Wolf and Wehner, 2000), and that the neural system processing visual compass information is a spatial low pass filter employing only a small low-acuity zone of the eye (Wehner and Labhart, in press). Hence, all neuroethological evidence at hand supports the view that the collar region of the ant's mushroom bodies is involved in the processing and/or storage of higher-order spatial information.

The ontogenetic plasticity of adult *Cataglyphis* brains reflects itself in age-dependent and task-related effects. In ants that have grown up in complete darkness and thus had been prevented from any visual orientation, the mushroom bodies increased with age. Five to six months old ants had significantly larger neuropile volumes than younger workers, while no significant volume changes did occur in the mushroom bodies during the ants' first 60 days of life (Fig. 2). This result corresponds with findings on carpenter ants (Gronenberg et al., 1996), in which age-related changes of the mushroom body volume were observed only between 10-day and 4-month old ants. The ants used in the present study were able to gain all aspects of experience during their maturation, except for visual experience. Even if they had been outside their artificial nest within the laboratory, they would not have been able to orient themselves visually during their outdoor trips, because the foraging arena was also kept in the dark. As all investigated brain structures exhibited the same (or at least a similar) pattern of neuropile enlargement during maturation, we assume that the observed neuropile expansion with increasing age is a developmental process independent of experience. This conclusion is corroborated by similar findings in bees that had been prevented from flying outside the hive (Withers et al., 1993), and in bees that had been socially isolated and reared in darkness (Fahrbach et al., 1998).

Little is known about the neural mechanisms that underlie age-related changes in the brain of social insects (Gronenberg, 1999). In honey bees neuropile growth has been shown to begin during the pupal stage and to continue throughout adult life (Farris et al., 2001), but neurogenesis ends in the mushroom bodies at day 5 of the pupal stage (Farris et al., 1999; Ganeshina et al., 2000), and Kenyon cell number remains constant with age (Fahrbach et al., 1995; Ganeshina et al., 2000; for ants see Gronenberg et al., 1996; Ishii et al., 2005). Increasing age has recently been found to be correlated with increasing complexity of the Kenyon cell dendrites (honey bees: Farris et al., 2001) and with enlarged brain synaptic boutons acquiring more synapses and vesicles in the lip region (ants: Seid et al., 2005). In *Cataglyphis*, however, we do not know yet how aging affects the final morphology of the mushroom body neuropiles.

Surprisingly, significant age-dependent increases of the total volume of the mushroom body as well as its constituent parts (lip, collar, peduncle plus vertical and medial lobes) occurred only at an age (more than 150 days in complete darkness) that lay beyond the one that foragers would usually reach in the field (about 34 days, i.e. 28 plus 6 days of indoor and outdoor life, respectively; Schmid-Hempel and Schmid-Hempel, 1984; Wehner et al., 2004). Hence, what we observe in dark-reared animals is a very slow process of size increase in the mushroom bodies. This process might be speeded up, if the animals start their foraging careers - and indeed it is (Fig. 3). A task-related increase of neuropile size occurs in the collar, the lip, and the peduncle plus mushroom body lobes as well. Furthermore, it is much more pronounced in small animals than in larger ones. Especially the visual input region, the collar, shows a massive volume

increase correlated with foraging activity. This corresponds with what has been found in honey bees (Durst et al., 1994; Withers et al., 1995). Unfortunately, however, in the current study we were not able to disentangle the effects of pure visual experience and actual foraging activity.

In the present account we have used body-size related allometric analyses throughout, in order to make our data useful for scientists working on brain allometry in general. In brain allometry studies the allometric coefficient a is a scaling factor describing the size of the brain compartment in question relative to body size. The exponent b describes how the relative brain size varies with body size. By determining the exponents b of the exponential relation between neuropile size and body size we could show that in this relation a strong negative allometry holds: b is much smaller than 1.0 (Tables 1 and 2). This means that the ratio of neuropile size to body size is not constant among the members of a colony, but is the larger, the smaller the animal. Taken together with the finding mentioned in the preceding paragraph, we can state that small animals do not only possess relatively larger mushroom bodies, but do also exhibit a larger increase of the volumes of their mushroom bodies as they engage in foraging. In the oldest (previous-year) foragers this trend seems to lead to an upper limit of the mushroom body size (about 0.03 mm^3). This limit is nearly reached by both small and large animals (with the exponent b being close to zero; $b = 0.13$, Table 2).

The fact that the smallest foragers showed the most massive increase in neuropile volume if compared to age-matched non-foragers leads to the conclusion that foraging with its need for an elaborate navigational toolkit requires a certain amount of a neural circuitry. This need for an appropriate size of the mushroom bodies for accomplishing foraging tasks might also be reflected in the phenomenon of precocious expansion of mushroom body neuropiles in carpenter ants (Gronenberg et al., 1996) and honey bees (Withers et al., 1993; Durst et al., 1994) that start to forage earlier than expected. Foraging experience was shown to enlarge the volume of mushroom bodies and to promote additional growth of Kenyon cell dendrites more substantially in experienced active foragers than in age-matched, less experienced foragers (Farris et al., 2001) or completely inexperienced workers (Withers et al., 1993; Durst et al., 1994; Gronenberg et al., 1996; O'Donnell et al., 2004). Similar effects were reported for *Drosophila*, in which the experience of light affected the mushroom bodies (Barth and Heisenberg, 1997) and the optic lobes (Barth et al., 1997). In the present study, both the mushroom bodies and the medulla were larger in foragers than in light-deprived non-foragers of similar age (Figs. 3 and 4). Even if the non-foragers had an age of 5–6 months, the group of hibernated (previous-year) foragers exhibited significantly larger neuropile volumes. Based on the observation of Fahrbach et al. (2003) that in honey bees the neuropile volume of the mushroom bodies, once expanded, stays stable during winter, we may conclude that the measured differences in neuropile volumes between *Cataglyphis* foragers and non-foragers is due to the ants' outdoor foraging activities. The foraging-dependent increase of mushroom body size might well be due to the ants' visual navigation performances. This might correlate with the concurrent significant increase in the size of the medulla, the largest visual neuropile in *Cataglyphis*. Ehmer and Gronenberg (2002, 2004) have shown that there are medullar neurons terminating in the collar region.

In conclusion, even during their short (in the mean 6-day) foraging lives workers of *Cataglyphis bicolor* exhibit substantial volume increases in all compartments of their mushroom bodies. These increases ride on a small age-dependent increase in mushroom body size. They are much more pronounced in small than in large animals, so that the negative allometry in the brain/body size relationship is much stronger in foragers than in age-matched non-foragers. It might well be the need for high-level navigational requirements that drives the substantial increase of neuropile volume even during the few days *Cataglyphis* is foraging within its desert habitat.

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