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AGE-DEPENDENT AND TASK-RELATED MORPHOLOGICAL CHANGES IN THE BRAIN AND THE MUSHROOM BODIES OF THE ANT *CAMPONOTUS FLORIDANUS*

WULFILA GRONENBERG, SILKE HEEREN AND BERT HÖLLDOBLER
Theodor Boveri Institut, Lehrstuhl für Verhaltensphysiologie und Soziobiologie der Universität, Am Hubland, D-97074 Würzburg, Germany

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Summary

Based on a brief description of the general brain morphology of *Camponotus floridanus*, development of the brain is examined in ants of different ages (pupa to 10 months). During this period, brain volume increases by approximately 20 % while the antennal lobes and the mushroom body neuropile show a more substantial growth, almost doubling their volume. In addition to the age-dependent changes, the volume of the mushroom body neuropile also increases as a consequence of behavioural activity associated with brood care and foraging. Foraging activity may lead to a more than 50 % additional increase in mushroom body neuropile volume. It is unlikely that the growth of mushroom body neuropile results from cell proliferation because no neurogenesis could be observed in adult ant brains.

Key words: ant, brain development, mushroom bodies, Kenyon cells, neuroplasticity, *Camponotus floridanus*.

Introduction

Comparative studies of phylogenetically closely related insect species have shown that species with relatively large adult individuals often exhibit more complex behavioural patterns, associated with the possession of more elaborate brains containing larger numbers of neurones, than species with smaller adults (Rensch, 1956; Neder, 1959; Rossbach, 1962; Cole, 1985). This general correlation between behavioural complexity and brain size is also reflected by the behavioural development of individuals. In hemimetabolous insects, the nervous system grows in every new larval stage, mainly by the addition of new sensory afferents. In holometabolous insects, the central nervous system is rebuilt and increases in size during pupal metamorphosis to provide the animal with the behavioural repertoire for reproduction.

While short-term changes in environmental conditions may result in modified neuronal activity, long-term environmental changes and behavioural modifications can also affect the morphology and size of a brain; synapses may be altered and additional neuronal processes or new neurones may develop. However, morphological changes in adult insect brains are much less obvious than those occurring during larval development or metamorphosis and, until recently, little was known about such changes.

Honeybees are particularly well suited for studying structural modification of the brain because during their adult life they undergo two major phases (nursing and foraging) that are distinguished by different sensory input and motor activities. In addition to earlier findings (Coss *et al.* 1980), recent research has addressed age- and experience-related changes in the brain of bees (Withers *et al.* 1993, 1995; Durst *et al.* 1994; Fahrbach *et al.* 1995). These studies showed that, while the overall brain volume remains almost constant, some brain compartments increase in size when bee nurses become foragers and leave the hive. These changes are particularly marked in the mushroom bodies, regions of neuropile concerned with higher associative functions and connected with learning and memory. All the evidence obtained from different research approaches on bees (e.g. Erber *et al.* 1980; Menzel, 1993) and *Drosophila melanogaster* (reviewed by Heisenberg, 1994) strongly suggests that the mushroom bodies play a key (although not exclusive) role in the neuronal control of adaptive behavioural modifications. Changes in the morphology of the mushroom bodies, which are correlated with behavioural changes in honeybees, are thought to be mediated by juvenile hormone (Withers *et al.* 1995).

Both bees and ants are known for their learning and memory capabilities and both show a rich behavioural repertoire which changes over time according to the tasks they fulfil within the cooperative system of the insect society (Hölldobler and Wilson, 1990). In addition, most ant species, like bees, exhibit a distinct age-polyethism, with younger workers performing tasks different from those of their older nestmates. However, ants generally live much longer and may take longer to mature than worker bees and, for this and for other reasons, the division of labour can be much more pronounced in ants than in bees.

Most of what we know about mushroom body function is derived from research on bees and *Drosophila melanogaster*. So far, only a few studies have attempted to correlate behavioural performance with brain morphology in ants (e.g. Vowles, 1964). In this paper, we address the question of how age and changes in long-term behavioural patterns can affect the morphology of the ant brain and of its subcompartments. To keep the subject simple, in this initial study we have excluded morphological caste differences and only compare morphologically indistinguishable sisters. We investigated the following questions. Does the volume of the brain or that of its subcompartments change with the age of the ant? If such a change occurs, how much is it affected by the tasks an ant has to perform (i.e. broodcare *versus* foraging)? Does such a change involve neurogenesis (the generation of new nerve cells) or does it result from sprouting and expansion of already existing neuronal processes?

Materials and methods

Animals and experimental colonies

Experiments were performed on *Camponotus floridanus* (Buckley). In order to exclude inter-colonial differences, all workers were taken from a single large colony collected near Tallahassee, Florida, USA, and reared and fed in the laboratory according to Carlin and Hölldobler (1986). From this stock colony, groups of marked workers (coloured with dots of gloss enamel paint; Revell Color) and brood were raised separately so that newly emerged workers could be identified and the age of all newly emerged adults would be known. Within colonies, the size of the ants may vary. While there is a size continuum, individuals can be ranked as minor or major workers. Unlike some other ant species, the size of the workers in *Camponotus floridanus* reflects more the condition and age of the colony than the task that an individual performs. Instead, these ants feature age polyethism so that, like honeybees, ants of different ages perform different tasks. In *C. floridanus*, ants stay inside the nest and care for the brood until the age of about 10 weeks, when they start to leave the nest and become foragers. To minimize inter-individual differences, all experiments were performed on minor workers (head width 0.6–0.8 mm).

To examine age-related differences and to separate them from the effects of experience normally gained during maturation, we formed artificial nurse groups of different age classes that did not have to leave the nest because they were provided for by older, paint-marked foragers. The artificial nurse groups had neither brood nor a queen to care for (thus minimizing their 'experience') and hence, literally, had nothing to nurse. We will refer to the broodless workers as 'idles'. Histology was performed on 'idles' of different age classes: 1 day, 10 days and 4, 6 and 8 months. For comparison, pupae of different developmental stages were also examined. The stages were judged by the colour of their eyes: unpigmented, light red, dark red and black.

To estimate the effect of the presence of brood, two groups of workers were supplied with eggs and larvae from the main colony. One of these groups was examined at the age of 1 month when they had been involved in brood care for some time but had not yet left the nest. They were thus comparable to normal nurses. To estimate the effect of the presence of brood on older workers, brood was added to a forager group 3 months prior to their examination, which took place at the age of 10 months. Ants from this group had thus experienced brood as well as foraging activities.

In order to assess changes related to foraging activity, 'idles' and nurses that had never left their nests were compared with foragers from the same age group that had experienced the foraging arenas. Since, under natural conditions, *C. floridanus* workers do not leave the nest before the age of 10 weeks, 'precocious' foragers were produced by depriving a group of newly eclosed workers of the help of more mature foragers. In order to increase their need for food, some groups were also supplied with brood which they had to feed. This stimulated a few workers to forage at the age of about 1 month. It was thus possible to study foragers of the age of 1, 4, 6, 8 and 10 months. Among these, the 1- and 10-month-old foragers had experience with brood whereas the other age groups did not.

Histology

Animals were decapitated, the head capsules cut open and the brains dissected out under saline (in mmol l^{-1}: 127 NaCl, 6.7 KCl, 2 $CaCl_2$, 4.8 TES, 3.5 sucrose; pH 7.0). Brains were then fixed for 2–3 h (2.5 % glutaraldehyde in 0.1 % cacodylate buffer), rinsed in buffer for several hours, impregnated in 1 % osmium tetroxide for 2 h, rinsed in water for 2 h, intensified in saturated aqueous ethylgallate, dehydrated in ethanol, plastic-embedded (Fluka Durcupan) and serially sectioned at 10 μm. Sections were then counterstained with Methylene Blue.

Fig. 1. The brain and the mushroom bodies of *Camponotus floridanus*. (A) Scanning electron micrograph of the brain (dorsal view with respect to the head capsule); boxed area indicates region sketched in B; *O*, optic nerve; *PC*, protocerebrum; *Al*, antennal lobe; *An*, antennal nerves. (B) Schematic drawing of the left half of the brain reconstructed from 15 μm serial sections and showing the antennal lobe, the optic lobe medulla (*M*) and lobula (*L*) and the mushroom body composed of the lateral (*Cl*) and medial (*Cm*) calyces, the peduncle (*P*), and the α and β lobes. The trajectory of a Kenyon cell is sketched within the mushroom body, and the hatched area indicates the Kenyon cell bodies (*Kcb*) around the calyces. (C) Vertical section through the median calyx of a mature worker (Nomarski optics) showing neuropile (*N*) wrapped by layers of almost uniformly sized Kenyon cell bodies. (D) Comparable section (calyx neuropile and cell bodies) from a second-stage pupa (Auramin-O-stained fluorescence micrograph); note the larger cell bodies (Kenyon cell neuroblasts *Kn*) in the centre of the calyx. (E,F) Scanning electron micrographs of a desheathed median calyx showing the neuropile and Kenyon cell body rind (cell bodies partly removed); the area outlined in E indicates the approximate region enlarged in F. (G) Calyx neuropile and cell bodies of a second-stage pupa (Orcein-stained vertical section) revealing dividing neuroblasts (mitotic chromosomes; metaphase spindle indicated by arrowhead). Scale bars, 100 μm in A, B; 25 μm in C, D, E, G; 10 μm in F.

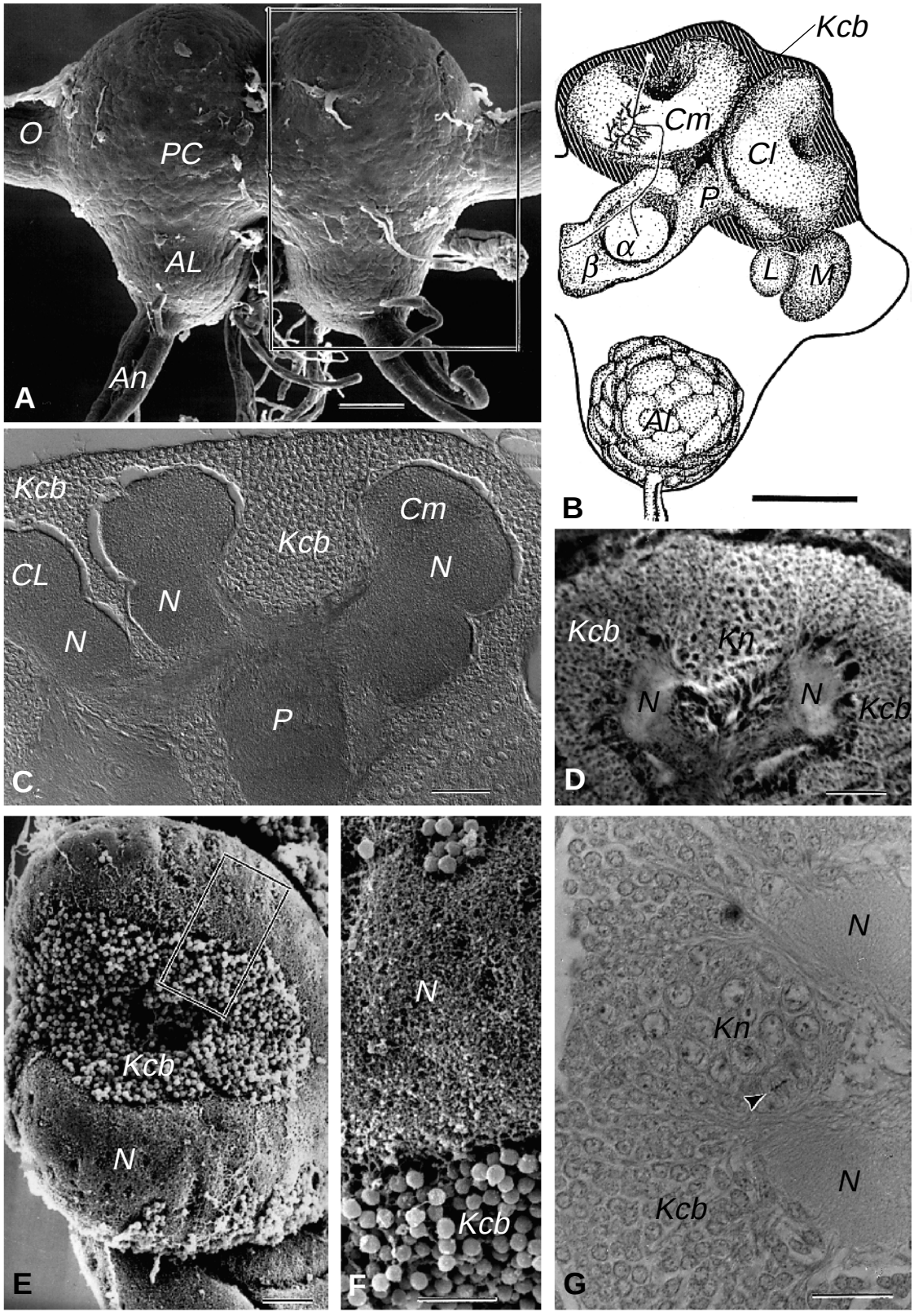

Fig. 1

To compare larval and adult tissue and to identify chromosomes, brains were fixed in Carnoy's fixative, dehydrated and paraffin-wax-embedded, serial-sectioned at 5 µm, dewaxed and stained with either 1 % acetic Orcein (Fig. 1G) or a fluorescent Feulgen analogue (0.25 % Auramin-O in SO_2-saturated water; Fig. 1D).

The brain morphology was also examined by scanning electron microscopy of glutaraldehyde-fixed and critical-point-dried specimens using a Zeiss DSM 962 scanning electron microscope (Fig. 1A). To reveal cell bodies and neuropile (Fig. 1E,F), some specimens were treated with trypsin prior to fixation and were then treated with 6 mol l^{-1} hydrochloric acid to dissolve dispensable connective tissue prior to critical-point drying (Matsuda and Uehara, 1984).

Evaluation

Microscopic images were video-recorded and digitally stored and evaluated by a computer equipped with a video card and appropriate software (Screen Machine, Fast Electronic) adapted for volume measurements (courtesy of Reinhard Wolf). From each microscopic section, the following regions were traced on the computer screen and the surface calculated: the entire brain, the antennal lobe, the medulla, the lobula and the mushroom body subcompartments (α lobe, β lobe, peduncle, calyces and cell body rind; indicated in Fig. 1B). On the basis of the section thickness, the volume of the respective structures was then calculated.

The density and size of Kenyon cell bodies were determined for nurses of different ages by counting cell body numbers in a 50 μm×50 μm square in the centre of the calyx cup and by measuring the thickness of cell body layers above the rim of the calyx.

Volumetric data obtained from different age classes were submitted to an analysis of variance (ANOVA), and the variances were then compared using the Student–Newman–Keuls test to establish significant differences between groups. To assess experience-related effects, data from foragers and nurses with brood were compared with data for 'idles' of the same or similar age class using a *t*-test. The *t*-test was also used to compare cell numbers and cell layer widths of different age classes. In each age class and treatment, 4–9 individuals were processed histologically and evaluated. A total of 66 individuals were examined.

Results

The adult brain

As a basis for the subsequent comparison of different experimental groups, some of the major morphological features of the brain of *Camponotus floridanus* must be described (Fig. 1). Despite large differences in head size of major and minor workers, the brains of large majors are only about 8 % bigger than those of minors. While in minors the brain makes up more than half of the head volume, in majors the brain makes up only a small fraction of the entire head volume (Gronenberg, 1996; the absolute brain volume is about 0.06 mm^3; Fig. 2).

The optic lobes, the central complex, the mushroom bodies and the diffuse (aglomerular) neuropile together constitute the protocerebrum (Fig. 1A). Unlike other Hymenoptera, in which the large optic lobes fill up the space between the eyes and the central brain, in most ants including *Camponotus floridanus* the optic lobes are very small (Fig. 1B) and the brain is connected to the small eyes by relatively thin optic nerves (Fig. 1A). The medulla, the second and largest optic ganglion, makes up less than 2 % of the total brain volume, and the lobula comprises only about 0.5 % (Fig. 3). The first optic ganglion, the lamina, has not been evaluated separately; it is larger than the lobula but smaller than the medulla.

While the optic lobes are small, the most prominent structures of the ant brain, the mushroom bodies, are very large and make up almost 40 % of the entire brain volume (Kenyon cells and neuropile in Figs 3, 4). As in other Hymenoptera (Kenyon, 1896; Hanström, 1928; Mobbs, 1982), each mushroom body is composed of two cup-shaped structures (the median and lateral calyces; Fig. 1E), each giving rise to a stalk or peduncle (Fig. 1B,C). The pedunculi fuse and form the perpendicularly arranged α and β lobes (Fig. 1B). The neuropile of the calyces is surrounded by an accumulation of cell bodies (Fig. 1B–F). These are the somata of the neurones that constitute the mushroom body, the Kenyon cells. As sketched in Fig. 1B, most Kenyon cells have their dendrites in one calyx and project bifurcating axons through the peduncle into the α and β lobes. Although there are slight differences in size between groups of Kenyon cell bodies (compare somata within the calyx cup with those outside the calyx in Fig. 1C), for practical purposes they will here be considered to be homogeneous in size. This enables them to be distinguished from the much larger proliferating precursors of Kenyon cells found in ant larvae (shown in Fig. 1G) and referred to below. In Figs 3 and 4, the volume occupied by all the Kenyon cells has been compared with the total neuropile volume of the mushroom bodies consisting of the calyces, the pedunculi and the α and β lobes. The neuropile occupies approximately twice as much space as the Kenyon cell bodies (Fig. 3).

The antennal lobes form another prominent structure of the ant brain (Fig. 1B). They receive the sensory afferent terminals of olfactory receptors located on the corresponding antenna. In *Camponotus floridanus*, the antennal lobes are subdivided into about 200 spherical compartments, the antennal glomeruli. They make up about 9 % of the total brain volume of *Camponotus floridanus* (Fig. 3). Owing to their glomerular organization, the antennal lobes can readily be discriminated from surrounding tissue. Together with the equally distinct neuropiles, the optic lobes and the mushroom bodies, their volume was individually assessed and compared in ants of different age and experience.

In contrast, the dorsal lobes and the diffuse protocerebral lobes cannot easily be distinguished from adjacent neuropile. In order to avoid ambiguities, they have not been evaluated separately. Similarly, the central complex has not been assessed independently in the present investigation. While it can be distinguished from surrounding tissue, the central complex is a primitive neuropile in evolutionary terms and is not believed to be involved in learning or memory (Homberg, 1987). Accordingly, the central complex, the diffuse

protocerebral lobes and the dorsal lobes have been omitted from Fig. 1B. However, their volume contributes to the measurement of total brain volume referred to in Figs 2–4.

Age-related changes

While in the previous section mean values for the volume of the brain and its different compartments are given, these measurements are not constant; they change with the development or age of the ants. This is demonstrated for similarly sized nurses of different age groups in Fig. 2. The smallest brains were found in pupae and in newly emerged workers (1 day old). Owing to the relatively small sample sizes, no mean values have been calculated for Figs 2 and 3 and the individual data are shown instead. However, the statistical analysis reveals that these young ants have significantly smaller brains than older ones. This apparent increase in brain size by 15–20 % is, of course, not associated with any growth of the ants, such as might be the case in hemimetabolous insects. The potential cause of this increase will be addressed later.

Does the age-dependent increase in brain volume uniformly affect all brain structures? Fig. 3 shows the volume changes of the different brain subcompartments over time. If the brains were growing homogeneously, all the graphs would run parallel to the abscissa. However, this is not the case for any of the structures. In the optic lobes (lobula and medulla), the relative volume appears first to decrease and then to increase. However, we do not know whether our data reflect the real development of optic lobes. The absolute sizes of the medulla and lobula are so small that technical errors, such as those that could result from the manual tracing technique, may substantially contribute to these values.

The same does not apply for the antennal lobes and the mushroom bodies. These are large structures, and the data show obvious trends, with a significant increase in the volume of the antennal lobes and of the mushroom body neuropile between 10 days and 4 months. Hence, superimposed on the

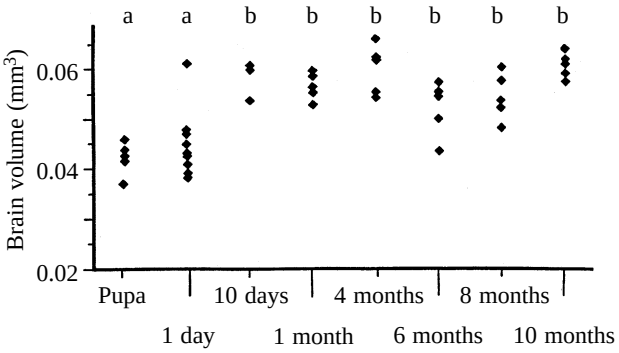

Fig. 2. Age-dependent differences in total brain volume of similarly sized nurses of different age classes ranging from the last pupal stage (pupa) to the age of (10 months). Each symbol represents one ant; different letters (a, b) denote significant differences between classes (P=0.05); ages marked by the same letter are not statistically significantly different (Student–Neuman–Keuls test; P<0.05).

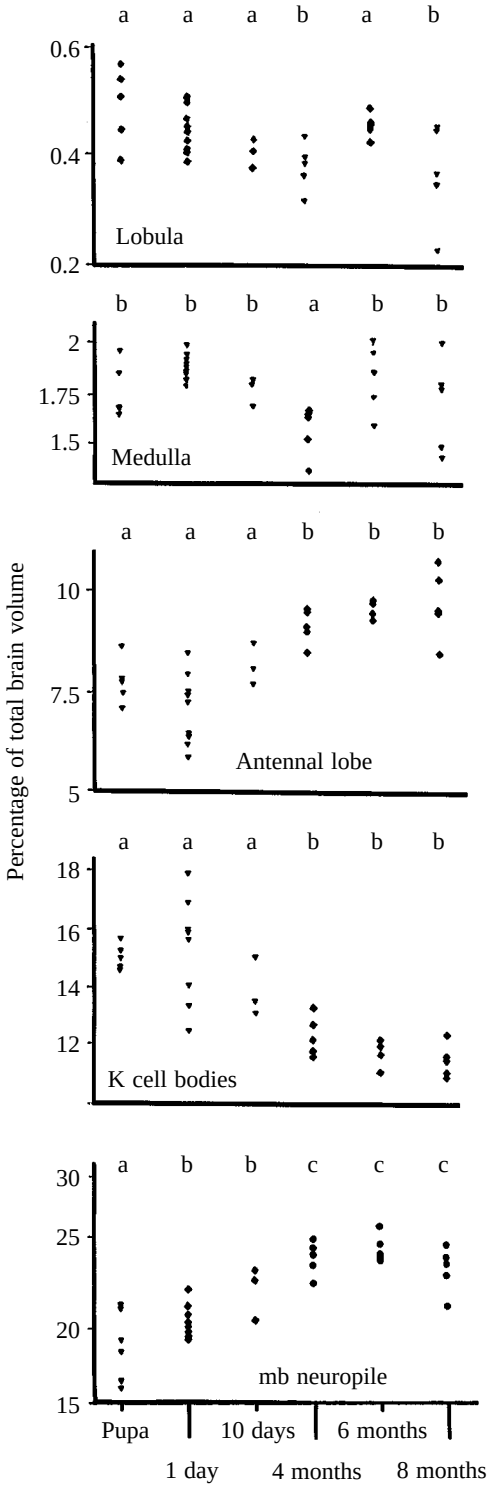

Fig. 3. Age-dependent differences in relative brain volume (ordinate; in %) of different brain regions (lobula, medulla, antennal lobe, Kenyon (K) cell body layer and neuropile of the mushroom body, mb) of similarly sized ‘idles’ of different age classes ranging from the last pupal stage (pupa) to the age of (8 months). Each symbol represents one ant; significant differences between mean values (Student–Neuman–Keuls test; P=0.01) within a panel are distinguished by different letters (a–c) and by different symbols. Note that the ordinate scaling is different for each block.

overall increase in brain size is a change in the proportion of the different brain compartments, among which the mushroom body neuropile shows the largest growth. Over a period of 6 months, its volume increases by approximately 30 % relative to the total brain volume or, in absolute terms, after about 6 months the mushroom body neuropile of 'idles' has doubled its volume compared with the last pupal stage (as demonstrated below, the increase in 'normal', undeprived workers is even larger). The absolute volume of the antennal lobes increases by about 90 % from 1-day-old adults to 10-month-old adults (note that Fig. 3 depicts the relative, not the absolute, volume). Interestingly, the growth of mushroom body neuropile is not accompanied by a comparable increase in Kenyon cell body volume. Instead, the rind of Kenyon cell somata around the calyces increases by only about 15 %, which leads to an actual decrease in its relative volume (since the entire brain volume increase is greater than the increase in volume of the Kenyon cell bodies; Figs 2, 3).

Task-related changes

We have also investigated whether certain behavioural situations, such as the presence or absence of brood and foraging activity, could affect the developmental patterns of the brain, independently of age-related changes.

Age-dependent changes in brain morphology were established using 'idles' (see Materials and methods). Lacking both brood and a queen, and fed by older workers, these ants had no motivation to engage in any significant activity. They lived within their dimly lit nest chambers without any need for much movement for months. Accordingly, their sensory input and behavioural activity were very limited.

The two age classes (1 month and 10 months) that received brood showed a more normal repertoire of activity. They tended and fed the larvae and thus had more sensory experience and showed more motor activity. These differences are reflected by the increased size of the mushroom body neuropile found in the 1-month-old and 10-month-old nurses compared with the 4-, 6- and 8-month-old 'idles' (Fig. 4). Both groups (open triangles in Fig. 4A) show significantly larger mushroom body neuropile volumes than their brood-care-deprived sister groups (open circles in Fig. 4A) at the age of 4, 6 and 8 months (P=0.01; statistical significance of differences between 'normal' nurses and 'idles' not indicated in Fig. 4A). Other brain structures, including the volume occupied by Kenyon cell bodies (Fig. 4B; no statistically significant differences between open triangles and open circles in any age group) are not affected by the presence of brood in the nest chambers.

The effect of brood care on nurse mushroom body development appears to be obvious. We subsequently analyzed the influence of foraging activity in greater detail, because foragers experience a greater variety of visual, olfactory and tactile stimuli in the foraging arenas and exhibit more motor activity.

When comparing nurses and foragers, no significant differences in shape and volume of the optic lobes or the antennal lobes or in total brain volume were found in any age class, and these structures are therefore not represented in Fig. 4. The relative volume occupied by the Kenyon cell bodies showed, however, significant differences between foragers and 'idles' for 6-month-old ants and between foragers and nurses at the age of 10 months, but the trend was not consistent for the 8-month-old workers (Fig. 4). Kenyon cell body volumes (Fig. 4B) are derived from the same foragers as those used to produce Fig. 4A. The minimum in mushroom body neuropile volume of the 8-month-old foragers matches the peak of their Kenyon cell body volume and may thus reflect fluctuations due to our small sample size.

As was the case for the presence of brood, a prominent effect of previous experience was only found in the development of

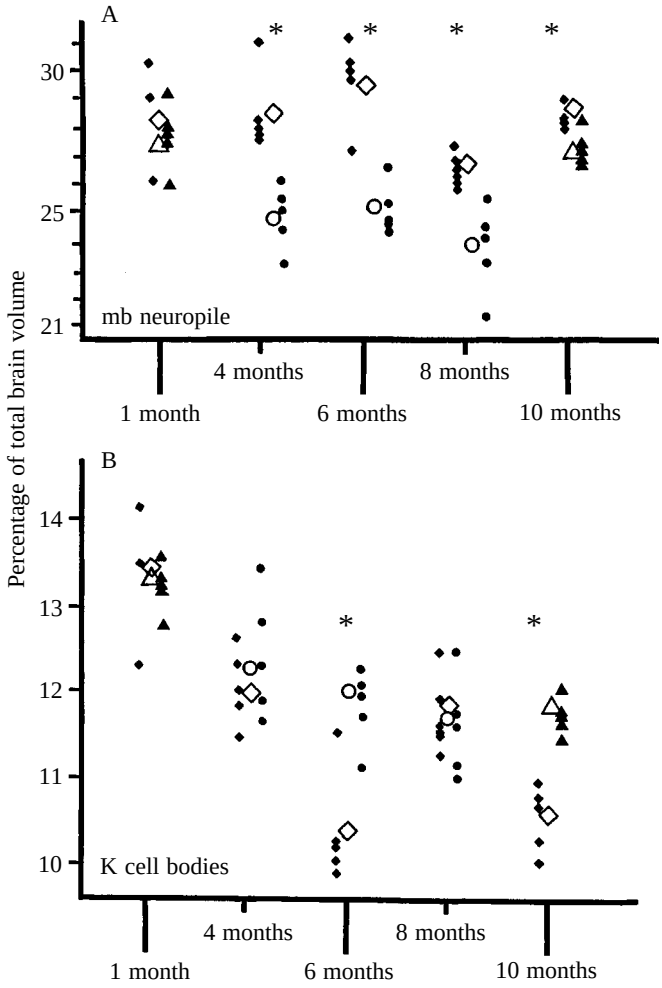

Fig. 4. Experience-dependent differences in the volume of the mushroom body (mb) neuropile (A) and Kenyon (K) cell body rind around the calyces (B) relative to overall brain volume (ordinate; in %). At each age (age classes from 1 month to 10 months), diamonds indicate foragers, circles at 4, 6 and 8 months indicate 'idles' and triangles at 1 and 10 months indicate nurses. Filled symbols denote single ants, open symbols denote the mean of the respective group; asterisks indicate significant differences between mean values of foragers and nurses of the respective age class (Student–Neuman–Keuls test; P=0.01).

mushroom body neuropile of foragers (Fig. 4A). In 1-month-old foragers, the neuropile is only slightly, and not significantly, larger than that of nurses (the data derived from nurses are the same as those in Fig. 3), while in the older ants (4–10 months) the difference is more pronounced and statistically significant (Fig. 4). However, the precocious foragers (1-month-old) are compared with 'normal' nurses (with brood care experience) while the 4-, 6- and 8-month-old foragers are compared with 'idles' (open circles in Fig. 4A). When compared with either younger (1 day, 10 days) or older (4–8 months) 'idles', the precocious foragers do show statistically significant differences in mushroom body neuropile volume.

In Fig. 4, the differences between mushroom body neuropile volume of foragers and nest workers appear greater for the 4- to 8-month-old workers than for the 1- and 10-month-old groups because, in these age classes, foragers are compared with 'idles' (open circles) rather than normal nurses (open triangles). Hence, these large differences for the 4- to 8-month-old test groups reflect both the effect of brood care and that of foraging activity. The effect of foraging activity alone can best be appraised from the smaller, yet statistically significant, difference for the 10-month-old group. It thus appears that, under natural conditions, brood care and foraging activity both contribute significantly to the development of the mushroom bodies.

Changes in the Kenyon cell body rind

After the first day of adulthood, the absolute volume occupied by Kenyon cell bodies remains almost constant, even though their relative volume decreases (Figs 3, 4). Comparing the number of Kenyon cell bodies in selected regions, significant differences (P=0.01) could be established only between pupae and ants more than 4 months old. In the older ants, the somata were packed more tightly (about 20 % more somata per volume) and the layer of cell bodies surrounding the calyces was significantly thinner (P=0.01). This is explained by the fact that the calyx neuropile grows much more extensively than the cell bodies and pushes the surrounding cell body rind outwards, thereby flattening the layer of somata. Thus, the relative decrease in Kenyon cell body volume appears to be because these cells do not continue to grow while the rest of the brain does. This finding also suggests that the disproportionate growth of the mushroom body may not be caused by cell proliferation.

To discount the possibility of Kenyon cell proliferation in adult ants, Feulgen- and Orcein-stained brains were examined to detect proliferative cells (neuroblasts) or mitotic cell stages. Both were found in young larvae (note the large cells in the centre of the calyx in Fig. 1D,G and the metaphase spindle with attached chromosomes indicating mitotic activity in Fig. 1G). However, no signs of cell proliferation were found in any ant older than the second pupal stage. We therefore suggest that neurogenesis is complete in young pupae and no new Kenyon cells that might contribute to mushroom body growth arise in adult ants. The absence of neurogenesis in adult ants or bees cannot, however, be definitely proved using the techniques employed in this paper.

Discussion

Our results show (1) that the overall brain volume during adult life (from the first day after eclosure to the age of 10 months) increases by 15–20 %; (2) that not all the brain compartments grow at the same rate, some (e.g. the optic lobes) appear to be fit for their function at birth, while others (the antennal lobes and mushroom bodies) continue to grow faster than the rest of the brain; (3) that the size of the mushroom bodies depends not only on the age but also on the behavioural activities of the ants; and (4) that neurogenesis is not a likely cause for brain growth and reorganization.

The significance of these structural adaptations of the brain and its components will become evident when they are compared with brain development in other insects.

Brain development

In contrast to hemimetabolous insects, in holometabolous insects the brain develops largely or entirely during the pupal stage. Accordingly, most previous developmental studies were focused on larval and pupal insects and did not closely examine the adult stages. However, at least two previous studies concerned with brain development in adult holometabolous insects revealed that in beetles the brain volume increases significantly (by about 50–100 %; Rossbach, 1962; Bieber and Fuldner, 1979). More recent, thorough studies have shown that, even in *Drosophila melanogaster* (which are relatively short-lived insects whose behaviour is not as complex as that of the social Hymenoptera), brain size can change during adulthood, depending on subtle environmental features such as population density (Heisenberg *et al.* 1995).

In adult honeybees, the overall brain volume does not change, but some of the brain subcompartments change their size depending on the age, as well as on the experience, of the animal (Withers *et al.* 1993). In agreement with our present results, the mushroom bodies play the most important role in these developmental changes in the brain of beetles, flies and bees (Rossbach, 1962; Bieber and Fuldner, 1979; Technau and Heisenberg, 1982; Withers *et al.* 1993, 1995; Durst *et al.* 1994; Fahrbach *et al.* 1995; Heisenberg *et al.* 1995). This suggests that the mushroom bodies may be important for adaptations to a changing environment or for adaptations to variations in individual tasks. Comparative neuroanatomical studies on many different arthropod species have also shown a positive correlation between the morphology of the mushroom bodies and the complexity of the behaviour of the respective taxon (e.g. Hanström, 1928; Rensch, 1956; Neder, 1959; the term 'behavioural complexity' is used quite vaguely in the literature). This trend culminates in the social Hymenoptera (Hanström, 1928). It is thus not surprising that in this study we found the largest volume changes in the neuropile of the mushroom bodies, which are the structures most closely connected with the adaptability and behavioural complexity of the ant.

The basis for postlarval mushroom body growth

Our results show that behavioural changes are correlated with an increase in mushroom body size. Comparative studies on different hemimetabolous insect species indicate that an expansion of mushroom body volume arises from an increase of the number of Kenyon cells (Rensch, 1956). This is also the case in the ontogeny of hemimetabolous insects: in cockroaches, new Kenyon cells are produced with each new developmental stage and, in addition, their fibre volume shows large increases (Neder, 1959). Similarly, work on adult crickets shows that the increase in mushroom body size is due to neurogenesis of Kenyon cells (Cayre *et al.* 1994). These findings imply that, in hemimetabolous insects, a number of neuroblasts persist in the adult stage and enable the animal to produce new Kenyon cells in order to adapt to changing environmental or behavioural requirements.

However, in holometabolous insects, postlarval growth has so far only been found in the mushroom bodies of Coleoptera, Diptera and Hymenoptera. Rove beetles and weevils show a significant increase in mushroom body volume during adult life (Rossbach, 1962; Bieber and Fuldner, 1979), which coincides with the development of brood-care behaviour. Depending on rearing conditions, the volume of mushroom body fibres in *Drosophila melanogaster* changes by about 20 % (Heisenberg *et al.* 1995). Kenyon cell proliferation may be involved but only plays a minor role in this phenomenon (Technau, 1984). In adult honeybees, the growth of Kenyon cell dendrites has been demonstrated directly from Golgi-impregnated material (Coss *et al.* 1980). Unlike hemimetabolous insects and Diptera, indications for neurogenesis of Kenyon cells have only been found in pupae and not in imagos of bees (Fahrbach *et al.* 1995) and of the primitive ant *Mesoponera caffraria* (Masson, 1970). While final verification can only be established by labelling the DNA of replicating cells, our present findings support the idea that, in adult Hymenoptera, the increase in the size of the mushroom body results exclusively from the growth of cell processes (axons and dendrites) and is not a consequence of neurogenesis.

Differences between bees and ants

Despite the fact that most of our data correspond to the recent findings from honeybees (Withers *et al.* 1993, 1995; Durst *et al.* 1994; Fahrbach *et al.* 1995), a difference seems to exist between bees and ants. In bees, the overall volume of the brain remains constant throughout adult development, whereas in ants the entire brain also increases in size. There is a second difference between the two groups: in the bee, the Kenyon cell body volume actually decreases with age, whereas in the ant it only decreases relative to the total brain volume. The increase in the brain volume of young ants may indicate that, at eclosion, they are less mature than bees (it takes *Camponotus floridanus* 3 months before they start foraging while honeybees fly and forage after 3 weeks).

Volumetric measurements show that the mushroom bodies of adult worker bees increase in size when foraging activity occurs and as a consequence of juvenile hormone treatment (Withers *et al.* 1993, 1995; Durst *et al.* 1994). This latter finding (Withers *et al.* 1995) indicates that, in the honeybee, foraging activity *per se* is not the reason for the growth of the mushroom body. Instead, physiological changes, such as an increase in juvenile hormone titre with a possible subsequent expansion of the mushroom bodies, may be induced by the additional visual stimuli that foraging bees experience when leaving the hive. This concept is strengthened by an interesting finding: the particular subcompartment of the mushroom body calyx which receives visual input, the collar (Mobbs, 1982), shows the largest volume change correlated with foraging activity of bees (Durst *et al.* 1994; Withers *et al.* 1995). It will be interesting to investigate whether similar differences in the development of mushroom body subcompartments specifically associated with vision also occur in ants. We suspect that this will not be the case in most ants since, while we have not examined this aspect specifically, it appears that in *Camponotus floridanus* (and probably most other ants) the collar region of the mushroom bodies is much smaller and less distinct than that of honeybees (Gronenberg, 1996). This qualitative finding reflects the fact that, in contrast to bees, vision plays only a minor role in the life of most ants. Accordingly, we expect that olfactory, rather than visual, experience will have the greatest impact on mushroom body growth. By the same token, it seems likely that, unlike bees, sensory experience in ants will affect the prominent lip region of the mushroom bodies where olfactory information is processed (Gronenberg, 1996).

Some of the questions only very recently addressed in bees, such as the caste-specific effects of experience on brain morphology and the involvement of juvenile hormone in mushroom body plasticity (Withers *et al.* 1995), have not been studied in ants. Since ant workers generally live much longer than bees, they can be subjected to different experimental regimes (which are expected to affect postlarval brain development) for extended periods. They can be reared under deprived conditions or, alternatively, be confronted with environments much more enticing and richer in stimuli than those tested in the present approach. Moreover, ants with striking morphological worker castes (minors and majors) may behave differently and may live in different sensory environments (e.g. leaf-cutting ant majors work outside cutting and transporting plant material or defending the colony, while small minors live permanently in the dark nest cultivating fungus). Yet despite their differences, within a colony they are sisters and genetically very similar. Ants thus offer a unique opportunity to assess the effects of a wide range of behavioural and morphological differences without the problems arising from genetic variability when comparing different species. For these reasons, we think that much can be learned about neuronal plasticity and the adaptation of the nervous system by studying the brain of ants. The present study is a first step in this direction.

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