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Volume Changes in the Mushroom Bodies of Adult Honey Bee Queens

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The volume of the mushroom bodies of the brains of honey bee queens (*Apis mellifera*) was estimated using the method of Cavalieri. Tissue sampled was obtained from queens in five different behavioral and reproductive states: 1-day-old virgin queens, 14-day-old virgin queens, 14-day-old instrumentally inseminated queens, 9- to 13-day old naturally mated queens, and 5-month-old naturally mated queens. There were significant volume changes within the mushroom bodies during the first 2 weeks of adult life. The volume occupied by the somata of the intrinsic neuronal population (Kenyon cells) of the mushroom bodies decreased by approximately 30% and the volume of the neuropil of the mushroom bodies increased between 25 and 50%. These volume changes are strikingly similar to those previously reported to occur for worker honey bees switching from hive activities to foraging (Withers, Fahrbach, & Robinson, 1993). However, in this study they were found even in queens that had no flight experience. In addition, queens exhibiting these volume changes were found to have low blood levels of juvenile hormone, while previous studies have shown that foraging worker honey bees have high hormone levels. These results suggest that some aspect of behavioral development common to both the queen and the worker castes is fundamental to protocerebral volume changes early in adulthood in honey bees. If juvenile hormone regulates this process, results from queens suggest that it may play an organizational role. © 1995 Academic Press, Inc.

In a typical honey bee colony, newly emerged (1-day-old) adult worker bees remain near their natal cell; workers 1 to 3 weeks of age rear brood, build comb, and tend the queen; and workers older than 3 weeks of age forage for pollen and nectar (Winston, 1987). This stereotyped division of labor by age is referred to as "age polyethism." We have demonstrated that this capacity for behavioral change in adult worker bees is correlated with anatomical plasticity in the honey bee brain. The two compartments of the mushroom bodies undergo simultaneous volume changes during adult behavioral development: a decrease in the volume occupied by the somata of the intrinsic cell population (the Kenyon cells) of the corpora pedunculata or mushroom bodies is accompanied by an increase in the volume occupied by the associated neuropil (Withers, Fahrbach, & Robinson, 1993). In colonies with a normal distribution of workers from 1 to 40 days of age, this decrease in the volume occupied by the Kenyon cell somata and increase in the volume of neuropil is strongly associated with foraging. One-day-old bees have a neuropil-to-neuronal somata volume ratio for the mushroom bodies of approximately 1:1; in foragers, this ratio is approximately 2:1 (Withers et al., 1993).

Age polyethism masks a striking behavioral flexibility that can be revealed by manipulations of colony population structure: a shortage of foragers will result in accelerated development of foraging, while a shortage of nurse bees will delay the transition to foraging (Robinson, 1992). For example, in colonies composed of a single cohort of young worker bees, some younger bees will forage (Robinson, Page, Strambi, & Strambi, 1989). The naturally occurring volume changes within the mushroom bodies can be accelerated by this manipulation so that precocious foragers 4 to 7 days of age show the configuration of normal-aged foragers typically 3 weeks of age or older (Withers et al., 1993). Thus, volume changes within the mushroom bodies are strongly associated with the performance of foraging in worker bees.

These findings suggest that an endocrine signal may trigger volume changes within the mushroom

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bodies. Juvenile hormone is known to regulate age polyethism in worker honey bees (reviewed by Robinson, 1992). Low blood titers are associated with activities performed in the hive and high titers with foraging (Robinson et al., 1989). Treatment of 1-day-old bees with juvenile hormone induces precocious foraging (Robinson, 1987). It is possible, therefore, that the anatomical plasticity of the mushroom bodies is controlled not solely by experience, but also by endocrine status.

The different castes resident within a honey bee colony permit us to broaden our consideration of the relationship between brain and behavioral plasticity beyond an analysis based solely on age polyethism in the worker bee. In the present study, we have explored the volumes of somal and neuropilar compartments of the mushroom bodies of queen honey bees. Although both workers and queens fly and share the same hive, the life history of a honey bee queen bears little resemblance to that of her daughter workers. At the time the queen emerges from her cell as an adult, she is already physically and behaviorally differentiated from workers (Ribbands, 1953; Butler, 1974; Winston, 1987). She has a shorter proboscis than worker bees, does not have specialized pollen collecting structures, and lacks the glands workers use to build comb and rear brood. During the first few days of adult life, a virgin queen remains in the hive. Honey bee queens become sexually mature at 5 or 6 days after emergence (Winston, 1987). Mating can occur at this time, although it may be delayed during periods of bad weather until as late as 14 days postemergence (Janscha, 1771; Oertel, 1940). The delay results from the fact that mating takes place outside the colony, during flight.

To mate, a queen will typically fly 2–3 km away from her colony. Once she has located a drone (male honey bee) congregation site, she will mate numerous times over a period of several days. Sperm obtained at these matings enters a sperm storage organ associated with the reproductive tract called the spermatheca; the sperm remain viable for years in this storage organ, and the queen will not mate again after her nuptial flights (Ribbands, 1953; Butler, 1974; Winston, 1987).

A queen bee may live and remain the colony's sole reproductively active female for several years. She does not fly after she mates, except possibly to leave the hive once with a reproductive swarm. The queen's longevity further differentiates her from the worker bees in the colony, as workers typically live approximately 2 months (Winston, 1987).

As in our earlier studies of the brains of worker bees, we compared volumes of the somal and neuropilar compartments of the mushroom bodies during the normal processes of behavioral development. Additionally, we have determined the importance of natural mating (and the flight that makes mating possible) by comparing the mushroom bodies of normally mated queens with unmated and instrumentally inseminated queens. The first manipulation permits us to detect volume changes in the mushroom bodies that occur independently of flight and mating; the second procedure distinguishes the effects of the presence of sperm in the sperm-storing spermatheca from the performance of mating behavior. We also studied the mushroom bodies of mature laying queens several months older than all of the other groups.

In contrast to the worker honey bee (Fluri, Lüscher, Wille, & Gerig, 1982; Robinson, Strambi, Strambi, Paulino-Simoes, Tozeto, & Negraes Barbosa, 1987; Robinson et al., 1989), the juvenile hormone profile throughout the life of a queen has not been determined. One intriguing exception to this is that mated queens older than two months of age are known to have very low levels of juvenile hormone (Fluri, Sabatini, Vecchi, & Wille, 1981; Robinson, Strambi, Strambi, & Feldlaufer, 1991); workers, as stated above, have high juvenile hormone levels when they are older. To gain further insight into the possible role of juvenile hormone in regulating the volume of the mushroom bodies, we performed hormone measurements on queens in parallel with the brain analyses.

METHODS

Queen Bees

Honey bee queens were reared according to standard commercial procedures at the Bee Research Facility of the University of Illinois at Urbana–Champaign (Laidlaw, 1977). Newly emerged adult virgin queens ($n = 12$) were collected from queen cells placed in an incubator. The brains of some newly emerged virgin queens were dissected within 4 h of emergence from their cells. Other newly emerged queens ($n = 8$) were each introduced into a colony and allowed to follow the normal course of behavioral development, including mating flights. These queens were checked once daily and were collected on the day they were first observed laying eggs in brood comb (mean age at dissection, 11 days). Two additional groups of newly emerged queens were placed in a "queen bank" until they were collected. [A queen bank is a colony designed

TABLE 1
Descriptions of Queen Bees Used in Volume Studies of the Mushroom Bodies

Group	n	Age	Mated?	Source colony[a]	Description
Virgins	12	2–4 h "1 day"	No	3 from Colony 13 9 from Colony 19	Emerged into a glass vial, in incubator
Banked	8	13–15 days	No	2 from Colony 13 6 from Colony 19	Emerged into a glass vial in incubator; stored for 2 weeks in a queen bank
Mated	8	9–13 days	Yes	Colony 13	Mated naturally; collected on the first day eggs were laid
Inseminated	10	13 days	I.I.[b]	Colony 13	Instrumentally inseminated; stored in a queen bank
Old laying	6	5 months	Yes	Unknown	Mated naturally in early May 1992; still laying when collected in September 1992

[a] Queens from the same colony are either super sisters (genetic relatedness of 0.75) or half sisters (genetic relatedness of 0.25). Queens from different colonies are unrelated.
[b] Instrumentally inseminated with semen from a single drone (Laidlaw, 1977).

for the storage of queens. The queens are placed in small individual cages within an otherwise queenless colony composed of a higher than usual proportion of worker bees less than a week of age. Younger workers tolerate foreign queens and care for them better than do older workers (Laidlaw, 1977).] The first of these banked queen groups (n = 8) was simply confined to the queen bank for 13–15 days before dissection. Queens in this group never flew, never mated, and never left the queen bank. The second group (n = 10) was also held in the queen bank and also never flew and never mated. These queens, however, were instrumentally inseminated on Day 10, each with semen from a single different drone bee (Laidlaw, 1977). A final group (n = 6) consisted of normally mated queens living in their own colonies. These individuals were collected at 5 months of age and were actively laying eggs at the time of collection. These groups are referred to in turn as newly emerged virgin queens, mated queens, banked queens, inseminated queens, and old laying queens. Table 1 summarizes the characteristics of these groups, including age at brain dissection and genetic relatedness.

Blood Samples, Tissue Preparation, and Histology

Queen bees were immediately placed on ice at the time of collection at the Bee Research Facility. Transit time to the laboratory was less than 1 h. Chilled bees were bled by insertion of an insect pin into the neck membranes, and hemolymph (blood) was collected into a calibrated capillary tube. Approximately 3 to 8 μl of hemolymph was removed at this time for radioimmunoassay of juvenile hormone, as described below. Brains were removed from the head capsule, rinsed in a bee saline (Huang, Robinson, Tobe, Yagi, Strambi, Strambi, & Stay, 1991), and immediately fixed by immersion into alcoholic Bouin's fixative. Brains were fixed at room temperature overnight, dehydrated in ethanol, cleared in toluene, and infiltrated with Paraplast in a Lipshaw vacuum infiltrator. Embedded brains were sectioned at a thickness of 10 μm on a rotary microtome. A complete set of sections from each brain was mounted onto gelatin-coated slides, dried overnight on a slide warmer, and then stained with Solvent Blue 38 (also referred to as Luxol Fast Blue: Sigma S 3382) followed by cresyl violet (Sigma C 1791), using a modification of the Klüver–Barrera method (Klüver & Barrera, 1953). Stained sections were rapidly dehydrated in ethanol, cleared in Hemo-De (Fisher), and coverslipped with Cytoseal (Stephens Scientific, Cornwall, NJ).

Volume Estimation

The Cavalieri method (Gundersen, Bagger, Bendtsen, Evans, Korbo, Marcussen, Møller, Nielsen, Nyengaard, Packenberg, Sorensen, Vesterby, & West, 1988) was used to estimate the volume of the mushroom bodies of the protocerebrum. *Camera lucida* drawings were made at a total magnification of $\times 300$ using a Zeiss microscope and drawing tube. One hemisphere, selected randomly, was drawn per brain. Sections were drawn by an observer blind to group identity. The first section in which the mushroom bodies appeared was identified; then a random number table was used to select which of the first four sections would be drawn; every fourth section thereafter was drawn, so that 10 of every 40 μm was sampled. This is a conservative sampling strat-

egy for our purposes, as preliminary studies on test brains ($n = 2$) from which every section was drawn demonstrated that sampling 10 of every 60 μm reliably produced volume estimates within ± 5% of the value obtained when cross-sectional areas of every section are summed. Cross-sectional areas were determined by using a simple point-counting method, using 10-mm grids (Gundersen et al., 1988). The Cavalieri method of volume estimation is the basis of many widely used stereological methods (Gundersen & Jensen, 1987; Møller, Strange, & Gundersen, 1990; West, 1993); it has been extensively validated, most definitively by studies in which the volume estimates were confirmed by fluid displacement (Michel & Cruz-Orive, 1988).

Anatomy and Nomenclature

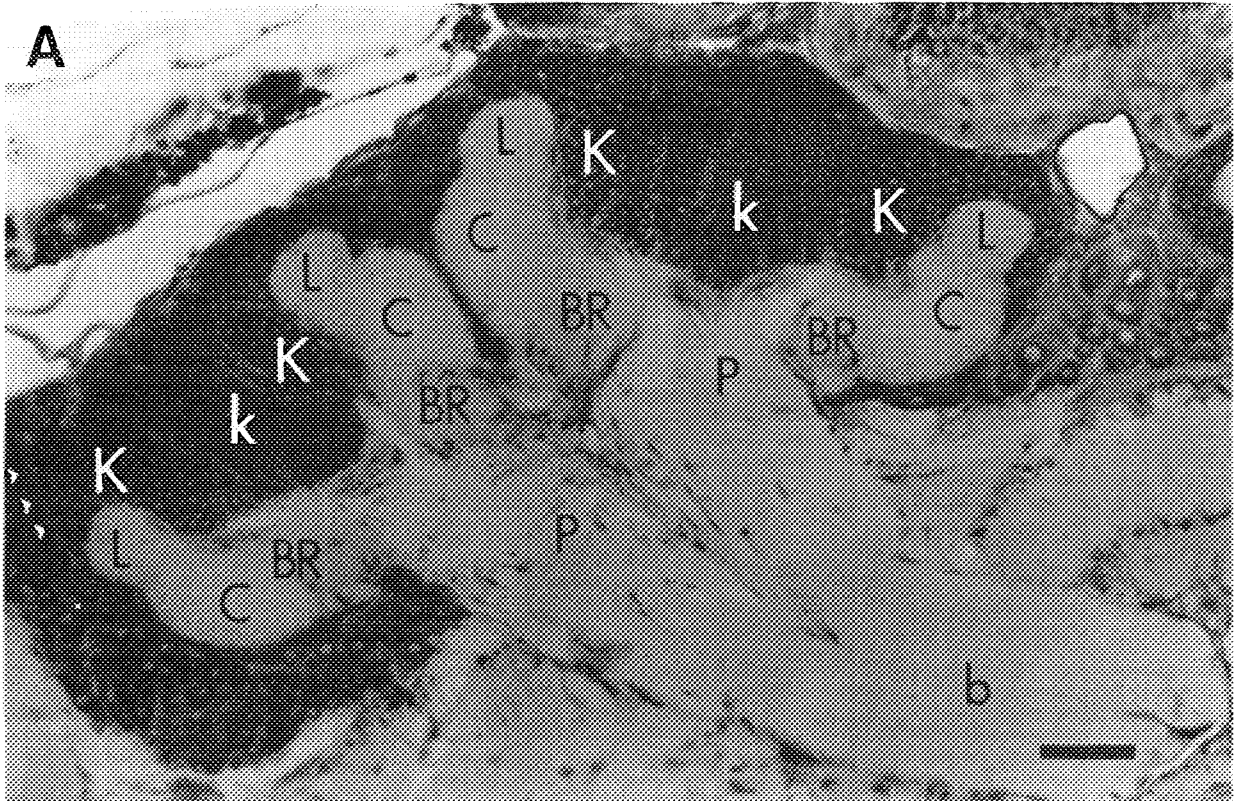
The mushroom bodies of the insect brain have been extensively described in honey bees and other insects (Kenyon, 1986a,b; Vowles, 1955; Weiss, 1974; Strausfeld, 1976; Mobbs, 1982, 1984, 1985; Schürmann, 1987). In the honey bee, they consist of a heterogeneous population of intrinsic neurons (the Kenyon cells) and an associated neuropil, which is divided into several distinct regions: the medial and lateral calyces, the peduncles, and the alpha- and beta-lobes. Each hemisphere contains a medial and a lateral population of Kenyon cells, each of which is associated, respectively, with the medial or lateral calyx. Detailed descriptions of Kenyon cell morphology can be found in Mobbs (1982). As is typical in arthropods, the neuronal somata of the Kenyon cells are completely separated from all neuropilar regions of synaptic contact. Note that the Kenyon cell body region contains both the Kenyon cell perikarya and the thin neurites that connect these perikarya to their dendritic arborizations in the neuropils of the calyces. The calycal neuropils are the input regions of the mushroom bodies, receiving both olfactory and visual information from the antennal lobe and optic lobe, respectively (Mobbs, 1985; Gronenberg, 1986). Each calyx comprises lip neuropil (receives olfactory information from the antennoglomerular tracts), collar neuropil (receives projections from the visual medulla and lobula), and basal ring neuropil (receives dual olfactory and visual input) (see Figs. 1A and 1B). The dendrites of the Kenyon cells arborize extensively in the calyces (Mobbs, 1982). Kenyon cell axons form the peduncles. The Kenyon cell axons divide at the base of the peduncle so that each cell sends one branch into the alpha-lobe and one branch into the beta-lobe of the protocerebrum, where the axons form their terminal arborizations. There are no apparent differences in the projections of the medial and lateral calyces.

The subdivisions of the calycal neuropil given by Mobbs (1982, 1985) for the worker honey bee brain are clearly visible in Paraplast sections of queen brains stained with Solvent Blue 38/cresyl violet (Figs. 1A and 1B), and we have adopted his nomenclature. No difference was detected at the light microscopic level in the organization of the mushroom bodies of queen bees compared with worker bees, although the mushroom bodies of the queen bee are, on average, considerably smaller than those of worker bees (Witthöft, 1967).

Radioimmunoassay of Juvenile Hormone

We used a radioimmunoassay (RIA) procedure previously validated for juvenile hormone extracted from worker honey bee hemolymph (Huang, Robinson, & Borst, 1994). Hemolymph (blood) from individual bees was mixed with 500 μl acetonitrile and stored at −20°C. Prior to RIA, juvenile hormone was extracted from each sample by adding 1 ml of 0.9% NaCl and 1 ml hexane to the acetonitrile–blood mixture. Samples were vortexed, cooled for 10 min on ice, vortexed a second time, and then centrifuged at 2000g for 5 min (4°C). The supernatant hexane phase containing the juvenile hormone was then removed. This extraction was then repeated. Pooled supernatants were dried in a vacuum centrifuge. To perform the RIA, 25 μl ethanol

FIG. 1. (A) Transverse section (10 μm) through the medial (right) and lateral (left) calyces of the mushroom bodies of a newly emerged honey bee queen, showing the Kenyon cells and the subregions of the neuropil. Arrowheads indicate the boundary of the Kenyon cell population of the lateral calyx. (B) Schematic diagram of the calyces of the mushroom bodies, viewed in transverse section and showing the boundaries of the neuropilar subregions used for volume estimation. The calyces are formed from three concentric zones of neuropil: the lip, the collar, and the basal ring. The basal ring is recognized in histological preparations by the rough, glomerular nature of its neuropil. A line interpolated between the notches near the dorsal margin of each calyx demarcates the lip subregion. The collar is then defined as the region bounded by the lip and the basal ring. The peduncle is formed of the axons of the Kenyon cells. These fibers bifurcate at the base of the joined peduncular stalks to form the alpha-lobe and the beta-lobe. The alpha-lobe, not seen in this section, would project perpendicularly out from the page and is shown by cross-hatching: as indicated, it is roughly circular in cross-section. Compare with the silver-stained preparation in Fig. 3a of Mobbs (1982). b, beta lobe; BR, basal ring; C, collar; k, compact region of Kenyon cells; K, Kenyon cells; L, lip; P, peduncle. Scale bar = 50 μm.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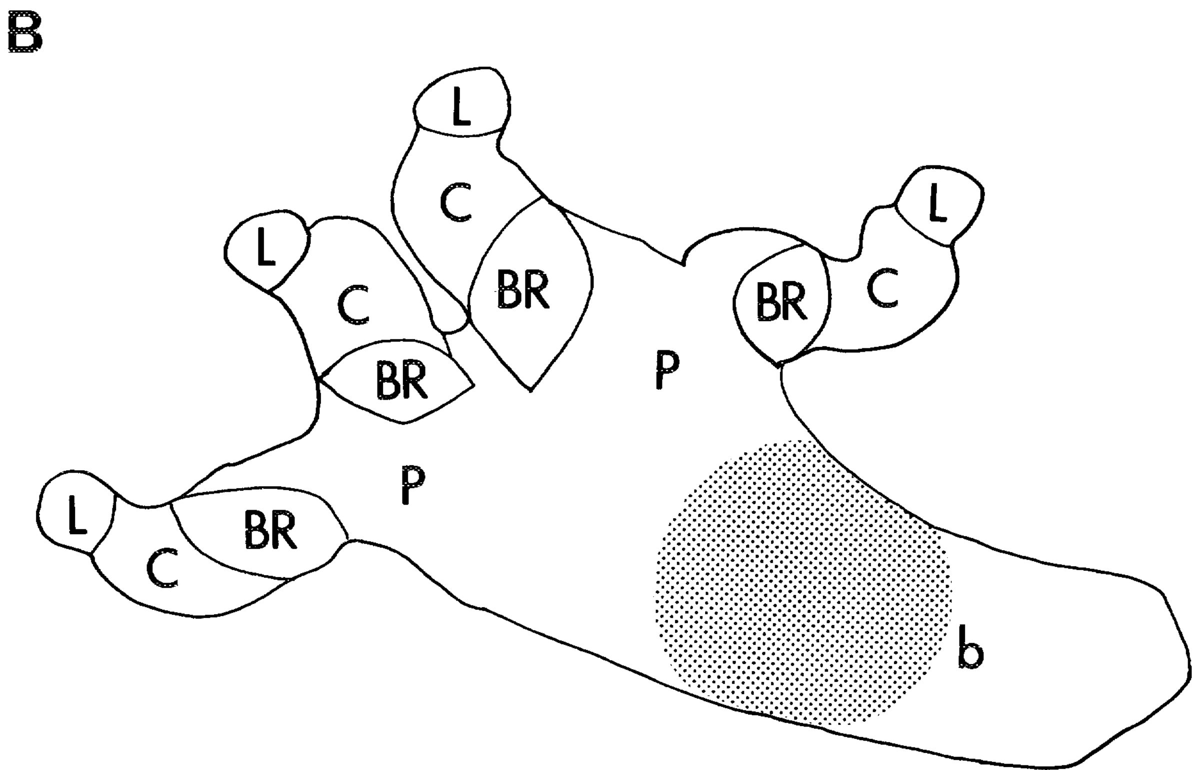
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was added to each extract, and a 2.5-μl aliquot was transferred to a tube containing 200 μl premixed antiserum (1:28,000) and 10,000 dpm of [10-^{3}H(N)]juvenile hormone (NEN, 629 Gbq/mmol). Incubation was for 2 h at room temperature. After cooling in an ice–water mixture for 10 min, unbound radiolabeled juvenile hormone was separated from bound juvenile hormone by adding dextran-coated charcoal for 2.5 min and centrifuging (at 2000g for 3 min, 4°C). Radioactivity in the supernatant was quantified by liquid scintillation spectrometry (Beckman LS600IC). A standard curve based on analyses of 0, 3, 10, 30, 100, 300, 1000, 3000, and 10,000 pg racemic juvenile hormone III (Sigma) was prepared for each assay. The juvenile hormone equivalents were adjusted by multiplying by 0.5 because the racemic juvenile hormone III used to generate the standard curve contains approximately 50% of each enantiomer and the antibody recognizes only the biologically active enantiomer (Hunnicutt et al., 1989). Juvenile hormone titers were quantified for all queens used for volume estimates except one banked inseminated queen. In order to interpret better the results obtained from the queens sampled for the anatomical study, hormone titers were quantified for five additional groups collected during the same field season: 1-, 2-, 3-, and 4-day-old banked virgins and 6-day-old queens captured while attempting to exit the hive, presumably to take a mating flight. Collection of these queens was facilitated by placing a plastic queen excluder grid over the hive entrance.

Statistical Analysis

Data were analyzed using the general linear modeling program SuperANOVA (Abacus Concepts, Inc.), after application of Bartlett's test for homogeneity of variances indicated that the data sets met the precondition of homoscedasticity (Sokal & Rohlf, 1981). A one-way analysis of variance was followed by comparisons of the means using the Student–Newman–Keuls test.

RESULTS

Total Volume of the Mushroom Bodies in Honey Bee Queens of Different Ages and Reproductive Status

Table 2 summarizes the total and regional volume estimates for all groups. There was no difference among the groups in total volume of the mushroom bodies (F = 1.8, df = 4, ns).

TABLE 2
Estimates of the Volume of the Mushroom Bodies in Honey Bee Queens[a] (Mean ± SE)

Group	n	Total volume	Kenyon cell somal region[b]	Neuropil[b]
Virgins	12	45.8 ± 1.8	22.9 ± 0.8[a]	22.9 ± 1.0[a]
Banked	8	43.6 ± 0.8	16.3 ± 0.3[b]	27.3 ± 0.6[a,b]
Mated	8	40.7 ± 2.5	14.5 ± 0.9[b]	26.1 ± 1.7[a,b]
Inseminated	10	44.7 ± 2.4	15.3 ± 0.9[b]	29.4 ± 1.5[b,c]
Old laying	6	49.2 ± 2.0	16.7 ± 0.4[b]	32.5 ± 1.6[c]

[a] $mm^3 \times 10^{-3}$. Estimates are based on a single brain hemisphere.
[b] Values marked with different letters indicate a difference significant at the $p < .05$ level (Student–Newman–Keuls test).

Volume of the Kenyon Cell Somal Region

Significant differences among groups were detected when the volume occupied by the neuronal somata of the Kenyon cells was considered independently of the total volume of the mushroom bodies (F = 20.8, df = 4, $p < .001$). Post hoc pairwise comparisons indicated that the volume occupied by the Kenyon cell somata (medial and lateral calyces combined, as they are not different in their pattern of connectivity) was greatest in the newly emerged virgins. All other groups had a significantly lower Kenyon cell somal volume, with an average reduction of approximately 30% (Table 2).

Volume of the Neuropil

Significant differences among groups were also observed in volume estimates for the neuropil of the mushroom bodies (F = 7.2, df = 4, $p < .001$). In contrast to the Kenyon cell body volume, the volume of the neuropil of the mushroom bodies was smallest in virgin queens and greatest in mated queens and old laying queens. Intermediate volumes were characteristic of the banked virgin queens and inseminated queens (Table 2).

Volume Differences of Subregions within the Neuropil

Changes in the total calycal volume paralleled the increase seen in total neuropil volume, with all other groups significantly larger than the newly emerged virgin queens (F = 13.8, df = 4, $p < .001$). In contrast, there was no difference among the groups in the volume of the alpha- and beta-lobes. Volume increases were detected in each of the calycal neuropil subdivisions: lip, collar, and basal ring (Table 3). No specific effect of mating on calycal volume was detected. The volume of the collar re-

TABLE 3
Neuropil Subregion Volume Estimates[a] (Mean ± SE)

Group	n	Calyx[b,c]	Lip[c]	Collar[c]	Basal ring[c]	Alpha- and beta-Lobes
Virgins	12	12.6 ± 0.6[a]	2.6 ± 0.2[a]	7.6 ± 0.3[a]	2.4 ± 0.2[a]	5.7 ± 0.2
Banked	8	16.0 ± 0.4[b]	3.7 ± 0.2[b]	9.2 ± 0.3[b]	3.0 ± 0.3[a,b]	6.7 ± 0.2
Mated	8	14.6 ± 0.7[b,c]	3.1 ± 0.1[a]	8.2 ± 0.6[a,b]	3.3 ± 0.2[a,b]	6.4 ± 0.4
Inseminated	10	17.2 ± 0.7[c]	3.8 ± 0.2[b]	9.6 ± 0.5[b]	3.7 ± 0.3[b]	7.2 ± 0.5
Old laying	6	19.2 ± 1.0[d]	4.3 ± 0.2[b]	11.3 ± 0.5[c]	3.7 ± 0.3[b]	6.9 ± 0.3

[a] $mm^3 \times 10^{-3}$. Estimates are based on a single brain hemisphere.
[b] "Calyx" refers to the input region of the neuropil of the mushroom bodies and comprises the lip, collar, and basal ring; the peduncle, alpha-lobe, and beta-lobe are not included.
[c] Values marked with different letters indicate a difference significant at the $p < .05$ level (Student–Newman–Keuls test).

gion of the neuropil was particularly sensitive to age and/or experience, with the old laying queens having a significantly greater volume than the banked, mated, or inseminated queens.

Ratios of Neuropil Volume to Kenyon Cell Somal Volume

The net effect of simultaneously shifting volumes within the neuronal and neuropilar subcompartments of the mushroom bodies during adult behavioral development can be summarized in the form of a neuropil volume to Kenyon cell somal volume ratio. There were highly significant differences between queen groups for this ratio (F = 109.0, df = 4, $p < .001$). The virgin queens had a ratio of 1, while the mated queens, instrumentally inseminated queens, and old laying queens all had a ratio of approximately 2. The banked virgin queens had a slightly lower ratio of approximately 1.7 (Fig. 2).

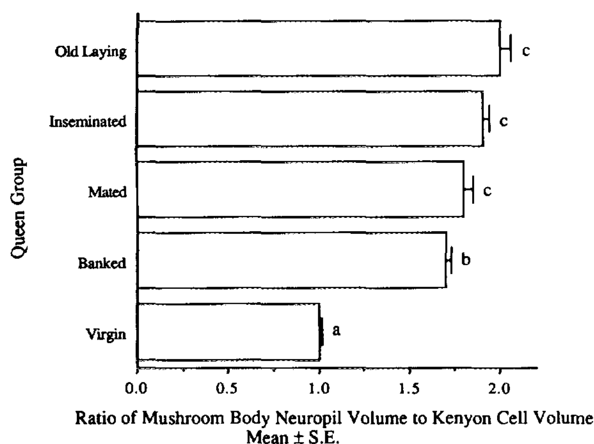

FIG. 2. Mean ratio (±SE) of neuropil volume to Kenyon cell body region volume for queen honey bees. Volume estimates and statistical analyses were performed as described in the text. The Student–Newman–Keuls test was used to determine which groups differed. Only bars marked with different letters differed significantly from one another ($p < .05$).

Radioimmunoassay Determination of Hemolymph Levels of Juvenile Hormone in Honey Bee Queens

Figure 3 shows the results of radioimmunoassay determination of blood juvenile hormone in honey bee queens. The low levels detected in the old laying queens are in agreement with those previously reported for reproductively active queens (Robinson et al., 1991). Surprisingly high levels of juvenile hormone were found in newly emerged and 1-day-old virgin queens. These levels approach those characteristic of foraging worker bees (Huang, Robinson, & Borst, 1994). Intermediate levels were characteristic of the other groups of honey bee queens.

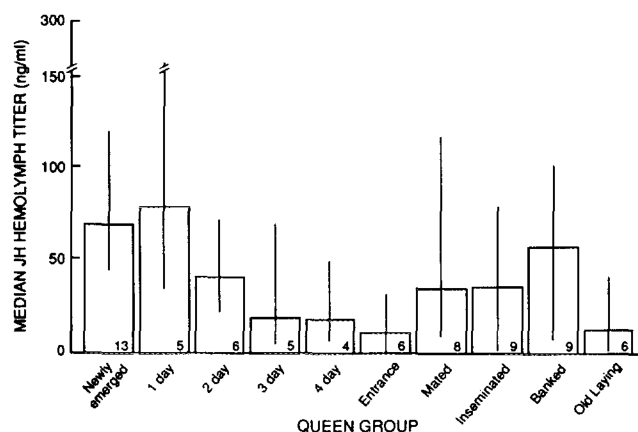

FIG. 3. Median juvenile hormone titers for queens (vertical bars indicate the range). Several additional groups were included in which brain measurements were not made: 1-, 2-, 3-, and 4-day-old virgins and 6-day-old queens captured while attempting to exit the hive, presumably to take a mating flight.

Correlation of Hemolymph Juvenile Hormone with the Ratio of the Volume of the Neuropil of the Mushroom Bodies to the Volume of the Kenyon Cell Somata

In order to explore a possible association between the structure of the mushroom bodies and the levels of juvenile hormone present at the time of sampling, we performed a correlational analysis of individual juvenile hormone levels with the ratios of neuropil volume to Kenyon cell body volume reported above. When all queens were used in this analysis, a statistically significant negative correlation between juvenile hormone levels and the volume ratio was observed ($r = -.43$, $p < .01$). This analysis was repeated without the youngest (newly emerged virgin queens) and the oldest groups (5-month-old laying queens) in order to examine the relationship between individual juvenile hormone levels and structure of the mushroom bodies of queens intermediate both in age and hormone level (mated queens, banked queens, and inseminated queens). A weak, statistically insignificant negative correlation described these data ($r = -.32$, ns).

DISCUSSION

We have demonstrated that a significant change in the structure of the mushroom bodies of the protocerebrum occurs during the adult life of honey bee queens. These changes, in which the volume occupied by the intrinsic neuronal population is significantly reduced while the volume occupied by the associated neuropil is significantly increased, occur between the 1st and 14th day of adult life, and are maintained in the oldest queen bees studied (5 months postemergence).

These results are similar to our previous findings in worker honey bees (Withers et al., 1993). The present findings indicate that these volume changes within the mushroom bodies are not caste-specific. The present study also demonstrates, for the first time, the stability of this reorganization. Once changed, the volume of the compartments of the mushroom bodies remains changed.

The volume of the mushroom bodies of worker and queen bees cannot be directly compared across the present and our earlier studies because of differences in the method of tissue preparation (Withers et al., 1993). We can, however, consider the neuropil to Kenyon cell somal region volume ratio in both studies. This ratio is always close to 1.0 in 1- to 3-day-old bees, regardless of whether the bee is a worker or a queen. Values of 1.5 to 1.7 are typical of nurse bees approximately 14 days of age and banked queens that have never flown. Values of approximately 2 are characteristic of forager bees (regardless of age) and of mated or inseminated queen bees 10 days of age or older.

We speculated that if the onset of foraging behavior could drive the reorganization of the mushroom bodies in the worker honey bee, then perhaps similarly important behavioral boundaries in the life of the queen bee might be reflected in differences in regional volumes within the mushroom bodies. Several clear transitions mark the life of the queen. These include mating; the presence of sperm in the spermatheca; and the onset of constant reproductive activity. The worker bee does not share any of this queen-specific behavioral development. The present study rules out the reproductive behavior of the queen as an important factor in determining the organization of the mushroom bodies in queen bees. There is, however, an aspect of behavioral development that is common to queens and workers. Like inexperienced foragers, virgin queens will make several orientation flights in the immediate vicinity of the nest before making any sustained flight.

Orientation flights are brief (less than 5 min in duration). They always precede true foraging or mating flights, and their function appears to be to familiarize bees with visual landmarks around the nest (Winston, 1987). At this time, bees may also learn the location of the hive entrance. Other investigators have suggested that the first orientation flights engage a specific aspect of plasticity in the calyces of the mushroom bodies of worker bees, namely spine head enlargement and spine stem shortening on the dendritic arborizations of Kenyon cells (Coss, Brandon, & Globus, 1980; Brandon & Coss, 1982). If, however, the present examples of volume changes within the mushroom bodies in worker bees and queen bees are at all dependent upon experience, this experience cannot be directly related either to orientation flight or to sustained flight, as it occurs in two groups of bees that have never flown (banked virgin queens and banked inseminated queens). This still leaves unexplored, for both workers and queens, the role of other forms of visual experience as well as exposure to specific chemosensory, tactile, and auditory inputs. Is visual experience outside the hive necessary? Banked and inseminated queens may have acquired limited visual experience when the hive was opened for experimental manipulations. A major role for visual experience in controlling the volume of the calycal

neuropils is, interestingly, not supported by the finding of differences in the volume of the collar region of neuropil between groups of queens with equivalent experience outside the hive (mated queens and old laying queens). The importance of some flight-independent aspect of experience is further supported by comparison of the two groups of banked queens. The members of one group remained virgins and never left the queen bank, while the inseminated group was removed from the queen bank on Day 10 for instrumental insemination. Although the inseminated group never flew, they are most similar to their same-age naturally mated counterparts in terms of volume of the neuropil of the mushroom bodies and neuropil volume to Kenyon cell somal volume ratio.

Another possible common factor is endocrine. In worker honey bees, increased levels of juvenile hormone production are always correlated with foraging behavior, even when colony manipulations provoke the premature onset of foraging (Robinson et al., 1989; Robinson, 1992). The present RIA results suggest that juvenile hormone might also regulate brain and behavioral development in honey bee queens, although with a different temporal pattern. One possibility is that high levels of juvenile hormone could have an organizational, as opposed to an activational effect, on the plasticity of the adult mushroom bodies. Clearly, high levels of juvenile hormone are not required to sustain the configuration of the mushroom bodies, which differed significantly between the 1-day-old queens and old laying queens. Also, hormone levels and state of the mushroom bodies were negatively or not significantly correlated in our analyses. We hypothesize that exposure to high levels of juvenile hormone at any time in life can permit, although not necessarily independently trigger, the observed changes within the mushroom bodies of foragers and queens. In fact, the typical hormone manipulation used to induce precocious foraging in workers can be interpreted as shifting the forager to a queen-like schedule of development by giving the worker brain its first exposure to high juvenile hormone levels on the first day of adult life.

The cellular basis of the observed volume changes is not yet clear. The death of Kenyon cells would offer a simple explanation for the decrease in neuronal volume within the mushroom bodies, yet in our histological preparations cell death is not in evidence: for example, no obvious pycnotic profiles are seen at any age under any experimental condition. Future studies will explore the possibility that individual Kenyon cell somata are decreasing in volume. We do not know if the changes in neuropil volume are caused by changes in the pattern of afferents or by proliferation of the processes of the intrinsic neurons of the mushroom bodies. Although it is rare for soma size to decrease in conjunction with growth of processes or increases in connectivity, this phenomenon has been reported to occur in the auditory cortex of Mongolian gerbils subjected to neonatal acoustic deprivation (McGinn, 1982). Finally, the significant postemergence decrease in the volume occupied by the somata of the Kenyon cells observed in all groups of bees so far studied has a parallel in the protocerebrum of newly eclosed *Drosophila melanogaster.* Ito and Hotta (1992) reported that the cortex of this region was thinner in 5-day-old flies than in 1-day-old flies. As in the present material, little histological evidence was obtained for neuronal death in the mushroom bodies.

A large body of literature indicates that differences in gross brain morphology typically reflect increases in dendrites and synapses (reviewed by Greenough & Chang, 1988; Greenough, Withers, & Wallace, 1990). Whether or not this is also the case in the honey bee is clearly the critical focus of our future investigations. The observed anatomical changes occur during periods of intense behavioral development for both workers and queens: both, for example, first venture from the physical and social world of their home colony at this time. Are the observed changes in brain structure related to observed behavioral transitions? If so, it appears to be possible to argue that at least some changes in the mushroom bodies precede the performance of novel behavior, as they can be detected in bees that have never flown. Intact mushroom bodies appear to be critical for the performance of olfactory learning tasks in flies and bees (reviewed by Davis, 1993; also see Erber, Masuhr, & Menzel, 1980; Heisenberg, 1980; Heisenberg et al., 1985) and in spatial orientation tasks in the cockroach (Mizunami & Strausfeld, 1991). The role of the mushroom bodies in *naturally occurring* behavioral development is less well defined.

In summary, we have used a within-species comparative approach to explore the causes of brain plasticity in the adult honey bee. Our results show clearly that volume changes within the mushroom bodies, although first demonstrated in foragers, are not confined to the worker caste. A feature common to the behavioral development of both castes, exposure to high levels of juvenile hormone in adult-

hood, may be of critical importance to the function of the mushroom bodies in the adult bee.

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