Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

Experience-Expectant Plasticity in the Mushroom Bodies of the Honeybee

Susan E. Fahrbach, Darrell Moore, Elizabeth A. Capaldi, et al.

Learn. Mem. 1998 5: 115-123
Access the most recent version at doi:10.1101/lm.5.1.115

References This article cites 39 articles, 10 of which can be accessed free at:
http://learnmem.cshlp.org/content/5/1/115.full.html#ref-list-1

Article cited in:
http://learnmem.cshlp.org/content/5/1/115.full.html#related-urls

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or **click here**

To subscribe to *Learning & Memory* go to:
http://learnmem.cshlp.org/subscriptions

Cold Spring Harbor Laboratory Press

Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

Experience-Expectant Plasticity in the Mushroom Bodies of the Honeybee

Susan E. Fahrbach,[1,3] Darrell Moore,[2] Elizabeth A. Capaldi,[1] Sarah M. Farris,[1] and Gene E. Robinson[1]

[1]Department of Entomology
University of Illinois at Urbana–Champaign
Urbana, Illinois 61801 USA
[2]Department of Biology
East Tennessee State University
Johnson City, Tennessee 37614 USA

Abstract

Worker honeybees (*Apis mellifera*) were reared in social isolation in complete darkness to assess the effects of experience on growth of the neuropil of the mushroom bodies (MBs) during adult life. Comparison of the volume of the MBs of 1-day-old and 7-day-old bees showed that a significant increase in volume in the MB neuropil occurred during the first week of life in bees reared under these highly deprived conditions. All regions of the MB neuropil experienced a significant increase in volume with the exception of the basal ring. Measurement of titers of juvenile hormone (JH) in a subset of bees indicated that, as in previous studies, these rearing conditions induced in some bees the endocrine state of high JH associated with foraging, but there was no correlation between JH titer and volume of MB neuropil. Treatment of another subset of dark-reared bees with the JH analog, methoprene, also had no effect of the growth of the MB neuropil. These results demonstrate that there is a phase of MB neuropil growth early in the adult life of bees that occurs independent of light or any form of social interaction. Together with previous findings showing that an increase in MB neuropil volume begins around the time that orientation flights occur and then continues throughout the phase of life devoted to foraging, these results suggest that growth of the MB neuropil in adult bees may have both experience-expectant and experience-dependent components.

[3]**Corresponding author.**

Introduction

The mushroom bodies (MBs) of the insect brain are critical for olfactory learning (Menzel et al. 1974; Erber et al. 1980; Heisenberg et al. 1985; Davis 1993; Debelle and Heisenberg 1994; Meller and Davis 1996). There is, however, growing evidence that a view of the MBs as only olfactory learning centers is too narrow. Inputs to the MBs not only arise in olfactory regions but also in the optic lobes and in the regions of the antennal lobes that receive mechanosensory information (Mobbs 1985). The MBs appear to be well developed in anosmic arthropod taxa (N.J. Strausfeld, R.S. Gomez, and L. Hansen, unpubl.). Recent studies have shown that in the cockroach *Periplaneta americana,* lesions of the MBs disrupt performance on tasks requiring visual learning (Mizunami et al. 1993). Intracellular recordings reveal that many MB extrinsic neurons are characterized by multimodal response patterns in both honeybee (Homberg 1984) and cockroach (Li and Strausfeld 1997).

The neuropil compartment of the MBs has been shown to exhibit volume plasticity in adult honeybees, carpenter ants, and fruit flies. In bees, an expansion of this neuropil accompanies adult behavioral maturation in workers, queens, and males (Withers et al. 1993; Durst et al. 1994; Fahrbach et al. 1995, 1997). Strikingly similar changes associated with foraging have been demonstrated in carpenter ant workers (Gronenberg et al. 1996). In fruit flies, both visual experience and complex-

LEARNING & MEMORY 5:115–123 © 1998 by Cold Spring Harbor Laboratory Press ISSN1072-0502/98 $5.00

Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

ity of the social environment affect the volume of the MB calyces (Heisenberg et al. 1995; Barth et al. 1997).

These volume changes do not reflect overall growth of the adult brain but are specific to the MB neuropil. Other brain regions show no volume changes at all during adult life, or display different temporal patterns of growth (Withers et al. 1993; Barth et al. 1997). The functional consequences of the MB volume changes are not known, but developmental plasticity of a brain center critical for learning on a scale that can be detected with relatively crude measures of volume is unlikely to be irrelevant for information processing or behavior.

Like many other social Hymenoptera, honeybees are characterized by division of labor on the basis of worker age, a phenomenon referred to as age polyethism (Robinson 1992). Worker honeybees typically do not begin to forage until they are ~3 weeks of age. Initial neuroanatomical studies found that the volume of the MB neuropil in foragers was significantly larger than that of 1-day-old bees or nurse bees (Withers et al. 1993). To determine whether this volume increase was associated with chronological age or behavioral status, we examined the MBs of young bees induced to become precocious foragers. The configuration of the MBs in precocious foragers resembled that of normal age foragers rather than that of same age nurse bees (Withers et al. 1993). This result was confirmed independently with a slightly different anatomical method (Durst et al. 1994). Induction of precocious forging in carpenter ants also produced an accelerated expansion of MB neuropil volume (Gronenberg et al. 1996).

The studies in honeybees suggested two different (but not mutually exclusive) scenarios for the observed increase in MB volume in foragers. First, it is possible that the experience of foraging directly sets in motion patterns of neural activity that lead to an increase in the volume of the MB neuropil. A possible, unproven consequence of this neuropil expansion might be better performance on tasks involving the MBs. This effect would be similar to that seen, for example, in the rodent cerebral and cerebellar cortices as a result of rearing in an enriched environment (Bennett et al. 1964; Greenough and Chang 1988) or learning a specific motor skill (Black et al. 1990). The second scenario is based on the correlation between high levels of the insect developmental hormone, juvenile hormone (JH) and foraging. Exposure to raised levels of JH during adult life could promote expansion of the MB neuropil (Robinson et al. 1989; Robinson 1992; Fahrbach 1997). This effect would be similar to that observed in steroid-sensitive regions of the vertebrate central nervous system during development of sex differences and as a consequence of seasonal changes in hormone titers (Breedlove 1992).

These hypotheses have been tested by manipulating experience or exposure to JH. Bees prevented from foraging had the same volume of MB neuropil as same-aged foragers from the same colony, demonstrating decisively that neither flight outside the hive nor foraging experience were essential for neuropil growth (Withers et al. 1995). This experiment left open a role for visual experience, as the nonforaging bees were frequently observed at the hive entrance. Also, this experiment did not address the role of JH, because the nonforaging bees were treated with the JH analog methoprene.

We have shown subsequently that exposure to JH is not likely to be a primary determinant of MB expansion in bees. Expansion of the MB neuropil can occur in bees developing with no adult exposure to JH (J.P. Sullivan, G.E. Robinson, and S.E. Fahrbach, unpubl.); also, foragers induced to revert to nursing experienced a significant reduction in JH but were no different from persistent foragers in the volume of MB neuropil (S.M. Farris, S.E. Fahrbach, and G.E. Robinson, unpubl.). Bees induced to prolong their tenure as nurses (a state of low to intermediate JH) also showed expansion of the MB neuropil (Withers et al. 1995). Additional evidence that changes in the MB neuropil volume are not directly responsive to JH comes from studies of queens and drones. Both queens and drones have a significant expansion of the MB neuropil early in adult life, although they have differing endocrine profiles (Fahrbach et al. 1995, 1997; Giray and Robinson 1996).

Taken together, these results suggest that a substantial fraction of the volume changes in the MB neuropil is a normal part of the adult life of the honeybee. These changes can be accelerated by induction of precocious foraging. Reliable occurrence of a developmental phenomenon in all members of a species does not, however, necessarily indicate lack of a role for experience. An event typically experienced by all members of a species could produce the same pattern of results. Exposure to light and patterned visual stimuli is an obvious example of such an input. This factor had never been controlled in our previous experi-

Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

ments. Another potentially important factor is the stimulation provided by social interactions. This factor is harder to define than exposure to light, but has been shown to influence the volume of the MB neuropil in fruit flies (Heisenberg et al. 1995). In this experiment we tested the hypothesis that visual experience is important for the expansion of the MB neuropil by comparing the MB neuropil volumes of dark-reared worker bees with those of their 1-day-old sisters. All bees were individually housed so that we could study the effects of visual deprivation in an extremely restricted rearing environment. Because previous studies had demonstrated that rearing in isolation can produce high JH titers in a subset of bees, we included radioimmunoassay (RIA) assessment of JH titers in a subset of dark-reared bees to explore further the possible effects of JH on brain plasticity.

Materials and Methods

ANIMALS AND DARK-REARING CONDITIONS

Worker honeybees were obtained from colonies reared at the Bee Research Facility, Department of Entomology, University of Illinois (Urbana–Champaign). To control for possible genetic variability in the rate of behavioral development, task preference, and brain structure, the colony that supplied bees for this experiment was derived from a queen instrumentally inseminated with semen of a single, unrelated drone (Laidlaw 1977). Therefore, all of the bees used within the experiment had an average genetic relatedness of 0.75 due to haplodiploidy (Page and Laidlaw 1988).

One-day-old bees were obtained by transferring, at 8 p.m., frames containing sealed combs of late stage pupae into a lightproof box that was placed overnight in a dark incubator (33°C, 95% relative humidity). The box was then protected from light during a transfer at 9 a.m. the next morning to an environmental chamber maintained at 25°C, 65% relative humidity, in constant darkness. In the dark chamber, the box was opened and newly emerged adults ($n = 96$) were placed individually into rectangular Plexiglas cages (Fig. 1). When necessary, a small, handheld flashlight (standard incandescent bulb, 2 C batteries) fitted with a red lens was used to check the placement of the bees. This light was kept a minimum of 0.5 m from the shelves holding the cages, and was used only intermittently, as needed. This should have produced very little, if any, stimulation of the photoreceptors as bees are characterized by red light insensitivity (Menzel and Blakers 1976; Camhi 1984). The cage contained "bee candy" (50:50 mixture of honey and powdered sugar) at one end and distilled water supplied via a drinking tube at the other end (both ad libitum). Bees could walk, but not fly, in these cages. The bees were not disturbed after being placed in their individual cages, and the environmental chamber remained closed for the duration of the experiment. These cages were designed for investigation of circadian rhythms in locomotor activity in honeybees (Moore and Rankin 1985). A motion detector placed halfway down the length of the cage permitted the recording of locomotor activity. All bees used for neuroanatomical analyses were active for the duration of the experiment.

At the time of introduction into the cages, half of the bees received topical treatment on the abdomen of 200 μg of methoprene dissolved in 5 μl of acetone; the remaining bees received acetone alone. This manipulation was included to test the hypothesis that exposure to JH is an important determinant of MB neuropil volume, as this experiment was conducted prior to our allatectomy studies. This dose of methoprene induces precocious foraging in worker honeybees when they are treated on the first day of adult life and reared in a hive (Robinson et al. 1989). Also at the time of introduction of bees into the cages, 15 additional bees were collected off the comb (in the dark) and immediately processed for histology as described below to serve as the 1-day-old comparison group.

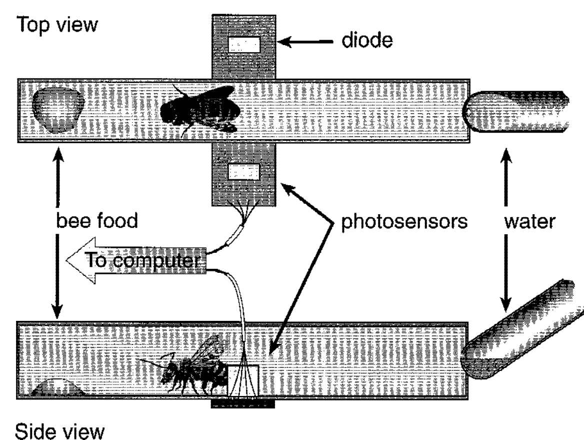

Figure 1: Design of cage used for rearing individual honeybees in darkness in social isolation. The cage is constructed of Plexiglas, with wire mesh ends. Dimensions are 12.5 cm long, 1.5 cm wide, and 2.0 cm high.

Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

Dark-reared bees were processed for histology at the end of 7 days as described below. Mortality was higher in the methoprene-treated group (70.8% survived 7 days) than in the acetone group (89.6% survived 7 days: $\chi^2 = 5.3$, $df = 1$, $P = 0.02$).

HISTOLOGY AND VOLUME ANALYSIS

To ensure that brains were fixed before exposure to light, a small amount of alcoholic Bouin's fixative was injected into the abdomen of 10 randomly selected methoprene-treated and 10 untreated bees before the lights were turned on in the environmental chamber at the termination of the experiment. A similar procedure was used for collecting the brains from 1-day-old bees at the start of the experiment. The brains were then taken into the light and dissected free of the head capsule as rapidly as possible. They were then immersed in aged alcoholic Bouin's fixative (Gregory et al. 1980) overnight at room temperature. Only brains that were yellow as a result of contact with the Bouin's fixative before lights on were used—application of this criterion resulted in discarding one brain obtained from a methoprene-treated bee. An additional group of bees ($n = 18$) was bled before injection with fixative. This required exposure to dim white light for ~2 min before the bee was injected with fixative. Chilled bees were bled by insertion of an insect pin into the neck membranes; hemolymph (blood) was collected into a calibrated capillary tube. Approximately 3–8 µl of hemolymph was removed at this time for RIA of JH.

Fixed brains were 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. A complete set of sections from each brain was mounted onto Fisher Superfrost Plus slides (Fisher 12-550-15), 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 and Barrera 1953). Stained sections were rapidly dehydrated in ethanol, cleared in Hemo-De (Fisher), and coverslipped with Cytoseal (Stephens Scientific, Cornwall, NJ). The Cavalieri method (Gundersen et al. 1988) was used to estimate the volume of the MB. Camera lucida drawings were made at a total magnification of 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 MB appeared was identified; then a random number table was used to select which of the first six sections would be drawn; every sixth section thereafter was drawn, so that 10 µm of every 60 µm was sampled. Preliminary studies on test brains ($n = 2$) from which every section was drawn demonstrated that sampling 10 µm out 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 15-mm grids (Gundersen et al. 1988). The Cavalieri method of volume estimation is the basis of many widely-used stereological methods (Gundersen and Jensen 1987; Møller et al. 1990; West 1993); it has been extensively validated, most definitively by studies in which the volume estimates were confirmed by fluid displacement (Michel and Cruz-Orive 1988).

The MBs have been described in detail in bees (Kenyon 1896; Mobbs 1982, 1984, 1985). The subdivisions of the calycal neuropil given by Mobbs for the bee brain are clearly visible in Paraplast sections stained with Solvent Blue 38/cresyl violet (Fig. 2), and we have adopted his nomenclature. 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).

Data were analyzed using the general linear modeling package SuperANOVA for the Macintosh (Abacus Concepts, Inc.) after application of Bartlett's test for homogeneity of variances indicated that the data sets met the precondition of homoscedasticity (Sokal and Rohlf 1981). Student *t*-tests were performed using StatView 4.5 for the Macintosh (Abacus Concepts, Inc.). A one-way analysis of variance was followed by comparisons of the means using the Student–Newman–Keuls test.

RIA

To determine the effects of individual rearing on the activity of the corpora allata, we used an RIA validated for JH extracted from worker honeybee hemolymph. Previous publications have described this assay in detail (Hunnicutt et al. 1989; Huang et al. 1994). Briefly, blood from individual bees was mixed with 500 µl of acetonitrile each and stored

Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

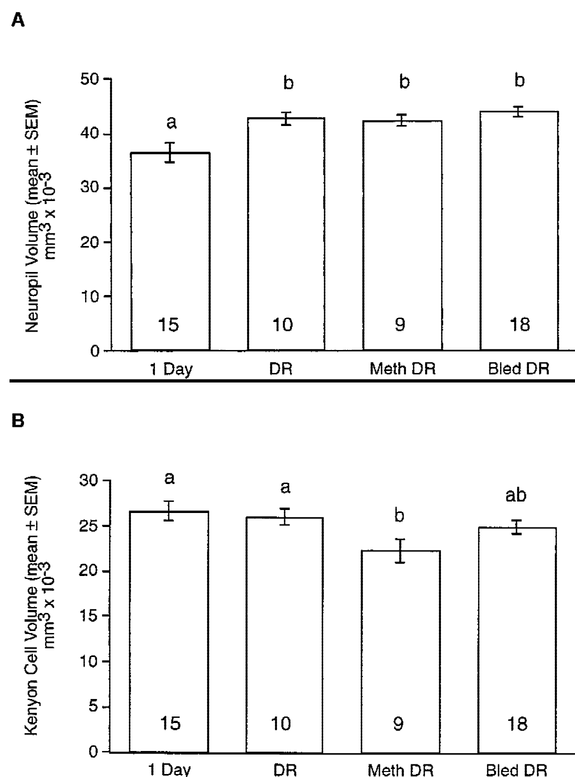

Figure 2: Effect of dark rearing on volume of the MB neuropil (*A*) and region occupied by the somata of the Kenyon cells (*B*). Numbers in bars indicate brains analyzed per group. Lowercase letters indicate significantly different groups ($P < 0.05$). (See text for details of statistical analysis and definitions of groups.)

at −20°C. Prior to RIA, JH was extracted from each sample by adding 1 ml of 0.9% NaCl and 1 ml of hexane to the acetonitrile–blood mixture. Samples were vortexed, cooled for 10 min on ice, vortexed a second time, and then centrifuged at 2000*g* for 5 min (4°C). The supernatant hexane phase containing the JH was then removed. This extraction was repeated. Pooled supernatants were dried in a vacuum centrifuge. To perform the RIA, 25 µl of ethanol was added to each extract, and a 2.5-µl aliquot was transferred to a tube containing 200 µl of premixed antiserum (1:28,000) and 10,000 dpm of [10^{-3} H(N)]–JH (NEN, 629 Gbq/mmole). Incubation was for 2 hr at room temperature. After cooling in an ice-water mixture for 10 min, unbound radiolabeled JH was separated from bound JH by adding dextran-coated charcoal for 2.5 min and centrifuging (at 2000*g* for 3 min at 4°C). Radioactivity in the supernatant was quantified by liquid scintillation spectrometry.

Results

EXPANSION OF THE MB NEUROPIL OCCURRED IN DARK-REARED WORKERS

The MBs from four groups of bees were subjected to volume analysis: 1-day-old bees ($n = 15$); 7-day-old dark-reared bees ($n = 10$); 7-day-old dark-reared bees treated with methoprene ($n = 9$); and 7-day-old dark-reared bees from whom blood samples were taken for RIA ($n = 18$). Significant differences among groups were observed in volume estimates for the MB neuropil ($F = 7.99$, $df = 3$, $P < 0.0002$; Fig. 3A). The volume of the total MB neuropil (calyces, peduncle, alpha and beta lobes) was significantly larger in 7-day-old dark-reared bees than in 1-day-olds (Student–Newman–Keuls test, $P < 0.01$). Treatment with methoprene on the first day of adult life did not affect this result. There was also no difference in MB neuropil volume as a result of a brief exposure to light (for the purpose of taking a blood sample) immediately before brain fixation. Compared with the 1-day-old bees, volume increases ranged from 18% to 21%. Significant differences were also observed in volume estimates for the region of the MB occupied by the somata of the Kenyon cells ($F = 2.94$, $df = 3$, $P = 0.0424$; Fig. 3B). In contrast to the neuropil effect, however, post hoc comparisons revealed that there was no difference between 1-day-old and 7-day-old dark-reared bees. Instead, the methoprene-treated group had a significantly smaller volume occupied by the somata of the Kenyon cells than both 1-day-olds (Student–Newman–Keuls test, $P < 0.05$) and untreated 7-day-old bees (Student–Newman–Keuls test, $P < 0.05$) and did not differ from the 7-day-old bees bled for RIA before brain fixation.

ALL REGIONS OF THE MB NEUROPIL INCREASED IN VOLUME EXCEPT THE BASAL RING

We compared the volume of the individual subcompartments of the MB neuropil in a subset of the brains subjected to volume analysis: 1-day-olds ($n = 10$), 7-day-old dark-reared ($n = 10$), and 7-day-old dark-reared, methoprene-treated bees ($n = 9$). The results revealed that all regions of the MB neuropil experienced growth under conditions of dark rearing in both methoprene-treated and untreated bees, with the exception of the basal ring, which was stable in volume (Fig. 3).

Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

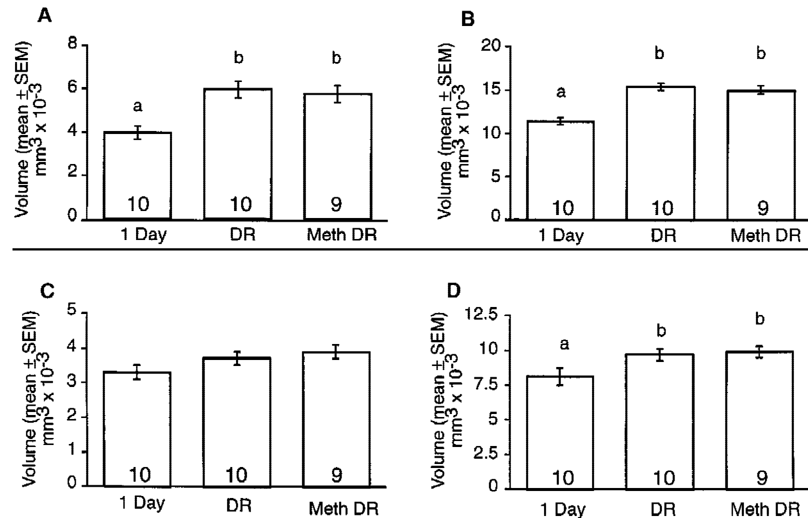

Figure 3: Effect of dark rearing on volume of the subcompartments of the MB calyces. (*A*) Lip; (*B*) collar; (*C*) basal ring; (*D*) peduncle. Numbers in bars indicate brains analyzed per group. Lower-case letters indicate significantly different groups ($P < 0.05$). (See text for details of statistical analysis and definitions of groups.)

SOME WORKERS REARED IN ISOLATION HAD JH TITERS EQUIVALENT TO FORAGERS

Previous studies have indicated that a subset of bees reared in isolation experience induction as precocious foragers (Huang and Robinson 1992). The mean JH titer (±S.E.M.) of 7-day-old dark-reared bees ($n = 18$) was 85 ± 18 ng/ml. Values above 100 ng/ml typically indicate forager status (Huang et al. 1994). Inspection of the present data revealed that five out of the 18 dark-reared bees for which JH titers were determined had forager-like values, whereas the remaining bees had lower titers similar to those of nurse bees. The high JH group, $n = 5$, had a mean ±S.E.M. of 191 ± 30 ng/ml (range = 150 to 311). In contrast, the low JH group, $n = 13$, had a mean ±S.E.M. of 45 ± 6 ng/ml (range = 14 to 76).

NO CORRELATION BETWEEN MB NEUROPIL VOLUME AND JH TITER IN INDIVIDUAL WORKERS

In addition to assessing the effects of methoprene treatment on MBs, we used the differences in JH titers induced by isolation rearing to explore a possible relationship between exposure to high levels of JH and growth of the MB neuropil volume. For the entire group of bees for which JH titers were determined, the Spearman rank correlation test revealed no association between JH titer and MB neuropil volume (ρ corrected for ties = 0.267, $P = 0.27$). Comparison of the MB neuropil volumes of the high JH group (45.1 ± 0.86 $mm^3 \times 10^{-3}$) versus the low JH group (43.6 ± 1.1 $mm^3 \times 10^{-3}$) revealed no difference ($t = -0.748$, $df = 16$, N.S.).

Discussion

The MB neuropil of honeybees reared in darkness in social isolation for 7 days was significantly larger than that of 1-day-old bees, demonstrating decisively that a major portion of the previously described volume increase associated with the transition to foraging is independent of flight experience, social contact, and visual stimulation. This study suggests that there is a programmed expansion of the MB neuropil in the first days of adult life of the honeybee. This form of brain plasticity can be described as experience-expectant (Black and Greenough 1986), in the sense that it occurs in every member of the species before what are presumably shared life experiences for that species.

Results from two other studies suggest that in addition to this experience-expectant brain plasticity, there are also at least two forms of experience-dependent plasticity that affect the MB neuropil. First, more experienced foragers (2 weeks foraging experience versus 1 week) have a larger volume of MB neuropil than less experienced foragers (S.E. Fahrbach, S.M. Farris, and G.E. Robinson, unpubl.). This result also could be a consequence of an age-dependent continuation of the expansion that begins early in life. Second, it is also clear that MB neuropil expansion is not related solely to chronological age, as precocious foraging is associated with accelerated growth of the MB neuropil (Withers et al. 1993; Durst et al. 1994; Gronenberg et al. 1996). A worker bee progressing through a typical age polyethism schedule experiences growth of the MB neuropil early in adult life, with later ex-

Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

pansion a consequence of some currently undetermined aspect of experience. Precocious foragers appear to experience both the experience-expectant and the experience-dependent phases of MB neuropil expansion simultaneously. Although we have speculated previously that the insect developmental hormone JH might regulate either or both of these phases of MB neuropil expansion, the present data and the results from studies of allatectomized bees (J.P. Sullivan, G.E. Robinson, and S.E. Fahrbach, unpubl.) suggest that any effect of JH on volume of the MB neuropil must be indirect.

Volume changes in the subcompartments of the MB neuropil were found in both olfactory (lip) and visual (collar) input regions of the MB calyx. Volume increases in the calyces may reflect either increases in the dendritic arborizations of the Kenyon cells, increases in the number of synapses formed by afferents from the primary sensory neuropils, or both. Interestingly, no volume increase was detected in the basal ring region, which receives both visual and olfactory inputs. It is therefore possible that the experience-expectant and experience-independent effects on MB neuropil volume are expressed differentially within the subcompartments of the calyx. Currently, however, we interpret the basal ring data cautiously, as it is the smallest neuropil compartment of the calyx and therefore possibly susceptible to volume underestimation. Increases in volume of the peduncle as well as in the alpha and beta lobes (data not shown) suggest that there may be new growth of axons or axon collaterals from the Kenyon cells. Although changes in the volume occupied by the somata of the Kenyon cells have been detected in this and earlier experiments, the significance of these changes is not known.

Functional correlates of the expansion in MB neuropil volume are as of yet unknown. There are changes in the behavior of honeybees that occur during the first week of life (switching from cell cleaning to other tasks, initiating orientation flights), but the extent to which these tasks depend on the MBs is not yet clear. In a laboratory assay designed to test ability to give a behavioral response to alarm pheromone, 4- and 7-day-old bees were more responsive than 1-day-old bees (Robinson 1987). This change in behavior may reflect the experience-expectant growth of the MB neuropil described in the present study.

Note that we have not ruled out a role for visual experience in forming the structure of the MB neuropil in the honeybee but, rather, have shown that there is growth independent of visual experience or social contact. There are numerous compelling reasons to continue investigations of the effects of visual experience on the MB neuropil of honeybees. Visual inputs to the MB calyces are well developed in the Hymenoptera (Mobbs 1985), and visual experience differentiates the experienced forager from the neophyte (Winston 1987). Disruption of the MBs has been shown to impair the performance of several species of insects on tasks requiring visual learning (Vowles 1967; Mizunami et al. 1993). It has been demonstrated recently that the volume of the calyces of the MBs of *Drosophila melanogaster* was significantly larger in newly eclosed flies reared for 4 days in constant light than in flies that spent the first 4 days of adult life in constant darkness (Barth and Heisenberg 1997). This effect could be detected in the ipsilateral calyx of monocularly deprived flies and was absent in flies bearing a mutation resulting in blindness, suggesting that the effect is mediated through the canonical visual pathway (Barth and Heisenberg 1997).

An additional observation, however, bears directly on the present data (Barth and Heisenberg 1997). A detailed study of the time course of volume changes in the *Drosophila* MB calyces showed that in both dark- and light-reared flies the MB calyces increased in volume during the first 6 hr of adult life. After this time, no further volume increase was seen in the dark-reared flies, whereas the flies reared in constant light showed light-dependent growth between 6 and 12 hr of adult life. The growth of the MB neuropil observed in the present study during 7 days of dark rearing is likely to be the counterpart of the early period of growth in the fly. This suggestion is consistent with the fact that adult honeybees have a much longer adult life than do fruit flies, characterized by a protracted process of behavioral maturation associated with social evolution. Future studies in the honeybee are needed to determine whether exposure to light alone is sufficient to account for later growth, as in the fly. It should also be noted that dark-rearing is a manipulation relevant to the normal development of the adult honeybee, as young bees spend almost all of their time within the hive. The hive interior is typically described as ''dark;'' recent measurements made at the Illinois Bee Research Facility have confirmed this assumption by showing that no more than 0.13% of incident sunlight can be detected inside a standard hive box (E.A. Capaldi and S.E. Fahrbach, unpubl.).

Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

The cellular changes that, summed together, cause the measurable changes in MB neuropil volume have not yet been described in either the fruit fly or the honeybee. These cellular changes can, in part, be profitably explored using bees dark-reared in social isolation. This rearing method produces reliable MB growth independent of visual or social experience, which can form the basis for comparison with bees experiencing further development of the MB neuropil during their careers as foragers.

Acknowledgments

We thank O. Jassim for assistance with the RIA, J. Kuehn for assistance with beekeeping, and S. Berlocher for the use of his environmental chamber. We also thank E. DeLucia for use of his L1-1000 Li-COR DataLogger Radiation Sensor for measurements of light levels inside hives. This research was supported by a National Science Foundation (NSF) award to S.E.F. and G.E.R., a National Institutes of Health (NIH) award to G.E.R., and a NSF Research Opportunity Award Supplement to D.M. E.A.C. was supported by a National Research Service Award from the NIH.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

Barth, M. and M. Heisenberg. 1997. Vision affects mushroom bodies and central complex in *Drosophila melanogaster. Learn. & Mem.* **4:** 219–229.

Barth, M., H.V.B. Hirsch, I.A. Meinertzhagen, and M. Heisenberg. 1997. Experience-dependent developmental plasticity in the optic lobe of *Drosophila melanogaster. J. Neurosci.* **17:** 1493–1504.

Bennett, E.L., M.C. Diamond, P. Krech, and M.R. Rosenzweig. 1964. Chemical and anatomical plasticity of the brain. *Science* **146:** 610–619.

Black, J.E. and W.T. Greenough. 1986. Induction of pattern in neural structure by experience: Implications for cognitive development. In *Advances in developmental psychology* (ed. M.E. Lamb, A.L. Brown, and B. Rogoff), pp. 1–50. Lawrence Erlbaum Associates, Hillsdale, NJ.

Black, J.E., K.R. Isaacs, B.J. Anderson, A.A. Alcantara, and W.T. Greenough. 1990. Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proc. Nat. Acad. Sci.* **87:** 5568–5572.

Breedlove, S.M. 1992. Sexual dimorphism in the vertebrate nervous system. *J. Neurosci.* **12:** 4133–4142.

Camhi, J. 1984. *Neuroethology: Nerve cells and natural behavior of animals.* Sinauer, Sunderland, MA.

Davis, R.L. 1993. Mushroom bodies and Drosophila learning. *Neuron* **11:** 1–14.

Debelle, J.S. and M. Heisenberg. 1994. Associative odor learning in *Drosophila* abolished by chemical ablation of mushroom bodies. *Science* **263:** 692–695.

Durst, C., S. Eichmuller, and R. Menzel. 1994. Development and experience lead to increased volume of subcompartments of the honeybee mushroom body. *Behav. Neural Biol.* **62:** 259–263.

Erber, J., T. Masuhr, and R. Menzel. 1980. Localization of short-term memory in the brain of the bee, *Apis mellifera. Physiol. Entomol.* **5:** 343–358.

Fahrbach, S.E. 1997. Regulation of age polyethism in bees and wasps by juvenile hormone. *Adv. Study Behav.* **26:** 285–316.

Fahrbach, S.E., T. Giray, and G.E. Robinson. 1995. Volume changes in the mushroom bodies of adult honey bee queens. *Neurobiol. Learn. & Mem.* **63:** 181–191.

Fahrbach, S.E., T. Giray, S.M. Farris, and G.E. Robinson. 1997. Expansion of the neuropil of the mushroom bodies in male honey bees is coincident with initiation of flight. *Neurosci. Lett.* **236:** 135–138.

Giray, T. and G.E. Robinson. 1996. Common endocrine and genetic mechanisms of behavioral development in male and worker honey bees and the evolution of division of labor. *Proc. Natl. Acad. Sci.* **93:** 11718–11722.

Greenough, W.T. and F.-L.F. Chang. 1988. Plasticity of synapse structure and pattern in the cerebral cortex. In *Cerebral cortex* (ed. A. Peters and E.G. Jones), pp. 391–440. Plenum Publishing, New York, NY.

Gregory, G.E., A.R. Greenway, and K.A. Lord. 1980. Alcoholic Bouin fixation of insect nervous systems for Bodian silver staining. I. Composition of "aged" fixative. *Stain Technol.* **55:** 143–149.

Gronenberg, W. 1986. Physiological and anatomical properties of optical input fibres to the mushroom body in the bee brain. *J. Insect Physiol.* **32:** 695–704.

Gronenberg, W., S. Heeren, and B. Holldöbler. 1996. Age-dependent and task-related morphological changes in the brain and mushroom bodies of the ant *Camponotus floridanus. J. Exp. Biol.* **199:** 2011–2019.

Gundersen, H.J.G. and E.B. Jensen. 1987. The efficiency of systematic sampling in stereology and its prediction. *J. Microsc. Oxf.* **147:** 229–263.

Gundersen, H.J.G., P. Bagger, T.F. Bendtsen, S.M. Evans, L. Korbo, N. Marcussen, A. Møller, K. Nielsen, B. Pakkenberg,

Downloaded from learnmem.cshlp.org on March 27, 2009 - Published by Cold Spring Harbor Laboratory Press

and F.B. Sørensen. 1988. The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* **96:** 857–881.

Heisenberg, M., A. Borst, S. Wagner, and D. Byers. 1985. *Drosophila* mushroom body mutants are deficient in olfactory learning. *J. Neurogenet.* **2:** 1–30.

Heisenberg, M., M. Heusipp, and C. Wanke. 1995. Structural plasticity in the *Drosophila* brain. *J. Neurosci.* **15:** 1951–1960.

Homberg, U. 1984. Processing of antennal information in extrinsic mushroom body neurons of the bee brain. *J. Comp. Physiol. A* **154:** 825–836.

Huang, Z.-Y. and G.E. Robinson. 1992. Honeybee colony integration: Worker-worker interactions mediate hormonally regulated plasticity in the division of labor. *Proc. Natl. Acad. Sci.* **89:** 11726–11729.

Huang, Z.Y., G.E. Robinson, and D.W. Borst. 1994. Physiological correlates of division of labor among similarly aged honey bees. *J. Comp. Physiol. A* **174:** 731–739.

Hunnicutt, D., Y.C. Toong, and D.W. Borst. 1989. A chiral specific antiserum for juvenile hormone. *Am. Zool.* **29:** 48A.

Kenyon, F.C. 1896. The brain of the bee. A preliminary contribution to the morphology of the nervous system of the arthropoda. *J. Comp. Neurol.* **6:** 133–210.

Klüver, H. and E. Barrera. 1953. A method for the combined staining of cells and fibers in the nervous system. *J. Neuropathol. Exp. Neurol.* **12:** 400–403.

Laidlaw, H.H. 1977. *Instrumental insemination of honey bee queens.* Dadant and Sons Press, Hamilton, IL.

Li, Y. and N.J. Strausfeld. 1997. Morphology and sensory modality of mushroom body extrinsic neurons in the brain of the cockroach, *Periplaneta americana. J. Comp. Neurol.* **387:** 631–650.

Meller, V.H. and R.L. Davis. 1996. Biochemistry of insect learning: Lessons from bees and flies. *Insect Biochem. Mol. Biol.* **26:** 327–335.

Menzel, R. and M. Blakers. 1976. Colour receptors in the bee eye—morphology and spectral sensitivity. *J. Comp. Physiol. A* **108:** 11–33.

Menzel, R., J. Erber, and T. Masuhr. 1974. Learning and memory in the honeybee. In *Experimental analysis of insect behaviour* (ed. L. Barton-Brown), pp. 195–217. Springer-Verlag, New York, NY.

Michel, R.P. and L.M. Cruz-Orive. 1988. Application of the Cavalieri principle and vertical sections method to lung: Estimation of volume and pleural surface area. *J. Microsc. Oxf.* **150:** 117–136.

Mizunami, M., J.M. Weibrecht, and N.J. Strausfeld. 1993. A new role for the insect mushroom bodies: Place memory and motor control. In *Biological neural networks in invertebrate neuroethology and robotics* (ed. R.D. Beer, R.E. Ritzman, and T. McKenna), pp. 199–225. Academic Press, New York, NY.

Mobbs, P.G. 1982. The brain of the honeybee *Apis mellifera* L. The connections and spatial organization of the mushroom bodies. *Phil. Trans. R. Soc. Lond B.* **298:** 309–354.

———. 1984. Neural networks in the mushroom bodies of the honeybee. *J. Insect Physiol.* **30:** 43–58.

———. 1985. Brain structure. In *Comprehensive insect physiology, biochemistry and pharmacology* (ed. G.A. Kerkut and L.I. Gilbert), pp. 299–370. Pergamon, Oxford, UK.

Møller, A., P. Strange, and H.J.G. Gundersen. 1990. Efficient estimation of cell volume using the nucleator and disector. *J. Microsc.* **159:** 61–71.

Moore, D. and M.A. Rankin. 1985. Circadian locomotor rhythms in individual honey bees. *Physiol. Entomol.* **10:** 191–197.

Page, R.E. and H.H. Laidlaw. 1988. Full sisters and super sisters: A terminological paradigm. *Anim. Behav.* **36:** 944–945.

Robinson, G.E. 1987. Modulation of alarm pheromone perception in the honey bee: Evidence for division of labor based on hormonally regulated response thresholds. *J. Comp. Physiol. A* **160:** 613–619.

———. 1992. Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* **37:** 637–665.

Robinson, G.E., R.E. Page, C. Strambi, and A. Strambi. 1989. Hormonal and genetic control of behavioral integration in honey bee colonies. *Science* **246:** 109–112.

Sokal, R.R. and J.F. Rohlf. 1981. *Biometry*. Freeman, New York, NY.

Vowles, D.M. 1967. Interocular transfer, brain lesions, and maze learning in the wood ant, *Formica rufa.* In *Chemistry of learning* (ed. W.C. Corning and S.C. Ratner), pp. 425–451. Plenum Press, New York, NY.

West, M.J. 1993. New stereological methods for counting neurons. *Neurobiol. Aging* **14:** 275–285.

Winston, M.L. 1987. *The biology of the honey bee.* Harvard University Press, Cambridge, MA.

Withers, G.S., S.E. Fahrbach, and G.E. Robinson. 1993. Selective neuroanatomical plasticity and division of labour in the honeybee. *Nature* **364:** 238–240.

Withers, G.S., S.E. Fahrbach, and G.E. Robinson. 1995. Effects of experience and juvenile hormone on the organization of the mushroom bodies of honey bees. *J. Neurobiol.* **26:** 130–144.

Received January 9, 1998; accepted in revised form May 15, 1998.