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Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies

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Abstract Levels of the biogenic amines dopamine, serotonin, and octopamine were measured in different brain regions of adult worker honey bees as a function of age-related division of labor, using social manipulations to unlink age and behavioral state. In the antennal lobes, foragers had higher levels of all three amines than nurses, regardless of age. Differences were larger for octopamine than serotonin or dopamine. In the mushroom bodies, older bees had higher levels of all three amines than younger bees, regardless of behavioral state. These correlative results suggest that increases in octopamine in the antennal lobes may be particularly important in the control of age-related division of labor in honey bees.

Key words *Apis mellifera* · Behavioural development · Division of labor · Dopamine · Serotonin · Octopamine

Introduction

Division of labor in colonies of the honey bee, *Apis mellifera*, is based on a stereotyped pattern of behavioral development by adult worker bees (Winston 1987). Adult bees work in the hive for the first 2–3 weeks performing tasks that include feeding the brood (''nursing'') and then switch to foraging for food outside the hive for the remainder of their 4- to 6-week life. Age-related division of labor also is characterized by a high degree of flexibility. Individual workers can accelerate, retard, or even reverse their behavioral development in response to changing colony needs (Robinson 1992).

Behavioral development in honey bees is thought to reflect changes in the responses of bees to stimuli that elicit the performance of each task (reviewed by Beshers et al. 1999). According to this hypothesis nurse bees are more responsive to stimuli that elicit the performance of brood care while foragers are more responsive to stimuli associated with foraging. Chemical stimuli are most likely of primary importance to honey bees; they live in a dark hive, apparently have limited auditory sensitivity but, like many species of social insect, possess keen chemical senses (Wilson 1971). Behaviorally related differences in response thresholds to chemical stimuli encountered in the beehive have been reported (Robinson 1987; Page et al. 1998). Behavioral development in honey bees also is thought to reflect differences in learning and memory. Foraging is probably a more cognitively demanding task than activities that are performed exclusively in the hive because it involves multimodal integration of information for navigation, efficient flower handling, and symbolic communication via the ''dance language'' (see Fahrbach and Robinson 1995).

Biogenic amines may play a role in the regulation of honey bee behavioral development. As discussed in Wagener-Hulme et al. (1999), the biogenic amines dopamine, serotonin, and octopamine are known to influence the expression of many types of behavior, presumably by modulating the responsiveness of animals to various behaviorally relevant stimuli. Treatment studies of honey bees revealed effects of biogenic amines on olfactory sensitivity and performance in a laboratory learning assay (Mercer and Menzel 1982; Macmillan and Mercer 1987; Hammer 1993). Previous studies (Harris and Woodring 1992; Taylor et al. 1992; Wagener-Hulme et al. 1999) have reported that older bees, notably foragers, had higher brain levels of all three amines than did younger bees that work in the

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hive. Using social manipulations to unlink chronological age and behavioral status, Wagener-Hulme et al. (1999) also found that octopamine was found to exhibit the most robust association between behavior and amine level, independent of age. These correlative results suggest that octopamine in particular is involved in the regulation of age-related division of labor in honey bees.

If octopamine and other amines are playing a causal role in the regulation of honey bee behavioral development, high levels in the brain might influence one or both of the following processes: response thresholds for foraging-related stimuli (Mercer and Menzel 1982; Bicker and Menzel 1989; Hammer et al. 1993) and aspects of learning and memory associated with foraging (Menzel and Müller 1996). These possibilities can be evaluated in a preliminary way by measuring biogenic amines in specific brain regions. Based on the description of behavioral development given above, two brain regions are of particular importance to our understanding of division of labor: the antennal lobes and the mushroom bodies. The antennal lobes are the primary processing center for olfactory stimuli (Homberg et al. 1989), and also are involved in early stages of olfactory learning and memory (Menzel and Müller 1996). If amine-mediated changes in olfactory processing are involved in regulating honey bee behavioral development, there should be behaviorally related differences in amine levels in the antennal lobes. The mushroom bodies are multi-modal integrative centers that are involved in learning and memory (Heisenberg et al. 1985; Erber et al. 1987). If there are amine-mediated changes in learning and memory in the mushroom bodies that occur (as a cause or consequence) during honey bee behavioral development, there should be differences in amine levels in the mushroom bodies that are related to behavioral status, age, or both.

Analyses of specific brain regions were conducted to gain a more detailed appreciation of the changes that occur in dopamine, serotonin, and octopamine levels during behavioral development. Results of these correlative studies also were used to gain further insight into the possible ways in which biogenic amines can regulate behavioral development in honey bees.

Materials and methods

Bees

Bees were obtained from three typical colonies, three single-cohort colonies, and three reversion colonies as described in Wagener-Hulme et al. (1999). Three of the colonies used by Wagener-Hulme et al. (1999) were also used here (colonies 30, 64, and 97). The following six behavioral groups were collected: control nurses (7–9 days old) and foragers (23–38 days old and a mixture of pollen and nectar foragers) from typical colonies, normal-age nurses and precocious foragers (all 7 days old) from single-cohort colonies, and reverted nurses and foragers (all 32–46 days old) from reversion colonies. Ages of bees were very similar to those used by Wagener-Hulme et al. (1999).

Brain dissection

Whole heads were partially freeze-dried at −10 °C and 0.5 mmHg for 145 min to facilitate dissection of brain regions (Müller and Altfelder 1991). To determine whether freeze-drying resulted in amine degradation, we measured amine levels in freeze-dried and fresh-frozen whole brains ($n = 10$ of each); no significant differences in amine levels were found (data not shown).

Brains were removed from approximately ten bees of each behavioral group per colony and separated into three regions: the mushroom bodies (paired medial and lateral calyces and Kenyon cell somata, but no peduncle), the antennal lobes, and the remainder of the proto/deutocerebrum. Optic lobes were removed and discarded. Dissections were performed on dry ice and brains were never allowed to thaw. Dissected brain regions from each bee were stored individually in 1.5-ml Eppendorf tubes at −80 °C until analyzed.

Sample preparation and high-pressure liquid chromatography

Sample preparation and high-pressure liquid chromatography (HPLC) were as described by Wagener-Hulme et al. (1999) except for the following changes. First, the sample buffer was composed of polished water, 15% methanol, 15% acetonitrile, and 200 ml l^{-1} of 1 mol l^{-1} EDTA, and did not include perchloric acid. Perchloric acid was eliminated to minimize background peaks that interfered with biogenic amine detection at the high sensitivity levels necessary to analyze brain regions from individuals bees (see Linn et al. 1995). Extraction of amines is less effective without perchloric acid; amine levels are < 50% of those obtained using an extraction buffer with perchloric acid. However, examination of the data in Figs. 1–3 indicates that our extraction clearly was no more variable than that described in Wagener-Hulme et al. (1999), so comparisons within this study between nurses and foragers are valid. Second, sample buffer (30 µl) containing the internal standards was added to each tube containing tissue while on ice (4 °C). Third, samples were not filtered. Fourth, each run contained 12 samples: either 6 samples of mushroom bodies (as defined above) and 6 samples of antennal lobes from one bee from each of the 6 behavioral groups or 12 samples proto/deutocerebra from two bees from each of the 6 behavioral groups. This was done to minimize the effects of variation in HPLC on intergroup comparisons. Detection limits were 15 pg for dopamine and serotonin, and 7.5 pg for octopamine.

Protein quantification

Protein amounts were measured for each sample because we assumed that there would be variation in dissection, making it more appropriate to express results on a per microgram protein basis. After centrifugation, the pellets of brain tissue were dissolved in 0.2 mol l^{-1} NaOH. Protein amount was quantified using standard methods, with a detergent-compatible protein assay (BioRad) using a Perkin-Elmer Lambda 3A UV/VIS Spectrophotometer at 750 nm.

Statistics

Measurements of dopamine, serotonin, and octopamine were made from ca. 150 samples of mushroom bodies, antennal lobes, and the remainder of the proto/duetocerebra (see Figs. 1–3). The samples were from 20 to 33 individual bees per behavioral group (9–12 bees per colony, three colonies). Four-way analysis of variance was performed (SAS/STAT System) to determine effects of source colony, colony type (typical, single-cohort or reversion), age (young or old), and behavior (nurse or forager) on levels of each amine. Source colony refers to the (typical) colony from which bees were obtained to make the experimental colony. Additional analyses were done by performing one-tailed t-tests on differences between nurses and foragers within each colony. Previous studies of whole brains (Harris and Woodring 1992; Taylor et al. 1992;

Wagener-Hulme et al. 1999) led us to make the *a priori* prediction that foragers have higher levels of biogenic amines in specific brain regions, regardless of age. We did not perform these analyses on nurses or foragers between colonies because such differences would be revealed by significant effects of colony type in the ANOVA and because the main focus of this experiment was to compare nurses and foragers within colonies.

Results

Mushroom bodies

Amounts of all three amines varied significantly with worker age, but not behavior (Table 1). There were significantly higher levels of all three amines in control foragers versus control nurses in typical colonies, but no differences between normal-age nurses and precocious foragers or reverted nurses and reversion colony foragers in the other two colony types (Fig. 1). No effects of colony type or source colony were found for any amine.

Antennal lobes

In contrast to the results for the mushroom bodies, amounts of all three amines in the antennal lobes varied significantly with worker behavior, but not age (Table 2). There were significantly higher levels of dopamine only in reversion colony foragers versus reverted nurses (Fig. 2). There were significantly higher levels of serotonin and octopamine in foragers versus nurses in all three colony types (Fig. 2). Differences between foragers and nurses were greater for octopamine than for serotonin (81.3 ± 11.3% and 41.4 ± 12%, respectively, higher in foragers than in nurses). There was no effect of colony type or source colony for any amine.

Remaining proto/deutocerebra

Amounts of all three amines varied significantly with worker behavior, but not age (Table 3). There were significantly higher levels of all three amines in control foragers versus control nurses and reversion colony foragers versus reverted nurses (Fig. 3). There was no effect of colony type or source colony for any amine (Table 3).

Protein amounts during behavioral development

There was relatively little variation between bees in the amount of protein in each brain region, as seen in the low standard errors for the entire data set [antennal lobes = 24.12 ± 0.93 μg, n = 143 (mean ± SE); mushroom bodies = 58.32 ± 1.4 μg, n = 143, remaining proto/deutocerebra = 82.0 ± 2.01 μg, n = 152]. Protein levels were stable during behavioral development. There were no effects of age, behavior, source colony or colony type on protein levels (Table 4, Fig. 4). Adding protein amounts from the three brain regions together results in amounts that are comparable to those from

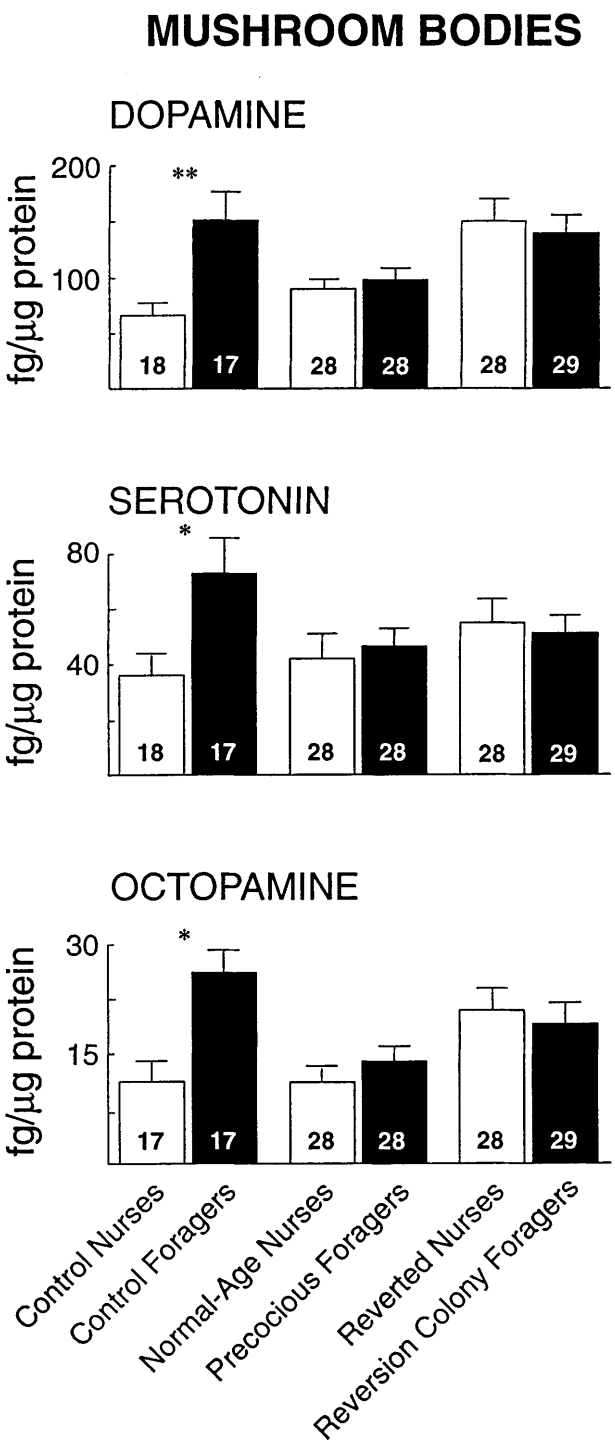

Fig. 1 Mean (+SE) concentrations of dopamine, serotonin, and octopamine in the mushroom bodies (calyces and Kenyon cell somata) of honey bees from typical colonies (control nurses and foragers), single-cohort colonies (normal-age nurses and precocious foragers), and reversion colonies (reverted nurses and reversion colony foragers). ANOVA results are in Table 1. Significant differences by one-tailed unpaired t-test are shown by *asterisks* (*$P < 0.05$, **$P < 0.01$; ***$P < 0.001$). Samples size is indicated in each bar

Table 1 Results of four-way ANOVA for concentrations of biogenic amines in the mushroom bodies of adult worker honey bees as a function of age, behavior, source colony, and colony type. Data in Fig. 1

Effect	*df*	*F*	*P*
A. DOPAMINE			
Age	1	7.1	**0.009**
Behavior	1	0.03	0.870
Source colony	2	0.03	0.968
Colony type	2	0.8	0.472
B. SEROTONIN			
Age	1	6.2	**0.014**
Behavior	1	0.2	0.645
Source colony	2	1.93	0.149
Colony type	2	1.55	0.215
C. OCTOPAMINE			
Age	1	5.7	**0.019**
Behavior	1	0.03	0.867
Source colony	2	0.6	0.568
Colony type	2	0.8	0.442

whole brains reported previously (Kokay and Mercer 1997), suggesting that only minimal amounts of tissue were lost during dissection.

Discussion

The principal significance of these results is that they demonstrate distinct patterns of changes in biogenic amine levels in different regions of the honey bee brain during behavioral development. Finding behaviorally related differences in amine levels in the antennal lobes is consistent with (but does not prove) the hypothesis that amines are involved in the regulation of division of labor in honey bee colonies.

Our results are in general agreement with those of Wagener-Hulme et al. (1999). Both studies show that

Table 2 Results of four-way ANOVA for concentrations of biogenic amines in the antennal lobes of adult worker honey bees as a function of age, behavior, source colony, and colony type. Data in Fig. 2

Effect	*df*	*F*	*P*
A. DOPAMINE			
Age	1	0.1	0.727
Behavior	1	5.2	**0.024**
Source colony	2	0.7	0.515
Colony type	2	1.2	0.293
B. SEROTONIN			
Age	1	1.1	0.299
Behavior	1	8.2	**0.005**
Source colony	2	1.8	0.171
Colony type	2	0.5	0.592
C. OCTOPAMINE			
Age	1	0.1	0.716
Behavior	1	10.6	**0.001**
Source colony	2	2.8	0.068
Colony type	2	0.2	0.803

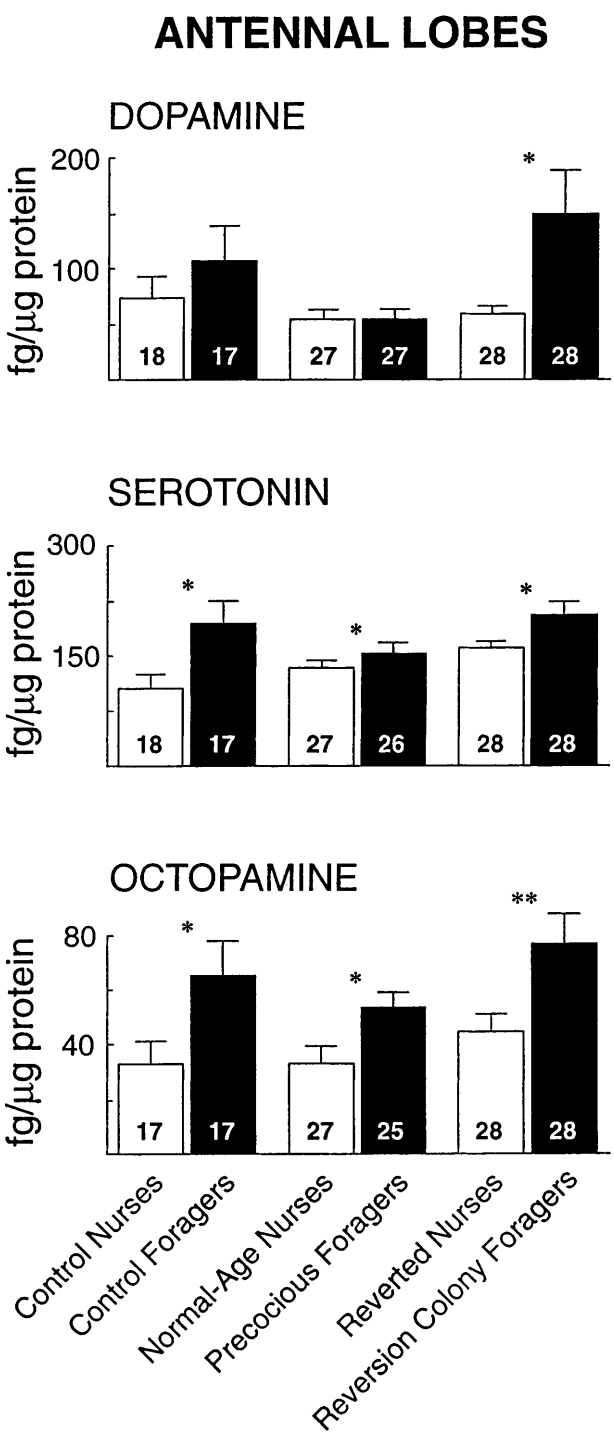

Fig. 2 Mean (+SE) concentrations of dopamine, serotonin, and octopamine in antennal lobes of honey bees. Behavioral groups, statistical analyses, and symbols as in Fig 1. ANOVA results are in Table 2

octopamine exhibits the strongest association between behavior and amine level, independent of age. This association is seen more clearly in this study; for example, Wagener-Hulme et al. (1999) reported that whole-brain levels of octopamine were not different in reverted nurses compared with reversion colony foragers. Another

Table 3 Results of four-way ANOVA for concentrations of biogenic amines in the proto/deutocerebra of adult worker honey bees as a function of age, behavior, source colony, and colony type. Data in Fig. 3

Effect	*df*	*F*	*P*
A. DOPAMINE			
Age	1	2.8	0.100
Behavior	1	11.7	**0.001**
Source colony	2	2.6	0.081
Colony type	2	0.3	0.772
B. SEROTONIN			
Age	1	1.9	0.176
Behavior	1	7.5	**0.007**
Source colony	2	1.4	0.261
Colony type	2	0.5	0.617
C. OCTOPAMINE			
Age	1	1.0	0.311
Behavior	1	10.3	**0.002**
Source colony	2	0.2	0.815
Colony type	2	1.3	0.266

notable difference is that Wagener-Hulme et al. (1999) found that whole-brain levels of serotonin did not vary with behavior independent of age, whereas in the present study levels of serotonin in the antennal lobes did vary with behavior. The reasons for these differences are not clear. It does not seem possible that differences in quantification (per brain versus per microgram protein) can explain the differences because protein levels did not differ between nurses and foragers in our study. One possibility is that absolute levels of amines in antennal lobes are so low that they would be obscured in whole-brain analyses. However, the patterns observed in the remaining proto/deutocerebra were similar to those of the antennal lobes and they contained the largest volume of brain tissue. Results from the proto/deutocerebra are difficult to interpret because less is known about the regions of the brain encompassed in those samples than

Table 4 Results of four-way ANOVA for levels of protein in antennal lobes, mushroom body calyces and somata, and remaining proto/deutocerebra of adult worker honey bees as a function of age, behavior, source colony, and colony type. Data in Fig. 4

Effect	*df*	*F*	*P*
A. DOPAMINE			
Age	1	3.7	0.057
Behavior	1	0.9	0.341
Source colony	2	1.0	0.362
Colony type	2	0.5	0.636
B. SEROTONIN			
Age	1	1.5	0.228
Behavior	1	2.0	0.160
Source colony	2	2.1	0.123
Colony type	2	0.3	0.723
C. OCTOPAMINE			
Age	1	0.1	0.760
Behavior	1	0.2	0.640
Source colony	2	0.1	0.934
Colony type	2	0.9	0.407

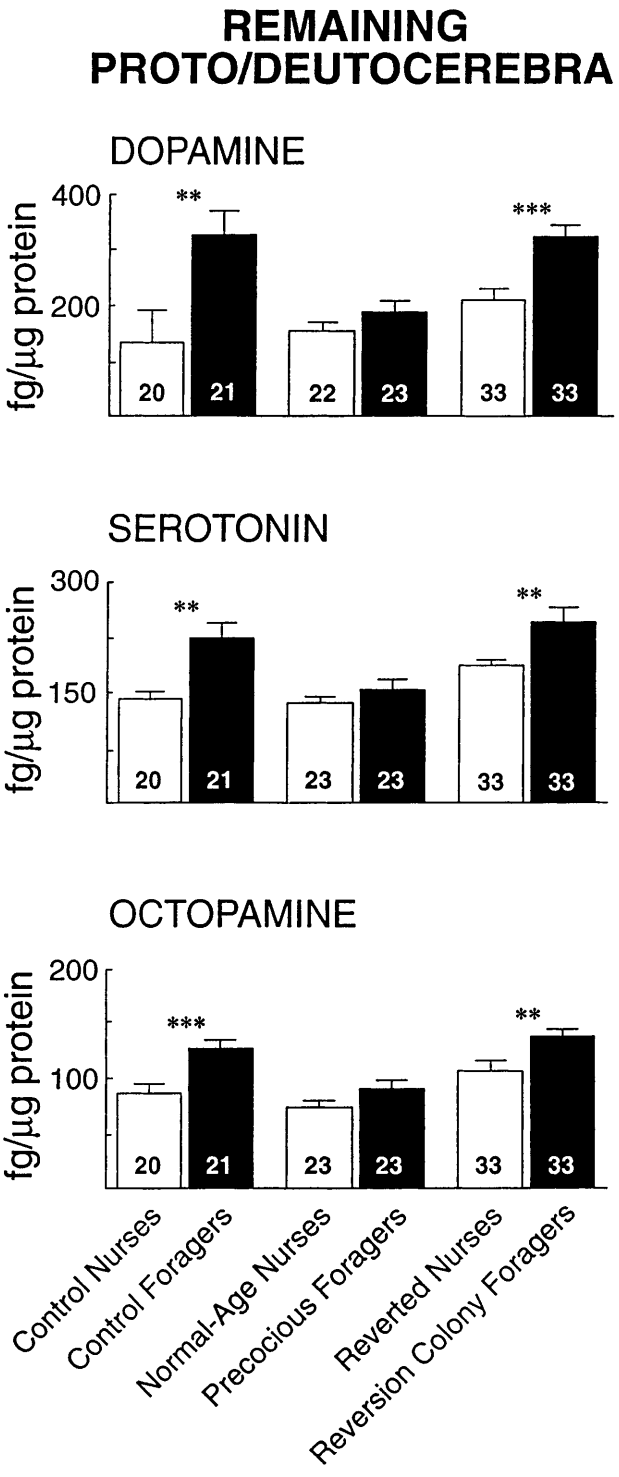

Fig. 3 Mean (+SE) concentrations of dopamine, serotonin, and octopamine in the remaining proto/deutocerebra of honey bees. Behavioral groups, statistical analyses, and symbols as in Fig. 1. ANOVA results are in Table 3

the antennal lobes and mushroom bodies. Another difference between the two studies is that Wagener-Hulme et al. (1999) found some effects of colony type and source colony on amine levels, but we did not. Comparisons of absolute levels of amines between the two

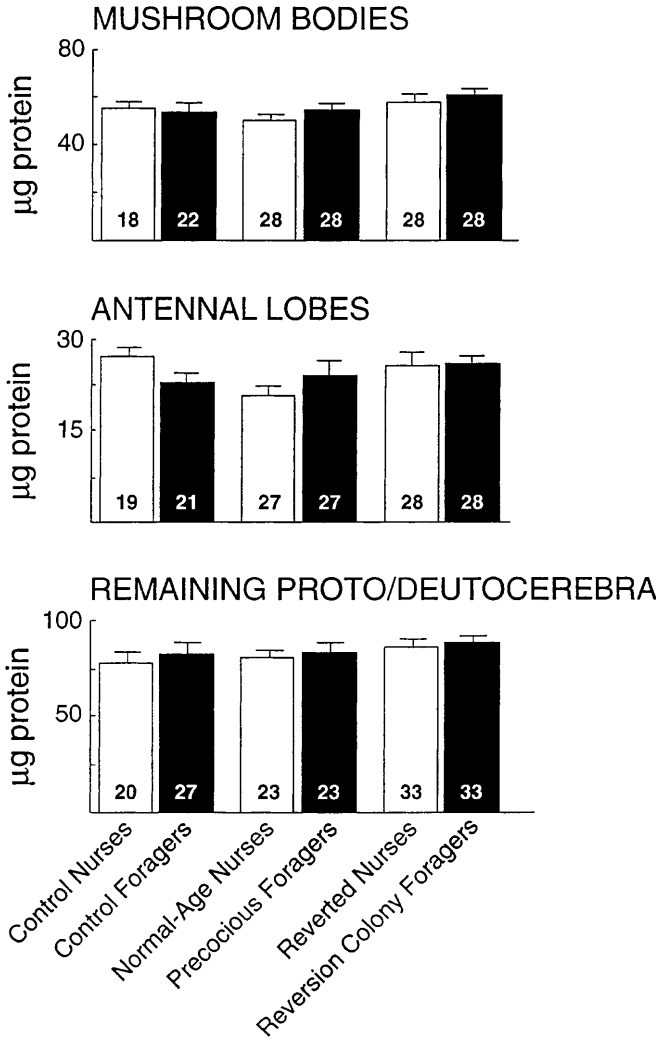

Fig. 4 Amounts of protein in samples of mushroom bodies (calyces and Kenyon cell somata), antennal lobes, and the remaining proto/deutocerebra of honey bees. Behavioral groups, statistical analyses, and symbols as in Fig. 1. ANOVA results are in Table 4

studies are difficult because of differences in extraction methods and quantification. We cannot explain the differences between the two studies but it is reassuring that the most robust findings of each study are consistent with one another: octopamine levels are closely associated with age-related division of labor in honey bees, and more so than are dopamine and serotonin.

If octopamine is playing a causal role in the regulation of honey bee behavioral development, high levels in the brain might influence one or both of the following processes: response thresholds for foraging-related stimuli (Mercer and Menzel 1982; Bicker and Menzel 1989; Hammer et al. 1993) and aspects of learning and memory associated with foraging (Hammer 1993; Menzel and Müller 1996). Our correlative, region-specific, analyses of biogenic amines were undertaken in part to gain further insight into these possibilities. If octopamine-mediated changes in olfactory processing are involved in regulating honey bee behavioral development, an unproven assumption, there should be behaviorally related differences in levels in the antennal lobes, which is what we found. Octopamine treatment to the brain is known to enhance responses to olfactory stimuli (Mercer and Menzel 1982).

If there are amine-mediated changes in learning and memory in the mushroom bodies that occur during honey bee behavioral development, also an unproven assumption, there should be differences in levels in the mushroom bodies that are related to behavioral status, age, or both; we found age-related changes. Laboratory studies have shown that octopamine treatment improves olfactory learning in foragers (Mercer and Menzel 1982; Macmillan and Mercer 1987). However, octopamine and serotonin act antagonistically in the modulation of olfactory learning in foragers (reviewed by Erber et al. 1993), while in our study octopamine and serotonin levels were both higher in old bees than in young bees. If our results have functional significance to honey bee behavioral development, one possibility is that the changes in the mushroom bodies are related to learning processes that are not directly related to the age at which they become nurses or foragers.

High levels of octopamine in the brains of forager bees may also be a consequence of octopaminergic regulation of the flight motor program, especially if high brain levels reflect high levels elsewhere in the CNS (Kozanek et al. 1988; cf. Linn et al. 1994). For example, high blood levels of octopamine are associated with flight in locusts (Goosey and Candy 1980; Bailey et al. 1984). Alternatively, high levels of octopamine in the brain may be a consequence of foraging. If either of these possibilities occur in honey bees, we might have expected no region-specific differences in amine levels, with foragers showing high levels throughout the brain. However, we have shown that changes in amine levels are region-specific.

Our results broadly agree with immunocytochemical analyses of biogenic amines in forager brains, the only group of bees examined in this way. For example, we found the highest concentrations of octopamine in the remaining proto/deutocerebra, followed by the antennal lobes and the mushroom bodies. Results from Kreissl et al. (1994) parallel these findings; the highest amount of octopamine immunoreactivity was in the central body and the protocerebral bridge, which would be found in our samples of remaining proto/deutocerebra. Kreissl et al. (1994) also found strong octopamine immunoreactivity throughout the antennal glomeruli, while the calyces of the mushroom bodies showed smaller numbers of stained processes and the Kenyon cell somata none at all. Comparisons of serotonin and dopamine immunoreactivity with our results also are consistent (Schürmann and Klemm 1984; Schäfer and Rehder 1989). The proximate cause(s) for increased amine levels in the honey bee brain may be related to one or more of the following possibilities: (1) increased synthesis, (2) decreased degradation, or (3) increased release into the brain from a source outside the measured regions. An increase in amine synthesis can be caused by individual aminergic neurons increasing their

activity, or by the birth of new aminergic neurons. However, using BrDU as a marker, no neurogenesis in adult honey bee brains was detected (Fahrbach et al. 1995).

Behaviorally related changes in octopamine levels in the antennal lobes are reminiscent of changes that occur in blood levels of juvenile hormone in honey bees. High levels of juvenile hormone are associated with foraging behavior under typical conditions, and using the same social manipulations as we used in this study, foragers have been shown to have higher blood levels of juvenile hormone than nurses regardless of age. In addition, removal of the corpora allata (glands that produce juvenile hormone) delays the onset of foraging, while treatment with juvenile hormone analogs causes precocious foraging (reviewed in Robinson 1998). It is intriguing that octopamine can stimulate production of juvenile hormone *in vitro* (Kaatz et al. 1994), and juvenile hormone analog treatment causes an increase in brain levels of octopamine, but not serotonin or dopamine (C. Wagener-Hulme and G. E. Robinson, unpublished observations). These results suggest that interactions between octopamine and juvenile hormone may be involved in the regulation of honey bee behavioral development, but additional studies are needed.

The role a particular neurochemical plays in behavioral regulation can be difficult to determine (e.g)., Monastirioti et al. 1996). We have demonstrated strong correlations between antennal lobe octopamine levels and behavior in the honey bee, but behavioral state has not been uncoupled from activity level, here or in Wagener-Hulme et al. (1999). Foragers were collected returning from a foraging trip and nurses were collected while performing their task in the hive; would nurses and foragers show the same amine levels when they were not currently active? It also is important to analyze other neuromodulators for evidence of antagonistic or synergistic effects on populations of neurons and behavioral output (e.g., Johnson and Harris-Warrick 1990). Treatment studies, especially with octopamine, are especially necessary to test the hypotheses suggested by the results of our correlative analyses.

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ERRATUM

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Biogenic amines and division of labor in honeybee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies

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After reviewing our figures in the original paper, we discovered that the *y*-axes in Figs. 1–3 are incorrect. All of the reported values are 10% of the actual values, because one "zero" was inadvertently left off each number on the *y*-axes (e.g., in Fig. 1 the range of the *y*-axis for dopamine should be 0–2000 fg μg^{-1} of protein). The original conclusions of the paper are not affected because all statistical analyses were performed on the correct data. It is still difficult to directly compare the levels of biogenic amines in honeybee brains reported in this paper to those in Wagener-Hulme et al. (1999), because dissection and HPLC techniques differed and values are expressed in this paper as femtograms per microgram of protein while in Wagener-Hulme et al. (1999) they are expressed as picograms per brain. However, the values of summed brain regions obtained from Figs. 1–3 with correct *y*-axes are now roughly comparable to those reported in Wagener-Hulme et al. (1999). We regret the error.

Reference

Wagener-Hulme C, Schulz DJ, Kuehn JC, Robinson GE (1999) Biogenic amines and division of labor in honey bee colonies. J Comp Physiol A 184: 471–479

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