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Biogenic amines and division of labor in honey bee colonies

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Abstract Brain levels of dopamine, serotonin, and octopamine were measured in relation to both age-related division of labor and inter-individual differences in task specialization independent of age in honey bee colonies. The only differences among similarly aged bees performing different tasks were significantly lower levels of dopamine in food storers than comb builders and significantly lower levels of octopamine in soldiers than foragers, but soldiers also were slightly younger than foragers. Differences associated with age-related division of labor were stronger. Older bees, notably foragers, had significantly higher levels of all three amines than did younger bees working in the hive. Using social manipulations to unlink chronological age and behavioral status, octopamine was found to exhibit the most robust association between behavior and amine level, independent of age. Octopamine levels were significantly lower in normal-age nurses versus precocious foragers and overage nurses versus normal-age foragers, but not different in reverted nurses versus reversion colony foragers. Dopamine levels were significantly lower in normal-age nurses versus precocious foragers, but higher in reverted nurses versus reversion colony foragers. Serotonin levels did not differ in any of these comparisons. These correlative results suggest that octopamine is involved in the regulation of age-related division of labor in honey bees.

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Introduction

Biogenic amines are among the best known neuromodulators in both vertebrates and invertebrates (Evans 1980; Bicker and Menzel 1989). They have been implicated in the control of numerous forms of behavior whose expression is influenced by changes in both environmental and physiological conditions. These include aggression (Summers and Greenberg 1995; Huber et al. 1997), feeding behavior (Long and Murdock 1983; Lent 1985), sexual behavior (Linn and Roelofs 1986), and learning and memory (Erber et al. 1993; Hasselmo 1995; Harvey 1996). Neuromodulators act by modifying the effects of hormones and neurotransmitters to modulate nervous system responses to various stimuli. Their broad distribution throughout the brain and other parts of the nervous system allow them to act on many neurons.

The biogenic amines dopamine, serotonin, and octopamine are found in the brain of the honey bee, *Apis mellifera* (Mercer et al. 1983; Schürmann and Klemm 1984; Schäfer and Rehder 1989; Fuchs et al. 1989; Schürmann et al. 1989; Brandes et al. 1990; Harris and Woodring 1992, 1995; Taylor et al. 1992; Kreissl et al. 1994). Applications of amines to bees affect sensitivity to olfactory stimuli and learning performance (Mercer and Menzel 1982; Mercer and Erber 1983; Macmillan and Mercer 1987; Michelsen 1988; Menzel et al. 1988). In addition, brain levels of amines vary with age (Fuchs et al. 1989; Harris and Woodring 1992) and behavioral state (Taylor et al. 1992), suggesting that they may be involved in controlling the complex changes in behavior that underlie colony division of labor.

Division of labor in honey bee colonies has been analyzed from behavioral, genetic, and endocrinological perspectives, but neurobiological studies of this complex behavioral system are only in their infancy. Division of labor in honey bee colonies is based on a pattern of

behavioral development by the individual worker bee (reviewed by Winston 1987). Bees work in the hive for the first 2–3 weeks of adult life performing such tasks as brood care and food processing, and then move to a phase of life dominated by foraging for nectar and pollen for their final 1–3 weeks. Age-related division of labor is flexible, however, and workers can respond to changing colony conditions by accelerating, delaying, or even reversing their behavioral development (Robinson 1992). Genetic and hormonal influences on honey bee behavioral development have been identified (reviewed by Page and Robinson 1991; Robinson 1992; Fahrbach and Robinson 1996; Fahrbach 1997; Robinson and Vargo 1997). Structural changes in two brain regions, the antennal lobes and the mushroom bodies, also have been detected in association with honey bee behavioral development (Withers et al. 1993; Durst et al. 1994; Winnington et al. 1996; Sigg et al. 1997; Fahrbach et al. 1998). However, it is not clear how these genetic, hormonal, and neural factors govern the changes in neural function that must underlie honey bee behavioral development.

A further division of labor also common in colonies of honey bees and other social insects involves specialization on different tasks by similarly aged workers (reviewed by Robinson 1992). For example, middle-age bees, 2–3 weeks old, perform a variety of tasks such as building comb, receiving nectar from incoming foragers, guarding the nest entrance, or removing corpses from the nest. Only a small percentage of a colony's workers act as guards (Moore et al. 1987) or undertakers (Visscher 1983; Trumbo et al. 1997); most bees apparently never perform these tasks. Even less is known of the underlying mechanisms for this form of behavioral differentiation than for age-related division of labor. Variation in worker genotype influences the likelihood of specializing on certain tasks at a given age, such as guarding or corpse removal (Robinson and Page 1988). In a survey of nine different groups of task specialists, the only endocrine correlates of division of labor among similarly aged workers that were detected were for guards and undertakers; both had high, forager-like, blood titers of juvenile hormone, higher than those of other middle-age bees (Huang et al. 1994). The neurobiology of specialization on different tasks by similarly aged workers is only beginning to be explored (Page et al. 1998).

The purpose of this study was to determine whether there are differences in dopamine, serotonin, or octopamine in the brain that occur in concert with age-related differences in behavior, interindividual differences in task specialization among similarly aged bees, or both. The rationale for this study is as follows. Bees live in a dense environment in which they are constantly exposed to a diverse array of stimuli that are each associated with different tasks, but they respond selectively, and differently, to these stimuli as a function of genotype, age, endocrine status, and colony needs. It is unlikely that this variation in behavior is based exclusively (if at all) on changes in peripheral perception of task-related stimuli (see Robinson 1987). It is more reasonable to hypothesize that CNS circuits are modulated in such ways as to increase the probability of responding to one set of stimuli while ignoring others (Beshers et al. 1999). Biogenic amines, with well-established roles as neuromodulators in other species and widespread distribution in the bee brain, are prime candidates for involvement in the control of division of labor in honey bees.

Previous studies have begun to correlate brain levels of biogenic amines in honey bees with age or behavior. The studies conducted to date have either focused on behaviorally identified bees of unknown age (Taylor et al. 1992), bees of known age but unknown behavioral status (Fuchs et al. 1989), or bees with inferred age differences (Harris and Woodring 1992). We measured brain levels of dopamine, serotonin, and octopamine from bees of both known age and behavior.

Materials and methods

General methods

Bees

Five typical colonies, each with a population of ~40 000 workers and a naturally mated queen, were used. To obtain large numbers of bees of known age, all (five to six) honeycombs containing old pupae were transferred from each colony to an incubator (33 °C). Workers emerging from these combs over each subsequent 24-h period were marked on the dorsal surface of the thorax with a spot of paint (Testor's PLA) and reintroduced to their natal colony. We marked ~5000–10 000 bees per colony, about 1000 bees per day. Colonies were otherwise maintained according to standard techniques at the University of Illinois Bee Research Facility, Urbana, Illinois. Experiments were performed from 1992 to 1994. Colonies were typical of North American populations of *Apis mellifera* (a mix of predominantly European subspecies, see Phillips 1915; Pellett 1938).

Collections

Bees of known age that were performing a particular behavior were collected into liquid N_2. We attached a thermos of liquid N_2 to a portable vacuum (Robinson and Page 1988) and sucked the bees of interest into the liquid N_2, where they were immediately frozen. This was done so that measurements of amine levels would reflect as much as possible the levels that existed naturally during the performance of the behavior. Because all bees were frozen within 1–2 s of collection, this technique also minimized the possibility of stress-induced changes in biogenic amines (Davenport and Evans 1984; Harris and Woodring 1992; Hirahima and Eto 1993). All bees were later transferred to a −80 °C freezer until dissection and analysis.

Brain dissection

Dissections were performed on a dissecting dish cooled by dry ice; brains remained frozen during the entire dissection procedure to minimize amine degradation. Hypopharyngeal glands, the subesophageal ganglion, and optic lobes were removed during dissection and discarded. Occasionally pieces of the brain were lost during dissection; because amine levels are expressed on a per brain basis (see below), partial brains were discarded and only intact brains (minus optic lobes) were analyzed. Each brain was then

placed individually in a 1.5-ml Eppendorf tube and stored (−80 °C) until analysis.

Sample preparation

Forty microliters of chilled perchloric acid (0.2 mol·l^{-1}) were added to each tube while on ice (4 °C). The perchloric acid contained dihydroxybenzylamine (DHBA) and synephrine as internal standards, at concentrations of 50 pg µl^{-1} and 25 pg µl^{-1}, respectively. DHBA was used to monitor recovery of dopamine and serotonin, and synephrine was used for octopamine. Each sample was sonified, rechilled for 20 min, and centrifuged at 13 000 rpm (15 000 *g*) for 10 min at 4 °C (Hermle 2360K centrifuge). The sample was transferred to a 0.22-µm nylon membrane filter in a new Eppendorf tube and centrifuged again for 6 min. The filtered sample was transferred to a microvial (precooled to 2 °C) for analysis by high-performance liquid chromatography (HPLC). All reference compounds were purchased from Research Biochemicals Incorporated.

Measurement of biogenic amines

The HPLC system consisted of a refrigerated Kontron automatic injector, a Shimadzu (LC-10AD) pump, a 80 mm × 4.6 mm high-efficiency reverse-phase catecholamine HR-80 column (#316 stainless steel, 3-µm spherical octadecylsilane packing), and a Coulochem II electrochemical detector coupled to a 2-channel model 5014 microdialysis analytical cell (ESA, inc.). Channel 1 of the detector was set at 425 mV for dopamine and serotonin and channel 2 was set at 650 mV for octopamine. The mobile phase (pH = 5.6) was composed of 15% methanol, 15% acetonitrile, 1.5 mmol·l^{-1} sodium dodecyl sulfate, 75 mmol·l^{-1} sodium phosphate monobasic, and trace amounts of triethylamine and EDTA (Sigma Chemical), with water purified by a Nanopure II system (Millipore). The flow rate of the mobile phase was 1 ml min^{-1}. Samples were analyzed in groups of either 24 or 48; external standards were run before and after the 24-sample runs, and before, midway through, and after the 48-sample runs.

Synephrine was used as an internal standard for brains from the last three colonies analyzed because we assumed it would increase the precision of octopamine quantification (like octopamine, synephrine is detected on Channel 2, while DHBA is detected on Channel 1). Because synephrine is a natural metabolite in some invertebrates (Osborne 1996), a preliminary study was performed to determine whether it can be used as an internal standard for honey bees. First, extracts from individual brains (n = 10) were split into two equal portions; half were run with a synephrine internal standard that was used to calculate octopamine levels and half without. The results suggested no presence of endogenous synephrine: there were no significant differences in octopamine amounts between the halves. Second, using synthetic synephrine as our reference compound, we could not detect endogenous synephrine in samples of bee brains (n = 16; limit of detection = 25 pg). These results indicate that synephrine can be used as an internal standard for octopamine quantification in honey bees, at least under the conditions described here.

Results are expressed on a per brain basis (minus optic lobes), as in Taylor et al. (1992). Limited data suggest that the amount of protein in the bee brain remains constant throughout adult behavioral development (Fuchs et al. 1989; Schulz and Robinson 1999). The overall volume of the bee brain does not change during behavioral development, though parts of the mushroom bodies and antennal lobes do (Withers et al. 1993; Durst et al. 1994; Winnington et al. 1996; Sigg et al. 1997).

Experiment 1: brain amines, age-related division of labor, and specialization on different tasks by similarly aged workers

Experiment 1 was a broad survey of bees performing one of nine different tasks. We explored whether there are differences in brain levels of biogenic amines that are associated with either age-related division of labor or with particular specializations on different tasks by similarly aged workers. We sampled "young," "middle-age," and "old" honey bee workers performing different tasks. Young bees were nurses or queen attendants; middle-age bees were food storers, comb builders, corpse removers (undertakers), and guards; and old bees were soldiers, pollen foragers, and nectar foragers. (Guards patrol the hive entrance while soldiers respond to a disturbance with stinging behavior; Breed et al. 1990.) The mean age of bees in each group is given in Table 1. We unintentionally collected soldiers that were significantly younger than both forager groups, but soldiers were still significantly older than middle-age bees.

Behavioral identification was after Huang et al. (1994), which in turn was based on descriptions in an extensive literature (references cited therein). For the sake of brevity, descriptions are given of the two most extensively analyzed behaviors in all three experiments, nursing and foraging. Both are thought to be performed by all bees; nurses and foragers also differ clearly in behavior, juvenile hormone titers, brain structure, and under typical conditions, age (Robinson 1998). Nurse bees were individuals with their heads in cells containing larvae. Returning pollen and nectar foragers were obtained by temporarily obstructing the hive entrance with a piece of 8-mesh hardware cloth. Pollen foragers were those with pollen loads in their corbiculae, while nectar foragers were initially identified as bees without pollen but with distended abdomens. The presence of a nectar load was then confirmed during brain dissection by squeezing the slightly thawed (now isolated) abdomen; any bee that did not possess a nectar load was eliminated from the sample. This method does not distinguish nectar foragers from water foragers, but the latter are generally rare (see Robinson and Page 1989).

Nurses, guards, nectar foragers, pollen foragers, and soldiers were collected from all five colonies; queen attendants and food storers, from four out of five colonies; comb builders from three out of five colonies; and undertakers from two out of five colonies. One-day-old adult bees also were collected from each colony.

Chromatogram analyses and amine quantification for bees sampled in 1992 (two of five colonies) were performed with Dynamax MacIntegrator II (Rainin Instrument) and EXCEL (Microsoft) software. EZChrom Chromatography Data System, version 6 (Scientific Software) was used in subsequent analyses.

Experiment 2: brain amines and chronological age versus behavioral status – single-cohort colonies

To uncouple chronological age from behavioral status, "single-cohort" colonies were used (Robinson et al. 1989). Brains were compared from normal-age nurses versus precocious foragers and overage nurses versus normal-age foragers. Nursing and foraging are among the easiest tasks to observe in small experimental colonies.

Single-cohort colonies were established as follows. One-day-old (0–24 h) bees were marked for 1–3 days until approximately 1500–2000 bees were obtained. A queen (unrelated to the workers), one frame of honey and pollen, and one frame of empty comb (for egg laying) were placed into the hive with the first group of marked bees. The colony was placed outside after the requisite number of bees was obtained. One frame of eggs (from another colony) also was placed in the hive at this time. The queen was released from her cage and the colony entrance opened 1 day after placing the colony outside. Precocious foraging occurs in single-cohort colonies due to the lack of older bees (Huang and Robinson 1992, 1996), and usually occurs when bees are 7–10 days old.

Observations began when the bees were 5 days old. Each colony was observed for two 1- to 2-h periods a day, once before noon (0900–1200 hours) and once in the afternoon (1500–1700 hours). Behavioral observations of nurses and foragers were as in Experiment 1. Forager samples consisted of a mixture of both nectar and pollen foragers. Precocious foragers and normal-age nurses were collected at 7–9 days of age and overage nurses and normal-age foragers at 21–24 days of age. To obtain average nurses, the emergence of new adults was prevented by replacing combs of developing pupae with combs of eggs and young larvae from other colonies. Care was taken to collect foragers at times when there

were no orientation flights occurring in front of the hive; these flights, easily distinguishable from foraging flights, are taken prior to the onset of foraging (Winston 1987). Five single-cohort colonies were established, one from each colony used in Experiment 1; the colonies in Experiment 1 were thus "source colonies" for the single-cohort colonies. Behavioral identification, brain dissection and amine quantification were as in Experiment 1. Samples of "control" nurses and (pollen) foragers taken from each source colony in Experiment 1 were used for comparative purposes here as well.

Experiment 3: brain amines and chronological age versus behavioral state – reversion colonies

Worker age and behavioral status also were uncoupled by creating colonies in which some foragers were induced to "revert" to nursing activities (Page et al. 1992; Robinson et al. 1992). Reversion colonies were made from three of the five colonies used in Experiment 1. After collections for Experiment 1 were completed, the remaining marked bees were allowed to age until they were 30–44 days old. At that point, the hive entrance was obstructed temporarily with a piece of 8-mesh hardware cloth and about 2000 age-marked bees were collected that were either pollen foragers, nectar foragers, or bees with worn wings (presumably due to foraging). They were vacuumed directly into a small hive containing one comb of uncapped larvae, one comb of honey and pollen, one empty comb, and the colony's original queen. When the 2000 bees were collected, the parent colony was closed and moved about 10 km away. The "reversion colony" was moved to the location of its parent colony to minimize the loss of its bees to other colonies in the apiary via "drifting." Reverted nurses and bees that continued to forage ("reversion colony foragers") were collected 2 days after colony establishment. Behavioral identification, dissection, and amine quantification were as in Experiment 1. Reverted nurses differ from overage nurses; the former have made the transition from nursing to foraging before once again resuming nursing activities, while the latter are assumed to continuously exhibit nursing behavior for a prolonged period of time.

Samples of control nurses and foragers taken from each source colony were again used, but in contrast to Experiment 2, these were additional samples that were not part of the data set for Experiment 1. Control nurses were collected approximately 3 weeks prior to establishing reversion colonies while control foragers were collected 1 week prior.

Statistical analyses

A total of 1320 bee brains were analyzed for dopamine, serotonin, and octopamine. Data from Experiment 1 were not normally distributed so analyses were performed on (natural) log-transformed data. In Experiment 1, two-way analysis of variance was used to examine the effects of age and source colony on brain levels of amines. Post-hoc multiple range tests (Student-Newman-Keuls test; $P < 0.05$ significance value) were used to analyze differences among the behavioral groups. In Experiments 2 and 3, four-way analysis of variance was used to examine the effect of behavior, age, source colony, and colony type (single cohort/reversion versus typical) on brain levels of amines. The effect of colony type was determined in Experiments 2 and 3 by including in the analyses normal age nurses and foragers collected from each source colony as described above. In Experiments 2 and 3, one-tailed *t*-tests also were used to examine the a priori hypothesis that levels of amines are higher in foragers than nurses in each colony type, regardless of worker age. Other pair-wise analyses, such as comparisons of foragers in typical versus single-cohort colonies, were not made; differences of this type would be detected as colony-type effects in the ANOVA, and our primary focus was to test for differences between nurses and foragers. EXCEL (version 4, Microsoft) and SAS were used for statistical analyses.

Because of the large amount of data collected in this study (five colonies, three amines, ten behavioral groups in Experiment 1 alone), the results from all colonies in each experiment were pooled, even though there were significant differences between source colonies (see Tables 2–4). Sample sizes for each behavioral group from each colony were similar (median = 23, range = 9–38). Careful inspection of the results indicate that the pooled colony data are an accurate reflection of the general patterns seen in the data from individual colonies. Differences between colonies are described for some of the key comparisons in the study.

Results

Experiment 1: brain amines, age-related division of labor, and specialization on different tasks by similarly aged workers

Brain levels of biogenic amines are shown in Fig. 1. There were highly significant source colony differences for dopamine, serotonin, and octopamine (Table 2). There also were highly significant age differences: older bees had higher levels of dopamine, serotonin, and octopamine than did younger bees. Significant age differences persisted even after 1-day-old bees were removed from the ANOVA ($P < 0.05$ for all three amines; data not shown).

There were significant behaviorally related differences for dopamine, serotonin, and octopamine (Fig. 1). The main trend was that older bees, notably foragers, had higher levels of amines than did bees working in the hive. For dopamine, nectar foragers were the only bees to differ significantly from all behavioral groups of young and middle-age bees. For serotonin, soldiers, pollen foragers, and nectar foragers differed significantly from all younger behavioral groups; for octopamine, it was pollen foragers and nectar foragers. As an indication of the inter-colony variation that existed in this study, in each of the five colonies nectar foragers had 10–37% higher levels of dopamine, 23–35% higher levels of serotonin, and 21–51% higher levels of octopamine than did nurses (data not shown).

Differences among similarly aged bees performing different tasks were rare. There were significantly lower levels of dopamine in food storers than comb builders

Table 1 Age of bees sampled in each behavioral group, in three age categories (plus 1-day-old bees): young (queen attendants and nurses), middle-age (food storers, comb builders, undertakers, and guards), and old (soldiers, nectar foragers and pollen foragers). Bees were sampled from five typical colonies. Groups that differed significantly from one another in age are indicated with different letters ($P < 0.05$, ANOVA and SNK post-hoc tests)

Behavioral group	Mean age ± SE (n = number of bees)	
1-day-old bees	1.0 (98)	a
Queen attendants	7.8 ± 0.2 (83)	b
Nurses	7.6 ± 0.1 (84)	b
Food storers	14.5 ± 0.5 (79)	c
Comb builders	16.6 ± 0.4 (62)	cd
Guards	18.6 ± 0.3 (108)	d
Undertakers	17.9 ± 0.3 (39)	d
Soldiers	27.1 ± 0.5 (84)	e
Nectar foragers	30.0 ± 0.6 (93)	f
Pollen foragers	29.9 ± 0.7 (69)	f

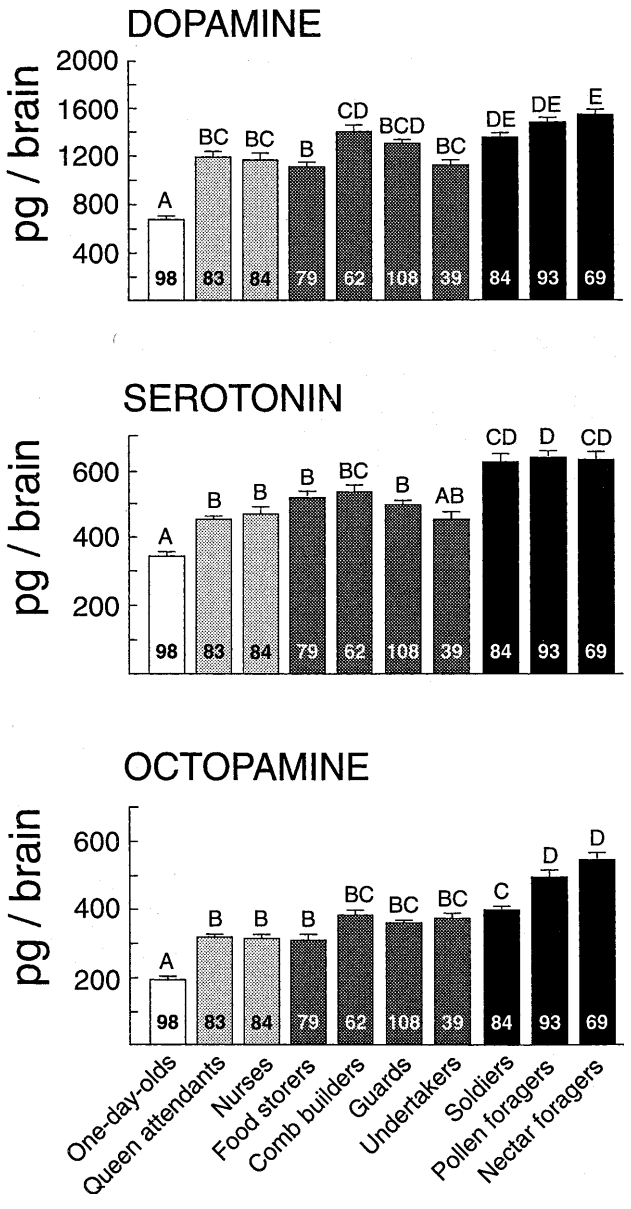

Fig. 1 Mean (+SE) brain levels of biogenic amines in adult worker honey bees of known age and behavioral status. Sample sizes indicated in each bar (data are from five colonies). Results of ANOVA in Table 2. Groups that differed significantly from one another in levels of biogenic amines are indicated with different letters above bars ($P < 0.05$, SNK post-hoc test)

and significantly lower levels of octopamine in soldiers than foragers (Fig. 1). However, soldiers also were significantly younger than foragers (Table 1).

Experiment 2: brain amines and chronological age versus behavioral state – single-cohort colonies

Results from Experiment 1 indicated significant age and behavior differences for all three amines. Older bees, notably foragers, had higher levels than did younger bees that work in the hive. Are these differences more closely associated with differences in worker age, behavioral state, or both? This issue was addressed in both Experiments 2 and 3.

Table 2 Results of two-way ANOVA for brain levels of biogenic amines in adult worker honey bees as a function of age and source colony. Data in Fig. 1

Effect	*df*	*F*	*P*
A. DOPAMINE			
Age	3	175.9	0.0001
Source colony	4	7.0	0.0001
B. SEROTONIN			
Age	3	124.4	0.0001
Source colony	4	81.9	0.0001
C. OCTOPAMINE			
Age	3	216.3	0.0001
Source colony	4	26.6	0.0001

Brain levels of biogenic amines for bees from single-cohort colonies are shown in Fig. 2. There were highly significant differences between source colonies for dopamine, serotonin, and octopamine (Table 3). There also were significant differences between colony types (single cohort versus typical) for serotonin and octopamine, but not dopamine. This result suggests that there were differences in octopamine and serotonin between nurses (or foragers) in single-cohort colonies and typical colonies but we did not subject these data to further analyses because the main focus of this experiment was to compare similarly aged nurses and foragers within colonies.

There were highly significant age-related differences for dopamine, serotonin, and octopamine. As in Experiment 1, older bees had higher levels of amines than did younger bees. There also were significant behaviorally related differences for octopamine and dopamine, with octopamine showing bigger differences. ANOVA revealed no behaviorally related differences for serotonin.

Pair-wise comparisons of nurses and foragers within single-cohort colonies and typical colonies were performed (Fig. 2). Based on the results of Experiment 1, we tested the a priori hypothesis that foragers have higher brain levels of amines than do nurses, regardless of age. For dopamine, levels were significantly higher in control foragers versus control nurses (already reported in Experiment 1) and in precocious foragers versus young nurses. There were no significant differences in dopamine levels between normal-age foragers and overage nurses. For serotonin, only the control foragers and nurses differed significantly. For octopamine, levels were significantly higher in all three comparisons: precocious foragers versus normal-age nurses, normal-age foragers versus overage nurses, and control foragers versus control nurses.

Experiment 3: brain amines and chronological age versus behavioral state – reversion colonies

Brain levels of biogenic amines from reversion colonies are shown in Fig. 2. There were significant differences

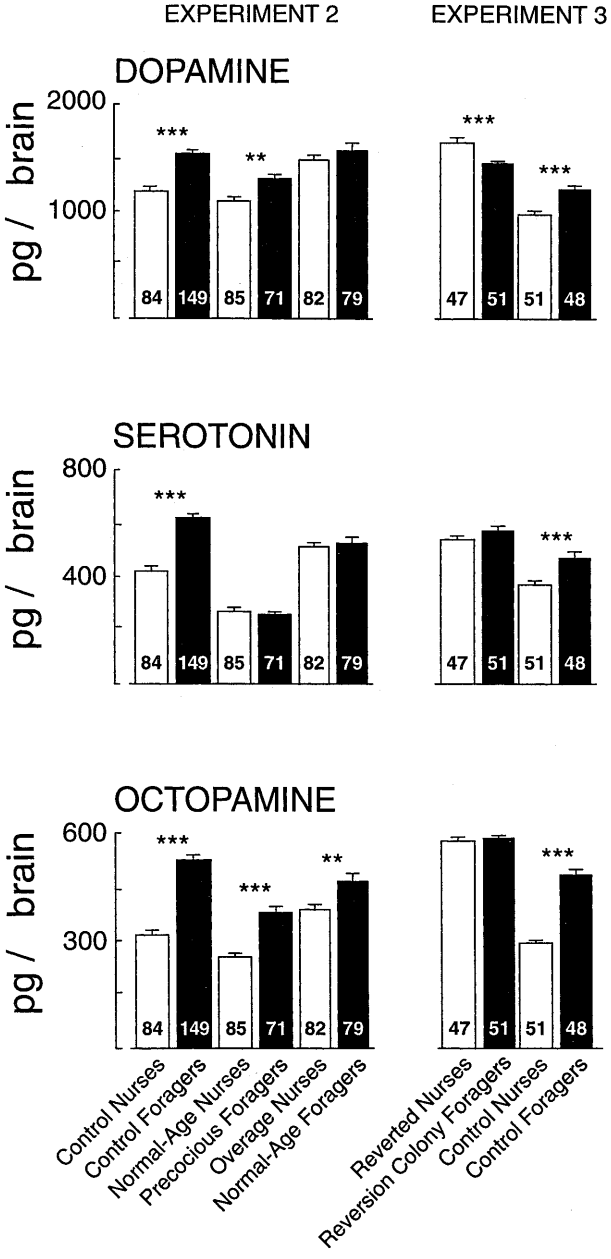

Fig. 2 Mean (+SE) brain levels of biogenic amines in foragers and nurses from single-cohort and reversion colonies. Control nurses and foragers were taken from the source colonies used to make the single-cohort and reversion colonies. In Experiment 2, these samples were also used in Experiment 1; in Experiment 3, separate samples from the same source colonies were used. Sample sizes indicated in each bar (data are from five single-cohort colonies and three reversion colonies). Results of ANOVA in Tables 3 and 4. *Asterisks* above bars denote results of one-tailed *t*-tests on each pair of nurse and forager groups based on the a priori hypothesis that levels of amines are higher in foragers than nurses in each colony type, regardless of worker age: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$

between source colonies for dopamine and octopamine (Table 4). There also were significant differences between colony types (reversion and typical colonies) for all three amines. These results again suggest that there were some differences between nurses or foragers in reversion colonies and typical colonies.

Table 3 Results of four-way ANOVA for brain levels of biogenic amines in adult worker honey bees as a function of age, behavior, source colony, and colony type. Data are from typical and single-cohort colonies. Data in Fig. 2

Effect	*df*	*F*	*P*
A. DOPAMINE			
Age	1	51.3	0.001
Behavior	1	5.8	0.017
Source colony	4	12.3	0.001
Colony type	1	0.0	0.992
B. SEROTONIN			
Age	1	29.9	0.001
Behavior	1	2.4	0.123
Source colony	4	3.9	0.004
Colony type	1	17.7	0.001
C. OCTOPAMINE			
Age	1	58.9	0.001
Behavior	1	43.3	0.001
Source colony	4	10.9	0.001
Colony type	1	12.9	0.004

There were highly significant age-related differences for dopamine, serotonin, and octopamine. Pair-wise analyses of nurses and foragers within reversion colonies and typical colonies reveal that this was largely a consequence of differences between control nurses and foragers.

There were highly significant behaviorally related differences for dopamine, but not for serotonin or octopamine. However, reverted nurses had significantly higher levels of dopamine than did foragers, a result not consistent with the results of Experiments 1 and 2.

Discussion

The principal significance of this study is that it demonstrates striking changes in brain levels of dopamine, serotonin, and octopamine during honey bee behavioral

Table 4 Results of four-way ANOVA for brain levels of biogenic amines in adult worker honey bees as a function of age, behavior, source colony, and colony type. Data are from typical and reversion colonies. Data in Fig. 2

Effect	*df*	*F*	*P*
A. DOPAMINE			
Age	1	36.5	0.001
Behavior	1	15.8	0.001
Source colony	2	8.9	0.001
Colony type	1	21.8	0.001
B. SEROTONIN			
Age	1	7.3	0.008
Behavior	1	1.4	0.235
Source colony	2	0.2	0.842
Colony type	1	19.8	0.001
C. OCTOPAMINE			
Age	1	41.3	0.001
Behavior	1	0.2	0.660
Source colony	2	3.2	0.042
Colony type	1	27.2	0.001

development. These results extend the findings of Fuchs et al. (1989), Harris and Woodring (1992), and Taylor et al. (1992).

One purpose of this study was to determine whether there are differences in dopamine, serotonin, or octopamine that are associated with interindividual differences in task specialization among similarly aged bees. There were significantly lower levels of dopamine in food stores than comb builders, but no other differences among other middle-age groups. The significance of this result is not clear. There also were significantly lower levels of octopamine in soldiers than foragers, but soldiers were significantly younger, even though they still were among the oldest bees. Higher levels of octopamine, at least in the blood, are typically associated with higher states of arousal in other insect species (Adamo et al. 1995), so soldiers might have been expected to have higher levels than foragers. However, both high or low levels of a particular biogenic amine can be associated with a particular behavior in different species. For example, low levels of serotonin are implicated in the expression of aggressive behavior in vertebrates while high levels play a similar role in invertebrates such as lobsters and crayfish (e.g., Huber et al. 1997). Our results do not rule out the possibility that there is a neurochemical basis to the specializations observed among similarly aged bees. Perhaps more sophisticated multivariate analyses of these three amines as a group (Adamo et al. 1997) or together with other neuroactive compounds would reveal distinct profiles of neurochemical differences between some of the groups studied here. Another possibility is that analyses need to be conducted on specific brain regions, rather than on the whole brain, to discern neurochemical correlates of interindividual specialization in honey bee colonies.

The second purpose of this study was to determine whether there are differences in dopamine, serotonin, or octopamine that are associated with age-related division of labor. These analyses show that brain amine levels can be influenced by source colony, colony type, age, and behavioral differences. Source colony differences in amine levels, already shown by Harris and Woodring (1992), may reflect environmental or genetic factors, or both. Effects of colony type were detected, such as higher levels of dopamine in nurses relative to foragers only in reversion colonies. These results were not consistent and reasons for such effects are not known, but our method of statistical analysis removes any confounding effect of colony type on the detection of age- and behaviorally related differences.

There is a strong age-related increase in dopamine, serotonin, and octopamine, but are all three related to behavioral development and division of labor? Age and behavior influences cannot be separated in Experiment 1 because bees performed different tasks at different ages. The relationships between amine levels and worker age and behavioral state are more clear from the results of Experiments 2 and 3. There are higher levels of octopamine in foragers than in nurses, independent of age, in comparisons of precocious foragers versus normal-age nurses and normal-age foragers versus overage nurses, but not in reversion colony foragers versus reverted nurses. This pattern for octopamine is much more suggestive of an involvement in division of labor than the pattern for dopamine and serotonin. There are consistent age-related differences in brain levels of serotonin and dopamine, but they are not tightly linked with behavioral status. These results suggest that dopamine and serotonin levels reflect previous experience or aging, rather than current behavioral state. In contrast, the association of high octopamine and foraging behavior suggests that increases in octopamine influence the likelihood of foraging, but treatment experiments are necessary to test this hypothesis.

One possibility is that high brain levels of octopamine lower response thresholds for foraging-related stimuli (Mercer and Menzel 1982; Bicker and Menzel 1989; Hammer et al. 1993) or influence aspects of learning and memory associated with foraging (Hammer 1993; Erber and Kloppenburg 1995; Kloppenburg and Erber 1995). High levels of octopamine in the brain also may be a consequence of octopaminergic regulation of the flight motor program (Bailey et al. 1984; Orchard et al. 1993; Weisel-Eichler and Liebersat 1996), if high brain levels reflect high levels elsewhere in the CNS (Kozanek et al. 1988; cf. Linn et al. 1994). Alternatively, high levels of octopamine in the brain may be a consequence of foraging. Insight into these possibilities and to the role of biogenic amines in the regulation of age-related division of labor in honey bee colonies in general can be gained by measuring biogenic amines in specific brain regions rather than in the whole brain. The companion paper (Schulz and Robinson 1999) reports the results of such analyses.

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