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Octopamine influences division of labor in honey bee colonies

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Abstract Forager honey bees have higher brain levels of octopamine than do bees tending larvae in the hive. To test the hypothesis that octopamine influences honey bee division of labor we treated bees orally with octopamine or its immediate precursor tyramine and determined whether these treatments increased the probability of initiating foraging. Octopamine treatment significantly elevated levels of octopamine in the brain and caused a significant dose-dependent increase in the number of new foragers. This effect was seen for precocious foragers in single-cohort colonies and foragers in larger colonies with more typical age demographies. Tyramine treatment did not increase the number of new foragers, suggesting that octopamine was exerting a specific effect. Octopamine treatment was effective only when given to bees old enough to forage, i.e., older than 4 days of age. Treatment when bees were 1–3 days of age did not cause a significant increase in the number of new foragers when the bees reached the minimal foraging age. These results demonstrate that octopamine influences division of labor in honey bee colonies. We speculate that octopamine is acting in this context as a neuromodulator.

Key words *Apis mellifera* · Division of labor · Honey bee · Neuromodulation · Octopamine

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Introduction

Honey bee colonies exhibit age-related division of labor, a consequence of individual bees changing jobs as they grow older. Young adults perform tasks in the hive such as feeding larvae ("nursing") while the oldest bees in a colony forage for nectar and pollen outside the hive. Division of labor in honey bee colonies is controlled by the interaction between the needs of the colony and the physiological states of the colony's workers (Franks and Tofts 1994; Robinson et al. 1994; Gordon 1996). The amount of food stores (Schulz et al. 1998; Fewell and Bertram 1999) and brood (Dreller et al. 1999; LeConte et al. 2001), and the age structure of the worker force (Huang and Robinson 1992, 1996) are among the colony-level factors that have been implicated in the control of honey bee division of labor. Individual-level genetic and endocrine factors that influence division of labor have been identified (reviewed by Robinson 1992; Robinson and Vargo 1997), and changes in brain structure (Fahrbach and Robinson 1996) and gene expression in the brain (Kucharski at al. 1998; Toma et al. 2000) also may be involved. However, the neurobiology of honey bee age-related division of labor is only beginning to be understood.

The biogenic amines octopamine, serotonin, and dopamine are well-known neuromodulators in both invertebrates and vertebrates (Roeder 1994; Seigel et al. 1999). Honey bee foragers have higher brain levels of all three amines than do nurses (Taylor et al. 1992; Wagener-Hulme et al. 1999). The association between amine levels and division of labor is particularly strong in the antennal lobes, especially for octopamine. Using social manipulations to obtain nurse bees and foragers that are the same age, Schulz and Robinson (1999) showed that foragers had higher antennal lobe levels of octopamine than did nurses, regardless of age. In contrast, octopamine levels in the mushroom bodies varied with bee age rather than nurse/forager status. These results suggest that octopamine is involved in the control of division

of labor, perhaps by modulating olfactory response-thresholds to task-related stimuli in the beehive (Robinson 1987b; Beshers et al. 1999). However, high brain levels of octopamine may either play a causal role in foraging, or occur as a consequence of foraging, or both.

This study used treatment experiments to test the hypothesis that octopamine is involved in the control of division of labor in honey bee colonies, and if so, to begin to explore its general mode of action.

Materials and methods

Four experiments were performed, each experiment replicated three to four times. In experiment 1 we treated bees orally with octopamine or its immediate precursor tyramine and determined whether these treatments increased the probability of initiating foraging. Tyramine was used to probe for the specificity of the octopamine effect. In experiment 2 we tested whether the octopamine effect was dose dependent. In experiment 3 we determined whether octopamine treatment also increases the probability of initiating foraging in typical colonies; experiments 1 and 2 were performed with small experimental "single-cohort colonies" for better efficiency and control of other variables. In experiment 4 we began to investigate how octopamine might work to influence division of labor by studying whether the octopamine effect was age dependent. We treated bees that were either too young to forage (1–3 days old) or just able to forage (4–6 days old) to explore whether octopamine treatment exerts long-term developmental effects or more short-term neuromodulatory effects.

Bees

Experiments were performed during the summers of 1998 and 1999 at the University of Illinois Bee Research Facility. Honey bees (*Apis mellifera* L.) were a typical North American mixture of predominantly European subspecies (Phillips 1915; Pellet 1938), and maintained according to standard procedures.

Single-cohort colonies

Single-cohort colonies were used in experiments 1, 2, and 4. A single-cohort colony is a small colony experimentally established entirely with 1-day-old adult bees. Some bees in single-cohort colonies become precocious foragers because of the lack of an existing foraging force (Robinson et al. 1989; Huang and Robinson 1992). Using single-cohort colonies allowed us efficiently to test whether a neurochemical affects division of labor, i.e., in trials that lasted approximately 1 week each. In contrast, experiments with typical colonies can last up to 3–4 weeks, which is the average age at onset of foraging in a typical colony (Michener 1974). Using single-cohort colonies also enabled us to precisely control colony age demography, genotypic composition, and population size of experimental colonies. Control of these colony variables was particularly important because the treatment was administered to whole colonies (see below).

To establish a single-cohort colony, ca. 1000–1250 1-day-old adult bees were obtained from frames of honeycomb containing old pupae from large colonies in our apiaries ("source colonies"). The frames were placed in an incubator at 33 °C until adults emerged. Each single-cohort colony in a trial was made up of a random mix of 1-day-old bees from two to eight source colonies so that all colonies in a trial had roughly equivalent genotypic compositions. It was necessary to use multiple source colonies to obtain a sufficient number of bees for the two to four single-cohort colonies used in each trial. Each bee was marked on the thorax with a spot of Testor's enamel paint, a different color for each colony.

Each colony in a trial contained the same number of 1-day-old bees, a single frame of honeycomb containing treated or control sucrose solution (see below), roughly the same amount of pollen, and a mated queen less than 1 year old obtained from the same commercial source. The location of treated and control colonies in the apiary was randomized from trial to trial to minimize possible microclimate effects on the number of bees foraging.

Biogenic amine treatment

Bees were allowed to feed ad libitum on sugar syrup containing octopamine or tyramine, which exposed them to neurochemical treatment on a chronic basis. A 50% sucrose solution treated with amine was placed in an otherwise empty honeycomb, and replenished daily so that bees never experienced a food shortage. Control bees were fed 50% sucrose with no amine. Chronic treatment was necessary because bees spend at least 4 days in the hive before initiating foraging, even precocious foragers in a starved single-cohort colony (e.g., Schulz et al. 1998). While direct injections of octopamine into the brain of honey bees have been used successfully for short-term behavioral assays (Hammer and Menzel 1998), a single injection elevates amine levels only for about 1–2 h (Linn et al. 1994). Repeated injections were rejected as probably too stressful, especially because octopamine brain levels rise in response to handling-related stress in bees (Harris and Woodring 1992). Oral treatment of octopamine did increase brain levels of octopamine (see Results), although it is not clear by what mechanism (see Discussion). Oral treatment served as a non-invasive method of chronically and reliably elevating brain levels of octopamine essential for this study. Harris and Woodring (1999) also developed a similar technique for caged bees in the laboratory. A disadvantage of this method is that it probably also resulted in other tissues besides the brain being exposed to increased amounts of octopamine, an issue we address in the Discussion.

Collection, dissection, and HPLC analysis

HPLC analyses were conducted to determine whether oral treatment of octopamine in the field elevates brain levels of octopamine. We also simultaneously tested for effects on levels of dopamine and serotonin; these data were obtained from the same chromatograms and served as an indicator of the specificity of the treatment effect. In one trial (trial 1 of experiment 2) bees fed one of four different doses of octopamine-treated sucrose solution were collected directly into liquid nitrogen for analysis (as in Wagener-Hulme et al. 1999). In a second trial (not used in behavioral studies), bees from a single-cohort colony treated either with octopamine (2.0 mg ml^{-1}) or control sucrose were collected for analysis. Bees were collected randomly from all parts of the comb when they were 5 days old, before the onset of any flight activity by the colony. Differences in numbers of bees analyzed for each amine result are because in a few chromatograms not all amine peaks were analyzable. All bees were stored at –80 °C until brain dissection and HPLC analysis. Whole bee heads were freeze-dried to facilitate dissection (as in Schulz and Robinson 1999). Brain dissections were conducted on dry ice and HPLC analyses performed on individual whole brains (minus optic lobes) as described in Wagener-Hulme et al. (1999). 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 ESA Catecholamine HR-80 column (no. 316 stainless steel, 3-μm spherical octadecylsilane packing), and an ESA Coulochem II electrochemical detector coupled to an ESA 2-channel model 5014 microdialysis analytical cell. Both external and internal standards were used for all samples, as in Wagener-Hulme et al. (1999). Results are expressed on a per brain basis (minus optic lobes), as in Taylor et al. (1992) and Wagener-Hulme et al. (1999). Chromatogram analyses and amine quantification were done with EZChrom Chromatography Data System, Version 6 (Scientific Software).

Experiment 1: effect of octopamine or tyramine treatment on the number of bees initiating foraging in single-cohort colonies

Three trials were performed, each with a matched pair of colonies, one fed only octopamine-treated sucrose (2.0 mg ml^{-1}) and one fed untreated sucrose (control). Trial 2 was performed blind with respect to treatment. Three additional trials were performed with three matched colonies: one fed octopamine-treated sucrose, one control, and one fed only tyramine-treated sucrose (2.0 mg ml^{-1}). Tyramine, chemically similar to octopamine as its ultimate precursor, was used to probe for the specificity of the octopamine effect. One of these trials (trial 3) also was performed blind with respect to treatment.

All colonies in each trial were observed for six periods throughout the day, from 0900 hours until foraging subsided at dusk. Hive entrances were blocked 5 min prior to observations, and returning foragers were identified by pollen loads on their corbiculae or distended abdomens reflecting a foregut full of nectar or water. To ensure that each forager was counted only once, it was marked with an additional spot of Testor's enamel paint on the abdomen as it attempted to enter the (blocked) hive entrance. Each observation period lasted 45–60 min, and consisted of segments of ca 5 min per colony, repeated until no more new foragers were observed. Hive entrances occasionally were opened to allow foragers to enter or exit the hive so as not to upset the flow of foraging. All returning foragers were counted and allowed to enter the hive by reopening the hive entrance. The order in which the colonies were observed in each observation period was randomized. There was a period of ca 60 min of undisturbed flight for each colony between each of the six daily observation periods.

Observations began when bees were 3 days old to ensure that we observed the very first foragers (extensive observation of bees from single-cohort colonies at this laboratory indicates that flight is rarely initiated prior to day 3). For each trial, the number of bees initiating foraging from each colony was determined for 3 days after the first forager was seen. The range of ages at which the first bees initiated foraging was 4–7 days old. In most cases, the first bees initiated foraging at 5 days old.

At the end of each trial a census was performed to determine the number of remaining bees in the colony that did not initiate foraging ("non-foragers"). Colonies were killed by freezing, and each bee was examined for the appropriate thoracic and abdominal paint marks and then counted.

Experiment 2: dose-dependent effects of octopamine treatment on the number of bees initiating foraging in single-cohort colonies

Four matched colonies were established in each trial, as in experiment 1. One was fed a low dose of octopamine (1.0 mg ml^{-1} in one trial, 0.5 mg ml^{-1} in two trials), one a medium dose (2.0 mg ml^{-1} as in experiment 1), one a high dose (5.0 mg ml^{-1}), and the fourth was the control (fed untreated sucrose). Three trials were performed, with methods as described for experiment 1. Tyramine was not tested further because it did not increase the number of bees initiating foraging in experiment 1 (see Results).

Experiment 3: effect of octopamine treatment on the number of bees initiating foraging in typical colonies

Single-cohort colonies provide an efficient means of conducting neurochemical treatment experiments in the field, but to more fully evaluate the role of octopamine in the control of division of labor it was important to determine whether effects are also detectable in more typical colonies. Two groups of approximately 1000 1-day-old bees were individually paint-marked and placed in separate cages that contained one honeycomb frame filled with either octopamine-treated (2.0 mg ml^{-1}) or untreated 50% sucrose solution. Both cages were put into an empty hive box placed on top of a typical ("host") colony. Each host colony occupied one standard Langstroth hive body, contained approximately 15,000 adult bees, five honeycomb frames of brood, four frames of honey and pollen, and a naturally mated queen. The cages were enclosed with a double screen that prohibited physical contact (i.e., feeding) between caged individuals and the residents of the colony but allowed colony odors to pass freely to the caged bees (to facilitate their acceptance after release). After 1 week of confinement, the cages were opened and the bees released into the host colony. Most of the caged bees survived confinement to be released into the colony; mortality was 18.5%, 2.9%, and 8.6% in each cage of octopamine-treated bees, and 17.7%, 1.7%, and 9.8% in each cage of control bees following release in trials 2, 3, and 4, respectively (dead bees were not counted in trial 1).

Beginning on the day that caged bees were released into the colony, we conducted 60-min observation periods (30 min per colony) three times daily; one in the morning (ca 0900–1000 hours), one at mid-day (ca 1300–1400 hours), and one in the evening (ca 1700–1800 hours). Data were collected for 3 days after the first paint-marked bee was seen foraging, during a total of nine observation periods. Hive entrances were blocked with a mesh screen and paint-marked foragers (identified as in experiment 1) were collected with a vacuum device and counted. Four trials were performed, each with marked bees from different source colonies. Each trial employed just one colony, because treatments were made to bees in cages prior to their introduction to the colony. Two colonies were used twice as host colonies, with a 3-week interval between uses. Trials 2 and 3 of this experiment were performed blind with respect to treatment.

Experiment 4: age-dependent effects of octopamine treatment on the number of bees initiating foraging in single-cohort colonies

We treated colonies when bees were either just able to forage (4–6 days of age) or still too young to forage (1–3 days of age) to explore whether octopamine treatment exerts long-term developmental effects or more short-term neuromodulatory effects. Four matched single-cohort colonies were established for each trial. Bees in the "negative control" colony were fed untreated 50% sucrose solution when they were 1–6 days of age. Bees in the "positive control" colony were fed sucrose solution containing 2.0 mg ml^{-1} octopamine when they were 1–6 days of age. Bees in the "early" treatment colony were fed octopamine-treated sucrose when they were 1–3 days of age and then were switched to untreated sucrose for days 4–6. Bees in the "late" treatment colony were fed untreated sucrose when they were 1–3 days of age and then were switched to octopamine-treated sucrose for days 4–6. Switching treatments was accomplished by removing the colony's sole honeycomb frame containing sucrose solution and replacing it with another frame containing the different solution.

This experiment was designed based on the following observations. Honey bees younger than 4 days of age have only rarely been observed foraging, even precocious foragers from starved single-cohort colonies (Schulz et al. 1998). Moreover, most 1- to 3-day-old honey bees appear to be unable to fly vigorously because their thoracic musculature has not fully developed (Fielding and Hepburn 1979). Treating 1- to 3-day-old bees therefore served as a method of assessing whether octopamine treatment early in life causes developmental effects that result in an increased likelihood of foraging at a later age. Treatment with juvenile hormone or juvenile hormone analog at 1 day of age has such effects on behavioral development (reviewed by Robinson and Vargo 1997; see also Sullivan et al. 2000). Three trials of this experiment were performed; forager counts and colony censuses were as described for experiment 1.

Statistical analyses

Effects of oral octopamine treatment on brain levels of octopamine, dopamine, and serotonin were confirmed to be of normal distribution with Komolgorov-Smirnov Normality Test and analyzed with one-tailed *t*-tests, based on the a priori assumption that oral treatment increases octopamine levels (Harris and Woodring 1999).

For behavioral experiments, differences in the distribution of foragers and non-foragers between colonies (experiments 1, 2, and 4) or groups (experiment 3) were determined with contingency table analyses (*G*-tests) on a trial-by-trial basis. In experiments with more than two colonies, subsequent pairwise analyses were performed with 2×2 *G*-tests. Within each trial, single-cohort colonies in experiments 1, 2, and 4 were made to be as similar as possible in terms of genotypic composition, population size, age demography, amount of comb and food, and queen age and source, thus making colony comparisons within a trial more appropriate.

In experiments 1, 2, and 4 the number of non-foragers was determined by counting during the census all marked bees that did not have the additional abdominal paint-mark used for foragers. In experiment 3 the number of non-foragers was determined by subtracting the number of marked bees collected as foragers from the total number of marked bees actually introduced to the colony (in trials 2, 3, and 4 dead bees were subtracted from the total when bees were released from their treatment cages; in trial 1 starting numbers were used). This was an efficient alternative to conducting full, destructive, censuses on the relatively large host colonies; it probably also gave an accurate count of the number of non-foragers at the end of the experiment because foraging began unexpectedly early in these colonies, only a few days after marked bees were released from their cages.

Results

Effects of oral octopamine treatment on brain levels of octopamine

Oral octopamine treatment resulted in a significant increase in brain levels of octopamine, while levels of serotonin and dopamine remained unchanged (Fig. 1). Bees treated with 2 mg ml^{-1}, the dose most effective in behavioral studies (see experiment 2 below), had brain octopamine levels that were roughly three times greater than untreated bees. However, not all treated bees had elevated brain levels of octopamine. Rather, as dose increased more bees showed elevated levels. We quantified this by calculating the percentage of bees that had octopamine levels 2 standard deviations or more above the mean of the control bees (Fig. 1A). For bees treated with 1.0 mg ml^{-1}, this value was 54.5%. For bees treated

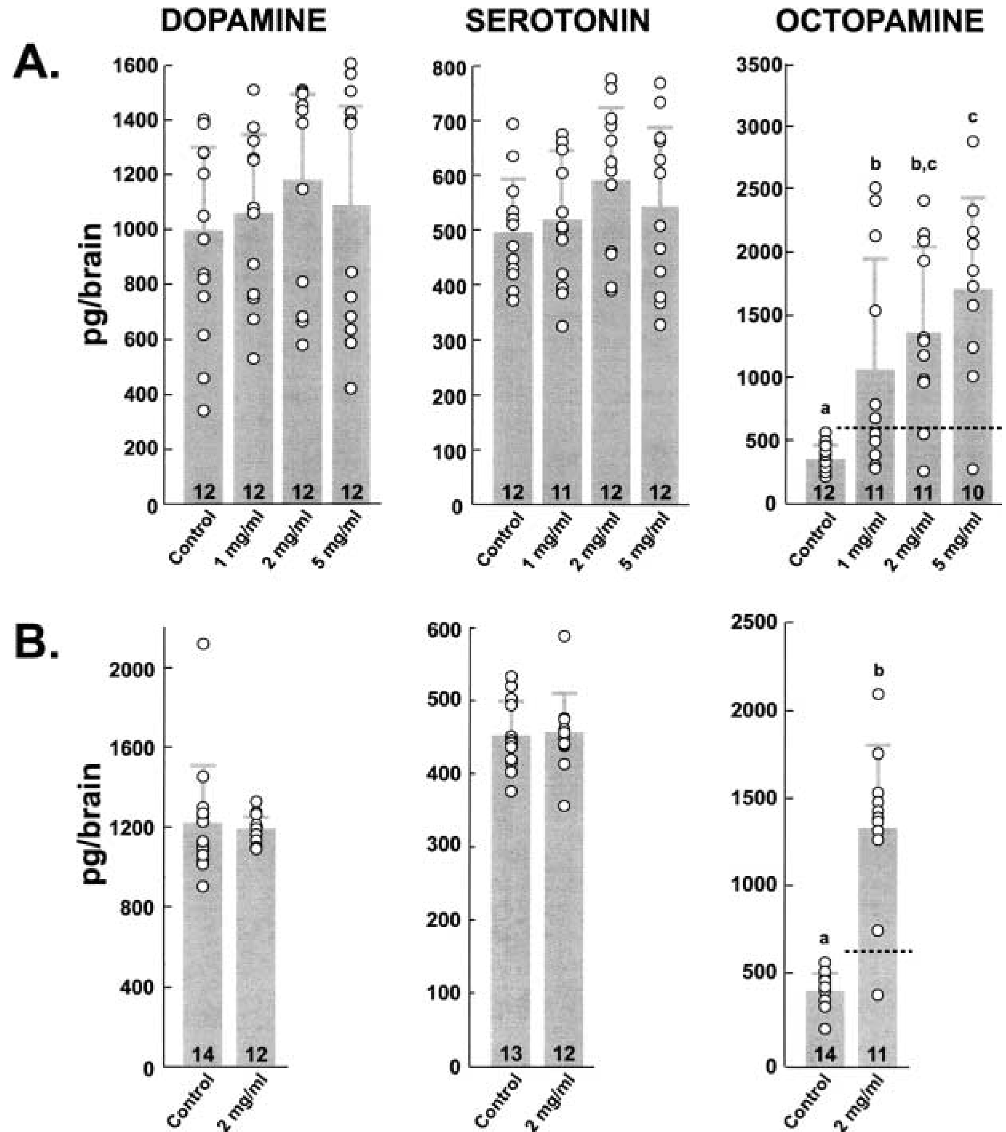


Fig. 1 Brain levels of biogenic amines in bees from single-cohort colonies treated with different doses of octopamine. *Bars* show mean ± SD for the individually plotted data points. Control colonies were fed 50% sucrose solution. **A** Bees were collected from colonies in experiment 2, trial 1, when they were 5 days old. **B** Bees were collected from other single-cohort colonies not used for behavioral data analysis in this paper. Statistical analyses: one-way *t*-tests with the a priori assumption that treatment increased octopamine levels. Different letters indicate significant ($P < 0.05$) differences between the groups. Numbers of individual brains analyzed are indicated in each bar. The *dashed line* on the octopamine graphs represents two standard deviations above the mean of the control bees

with 2.0 mg ml^{-1} and 5.0 mg ml^{-1}, it was 81.8% and 90%, respectively.

Experiment 1: effect of octopamine or tyramine treatment on the number of bees initiating foraging in single-cohort colonies

Octopamine treatment caused a significant increase in the number of bees initiating foraging in 6 out of 6 trials (Fig. 2A, octopamine versus control; Fig. 2B octopamine versus tyramine versus control). These results, coupled with those from replicates of this experiment performed as part of experiments 2 and 4, indicate that there actually was a significant increase in the number of bees initiating foraging in 12 out of 12 trials, conducted over 2 years (Figs. 2, 3, 5). The number of bees foraging from octopamine-treated colonies on the first 3 days of foraging activity never exceeded 5–10% of the adult population, but octopamine-treated colonies had two to eight times more foragers during this period than did control colonies.

Tyramine treatment did not cause an increase in the number of bees that initiated foraging (Fig. 2B). Octopamine-treated colonies had significantly more foragers than both tyramine-treated and control colonies. Tyramine-treated colonies had significantly fewer foragers than control colonies in two out three trials.

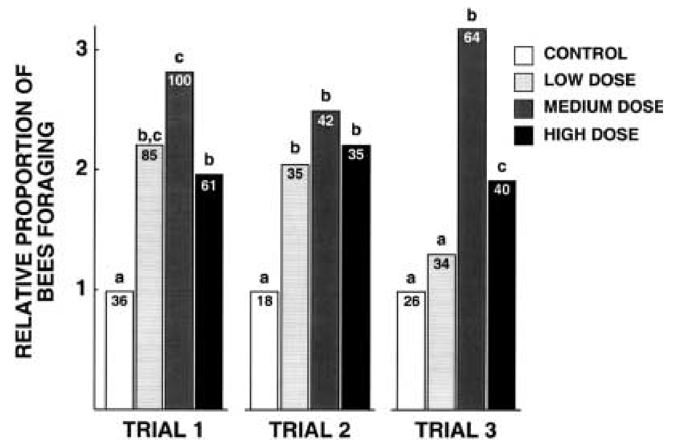


Fig. 3 Dose-dependent effects of octopamine on the number of bees initiating foraging in single-cohort colonies. *Control*: fed untreated sucrose; *Low Dose*: 1.0 mg ml^{-1} in trial 1, 0.5 mg ml^{-1} in trials 2 and 3; *Medium Dose*: 2.0 mg ml^{-1} (the dose used in all other experiments); and *High Dose*: 5.0 mg ml^{-1}. Notation and statistical analyses as in Fig. 2. Results for 3×2 *G*-tests were $P < 0.001$ for trials 1 and 3, and $P < 0.005$ for trial 2

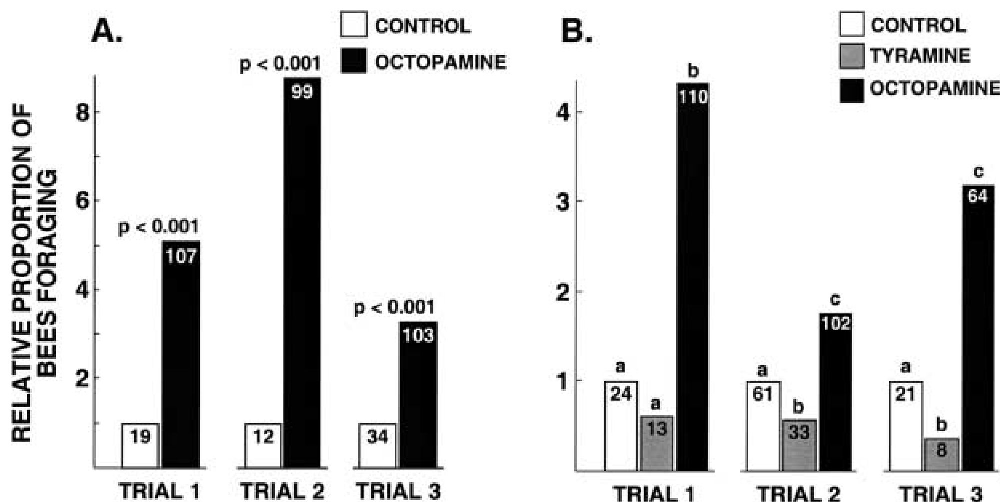
Experiment 2: dose-dependent effects of octopamine treatment on the number of bees initiating foraging in single-cohort colonies

Dose-dependent effects were detected. All doses increased the number of bees that initiated foraging relative to control colonies, but the medium dose (2 mg ml^{-1}) was more potent than either the low or high doses (Fig. 3). This was the dose used in all other experiments.

Experiment 3: effect of octopamine treatment on the number of bees initiating foraging in typical colonies

Octopamine treatment caused a significant increase in the number of bees initiating foraging in typical colonies in three out of four trials (Fig. 4). The onset of foraging was surprisingly soon after release from treatment cages into host colonies. Some treated and control bees began foraging when they were 8–9 days of age.

Fig. 2 Effect of octopamine or tyramine treatment on the number of bees initiating foraging in single-cohort colonies. The proportion of foragers in each colony was determined. To calculate the relative proportion of bees foraging, these data were then normalized within each trial with the proportion of foragers from the control colony. The number of foragers for each colony is indicated in each bar. Statistical analyses: **A** 2×2 *G*-tests (performed on the actual distributions of foragers and non-foragers); **B** 3×2 *G*-tests (all $P < 0.001$), followed by pairwise 2×2 *G*-tests. *Different letters* indicate significant ($P < 0.05$) differences between the groups



Experiment 4: age-dependent effects of octopamine treatment on the number of bees initiating foraging in single-cohort colonies

Colonies fed octopamine-treated sucrose on days 1–6 had a significantly greater number of bees that initiated foraging than did control colonies in three out of three trials, replicating the results seen in earlier experiments. Age-dependent effects of octopamine treatment were detected in these same trials (Fig. 5). In three out of three trials, colonies treated with octopamine on days 1–6 had a significantly greater number of bees that initiated foraging than did colonies treated on days 1–3. Colonies treated with octopamine on days 4–6 had just as many foragers as did colonies treated on days 1–6 (in two out of three trials). Colonies treated on days 4–6 had significantly more foragers than colonies treated on days 1–3 (in two out of three trials) or control colonies (in three of three trials). Colonies treated on days 1–3 did not have more foragers than control colonies.

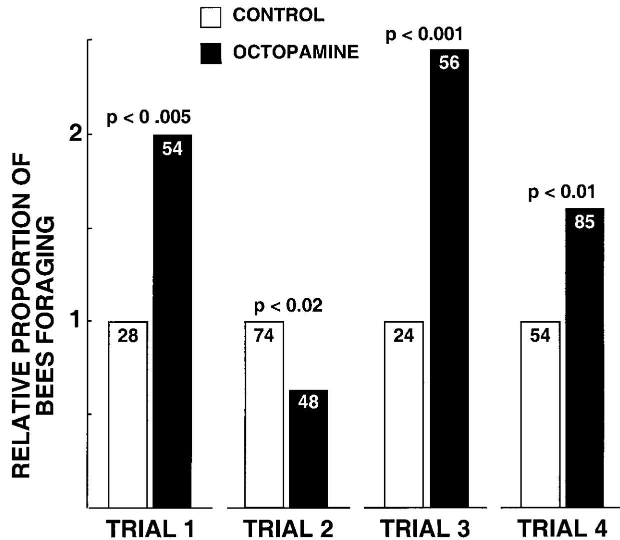


Fig. 4 Effect of octopamine treatment on the number of bees initiating foraging in typical colonies. Notation and statistical analyses as in Fig. 2

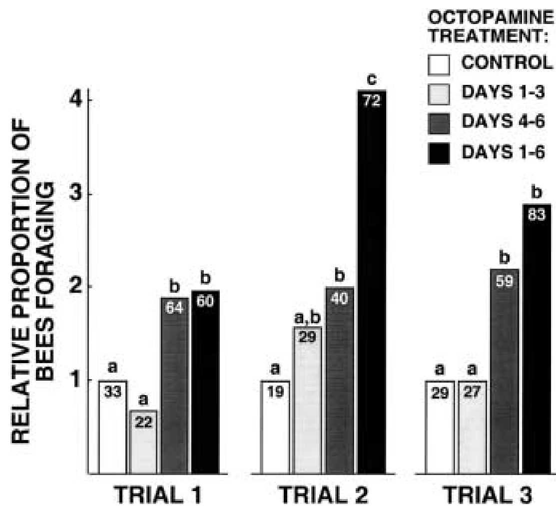


Fig. 5 Age-dependent effects of octopamine treatment on the number of bees initiating foraging in single-cohort colonies. Treatments: untreated sucrose on days 1–6 days (control); octopamine-treated sucrose on days 1–3 and untreated sucrose on days 4–6; untreated sucrose on days 1–3 and octopamine-treated sucrose on days 4–6; and octopamine-treated sucrose on days 1–6. Notation and statistical analyses as in Fig. 2. Results for all 3×2 *G*-tests were $P < 0.001$

Discussion

These results demonstrate that octopamine influences division of labor in honey bees. They suggest that previous findings of higher brain levels of octopamine in forager honey bees relative to hive bees (Harris and Woodring 1992; Schulz and Robinson 1999; Wagener-Hulme et al. 1999) reflect a pre-foraging elevation that increases the likelihood of switching from working in the hive to foraging. Juvenile hormone influences the age at which bees shift from hive work to foraging (e.g., Sullivan et al. 2000), but it is not known whether octopamine and juvenile hormone influence honey bee behavioral development as parts of the same or different neural pathways.

Oral treatment provides a non-invasive method of chronically elevating brain levels of octopamine in the field, but it is not clear by what mechanism. Did the ingested octopamine ultimately pass through the gut and the blood-brain barrier, or trigger some endogenous response? Analyzing these possibilities would be interesting, now that both brain and behavioral effects are known. Insects possess a blood-brain barrier of glial and perineurial cells which covers the entire central nervous system (reviewed by Carlson et al. 2000), but this barrier is not absolute. Fatty acids (Eldefrawi and O'Brien 1966), aliphatic alcohols (Eldefrawi and O'Brien 1967), and even the neurotransmitter acetylcholine (Treherne and Smith 1965) are able to penetrate the central nervous system. Injection of amines into the hemocoel has been used successfully for many years (e.g., Long and Murdock 1983; Lent and Dickinson 1984; Robinson et al. 1999), and Linn et al. (1994) showed that injections increase brain levels of biogenic amines. Penetration of an ingested amine through the midgut has not been studied directly. While we do not know the mechanism by which brain levels were elevated by oral treatment, octopamine had striking behavioral effects. The simplicity of this method should allow it to be used to study neurochemical regulation of insect behavior in a variety of contexts because many species of insects readily ingest sucrose solutions.

One disadvantage of oral treatment is that it probably also results in other tissues besides the brain being exposed to increased amounts of octopamine. This raises the question of whether the exogenous octopamine acted in the brain or the peripheral nervous system in our experiments to cause the dramatic effects on division of labor that we observed. Octopamine is known to

act peripherally as both a neuromodulator and a neurohormone, especially in insect flight. Injections of octopamine into thoracic ganglia release complete flight-motor patterns in moths and locusts (Sombati and Hoyle 1984; Claasen and Kammer 1986). Therefore, it is possible that octopamine affected the peripheral nervous system to influence foraging behavior. However, we think it is likely that the initiation and overall control of foraging in honey bees takes place in the brain. Foraging is a complex process that includes navigation and landmark learning, manipulation of a variety of complex flower types, and communication among bees about floral resources (Winston 1987). While the execution of some these activities involves the peripheral nervous system, their integration almost certainly occurs in the brain. To determine whether octopamine is acting centrally or peripherally to affect division of labor requires a technique for chronic but specific treatment of the brain. Hammer and Menzel (1998) were able to specifically treat the antennal lobes of honey bees with octopamine by direct injection, but this form of treatment provides only a short-term elevation of octopamine. Perhaps it would be possible to inject slow-release microcapsules (Jain et al. 2000) of octopamine directly into the brain.

Octopamine is known to alter sensitivity to chemical stimuli in honey bees (Mercer and Menzel 1982), moths (Linn and Roelofs 1986; Pophof 2000), and flies (Long and Murdock 1983). We speculate that as more octopamine is released by neurons in the brain the bee becomes more responsive to stimuli that lead to taking the very first foraging trip, or to stimuli that elicit the performance of each foraging trip, or both. This speculation is based on the idea that the regulation of division of labor occurs as a result of differences in sensitivity to task-related stimuli, many of them chemical or mechanical in the dark beehive (Robinson 1987b; Beshers et al. 1999). Some of the stimuli associated with foraging are known, such as those associated with brood (Free 1967), food stores (Fewell and Bertram 1999), and dance language (von Frisch 1967; Seeley 1995), but it is not known whether these stimuli also stimulate the bee to take its very first foraging trip. A better understanding of the stimuli that elicit foraging behavior in honey bee colonies is necessary.

Our working model for neuroethological analyses of division of labor in honey bee colonies is that changes in the likelihood of shifting from hive activities to foraging are the result of a long-term process of behavioral development coupled with short-term neuromodulatory changes that affect responsiveness to task-related stimuli. These changes in responsiveness in turn influence the probability that a bee will initiate foraging. Behavioral development, from hive activities to foraging, typically takes place over a time scale of days, and factors that affect behavioral development have comparable time scales. For example, adult bees with the sole glandular source of juvenile hormone removed show a delay of several days in the age at which they shift to foraging; this delay can be eliminated by juvenile hormone analog treatment at 1 day of age, even though the onset of foraging may not occur for 10–30 days later (Sullivan et al. 2000). Two aspects of our results suggest that octopamine is not acting similarly, but functions instead as a more short-term neuromodulator. They are discussed in the following two paragraphs.

First, age-dependent effects of octopamine treatment were detected; bees treated when they were just able to forage (4–6 days old) showed the octopamine effect, but bees treated when they were too young to forage (1–3 days of age) did not. Most 1- to 3-day-old bees cannot fly vigorously because their thoracic musculature has not fully developed (Fielding and Hepburn 1979); for them to be affected by octopamine treatment would have required a latency of at least a few days, but this did not happen. In contrast, the bees treated when they were 4–6 days old were capable of foraging (e.g., Schulz et al. 1998), so the treatment effect could have been relatively more immediate. Another interpretation of these results is that 1- to 3-day-old bees were not able to respond to octopamine treatment because of developmental differences in the quantity or activity of octopamine receptors; developmental profiles for octopamine receptors in bees have not been published.

Second, more octopamine-treated bees initiated foraging surprisingly soon after being released into typical colonies. Some treated and control bees in colonies that we assumed had typical age demographies initiated foraging as young as 8 days of age, which was very similar to the ages at which octopamine-treated bees began foraging in single-cohort colonies. This is much younger than when most bees initiate foraging in typical colonies (ca. 21 days of age; Michener 1974), suggesting that aspects of the treatment in addition to octopamine exposure affected the likelihood of initiating foraging. Nevertheless, a clear effect of octopamine treatment was seen. In contrast, bees treated at 1 day of age with juvenile hormone analog started foraging in typical colonies when they were about 14 days of age (Robinson 1987a) and at about 7 days of age in single-cohort colonies (Robinson et al. 1989; Withers et al. 1995). Juvenile hormone treatment thus exerts its influence over a longer time scale in a manner that seems to be dependent upon social context, i.e., the age structure of the colony (Huang and Robinson 1996). In contrast, octopamine treatment exerts its influence over a shorter time scale and seems less sensitive to variation in colony age demography.

The effects of octopamine treatment on honey bees are not, however, completely independent of other aspects of social environment. Treatment caused an initial two- to eightfold increase in the size of the colony's foraging force, but still only 5–10% of the bees from treated colonies acted as foragers during that time. Because all bees had equal exposure to the octopamine treated sucrose, their only source of food, these results are puzzling, at least from the perspective of the neuroethology of the individual bee. We suggest that these results are consistent with the realization that division of

labor in honey bee colonies is controlled by both the needs of the colony and the physiological states of the colony's workers (Franks and Tofts 1994; Robinson et al. 1994; Gordon 1996). For a colony to survive there are other jobs that must be performed besides foraging. It appears that changing one neurobiological factor in individuals, at least octopamine, cannot completely overcome the regulatory processes that maintain division of labor between hive bees and foragers. Because only some of these processes are understood (see Robinson and Huang 1998; Beshers et al. 1999), this explanation is unsatisfactorily vague. An alternative perspective is that there are neural or neuroendocrine factors remaining to be discovered, more essential than octopamine, that if used in a treatment experiment would significantly disrupt social organization and cause a much greater fraction of the bees in a colony to forage. It is also possible that although bees have equal access to treated sucrose solution, the distribution of the chemical may be uneven due to the dynamics of trophallaxis (social feeding). While most treated bees tested had elevated levels of octopamine in their brains, a broad range of levels was found (see Fig. 1). For example, the brains of bees treated with 2.0 mg ml^{-1} of octopamine had 236.1–2408.4 pg of octopamine. Individual bees do not feed constantly, and therefore fluctuations in brain levels of octopamine are almost certainly occurring. Our data show that not all bees from colonies orally treated with octopamine have similar levels in their brains, and some do not even have levels higher than control bees. Perhaps it is only the 5–10% of bees with the highest levels of artificially elevated brain octopamine that become foragers. There is not enough information available to further evaluate any of these three speculative explanations.

Our focus on octopamine is not meant to suggest that other neurochemicals are not involved in the control of division of labor in honey bee colonies. Correlations between behavioral state and brain levels were strongest for octopamine in studies by Wagener-Hulme et al. (1999) and Schulz and Robinson (1999), so we proceeded accordingly. However, these studies also showed that foragers had higher levels of serotonin than nurses in their antennal lobes, so treatment experiments with serotonin should be performed. Our relatively small set of results for tyramine treatment indicated that this amine did not increase the likelihood of initiating foraging. This speaks to the specificity of the octopamine treatment effect because tyramine is the precursor of octopamine and as such is quite similar structurally. Tyramine treatment actually caused a significant decrease in the number of bees initiating foraging in two out of three trials. Further studies with tyramine should also be done, including a determination of the effects of tyramine treatment on brain levels of both tyramine and octopamine. In general, treatments with cocktails of amines and other compounds might give a fuller understanding of the neurochemical regulation of division of labor, including the important sites of action in the brain and possible interactions with the endocrine system.

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