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Juvenile Hormone and Octopamine in the Regulation of Division of Labor in Honey Bee Colonies

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Forager honey bees have high circulating levels of juvenile hormone (JH) and high brain levels of octopamine, especially in the antennal lobes, and treatment with either of these compounds induces foraging. Experiments were performed to determine whether octopamine acts more proximally than JH to affect the initiation of foraging behavior. Bees treated with octopamine became foragers more rapidly than bees treated with the JH analog methoprene. Bees treated with methoprene showed an increase in antennal lobe levels of octopamine, especially after 12 days. Bees with no circulating JH (corpora allata glands removed) treated with octopamine became foragers in similar numbers to bees with intact corpora allata. These results suggest that JH affects the initiation of foraging at least in part by increasing brain levels of octopamine, but octopamine can act independently of JH. Effects of JH that are not related to octopamine also are possible, as bees treated with both octopamine and methoprene were more likely to become foragers than bees treated with only octopamine or methoprene.

Behavioral plasticity in vertebrates involves many factors, including both endocrine and neurochemical factors and interactions between them (e.g., Brain and Haug 1992; Mani, Allen, Clark, Blaustein, and O'Malley, 1994; Cologer-Clifford, Simon, Richter, Smoluk, and Lu, 1999). Invertebrates show considerable plasticity as well, influenced by hormonal (e.g., Maleszka and Helliwell, 2001; Sigg, Thompson, and Mercer, 1997; Walikonis, Schoun, Zacharias, Henley, Coburn, and Stout, 1991) and neurochemical factors (e.g., Erber *et al.*, 1993; Linn *et al.*, 1992), but the interaction of both hormones and neurochemicals in the regulation of invertebrate behavioral plasticity has been studied in relatively few species (Gammie and Truman, 1997; 1999; Kravitz, 2000). We studied the interaction between juvenile hormone and octopamine in the regulation of honey bee division of labor, a complex social behavioral system characterized by plasticity among individual workers.

Division of labor in honey bee colonies is based on a stereotyped pattern of behavioral development by adult workers (Winston, 1987). Young bees work in the hive for the first 2–3 weeks, performing such tasks as feeding brood (nursing), while older bees forage for nectar and pollen outside of the hive for the remainder of their 4 to 6-week lives. The regulation of this age-related division of labor involves several physiological factors (Robinson, 1998), including juvenile hormone and the neurochemical octopamine (Wagener-Hulme, Kuehn, Schulz, and Robinson 1999; Schulz and Robinson, 1999, 2001). Circulating levels of juvenile hormone (JH) are high in foragers compared to nurses, and treatments with the JH analog methoprene (JHA) also result in precocious forager development (reviewed by Bloch, Wheeler, and Robinson, 2002). Recently it was discovered that JH does not activate foraging but rather somehow is involved in controlling the pace at which bees develop into foragers (Sullivan, Jassim, Fahrbach, and Robinson, 2000). Bees that have had their corpora allata (the sole source of JH) surgically removed still become foragers, but at significantly delayed ages.

The biogenic amine octopamine also has been implicated in the regulation of honey bee division of

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labor. Levels of octopamine are higher in the brains of foragers than nurses (Wagener-Hulme *et al.,* 1999), particularly in the antennal lobes (Schulz and Robinson, 1999), and oral treatments with octopamine induce precocious foraging (Schulz and Robinson, 2001). Octopamine is thought to act as a neuromodulator in honey bee division of labor, modulating responsiveness to olfactory stimuli that trigger the performance of certain tasks (Schulz and Robinson, 2001).

While both JH and octopamine induce precocious foraging, they appear to act differently and over different time scales. One-day-old bees treated with JHA become precocious foragers when introduced into a typical colony, but not until about 2 weeks of age (Robinson, 1987a; Sullivan *et al.,* 2000). In contrast, octopamine-treated bees introduced into a typical colony become foragers sooner, as early as 7 days of age (Schulz and Robinson, 2001). JHA treatment is active even as a single dose administered when bees are not even capable of flight, let alone foraging. In contrast, a successful octopamine treatment must be chronically administered to bees already capable of foraging (Schulz and Robinson, 2001); bees that are 1–3 days old chronically treated with octopamine are not affected. Together these results suggest that JH may act early in the process of forager development, while octopamine acts more proximally as a short-term releaser of foraging.

JH and octopamine might act to influence honey bee foraging behavior by one or more of the following three possibilities: JH might affect octopamine, octopamine might affect JH, or they may act separately. These possibilities are not mutually exclusive, as JH and octopamine could have reciprocal actions or act on multiple targets necessary for foraging, some independently and some in concert. There already is evidence for an effect of octopamine on JH in honey bees; octopamine application to isolated corpora allata from adult worker bees results in an increase in JH biosynthesis (Kaatz, Eichmüller, and Kreissl, 1994). However, given earlier findings suggesting a neuromodulatory role of octopamine on foraging (Schulz and Robinson, 2001), we were interested in octopamine dynamics in the brain. We predicted that if octopamine acts more proximally than JH in the regulation of foraging, then (1) treatment with JHA should increase levels of octopamine in the antennal lobes prior to the onset of foraging and (2) octopamine treatment should be able to exert an effect on the initiation of foraging even in the absence of circulating JH.

Before testing these predictions, we first confirmed that octopamine acts more rapidly than JH to affect foraging, something that had never been done in the same study. We also explored the possibility of redundant or synergistic effects of JH and octopamine by combination treatments.

MATERIALS AND METHODS

General Methods

Bees. Experiments were performed during the summers of 1999, 2000, and 2001 at the University of Illinois Bee Research Facility. Honey bees (*Apis mellifera* L.) were a typical North American mixture of predominantly European subspecies and maintained according to standard procedures.

Single-cohort colonies. A single-cohort colony is a small colony experimentally established with 1-day-old adult bees. About 5–10% of the bees in a single-cohort colony become precocious foragers because of the lack of an existing foraging force (Robinson, Page, Strambi, and Strambi, 1989; Huang and Robinson, 1992). Using single-cohort colonies allows us to control important variables such as colony size and age demography and efficiently test whether a treatment affects division of labor (e.g., Schulz and Robinson, 2001) in trials that last approximately 1 week each. Octopamine and JHA cause similar effects in both single-cohort colonies and more typical colonies (Sullivan *et al.,* 2000; Schulz and Robinson, 2001).

One-day-old adult bees were collected upon emergence from frames of sealed brood taken from large ("source") colonies in our apiaries in the field. 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 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 comb containing treated or control sucrose solution (see below), roughly the same amount of pollen (~50 g), and a mated queen less than 1 year old obtained from the same commercial source.

Foraging observations. All colonies in each trial were observed for six periods throughout the day, from 0900 h until foraging subsided at dusk. Hive entrances were blocked 5 min prior to observations,

and returning foragers were identified by brightly colored pollen loads on their hind legs 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 too much. 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 foraging has never been seen to be initiated prior to day 4; Schulz and Robinson, 1998).

At the end of each trial a complete census was performed to determine the number of remaining bees in the colony that did not initiate foraging (nonforagers). Colonies were killed by freezing, and each bee was examined for the appropriate thoracic and abdominal paint marks and then counted. Destructive censusing is necessary to achieve the highest accuracy, and these experimental colonies are too small to compete with larger colonies for long-term survival.

Oral treatments with octopamine or methoprene. Bees were allowed to feed *ad libitum* on 50% sucrose (w/w) solution containing octopamine (Sigma Chemical Co., St. Louis, MO) or the JH analog methoprene (Wellmark International). The same batch of methoprene (280-181-1) was used throughout. Previous studies have demonstrated that methoprene reliably mimics the effects of JH on adult behavior and physiology in honey bees (see Bloch *et al.,* 2002). Oral treatment with methoprene produces results similar to topical treatment (see Beetsma and ten Houten, 1975; Robinson and Ratnieks, 1987). Treated sucrose solution was placed in an otherwise empty honeycomb and replenished daily so that bees never experienced a food shortage. Control colonies were fed 50% sucrose with no treatment.

The octopamine (2.0 mg/ml) dose used has been shown to induce foraging (Schulz and Robinson, 2001). A 4.0 mg/ml solution with methoprene resulted in a stable suspension readily consumed by the bees. The methoprene dose was based on experiments with small groups of bees (Robinson and Ratnieks, 1987).

Topical treatment with methoprene. We used a topical treatment of 200 μg of methoprene dissolved in 5 μl acetone applied to the dorsal abdomen of each bee. This dose of methoprene consistently causes precocious behavioral development in honey bees (Robinson, 1985, 1987a; Robinson *et al.,* 1989; Sullivan *et al.,* 2000). Control bees were treated on the dorsal abdomen with 5 μl acetone alone; no effects on behavioral development have been found with acetone treatment of adults (Robinson, 1985). Treated bees were maintained in separate cages at 33°C for at least 1 h after treatment to prevent the possible transfer of methoprene to untreated bees.

Corpora allata removal. To obtain bees without circulating JH, we removed the sole glandular source of JH in honey bees, the corpora allata. Surgery was performed on bees less than 2 h after adult emergence. Detailed methods are described in Sullivan *et al.* (2000). Briefly, bees were anesthetized on ice and immobilized on a dissecting stage, and their corpora allata were removed through a small incision in the back of the head. Sham-allatectomized (sham) bees were treated identically but their corpora allata were moved gently and not removed. "Untreated" bees were cold-anesthetized but otherwise unmanipulated. Bees were placed in a small holding cage for 12 h at 33°C following surgery to allow full recovery before being introduced into a colony. The effects of this procedure have been verified by radioimmunoassay; allatectomized bees have undetectably low circulating JH (Sullivan *et al.,* 2000).

Collection, dissection, and HPLC analysis. Bees for HPLC analysis were collected directly into liquid nitrogen (Wagener-Hulme *et al.,* 1999). All bees were stored at −80°C until brain dissection and HPLC analysis. Whole bee heads were partially freeze-dried to facilitate dissection (as in Schulz and Robinson, 1999). Quantification of amine levels in antennal lobes was performed as in Schulz and Robinson (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 (3-μm octadecylsilane packing), and an ESA Coulochem II coulometric electrochemical detector coupled to an ESA Model 5014B 2-channel microdialysis analytical cell. Both external and internal standards were used for all samples. Chromatogram analyses and amine quantification were performed with EZChrom Chromatography

Data System v6.8 (Scientific Software). Antennal lobe results are expressed as a concentration of octopamine per protein in the sample to account for any differences in dissection. Quantification of protein was performed as in Schulz and Robinson (1999), using a kit based on the Lowry method (Bio-Rad).

Statistical analyses. Differences in the distribution of foragers and nonforagers between treated and control colonies or between treatment groups within colonies were determined with pairwise contingency table analyses (*G* tests) on a trial by trial basis. Within each trial, single-cohort colonies 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 appropriate. When necessary, differences in the age distribution of foragers were determined by Mann–Whitney *U* tests performed on the total counts for each trial.

Data for levels of octopamine in antennal lobes were confirmed to be of normal distribution by the Kolmogorov–Smirnov Normality test and then analyzed with ANOVA. Post hoc comparisons following ANOVA were analyzed by Fisher's LSD.

Experiments

Timing of octopamine and juvenile hormone analog effects on forager development. Two trials were performed, each consisting of three single-cohort colonies: one fed methoprene in sucrose solution, one fed octopamine in sucrose solution, and one fed sucrose alone (control). Foraging was then observed for 4 days after the first colony in each trial began foraging. We hypothesized that if octopamine acts more proximally than JH to affect the initiation of foraging, then octopamine-treated bees should become foragers earlier than methoprene-treated bees.

Effects of juvenile hormone analog treatment on levels of octopamine in the antennal lobes. Two trials were performed, each with topically treated bees from different source colonies. Treated bees were introduced into a colony with a typical age demography that contained ca. 10,000 workers. Bees were introduced into a more typical colony to determine whether JH affects octopamine over a longer period of time than can be studied in a single-cohort colony. This colony was housed in a glass-walled observation hive containing four frames of honeycomb. The glass walls of this hive consisted of hinged doors (three doors per frame per side) that could be opened to collect bees without disturbing the entire colony. The same colony was used for both trials, separated by 3 weeks.

TABLE 1
Groups Established to Test the Effects of JHA on Octopamine Levels in the Antennal Lobes

Treatment	Flight experience	Group name
Control	No	Octopamine big-back
Control	Yes	Octopamine non-big-back
Methoprene	No	Methoprene big-back
Methoprene	Yes	Methoprene non-big-back
Acetone	No	Acetone big-back
Acetone	Yes	Acetone non-big-back

If methoprene treatment can influence octopamine levels in the antennal lobes, we were interested to know if the effect was indirect, i.e. mediated by methoprene-induced foraging. To restrict the flight of methoprene-treated bees, we glued a plastic disc to the dorsal thorax of these bees that, when combined with a modified hive entrance, prevented bees from leaving the hive (as in Withers, Fahrbach, and Robinson, 1995). These "big-back" bees had no flight experience. Bees without discs on their thoraces (non-big-back bees) exited and entered the hive freely. Because the modified entrance restricted hive ventilation, the hive was kept indoors in a temperature-controlled environment (ca, 25°C) but bees were allowed to fly freely through a tube attached to the outside; this prevented heat stress. Bees from each experimental group (n = 20) were collected for octopamine analysis from inside the hive when they were 6 and 12 days old at 00:00 (midnight). At this time, all flight activity had ceased for at least 4 h, minimizing the chance that brain differences between big-back and non-big-back bees might be due to recent flight activity (Goosey and Candy, 1980). The six experimental groups introduced into the host colony are listed in Table 1.

Effects of octopamine treatment on bees without juvenile hormone. Two single-cohort colonies were established for each trial. One colony was treated with octopamine in sucrose solution and one was fed sucrose alone (untreated). The octopamine-treated colony contained 100 individually number-tagged bees (Opalithplättchen, Graz) of each of the following focal groups: (A) allatectomized, (B) sham-allatectomized, (C) methoprene treated (topical), and (D) controls. Fifty 1-day-old bees of each group were added each day to establish the colony over the first 2 days. On the third day, 700 one-day-old bees from the same genetic sources used for the four groups were paint-marked

and added to bring the colony population to 1100. Bees in the focal groups were therefore up to 2 days older than the rest of the bees, increasing their likelihood of precocious foraging (Page, Robinson, Britton, and Fondrk, 1992; Jassim, Huang, and Robinson, 2000). The untreated colony was established with an equal population of bees from the same source colonies and made over 2 days with same age demography as the octopamine-treated colony, but it contained no focal bees. This untreated colony was used to determine whether there was an overall effect of octopamine in the treated colony. Limitations on the number of allatectomized and sham-allatectomized bees that can be produced in a timely fashion prevented use of a more balanced experimental design, with bees from the focal groups in both the treated and the control colonies. Octopamine treatments were not replenished once 3 days of foraging had been observed. Two trials of this experiment were performed.

Foraging observations began when either colony in a trial began to forage and were then continued for the first 3 days of foraging on both colonies to establish an overall effect of octopamine treatment. Foraging observations were continued only on the octopamine treatment colony for an additional 5 days, for a total of 8 days of observation. During this time, as foragers brought in fresh nectar and the octopamine was not replenished, we assumed that the octopamine treatment was diluted and rendered ineffective. Each day a census of the focal bees in the octopamine-treated colony was performed to account for any mortality within the treatment groups.

Redundant or additive effects of octopamine and juvenile hormone analog treatment. Results of Trial 1 in the previous experiment showed that bees treated with both octopamine and methoprene were more likely to become foragers than bees treated with octopamine alone. These results suggested that methoprene and octopamine are not acting redundantly. To further examine this possibility, we performed an additional experiment using a combination of both octopamine and methoprene treatment.

Three trials of this experiment were performed. For each trial, two single-cohort colonies were established, one fed octopamine in sucrose and one fed sucrose alone. Each single-cohort colony was established with two groups of 1-day-old adult bees ($n = 110$ of each), one treated topically with methoprene (in acetone), and the other treated with acetone alone. After 2 days, 900 untreated bees were introduced into each colony, for a total colony population of 1120 bees. Thus each trial contained four focal groups, two in each colony: (A) octopamine + methoprene, (B) octopamine + acetone, (C) methoprene, and (D) acetone. Foraging observations were performed for the first 3 days after the onset of foraging by either colony in a trial.

RESULTS

Timing of Octopamine and Juvenile Hormone Analog Effects on Forager Development

In both trials of this experiment, the previously reported behavioral effects of octopamine and methoprene were observed. Bees treated with either octopamine or methoprene were more likely to become foragers than control bees (Fig. 1). The age distribution of foragers in the octopamine-treated colonies was younger than in the methoprene-treated colonies during the first 4 days of foraging (Fig. 1). Differences in both trials were significant ($P < 0.05$); this result was stronger in Trial 1 and most obvious on the first day of foraging. There were no differences in either trial in the total number of bees foraging after 4 days in the octopamine- and methoprene-treated colonies.

Effects of Juvenile Hormone Analog Treatment on Octopamine in the Antennal Lobes

Data are reported in Fig. 2, and ANOVA results for these data are summarized in Table 2. There was a significant effect of age ($P < 0.001$) on levels of octopamine in the antennal lobes; older bees had higher levels, which is consistent with previous studies (Schulz and Robinson, 1999). As indicated in Fig. 2 and Table 2, there also was a significant effect of methoprene treatment on levels of octopamine in the antennal lobes ($P < 0.005$). Twelve-day-old methoprene-treated bees had significantly more octopamine in their antennal lobes than either control or acetone-treated bees (Fig. 2). Six-day-old methoprene-treated bees had significantly higher levels of octopamine in their antennal lobes than acetone-treated, but not control, bees. There were no significant effects of flight experience on octopamine levels in the antennal lobes. This result indicates that flight was not necessary for the either the methoprene-induced or the age-related increase in octopamine.

Effects of Octopamine Treatment on Bees without Juvenile Hormone

In both trials, the basic octopamine treatment effect (early foraging) was observed. Bees from the octopam-

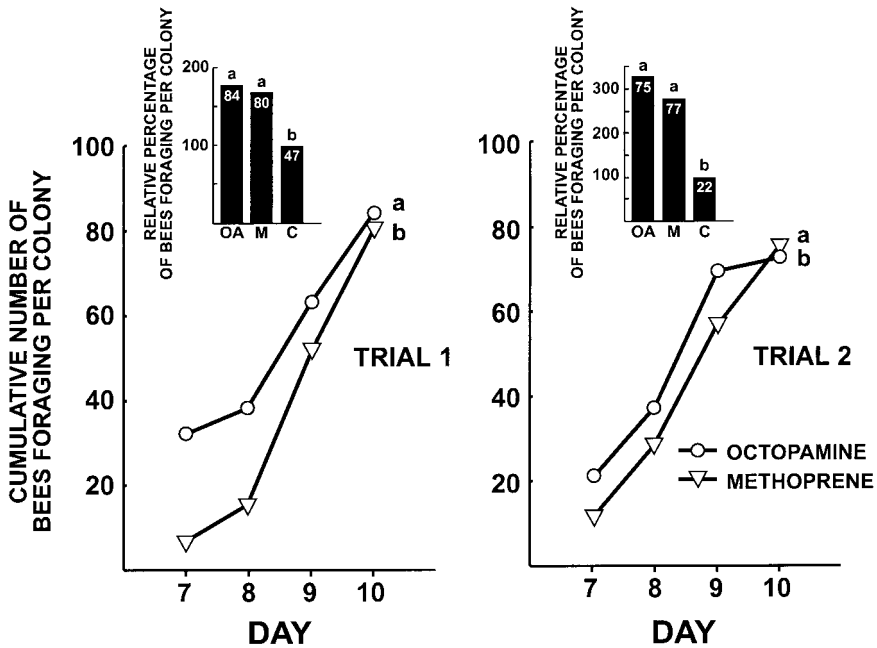

FIG. 1. Cumulative number of bees foraging over the first 4 days of foraging in colonies treated with octopamine or the juvenile hormone analog methoprene. Different letters associated with line graphs represent significant differences in the age distribution of foragers between colonies ($P < 0.05$; Mann–Whitney *U* test). (Insets) Relative percentage of bees initiating foraging in colonies fed octopamine (OA), methoprene (M), or control sucrose (C). To calculate the relative percentage, the percentage of bees foraging in each colony was normalized within each trial with the percentage of bees foraging from the control colony. Actual numbers of bees foraging in each colony are reported in the bars. Statistical analyses are pairwise 2×2 pairwise *G* tests performed on the actual distribution of foragers and nonforagers.

ine-treated colony were significantly more likely to become foragers over the first 3 days of foraging than were bees from the untreated colony ($P < 0.01$; data not shown). The basic methoprene treatment effect (early foraging) was observed in Trial 1, but not Trial 2 (Fig. 3).

Bees responded to octopamine treatment even in the absence of JH. Allatectomized bees in the octopamine-treated colony did not show a delay in the onset of foraging as they do in colonies not treated with octopamine (Sullivan *et al.*, 2000). There were no differences between sham, control, and allatectomized bees in the number of foragers during the first 3 days of foraging (Fig. 3), which is when the colony was being treated with octopamine. However, after 8 days of foraging, 5 days after the octopamine treatment was stopped, an allatectomy deficit was observed. In both trials, the methoprene, sham, and control groups had significantly more foragers than the allatectomized group.

In the octopamine-treated colony, there were significantly more methoprene-treated bees foraging than sham and allatectomized bees in both trials (Fig. 3). In Trial 1, but not in Trial 2, there also were significant differences between methoprene-treated and control bees. Methoprene typically causes precocious foraging (Jaycox, Skowronek, and Gwynn, 1974; Robinson, 1985, 1987a; Sullivan *et al.*, 2000); perhaps because the control bees in our experiments also were octopamine treated (Fig. 3, "OA only"), this may have reduced the typically robust difference between methoprene-treated and control bees.

Redundant or Additive Effects of Octopamine and Juvenile Hormone Analog Treatment

In three out of three trials, the basic octopamine treatment effect (early foraging) was observed; significantly more octopamine-treated bees became foragers than control bees ($P < 0.01$; data not shown). In five out of six comparisons over three trials, significantly more methoprene-treated bees became foragers than acetone-treated bees (Fig. 4). Bees treated with both octopamine and methoprene were significantly more likely to become foragers than those treated with octopamine or methoprene alone (Fig. 4). These results suggest that octopamine and methoprene treatments are not redundant.

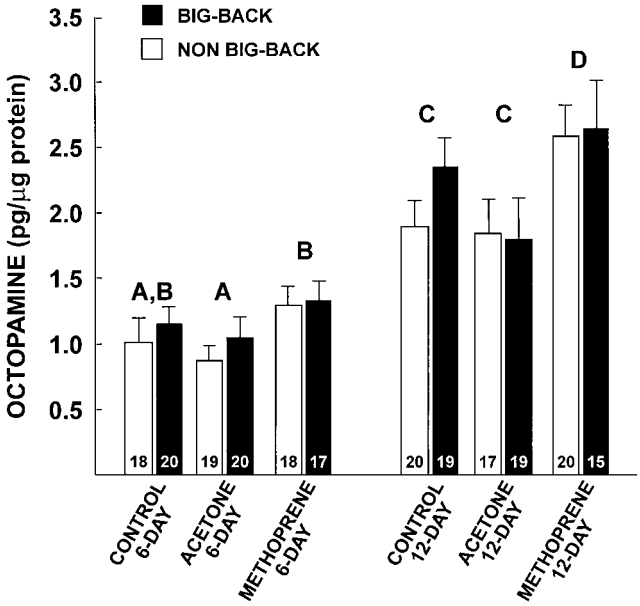

FIG. 2. Levels of octopamine +/− SEM in the antennal lobes of bees treated topically with methoprene as 1-day-old adults. Number of individual pairs of antennal lobes analyzed are noted in each bar. ANOVA (Table 2) revealed no significant effect of trial, and so the two trials were pooled. ANOVA also revealed no effect of flight experience, so big-back and non-big-back groups for each treatment and age were pooled for subsequent analyses between treatment/age groups (ANOVA $P < 0.001$). Different letters represent significant differences ($P < 0.05$) revealed by post hoc LSD analysis.

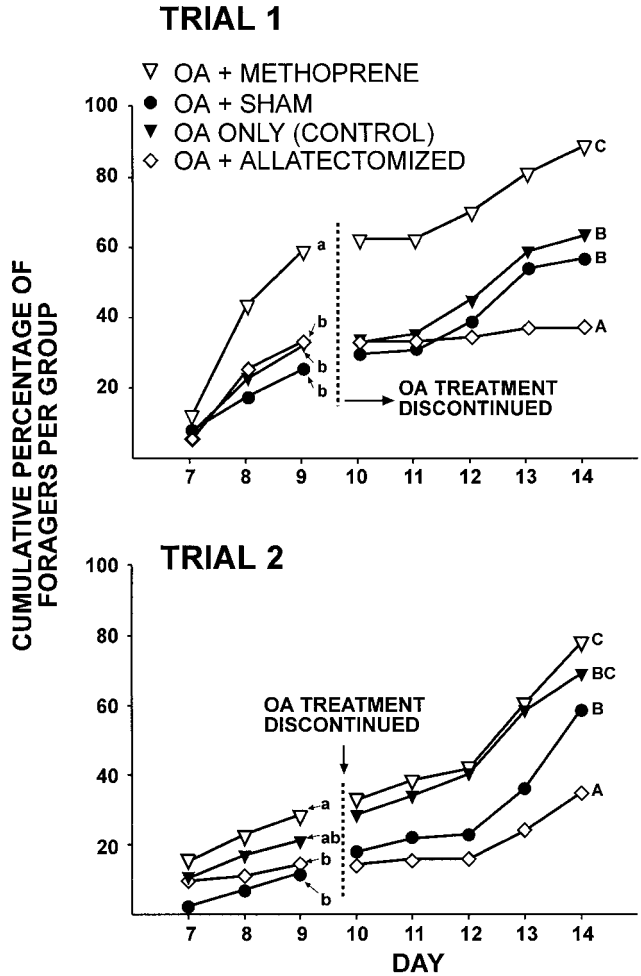

FIG. 3. Cumulative percentage of bees foraging from each octopamine-treated (OA) focal group in the octopamine-treated colonies only. Percentages based on a starting population of 100 bees per group and adjusted for mortality during daily censuses of focal groups. Different letters represent significant differences ($P < 0.05$; pairwise 2×2 G tests) in the distribution of foragers and nonforagers for the 2 days on which this analysis was performed (days 9 and 14). Dashed lines represent the point at which octopamine treatments ceased to be replenished.

DISCUSSION

These results allow us to begin to develop an understanding of the integration of endocrine and neurochemical factors that influence division of labor in honey bee colonies. Octopamine and JH interact to influence the initiation of foraging, with octopamine acting more proximally than JH. Our conclusions are based on the following three major findings of this study.

First, treatments with octopamine acted more rapidly to influence foraging than did treatments with JHA. This is consistent with previous studies suggesting that octopamine works over a short-term time scale to affect the initiation of foraging (Schulz and Robinson, 2001) while JH acts over a longer time scale to affect the rate at which bees develop into foragers (Robinson and Vargo, 1997; Sullivan *et al.*, 2000).

Second, JHA treatment elevated levels of octopamine in the antennal lobes prior to the onset of foraging. While experiments on the effects of octopamine on foraging behavior have not been able to target the antennal lobes specifically (Schulz and Robinson,

TABLE 2

ANOVA Results for Effects of JHA on Octopamine Levels in the Antennal Lobes (Data in Fig. 2)

Effect	*df*	*F*	*P*
Age	1	73.110	<0.0001
Treatment	2	6.896	0.0013
Flight experience	1	1.300	0.2556
Trial	1	0.0002	0.9884

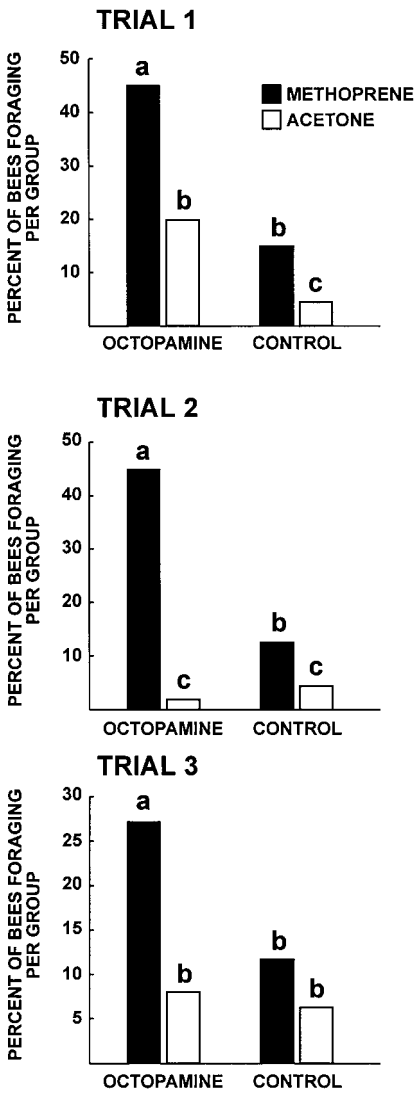

FIG. 4. Percentage of bees foraging in colonies containing bees treated with both octopamine and methoprene. Letters indicate significant differences in the distribution of foragers and nonforagers between treatment groups (2×2 pairwise Fisher's exact test).

2001), the antennal lobes have been implicated as a key brain region in octopamine-related regulation of foraging. Foragers have higher levels of octopamine in their antennal lobes than do nurses (Schulz and Robinson, 1999); this elevation is tightly linked with foraging and occurs regardless of age, colony type, flight activity, and amount of foraging experience. In addition, we showed here that the foraging-related increase in the antennal lobes occurs prior to the onset of foraging.

Third, while bees without circulating JH developed into foragers at significantly older ages than did intact bees (Sullivan *et al.*, 2000), allatectomized and intact bees treated with octopamine became foragers at similar ages. This suggests that bees were able to respond to octopamine treatment without a functioning JH system, which enabled the octopamine treatment to "rescue" the allatectomy-induced delay. However, allatectomized bees clearly showed delayed foraging after octopamine treatment was discontinued. Together, these results strongly suggest that octopamine acts more proximally than JH to affect foraging behavior.

Our hypothesis for how octopamine and JH are involved in the regulation of foraging is based on a response-threshold model for division of labor. We hypothesize that the proximate explanation for why there is division of labor is because workers have different response thresholds to task-related stimuli (Beshers, Robinson, and Mittenthal, 1999). Biogenic amine neuromodulators are known to affect response thresholds to behaviorally relevant stimuli (e.g., Kloppenburg and Heinbockel, 2000), and treatment with octopamine increases responses to some foraging-related stimuli in honey bees (A. B. Barron, D. J. Schulz, and G. E. Robinson, in press). Task-related stimuli in honey bee colonies are likely to include olfactory and tactile cues, as bees live in enclosed, dark cavities. Olfactory and tactile stimuli are processed via the antennal lobes. We suggest that response thresholds of bees at different stages of behavioral development are influenced by octopamine and other neuromodulators in the antennal lobes. JH may pace honey bee behavioral development by altering levels of these neuromodulators. One experimental prediction of this scenario is that allatectomized bees that become foragers (Sullivan *et al.*, 2000) should have elevated levels of octopamine in their antennal lobes. This has not been determined yet.

Our results suggest that JH and octopamine also may have independent actions involved in the regulation of foraging. Combined treatments with methoprene and octopamine were more effective than treatments with either compound alone. This could be the result of additive effects or synergistic effects. JH already is known to have multiple effects related to division of labor. For example, bees treated with methoprene become more responsive to alarm pheromone (Robinson, 1987b), their hypopharyngeal glands switch from producing food for larvae to enzymes involved in processing nectar (Sasagawa, Sasaki, and Okada, 1989), and allatectomized bees show deficits in flight muscle metabolism (J. P. Sullivan, J. Harrison, J. H. Fewell, S. E. Fahrbach, and G. E. Robinson,

unpublished results). In other insects, circulating octopamine is elevated in association with long-distance flight (reviewed by Orchard, 1982). Perhaps treatments with both octopamine and JHA influence processes independently to further increase the likelihood that a bee will become a forager.

The interaction between JH and octopamine in the regulation of foraging behavior is similar in some respects to the control of aggressive behavior in vertebrates by endocrine and neurochemical factors. Elevated testosterone increases aggression in rats, causing a decrease in serotonin. Treatments with testosterone require long-term administration to be effective, while treatments targeting serotonin act more rapidly (Breuer, McGinnis, Lumia, and Possidente, 2001; Bonson, Johnson, Fiorella, Rabin, and Winter, 1994; Cologer-Clifford *et al.*, 1999). Testosterone treatments result in a depletion of serotonin in the brain and increased aggression, but even in the presence of elevated testosterone, serotonin is the more proximal factor affecting aggression; rats with elevated testosterone treated with serotonin agonists show a decrease in aggression (Cologer-Clifford *et al.*, 1999; Bonson, and Winter 1992). In honey bees, our results show that octopamine treatment can rescue the allatectomy-induced delay in forager development and thus act as a proximal factor that affects foraging. It is not known whether JH acts directly to influence octopaminergic neurons; this issue is complicated by the fact that no JH receptor has yet been characterized. More research is necessary to provide a better understanding of how JH influences octopamine in the antennal lobes and how octopamine influences response thresholds to task-related stimuli.

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