Biogenic Amines in the Antennal Lobes and the Initiation and Maintenance of Foraging Behavior in Honey Bees

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ABSTRACT: **Previous findings showed that high levels of octopamine and serotonin in the antennal lobes of adult worker honey bees are associated with foraging behavior, and octopamine treatment induces precocious foraging. To better characterize the relationship between amines and foraging behavior in honey bees, we performed a detailed correlative analysis of amine levels in the antennal lobes as a function of various aspects of foraging behavior. Flight activity was measured under controlled conditions in a large outdoor flight cage. Levels of octopamine in the antennal lobes were found to be elevated immediately subsequent to the onset of foraging, but they did not change as a consequence of preforaging orientation flight activity, diurnal pauses in foraging, or different amounts of foraging experience, suggesting that octopamine helps to trigger and maintain the foraging behavioral state. In contrast, levels of serotonin and dopamine did not show changes that would implicate them as either causal agents of foraging, or as neurochemical systems affected by the act of foraging. Serotonin treatment had no effect on the likelihood of foraging. These results provide further support for the hypothesis that an increase in octopamine levels in the antennal lobes plays a causal role in the initiation and maintenance of the behavioral state of foraging, and thus is involved in the regulation of division of labor in honey bees.** © 2002 Wiley Periodicals, Inc. J Neurobiol 54: 406–416, 2003

Keywords: **octopamine; serotonin; antennal lobes; foraging behavior**

INTRODUCTION

There are potent reciprocal relationships between biogenic amines and behavior. Dopamine, serotonin, and octopamine regulate diverse behaviors, whereas levels of circulating brain amines are in turn influenced by the performance of specific behaviors. For example, biogenic amines have been implicated in the regulation of aggression (Adamo et al., 1995; Brain and Haug, 1992; Kravitz, 2000; Stevenson et al., 2000), feeding behavior (Leibowitz and Alexander, 1998; Long and Murdock, 1983; Lent, 1985), and learning and memory (Erber et al., 1993; Hasselmo, 1995). In addition, long distance flight elevates blood levels of octopamine in locusts (Goosey and Candy, 1980), and the performance of cognitive tasks can increase the release of dopamine in the mammalian brain (Feenstra et al., 2001; Fried et al., 2001). This study addressed the question of whether there is a reciprocal relationship between biogenic amines and foraging behavior in the honey bee, *Apis mellifera*.

Division of labor in honey bee colonies is based on

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a pattern of behavioral development in which adult worker bees progress through different specialized behavioral groups as they grow older (Winston, 1987). Young bees work in the hive for the first 2 to 3 weeks of life performing such tasks as brood care (nursing) and then become foragers and collect nectar and pollen. Bees take hundreds of individual foraging trips during their 4–6 week adult life, and forager bees perform little or no other work in the hive besides foraging. They are inactive at night, while younger bees work around the clock (Bloch and Robinson, 2001), and they also remain inactive on days when foraging is not possible or relatively unprofitable (Seeley, 1995). Therefore, becoming a forager can be seen as entering into a long-term, stable behavioral state.

The transition to foraging in honey bees involves changes in brain levels of biogenic amines. Foragers have higher brain levels of dopamine, serotonin, and octopamine than bees that work in the hive (Taylor et al., 1992; Wagener-Hulme et al., 1999). This relationship is most consistent for octopamine and serotonin in one particular region of the brain, the antennal lobes. Foragers have more serotonin and octopamine in their antennal lobes than do nurses (Schulz and Robinson, 1999). Bees induced to forage precociously show high, forager-like levels of serotonin and octopamine in their antennal lobes, and bees that revert to nursing after foraging show lower, nurselike, levels in the antennal lobes (Schulz and Robinson, 1999). Because it is thought that many behaviors related to division of labor are evoked by chemosensory stimuli, these results suggest that biogenic amines play a strong role in the organization of this social system. This is consistent with the finding that bees treated with octopamine were more likely to become precocious foragers (Schulz and Robinson, 2001). However, these oral octopamine treatments did not specifically target the antennal lobes; there is as yet no chronic and specific treatment that allows bees to be studied under natural conditions in the field. Therefore, one goal of this study was to determine whether levels of octopamine in the antennal lobes exhibit a pattern consistent with what would be expected if octopaminergic activity in the antennal lobes influences the onset and maintenance of the long-term, stable behavioral state of foraging, rather than more transient changes in foraging or even nonforaging flight activity. To better characterize the relationship between octopamine and foraging behavior in honey bees, we dissected foraging behavior into subcomponents. If octopamine in the antennal lobes plays a causal role in the initiation of foraging, then levels of octopamine in the antennal lobes should: increase prior to the onset of foraging; not be elevated during preforaging orientation flights; and remain high in inactive foragers.

It also is possible that octopamine in the antennal lobes is influenced by the performance of foraging behavior. We tested for this reciprocal relationship between octopamine and foraging by determining whether levels in the antennal lobes were affected by amount of foraging experience. The antennal lobes have been implicated in olfactory learning in honey bees (Grünbaum and Müller, 1998; Hammer and Menzel, 1998; Müller, 2000), and foraging presumably relies heavily on olfactory learning (Menzel and Müller, 1996). A role for octopamine in this process might be reflected in variation in octopamine levels in antennal lobes associated with differences in foraging experience. We also made parallel measurements of serotonin and dopamine in all of the conditions mentioned above to more fully explore the possible relationship between foraging behavior and biogenic amines.

Prior to this study, treatment experiments with serotonin had not yet been performed. Another goal of this study was to determine whether serotonin, which shows a pattern similar to octopamine in terms of antennal lobe levels and foraging, also plays a causal role in the initiation of foraging. To investigate this possibility, treatment experiments were conducted with serotonin alone as well as with "cocktails" (e.g., Mesce et al., 2001) of serotonin and octopamine to determine whether these compounds act synergistically to influence foraging.

MATERIALS AND METHODS

Experiments were performed during the summer of 1999 at the University of Illinois Bee Research Facility. Honey bees (*Apis mellifera* L.) were a typical North American mixture of predominantly European subspecies and were maintained according to standard bee keeping procedures (e.g., Morse, 1990).

Single-Cohort Colonies

A single-cohort colony is a small colony experimentally established entirely with 1-day-old adult bees. Before bees become foragers, orientation flights are taken by most bees in the colony simultaneously when they are about 4 days old (personal observation). Some bees in single-cohort colonies then become precocious foragers because of the lack of an existing foraging force (Robinson et al., 1989; Huang and Robinson, 1992). Thus single-cohort colonies allow us to easily examine effects of flight independent of foraging, and their small size also allows us to monitor a colony's entire

foraging force. Foragers from single-cohort colonies are similar to those in more typical colonies in circulating juvenile hormone levels (Robinson et al., 1989), brain levels of biogenic amines (Wagener-Hulme et al., 1999; Schulz and Robinson, 1999), and brain mRNA levels of the *period* (Toma et al., 2000) and cyclic-GMP dependent protein kinase genes (Ben-Shahar et al., 2002).

Each single-cohort colony was established with about 1000–1250 1-day-old adult bees 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; we assumed that all colonies in a trial had roughly equivalent genotypic compositions. Each bee was marked on the thorax with a spot of Testor's enamel paint, a different color for each single-cohort colony.

All single-cohort colonies in a trial contained the same number of 1-day-old bees from the same source colonies, a single frame of honeycomb containing honey, pollen, and empty comb for brood rearing, and a mated queen less than 1 year old obtained from the same commercial source.

Experiment 1: Effects of Foraging Initiation and Differing Amounts of Foraging Experience on Amines in the Antennal Lobes

This experiment addressed the issues of whether amine levels in the antennal lobes increase prior to the onset of foraging and whether they are affected by amount of foraging experience. To determine whether antennal lobe biogenic amine levels increase prior to foraging or vary with foraging experience, we collected bees of the same age that had been foraging for different numbers of days, and therefore presumably had different amounts of foraging experience. Three trials of this experiment were performed.

The single-cohort colonies in this experiment were each housed in a large outdoor mesh flight cage (6 m × 20 m × 3 m) to limit the bees to one foraging source so that we could observe all of the foragers in a colony. The colony was placed approximately 17 m from the sole food source in the flight cage, a sucrose feeder (50% w/w) and a dish of freshly ground pollen from unknown floral sources. Foragers landing at the feeders were paint-marked on their abdomens with a spot of Testor's enamel paint. Different color paint was used for each day of foraging, and every forager that landed at the feeder was paint-marked from the very first forager seen until the collections were performed. When an observer was not present, the feeder was removed from the flight cage and the entrance of the colony blocked with artificial rain (Huang and Robinson, 1996; Elekonich et al., 2001) to prevent the bees from foraging and gaining experience in the absence of an observer.

In all trials, all groups were collected at the same time to ensure that all bees collected were the same age. In Trial 1, foragers with zero (bees on their first successful foraging flight), 1, 2, 3, and 4 days of experience were collected when they were 12 days old. In Trial 2, foragers with 1, 2, 3, and 4 days of experience were collected when they were 13 days old. In Trial 3, foragers with 2, 3, 4, 5, and 6 days experience were collected when they were 12 days old. All foragers were collected while at the feeder (actively foraging). In each trial, same-aged nurse bees also were collected at the same time for comparison with forager groups. Nurses were identified as bees on the comb with their heads in cells containing larvae. The earliest we can collect foragers is on their first foraging flight, as this group is identified solely by the performance of the behavior. However, we dissociated flight from foraging in young bees by examining orientation flights (see Experiment 2).

In Trial 3, a second single-cohort colony was established to be as similar as possible to the colony placed in the flight cage. This colony had the same number of bees, from the same random mix of source colonies, and was established at the same time as the colony in the flight cage. This colony was placed outside of the flight cage so that we could determine whether the flight cage environment affected biogenic amine levels in the antennal lobes of foragers. Foragers in this "field colony" were free to forage on any floral source in the area, and potentially could fly much greater distances. Nurses and foragers from the field colony and the colony in the flight enclosure were collected when they were 14 days old. Foragers in the field colony were collected returning to the hive entrance and identified by pollen loads on the corbiculae of their hind legs.

Experiment 2: Effects of Orientation Flight Activity on Amine Levels in the Antennal Lobes

This experiment addressed the issue of whether amine levels in the antennal lobes fluctuate transiently during preforaging orientation flights. This was accomplished by collecting bees from single-cohort colonies before, during, and after orientation flights. Orientation flights are easily distinguished at the hive entrance from foraging flights. They are taken by young bees prior to the onset of foraging to learn, among other things, the location of the hive relative to prominent landmarks (Capaldi et al., 2000; Winston, 1987). We collected bees from two different colonies either 6 h prior to orientation flights ("before"), during orientation flights ("during"), or 18 h after orientation flights ("after"). Bees collected prior to orientation flights were collected randomly from inside the hive. During orientation flights, some bees were collected immediately while others were paint-marked on their abdomens with a spot of Testor's enamel paint. Bees collected after orientation flights were identified by paint marks on their abdomens and collected from inside the hive. Foragers also were collected for comparison.

Experiment 3: Effects of Foraging Flight Activity on Amine Levels in the Antennal Lobes

This experiment addressed the issue of whether levels of octopamine in the antennal lobes remain high in inactive foragers. We collected foragers, active and inactive, from two different typical, large (10,000+ bees) field colonies with multiply mated queens. Successful foragers of unknown age were identified at the hive entrance by pollen loads on their corbiculae. Some of these bees were collected immediately as active foragers at 1600, while others were paint-marked on the abdomen with a spot of Testor's enamel paint. The following morning at 0400, before sunrise and before any flight activity resumed, bees previously paint-marked as returning foragers were collected from inside the hive as inactive foragers.

Experiment 4: Effects of Serotonin, 5-Hydroxytryptophan, or Octopamine-Serotonin Cocktail Treatments on the Initiation of Foraging

Previous results showed that levels of serotonin, but not dopamine, in the antennal lobes often were significantly higher in foragers than in nurses, but not as consistently or as robustly as for octopamine (Schulz and Robinson, 1999). To investigate whether serotonin, like octopamine (Schulz and Robinson, 2001), also plays a causal role in the initiation of foraging, the following experiment was performed. Using the same technique as Schulz and Robinson (2001), we treated single-cohort colonies orally either with 50% sucrose solution containing serotonin or its immediate precursor 5-hydroxytryptophan (5-HTP; Sigma Chemical Co., St. Louis, MO) placed directly in the honeycomb of the colony. Six trials were performed, each with a closely matched pair of colonies, one fed only serotonin (Trials 1–3) or 5-HTP (Trials 4–6) in 50% sucrose solution, and one fed only untreated sucrose (control). Treated colonies were fed 2 mg/mL of serotonin or 5-HTP, except for Trial 3, in which the colonies were fed 4 mg/mL serotonin. Effects of serotonin and 5-HTP treatment on brain levels of serotonin, octopamine, and dopamine were examined with HPLC as in Schulz and Robinson (2001).

Because both octopamine and serotonin are elevated in the antennal lobes of foragers, we also treated with octopamine and serotonin cocktails to determine if these compounds act synergistically to influence the initiation of foraging. Two trials were performed, each with four colonies. One colony was treated with control 50% sucrose, one with octopamine (2 mg/mL), one with either serotonin (Trial 1) or 5-HTP (Trial 2; 2 mg/mL), and one treated with both octopamine and serotonin (Trial 1) or both octopamine and 5-HTP (Trial 2; 2 mg/mL each).

All colonies in each trial were observed for six periods throughout the day, from 0900 hours until foraging subsided at dusk (as in Schulz and Robinson, 2001). For each trial, the number of bees initiating foraging from each colony was determined for 3 days after the first forager was seen in either colony. 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 ("nonforagers").

Collection, Dissection, and HPLC Analysis

Bees were collected directly into liquid nitrogen (as in Wagener-Hulme et al., 1999), and were stored at −80°C until brain dissection and HPLC analysis. Whole bee heads were partially lyophilized to facilitate dissection of antennal lobes (as in Schulz and Robinson, 1999). Equal numbers of bees from each group in a trial were represented in each HPLC run to control for day-to-day variation (standards also were used as described below).

Quantification of amines in antennal lobes was performed by HPLC with electrochemical detection as in Schulz and Robinson (1999), except that samples were extracted in 20 μL of 0.2 *M* perchloric acid (see Wagener-Hulme et al., 1999). Internal standards (synephrine and dihydroxybenzylamine) were used for all samples, and each run was calibrated with external standards (octopamine, dopamine, and serotonin). Chromatogram analyses and amine quantification were done with EZChrom Chromatography Data System v6.8 (Scientific Software). Results are expressed as a concentration of amines 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

Measurements of levels of octopamine, dopamine, and serotonin in antennal lobes were tested for normality with the Komolgorov-Smirnov Normality Test. When necessary, data were normalized by log transformation. The data then were analyzed by ANOVA with Fisher's LSD posthoc analyses. In Experiment 1, a one-way ANOVA was performed on the data from each individual trial to determine if there was an effect of behavior on levels of biogenic amines. Because not all groups analyzed in this experiment were represented in each trial, an overall ANOVA could not be performed. A subsequent one-way ANOVA was performed only for the forager groups in each trial to determine if there was an effect of foraging experience on levels of biogenic amines. The foragers and nurses collected from both inside the flight enclosure and in the field colony were analyzed with two-way ANOVA for effects of behavior and location.

For Experiment 4, differences in the distribution of foragers and nonforagers between colonies were determined with contingency table analyses (2×2 *G*-tests) on a trial-by-trial basis.

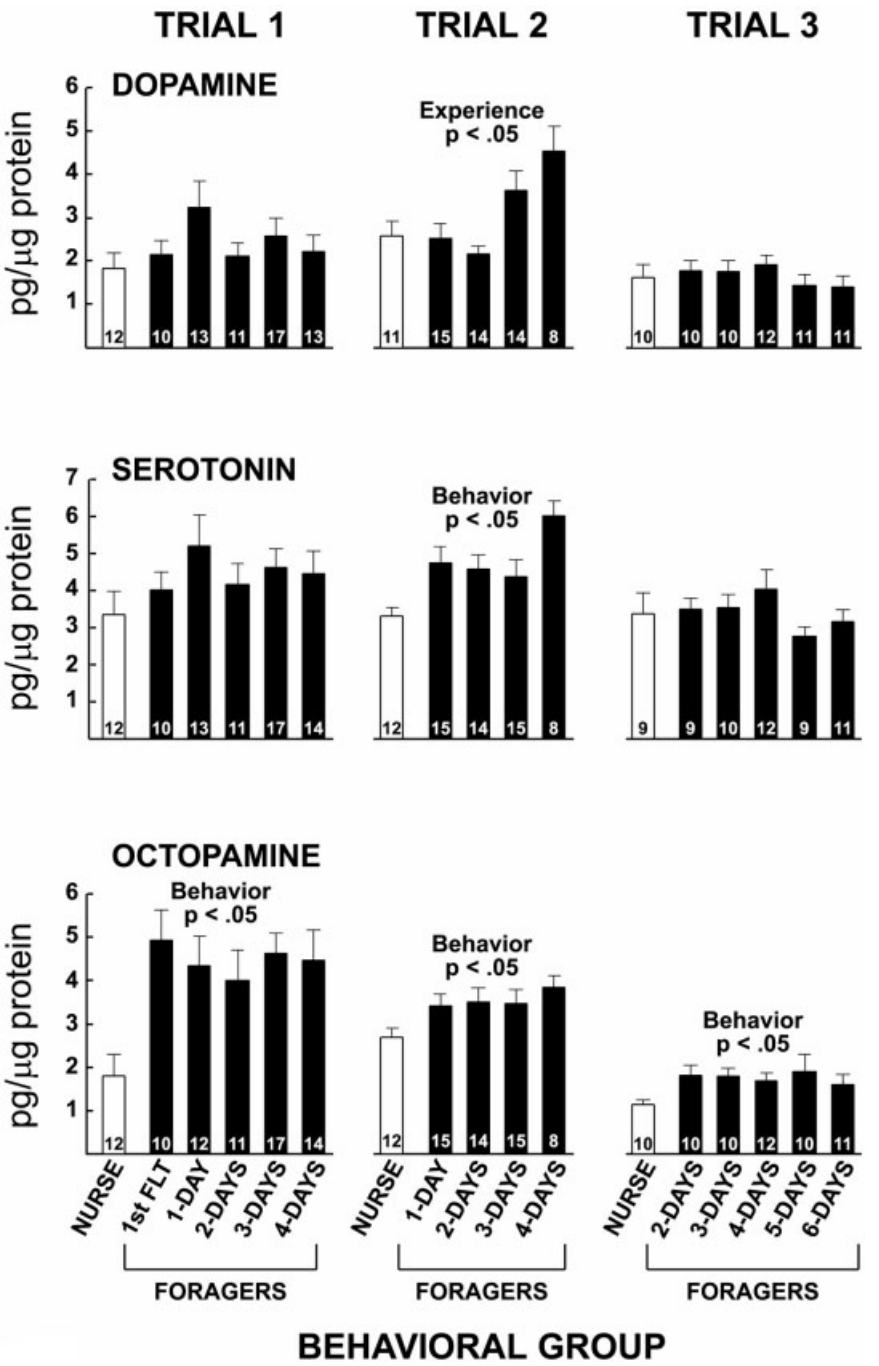

Figure 1 Biogenic amine levels in the antennal lobes of nurses and foragers with differing amounts of foraging experience (0–6 days). Bees with zero days foraging experience were collected on their very first foraging flight ("1st FLT" in Trial 1). Bars show mean ±S.E.M. Results for each trial were analyzed with ANOVA to determine whether differences in amine levels were due to variation in behavioral state ("behavior", i.e., nurse or forager), amount of foraging experience ("experience"), or both. Numbers in bars indicate numbers of antennal lobe pairs analyzed individually.

RESULTS

Experiment 1: Effects of Foraging Initiation and Differing Amounts of Foraging Experience on Amines in the Antennal Lobes

We expected octopamine would increase in the antennal lobes prior to the onset of foraging, and remain constantly elevated in foragers regardless of the extent of foraging experience. This is what was found. In three out of three trials, all foragers had significantly ($p < .001$) higher levels of octopamine in their antennal lobes than did nurses (Fig. 1), even bees on their very first successful foraging flight (Trial 1). There were no differences between nurses and foragers for dopamine and differences for serotonin in only one of three trials (Trial 2). There were no consistent effects of amount of foraging experience on amine levels (Fig. 1).

No formal analysis was performed between trials, but obvious intertrial differences exist in overall amounts of amines. These differences could reflect effects of genotype of the bees in each trial, changes in environmental conditions, or any combination of factors. Such differences between trials have been reported previously (Wagener-Hulme et al., 1999), and the overall amine levels reported in this study are consistent with other studies (Schulz and Robinson, 1999; Schulz et al., 2002).

Foragers in both colonies inside and outside the flight enclosure had significantly higher levels of dopamine, serotonin, and octopamine in their antennal lobes than nurses (Fig. 2). These differences were most consistent and robust for octopamine, which agrees with previous results (Schulz and Robinson, 1999). There were no differences between the colonies located inside and outside the flight enclosure in overall levels of octopamine and serotonin in antennal lobes (Fig. 2). Bees inside the flight enclosure had significantly higher levels of dopamine in their antennal lobes than bees outside the enclosure (Fig. 2).

Experiment 2: Effects of Orientation Flight Activity on Amine Levels in the Antennal Lobes

We expected that octopamine levels in the antennal lobes would not fluctuate transiently during orientation flights, and this is what was found. In two out of two trials, there were no differences in octopamine in the antennal lobes for bees collected before, during, or after orientation flights. Foragers had significantly higher levels of octopamine in the antennal lobes compared to these three groups of bees (Fig. 3). The same pattern of results was obtained for serotonin and dopamine (Fig. 3).

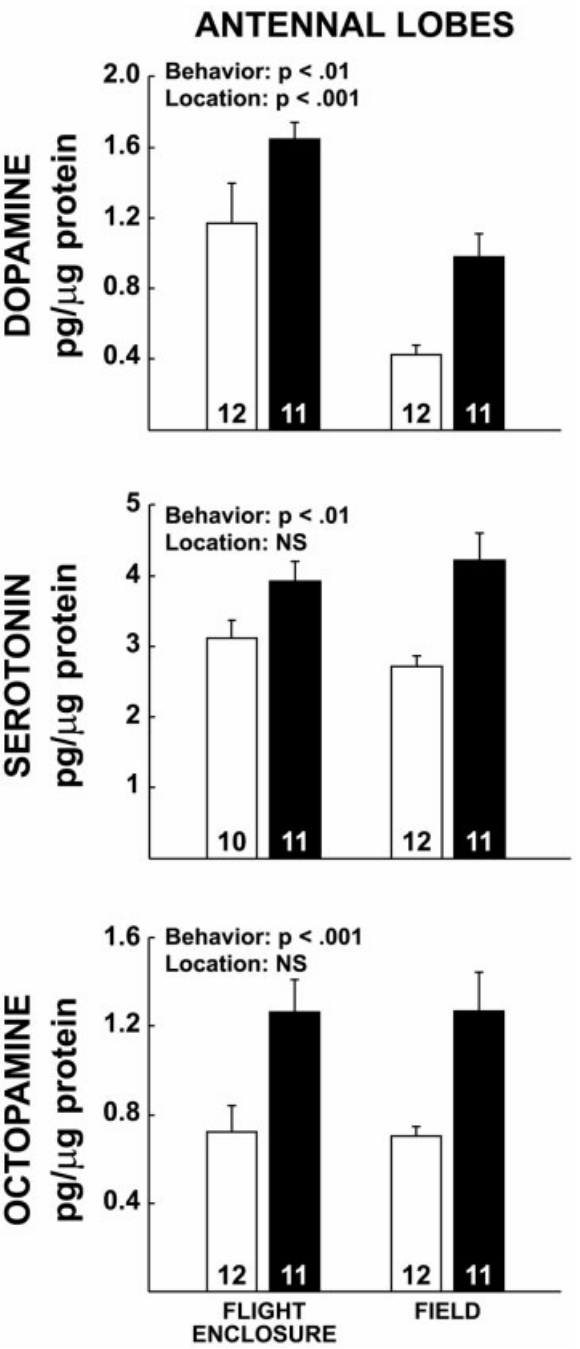

Figure 2 Effects of confinement in a large flight cage on biogenic amine levels in the antennal lobes of foragers and nurses. Bars show mean ±S.E.M. (numbers in bars as in Fig. 1). Statistical results from two-way ANOVA to determine whether differences in amine levels are due to behavior (nurse vs. forager), location (flight cage or free flying), or both.

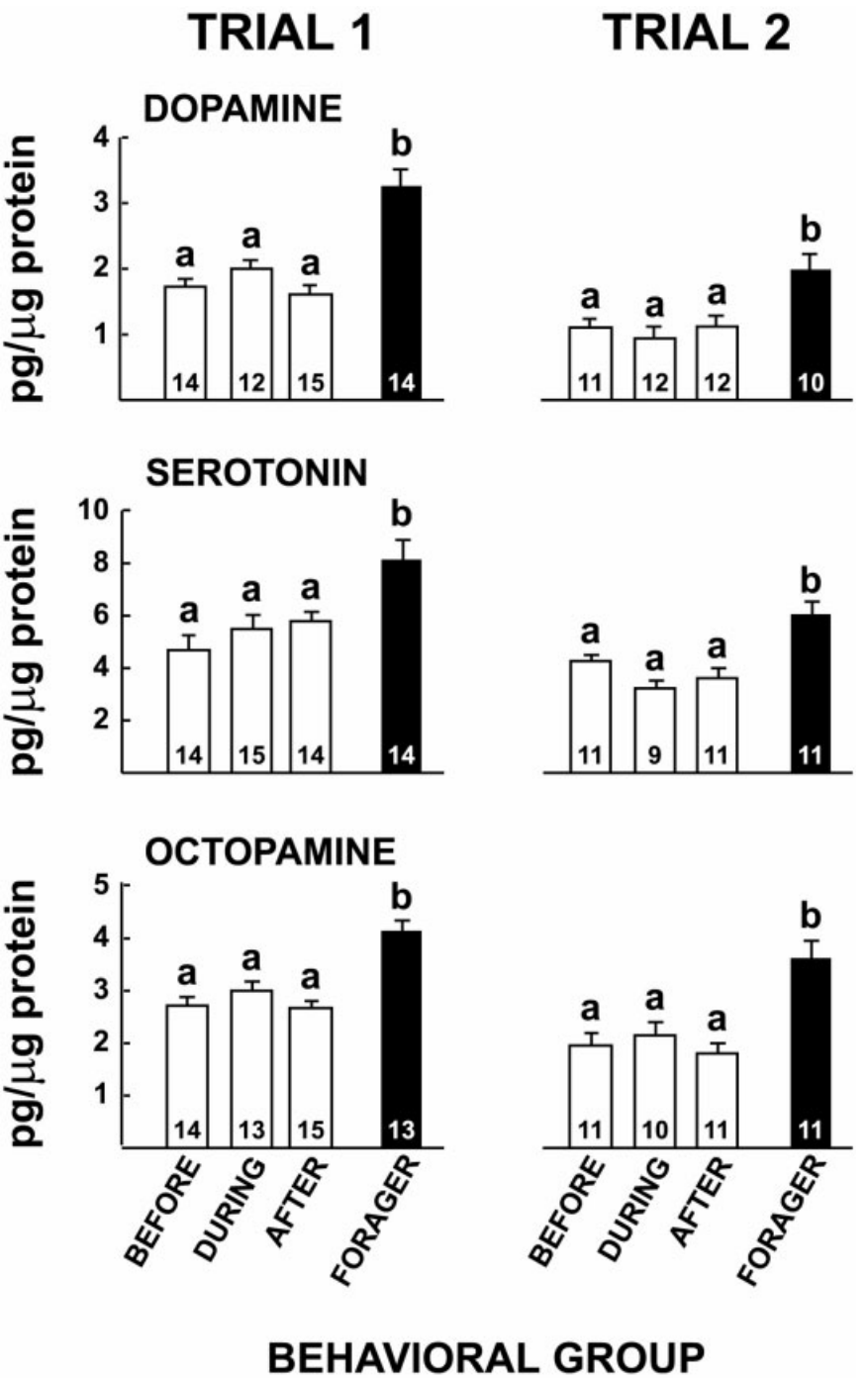

Figure 3 Biogenic amine levels in the antennal lobes of bees collected before, during, and after orientation flights. Forager group collected from the same colonies included for comparative purposes. Bars show mean ±S.E.M. (numbers in bars as in Fig. 1). Letters represent significant differences ($p < .01$) between groups as revealed by ANOVA with posthoc Fisher's LSD tests.

Table 1 ANOVA Results for Comparisons of Biogenic Amine Levels in the Antennal Lobes of Active and Inactive Foragers

Dopamine		
Factor	*F*-value	*p*-value
Activity	1.189	.2819
Trial	2.008	.1640
Activity*trial	0.001	.9770
Serotonin		
Factor	*F*-value	*p*-value
Activity	10.827	.0020
Trial	61.520	<.0001
Activity*trial	6.315	.0158
Octopamine		
Factor	*F*-value	*p*-value
Activity	.001	.9697
Trial	16.417	.0002
Activity*trial	0.720	.4008

Experiment 3: Effects of Foraging Flight Activity on Amine Levels in the Antennal Lobes

We expected that foragers would have elevated levels of octopamine in their antennal lobes regardless of whether they were actively foraging or not, which is what we found. There were no consistent differences between active and inactive foragers in levels of octopamine in the antennal lobes (Table 1), and a similar pattern was obtained for dopamine. Inconsistent results were obtained for serotonin. In Trial 1 there were significant effects of both activity and trial, as well as an activity by trial interaction, but these differences were not seen in Trial 2.

Experiment 4: Effects of Serotonin, 5-HTP, or Octopamine-Serotonin Cocktail Treatments on the Initiation of Foraging

If serotonin plays a causal role in the initiation of foraging similar to octopamine, then we would expect serotonin treatments to increase foraging in a manner similar to octopamine treatments (Schulz and Robinson, 2001). This did not occur, even though oral treatment with serotonin or 5-HTP (2 mg/mL) did result in a significant increase in brain levels of serotonin (while levels of octopamine and dopamine remained unchanged; data not shown). In five out of six trials, neither serotonin nor 5-HTP treatment caused an increase in the number of bees that initiated foraging [Fig. 4(A,B)]. In two out of two trials, octopamine-treated colonies and colonies treated with both octopamine and serotonin or 5-HTP had significantly more foragers than both control and bees treated with serotonin or 5-HTP alone. However, there were no differences between colonies treated with octopamine and colonies treated both with octo-

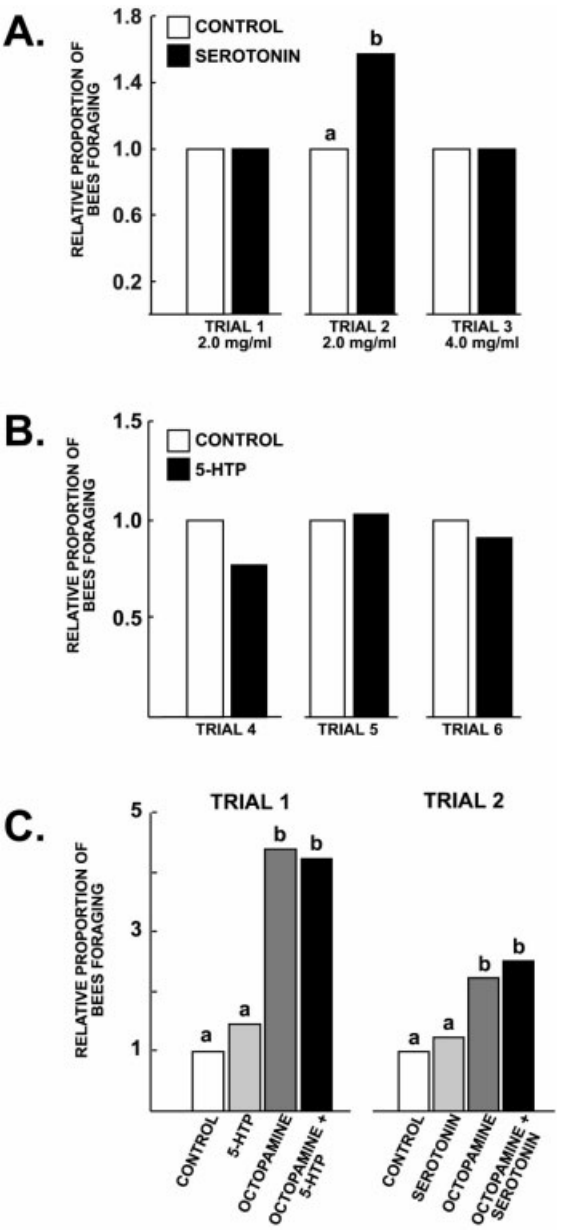

Figure 4 Effects of serotonin treatment on the likelihood of initiating foraging in honey bee colonies. (A) Serotonin (doses as shown), (B) 5-HTP (2 mg/mL), or (C) octopamine+serotonin (2 mg/mL each). The actual proportion of foragers in each colony was determined and used to calculate the relative proportion of bees foraging by normalizing within each trial with the values from the control colony. Different letters indicate significant differences ($p < .05$) between the groups in each trial as revealed by pairwise 2×2 *G*-tests.

pamine and serotonin or octopamine and 5-HTP [Fig. 4(C)].

DISCUSSION

Levels of octopamine in the antennal lobes were found to be elevated immediately subsequent to the onset of foraging, the earliest point at which we can sample foraging-related changes in octopamine, but they did not change as a consequence of flight activity or foraging experience. These results suggest that octopamine increases in the antennal lobes prior to the onset of foraging to trigger foraging behavior, and remains elevated to maintain the forager behavioral state. Levels of octopamine do rise in bees as they age, prior to the onset of foraging (Wagener-Hulme et al., 1999), which is consistent with this notion. In contrast, levels of serotonin and dopamine did not show changes that would implicate them as either a causal agent of foraging, or as something affected by the act of foraging, and serotonin treatments had no effect on the likelihood of foraging. These results provide further support for the hypothesis that an increase in octopamine levels in the antennal lobes plays a causal role in the initiation and maintenance of the behavioral state of foraging, and thus is involved in the regulation of division of labor in honey bees (Schulz and Robinson, 2001).

Some animals have extensive behavioral repertoires and make numerous, short-term switches from one behavior to another throughout their life. The evolution of division of labor in social insects, however, has involved an organization of the behavioral repertoire into a set of long-term, stable behavioral states, characterized by the consistent performance of a subset of the entire repertoire over a sustained period of time (Oster and Wilson, 1978). Foraging in honey bees is one such behavioral state. A bee's foraging career may span as much as 2 weeks (out of an adult lifespan of 4–6 weeks), and foragers rarely perform other tasks, even when foraging is not possible such as during heavy rain or at night (Bloch and Robinson, 2001; Moore et al., 1998). The foraging state in honey bees is associated with long-term physiological changes in endocrine activity (Huang et al., 1991, 1994), exocrine gland secretions (Winston, 1987), oxygen metabolism (Harrison, 1986), neuroanatomy (Withers et al., 1993, 1995; Durst et al., 1994; Sigg et al., 1997), gene expression in the brain (Toma et al., 2000; Shapira et al., 2001; Kucharski et al., 1998; Ben-Shahar et al., 2002), and octopamine in the antennal lobes (Schulz and Robinson, 1999, 2001).

It is not known how octopamine in the antennal lobes influences the foraging behavioral state. One hypothesis is that octopamine modulates responsiveness to specific task-related stimuli, making bees more likely to respond to foraging-related stimuli (see Beshers et al., 1999; Schulz and Robinson, 2001). This hypothesis is consistent with findings that octopamine treatments increase responsiveness to brood pheromone, a cue known to influence foraging behavior, resulting in an increase in the number of bees that forage (Barron et al., 2002). This hypothesis, however, is not completely consistent with the generally accepted role of octopamine as a more general excitatory neurochemical that increases arousal (Corbet, 1991). Alternatively, octopamine may act in honey bees through a general arousal mechanism, with the effects of octopamine influenced by aspects of the bee's social environment. For example, because the probability of performing a task in a beehive is determined both by responsiveness to the task-related stimulus and the probability of encountering the stimulus (Robinson, 1987), octopamine may cause stronger effects for foraging than other tasks if bees with higher levels of octopamine are more likely to encounter foraging-related stimuli. Octopamine is known to affect other aspects of bee behavior in ways that are consistent with the general arousal model (Erber et al., 1993; Robinson et al., 1999). There is not enough information at the present time to distinguish between the two alternatives with respect to honey bee division of labor.

The neurobiology of long-term behavioral states in vertebrates has been studied extensively for reproductive and parental behaviors (Crews, 1987; Wingfield et al., 1997; Wang and Buntin, 1999). As with honey bee foraging, the initiation and maintenance of long-term states of reproductive and parental behavior are also regulated neurochemically, in some cases involving amines (Mani et al., 1994; Wang et al., 1998). In addition, many behavioral states influenced by biogenic amines are relatively short term. For example, the influence of serotonin on states of hunger and satiation in leeches (Lent and Dickinson, 1984) and aggression in lobsters (Kravitz, 2000) acts over time scales of hours or days. Little is known about the neural mechanisms that have been modified to prolong behavioral states in evolution, though, in the case of reproductive and parental care, such developments would have profound effects on the social organization of the species. Insight into this issue might be possible with more studies on how neurochemicals initiate and maintain long-term behavioral states.

Oral treatments indicate that octopamine plays a causal role in the transition to foraging (Schulz and

Robinson, 2001), but these treatments do not specifically target the antennal lobes. Our study reports a detailed analysis of the relationship between octopamine levels in the antennal lobes and division of labor in the honey bees. Our results suggest that octopamine, but not serotonin, in the antennal lobes plays a causal role in both the initiation and maintenance of foraging behavior in honey bee colonies. To determine whether octopamine truly influences the division of labor via activity in the antennal lobes, a technique for long-term elevation of octopamine specifically in antennal lobes must be developed.

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