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Octopamine influences honey bee foraging preference

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Abstract

Colony condition and differences in individual preferences influence forage type collected by bees. Physiological bases for the changing preferences of individual foragers are just beginning to be examined. Recently, for honey bees octopamine is shown to influence age at onset of foraging and probability of dance for rewards. However, octopamine has not been causally linked with foraging preference in the field. We tested the hypothesis that changes in octopamine may alter forage type (preference hypothesis). We treated identified foragers orally with octopamine or its immediate precursor, tyramine, or sucrose syrup (control). Octopamine treated foragers switched type of material collected, control bees did not. Tyramine group results were not different from the control group. In addition, sugar concentrations of nectar collected by foragers after octopamine treatment were lower than before treatment, indicating change in preference. In contrast, before and after nectar concentrations for bees in the control group were similar. These results, taken together, support the preference hypothesis.

Keywords

biogenic amine; *Apis mellifera*; nectar forager; water forager

Adaptive behavior results from appropriate regulation of responses to external and internal stimuli. Honey bee worker behavior is regulated both in the long-term, over weeks (reviewed in Robinson 1992, 2002), and in the short-term, within minutes to days (reviewed by Seeley 1995, Page and Erber 2002). The long-term changes in worker behavior underlies the age-related division of labor in the colony, where younger workers perform jobs inside the colony, and workers at about three-weeks of age switch to jobs outside the colony such as foraging for resources (e.g. Winston 1987). Foragers collect pollen for protein source, nectar for energy, water for temperature regulation, and plant resin (propolis) for nest repair (Winston 1987). Based on environmental and colony conditions, allocation of foragers to different resources such as pollen vs nectar could change within hours (Seeley 1995, Page and Erber 2002, e.g. Fewell and Page 1993).

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The physiological bases of long-term behavioral change is well studied. Genetic (e.g. Giray and Robinson 1994), neuroendocrine (Fahrbach et al. 1995, 1997, Whithers et al. 1993, Giray and Robinson 1996, Giray et al. 1999, 2000), and molecular mechanisms have been elucidated that underlie the switch from nest jobs to outside jobs, typically over a three-week period of behavioral development (reviewed by Robinson 1992, Robinson and Vargo 1997, Giray 2000, Robinson 2002, Whitfield et al. 2003). However, physiological bases of behavioral regulation independent of age is only beginning to be studied (Taylor et al. 1992, Huang et al. 1994, Humphries et al. 2003). The role of colony-level stimuli such as nest conditions, brood pheromone (Pankiw et al. 1998), and worker-worker interactions in altering forager behavior is known. Through what physiological mechanisms these stimuli translate to different behaviors has not been studied. Taylor et al. (1992) demonstrated significant differences in biogenic amines in the brain of pollen and nectar foragers (but see Wagener-Hulme et al. 1999). Huang et al. (1994) found differences in juvenile hormone titers in similarly aged workers performing jobs such as guarding the nest and storing nectar. However, there are no systematic, causal examination of any physiological mechanism for short-term regulation of honey bee foraging behavior.

For short-term regulation of foraging behavior, octopamine is one of the most promising candidates as indicated by physiological studies on octopamine, and its behavioral effects. Octopamine has been shown to influence response threshold to sucrose in the honey bee (reviewed in Page and Erber 2002) and other insects (reviewed by Roeder 1999, 2005). Evidence for role of octopamine in learning and memory in bees include neuroanatomy (Mercer et al. 1983) and behavioral pharmacology (Hammer and Menzel 1998, Menzel et al. 1999, Scheiner et al. 2002). In honey bees, octopamine has been demonstrated to have brain levels correlated with age and behavioral specialization of bees, for instance for onset of foraging (Schulz and Robinson 1999, Wagener-Hulme et al. 1999) and performance of hygienic behavior (Spivak et al. 2003). In addition, in experiments where octopamine effects on shift from nest jobs to foraging was studied, octopamine applications were effective only a few days before onset of foraging behavior, indicating a possible neuromodulatory role for octopamine (Schulz and Robinson 2001). Further studies on octopamine-mediated increase in sensitivity to foraging-related stimuli support the neuromodulatory effect (Barron et al. 2002). Recently, Barron et al. (2007a) have shown that octopamine modulates dance behavior of honey bees such that bees exposed to octopamine treatment are more likely to dance for rewards than control bees.

We tested effect of octopamine on short-term changes in foraging behavior. We hypothesized that octopamine affects forager preference since octopamine is thought to influence reward representation as shown by change in probability for dance in response to the same reward (Barron et al. 2007a).

We hypothesize that foragers exposed to greater amounts of octopamine may forage for materials that the foragers previously would be less likely to collect (preference hypothesis). One implication of this hypothesis is that individual bees may shift towards individually less rewarding resources, such as from nectar to water, under conditions leading to increased intrinsic octopamine levels, such as stress (see Roeder 2005). Nevertheless, a shift in individual foraging preference may be adaptive at the colony level. For instance individual

bees switching from nectar to water would be important in colony response to environmental conditions such as excessive heat (water is used to dissipate heat, rev. in Winston 1987, e.g. Lindauer 1954 as cited in von Frisch 1967).

To perform tests on octopamine effect on forager preference, we treated identified nectar foragers orally with octopamine or its immediate precursor, tyramine. In the following one to three days we examined changes in material type and amount collected by the foragers in the control and treatment groups. Forage amounts were determined because octopamine is known to influence motor function (e.g. in the honey bee, Fussnecker et al. 2006), flight and flight metabolism in insects (see rev. Libersat and Pflueger 2004, Roeder 1999, 2005). For foragers returning with fluid loads, that could be nectar or water, we determined the sugar concentration of the load and compared before and after treatment concentrations.

Materials and methods

Bees

We used colonies maintained at the University of Puerto Rico Bee Research Facility at the Gurabo Experimental Agriculture Station. Experiments were performed at the Experimental Station grounds at Gurabo, PR or at the Institution for Tropical Architecture (Casa Klumb) grounds at Rio Piedras, PR. In all experiments 9–10 frame, 1 story hives have been used. These hives contained about 18–20000 worker bees, a naturally mated queen, and frames with honey and pollen, young brood (2 frames), capped older brood (2 frames), and empty frames (1–2). Effect of young brood, colony pollen and nectar stores, and availability of storage area on foraging behavior is known (rev. in Seeley 1995, see: Dreller et al. 1999, Pankiw and Page 2001, Pankiw et al. 1998, Camazine 1993, Schulz et al. 1998). By quantifying hive contents before including colonies in experiments we diminished differential effect of these factors on foraging behavior of experimental bees.

Entrance observations

Colonies were fitted with an entrance ramp exactly matching the hive entrance in width, and extending (45cm) outside. The ramps were covered with a Plexiglas top at a height (1cm) allowing one bee to walk freely between the ramp and the cover. To prevent bees from walking upside down, the Plexiglas surface facing the ramp was coated with a thin layer of Vaseline before the observations started. The departure, arrival times, and forage type of bees were recorded using a tape recorder. Data were later transcribed and analyzed using statistical analysis software JMP. Observations were terminated on the third day after treatment, and all remaining tagged foragers were collected. This assured that we would focus on short-term changes in foraging behavior that could be accounted for by the octopamine treatment. In other studies, octopamine treatment by feeding was shown to be effective no more than 3 to 4 days before the behavioral observation (e.g. Schulz and Robinson 2001).

When returning foragers were to be collected the entrance of the ramp was closed with an 8-mesh (3.2 mm x 3.2 mm mesh size) wire-screen. Foragers landing on the screen were allowed to climb into plastic vials for collection. Foragers that would be kept for further

analysis were frozen in the vials. Later their pollen load and nectar load were collected following dissection, and amount of nectar and pollen, and nectar concentrations were determined. Nectar was quantified by collecting into calibrated capillary tubes from the opening of the dissected crop.

Treatment experiments in field colonies—Three treatment experiments were performed in the field: Optimum dose (1 colony), octopamine treatment (3 colonies), tyramine effect (2 colonies) experiments. Foragers were collected at the beginning of treatment experiments and chilled in a cooler containing crushed ice. Honey crop content of these foragers were examined by inducing them to regurgitate small amount of nectar (e.g., Pankiw and Page 2000, Giray et al. 1999). We determined sugar concentration of crop content by use of a Brix hand-held refractometer (except in optimum dose study). Foragers were classified as pollen and nectar foragers if they carried pollen and nectar, pollen foragers if they carried only pollen on their hind legs, nectar foragers if they carried only nectar in the crop, and water foragers if the crop content sugar concentration was less than 10%. Liquid in the crop was identified as nectar if it had higher than or equal to 10% sugar concentration. This criterion was used because returning nectar foragers were shown to respond, on average, to 10% sucrose concentration (rev. in Scheiner et al. 2004) and in the field nectar foragers collect nectar with at least about 12% sucrose concentration (Seeley 1986, rev. in Seeley 1995).

Foragers were anesthetized by chilling (appears to prevent handling effect on intrinsic biogenic amines, Pankiw and Page 2003) and tagged with colored number tags to identify them individually. The tag numbers and treatments were randomized to allow blind observations. In all treatments chemicals to be tested were dissolved in 50% (w/v) sucrose solution. Foragers were placed in plastic screen cages (JZBZ^{TM}) fitted with a modified 500 μ l Eppendorf^{TM} tube. The tube was used as a feeder and filled with 200 μ l of the feeding solution. Foragers (n=10) were tested and found to be consuming on average 25 μ l of the 50% sucrose solution in 8 hours from the tubes in these cages. Bees in individual cages were fitted in a modified bee frame with three shelves. The frame with caged bees was fitted in a wire screen box to prevent food exchange between caged bees and bees in the colony. The screen box was then placed inside the colony. After overnight feeding (10–14 hrs) bees were released inside a hive super (box) placed above the colony. Overnight recovery and feeding may allow potential effect of anesthesia on foraging to wear off. Pankiw and Page (2003) showed that one hour after anesthesia no effect of handling on sucrose response thresholds were detectable. We made sure no bees from the colony had access to the tube feeders in the plastic cages. Entrance observations started one hour after release of caged foragers. In all studies, foragers were observed (and collected when needed) for 2 hours (900–1100 hours $\pm$ 30 min) in the morning and 2 hours in the afternoon (1400–1600 hours $\pm$ 30 min).

This method of treatment and observation helped us control the treatment dose, and limit observation to individually tagged and treated bees only when neuromodulatory effects of octopamine are expected. However, the sample sizes are smaller than whole colony treatment experiments (e.g. Schulz and Robinson 2001).

Optimum dose for octopamine application

In a pilot experiment we applied 3 different doses of octopamine to determine an optimum application dose. Previously 1mg/ml of oral octopamine application was shown to be effective for initiation of foraging behavior under conditions of free consumption by bees in a colony (Schulz and Robinson 2001). We used doses at or lower than 1 mg/ml for no-choice consumption in cages. We tested 0 (control group), 0.125, 0.5 and 1mg/ml dosages. The 0.5 and 1mg/ml dosages lead to comparable amounts of total octopamine ingested by bees as in another honey bee study where octopamine was administered orally (10 μ l of 2mg/ml dose, Spivak et al. 2003). The foragers were identified only as “non-pollen” or “pollen” foragers at the onset of the experiment. Sugar concentration for honey stomach or crop content was not determined for non-pollen foragers, and pollen foragers were not examined to determine presence of nectar in the crop. As a result “non-pollen” foragers in this experiment may include foragers collecting water or nectar, and “pollen” foragers may include foragers collecting only pollen and those collecting nectar and pollen.

All groups started with 50% “pollen” foragers and 50% “non-pollen” foragers. In the control group no change in the proportion of pollen and nectar foragers were observed. We demonstrated that greater percentages of foragers in the treatment groups shifted to collecting nectar or water with increased octopamine application. In the highest dose of 1mg/ml octopamine treatment group, 80% of foragers collected nectar or water ($n = 52$, $df = 1$, $X^2 = 4.022$, $p=0.045$). We used a regression of proportion of non-pollen and pollen foragers because octopamine effects on sucrose response threshold (reviewed in Page and Erber 2002), and on onset of foraging (Schulz and Robinson 2001, for doses less than 2mg/ml) show a linear relation with dose.

For bees observed over the course of the three days, we recorded time on foraging flights as another measure for forage type for non-pollen foragers. Water foragers usually take trips shorter than 10 minutes, nectar foragers take trips longer than 10 minutes (rev. in Gary 1992). For this measure only the pooled results for the octopamine treated bees differed from the bees in the control group. There were 8 non-pollen foragers in the octopamine treatment group with shorter than 10 min. roundtrip times, whereas there were none in the control group (G-test: $N_{\text{treatment}}=21$, $N_{\text{control}}=6$; $G^2=4.905$ $df=1$, $P=0.027$). These preliminary results suggest that some octopamine treated non-pollen foragers may be collecting water.

Octopamine effect on foragers

In the second experiment, we compared the 1mg/ml octopamine and control (fed 50% w/w sucrose syrup) groups initially composed of nectar (Colonies 1–3) foragers only, identified according to above described criterion. In all colonies we classified the material collected before and after treatment as water and nectar. In these experiments pollen foragers observed after treatment were not considered because of small numbers switching to pollen and because we could not identify a clear way to determine reward value representation in pollen vs nectar foragers. In 2 colonies (1 and 3) we also measured amount of nectar collected. In colony 1 we were able to collect foragers only on the first day of observations

due to weather conditions. In Colony 3, we also recorded the sugar concentrations of material collected before and after the treatment for individual bees.

Data from each colony were analyzed by G-tests on frequency of water foragers and nectar foragers in treatment and control bees. We also calculated a combined probability for octopamine treatment effect on shift to water foraging for all three colonies. For material amount differences, we compared by t-test the forage amount collected by bees in different treatment groups in colonies 1 and 3.

Tyramine effect experiment

In one experiment, where results were pooled from observations on two colonies that were run simultaneously, we examined specificity of the octopamine effect.

We fed 1mg/ml tyramine in sugar syrup (50% w/v), 1mg/ml octopamine in sugar syrup and just sugar syrup (control) to three groups of foragers and compared water and nectar foragers after treatment. Analysis was a G-test on frequency of water and nectar foragers in each treatment group.

Results

Octopamine effect on nectar foragers

In all colonies, after the treatment the control bees were more likely to collect nectar than bees in the octopamine treatment group (Figure 1). In colony 1 the difference was close to significant ($G^2 = 3.480$, $df = 1$, $P = 0.06$), where the sample size was the smallest due to inclement weather in two last days of observation. In colony 2 ($G^2 = 4.481$, $df = 1$, $P = 0.04$) and colony 3 ($G^2 = 10.690$, $df = 1$, $P = 0.001$) the difference was significant below the 5% level. Overall, as indicated by a combined analysis of the three colonies, there was a highly significant octopamine effect on foraging preference: octopamine treated bees were more likely to collect water than control bees (Combined probability analysis ; $X^2=25.690$, $df=6$, $P < 0.001$, Sokal and Rohlf 1995).

Control bees did not change their preference. A control bee that collected nectar with high or low sugar concentration before the experiment, did the same after the experiment. This lead to a significant positive correlation for sugar concentration of nectar collected by bees in the control group before and after the experiment (Colony 3; $r=0.67$, $p=0.02$, $n=15$; Figure 2 Control). In contrast, in the octopamine treatment bees, the 'nectar' collected after the experiment had lower sugar concentration (one-tailed paired t-test : $t=1.85$, $df=17$, $P < 0.05$). This lead to a lack of correlation for sugar concentration of nectar collected by bees in the octopamine treatment group before and after the experiment (Figure 2 Octopamine; $r=0.14$, $p=0.72$, $n=18$, power test: least n for statistical significance is 527).

The nectar loads of bees in the octopamine treatment group were greater than bees in the control group in 1 of 2 colonies only (Figure 3). Nectar concentration effect on nectar load was tested. Although a positive correlation exists between nectar load and nectar sugar concentration, this was not statistically significant (results not shown). Water foragers

collected generally smaller loads than nectar foragers. Water foragers were excluded in nectar load analyses.

Tyramine effect

In the tyramine effect experiment, similar to the octopamine effect experiment, there were significant differences in proportion of water foragers across treatment groups (G-test: $G^2 = 8.364$, $df = 2$, $P < 0.02$). In post-hoc comparisons, there were significantly more water foragers in the octopamine treatment group, but there was no significant difference between the control and the tyramine groups (Figure 4). The smaller number of bees observed foraging in the tyramine group may indicate a different effect of tyramine on flight (see also Schulz and Robinson 2001).

Discussion

These results show that octopamine influences the type of material collected by typical foragers under natural conditions. In the optimum dose experiment, significantly greater number of octopamine treated non-pollen foragers took shorter flights, probably collecting water (see Gary 1992). This inference was confirmed in experiments where forage materials were directly determined. Overall, foragers treated with octopamine were more likely to collect water than foragers in the control groups (Figures 1 and 4). These results are consistent with change in foraging preference (Preference Hypothesis). Octopamine effect on forage load (amount of nectar) was variable.

Octopamine is emerging as an important mechanism regulating division of labor in social insects (dominance in bumble bees: Bloch et al. 2000; age-related division of labor—onset of foraging in honey bees: Schulz and Robinson 1999, 2001, Baron et al. 2002, dance elicitation to rewards: Barron et al. 2007a; ants: Boulay et al. 2000). Our results support the hypothesis that octopamine is involved in short-term regulation of forager behavior in honey bee colonies, likely through effects on reward representation.

Typically, in a social insect colony, individuals perform different set of jobs at different ages. However, same-age individuals could perform different jobs within the age-associated set of jobs based on their differences in response thresholds (pollen vs nectar foragers Page and Erber 2002, Scheiner et al. 2004). Bees could also switch between jobs performed at the same age group based on changes in levels of stimuli in the colony (e.g. switching nectar sources; shift to water foraging due to increased temperatures, rev. in Seeley 1995, Winston 1987, Lindauer 1954 as cited in von Frisch 1967). Our results are consistent with the idea that octopamine may be involved in regulation of switch between materials collected by different foragers. However, other neurochemicals may also be important for short-term regulation of foraging behavior. There is evidence for increased titers of dopamine and serotonin in brains of honey bee foragers as well (Taylor et al. 1992, Wagener-Hulme et al. 1999, Schulz and Robinson 1999). Perhaps, part of the variability in response of octopamine treated bees could be explained with such interplay between different neurochemicals that modulate behavior in response to environmental and colony stimuli (not all octopamine treated bees became water foragers!). Further examination of effects of different colony

conditions and neurochemicals by themselves and in combination could be useful in understanding short-term regulation of foraging behavior (see Schulz and Robinson 2001)

The effect of octopamine on forage amount was variable. The difference between bees in the octopamine treatment and control groups changed in different colonies: only 3 μ l average difference in Colony 3 vs over two-fold difference in Colony 1. This may be partly due to large variance in nectar load of bees. Nectar load may reflect an optimization decision by bees based on colony conditions, reward representation and distance of the nectar source (rev. in Seeley 1995). Therefore differences in field conditions would influence our ability to detect effect of octopamine on nectar load. In fact, in the recent study by Barron et al. (2007a) whole bees were weighed at departure and return from the field and octopamine treated and control bees were found to have similar weight change (inferred to be forage load).

The experiment with tyramine treatment indicates that effect on material type is specific to octopamine. If the octopamine effects were not specific, we would have expected similar change in forage type with tyramine application. Octopamine treatment resulted in the greatest change in type of material collected. However, tyramine may also have a slight effect on shift to water foraging (33% of tyramine group bees collected water and only 13 % of the control bees). Although not statistically significant, the likely intermediate effect of tyramine on foraging preference (see Figure 6) may be biologically significant since there is a tyramine receptor in the brain (Blenau et al. 2000, Mustard et al. 2005). However, in a pilot study, admittedly with limited sample size, we did not see any shift to water foraging in the tyramine treated bees (unpublished results). Further studies on tyramine and octopamine, and their receptors could provide information on how biogenic amines regulate behavior (see also Roeder 2005).

There is a body of work demonstrating genetic effects on job performance for the set of jobs performed by bees at a particular age group (e.g. Robinson and Page 1988). For instance, soldiers and foragers are same age workers, but are genetically distinct individuals from different subfamilies, sharing the queen mother but sired by different drone fathers (Breed et al. 1990). For foraging tasks at least pollen foragers and nectar foragers are genetically distinct as indicated by separate selection experiments (Page and Fondrk 1995, Helmich et al. 1985), and by subfamily allocation to jobs assessed by genetic markers (e.g. Calderone et al. 1989, Dreller et al. 1995). Understanding genetic differences in behavior may require understanding physiological bases of differences in behavior (Giray et al. 1999, Giray et al. 2001). Some of the genetic differences in foragers collecting different materials may involve the biogenic amine octopamine or its receptors (Grohman et al. 2003, rev. in Evans and Maqueira 2005, see also Page and Erber 2002). Similar genetic differences in either production of or sensitivity to a regulatory chemical, juvenile hormone, appears to be important for genetic differences in rate of behavioral development from nest jobs to outside jobs across subfamilies and races of bees (Robinson et al. 1989, Giray and Robinson 1994, Giray et al. 1999). Similarly, work on genetic differences in octopamine system and its correlates to behavioral differences could add to our integrative understanding of genetic and neurochemical mechanisms of behavioral regulation.

Other researchers demonstrated that oral treatment of octopamine leads to increase in brain octopamine titers (Schulz and Robinson 1999, 2001; Barron et al. 2007b). However, one important caveat in inferring a role for octopamine in short-term integrative regulation of naturally occurring foraging behavior in honey bees remains. We have no studies measuring and comparing titers for octopamine in the brain of foragers collecting the different materials. Information on other biogenic amines and higher octopamine titers in brains of foragers in comparison to nurses (Schulz and Robinson 1999, Wagener-Hulme et al. 1999) are in agreement with a role for octopamine in regulation of foraging behavior.

The combined evidence based on previous work and this study argue for a role for octopamine in short-term regulation of foraging behavior. Octopamine appears to shift foragers to different resources, probably through altered reward representation. This shift may be critically important under stressful colony conditions, such as a shift to water in heat stress, and to pollen when brood population is increasing in early spring (Seeley 1995). Nevertheless, further research, such as octopamine titer measurements and studies employing RNAi for octopamine receptor (e.g., Farooqui et al. 2003) are needed to further elaborate the causal link between octopamine and short-term regulation of foraging behavior.

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Figure 1.

Proportion of identified nectar foragers returning to the colony with nectar or water after octopamine (1mg/ml octopamine in 50% w/v sucrose solution) or control (50% w/v sucrose solution) treatment in each colony. P values refer to non-parametric statistics comparing frequencies of nectar and water foragers in each treatment group for each colony.

Figure 2.

Comparison of sugar concentration in nectar collected before and after treatment (Colony 3). Bees in the control group did not change their preference. A control bee that collected nectar with high or low sugar concentration before the experiment, did the same after the experiment. This led to a significant positive correlation for sugar concentration of nectar collected by bees in the control group before and after the experiment. In contrast, the bees in the octopamine treatment group collected 'nectar' with lower sugar concentration after the experiment (one-tailed paired t-test : $t=1.85$, $df=17$, $P < 0.05$). This led to a lack of correlation for sugar concentration of nectar collected by bees in the octopamine treatment group before and after the experiment. N is 14 in the control group, and 18 in the octopamine group.

Figure 3.

Nectar load collected by bees in control and octopamine treatment groups. In Colony 1, foragers in the octopamine treatment group collected greater nectar loads than bees in the control group (t-test: $t = 2.52$, $df = 1$, $P < 0.03$). In Colony 2 there were no significant differences across the groups (t-test: $t = 0.7$, $df = 1$, $P = 0.50$). Bars indicate mean ($\pm$ SE) forage load for groups. Asterisk refers to significant difference across the treatment and control group at 5% level. NS means the difference is statistically not significant.

Figure 4.

Proportion of identified nectar foragers returning to the colony with nectar or water after octopamine (1mg/ml in 50% w/v sucrose solution), tyramine (1mg/ml in 50% w/v sucrose solution) or control (50% w/v sucrose solution) treatment. Letters above columns refer to non-parametric statistical analyses. Groups marked by different letters differ at the 5% significance level.