SHORT COMMUNICATION

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The effect of octopamine on behavioral responses of free-foraging bumblebees to a change in food source profitability

Abstract The invertebrate neuromodulator octopamine is known to be involved in bees' associative learning, enhancing the responsiveness of a bee to a conditioned stimulus. In this study, we tested the effect of octopamine on the choice behavior of free-flying bumblebees using a two-phase experiment in an array of artificial flowers. During the first phase of the experiment, the bee was allowed to collect octopamine-laden sugar water from two types of equally rewarding flowers (yellow versus blue). In the second phase, one type of flower was set to be unrewarding. The behavior of the bee (proportion of visits to the unrewarding flowers) over the two phases was fitted to a sigmoid regression model. Our results show that octopamine had no significant effect on the bees' equilibrium choice or on the overall rate of the behavioral change in response to the change in reward. Rather, octopamine significantly affected the time interval between the change in reward status and the initiation of behavioral change in the bee.

Introduction

Neuromodulators such as biogenic amines and peptides are substances that elicit and modify behavior patterns. They orchestrate behavior by acting simultaneously at

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many synaptic levels in both the central and peripheral nervous system, and also by a broader regulation of metabolism (Bicker and Menzel 1989; Roeder 1999). In social insects, one biogenic amine, octopamine, has been implicated in a variety of behaviors. In honeybees, for instance, octopamine is involved in the regulation of division of labor (Schulz and Robinson 1999); oral administration of octopamine to honeybee workers results in increased foraging activity (Schulz and Robinson 2001). Octopamine is likewise thought to be involved in the regulation of trophallaxis in ants and the establishment of social status in bumblebees (Bloch et al. 2000; Boulay et al. 2000).

One intensively studied behavior in bees for which the role of octopamine has not been evaluated to date is floral choice. One reason to anticipate a role for octopamine in the context of floral choice is that, in generalist bees, floral preferences are formed and modified through learning. In such bees, there is ample evidence that octopamine is involved in cue learning with nectar rewards. Mercer and Menzel (1982), for example, showed that responsiveness to the conditioned stimulus (CS), in this case orange scent, in a proboscis extension reflex (PER) conditioning paradigm was enhanced by octopamine, closely resembling the effect of sensitization. Later, Hammer (1993) identified a neuron in the subesophageal ganglion of the honeybee, termed VUM_{mx1}, which mediated the reinforcing function of sugar in odor-conditioning of the proboscis extension response. This neuron belongs to a group of octopamine-immunoreactive neurons (Kreissl et al. 1994), and it was consequently hypothesized that octopamine is released by VUM_{mx1} under sugar stimulation and mediates the effect of CS reinforcement in PER odor conditioning. In support of this hypothesis, Hammer and Menzel (1998) showed that the reinforcing effects of sugar stimulation could be substituted by odor-coupled injection of octopamine into either of two brain regions, the antennal lobe or the calyces of the mushroom bodies.

Despite the detailed description of the role of octopamine in associative learning in bees under highly

controlled conditions, little is known about how octopamine affects the more complex choice behavior exhibited by free-foraging animals. To address this question, we trained individual bumblebee workers to collect octopamine-laden sugar water from six artificial flowers of two colors, blue and yellow. Our experiment was not designed to identify a specific mechanism by which octopamine affects choice behavior, or specific sites in the brain involved in an effect on behavior. Rather, we attempted to define in detail at the effect of octopamine on the behavior of the free-foraging bee. We view this approach as complementary to studies carried out in restrained bees (see Mercer and Menzel 1982; Hammer 1993; Hammer and Menzel 1998), which concentrate on specific sites of action and neurological pathways where octopamine affects associative or non-associative learning.

Methods

The experiment took place in a small flight chamber (1.9×3.0×1.8 m) illuminated only by fluorescent light, in October–November 2001. A commercial bumblebee hive (purchased from Koppert Biological Systems) was placed outside the chamber, and foragers were allowed to enter the chamber through a short tube. During the experiment, only one forager was allowed into the room, but during all other times, bees had free access to the flight chamber and were allowed to feed on sugar water (30%) from two feeders placed on the experimental table. Temperature in the room ranged between 25 and 30°C. Artificial flowers (three blue and three yellow) were arranged as a circle of alternating colors around the edge of a round table (diameter 1.2 m). Each "flower" consisted of a small container that holds about 10 ml of sugar water. A cork with a magnetic collar floats on the sugar solution and prevents access by the bee. The bee instead has access to a small cup (4 μl) mounted on top of the cork. This cup is filled with sugar water by activating an electromagnet, which pulls the cup down into the container. The cup is filled with sugar water and, upon deactivating the electromagnet, returned to its original position, a process that takes 3 s to complete. Refilling of each of the six flowers is controlled at a user-specified time interval (termed the refilling interval). A second simultaneously running program detects the bee's probing into flowers and records the exact time of probing and the specific flower visited.

Each individual bee (a total of 39, all from the same colony) was tested in a two-phase experiment. In the first phase, the two types of flowers were equally rewarding. The refilling interval was set to 60 s for all six feeders and each bee was allowed to complete 180 visits (this particular time interval between refilling of the flowers allows the foraging bee to find a rewarding flower in about 70% of her visits). During this phase, the bee learned how to collect sugar water from the feeders. In the second phase, the three yellow feeders were turned off (gave no reward) and the refilling interval for the three blue feeders was set to 30 s. At these values, the overall availability of sugar water in the system was the same as in the first phase, but the two color types differed in reward. The bee was allowed to perform an additional 420 visits, and the change in the proportion of visits to the yellow feeders was recorded. Completing the 600 visits (both phase 1 and 2) required a bee to perform an average of 19.5 bouts (about 30 visits each) and took about 2 h to complete.

Using this paradigm, the pattern in floral choice was determined for bees fed variable levels of octopamine. Octopamine (Sigma) was dissolved in sugar water (30% sucrose) at concentrations of 0, 2, 5, and 8 mg/ml [a concentration range used by Schulz and Robinson's (2001) study on honeybees]. Feeders were then filled with octopamine-containing sugar water of the desired concentration. Any given test bee was fed at one, and only one, level of octopamine in all feeders. In addition to the four treatment groups of bees tested over two phases, a control group of bees was allowed to complete a series of 600 visits at 0 mg/ml of octopamine, with the refilling intervals held constant at 60 s for both feeder types.

To analyze the behavioral response to the change in feeder profitability, visits of individual bees were arbitrarily binned into sets of 30 successive visits. For each set, the proportion of visits to the yellow feeder was calculated. The relationship of the proportion of visits to the yellow feeder (Y) to the number of visits made (X) was analyzed using a sigmoid model:

$$Y = Y_0 + \frac{a}{1 + e^{-\left(\frac{X-X_0}{b}\right)}}$$

where a is the amplitude, the distance between two asymptotes; b is the rate of transition between the asymptotes; X_0 is the value of the inflection point, equal to the number of visits at which 50% of the transitions between the two asymptotes have occurred; and Y_0 is the value of the second asymptote. This model was used because it described very well the steady-state value of choice in each phase as well as the transition from one steady-state value to the other. Preference under steady-state conditions is described by Y_0, the steady-state value of choice in the second phase, as well as Y_0-a (the difference between Y_0 and a), which is equal to the steady-state value of choice in the first phase. An effect of octopamine on these parameters thus reflects an effect on floral choice. The transition between the two states is described by parameters b, the rate of change from one state to the other, and X_0, the inflection point of the transition. An effect of octopamine on the b parameter reflects an effect on learning while an effect of octopamine on X_0 reflects an effect on the bee's arousal.

Results

During the first phase, when the refilling interval was identical for blue and yellow feeders, the mean proportion of visits to the yellow feeders was 0.51±0.02 ($n=34$). Although this value is significantly different from the expected proportion of visits (one sample t-test, $P=0.007$), the deviation is so small that, practically speaking, the bees visited the two types of flowers at almost equal frequencies. Following the change in the refilling interval, bees showed a gradual shift in the proportion of visits to the now-unrewarding yellow feeders and, by the end of the second phase, almost no visits were made to these feeders (Fig. 1).

Using sigmoid regression analysis (SigmaPlot 4.0), the four model parameters were estimated for each bee. The effect of treatment (octopamine concentration in the sugar water) on these estimates was tested using linear regression. Overall, only the effect of octopamine treatment on inflection point was significant (Y_0, the second asymptote, $F_{3,25}=0.381$, $P=0.54$; a, the amplitude of change in states, $F_{3,25}=0.09$, $P=0.75$; b, the rate of transition between the two asymptotes, $F_{3,25}=0.014$, $P=0.91$; X_0, the inflection point, $F_{3,25}=8.23$, $P=0.008$). Thus, our results show that octopamine does not affect the steady-state values of preference (for example, by altering the perception of differences in reward), but rather affects the manner in which preference changes following a change in reward schedule. In particular, octopamine affected the parameter X_0, the inflection point of the sigmoid relationship. Given

Fig. 1 Change in the proportion of visits to the three yellow feeders. During the first 180 visits, all six feeders were re-filled with sugar water every 60 s. After this period, the three yellow feeders were shut-off and did not refill again. The three blue feeders were refilled every 30 s. The figure presents the change in the proportion of visits to the yellow feeders for different concentrations of oc-topamine in the sugar water
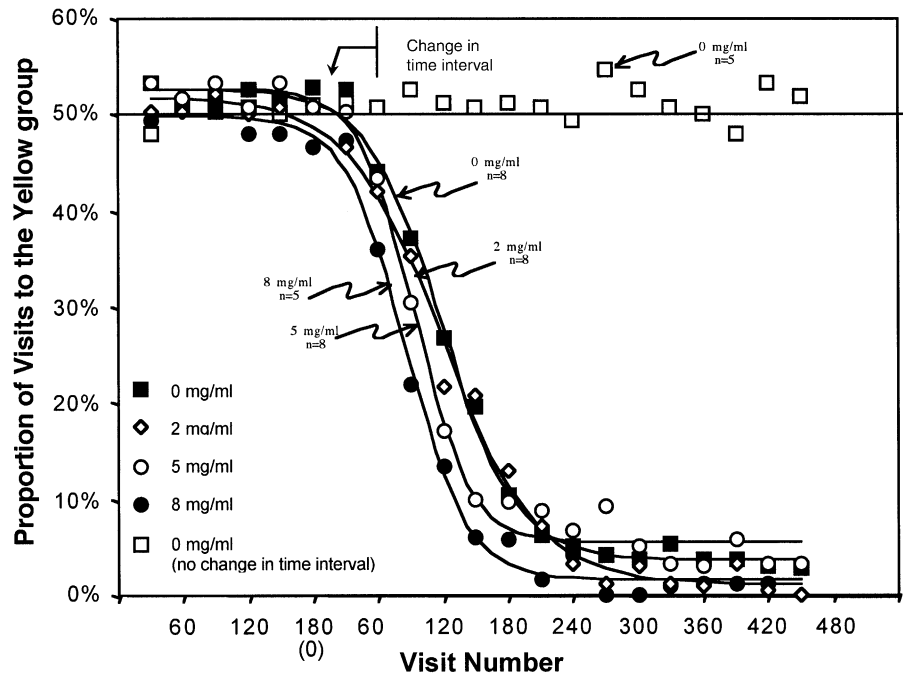
that octopamine did not affect either the asymptotic values of preference or the rate of transition from one asymptotic value to the other, the effect on inflection point amounts to an effect on the initiation of transition from the steady-state preference in the first phase. In short, bees fed at higher levels of octopamine appear to respond earlier to the change in reward schedule.

Discussion

Given these results, at least two questions arise. First, does variation in the level of octopamine at the feeders result in differences in octopamine levels in the brain? Foragers were first exposed to octopamine when the experiment began and testing began about 30 min after the end of the first phase. The octopamine-laden sugar water was carried by the forager to the colony in their crop where no sugar is absorbed (Crailsheim 1988). Nevertheless, according to Visscher et al. (1996), the sugar reserves in the honeybee hemolymph (about 2 mg) allow a worker to fly for about 12 min, after which it releases some nectar from the crop into the midgut, where the sugar is absorbed. If this value is similar for bumblebees, then by the end of the first phase of the experiment the forager is probably releasing some of the octopamine sugar water from the crop into the midgut. Schulz and Robinson (2001) used a similar technique to administer octopamine to bees and to measure its effect on division of labor in free-foraging honeybees. Using HPLC analysis, they showed that bees fed sugar water with octopamine had significantly higher levels of this biogenic amine in the brain; however, it was not clear whether the orally administered octopamine reached the brain. Ingested octopamine may conceivably pass through the gut and the blood–brain barrier; alternatively, it may generate endocrine responses which stimulate octopamine production in the brain. The bees in their experiments were fed octopamine chronically for a few days; in our experiment, bees were allowed to feed on octopamine-laden sugar water for only about 30 min before tests began (the approximate duration of phase 1). Given the difference in time frame, and lacking information about how long orally administered octopamine takes to reach the brain, it would be desirable to assess octopamine titers in the brain under our procedure.

A second question that needs to be addressed further is whether or not the effect of octopamine observed here reflects an effect on learning. As discussed above, octopamine has been shown to have a dual effect on learning in honeybees. It has a direct effect on associative learning wherein it can substitute for the unconditioned stimulus (Hammer and Menzel 1998), and an indirect effect wherein it increases sensitization (Mercer and Menzel 1982). Our results show no effect of octopamine on acquisition (b and Y_0 parameters in the sigmoid equation); rather, octopamine affected the choice process indirectly by shortening the time lag between the change in profitability (when information about relative rewards start to accumulate) and the initiation of behavioral change. This may explain why the only affected parameter is the inflection point (X_0). These findings are fully consistent with the effect of brain-injected octopamine found by Mercer and Menzel (1982) using the PER paradigm with restrained honeybees.

References

Bicker G, Menzel R (1989) Chemical codes for the control of behaviour in arthropods. Nature 337:33–39

Bloch G, Simon T, Robinson GE, Hefetz A (2000) Brain biogenic amines and reproductive dominance in bumble bees (*Bombus terrestris*). J Comp Physiol A 186:261–268

Boulay R, Soroker V, Godzinska JE, Hefetz A, Lenoir A (2000) Octopamine reverses the isolation-induced increase in trophallaxis in the carpenter ant *Camponotus fellah*. J Exp Biol 203:513–520

Crailsheim K (1988) Regulation of food passage in the intestine of the honeybee (*Apis mellifera* L.). J Insect Physiol 34:85–90

Hammer M (1993) An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. Nature 366:59–63

Hammer M, Menzel R (1998) Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. Learning Memory 5:146–156

Kreissl S, Eichmuller S, Bicker G, Rapus J, Eckert M (1994) Octopamine-like immunoreactivity in the brain and subesophageal ganglion of the honeybee. J Comp Neurol 348:583–595

Mercer AR, Menzel R (1982) The effect of biogenic amines on conditioned and unconditioned responses to olfactory stimuli in the honeybee *Apis mellifera*. J Comp Physiol A 145:363–368

Roeder T (1999) Octopamine in invertebrates. Prog Neurobiol 59:533–561

Schulz DJ, Robinson GE (1999) Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. J Comp Physiol A 184:481–488

Schulz DJ, Robinson GE (2001) Octopamine influences division of labor in honey bee colonies. J Comp Physiol A 187:53–61

Visscher PK, Crailsheim K, Sherman G (1996) How do honey bees (*Apis mellifera*) fuel their water foraging flights? J Insect Physiol 42:1089–1094