Animal Behaviour 100 (2015) 144–150

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Effect of octopamine manipulation on honeybee decision making: reward and cost differences associated with foraging

Tugrul Giray [a, *], Charles I. Abramson [b], Ana Chicas-Mosier [b], Tiyi Brewster [c], Christine Hayes [d], Karianne Rivera-Vega [e], Maya Williams [f], Harrington Wells [g]

[a] Department of Biology, University of Puerto Rico, Rio Piedras, San Juan, PR, U.S.A.
[b] Department of Psychology, Oklahoma State University, Stillwater, OK, U.S.A.
[c] Department of Biology, Clarkson University, Potsdam, NY, U.S.A.
[d] Department of Biology, Bowdoin College, Brunswick, ME, U.S.A.
[e] Department of Biological Sciences, Fordham University, Bronx, NY, U.S.A.
[f] Department of Biology, The College of New Jersey, Ewing, NJ, U.S.A.
[g] Department of Biological Science, University of Tulsa, Tulsa, OK, U.S.A.

ARTICLE INFO

Article history:
Received 24 May 2014
Initial acceptance 2 September 2014
Final acceptance 6 November 2014
Available online 29 December 2014
MS. number: A14-00428R

Keywords:
bioamines
foraging
honeybee
mianserin
octopamine

Neuromodulators have been shown to influence behavioural response in a context-dependent manner. To understand the nature of this effect we presented honeybee foragers with a foraging choice problem and fed them octopamine, its antagonist (mianserin), or simply sucrose (treatments). The test situation caused bees to deal with both cost variable (effort or work to reach the reward) and reward variable (sucrose molarity) problems simultaneously, where cost was varied by altering stamen length. High work (cost) was paired with a high reward, and low work was paired with a low reward, using blue versus white flowers as a colour cue. Regardless of treatment, roughly a third of the control bees maximized energy gain by choosing high-reward/high-work flowers (energy maximizers), but another third of the foragers consistently chose flowers that minimized work and consequently minimized reward (work minimizers). The remaining foragers seemed unable to solve the reward–cost problem and showed high fidelity to a flower colour (colour constant) even though doing so resulted in a change in cost and reward between experimental test phases. Ingestion of octopamine or its antagonist did not alter the frequency of each type of response in the forager population. However, error rate was altered in bees following energy maximization or work minimization strategies when ingesting octopamine or its antagonist. Although octopamine and mianserin affect the behaviour of honeybees, they do not appear to determine the foraging strategy of individuals.

Work on neuromodulators is now shedding light on how bioamines alter behaviour, including modification of learning and memory (Schroll et al., 2006; Schwaerzel et al., 2003; Unoki, Matsumoto, & Mizunami, 2005, 2006; Vergoz, Rousel, Sandoz, & Giurfa, 2007) in diverse invertebrates. In the particular case of the influence of dopamine and octopamine on learning, the idea that each is involved in either just appetitive or aversive memory formation (Kaczer & Maldonado, 2009; Klappenbach, Maldonado, Locatelli, & Kaczer, 2012) is changing. Thus, complex environmental situations, which may be the norm (reviewed in: Cnaani, Thompson, & Papaj, 2006; C. E. Sanderson, Orozco, Hill, & Wells, 2006), present intriguing test situations for the emerging neuromodulator model (Kaczer, Klappenbach, & Maldonado, 2011). One prediction is that our understanding of adaptive behavioural responses to aversive and appetitive stimuli would greatly benefit from studies on neuromodulator effect on reward and punishment pathways under natural conditions (Agarwal, Giannoni Guzmán, Morales-Matos, Del Valle Díaz, Abramson, & Giray, 2011; Barron, Søvik, & Cornish, 2010; Giray, Galindo-Cardona, & Oskay, 2007). Here we present such a study using honeybees as a model insect system where foraging decisions involve both negative and positive factors controlled on artificial flower patches.

In honeybees, ingestion of octopamine analogues and antagonists seemingly results in discounting punishment in laboratory assays (Agarwal et al., 2011) and in discounting reward quality as measured in nectar brought back to the hive (Giray et al., 2007). Yet, how these neuromodulators mitigate flower-visiting decisions and

* Correspondence: T. Giray, Department of Biology, University of Puerto Rico, Rio Piedras, San Juan, PR 00931, U.S.A.
E-mail address: tgiray2@yahoo.com (T. Giray).

http://dx.doi.org/10.1016/j.anbehav.2014.11.018
0003-3472/

flower-handling differences are unknown (Giray et al., 2007). Such information may help us understand the decision process of free-flying foragers choosing among alternative flowers (Abramson et al., 2012, 2008; Cakmak et al., 2010).

Honeybees present a model invertebrate for studying behavioural and ecological aspects of foraging through coupling the control of artificial flower patches in a free-flying natural environment (e.g. see: Amaya-Márquez, Hill, Abramson, & Wells, 2014; Avarquès-Weber & Giurfa, 2013; von Frisch, 1967; Menzel, 2001; S. W. Sanderson et al., 2013; Seeley, 1995; Srinivasan, 2010; Wells & Wells, 1986). Furthermore, neuromodulation might be expected to have its greatest effect on honeybee foraging in situations where the problem is difficult to solve. In fact, neuromodulation may underlie differences in behavioural plasticity among subspecies of honeybees (see Barron, Maleszka, Vander Meer, & Robinson, 2007; Giray et al., 2007). Subspecies of bees with different foraging environments (e.g. short-term versus long-term resource availability) show differences in plasticity such that, in artificial flower experiments, subtropical subspecies do not switch flower morphs upon a change in reward probability, whereas temperate subspecies switch flower choice with some probability (~25%) (Cakmak et al., 2010). Studies on neuromodulator effects on resource choice (Giray et al., 2007) and differences in flower constancy across honeybee subspecies (Cakmak et al., 2010) may connect the dots between neural and genetic mechanisms and theory that predicts increased specialization with increased environmental choices (reviewed in Cakmak et al., 2009). Although the difficult flower morph–reward association problem presented in Cakmak et al.'s study was artificial, similar or more difficult problems are likely to occur under natural conditions, such as presented by cheating and rewarding flowers where no reward versus a high reward could be associated with variable or constant floral morphology and fragrance (Ackerman, Cuevas, & Hof, 2011). Indeed, in Cakmak et al.'s (2009) study some foragers maximized energy gain by choosing high-reward, high-effort flowers (3–4 times the number of J/s), but other foragers consistently chose flowers that minimized work and consequently minimized reward. The remaining foragers showed high fidelity to a flower colour (some to blue and others to white flowers) even though doing so resulted in a change in cost and reward between experimental test phases. One hypothesis is that individual differences in bioamine neuromodulation underlie alternative solutions to complex foraging problems.

In simple reward situations, ingestion of the biogenic amine octopamine should cause bees to accept a lower reward in theory because the appetitive neuropathway is upregulated (i.e. all rewards appear good) and the aversive memory pathway is impaired (i.e. all flower morphologies appear easy). This is consistent with the finding that octopamine treatment makes bees more likely to dance for minimal rewards (Barron et al., 2007) and corresponds to foraging for rewards with low sugar concentration in the field (Giray et al., 2007). There are two components to flower choice in energy–work problems. In Cakmak et al.'s (2009) study, flowers with long stamens delayed reaching the reward and acted as an aversive stimulus compared with short-stamen flowers. This is because the physical effort in obtaining a reward can act as a punishing stimulus (see Discussion). Furthermore, 'reward' was measured by the difference in sugar solution quality, while flower colour was the conditional stimulus (CS). In general, mianserin should reduce reward value, yet leave the effect of the aversive stimulus unaltered (Agarwal et al., 2011), and this should be reflected in more foragers avoiding the aversive situation (long stamens). Mianserin treatment should result in more foragers preferring the low-work, low-reward flowers. Octopamine should lead to enhancing reward, and thus make it seem less important to the forager to track the greater reward (higher-molarity reward). In addition, octopamine interferes with aversive learning (Agarwal et al., 2011), and thus, should result in increasing the number of foragers not able to solve the problem.

METHODS

We used the experimental design of Cakmak et al. (2009), but added ingestion of octopamine or its antagonist (mianserin) by foragers. Cakmak et al. examined the effect of complex problems on the decision process of free-flying foragers choosing which flowers to visit. Artificial flower patches were utilized to control experimental conditions. Complexity was created by varying both reward and cost (time).

Flower Patch and Bees

We used the same flower and flower patch design as in Cakmak et al. (2009). This design utilizes 36 square Plexiglas flowers (18 blue and 18 white) arranged randomly as to colour on a Cartesian lattice in a flower patch approximately 0.36 m^2. Cost is altered by changing 'stamen' length (i.e. straight pin length), where several rings of stamens surrounded the 'nectary'. Not only does it take a forager longer to wiggle through the long stamens (short stamens they simply walk over), but also some entrance points are impassable and the forager has to back out and try again (see: Cakmak et al., 2009; also see Supplementary Material: Video S1 shows a bee visiting a blue flower with long stamens; Video S2 shows a bee visiting a white flower with short stamens).

Each trial of an experiment utilized a new set of uncaged free-flying, naïve honeybees (*Apis mellifera*) that had no previous experience with the artificial flower patch or the cost–reward problem. These bees were trained to the flower patch following the methods of Wells and colleagues (e.g. Cakmak et al., 2009; C. E. Sanderson et al., 2006; Wells & Wells, 1986). Four or fewer bees were used in each trial of the experiment, each uniquely marked (e.g. see Seeley, 1995) with Testor's™ enamel paint. Any additional bees that visited the flower patch were removed from the system. Because of differing return-trip times, there were only one or two bees on the flower patch at a time, which mimicked a natural foraging environment.

Bees were captured upon their first return visit to the flower patch from the hive as they landed on the first flower but before they could reach the nectary. Immediately upon capture, on this second trip to the flower patch, a bee was held by its wings and fed 10 μl of one of three solutions: (1) 1 μg/μl of octopamine in 0.5 M sucrose solution; (2) 1 μg/μl of mianserin in 0.5 M sucrose solution; or (3) 0.5 M sucrose solution. Bees that would not drink the solution were removed from the system. After drinking the reward, bees were held for 15 min in a cage and then released. We minimized the time that bees were held to maximize forager return rate (Craig et al., 2012). Time taken for the first return to the flower patch after being released ranged from 10 to 20 min for bees that returned to the flower patch.

In our experience, and as reported previously, octopamine remains stable in sugar solutions, providing consistent effects on behaviour and measurable and tractable changes in haemolymph and brain octopamine titres (Agarwal et al., 2011; Barron et al., 2007; Giray et al., 2007; Scheiner, Plückhahn, Oney, Blenau, & Erber, 2002; Schulz, Barron, & Robinson, 2002). Mianserin has also been shown to be stable in terms of its effects, even in overnight feeding experiments, followed by behavioural tests (e.g. Agarwal et al., 2011; see also Vergoz et al., 2007).

Solutions were prepared daily and brought to room temperature just before feeding to the bees. Feeding periods were within 30 min of solution preparation (see above). The return time or return

success did not change with treatment in this or in previous studies (return time was 10–20 min, and was similar across treated and control-treated bees, ~90% return rate). All trials of the experiment lasted 2–3 h, with part of this time before treatment and only part (1–2 h) after treatment. These time spans are shorter than other field or laboratory treatments with oral doses of octopamine or mianserin where robust behavioural effects were also observed by two of the authors in this study and by other researchers in independent studies (Agarwal et al., 2011; Barron et al., 2007; Giray et al., 2007; Scheiner et al., 2002; Schulz et al., 2002). It is fortunate that oral feeding works well in honeybees, to the extent that even in almost industrial settings, such as filling of honeybee combs, using a sugar feed filler, or applying the chemical to whole colonies, robust effects have been observed at the onset of foraging behaviour, with effects on individual bees lasting up to 3 days (Schulz et al., 2002). In addition, Barron et al. (2007) demonstrated that oral and topical applications result in comparable levels of octopamine in the brain and last for over 12 h after one application. The dose used in this study (1 μg/μl of octopamine or mianserin) is the same effective oral treatment dose as that determined in previous studies (e.g. Agarwal et al., 2011; Giray et al., 2007).

The Experiment

Each run of the experiment used a new set of bees and had two test phases given in sequence without interruption upon return of a forager to the flower patch. The flower colour sequence that each bee visited was recorded during each test phase. In test phase 1, each bee was presented with a flower patch of 18 blue long-stamen flowers and 18 white short-stamen flowers randomly arranged with respect to colour. The blue long-stamen flowers offered foragers 4 μl of 2 M unscented sucrose reward and the white short-stamen flowers offered foragers 4 μl of 0.5 M unscented sucrose reward. In test phase 2, we switched the flower colour associated with the long-stamen high reward (from blue to white) to demonstrate that foragers were truly responding to the cost and reward associated with flower colour. Half of the bees received test phase 2 before test phase 1.

Stage 1: testing for alternative foraging strategies

We first tested whether forager response was uniform among foragers following the analysis used by Cakmak et al. (2009). We tested forager response for fit to a normal distribution via the Shapiro–Wilk test (Sall & Lehman, 1996) using arcsine square-root-transformed relative frequency of visits to blue flowers (Sokal & Rohlf, 1995). Then we tested for a limited number of distinct foraging strategies by fitting bees' visits in test phases 1 and 2 to a Poisson distribution using Kolmogorov–Smirnov goodness-of-fit test (following Sokal & Rohlf, 1995).

Stage 2: testing for neuromodulator effect

Next, we tested for neuromodulator effect on behaviour. We examined whether the neuromodulator agonist or antagonist changed the distribution of foragers among the distinct foraging strategies illuminated in the stage 1 analysis. To do this we used a chi-square test of homogeneity (Sokal & Rohlf, 1995). Next we tested whether the drugs affected error rates seen in the bees following the energy maximization or work minimization strategy. The magnitude of difference between test phases 1 and 2 represents learning level, but direction is defined by foraging strategy. We tested the absolute value of difference in relative frequency of visits to blue flowers (based on arcsine square-root-transformed data) between test phases 1 and 2 using a two-way ANOVA with drug effect (octopamine, mianserin and sugar), group effect (energy maximizer and work minimizer) and interaction effect (Sall & Lehman, 1996). See Sokal and Rohlf (1995) for a detailed discussion of partitioning the variance and related degrees of freedom associated with two-way ANOVAs.

When we tested the effect of drug treatment on energy maximization and work minimization we looked at the situation when rewards and/or aversive stimuli associated with conditioning cues are reversed. Colour constant bees do not use differences in reward or aversive stimuli to make decisions (e.g. Cakmak et al., 2009; C. E. Sanderson et al., 2006; Wells & Wells, 1986), and thus, we used a separate analysis to examine drug effect in this group of bees. Here we tested for a change in the bees' degree of flower colour fidelity. We tested the absolute value of difference in relative frequency of visits to blue flowers (based on arcsine square-root-transformed data) between test phases 1 and 2 using a one-way ANOVA with drug effect (octopamine, mianserin and sugar) as the factor.

RESULTS

Data analysis occurred in two stages. The first demonstrated that forager response was not uniform among foragers (see Alternative Foraging Strategies), which is a necessary prerequisite for interpretation of drug effects. The second stage showed a neuromodulator effect on behaviour (see Neuromodulator Effect).

Alternative Foraging Strategies

Like the results reported by Cakmak et al. (2009), response was neither uniform nor random across bees in the current study. The relative frequency of blue flower choice was not normally distributed in either test phase 1 (Shapiro–Wilk test: $W_{120} = 0.9221$, $P < 0.0001$) or test phase 2 ($W_{120} = 0.9226$, $P < 0.0001$). Furthermore, the relative frequency of individual foragers' visits to blue flowers (test phase 1 versus test phase 2) was not Poisson distributed (Kolmogorov–Smirnov test for fit to distribution: $D_{100} = 0.148$, $P < 0.05$; Fig. 1) as would be expected if there were no relation between flower choice in each of the two test phases. Four distinct patterns are seen in Fig. 1: (1) fidelity to blue flowers in both test phases; (2) fidelity to white flowers in both test phases; (3) fidelity to blue flowers in test phase 1 and fidelity to white flowers in test phase 2; and (4) fidelity to white flowers in test phase 1 and fidelity to blue flowers in test phase 2.

Thus, the bees' response to the test situation fell into three distinct categories. Roughly a third of the foragers chose the flower colour that gave the greatest energy reward (long-stamen, high-molarity reward). In contrast, about a third of the bees showed fidelity to the flower requiring the least effort to visit (short-stamen, low-molarity reward). The remaining bees were flower-colour constant across the two test phases, some to blue and others to white. They chose flowers to visit based solely on colour, ignoring both reward received and effort required to reach the nectary. In effect, they could not solve the cost–reward problem.

Neuromodulator Effect

First, we examined whether the neuromodulator agonist or antagonist changed the distribution of foragers among the distinct foraging strategies. There was no significant difference among bees with respect to whether they received mianserin, octopamine or simply sugar (chi-square test: $\chi^2_4 = 3.320$, $P > 0.25$; Table 1).

Second, we tested whether these treatments affected the error rate of bees following the energy maximization or work minimization strategy. Error rate is the percentage of visits to the flower that does not fit the predominant choice or apparent strategy of the bee in a particular trial. For instance, bees following an apparent energy maximization strategy predominantly visit long-stamen

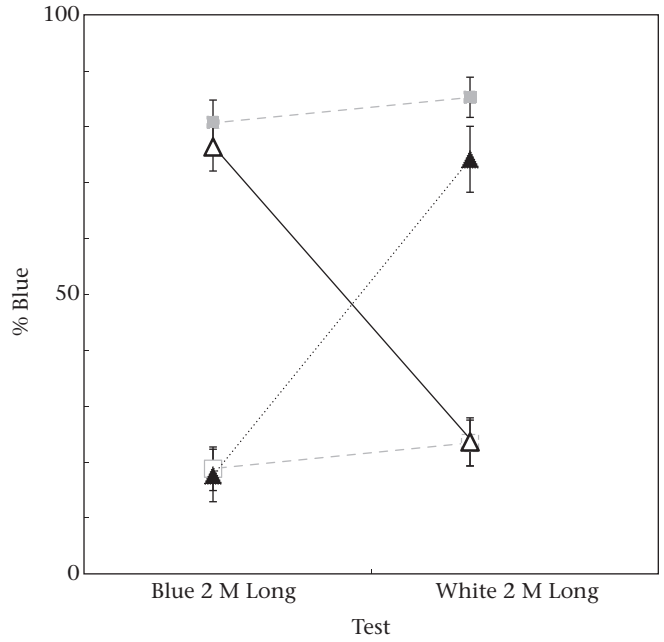

Figure 1. Flower choice by honeybees visiting patches of blue and white flowers where stamen length and reward molarity varied between flower colours in test phases 1 and 2. Test phase 1 offered bees short-stamen white flowers with 0.5 M sucrose rewards and long-stamen blue flowers with 2 M sucrose rewards. Test phase 2 offered bees long-stamen white flowers with 2 M sucrose rewards and short-stamen blue flowers with 0.5 M sucrose rewards. Mean percentage ± SE of blue flowers visited by treatment is shown for each subpopulation of foragers. In test phases 1 and 2, some bees (Δ, $N = 44$) based flower choice on net reward, while others (▲, $N = 43$) minimized handling time, and still others remained constant to a flower colour ($N = 33$; fidelity to blue flowers: ■, $N = 16$; fidelity to white flowers: □, $N = 17$).

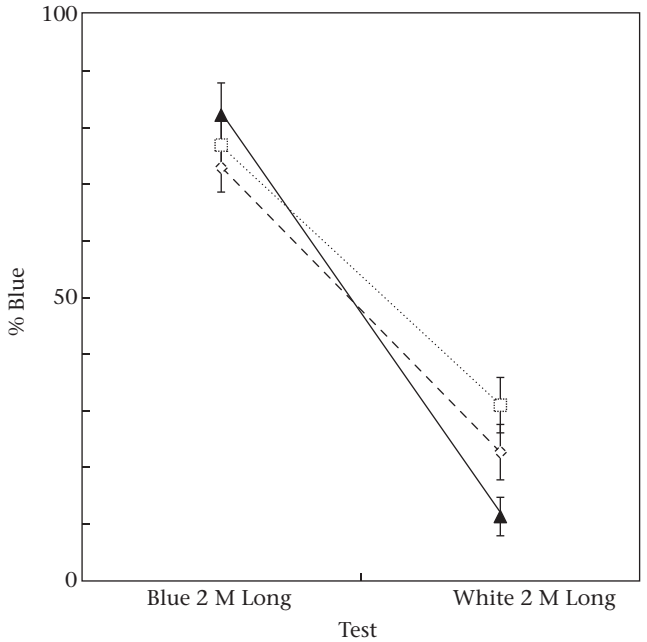

Figure 2. Flower choice by honeybees following the energy maximization 'strategy' when visiting patches of blue and white flowers where stamen length and reward molarity varied between flower colours in test phases 1 and 2 (as described in Fig. 1). Mean percentage ± SE of blue flowers visited by treatment is shown for foragers given sugar solution (▲, $N = 9$), octopamine (□, $N = 17$) or mianserin (◊, $N = 18$).

flowers offering the 2 M reward; however, in the 100 individual trips recorded, each individual also may visit several short-stamen flowers offering the 0.5 M reward. Error rates ranged from 5% to 30% (see Figs. 2–3). The magnitude of difference represents the learning level, but the direction is defined by the group. We eliminated the colour-constant group because, by definition, these bees showed no change between test phases 1 and 2. Test for change used the absolute value of difference (based on arcsine square-root-transformed data) in a two-way ANOVA with treatment (octopamine, mianserin or sugar), group (energy maximizer or work minimizer) and interaction effects. Treatment effect (ANOVA: $F_{2,81} = 1.749$, $P = 0.18$) and group effect (ANOVA: $F_{1,81} = 1.829$, $P = 0.18$) were not significant by themselves, but there was a significant interaction effect (ANOVA: $F_{2,81} = 9.874$, $P < 0.001$).

To understand the nature of this interaction effect we plotted visits to blue flowers for test phases 1 and 2 for both the energy maximizer (Fig. 2) and the work minimizer (Fig. 3) groups. For the energy maximizer group, both antagonist and agonist increased error rate compared to bees given only sugar water. For the work minimizer group, the agonist group had improved performance (i.e. lower error rate). Tukey HSD and HSU Dunnett post hoc tests were performed and results were the same for the two statistics. A significant difference was observed between forager responses of the mianserin and sugar treatments when long-stamen white flowers held the higher reward (energy maximizers: Dunnett: $P = 0.0100$;

Table 1
Number of bees following each foraging 'strategy' when given mianserin, octopamine or sugar solution

	Mianserin	Octopamine	Sugar
Energy maximizers	18	17	9
Work minimizers	13	18	12
Colour constant (to blue or white)	16	9	8
Total	47	44	29

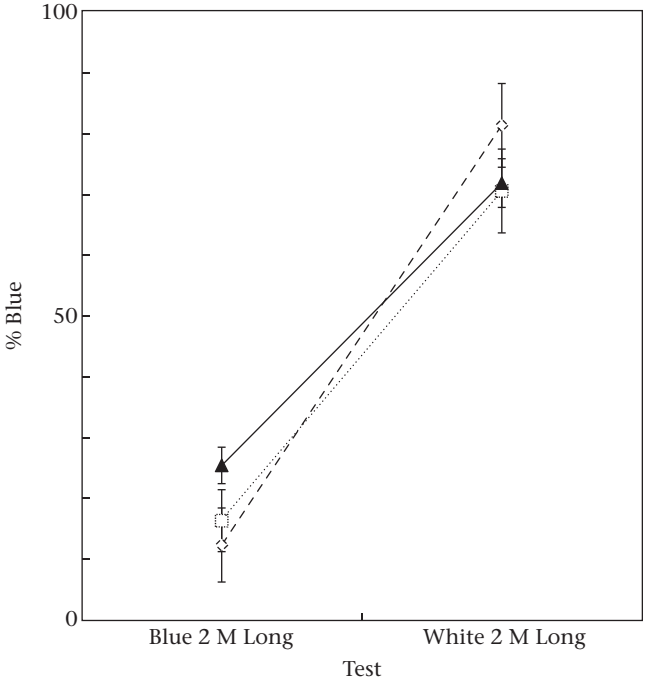

Figure 3. Flower choice by honeybees following the work minimization 'strategy' when visiting patches of blue and white flowers where stamen length and reward molarity varied between flower colours in test phases 1 and 2 (as described in Fig. 1). Mean percentage ± SE of blue flowers visited by test phase is shown for foragers given sugar solution (▲, $N = 12$), octopamine (□, $N = 18$) or mianserin (◊, $N = 13$).

Fig. 2) and when long-stamen blue flowers held the higher reward (work minimizers: Dunnett: $P = 0.0201$; Fig. 3).

Drug treatment (octopamine, mianserin or sugar) had no effect on flower colour fidelity across test phases for the colour-constant bees (ANOVA: $F_{2,30} = 0.2992$, $P = 0.7436$), as might be expected since these foragers did not appear to use the difference in either reward or punishment to make flower visit decisions.

DISCUSSION

As in a previous study without neuromodulator treatment (Cakmak et al., 2009), we found that three distinct behavioural groups of foragers occurred when bees were presented the choice between high-reward/high-work and low-reward/low-work flowers. The first group included flower-colour-constant bees (some to blue flowers, others to white flowers) that made flower choices irrespective of reward (2 M versus 0.5 M sucrose) or flower-handling difficulty. The second group included individuals that maximized energy by visiting flowers that provided high reward (2 M sucrose) in a difficult-to-handle flower. The third group minimized work by choosing flowers with short stamens even though the nectar reward was only 0.5 M sucrose.

We expected bees treated with octopamine or mianserin to switch to the work minimizer strategy based on the model that bioamine neuromodulation underlies alternative solutions to complex foraging problems seen in populations of honeybees. The prediction for octopamine is based on its association with stimulating reward neuropathways and damping aversive neuropathways (see Agarwal et al., 2011), as seen in proboscis extension reflex (PER) experiments using harnessed bees and observations of dancing for rewards with lower sugar concentration (Barron et al., 2007; Giray et al., 2007). The prediction that a greater number of work minimizer foragers will occur with mianserin treatment stems from the blocking of octopamine's effect, restoring aversive learning performance in an electric shock association assay (Agarwal et al., 2011). In contrast to theory, our results did not show a significant increase in work minimizers when treated with neuromodulators in this study. In fact, we were unable to show any significant change in numbers of bees following each foraging strategy.

One potential explanation of these results could be that difficulty in accessing reward may not be equivalent to “punishment”. The use of flowers with long stamens as a punishing stimulus is, perhaps, unorthodox in the honeybee literature where electric shock is often the stimulus of choice (Abramson, 1986; Agarwal et al., 2011; Dinges et al., 2013). Our rationale behind the use of long stamens is based on the small, yet consistent, vertebrate literature showing that the physical effort in obtaining a reward can act as a punishing stimulus in chickens (Sumpter, Temple, & Foster, 1998), pigeons (Chung, 1965), mice (Zarcone, Chen, & Fowler, 2007), rats (Alling & Poling, 1995) and humans (Miller, 1968, 1970). These and other studies (for a review of the early literature see Friman & Poling, 1995) suggested to us that response effort might produce effects similar to the response-contingent presentation of an aversive stimulus such as shock.

In fact, our study showed that error rates were altered when bees received the neuromodulator treatments, even though the frequency of forager strategies used to “solve” the problem did not change. Foragers that “solved” the energy–work problem by maximizing energy or minimizing work behaviour showed differences in their performance in repeat tests after treatment with octopamine or its antagonist mianserin. When treated with either octopamine or mianserin, energy maximizers made more “mistakes” relative to bees in the control sucrose treatment group in that they chose low-reward/low-work flowers with increased frequency. In contrast, mianserin treatment resulted in work minimizers performing better than either the control or the octopamine foragers. They chose low-reward/low-work flowers more frequently than did bees in the sucrose control treatment. Octopamine foragers presented a more complex response in the work minimizer group. They were nearly as proficient in choosing the low-reward/low-work flower as mianserin treatment bees when it was associated with blue flowers, but they performed only as well as the control bees when white flowers were associated with low-reward/low-work. This may be related to the fact that our white flowers were not “bee-white” (Hill, Wells, & Wells, 1997; Hill, Hollis, & Wells, 2001), and this may reflect subtle visual effects of treatments (but see below).

One potential explanation, but only for increased error rates, could be interference in the assay due to sensory and motor effects of octopamine. We have tested previously the motor effects at the dose used in this study and found no significant effects on locomotor activity (Agarwal et al., 2011). Sensory perception was also tested, although for a nonvisual stimulus, with no discernible effect (Agarwal et al., 2011). In the current study, the standard and robust protocol allows elimination of these effects of octopamine on error rate. For instance, no change in visits to flowers was observed for individuals not able to solve the problem, and instead these individuals visited predominantly a single colour morph, independent of the reward or difficulty (see Results). This result rules out a possible change in error rate due to visual sensory effects. In addition, return times, bout length and visit frequency did not differ before and after treatment (data not shown). Thus, the sensory and motor effects of octopamine, which are usually measurable for direct application of octopamine to the brain and in simplified test conditions of fixed bees (e.g. Erber & Kloppenburg, 1995), may have been minimized in the complex field test conditions of the current experiment. A suggestive experiment is the link between sucrose sensitivity and light sensitivity of genetically selected bees, but these are not directly linked to octopamine and involve laboratory assays of bees walking to light at very low intensities (Tsuruda & Page, 2009).

The principal conclusion from our results is that treatment with the biogenic amine octopamine and its antagonist modify foraging behaviour of bees, consistent with modulation of reward and aversive learning, but do not alter the foraging strategy of individual bees. In retrospect, this is consistent with results from aversive-learning assays in the laboratory, in which only the rate of learning was altered, yet individuals did learn to avoid colour associated with shock in all neuromodulator agonist and antagonist treatments (Agarwal et al., 2011). Overall, this suggests that octopamine increases behavioural plasticity, but not to the extent of changing basic strategy. This result may reflect the emerging idea that dopamine is involved in both reward and aversive learning and that the role of octopamine is modulatory (see Kim, Lee, & Han, 2007; Waddell, 2013). However, single bioamine differences may not be the answer to major differences in forager response to complex problems.

The experimental design used here could be useful in future work to delimit the effect of bioamine and other neuromodulators involving insects' responses to complex problems. This may involve a series of just reward differences (Barron et al., 2007; see also: Giray et al., 2007), just effort differences (e.g. Agarwal et al., 2011; Vergoz et al., 2007), and different combinations of these (Cakmak et al., 2009). The foraging problems could be coupled to treatments with other biogenic amine agonists and antagonists (Kaczer et al., 2011; Giray et al., 2007; Vergoz et al., 2007; see also Giurfa, 2013), measurements of titres of intrinsic biogenic amines (e.g. Schulz, Elekonich, & Robinson, 2003) and density of their receptors in the brain (e.g. Humpfries et al., 2003). Another important piece of

the bioamine role in problem solving can be provided by comparing ecologically and behaviourally differing honeybee populations or subspecies (Cakmak et al., 2010) in a common garden setting (Kence, Oskay, Giray, & Kence, 2013) for behavioural and biogenic amine differences, since differences in the reward and aversive pathway may underlie different foraging strategies observed across subspecies. Current work demonstrates the increasingly sophisticated understanding of foraging choice plasticity accessible through use of controllable complex foraging situations.

Acknowledgments

This research was supported by the National Science Foundation (grant DBI0851651) and a University of Puerto Rico FIPI grant (2012-0051) to T.G. The University of Central Oklahoma's Office of Academic Affairs supported C.I.A. and H.W. The research was made possible through logistical support by the Beekeeping Research Center of Uludag University. We thank National Science Foundation Research Experience for Undergraduates (NSF-REU) students Eddie Pérez Claudio and Yoselyn Rodríguez-Crúz for the supplementary video clips of bees visiting flowers with short and long stamens.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2014.11.018.

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