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

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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

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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