Cocaine affects foraging behaviour and biogenic amine modulated behavioural reflexes in honey bees

In humans and other mammals, drugs of abuse alter the function of biogenic amine pathways in the brain leading to the subjective experience of reward and euphoria. Biogenic amine pathways are involved in reward processing across diverse animal phyla, however whether cocaine acts on these neurochemical pathways to cause similar rewarding behavioural effects in animal phyla other than mammals is unclear. Previously, it has been shown that bees are more likely to dance (a signal of perceived reward) when returning from a sucrose feeder after cocaine treatment. Here we examined more broadly whether cocaine altered reward-related behaviour, and biogenic amine modulated behavioural responses in bees. Bees developed a preference for locations at which they received cocaine, and when foraging at low quality sucrose feeders increase their foraging rate in response to cocaine treatment. Cocaine also increased reflexive proboscis extension to sucrose, and sting extension to electric shock. Both of these simple reflexes are modulated by biogenic amines. This shows that systemic cocaine treatment alters behavioural responses that are modulated by biogenic amines in insects. Since insect reward responses involve both octopamine and dopamine signalling, we conclude that cocaine treatment altered diverse reward-related aspects of behaviour in bees. We discuss the implications of these results for understanding the ecology of cocaine as a plant defence compound. Our findings further validate the honey bee as a model system for understanding the behavioural impacts of cocaine, and potentially other drugs of abuse.

Eirik Søvik[1,2], Naïla Even[1], Catherine W. Radford[1], and Andrew B. Barron[1]

1. Department of Biological Sciences, Macquarie University, Sydney, Australia

2. Department of Biology, Washington University, St. Louis, U.S.A.

Running title: Behavioural effects of cocaine in bees

Corresponding author: Eirik Søvik

Department of Biology

Washington University in St. Louis

Campus Box 1137

One Brookings Drive

St. Louis, MO 63130-4899

Telephone: +1 (314) 935 3485

Fax: +1 (314) 935 4432

Email: eirik.sovik@gmail.com

Introduction

Humans and mammals consume drugs of abuse because they make them feel good (Siegel, 2005). This presents an unusual paradox (Sullivan, Hagen & Hammerstein, 2008), since many of the drugs of abuse are naturally occurring plant-derived compounds, and the evolutionary explanation given for the existence of most plant-derived drugs of abuse, is that they evolved as a defence mechanism to deter herbivory (Sullivan et al., 2008). It therefore makes no sense that these compounds should be consumed for their rewarding properties and may even be consumed compulsively. An explanation given for this apparent paradox is that plants evolved to deter herbivores insects (Nathanson et al., 1993), not mammalian ones. This argument assumes that the neurochemical pathways affected by drugs of abuse do different things in these two animal groups such that drugs of abuse are lethal to insects, but rewarding to mammals. By this argument drug reward is viewed as an evolutionary side-effect as mammals are not seen as the co-evolved target of these plant defence compounds. If this explanation is correct, drugs of abuse should not be rewarding to insects.

For a while there was some support for the idea that the neurochemical pathways signalling reward and aversion differed between insects and mammals, however this view is now being revised (Waddell, 2013). The predominant belief was that dopamine, which signals reward in mammalian nervous systems (Schultz, 2007), signalled aversive stimuli in insects (Schwaerzel et al., 2003; Vergoz et al., 2007; Honjo & Furukubo-Tokunaga, 2009; Nakatani et al., 2009). However, as more precise genetic tools have become available for studying reward circuitry in insects, it has become clear dopamine plays a role in reward signalling in insects as well (Waddell, 2013).

Despite the similarity in neurochemical reward pathways, very few studies have examined the possibility of drug reward in insects (Søvik & Barron, 2013). The most convincing evidence

that a psychostimulant drug can affect the reward system of an insects comes from the finding that following treatment with cocaine, bees are more likely to do a recruitment dance that is highly correlated with perceived reward value of a foraging site (Barron et al., 2009). This indicated that cocaine affected the perceived value of the floral resources collected.

Consequently, we investigated the effects of cocaine on reward related behaviours in honey bees. We examined whether honey bees developed a preference for a location in which they had been treated with cocaine, and whether cocaine altered foraging activity. Further, we explored the effects of cocaine on a simple appetitive reflex, sucrose responsiveness (Scheiner, Page Jr & Erber, 2001, 2004). Lastly, to test if the behavioural effects were limited to reward related behaviours we examined the effects of cocaine on responsiveness to punishing electric shock using the sting extension reflex (Roussel et al., 2009; Giray et al., 2014; Tedjakumala, Aimable & Giurfa, 2014). We discuss our findings in terms of understanding the actions of cocaine on insects and the implications of this for reconciling the ecological and neurobiological roles of cocaine.

Materials & Methods

Subjects

All experiments were performed at Macquarie University, Sydney, Australia. Bees used were of the standard commercially available strains in Australia, and reared according to standard bee keeping practices. For foraging experiments, a colony containing approx. 5000 bees was housed in a 400 m^2 flight enclosure.

Pharmacological treatments.

For topical application, 3 μg freebase cocaine dissolved in 1 μL dimethylformamide (DMF) was applied to the dorsal thorax of bees using a glass microcapillary. This was the same non-toxic dose that increased dance rate in the study by Barron et al. (2009). DMF is a solvent that can penetrate bees' cuticle and allows cocaine to pass into the haemocoel (Barron et al., 2007). This method has previously been used for administering cocaine to honey bees (Barron et al., 2009; Søvik, Cornish & Barron, 2013). As a control, bees were treated with DMF alone in the same manner.

For volatilised treatments, freebase cocaine was dissolved in ethanol, and carefully pipetted onto a nichrome wire filament connected to a power source (McClung & Hirsh, 1998). Ethanol was evaporated from the filament at room temperature. To treat bees, a single bee was kept in a 50 cm^3 airtight container encapsulating the filament. The filament was heated for 10 s and bees were kept in the container, exposed to volatilised cocaine, for one minute. Unlike vertebrates, insects have an open gas exchange system that transports oxygen directly to tissues where it is needed in the gaseous phase, bypassing the haemolymph. Air is taken in through spiracles in the thorax and abdomen, passed through trachea, before gas exchange takes place via tracheoles (Chapman, 2013). This system allows volatilised cocaine to be delivered directly to cells throughout the bee nervous system. As a control, pure ethanol was applied to the filament, allowed to evaporate, and the clean filament was used for treatments using the method outlined above (for details see Søvik et al., 2013). All reagents were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Effects of cocaine on honey bee foraging preferences.

To examine if bees developed a preference for a feeder associated with cocaine treatment, 60 individually paint-marked bees were trained to two *ad libitum* 1.5 M sucrose feeders placed at the

93 closed ends of two 2 m long tunnels that intersected at a 45° angle (Fig 1). The walls and floor of
94 the tunnels were solid opaque plastic; the ceiling was covered with mesh. From the perspective of
95 approaching from the hive, the entrance to the left tunnel and the walls surrounding the feeder in
96 the left tunnel were marked with horizontal green and white stripes, the entrance to the right
97 tunnel and the walls surrounding the feeder in the right tunnel were marked with vertical blue and
98 white stripes. The tunnels created two visually distinct and spatially separated environments in
99 which feeders were located. Bee's choice of feeder could easily be assayed visually by observing
100 which tunnel they entered and which feeder they alighted on. The colours blue and green were
101 chosen because bees have distinct photoreceptors for these two colours (Chittka & Menzel,
102 1992), further, the 90^{o} difference in orientation of the striped patterns is easily differentiated by
103 honey bees (Frisch, 1971) and was added to make the tunnels even more distinctive. This design
104 was chosen in order to increase the distinctiveness of the two tunnels (i.e. in order to make it as
105 easy as possible for the bees to tell the two tunnels apart). This allowed detecting changes in
106 preference rather than discriminatory abilities.

107 Bees were trained and tested in a five-day protocol. On day one of a trial, bees were
108 trained to use both tunnels by alternating the availability of tunnels every 15 min while
109 progressively stepping a 1.5 M sucrose feeder deeper into each tunnel over a 4 h period. Bees
110 were released from the tunnel after feeding by lifting the mesh.

111 On day two, bees were further trained to use the tunnels by alternating the availability of
112 the tunnels every 30 min for 3 h, then simultaneously opening both tunnels to provide bees with a
113 free choice of feeders for 1 h. During this time the number of visits of each bee to each feeder
114 was recorded. These were converted to a preference index as follows:

$$\text{Preference index} = \frac{(\text{number of visits to green tunnel} - \text{number of visits to blue tunnel})}{\text{total number of visits}}$$

This preference index is similar to that used for aversive conditioning by Vergoz et al. (2007), but because individual bees varied in the total number of visits made, we divided difference in visits made by the total number of visits to allow for comparison between bees. At this stage the median preference index was not significantly different from zero (Wilcoxon signed rank test. $W = 456$, $p = 0.166$, $n = 75$) indicating there was no preference toward either tunnel.

On days three and four of a trial, bees had access to the green tunnel only for 2 h a day, which offered a 1 M sucrose feeder. Bees were randomly assigned to cocaine or control treatment groups. We used the slower topical treatment method so that cocaine would persist in bees' systems for the majority of their time interacting with the tunnels (previous work suggested topical cocaine treatment influenced bee behaviour for approximately 1.5 h following treatment (Barron et al., 2009), whereas the effects of volatilised treatment appeared to be shorter in duration). Bees were treated with either 1 µl DMF containing 3 µg cocaine in or 1 µl DMF alone on their first visit to the feeder each day.

With this assay design bees had more opportunities to visit the green tunnel than the blue tunnel, and therefore had more reinforcing experiences in the green tunnel than the blue tunnel. Thus, expected all bees to develop a weak preference for the green tunnel. However, the aim of this experiment was to test whether cocaine treatment affected the magnitude of the preference for the green feeder.

On day five of a trial, all bees were given simultaneous access to both tunnels for 1 h to test the preference of bees for the different tunnels. The number of visits by each bee to each tunnel was recorded. During the test both tunnels contained empty feeders, and once bees had reached the end of a tunnel they were released. The number of visits to each tunnel by each bee was converted to a preference index as described. Five replicate trials of this experiment were performed. For analysis data from all trials were pooled.

Effect of volatilised cocaine on foraging rate.

Previously, Barron et al. (2009) did not find a difference in foraging rate between bees treated with cocaine and controls, using the topical treatment method. As topical treatment is rather slow (Barron et al., 2007) and rate of cocaine delivery to the central nervous system affects the magnitude of behavioural responses (Samaha & Robinson, 2005), we decided to test if the number of foraging trips was affected following the more rapid volatilised treatment method (Søvik et al., 2013). In a flight cage bees were trained to visit an *ad libitum* sucrose feeder where they were given individually distinctive paint marks. Bees that returned five times after being marked were caught and treated with 5 µg volatilised freebase cocaine or control. We chose 5 µg as this was the highest volatilised dose previously tested that did cause deleterious motor effects (Søvik, 2013). Bees were assigned to treatment groups randomly. The number of visits treated bees made to the feeder in the 40 min following treatment were recorded. Sucrose concentration, has previously been shown to affect foraging rate in bees (Seeley, 1995), therefore we studied responses of bees to both a low (0.5 M) and a high (2.0 M) sucrose solution.

Effects of volatilised cocaine on sucrose responsiveness.

To test if volatilised cocaine affected sucrose responsiveness we used cage-reared bees of known age and social history. Upon emergence, bees were placed in mesh cages (20 x 16 x 3 cm) with *ad libitum* access to honey. The cages contained eighty bees each, and were kept at 34ºC for 6 days. When bees were 7 days old, they were fastened individually in an 8 mm tube in a way that prevented the bees from escaping but allowed the proboscis and antenna to move freely (Bitterman et al., 1983). This method is most commonly used for proboscis extension learning experiments (Felsenberg et al., 2011), but has also been used to measure bees' responsiveness to sucrose (Scheiner et al., 2004). Once harnessed, bees were treated with 0 or 10 µg volatilised cocaine and tested for sucrose responsiveness. The 10 µg was chosen based on an initial pilot

experiment suggesting that this dose was sufficient to elicit increased responsiveness to sucrose (E.S. unpublished data). We repeated this experiment with 0, 5, 10, 20, or 50 µg volatilised cocaine to examine if the effect seen with 10 µg was dependent on the cocaine dose used.

The sucrose responsiveness test consisted of touching a drop of 10 % sucrose solution to the antennae of bees 3 min after drug exposure, and recording whether or not the proboscis was extended. After the test, bees were tested for their response to water and honey. Bees responding to water, or failing to respond to honey were excluded from the analysis.

Effects of volatilised cocaine on responsiveness to electric shocks.

To examine effects of cocaine on responsiveness to electric shock, bees were fastened between two conducting brass plates with a piece of electrical tape (for details see Vergoz et al. 2007). After treatment with 0, 5, 10, 20 or 50 µg volatilised cocaine, brass plates were connected to an electrical supply, and bees were shocked with gradually increasing voltage (0.5 V every 5 minutes) from 0-10 V. The first voltage at which a bee extended its stinger (a reflexive response) was recorded for each bee. Testing occurred in front of an extraction fan so no alarm pheromone would linger in the testing room and affect bees yet to be tested (Vergoz et al., 2007). Comparisons between groups were based on EV_{50} (half maximal effective voltage): the point at which half of all bees in the treatment group extended their stingers.

Results

Effects of cocaine on honey bee foraging preferences.

Repeatedly treating bees with 3 µg cocaine in DMF at a sucrose feeder enhanced bees' preference for that feeder in a choice assay when compared to bees treated with DMF as a control (Mann-Witney test: $U = 2185$, $p = 0.0038$; Effect size: $r = -0.25$). Treating bees with cocaine at a feeder

while they were foraging resulted in a greater preference for that feeder in a free-choice test when compared to bees treated with DMF (vehicle control) while foraging at the feeder (Fig 2A).

Effect of volatilised cocaine on foraging rate.

Bees treated with 5 µg volatilised cocaine once at a 0.5 M feeder made significantly more return visits to the feeder in the 40 min following treatment, than controls (t_{70} = 5.0710, p = 0.00003; Effect size: d = 0.9905; Fig 2B). Bees treated with cocaine at a 2 M feeder showed no increase in visitations after cocaine treatment (t_{70} = -0.2087, p = 0.8353; Effect size: d = 0.0399; Fig 2B). Demonstrating that bees altered the rate at which they returned to a low quality feeder following volatilised cocaine treatment, but not to a high quality feeder (Fig 2B).

Effects of volatilised cocaine on sucrose responsiveness.

Treatment with 10 µg of volatilised cocaine increased bees responsiveness to sucrose (χ^2 = 6.0268, df = 1, p = 0.0141; Effect size: d = 0.6331; Fig 3A). The effect was dependent on the cocaine dose. Bees treated with 5 and 10 µg of cocaine were significantly more responsive to sucrose than controls (χ^2 = 14.089, df = 4, p = 0.0070; Fig 3B), while bees treated with 20 or 50 µg of cocaine did not differ from controls. The control treatment differed quite markedly between two experiments; however, this is likely because the two experiments were performed at different times of the year. Sucrose responsiveness varies with season and environmental conditions. The important aspect is the different between the cocaine treated bees and the control treated bees in a given experiment.

Effects of volatilised cocaine on responsiveness to electric shocks.

Cocaine affected bees' responsiveness to shock in a dose dependent manner (Fig 3C). We used the EV_{50} for statistical comparisons. All bees treated with cocaine were significantly more

sensitive to electric shock than control treated bees ($F_{4,40} = 5.4$, $p = 0.0015$; Fig 3C). There were no differences between the cocaine treatment groups with the exception of bees treated with 50 µg cocaine. The bees treated with 50 µg were significantly more sensitive than all other cocaine treated groups The EV_{50} of cocaine treated bees (50µM EV50 = 2.1; 20µM EV50= 3.5; 10µM, EV50= 2.6; 5µM EV50= 3.1) was lower than in control treated bees (EV50=5.3).

Discussion

In two separate experiments we observed that cocaine administration affected aspects of foraging decisions. Cocaine treatment increased the preference for a feeding location, and the rate of visitation at a sucrose feeder (Fig 2). Further, cocaine caused increased responsiveness to sucrose (Fig 3A and B). These findings, as well as those of Barron et al. (2009), lends support to the hypothesis that cocaine alter reward responses across divergent animal groups. However, we also found that cocaine made bees more responsive to electric shock (Fig 3C). Thus, the effect of cocaine is not limited to reward-related behaviours. Rather cocaine altered a range of behavioural responses, all, at least partially, modulated by octopaminergic or dopaminergic signalling. This is consistent with cocaine broadly interfering with octopaminergic and/or dopaminergic signalling in honey bees.

Our experiments indicate that cocaine alters the perceived concentration of sucrose in honey bees. Previous studies have shown that bees form stronger associations when rewarded with higher sucrose concentrations compared to lower ones (Loo & Bitterman, 1992). This can potentially explain the increased response rate to 10 % sucrose. Interestingly, cocaine only caused bees to increase their visitation rate at the low sucrose concentration feeder. This could be because with high sucrose concentrations, the relative change in perceived sucrose concentration is lower than with low sucrose concentrations.

This study provides further support to the bold claim that the neurochemicals modulating reward systems are broadly conserved across diverse animal phyla (Barron, Søvik & Cornish, 2010; Waddell, 2013), and therefore despite certain differences in specific neurochemistry and transporter affinities, diverse reward systems appear susceptible to disruption by the same drugs (Søvik & Barron, 2013). By 'broad conservation' we do not imply that the reward processing circuitry present in insects and mammals was present in the last common ancestor of these groups, but rather that biogenic amines may have performed functions in the common ancestor that predisposed them to become modulators of reward systems in most animal phyla (Barron et al., 2010).

We believe that this is not necessarily contradictory to the ecological function of cocaine as a deterrent compound inhibiting herbivory of the coca plant. Cocaine also enhanced responsiveness to electric shock (Fig 3C), and our previous work has shown cocaine profoundly damaged motor systems, coordination and locomotion in bees (Søvik et al., 2013). Similar findings have been reported for other insects, emphasising the insecticidal properties of cocaine (Nathanson et al., 1993). The effects of cocaine on insects are therefore extremely dose dependent. The rewarding effects reported here were seen at very low doses only. When herbivores ingest plant tissues containing cocaine, they quickly ingest enough to interfere with their motor system, and thus cannot continue feeding (Nathanson et al., 1993).

In mammals it is also seen that in recreational drug use, drugs are usually administered in ways that bypass the gut and achieve rapid delivery of a very low and controlled dose to the central nervous system in order to maximise the hedonic effects while minimising the toxic effects (Hagen et al., 2009).

Given the similarities observed in drug responses between vertebrate and invertebrates, it might be possible to use simple invertebrate animals as models for studying aspects of drug reward. While much important work is being done with mammalian models, many other fields of

neuroscience have benefitted greatly from the advantages of relatively simple invertebrate model systems (Burne et al., 2011). Previous work with *Drosophila* has highlighted the importance of circadian regulation (Andretic, Chaney & Hirsh, 1999; Abarca, Albrecht & Spanagel, 2002) and LIM-only proteins (Heberlein et al., 2009; Lasek et al., 2010) for the formation of sensitisation. However, invertebrate research has so far not been particularly concerned with drug reward (Søvik & Barron, 2013). Given the importance of drug reward in human drug use (Siegel, 2005), this should be a key area for future investigations. Honey bees, spend the majority of their time searching out natural rewards in their environments and have a long history as a model organism for studying the neurobiology of natural rewards (Perry & Barron, 2013). Considering the similarities in responses to cocaine between humans and bees, we can now capitalise on the potential of the honey bee as a simple invertebrate model organism to study drug reward.

Acknowledgements

We would like to thank Falk von Hollen for helping out with the foraging preference experiments, Lea Denneulin and Flavie Rongère for helping with the shock responsiveness experiment.

282

283 **References**

284

285 Abarca C, Albrecht U, Spanagel R. 2002. Cocaine sensitization and reward are under the
286 influence of circadian genes and rhythm. *Proceedings of the National Academy of Sciences*
287 *of the United States of America* 99:9026–9030.

288 Andretic R, Chaney S, Hirsh J. 1999. Requirement of circadian genes for cocaine sensitization in
289 *Drosophila*. *Science* 285:1066–1068.

290 Barron AB, Maleszka R, Helliwell PG, Robinson GE. 2009. Effects of cocaine on honey bee
291 dance behaviour. *The Journal of experimental biology* 212:163–168.

292 Barron AB, Vander Meer RK, Maleszka J, Robinson GE, Maleszka R. 2007. Comparing
293 injection, feeding and topical application methods for treatment of honeybees with
294 octopamine. *Journal of insect physiology* 53:187–194.

295 Barron AB, Søvik E, Cornish JL. 2010. The roles of dopamine and related compounds in reward-
296 seeking behavior across animal phyla. *Frontiers in behavioral neuroscience* 4:1–9.

297 Bitterman ME, Menzel R, Fietz A, Schäfer S. 1983. Classical conditioning of proboscis extension
298 in honeybees (*Apis mellifera*). *Journal of comparative psychology* 97:107–119.

299 Burne T, Scott E, van Swinderen B, Hilliard M, Reinhard J, Claudianos C, Eyles D, McGrath J.
300 2011. Big ideas for small brains: what can psychiatry learn from worms, flies, bees and fish?
301 *Molecular psychiatry* 16:7–16.

302 Chapman RF. 2013. *The Insects: Structure and Function*. Cambridge, UK: Cambridge University
303 Press, UK.

304 Chittka L, Menzel R. 1992. The evolutionary adaptation of flower colours and the insect
305 pollinators' colour vision. *Journal of Comparative Physiology A* 171:171–181.

306 Felsenberg J, Gehring KB, Antemann V, Eisenhardt D. 2011. Behavioural pharmacology in
307 classical conditioning of the proboscis extension response in honeybees (*Apis mellifera*).
308 *Journal of visualized experiments* 47:e2282.

309 Frisch K von. 1971. *Bees, Their Vision, Chemical Senses, and Language*. Itacha, NY: Cornell
310 University Press.

311 Giray T, Agosto-rivera JL, Stevison BK, Giannoni-guzma MA, Freeman B, Ricci P, Brown EA,
312 Abramson CI. 2014. Ethanol-Induced Effects on Sting Extension Response and Punishment
313 Learning in the Western Honey Bee (Apis mellifera). 9:1–8.

Hagen EH, Sullivan RJ, Schmidt R, Morris G, Kempter R, Hammerstein P. 2009. Ecology and neurobiology of toxin avoidance and the paradox of drug reward. *Neuroscience* 160:69–84.

Heberlein U, Tsai LT-Y, Kapfhamer D, Lasek AW. 2009. *Drosophila*, a genetic model system to study cocaine-related behaviors: a review with focus on LIM-only proteins. *Neuropharmacology* 56 Suppl 1:97–106.

Honjo K, Furukubo-Tokunaga K. 2009. Distinctive neuronal networks and biochemical pathways for appetitive and aversive memory in *Drosophila* larvae. *The Journal of neuroscience* 29:852–62.

Lasek AW, Kapfhamer D, Kharazia V, Gesch J, Giorgetti F, Heberlein U. 2010. *Lmo4* in the nucleus accumbens regulates cocaine sensitivity. *Genes, brain, and behavior* 9:817–824.

Loo SK, Bitterman ME. 1992. Learning in honeybees (*Apis mellifera*) as a function of sucrose concentration. *Journal of Comparative Psychology* 106:29–36.

McClung C, Hirsh J. 1998. Stereotypic behavioral responses to free-base cocaine and the development of behavioral sensitization in *Drosophila*. *Current Biology* 8:109–112.

Nakatani Y, Matsumoto Y, Mori Y, Hirashima D, Nishino H, Arikawa K, Mizunami M. 2009. Why the carrot is more effective than the stick: different dynamics of punishment memory and reward memory and its possible biological basis. *Neurobiology of learning and memory* 92:370–80.

Nathanson JA, Hunnicutt EJ, Kantham L, Scavone C. 1993. Cocaine as a naturally occurring insecticide. *Proceedings of the National Academy of Sciences of the United States of America* 90:9645–9648.

Perry CJ, Barron AB. 2013. Neural mechanisms of reward in insects. *Annual Review of Entomology* 58:543–562.

Roussel E, Carcaud J, Sandoz J-C, Giurfa M. 2009. Reappraising social insect behavior through aversive responsiveness and learning. *PloS one* 4:e4197.

Samaha A-N, Robinson TE. 2005. Why does the rapid delivery of drugs to the brain promote addiction? *Trends in pharmacological sciences* 26:82–87.

Scheiner R, Page Jr RE, Erber J. 2001. The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (*Apis mellifera* L.). *Neurobiology of learning and memory* 76:138–50.

Scheiner R, Page Jr RE, Erber J. 2004. Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). *Apidologie* 35:133–142.

Schultz W. 2007. Behavioral dopamine signals. *Trends in neurosciences* 30:203–210.

347 Schwaerzel M, Monastirioti M, Scholz H, Friggi-Grelin F, Birman S, Heisenberg M. 2003.
348 Dopamine and octopamine differentiate between aversive and appetitive olfactory memories
349 in *Drosophila*. *The Journal of neuroscience* 23:10495–10502.

350 Seeley TD. 1995. *The Wisdom of the Hive*. Cambridge, Massachusetts: Harvard University Press.

351 Siegel RK. 2005. *Intoxication: The universal drive for mind-altering substances*. Rochester,
352 Vermont: Park Street Press.

353 Søvik E. 2013. Reward processing and responses to drugs of abuse in the honey bee, *Apis*
354 *mellifera*. Macquarie University, Australia.

355 Søvik E, Barron AB. 2013. Invertebrate models in addiction research. *Brain, behavior and*
356 *evolution* 82:153–165.

357 Søvik E, Cornish JL, Barron AB. 2013. Cocaine tolerance in honey bees. *PLoS One* 8:e64920.

358 Sullivan RJ, Hagen EH, Hammerstein P. 2008. Revealing the paradox of drug reward in human
359 evolution. *Proceedings of the Royal Society B: Biological Sciences* 275:1231–1241.

360 Tedjakumala SR, Aimable M, Giurfa M. 2014. Pharmacological modulation of aversive
361 responsiveness in honey bees. *Frontiers in behavioral neuroscience* 7:221.

362 Vergoz V, Roussel E, Sandoz J-C, Giurfa M. 2007. Aversive learning in honeybees revealed by
363 the olfactory conditioning of the sting extension reflex. *PloS one* 2:e288.

364 Waddell S. 2013. Reinforcement signalling in *Drosophila*; dopamine does it all after all. *Current*
365 *opinion in neurobiology* 23:324–329.

366

Figure 1

Schematic of experimental set-up used for foraging preference experiment.

In the foraging preference experiment, bees were trained to two tunnels. One was blue with vertical stripes while the other was green with horizontal stripes. The difference between the two tunnels was to make it as easy as possible for the bees to tell to two tunnels apart.

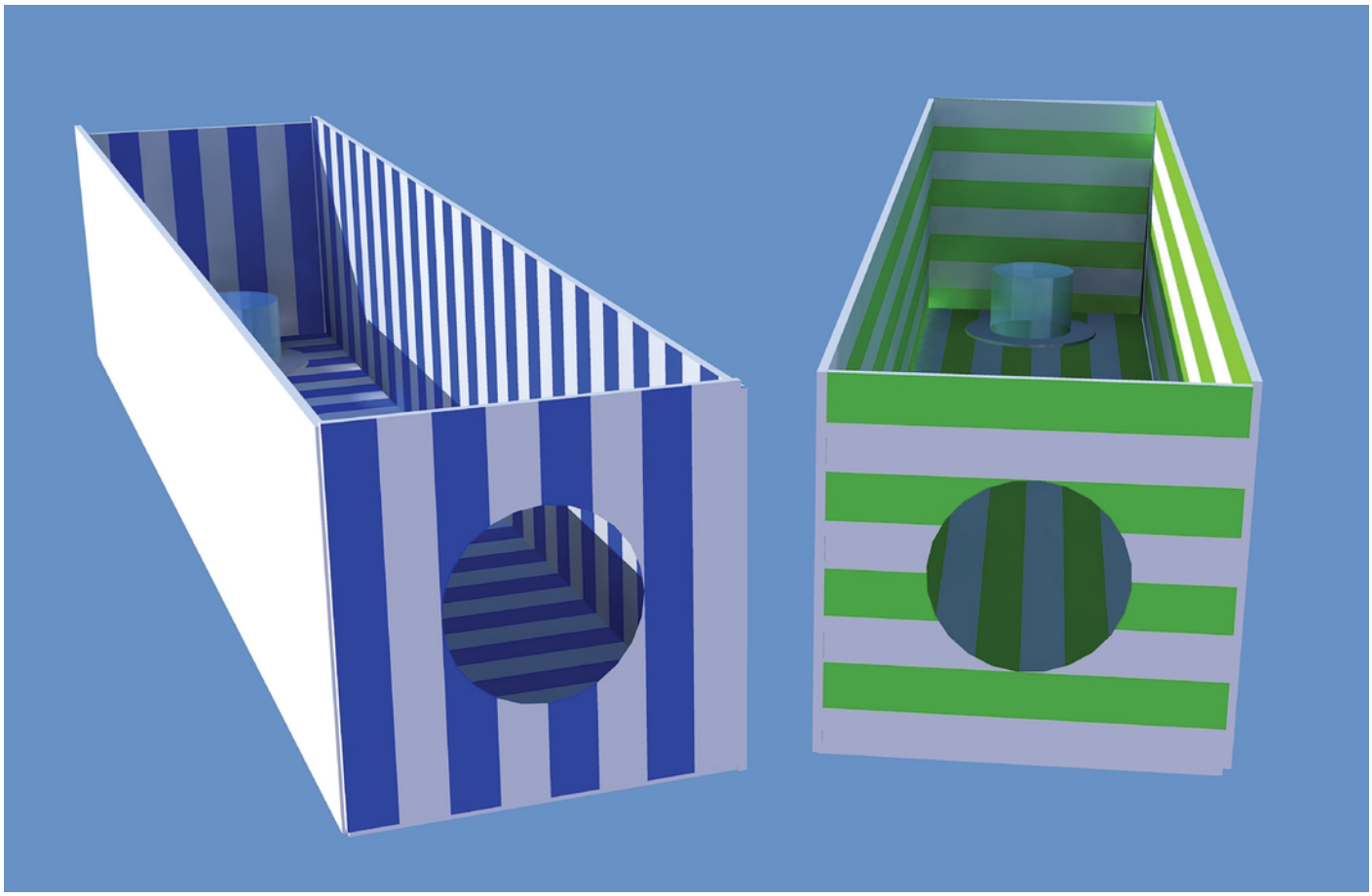

Figure 2

Foraging behaviour in honey bees following cocaine administration.

A. Scatter plot showing the effect of topical cocaine treatment on preference for the green arm. Each point represents one bee. Dotted lines mark median values for each treatment group. The preference for the green arm was significantly higher for cocaine-treated than control-treated bees (Mann-Witney U = 2185, p = 0.0038). **B.** Effect of volatilised cocaine treatment on visitation rate at a sucrose feeder (error bars represent standard error). Bees treated with volatilised cocaine (grey bars) increased their rate of foraging relative to controls (white bars) when foraging at a 0.5 M sucrose feeder (t_{70} = 5.0710, p = 0.00003), but not at a 2 M sucrose feeder (t_{70} = -0.2087, p = 0.8353).

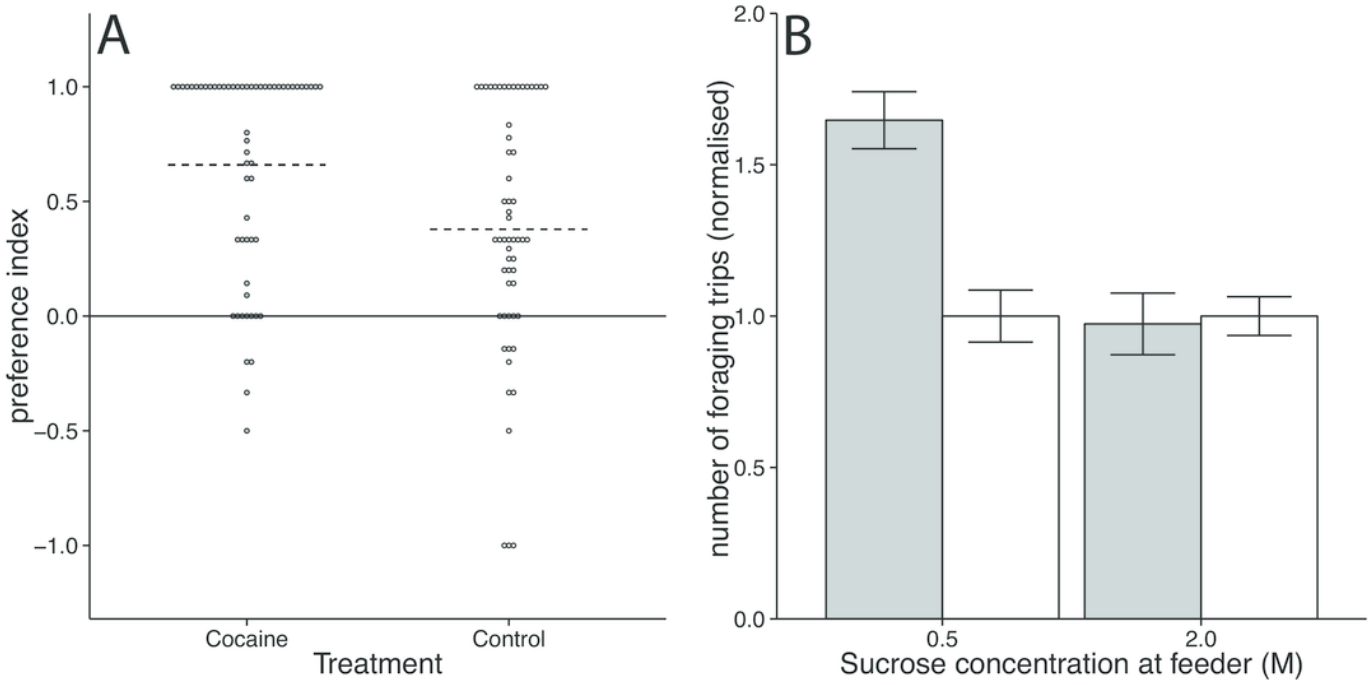

Figure 3

Behavioural responsiveness following cocaine administration in honey bees.

Proportion of bees responding to 10 % sucrose following treatment with 0 or 10 µg of volatilised cocaine (error bars represents standard error and letters denote statistically different groups). There was a significant increase in sucrose responsiveness in bees treated with 10 µg cocaine relative to control (χ^2 = 6.1013, df = 1, p = 0.0135). **B.** Proportion of bees responding to 10 % sucrose following treatment with 0, 5, 10, 20, or 50 µg of volatilised cocaine. There was a dose-dependent relationship between cocaine dose and sucrose responsiveness (χ^2 = 14.089, df = 4, p = 0.0070). **C.** Shock responsiveness of bees following cocaine administration. Curves are based on weibull distributions of shock responsiveness for each group. Comparisons are based on estimates of EV_{50} for 40 bees per group ($F_{4,40}$ = 5.4, p = 0.0015). Pairwise comparisons found that the 50 µg group was different from all other groups, while the remaining cocaine treated groups were different from controls.