

1 **Title: Unexpected rewards induce dopamine-dependent positive emotion-like**
2 **state changes in bumblebees**

3 **Authors:** Clint J. Perry*, Luigi Baciadonna, Lars Chittka

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5 **Affiliations:**

6 ¹Department of Biological and Experimental Psychology, School of Biological and Chemical
7 Sciences, Queen Mary University of London, London E1 4NS, UK

8
9 * To whom correspondence should be addressed: clint.perry@qmul.ac.uk

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11 **Abstract:**

12 Whether invertebrates exhibit positive emotion-like states and the mechanisms underlying such
13 states remain poorly understood. We demonstrate that bumblebees exhibit dopamine-dependent
14 positive emotion-like states across behavioral contexts. After training on one rewarding and one
15 unrewarding cue, bees that received pre-test sucrose responded in a positive manner towards
16 ambiguous cues. In a second experiment, pre-test consumption of sucrose solution resulted in a
17 shorter time to re-initiate foraging after a simulated predator attack. These behavioral changes
18 were abolished with topical application of the dopamine-antagonist fluphenazine. Further
19 experiments establish that pre-test sucrose is not simply causing bees to become more
20 exploratory. Our findings reveal a new opportunity to understanding the fundamental neural
21 elements of emotions and may alter our view of how emotion states affect decision-making in
22 animals.

23 **Main Text:**

24 Emotions are transient subjective states, underpinned by physiological, behavioral and
25 cognitive phenomena, triggered by appraisal of environmental situations (1–3). Our conceptual
26 understanding of emotion is largely based on human subjective experiences, i.e. what we ‘feel’,
27 assessed directly through verbal reports. In animals, similar emotion-like states can be inferred

28 through observable, quantifiable parameters. To ensure the criteria of emotion-like states are
29 met, and to distinguish these from other forms of environmentally induced states, perhaps driven
30 by learning, we must quantify the range of physiological, behavioral and cognitive phenomena
31 that occur in response to environmental factors similar to those studied in humans (4).

32 The majority of work on animal emotions focuses on mammals and almost exclusively
33 on negative emotions (5). The idea that invertebrates may exhibit basic forms of emotion is
34 increasingly accepted (6–8), and given the assumed adaptive function of emotions (to coordinate
35 the individual’s cognitive and behavioral resources towards fitness-relevant priorities (1, 2, 9)),
36 we might expect that a diversity of emotion-like states, including positive ones, exist across
37 phyla, albeit not necessarily consciously so (9–11).

38 In humans, consumption of sweet snacks can induce positive emotions (12–14). Here, we
39 examine whether a small amount of pre-test sucrose solution causes bumblebees (*Bombus*
40 *terrestris*) to behave in a way indicative of an induced positive emotion-like state.

41 In Experiment 1.1, we utilized the well-established judgment bias paradigm, where
42 subjects associate one cue with a positive event and another cue with a negative event (15).
43 Subjects in a positive emotion state tend to respond to ambiguous (intermediate) stimuli as if
44 predicting the positive event (4).

45 We trained bees on a go/no-go task, to enter a cylinder beneath a colored placard (e.g.
46 blue) on one side of an arena where they would find 30% sucrose solution (Fig. 1A-B). On
47 alternate trials, bees learned to *not* enter a cylinder at the opposite side of the arena under a
48 placard of different color without reward (e.g. green, water). The latency from the time bees
49 entered the arena to the time they entered the presented cylinder was recorded.

50 We then examined bees' response ('judgment') to ambiguous information (intermediate
51 color and location; Fig. 1C). Half of the trained bees, randomly selected, found for the first time,
52 and drank, a 5 μ l droplet (equivalent to <5% of stomach capacity) of 60% sucrose solution in the
53 tunnel leading to the arena or received no reward. Bees that consumed sucrose solution prior to
54 making a decision took less time to enter the chamber of the middle (M) ambiguous stimulus
55 (Fig. 1D; Tables S1-S2; Supplementary Materials).

56 Could it be that when a bee consumed the small reward, rather than a positive emotion-
57 like state, a higher expectation of subsequent reward resulted in greater exploration of novel
58 stimuli? Indeed, previous work indicates that honeybees' foraging choices are controlled by short
59 term memories initiated by rewards just experienced (16, 17). However, bees tested on stimuli
60 not intermediate to the trained stimuli (novel in terms of color, position and number; Experiment
61 1.2; Fig. 1E), exhibited no difference in choice time (Fig. 1F; Table S3) or number of choices
62 (Fig. 1G; Table S4) between conditions, indicating that pre-decision sucrose consumption did not
63 cause a general increase in expectation of reward.

64 Consumption of sucrose solution may simply make bees more excited or active, resulting
65 in faster decisions for ambiguous stimuli. Thorax temperature increased after consumption of 5 μ l
66 60% sucrose solution ($n = 72$, $t_{70} = 6.78$, $p = 3.12e^{-9}$; Experiment 2.1; Fig. S1 A,B; Supplemental
67 Materials) denoting increased metabolic rate. But this did not transfer to increased activity.
68 Sucrose and control bees ($n = 24$) showed no difference in flight time ($t_{22} = 0.666$, $p = 0.512$) or
69 speed ($t_{22} = 0.241$, $p = 0.812$) to a feeder (Experiment 2.2; Fig. S1 C, D; Supplemental
70 Materials), and when the feeder was removed, speed during a 120s flight also did not differ
71 between groups ($n = 24$, $t_{22} = -0.403$, $p = 0.691$; Experiment 2.3; Fig. S1 E), suggesting that
72 unexpected rewards did not affect bees' overall activity level.

73 It has been argued that one characteristic of emotions across species is generalization – a
74 property whereby an induced emotion state operates across behavioral contexts (9). To examine
75 whether these behavioral results were similar across contexts we tested whether an unanticipated
76 reward would change bees' reaction to later aversive stimuli (Experiment 3). We trained bees to
77 forage at a feeder containing 30% sucrose solution. After training and on their next foraging trip,
78 bees were held temporarily in the tunnel connecting the hive and arena. Bees would either
79 receive an unanticipated 5µl droplet of 60% sucrose solution or nothing (control). After a 10-
80 second delay, a predator attack was simulated. At natural flowers, bees are sometimes ambushed
81 by sit-and-wait predators such as crab spiders; bees often escape after a brief struggle, allowing
82 them to modify their subsequent behavior to cope with such threat (18). Mimicking such an
83 attack, the bee was captured by a trapping mechanism, applying constant pressure for three
84 seconds by a stamp-shaped device softened with a sponge connected to a micro-servo (Fig. 2A;
85 (18)). The bee was subsequently released and the time it took to commence foraging was
86 recorded.

87 Sweet food can increase positive emotions and improve negative mood in human adults,
88 and reduce crying and grimacing of newborns in response to aversive stimuli (12–14). If
89 drinking an unexpected sucrose solution caused a positive emotion-like state in bees, we predict
90 that, following consumption, bees' aversive reaction to the 'predator' would be attenuated.
91 Indeed, bees that consumed sucrose solution prior to the 'attack' took less time to re-initiate
92 foraging ($n = 35$, $t_{33} = -3.70$, $p = 7.87e^{-04}$; Fig. 2B).

93 The insect reward system parallels that of mammals in several aspects, including some of
94 the neurochemicals involved (19). In mammals, several neurotransmitters play key roles in both
95 reward processing and emotions. We asked if the biogenic amines linked to reward processing in

96 the insect brain might be involved in the behaviors suggestive of emotion-like states here
97 observed. We topically treated bees (20, 21) with antagonists of biogenic amines Octopamine
98 (OA; antagonist: mianserin; n = 20), Dopamine (DA; antagonist: fluphenazine; n = 20) and
99 Serotonin (5-HT; antagonist: yohimbine; n = 20) and determined their effect on behavior induced
100 by pre-decision sucrose. Bees were trained as in Experiment 3. Fifteen minutes after antagonist
101 application, or vehicle control (DMF; n = 20), bees consumed, for the first time, a 5 μ l droplet of
102 60% sucrose solution. Following this, bees were subjected to a simulated predator attack and the
103 time taken to return to foraging was recorded (Experiment 4.1). Only bees treated with the DA-
104 antagonist took longer to begin foraging than control bees (ANOVA: n = 96, df = 4, F = 3.48, p
105 = 0.011; Tukey posthoc: p = 0.039; Fig. 3A). We speculate that this is a consequence of brain
106 dopamine signals responding to unexpected reward (22–25). To ensure that the DA-antagonist
107 was not simply interacting with pathways mediating normal response to the aversive stimulus,
108 bees were topically treated with DA-antagonist without receiving pre-test sucrose. The time to
109 begin foraging for these bees was similar to both bees treated with DA-antagonist treated + pre-
110 decision sucrose and control bees receiving no pre-test sucrose (n = 16; Fig. 3A and Fig. 2B:
111 Control).

112 We explored whether blocking DA had similar effects on the observed cognitive
113 consequences of pre-decision reward in the judgment bias paradigm. Bees were trained as in
114 Experiment 1.1 and then treated with either DA-antagonist or DMF 15 minutes prior to
115 consuming an unexpected 5 μ l of 60% sucrose solution and testing. Compared to control, DA-
116 antagonist-treated bees took longer to enter the middle (M) ambiguous stimulus chamber
117 (Experiment 4.2; Fig. 3B; Table S5-S6).

118 Recent evidence suggests clear roles of DA in reward related processes in invertebrates
119 (23), including motivation for reward (25) nutritional value of reward (22) and arousal (26). Our
120 results corroborate DA's role in the neuronal processes mediating reward signals, in bees. An
121 intriguing prospect of research would be whether similar circuits controlling wanting, hunger,
122 nutritional valuation and/or arousal underpin the emotion-like states indicated by our results in
123 bees.

124 The behaviors displayed by bumblebees in response to a small amount of pre-decision
125 sucrose conform to criteria commonly applied to mammals for internal emotion-like states
126 interacting with decision-making: positive judgment bias to ambiguous stimuli and attenuated
127 response to negative stimuli. Whether common neural processing features evolved independently
128 or an ancient role of biogenic amines evolved to serve similar functions, new findings (including
129 ours) support the hypothesis that the fundamental elements of emotion exist in many species (9).

130 Our results lend support to the notion that invertebrates may have states that fit the
131 criteria defining emotion (1, 9). The adaptive function of emotion is thought to be the integration
132 of information about environment and body to modulate decisions and behavior (9).
133 Understanding and investigating the basic features of emotion states will bring us a step closer to
134 determining the brain mechanisms underlying emotion across taxa.

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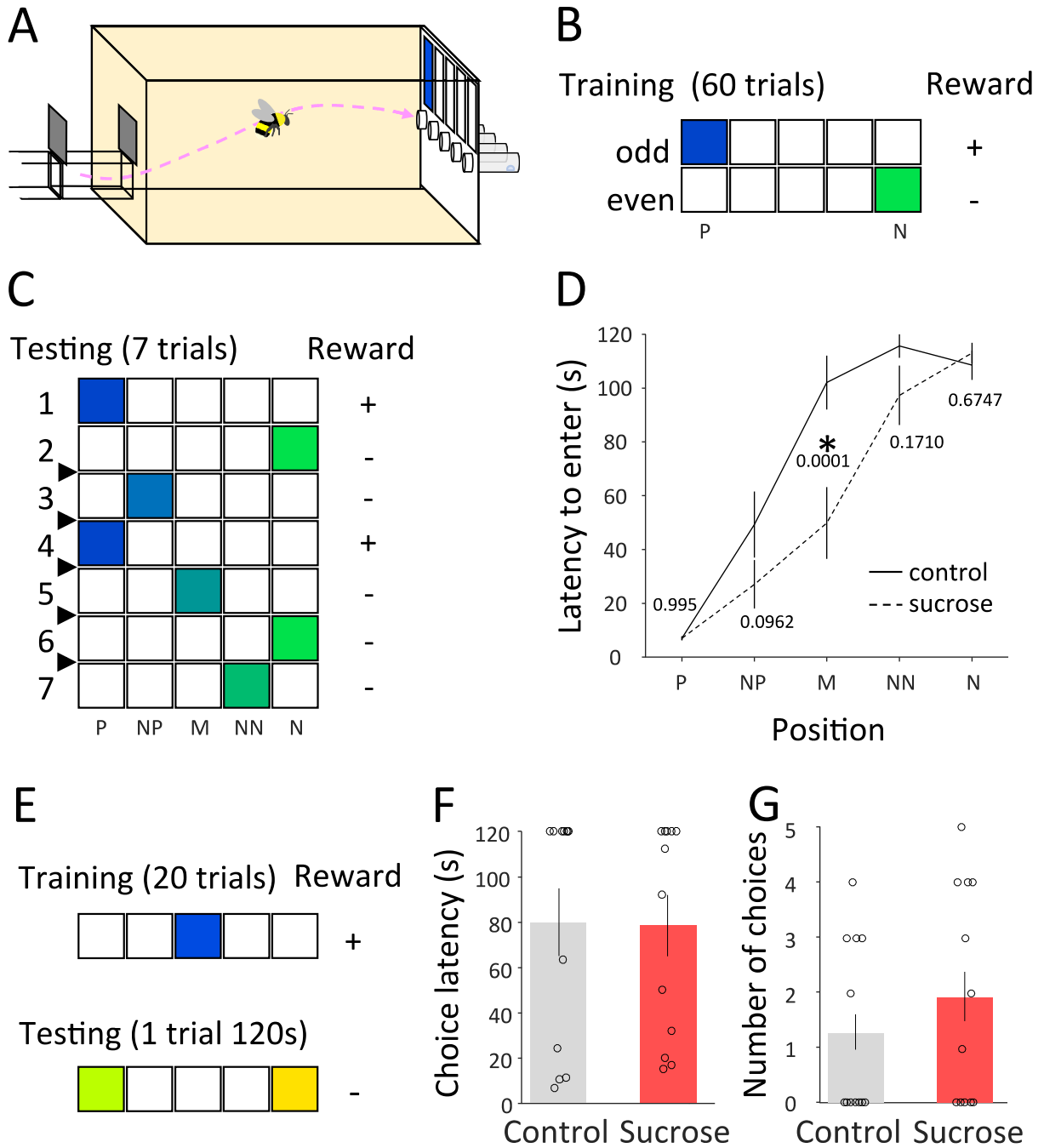
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203 experiments. C.J.P and L.B. conducted the experiments and carried out behavioral data analysis.
204 The manuscript was written by C.J.P., L.B. and L.C.



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208 **Fig. 1. Judgment bias to ambiguous stimuli.** (A) Set up for Experiment 1.1. (B-C) Each row

209 shows a ‘bee’s eye view’ of placards within arena. (B) Training stimuli for one of four

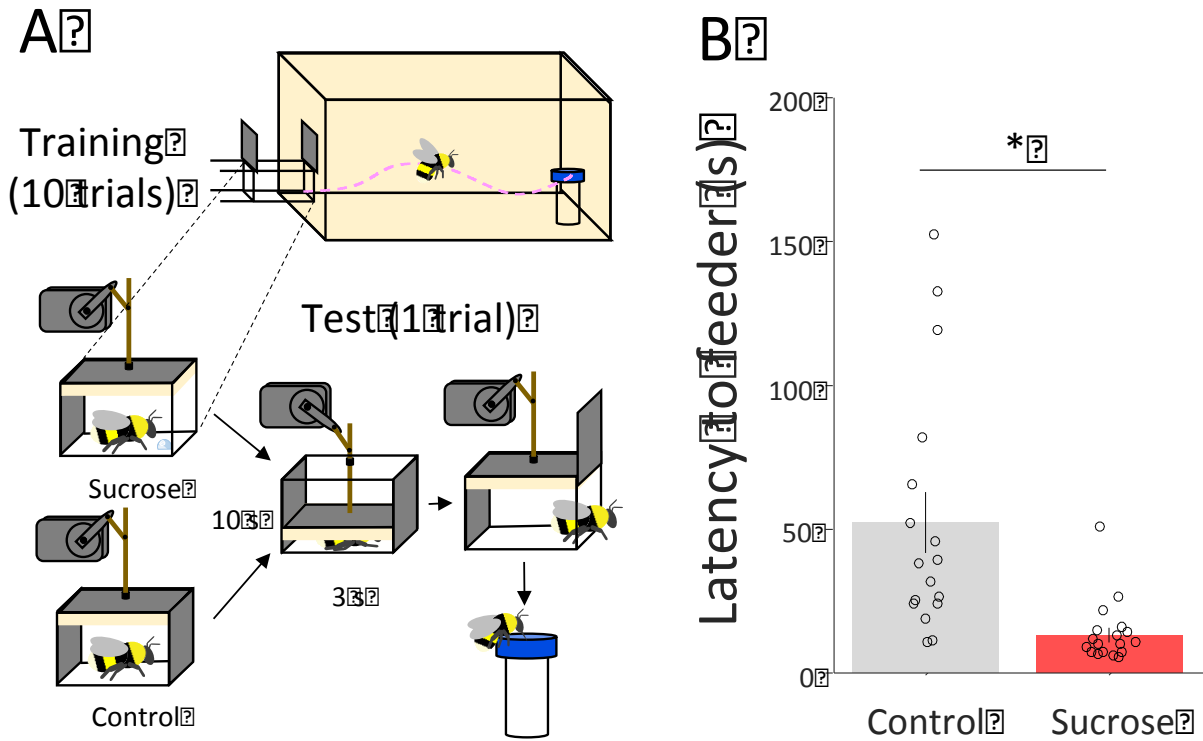
210 counterbalanced orientations (Fig. S2; N = Negative, P = positive). Bees (n = 24) were trained

211 find sucrose solution in a cylinder under one placard and avoid another. Only one cylinder was
212 accessible on any one trial: odd trials were rewarded and even trials unrewarded. **(C)** Testing
213 procedure. Half the bees received pre-test sucrose (arrowheads). After two ‘reminder trials’, bees
214 were tested on three ambiguous stimuli alternated between trained stimuli. Order was
215 counterbalanced (Fig. S3). **(D)** Results of Experiment 1.1. The sucrose group took less time to
216 enter the middle position (M) than the control group. Numbers are p values. **(E)** Training
217 procedure for Experiment 1.2. Bees (n = 24) were trained to find a reward under a blue placard,
218 and subsequently tested on two novel stimuli. **(F-G)** Results for Experiment 1.2. **(F)** Latency to
219 feeder and **(G)** number of choices did not differ between groups. Here and elsewhere, bars =
220 mean, open circles = individual bees. Here and elsewhere, error bars = s.e. Generalized linear
221 modeling analyses in Tables S1-S4.

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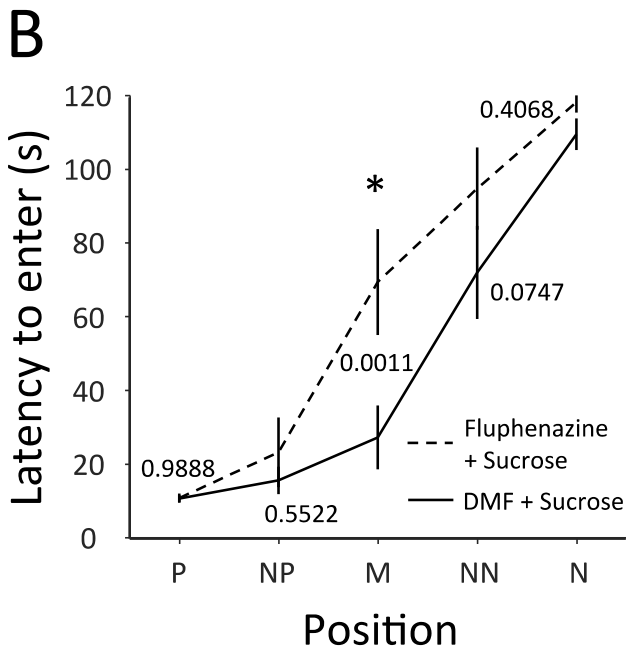
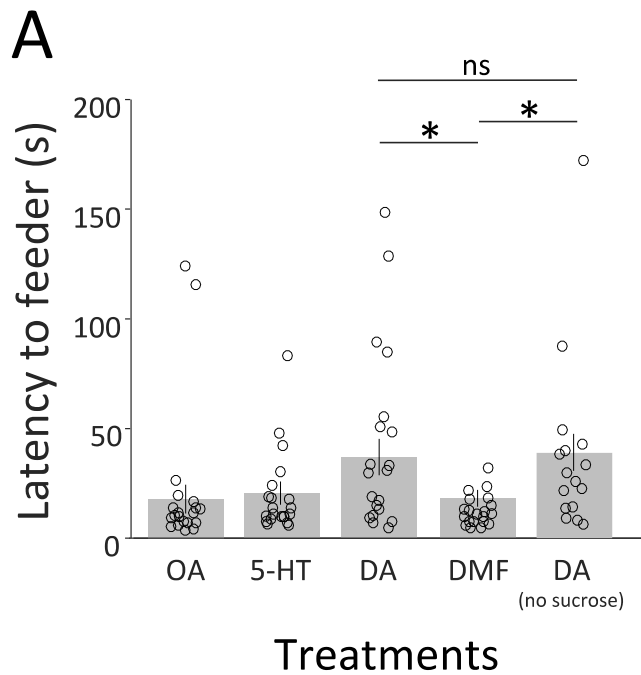
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226 **Fig. 2. Attenuation of response to aversive stimuli.** (A) Training and test procedure for
227 Experiment 3. Bees ($n = 35$) were trained to feed at a 30% sucrose solution feeder. Subsequently,
228 bees consumed $5\mu\text{l}$ of 60% sucrose solution prior to a simulated predator attack. (B) Results of
229 predation experiment. Sucrose group bees took less time to resume foraging behavior than the
230 control group ($t_{33} = -3.70$, $p = 7.87e^{-04}$).



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232

233 **Fig. 3. Results of experiments blocking biogenic amines. (A)** Results for Experiment 4.1:

234 Predation. Dopamine (DA), but not Octopamine (OA) or Serotonin (5-HT) antagonist-treated

235 bees took more time to resume foraging behavior than DMF-treated bees (n = 96; t-test with

236 Bonferroni correction; DA: $t_{38} = 3.14$, $p = 0.003$; OA: $t_{38} = 1.19$, $p = 0.241$, 5-HT: $t_{38} = 1.16$, $p =$
237 0.113). **(B)** Results for Experiment 4.2: Judgment Bias. Fluphenazine (DA-antagonist) treated
238 bees took more time to enter the middle position (M) than vehicle (DMF) treated bees. Numbers
239 indicate p values. Generalized linear modeling analyses in Tables S5-S6.

240

241 **Supplementary Materials:**

242 Materials and Methods

243 Figures S1-5

244 Tables S1-6

245 Reference 27