Unexpected rewards induce dopamine-dependent positive emotion-like state changes in bumblebees.
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Title: Unexpected rewards induce dopamine-dependent positive emotion-like state changes in bumblebees

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

Main Text:
Emotions are transient subjective states, underpinned by physiological, behavioral and cognitive phenomena, triggered by appraisal of environmental situations (1–3). Our conceptual understanding of emotion is largely based on human subjective experiences, i.e. what we ‘feel’, assessed directly through verbal reports. In animals, similar emotion-like states can be inferred
through observable, quantifiable parameters. To ensure the criteria of emotion-like states are met, and to distinguish these from other forms of environmentally induced states, perhaps driven by learning, we must quantify the range of physiological, behavioral and cognitive phenomena that occur in response to environmental factors similar to those studied in humans (4).

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

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

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

We trained bees on a go/no-go task, to enter a cylinder beneath a colored placard (e.g. blue) on one side of an arena where they would find 30% sucrose solution (Fig. 1A-B). On alternate trials, bees learned to not enter a cylinder at the opposite side of the arena under a placard of different color without reward (e.g. green, water). The latency from the time bees entered the arena to the time they entered the presented cylinder was recorded.
We then examined bees’ response (‘judgment’) to ambiguous information (intermediate color and location; Fig. 1C). Half of the trained bees, randomly selected, found for the first time, and drank, a 5µl droplet (equivalent to <5% of stomach capacity) of 60% sucrose solution in the tunnel leading to the arena or received no reward. Bees that consumed sucrose solution prior to making a decision took less time to enter the chamber of the middle (M) ambiguous stimulus (Fig. 1D; Tables S1-S2; Supplementary Materials).

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

Consumption of sucrose solution may simply make bees more excited or active, resulting in faster decisions for ambiguous stimuli. Thorax temperature increased after consumption of 5µl 60% sucrose solution (n = 72, t_{70} = 6.78, p = 3.12e^-9; Experiment 2.1; Fig. S1 A,B; Supplemental Materials) denoting increased metabolic rate. But this did not transfer to increased activity.

Sucrose and control bees (n = 24) showed no difference in flight time (t_{22} = 0.666, p = 0.512) or speed (t_{22} = 0.241, p = 0.812) to a feeder (Experiment 2.2; Fig. S1 C, D; Supplemental Materials), and when the feeder was removed, speed during a 120s flight also did not differ between groups (n = 24, t_{22} = -0.403, p = 0.691; Experiment 2.3; Fig. S1 E), suggesting that unexpected rewards did not affect bees’ overall activity level.
It has been argued that one characteristic of emotions across species is generalization – a property whereby an induced emotion state operates across behavioral contexts (9). To examine whether these behavioral results were similar across contexts we tested whether an unanticipated reward would change bees’ reaction to later aversive stimuli (Experiment 3). We trained bees to forage at a feeder containing 30% sucrose solution. After training and on their next foraging trip, bees were held temporarily in the tunnel connecting the hive and arena. Bees would either receive an unanticipated 5µl droplet of 60% sucrose solution or nothing (control). After a 10-second delay, a predator attack was simulated. At natural flowers, bees are sometimes ambushed by sit-and-wait predators such as crab spiders; bees often escape after a brief struggle, allowing them to modify their subsequent behavior to cope with such threat (18). Mimicking such an attack, the bee was captured by a trapping mechanism, applying constant pressure for three seconds by a stamp-shaped device softened with a sponge connected to a micro-servo (Fig. 2A; (18)). The bee was subsequently released and the time it took to commence foraging was recorded.

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

The insect reward system parallels that of mammals in several aspects, including some of the neurochemicals involved (19). In mammals, several neurotransmitters play key roles in both reward processing and emotions. We asked if the biogenic amines linked to reward processing in
the insect brain might be involved in the behaviors suggestive of emotion-like states here observed. We topically treated bees (20, 21) with antagonists of biogenic amines Octopamine (OA; antagonist: mianserin; n = 20), Dopamine (DA; antagonist: fluphenazine; n = 20) and Serotonin (5-HT; antagonist: yohimbine; n = 20) and determined their effect on behavior induced by pre-decision sucrose. Bees were trained as in Experiment 3. Fifteen minutes after antagonist application, or vehicle control (DMF; n = 20), bees consumed, for the first time, a 5μl droplet of 60% sucrose solution. Following this, bees were subjected to a simulated predator attack and the time taken to return to foraging was recorded (Experiment 4.1). Only bees treated with the DA-antagonist took longer to begin foraging than control bees (ANOVA: n = 96, df = 4, F = 3.48, p = 0.011; Tukey posthoc: p = 0.039; Fig. 3A). We speculate that this is a consequence of brain dopamine signals responding to unexpected reward (22–25). To ensure that the DA-antagonist was not simply interacting with pathways mediating normal response to the aversive stimulus, bees were topically treated with DA-antagonist without receiving pre-test sucrose. The time to begin foraging for these bees was similar to both bees treated with DA-antagonist treated + pre-decision sucrose and control bees receiving no pre-test sucrose (n = 16; Fig. 3A and Fig. 2B: Control).

We explored whether blocking DA had similar effects on the observed cognitive consequences of pre-decision reward in the judgment bias paradigm. Bees were trained as in Experiment 1.1 and then treated with either DA-antagonist or DMF 15 minutes prior to consuming an unexpected 5μl of 60% sucrose solution and testing. Compared to control, DA-antagonist-treated bees took longer to enter the middle (M) ambiguous stimulus chamber (Experiment 4.2; Fig. 3B; Table S5-S6).
Recent evidence suggests clear roles of DA in reward related processes in invertebrates (23), including motivation for reward (25) nutritional value of reward (22) and arousal (26). Our results corroborate DA’s role in the neuronal processes mediating reward signals, in bees. An intriguing prospect of research would be whether similar circuits controlling wanting, hunger, nutritional valuation and/or arousal underpin the emotion-like states indicated by our results in bees.

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

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

References and Notes:


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Fig. 1. Judgment bias to ambiguous stimuli. (A) Set up for Experiment 1.1. (B-C) Each row shows a ‘bee’s eye view’ of placards within arena. (B) Training stimuli for one of four counterbalanced orientations (Fig. S2; N = Negative, P = positive). Bees (n = 24) were trained...
find sucrose solution in a cylinder under one placard and avoid another. Only one cylinder was accessible on any one trial: odd trials were rewarded and even trials unrewarded. (C) Testing procedure. Half the bees received pre-test sucrose (arrowheads). After two ‘reminder trials’, bees were tested on three ambiguous stimuli alternated between trained stimuli. Order was counterbalanced (Fig. S3). (D) Results of Experiment 1.1. The sucrose group took less time to enter the middle position (M) than the control group. Numbers are p values. (E) Training procedure for Experiment 1.2. Bees (n = 24) were trained to find a reward under a blue placard, and subsequently tested on two novel stimuli. (F-G) Results for Experiment 1.2. (F) Latency to feeder and (G) number of choices did not differ between groups. Here and elsewhere, bars = mean, open circles = individual bees. Here and elsewhere, error bars = s.e. Generalized linear modeling analyses in Tables S1-S4.
Fig. 2. Attenuation of response to aversive stimuli. (A) Training and test procedure for Experiment 3. Bees (n = 35) were trained to feed at a 30% sucrose solution feeder. Subsequently, bees consumed 5μl of 60% sucrose solution prior to a simulated predator attack. (B) Results of predation experiment. Sucrose group bees took less time to resume foraging behavior than the control group (t_{33} = -3.70, p = 7.87e^{-04}).
Fig. 3. Results of experiments blocking biogenic amines. (A) Results for Experiment 4.1: Predation. Dopamine (DA), but not Octopamine (OA) or Serotonin (5-HT) antagonist-treated bees took more time to resume foraging behavior than DMF-treated bees (n = 96; t-test with
Bonferroni correction; DA: $t_{38} = 3.14, p = 0.003$; OA: $t_{38} = 1.19, p = 0.241$, 5-HT: $t_{38} = 1.16, p = 0.113$). (B) Results for Experiment 4.2: Judgment Bias. Fluphenazine (DA-antagonist) treated bees took more time to enter the middle position (M) than vehicle (DMF) treated bees. Numbers indicate p values. Generalized linear modeling analyses in Tables S5-S6.

Supplementary Materials:

Materials and Methods

Figures S1-5

Tables S1-6

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