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Research report

Consumption of an acute dose of caffeine reduces acquisition but not memory in the honey bee

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ABSTRACT

Caffeine affects several molecules that are also involved in the processes underlying learning and memory such as cAMP and calcium. However, studies of caffeine's influence on learning and memory in mammals are often contradictory. Invertebrate model systems have provided valuable insight into the actions of many neuroactive compounds including ethanol and cocaine. We use the honey bee (*Apis mellifera*) to investigate how the ingestion of acute doses of caffeine before, during, and after conditioning influences performance in an appetitive olfactory learning and memory task. Consumption of caffeine doses of 0.01 M or greater during or prior to conditioning causes a significant reduction in response levels during acquisition. Although bees find the taste of caffeine to be aversive at high concentrations, the bitter taste does not explain the reduction in acquisition observed for bees fed caffeine before conditioning. While high doses of caffeine reduced performance during acquisition, the response levels of bees given caffeine were the same as those of the sucrose only control group in a recall test 24 h after conditioning. In addition, caffeine administered after conditioning had no affect on recall. These results suggest that caffeine specifically affects performance during acquisition and not the processes involved in the formation of early long term memory.

1. Introduction

Caffeine is widely used as a chemical stimulant that reportedly affects mood, alertness and attention. Evidence supporting its influence on learning and memory is often contradictory; some studies report memory improvement after caffeine ingestion whereas others suggest that caffeine consumption has little or no effect on learning and memory [1]. Studies in humans are complicated due to the widespread chronic use of caffeine, making the use of animal models particularly useful for studying the actions of caffeine. The ambiguity regarding caffeine's effect on learning and memory processes could be a result of differences in administration of doses before, during, and after learning tasks. Furthermore, caffeine may affect different types of learning (for example, passive versus intentional) in different ways [2].

Invertebrates have successfully been developed as model organisms used to study the effects of drugs of abuse such as ethanol [3–5] and cocaine [6–10]. Therefore, invertebrate models have great potential for providing insight in to the effects of caffeine on learning and memory and the mechanisms underlying caffeine's action. In fruit flies, caffeine increases arousal, measured as periods of walking activity, and decreases the amount of time flies spend sleeping [11,12]. Chronic treatment with caffeine (consumption of caffeine for at least 20 h before conditioning) reduces visual learning performance in flies in conditioning paradigms where an aversive stimulus is paired with a visual cue [13,14].

The wide range of learning assays available and the ability to manipulate the molecular and neural processes in the brain make the honey bee an excellent system for investigating the effects of caffeine on learning and memory [15,16]. The presence of caffeine in the nectar and pollen of several plant species [17,18] suggests that honey bees may encounter caffeine when foraging. Free flying honey bees willingly consume sucrose solutions containing a range of caffeine concentrations similar to those found in citrus nectar (12.5–100 ppm, or 0.07–0.5 mM) and show a preference for sucrose solutions containing 25 ppm (approximately 0.13 mM) caffeine over solutions of sucrose alone [19]. Caffeine had no effect on acquisition, but increased long term memory retention in an appetitive visual learning task in the honey bee [20]. Furthermore, treatment with caffeine increased the performance of bees in a delayed match to sample assay [20]. Caffeine also increases long term memory when given to bees before a single olfactory training trial [21].

Abbreviation: PER, proboscis extension reflex.

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The studies in bees are in contrast to the work in *Drosophila* where caffeine decreased acquisition [13,14]; however, there are several significant differences. First, the studies in flies used chronic treatment with consumption of caffeine taking place over more than 20 h, while the studies in honey bees used a single acute treatment. Second, the flies consumed the caffeine while in the bees the caffeine was delivered through the cuticle using dimethyl formamide [20] or via injection [21]. Third, the studies in *Drosophila* used an aversive stimulus during conditioning, whereas the bees were conditioned using an appetitive reward. Different molecular mechanisms underlie aversive and appetitive conditioning [22], and it is possible that caffeine interacts differently with these two systems.

To investigate the effects of caffeine consumption on learning, we examine the effects of caffeine on acquisition and memory recall using olfactory appetitive learning. Honey bees can be trained to associate an odor (the conditioned stimulus, CS) with a reward solution (the unconditioned stimulus, US) so that a bee will extend its proboscis (mouthparts) in response to the odor [23]. We test whether ingesting caffeine during conditioning influences the rate of acquisition and memory recall. Furthermore, we examine if caffeine administered prior to conditioning affects acquisition and whether caffeine administered immediately after conditioning influences recall. In addition, we use behavioral assays to determine if bees can detect caffeine via gustatory sensilla on the antennae and proboscis.

2. Materials and methods

2.1. Subjects

The honey bees *(Apis mellifera carnica)* used in this study were collected from colonies maintained at Arizona State University or at Newcastle University. Individual adult foragers were captured at the colony entrance in small glass vials and chilled at 4 °C until immobile. Each bee was then restrained in a short tube (harness) using a strip of duct tape placed between the head and thorax. Care was taken so that the bee could still freely move its antennae and proboscis. Bees were fed 1.0 M sucrose solution until satiated, and were left in a humid box (a perforated plastic box containing wet paper towels) at room temperature overnight (18–24 h) before being used for experiments.

2.2. Associative olfactory conditioning

Honey bees were classically conditioned to associate an odor with a sucrose reward for 6 or 12 trials with a 5–6 min inter-trial interval [23]. During a conditioning trial, each bee was exposed to a 4 s odor pulse. The odors used were 1-hexanol or 2-octanone (98% purity, Sigma–Aldrich, St. Louis, MO, USA) diluted to 2.0 M in hexane. A 3 μl drop of odor was placed on a thin strip of filter paper within a glass tube. Odors were delivered using an automated system that causes air to flow through the odor tube and across the bee's antennae as described in Mustard et al. [24]. Three seconds after the onset of the odor, the bee's antennae were stimulated with the unconditioned stimulus (US) (e.g. 1.0 M sucrose solution). When the bee responded by extending its proboscis, it was fed 0.4 μl of the indicated reward solution (sucrose alone or sucrose plus caffeine) using a micrometer syringe (Gilmont Industries). If a bee extended its proboscis in response to odor but prior to antennal stimulation, this was recorded as a positive (conditioned) response. After conditioning, each subject was presented with an unreinforced test trial with the conditioned odor and with an unreinforced test trial with a novel odor. Presentation order of the test odors was randomized across subjects. Recall tests were performed 5 min after the final conditioning trial (the immediate recall test) and 24 h later. Bees were fed to satiation with 1.0 M sucrose and left in a humid box between the immediate and 24 h recall tests.

In experiment 1, the antennae were stimulated with 1.0 M sucrose, while the reward solution fed to the bee on each of the 12 conditioning trials contained 1.0 M sucrose plus the indicated concentration of caffeine. For the prefeeding and postfeeding experiments, the solution used to stimulate the antennae and as the reward during the 6 conditioning trials was 0.5 M sucrose. During the prefeeding experiment, the bees were given 5 μl of 0.5 M sucrose plus the indicated concentration of caffeine 30 min before conditioning began. The effects of caffeine were first observed between training trials 4 and 6 in experiment 1, which corresponds to 20–30 min after the initial dose of caffeine was consumed. One reason for this may be that the caffeine needed time to be absorbed and enter the hemolymph of the bee. A similar delay of effects for 20–30 min after consumption has been observed for other compounds [24,25]. To give the caffeine time to be distributed throughout the bee, conditioning did not begin until 30 min after consumption of the caffeine. For the postfeeding experiment, all bees were conditioned with 0.5 M sucrose over 6 trials. Immediately after the last conditioning trial, bees were given a 5 μl dose of 0.5 M sucrose plus the indicated amount of caffeine. Bees were then tested for their response to the conditioned odor and novel odor 5 min and 24 h after consuming the caffeine solution.

2.3. Antennal gustatory response assay

When the antennae of a honey bee contact a stimulatory substance such as sucrose, the honey bee will reflexively extend its proboscis to consume the solution [23], the proboscis extension reflex (PER). A modified version of the sucrose response threshold test for the proboscis extension reflex [26] was used to determine if honey bees could detect caffeine in sucrose solution. Bees were prepared as described above. The antennae of each subject were presented with a series of 1.0 M sucrose solutions containing caffeine. The concentrations of caffeine used were 0 (sucrose alone), 0.0001 M, 0.001 M, 0.01 M, and 0.1 M, which were presented in order of increasing concentration. As in Page et al. [26], after each stimulus presentation, water was presented to the antennae to control for stimulus sensitization. The inter-stimulus interval for each stimulus presentation was 5–6 min.

2.4. Proboscis gustatory response assay

The ability of the honey bee to detect caffeine in sucrose solution with the proboscis was assessed by testing whether bees would drink solutions containing caffeine. Bees were harnessed, fed, and left for 24 h as described above. To synchronize the motivational state of the bees, 30 min prior to testing, each bee was fed 5 μl of 1.0 M sucrose. To provoke proboscis extension, the antennae were stimulated with 1.0 M sucrose. A series of three trials then ensued during which the proboscis was presented with a droplet of solution. If the bee consumed the entire droplet, a positive response was recorded. During the first trial, the proboscis of each subject was presented with a 0.4 μl of a solution containing 1.0 M sucrose, during the second trial, a droplet of deionized water was presented, and during the third, a droplet of 1.0 M sucrose containing caffeine (0.0001, 0.001, 0.01 or 0.1 M) was presented. Because ingestion of the caffeine dose could influence behavior, different groups were used to assess each caffeine concentration.

2.5. Sucrose consumption assay

The reduction in response levels observed during acquisition and immediate recall could be due to a reduction in the motivation of the bees to feed. To examine if caffeine consumption affected motivation to feed, the amount of 0.5 M sucrose solution a honey bee would consume after being fed a dose of caffeine was measured. The day after being harnessed and fed as described above, each bee was fed 5 μl of one of three solutions: 0.5 M sucrose solution, or 0.5 M sucrose solution containing either 0.001 M or 0.01 M caffeine. 5 μl of solution was used because that is the amount of reward solution consumed during the 12 trial conditioning protocol. Any bee that did not consume the entire dose was discarded. Thirty min or 24 h after feeding the caffeine or control solution, the amount of 0.5 M sucrose solution each bee would willingly consume was measured using a Gilmont micrometer syringe.

2.6. Data analysis

The response variable measured during conditioning, testing, and the gustatory assay (proboscis extension) was scored as a binary variable. All learning data were analyzed using logistic regression (lreg) with post hoc multiple comparisons (mc). For the acquisition data, models were tested using 2-way interaction terms including caffeine treatment vs. conditioning trial number to test whether there were differences in the rate of acquisition. Antennal taste assay data were analyzed using repeated-measures logistic regression (RM lreg) whereas proboscis taste assay data were analyzed using logistic regression (lreg). Sucrose consumption data were analyzed using analysis of variance (ANOVA).

3. Results

3.1. Does consumption of caffeine in the reward solution affect appetitive olfactory learning?

Honey bees conditioned with sucrose solutions containing high concentrations of caffeine were less likely to exhibit conditioned proboscis extension during acquisition than bees conditioned with sucrose only (Fig. 1). Bees conditioned with reward solutions containing caffeine at concentrations greater than 0.0001 M exhibited a lower average asymptotic level of learning than the 1.0 M sucrose control group (main effect: lreg, $\chi^2_4 = 82.1, P < 0.001$). This was partially explained by the fact the presence of caffeine in the reward increased the likelihood that a bee would not respond to the odor