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Short communication

Caffeine has no effect on eyeblink conditioning in mice

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ABSTRACT

Caffeine is one of the most widely used drugs in the world. In the brain, caffeine acts as an antagonist for the adenosine A_1 and A_{2B} receptors. Since A_1 receptors are highly concentrated in the cortex of the cerebellum, we hypothesized that caffeine could potentially affect learning tasks that require the cerebellar cortex, such as eyeblink conditioning. To test this hypothesis, we examined the effect of low (5 mg/kg) and high (50 mg/kg) doses of caffeine, injected intraperitoneally before training, on eyeblink conditioning in mice. The results show that, at the dosages we used, caffeine affects neither the rate of acquisition, nor the timing of the onset or peak of the conditioned blink responses. Therefore, we conclude that caffeine neither improves nor worsens performance on eyeblink conditioning.

1. Introduction

Approximately 80–90% of all humans use caffeine on a regular basis. The average daily caffeine intake is ~75 mg worldwide but can be as high as 400 mg in Scandinavia [1]. The popularity of caffeine has been linked to its cognitive benefits, such as increased alertness and improved ability to concentrate on various tasks [2]. Caffeine also seems to have a beneficial effect on patients with dementia [3], as well as Parkinson's disease [4]. Due to its hydrophobic nature, caffeine can cross the blood brain barrier and reach targets in the brain [5,1]. At non-toxic concentrations, caffeine acts as an antagonist of the G-protein coupled A_1 and A_{2B} adenosine receptors [1]. While the A_{2B} receptor is found mostly in the striatum, the A_1 receptor is found throughout the brain with high concentrations in the hippocampus, cerebral cortex, and cerebellar cortex [6]. Previous studies indicate that caffeine affects several types of behaviors and learning. Dosages between 30 and 100 μM/kg (5.8–19.4 mg/kg) increase locomotion in mice [7]. The reaction time in humans slightly improves after ingestion of 300 mg (~4.3 mg/kg) of caffeine [8]. More surprisingly perhaps, caffeine decreases the response rate of rats trained to press for food pellets with a 30-s interval [9]. Caffeine can also affect memory. Floral nectar containing caffeine helps honey bees to remember the location of a flower [10]. Humans likewise show improved recall of faces if they ingest 200 mg (~2.9 mg/kg) of caffeine after looking at those faces [11].

Caffeine has also been suspected to play a role in eyeblink conditioning. In eyeblink conditioning, a neutral, conditional stimulus (CS), such as a tone or a light, is paired with a blink eliciting, unconditional stimulus (US), such as a corneal air puff or a periorbital electrical shock. After repeated CS-US pairings, presenting the CS will elicit a conditioned blink response (CR). Eyeblink conditioning is dependent on the cerebellar cortex [12,13], which has a high concentration of A_1 receptors. Other adenosine targets in the brain, including the motor cortex [14], and the hippocampus [15], have also been shown to influence performance in eyeblink conditioning. Caffeine also modulates LTP and LTD, [16,17], two types of synaptic plasticity that play a role in cerebellar motor learning [18,19]. So far, experiments testing the effect of caffeine on eyeblink conditioning have had mixed results. Winsky and Harvey showed that, for conditioning of the nictitating membrane responses in rabbits, an adenosine agonist analog (L-Pia) retards the acquisition of CRs [20]. Caffeine, on the other hand, did not affect conditioning, except for a very high dose (58 mg/kg), which resulted in a small but significant improvement in the rate of CRs [21]. Tests in humans show that a low dose of caffeine (2 mg/kg) has a negative effect on conditioning whereas a slightly higher dose (5 mg/kg), has no effect [22]. Given that experiments so far have been few and difficult to interpret, we designed an experiment to test how the acquisition and shape of CRs in mice are affected by small (5 mg/kg) and large (50 mg/kg) doses of caffeine.

2. Method

2.1. Subjects and surgery

Subjects were 33 C57Bl/6 mice, 12–24 weeks old, individually housed in cages with 12 h light/dark cycles and access to food ad libitum. Prior to surgery, the mice were anesthetized in a mixture oxygen

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and 5% (vol/vol) isoflurane and a systemic analgesic, Rimadyl (Pfizer, 5 mg/ml), was administered sub-cutaneously. To maintain the anesthesia during surgery the isoflurane was set to 2%. After injection of a local anesthetic (lidocaine 10%), the tissue and membranes covering the skull were stripped completely, thus revealing the bone underneath. To fix the head of the mice during conditioning a messing pedestal (8 × 5 × 3 mm) was attached to the skull using Optibond All-In-One (Kerr) and Charisma (Heraeus Kulzer). The mice were given two days to recover after the surgery with a post-analgesic injection of Rimadyl (Pfizer, 5 mg/kg) after 24 h. For more details of the methods see Ref. [23]. All experiments were approved by the Dutch Ethical Committee for animal experiments and were in carried out in accordance with the institutional animal care and use committee guidelines.

2.2. Training

The mice were habituated in two 30-min sessions on the three days preceding the training. Approximately 15 min before each training session, a neodymium magnet (1.5 × 0.7 × 0.5 mm) was placed on the mouse's lower left eyelid using superglue (cyanoacrylate) after which the mouse received an intraperitoneal injection of saline or saline mixed with caffeine. Of the 33 mice, nine were injected with a small dose of caffeine (5 mg/kg), 10 received a large dose (50 mg/kg), and the remaining 14 mice were injected with saline and served as controls. The CS was a green LED light that was on for 280 ms and co-terminated with the US which was a 30 ms, 30 psi (at the source) air puff aimed at the center of the left eye. The training consisted of 10 sessions, lasting 40–60 min, on ten consecutive days. Each session was divided into 20 blocks each consisting of 10 paired trials and 1 CS alone trial, resulting in a total of 220 trials, 200 paired and 20 CS alone, per session. The interstimulus interval (ISI) was 250 ms, and the intertrial interval (ITI) was 10 ± 2 s. Before each session, we verified that the air puff elicited a clear blink response. Eyelid movements were recorded with the magnetic distance measurement technique (MDMT), in which a magnetic sensor is used to measure movements of the small magnet on the eyelid.

2.3. Analysis

The MDMT signal, sampled at 1000 Hz, was analyzed offline using custom made scripts in Matlab (Mathworks). On each trial, we recorded the CR amplitude, defined as the mean amplitude in the last 5 ms of the CS-US interval (corresponding to 245–249 ms in all figures). Trials were categorized as invalid if the mice moved their eyelid by more than 10% of the UR amplitude 50 ms before or after CS onset. On valid trials, a response was categorized as a CR if the mean amplitude in the 5 ms preceding the US was more than 10% of the average UR amplitude for that session (see Fig. 1). This procedure did sometimes fail to detect responses that we, upon visual inspection, categorized as CRs. However, to avoid experimenter bias we decided not to make corrections manually. The peak latency was defined as the earliest time, > 100 ms after CS onset, when the amplitude was > 95% of the maximum amplitude on that trial. The onset was defined as the latest point > 50 ms after the CS onset, when the amplitude < 5% of the maximum. To test if caffeine affected the learning process, we performed two repeated measures ANOVA with CR percentage or CR amplitude on successive sessions as the within factor and treatment as the between subjects' factor (Fig. 2A–B). We also performed six one-way ANOVAs to test if the CR percentage, onset latency, peak latency or the standard deviation of the onset and peak latency was affected by caffeine. The topographical analysis was based on all CRs in CS alone trials in sessions 6–10. All variables were approximately normally distributed according to the Lilliefors test (all $p > 0.05$). All statistical comparisons and results are summarized in Table 1.

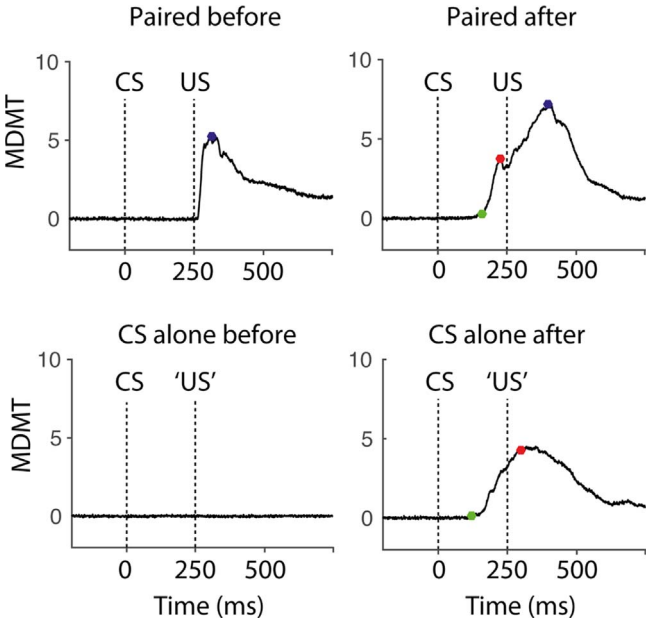

Fig. 1. MDMT traces on single paired (top row) and CS alone trials (bottom row) before (left) and after (right) eyeblink conditioning in one mouse. Initially, mice blink in response to the US, but not the CS. After training, mice blinks to the CS, independent of whether the US is given. Colored dots denote the UR (blue), the CR onset (green) and the CR peak (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Caffeine does not result in faster acquisition

Mice injected with a low dose (5 mg/kg), or a high dose (50 mg/kg) of caffeine, did not acquire CRs faster nor did they reach a higher percentage of CRs than littermates receiving saline injections (see Figs. 2 and 3). A repeated measures ANOVA revealed a significant effect of training. That is, the percentage of CRs increased as a result of training, $F(9270) = 95$, ($p < 0.0001$). However, there was no evidence that the learning rate depended on the amount of caffeine the mice received before training, $F(18,270) = 1.13$, $p = 0.325$. A second repeated measures ANOVA showed that amplitude in the last 5 ms of the CS-US interval increased as a result of training, $F(9270) = 45.5$, $p < 0.0001$. However, like the CR percentage, the amplitude was not affected by caffeine dosage, $F(18,270) = 1.14$, $p = 0.31$. To sum up, mice produced more CRs as training progressed, however, caffeine did not affect how fast the mice learned or the amplitude of the responses.

3.2. Caffeine does not affect the timing of conditioned responses

Even though caffeine does not influence the acquisition or the rate of CRs, it might still influence the topography of the CRs. For example, caffeine might cause mice to blink with a shorter or a longer delay, or cause increased or decreased variability in the timing of the CRs. To test if caffeine altered the timing of the CRs, we did four one-way ANOVAs on the latency to CR onset, the peak latency, and the standard deviation of these two variables (see Table 1 for details). The results revealed no significant effects on the timing of the CRs (all $p > 0.14$).

4. Discussion

Mice injected intraperitoneally with 5 mg/kg or 50 mg/kg of caffeine, before training sessions, do not acquire CRs faster or reach a higher CR percentage than mice injected with saline. Neither did we find any evidence suggesting that the latency to CR onset or CR peak, or the variance of these two variables, are affected by caffeine injections.