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Caffeine increases food intake while reducing anxiety-related behaviors

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ABSTRACT

The objective of this study was to determine the effects of different doses of caffeine on appetite and anxiety-related behavior. Additionally, we sought to determine if withdrawal from chronic caffeine administration promotes anxiety. In this study, we utilized rodent open field testing and feeding behavior assays to determine the effects of caffeine on feeding and anxiety-related behavior (n = 8 mice; 4–8 weeks old). We also measured 2 h and 24 h food intake and body-weight during daily administration of caffeine (n = 12 mice; 4–8 weeks old). To test for caffeine withdrawal induced anxiety, anxiety-related behavior in rodents was quantified following withdrawal from four consecutive days of caffeine administration (n = 12 mice; 4–8 weeks old). We find that acute caffeine administration increases food intake in a dose-dependent manner with lower doses of caffeine more significantly increasing food intake than higher doses. Acute caffeine administration also reduced anxiety-related behaviors in mice without significantly altering locomotor activity. However, we did not observe any differences in 24 h food intake or body weight following chronic caffeine administration and there were no observable differences in anxiety-related behaviors during caffeine withdrawal. In conclusion, we find that caffeine can both increase appetite and decrease anxiety-related behaviors in a dose dependent fashion. Given the complex relationship between appetite and anxiety, the present study provides additional insights into potential caffeine-based pharmacological mechanisms governing appetite and anxiety disorders, such as bulimia nervosa.

1. Introduction

The most broadly consumed behaviorally active substance worldwide is caffeine (Fredholm, Bättig, Holmén, Nehlig, & Zvartau, 1999). Americans consume a daily average of 165 mg of caffeine from beverages alone (Mitchell, Knight, Hockenberry, Teplansky, & Hartman, 2014). Additionally, it has been reported that 85% of Americans will consume a minimum of one caffeinated drink per day (Mitchell et al., 2014). Given that 34.9% of Americans over 20 years of age are considered obese, it is paramount to address the possible effects of caffeine on food intake (Ogden, Carroll, Kit, & Flegal, 2014). Interestingly, the effects of caffeine on food intake are controversial. For instance, a study by Retzbach, Dholakia, and Duncan-Vaidya (2014) showed that caffeine increases lever pressing for sucrose, suggesting that caffeine can increase appetitive behavior and feeding. This increase in lever pressing was accompanied by an increase in c-fos expression in the nucleus accumbens, a critical brain region for reward (Berridge & Kringelbach, 2015) and hedonic control of feeding behavior (O'Connor et al., 2015; Castro & Berridge, 2014). Furthermore, a study by Bonaventura et al. (2012) showed that both high-palatable and low-palatable food intake decreased in rats when A_{2A} adenosine receptors were activated using A_{2A} agonists. Since caffeine exerts its effect by blocking A_{2A} receptors (Ribeiro & Sebastiao, 2010), this finding suggests that caffeine can stimulate food intake. Conversely, Pettenuzzo et al. (2008) found that chronic caffeine administration in rats does not affect the consumption of rat chow, but decreases palatable food intake. Other studies have shown that caffeine has no effect on appetite sensations or energy intake, while at the same time it has been shown that caffeine decreases both consumption of powdered food and body weight in rats (Gavrieli et al., 2011; Racotta, Leblanc, & Richard, 1994; Schubert et al., 2014). A similar effect has been observed in

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humans where caffeine was shown to intensify the anti-appetitive effects of nicotine (Jessen, Buemann, Toubro, Skovgaard, & Astrup, 2005). In addition to these findings, Gavrieli et al. (2013) showed that consumption of caffeinated coffee reduced energy intake in obese individuals. These disparate findings may be explained by the doses of caffeine administered and/or the routes of caffeine administration (injection vs. oral consumption).

The neural mechanisms for caffeine's effects on feeding are incompletely understood. However, caffeine has been shown to increase excitatory synaptic input to agouti-related peptide (AgRP) neurons located in the hypothalamic arcuate nucleus (Yang, Atasoy, Su, & Sternson, 2011). When activated, AgRP neurons increase feeding behavior by driving consummatory and appetitive behaviors (Aponte, Atasoy, & Sternson, 2011; Sternson & Atasoy, 2014). Additionally, caffeine can both increase dopamine levels and activate dopamine neurons in the nucleus accumbens, a brain region critical for motivation and feeding behaviors (O'Connor et al., 2015; Retzbach et al., 2014; Solinas et al., 2002). Taken together, emerging evidence suggests that caffeine increases feeding behavior, at least in part, by increasing the activity of appetite promoting AGRP neurons in the arcuate nucleus and dopamine neurons in the nucleus accumbens. We therefore proposed that acute administration of caffeine would increase food intake, depending on the dose of caffeine administered.

Beyond caffeine's effects on feeding, recent reports have implicated caffeine in anxiety-related behaviors. For example, Maximino, Lima, Olivera, Picanço-Diniz, and Herculano (2011) showed that caffeine can induce an anxiety-like behavioral response in zebrafish in a dose-dependent manner by blocking A_1 receptors. However, a recent report showed that caffeine can have anxiolytic effects in rats in the open field test and elevated plus maze (Hughes, Hancock, Henwood, & Rapley, 2014). Consistently, caffeine administration can exert anxiolytic or anxiogenic effects in rats depending on the anxiety test employed, the rat strain, and the sex of the rat (Hughes & Hancock, 2016). Caffeine has also been reported to have anti-depressive like qualities. For example, a recent study demonstrated that chronic pre-treatment with caffeine prior to social defeat stress in mice can reduce the subsequent development of depressive and anxiogenic like phenotypes (Yin et al., 2015). This effect was prevented when caffeine was co-administered with a selective dopamine D1 receptor antagonist, indicating that caffeine reduces stress induced anxiety and depressive phenotypes via dopaminergic signaling. By contrast, caffeine withdrawal is known to cause a large number of behavioral effects including: irritability, sleepiness, nervousness, restlessness, and anxiety (Dews, O'Brien, & Bergman, 2002).

Due to the known interactions between anxiety-related behaviors and feeding, the current study investigated how both acute and chronic caffeine administration affects feeding and anxiety in AgRP-IRES-Cre transgenic mouse, a common transgenic animal for feeding studies (Maniam & Morris, 2012; Hardaway, Crowley, Bulik, & Kash, 2015). Based on the known reciprocal interaction between feeding and anxiety behavior (Hardaway et al., 2015; Maniam & Morris, 2012), we hypothesized that low doses of caffeine would exert an anxiolytic effect and increase feeding while high doses would have an anxiogenic effect and decrease feeding. To elucidate the effects of caffeine on feeding behavior and anxiety, we measured free access food intake and open field exploratory behavior following acute caffeine administration. We also tested for caffeine withdrawal induced anxiety by performing open field behavioral testing following withdrawal from chronic caffeine administration.

2. Methods

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of State University of New York Upstate Medical University, according to US National Institute of Health's Guide for the Care and Use of Laboratory Animals. AgRP-IRES-Cre transgenic mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). These mice do not have any known phenotypes when Cre recombinase is not active, as used in this study. AgRP-IRES-Cre mice were used to maintain consistency with potential future studies which will use Cre-loxP recombination strategies to manipulate hypothalamic AgRP neurons to determine their role in caffeine induced feeding behavior. Male and female mice between the ages of 4 and 8 weeks were used for all experiments. Mice were maintained on a 12 h light/dark cycle (light onset at 6:30 a.m.) and housed in ventilated cages with free access to water and standard rodent chow (LabDiet: 5008 Formulab Diet). All behavioral experiments were conducted in the home cage unless otherwise noted.

2.1. Feeding behavior assays and caffeine treatment

Prior to feeding behavior experiments, mice were single caged and habituated for one week. Animals were handled daily for the week prior to beginning experiments to habituate them to behavioral assays. All mice were handled by picking them up by their scruff multiple times to mimic handling conditions seen during i.p. injections. Handling was performed to reduce stress responses in response to i.p. injections. Food intake was measured at the indicated time-points by briefly removing the food from the hopper and obtaining its weight. For the acute caffeine intake experiment, 8 mice were used and received the same treatment. Intraperitoneal (i.p.) injections were performed daily at 9:00 a.m. for 8 days. Mice received saline injections (0.9% saline; 200 μl) on days 1–3. Saline injections were performed to obtain baseline food intake measurements prior to caffeine administration. On days 4–8, the mice received an injection of caffeine (Tocris Bioscience, Bristol, UK) every other day with increasing doses (6 mg/kg, 12 mg/kg, and 24 mg/kg; dissolved in 200 μl 0.9% saline) and received saline injections on the days where caffeine was not administered. Caffeine injections were administered every other day to prevent habituation to caffeine treatment. Food was replaced daily with approximately 20 g of fresh standard chow. Food intake was measured at various time points following injections: 30 min, 1 h, 2 h, and 5 h. Average food intake and body weight were calculated for each mouse.

For chronic caffeine administration, 12 mice were used and were separated into two equal groups. Animals were assigned to control (saline injections) or experimental groups (caffeine injections). Injections were administered at 1:45 p.m. and 2:15 p.m. for all 10 days. On days 1–2, both of the groups received saline injections at each of the two indicated time-points. Saline injections were administered to obtain a baseline measure of food intake prior to treatment. On days 3–6, the experimental group received a saline injection followed by a caffeine injection (20 mg/kg in 200 μl 0.9% saline), while the control received two saline injections. Both groups received two saline injections on days 7–10. Saline was administered on days 7–10 to maintain consistency in injection conditions while testing for behavioral symptoms of caffeine withdrawal. Food was replaced daily with approximately 15 g of fresh standard chow. Body weight was measured immediately following the first injection each day. Food intake was measured at various time points following the second injection: 30 min, 1 h, 2 h, and 24 h. Average food intake and body weight were calculated for each mouse.