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Adolescent caffeine consumption increases adulthood anxiety-related behavior and modifies neuroendocrine signaling

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ABSTRACT

Caffeine is a commonly used psychoactive substance and consumption by children and adolescents continues to rise. Here, we examine the lasting effects of adolescent caffeine consumption on anxiety-related behaviors and several neuroendocrine measures in adulthood. Adolescent male Sprague-Dawley rats consumed caffeine (0.3 g/L) for 28 consecutive days from postnatal day 28 (P28) to P55. Age-matched control rats consumed water. Behavioral testing for anxiety-related behavior began in adulthood (P62) 7 days after removal of caffeine. Adolescent caffeine consumption enhanced anxiety-related behavior in an open field, social interaction test, and elevated plus maze. Similar caffeine consumption in adult rats did not alter anxiety-related behavior after caffeine removal. Characterization of neuroendocrine measures was next assessed to determine whether the changes in anxiety were associated with modifications in the HPA axis. Blood plasma levels of corticosterone (CORT) were assessed throughout the caffeine consumption procedure in adolescent rats. Adolescent caffeine consumption elevated plasma CORT 24 h after initiation of caffeine consumption that normalized over the course of the 28-day consumption procedure. CORT levels were also elevated 24 h after caffeine removal and remained elevated for 7 days. Despite elevated basal CORT in adult rats that consumed caffeine during adolescence, the adrenocorticotropic hormone (ACTH) and CORT response to placement on an elevated pedestal (a mild stressor) was significantly blunted. Lastly, we assessed changes in basal and stress-induced *c-fos* and corticotropin-releasing factor (*Crf*) mRNA expression in brain tissue collected at 7 days withdrawal from adolescent caffeine. Adolescent caffeine consumption increased basal c-*fos* mRNA in the paraventricular nucleus of the hypothalamus. Adolescent caffeine consumption had no other effects on the basal or stress-induced *c-fos* mRNA changes. Caffeine consumption during adolescence increased basal *Crf* mRNA in the central nucleus of the amygdala, but no additional effects of stress or caffeine consumption were observed in other brain regions. Together these findings suggest that adolescent caffeine consumption may increase vulnerability to psychiatric disorders including anxiety-related disorders, and this vulnerability may result from dysregulation of the neuroendocrine stress response system.

1. Introduction

Caffeine is the most widely used psychostimulant in the world (Rath, 2012; Warzak et al., 2011). Caffeine consumption has risen in recent years, especially among children and adolescents (Temple, 2009). In fact, 75% of children 5 years or older in the United States consume caffeine on a daily basis (Ahluwalia and Herrick, 2015), and daily caffeine consumption in 9–17 year olds has more than doubled since 1980 (Frary et al., 2005). The rise in caffeine consumption is compounded by the fact that energy drinks that are increasingly marketed to children and young adults contain extremely high concentrations of caffeine.

Although caffeine is thought to be relatively safe, epidemiological studies suggest that caffeine consumption is linked to anxiety disorders. For example, oral caffeine administration precipitates panic attacks in adults diagnosed with panic disorder, generalized social anxiety disorder, and performance social anxiety disorder (Nardi et al., 2009). Genetic studies have linked panic disorder and agoraphobia with single nucleotide polymorphisms

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in the adenosine A_{2A} receptor, a primary target antagonized by caffeine (Deckert et al., 1998; Lam et al., 2005). Animal studies also report caffeine-induced anxiety in adult rats as measured by the elevated plus maze, light dark box (El Yacoubi et al., 2000), and open field (Noschang et al., 2009) during acute caffeine administration and chronic caffeine consumption. Withdrawal-induced anxiety was also observed 48 h following chronic caffeine in adult animals (Bhattacharya et al., 1997). While there is substantial evidence to suggest a relationship between caffeine consumption and anxiety in adults, little research has specifically examined the lasting effects of caffeine consumption during adolescence and adulthood.

Adolescence is a developmental period characterized by the maturation of the brain during which numerous endogenous and environmental factors impact the maturation process (Arain et al., 2013; Wahlstrom et al., 2010). Here, we explore the notion that adolescent caffeine consumption is an environmental factor that may increase the risk of developing neuropsychiatric conditions such as anxiety. There appears to be a relationship between anxiety and caffeine consumption occurring during early life. A recent study reports that higher levels of caffeine intake in children in the United Kingdom were associated with an increased risk of anxiety (Ruxton, 2014). Similarly, energy drink consumption in Australian young adult males correlates with self-reported anxiety (Trapp et al., 2014). Rats administered acute caffeine also display increased anxiety while consuming caffeine during adolescence (Ardais et al., 2014). Thus, there is substantial evidence suggesting that caffeine consumption can influence anxiety in both adolescent and adults, although no studies have examined the long-term effects of chronic adolescent caffeine consumption on anxiety-related behaviors.

The anxiogenic nature of acute caffeine administration has prompted several studies to examine the effect of acute caffeine administration on hypothalamic–pituitary–adrenal (HPA) axis activation. Although there is inconsistent evidence to support a causal role of HPA functioning in the manifestation of anxiety behavior (Armario et al., 2012), the activity of the HPA axis is thought to significantly impact the pathogenesis of stress-related disorders such as depression and anxiety (Jezova and Hlavacova, 2008; McEwen, 2005). The fact that acute caffeine administration increases HPA activity supports a potential link between caffeine-induced anxiety and HPA axis function. In humans, acute caffeine injected into sleeping subjects significantly increases plasma adrenocorticotropin hormone (ACTH) and cortisol (Lin et al., 1997). Studies in rats corroborate this link where higher doses of caffeine elevate plasma ACTH (Patz et al., 2006) and corticosterone (CORT) levels (Nicholson, 1989; Patz et al., 2006). Caffeine also increases levels of the stress-related neuropeptide, corticotropin-releasing factor (CRF), in cultured hypothalamic neurons (Nicholson, 1989). Pharmacological blockade of endogenous CRF release attenuates the increases in plasma CORT seen in response to acute caffeine (Nicholson, 1989). Together, these studies suggest that caffeine enhances both anxiety and the activity of the HPA axis.

The effect of chronic caffeine consumption, especially in adolescents, on anxiety and HPA axis function remains unclear. The present studies were designed to assess the effects of adolescent caffeine consumption on anxiety and HPA axis function in adulthood and in the absence of caffeine. We first conducted several tests of anxiety-related behaviors following 28 days of caffeine consumption occurring during adolescence (postnatal days 28-55; P28-55) or adulthood (P67-94). Based on the observation that adolescent, but not adult, caffeine consumption produced significant lasting enhancements in anxiety-related behaviors, we further characterized the effect of chronic caffeine consumption during adolescence on concurrent and subsequent HPA axis function to determine whether dysregulation of HPA axis activity was associated with enhanced anxiety behavior.

2. Materials and methods

2.1. Rats and housing

Male Sprague-Dawley rats (Charles River) were received on either P21 (adolescent studies) or P60 (adult studies) and pair housed with ad libitum food and water. Separate cohorts of rats were used for each experimental procedure, except where noted. All experimental procedures were conducted during the light period of a 12 h light/dark cycle and were completed in accordance with the guidelines established by the National Institutes of Health and approved by the Institutional Animal Care and Use Committee at the University of Colorado Boulder.

2.2. Caffeine consumption procedure

Seven days after arrival, caffeine-consuming rats were given access to a single bottle containing caffeine in water (0.3 g/L) for 28 days based on previously published procedures (Ardais et al., 2014; O'Neill et al., 2015). Caffeine consumption procedures occurred continuously between P28-P55 in adolescent studies and P67-94 in adult studies. Age-matched control groups continued to receive water throughout the procedure. Caffeine and water consumption were recorded by measuring the amount of fluid consumed throughout the procedure. To avoid the potential stress effects of housing rats singly during adolescence, rats were pair-housed throughout the procedure. Total consumption of water and caffeine for each cage were divided by in half to estimate individual fluid consumption. Caffeine intake (mg/kg/day) was further estimated by adjusting the intake by each animal's individual body weight. Following 28 days of caffeine exposure, the caffeine solution was replaced with water for the remainder of the experiment. Behavioral testing was initiated at least 7 days (unless specified otherwise) after the last caffeine consumption (adolescent studies: P62-69; adult studies: P102-109). Likewise, brain tissue and blood were collected in the absence of caffeine between P62-P69 (except where noted), periods corresponding to adulthood. Behavioral testing and blood sampling were completed in separate cohorts.

2.3. Behavioral tests

2.3.1. Elevated plus maze

Rats were subjected to the elevated plus maze between P62-67 (adolescent studies) or P101-109 (adult studies). An additional cohort of adolescent and adult rats was tested on the elevated plus maze during the last week of caffeine consumption (adolescent: P50-55; adult: P90-94) to evaluate the direct effects of caffeine consumption on anxiety measures. The elevated plus maze consisted of four arms (50 × 10 cm each) joined by a central platform (10 × 10 cm). Two arms are enclosed with 40 cm high walls, while the other two are "open". The entire apparatus was elevated 75 cm from the floor. The elevated plus maze procedures were conducted in a fully lit room. Rats were put in the center of the maze and allowed to explore the arms for 5 min. Time spent in the open arms was recorded manually by a technician who was blind to the experimental groups. Open arm time was defined as more than half of the rat's body being in the open arm.

2.3.2. Social interaction

Rats were tested in a social interaction test between P62-67 (adolescent studies) or P101-109 (adult studies). Each rat was allocated a standard plastic tub cage with a wire lid and bedding located in a designated testing room. Rats were placed in the test cage