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Adolescent intake of caffeinated energy drinks does not affect adult alcohol consumption in C57BL/6 and BALB/c mice

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ABSTRACT

The rise in marketing and mass consumption of energy drink products by adolescents poses a largely unknown risk on adolescent development and drug reward. Yet, with increasing reports of acute health issues present in young adults who ingest large quantities of energy drinks alone or in combination with alcohol, the need to elucidate these potential risks is pressing. Energy drinks contain high levels of caffeine and sucrose; therefore, exposure to energy drinks may lead to changes in drug-related behaviors since caffeine and sucrose consumption activates similar brain pathways engaged by substances of abuse. With a recent study observing that adolescent caffeine consumption increased cocaine sensitivity, we sought to investigate how prolonged energy drink exposure in adolescence alters alcohol use and preference in adulthood. To do so, we utilized three different energy drink exposure paradigms and two strains of male mice (C57BL/6 and BALB/c) to monitor the effect of caffeine exposure via energy drinks in adolescence on adult alcohol intake. These paradigms included two models of volitional consumption of energy drinks or energy drink-like substances and one model of forced consumption of sucrose solutions with different caffeine concentrations. Following adolescent exposure to these solutions, alcohol intake was monitored in a limited-access, two-bottle choice between water and increasing concentrations of alcohol during adulthood. In none of the three models or two strains of mice did we observe that adolescent 'energy drink' consumption or exposure was correlated with changes in adult alcohol intake or preference. While our current preclinical results suggest that exposure to large amounts of caffeine does not alter future alcohol intake, differences in caffeine metabolism between mice and humans need to be considered before translating these results to humans.

Introduction

Consumption of highly caffeinated energy drinks has increased rapidly over the last decade. Between the years of 2002–2006, sales of energy drinks grew at a rate of 55% annually with sales in the United States reaching $5.4 billion in 2006 (Arria & O'Brien, 2011; Reissig, Strain, & Griffiths, 2009). The consumption of energy drinks is particularly prevalent in adolescents and young adults, with reports of more than 30% of this population consuming these drinks on a regular basis (Howland, Rohsenow, Calise, Mackillop, & Metrik, 2011). Adolescents and young adults are selectively targeted by energy drink manufacturers and marketing, thus increasing the desire and probability of consumption in this age group (O'Brien, McCoy, Rhodes, Wagoner, & Wolfson, 2008; Reissig et al., 2009; Temple, 2009). Importantly, the current generation of energy drinks contains much higher caffeine concentrations (ranging from 9 to 30 mg/oz, Reissig et al., 2009) than standard caffeinated sodas such as cola which generally contain roughly 3 mg/oz caffeine (Reissig et al., 2009).

Several reports have indicated that caffeine can induce behavioral effects commonly associated with drugs of abuse, such as increased self-administration, reward, withdrawal, and tolerance (Temple, 2009). Adolescent caffeine consumption produces cross-sensitized responses to methylphenidate (Boeck et al., 2009), increased self-administration of nicotine (Tanda & Goldberg, 2000), increased cocaine sensitivity (O'Neill et al., 2015), and increased self-administration of alcohol after caffeine exposure (Kunin, Gaskin, Rogan, Smith, & Amit, 2000). This is not unexpected as caffeine induces dopamine release in brain regions that process behavioral reinforcement in ways similar to those of drugs of abuse (Garrett & Griffiths, 1997; Pierce & Kumaresan, 2006; Robinson &

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Berridge, 2000). As the adolescent brain is still under development, adolescent exposure to highly caffeinated energy drinks could potentially influence short- and long-term behaviors, specifically relating to drug reward and consumption. Brain structures such as the prefrontal cortex, which is important for the rewarding value of taste (Rolls, 2000) and decision making/reward-guided learning (Rushworth, Noonan, Boorman, Walton, & Behrens, 2011), are still developing throughout adolescence (Casey & Jones, 2010; Dahl, 2004; Spear, 2000). As a result of this continued development, adolescents are known to demonstrate less impulse control than adults (Dahl, 2004; Romer, 2010) and exhibit increased susceptibility to drugs of abuse (O'Neill et al., 2015; Spear, 2000; Stone, Becker, Huber, & Catalano, 2012).

A relatively small number of human studies have reported correlations between energy drink consumption and negative alcohol outcomes (Arria et al., 2010, 2011; Ferreira, de Mello, Pompéia, & de Souza-Formigoni, 2006; Kozlowski et al., 1993; McKetin, Coen, & Kaye, 2015; Oteri, Salvo, Caputi, & Calapai, 2007). However, interpretation and applicability of these studies are heavily limited to user self-reports or acute in-laboratory behavioral tests, which limit the evaluation of objective long-term consequences of energy drink exposure in adolescence on adult alcohol consumption. To better understand the consequences and potential risks of adolescent consumption of caffeinated energy drinks, rodent animal models provide a convenient way to conduct developmental studies in a much shorter time period as adolescence in rodents takes weeks versus years in humans (Spear, 2004).

In order to study the impact of prolonged adolescent exposure to energy drinks on adult alcohol intake in a well-controlled manner, we developed three different rodent models for energy drink consumption or exposure during adolescence. We used C57BL/6 mice that readily consume large quantities of alcohol or sucrose solutions and BALB/c mice, who consume lower levels of alcohol (Pothion, Bizot, Trovero, & Belzung, 2004; Yoneyama, Crabbe, Ford, Murillo, & Finn, 2008). Adolescent male mice were exposed to 'energy drinks' (actual energy drinks or caffeinated sucrose solutions mimicking energy drink concentrations) either voluntarily using a continuous-access or limited-access two-bottle choice paradigm or involuntary by oral gavage of the caffeinated sucrose solutions. Directly following adolescent 'energy drink' exposure, alcohol intake and preference was measured in the young adult mice. No correlations between adolescent caffeinated energy drink exposure and adult alcohol consumption were observed in any of our exposure paradigms, concluding that adolescent exposure to caffeinated energy drinks in male C57BL/6 or BALB/c mice does not affect adult alcohol consumption. Reconciling our negative results to those observed in human correlations between energy drink use and alcohol intake requires further exploration.

Materials and methods

Animals

Male C57BL/6 and BALB/c wild-type inbred mice were purchased from Harlan (Indianapolis, IN, USA). For Experiments 1 and 2, animals were single-housed in double grommet, ventilated Plexiglas® cages throughout testing. In Experiments 3 and 4, animals were group-housed throughout adolescence and moved to single housing in double grommet, ventilated Plexiglas® cages for adult alcohol intake testing. All mice were housed in a 12 h reverse dark–light cycle to allow energy drink exposure and alcohol intake studies to be conducted during each animal's active light cycle. The temperature of the housing room was maintained at 21 °C; food and water were provided *ad libitum* throughout all Experiments.

Mice were 4-weeks-old, 30-days postnatal (P30) when shipped and allowed to acclimate for 7–10 days prior to the Experiment initiation. Experiments started when mice were approximately 40 days old and could be described as being in mid-adolescence (Adriani et al., 2004; Spear, 2000). On Fridays, cages were changed and the animals were weighed. All procedures were approved by the Institutional Animal Care and Committee and performed in an Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) -certified facility in accordance with the National Institutes for Health Guide for Care and Use of Laboratory Animals.

Drugs and solutions

The alcohol solutions were prepared by diluting 200 proof ethanol (Goldshield, Hayward, CA, USA) in reverse-osmosis filtered water to produce 1%, 3%, 6%, 12% and 20% (vol/vol) ethanol solutions. Red Bull® (Santa Monica, CA, USA), Monster® (Corona, CA, USA), and NOS® (Coca-Cola Company, Atlanta, GA, USA) were decarbonated and filter-sterilized prior to consumption. Sucrose (Fisher Scientific, Pittsburgh, PA, USA) + caffeine (Sigma–Aldrich, St. Louis, MO, USA) and sucrose + quinine (Sigma–Aldrich) solutions were prepared in reverse-osmosis filtered water and filter-sterilized.

Experiment 1: voluntary continuous-access to energy drinks in adolescence in C57BL/6 male mice

To model the effects of adolescent energy drink consumption on future alcohol consumption in mice, we provided groups of adolescent C57BL/6 mice ($n = 6$ per group) with continuous-access (24 h/day) to a two-bottle choice consisting of water and one of three different energy drinks (Red Bull®, Monster®, or NOS®) for 10 consecutive days. The water group was exposed to two-bottles of water to control for the two-bottle access option. Of the three energy drinks, NOS® has the highest caffeine concentration (550 mg/L versus 326 mg/L for Red Bull® and Monster®). To control for additional ingredients present in energy drinks such as vitamins, taurine, ginseng, and guarana, an additional two groups of mice ($n = 6$ per group) had continuous-access to either water and a sucrose + caffeine solution containing an amount of caffeine that was equivalent to the amount present in Red Bull® and Monster® solutions (326 mg/L caffeine, $n = 6$ per group). The Red Bull®, Monster®, and NOS® energy drink solutions contained 115–120 g/L of sugar, predominantly made up of sucrose (although Western Red Bull® also contains glucose). Therefore, we used 120 g/L sucrose for our control solution with the exception of the water control solution. The weights of the bottles were measured to the nearest 0.1 g and replaced afterward.

At the end of the 10 day period, the energy drink solutions were exchanged for alcohol solutions. Mice were presented with water and solutions of increasing alcohol concentration (3%, 6%, 12%, and 20%) in a two-bottle choice, limited-access paradigm for a 4 h period (11:00 AM to 3:00 PM) for 4 days each for 16 consecutive days (see Fig. 1 for timeline). The weight of each bottle was measured to the nearest 0.1 g at the end of the alcohol access and bottle location was reversed every day to prevent habit formation. Throughout, combined alcohol intake per alcohol concentration is denoted as 'total intake,' whereas combined intake of alcohol during the entire 4-week period is denoted as 'cumulative intake.'

Experiment 2: voluntary limited-access to energy drinks in adolescence in C57BL/6 male mice

A two-bottle limited-access (4 h/day, 5 days/week) paradigm was performed during which adolescent C57BL/6 male mice ($n = 6$