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CAFFEINE TRIGGERS BEHAVIORAL AND NEUROCHEMICAL ALTERATIONS IN ADOLESCENT RATS

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Abstract—Caffeine is the psychostimulant most consumed worldwide but concerns arise about the growing intake of caffeine-containing drinks by adolescents since the effects of caffeine on cognitive functions and neurochemical aspects of late brain maturation during adolescence are poorly known. We now studied the behavioral impact in adolescent male rats of regular caffeine intake at low (0.1 mg/mL), moderate (0.3 mg/mL) and moderate/high (1.0 mg/mL) doses only during their active period (from 7:00 P.M. to 7:00 A.M.). All tested doses of caffeine were devoid of effects on locomotor activity, but triggered anxiogenic effects. Caffeine (0.3 and 1 mg/mL) improved the performance in the object recognition task, but the higher dose of caffeine (1.0 mg/mL) decreased the habituation to an open-field arena, suggesting impaired non-associative memory. All tested doses of caffeine decreased the density of glial fibrillary acidic protein and synaptosomal-associated protein-25, but failed to modify neuron-specific nuclear protein immunoreactivity in the hippocampus and cerebral cortex. Caffeine (0.3–1 mg/mL) increased the density of brain-derived neurotrophic factor (BDNF) and proBDNF density as well as adenosine A_1 receptor density in the hippocampus, whereas the higher dose of caffeine (1 mg/mL) increased the density of proBDNF and BDNF and decreased A_1 receptor density in the cerebral cortex. These findings document an impact of caffeine consumption in adolescent rats with a dual impact on anxiety and recognition memory, associated with changes in BDNF levels and decreases of astrocytic and nerve terminal markers without overt neuronal damage in hippocampal and cortical regions. © **2014 IBRO. Published by Elsevier Ltd. All rights reserved.**

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Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; DG, dentate gyrus; PBS, phosphate-buffered saline; PND, postnatal days.

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INTRODUCTION

Caffeine is the most popular psychostimulant substance consumed worldwide, being found mainly in coffee and its psychostimulant effect is due to the antagonism of central adenosine A_1 and A_{2A} receptors (A_1R and $A_{2A}R$) (Fredholm et al., 1999). The actions of caffeine include the maintenance of alertness and arousal, decreased reaction times and increased vigilance and attention (Brice and Smith, 2002; Childs and de Wit, 2006). It is a matter of debate if caffeine has net cognitive enhancing properties or if its acute intake simply promotes a relief of withdrawal symptoms (Childs and de Wit, 2006; Rogers et al., 2013). Studies from our group and others found that the acute administration of caffeine improves the performance of adult rodents in various learning and memory tasks (Angelucci et al., 1999; Costa et al., 2008a; Botton et al., 2010), whereas chronic caffeine administration prevents mnemonic deficits in experimental models of Alzheimer's disease as well as age-related cognitive decline (e.g. Prediger et al., 2005; Arendash et al., 2006; Dall'Igna et al., 2007; Costa et al., 2008b; Espinosa et al., 2013; Sallaberry et al., 2013).

Notably, the impact of caffeine consumption during adolescence remains poorly investigated (Porciúncula et al., 2013). Thus is of particular relevance since there has been a substantial increase in caffeine consumption among children and adolescents over the past four decades (Harnack et al., 1999). This phenomenon has been associated with a recent surge of energy drink sales (Heckman et al., 2010), the search for better cognitive performance among college students and shifts in the circadian rhythm (Taylor et al., 2011). Caffeine is the main active ingredient of energy drinks and many of them contain 70–80 mg per 8-oz serving (~3 times the concentration in cola drinks) (Reissig et al., 2009; Seifert et al., 2011) prompting the emergence of caffeine intoxication (Clauson et al., 2008). While moderate caffeine use is "generally recognized as safe" by the FDA, this classification is largely based on studies conducted in adults. In fact, healthy people can tolerate moderate ingestions of caffeine, but heavy caffeine consumption has been associated with serious adverse health effects (Reissig et al., 2009; Seifert et al.,

2011). However, the threshold dose for caffeine intoxication in adolescents is unknown.

This question is particularly pertinent since adolescence is classically defined in rodents as the critical final period of cerebral maturation (Arain et al., 2013) and recent studies have shown that caffeine negatively impacts on neuronal migration and wiring of brain circuits during early development (Silva et al., 2013). Thus, we now evaluated the safety and behavioral impact of caffeine intake during adolescence by testing the impact of three different doses of caffeine in order to mimic low, moderate and moderate/high consumption in humans (Fredholm et al., 1999). Furthermore, we also gauged the impact of caffeine consumption on the density of proteins associated with cognition and synaptic integrity, such as BDNF, SNAP-25, NeuN and GFAP.

EXPERIMENTAL PROCEDURES

Animals

According to previous studies, the time window from postnatal days (PND) 28–60 is considered as the prototypic period during which rats of most breeding stocks exhibit typical adolescent characteristics (Spear, 2000; Schneider, 2013). In this study, male Wistar rats (28-day-old) were maintained at five per cage under a 12-h light/dark cycle (lights on at 7:00 A.M.), constant temperature (22 ± 1 °C) and with free access to food and water beverages (see below). All experimental procedures were designed to minimize the number of animals used and their suffering and were approved by the Committee on the Ethics of Animal Experiments of the Federal University of Rio Grande do Sul (CEUA-UFRGS – Protocol number 20332).

Caffeine treatment

The animals received caffeine (0.1, 0.3 or 1.0 g/L) dissolved in tap water only during the dark cycle, which is their active period, to mimic the pattern of caffeine consumption in humans. During the light cycle, the rats received water *ad libitum*. The night treatment with caffeine was maintained throughout the behavioral tasks (carried out during the day) to avoid both the acute impact of caffeine as well as the effects of caffeine withdrawal, which develops over a period of 24–48 h (Finn and Holtzman, 1986; Johansson et al., 1993). Although we did not quantify the plasma levels of caffeine, the chosen treatment regimens are thought to correspond to a low, moderate and high caffeine intake in humans, with effects believed to be mainly operated through antagonism of adenosine receptors (Fredholm et al., 1999). Fig. 1 summarizes the timeline of treatments and all the subsequent tests and manipulations of the rats in this study.

Behavioral analysis

All behavioral tests were performed between 7:00 A.M. and 12:00 P.M. The behavioral analysis was recorded by using a computer-operated tracking system (Any-maze, Stoelting, Woods Dale, IL) and was ranked by two observers blinded to the treatments. Since our tracking video system can record simultaneously 4 animals, rats from different groups were analyzed in parallel to decrease the impact of shifts of their circadian rhythm.

Open field. Rats (PND 48 and 49) were exposed to an open-field arena during two days in order to evaluate locomotor activity and non-associative learning. The first day corresponded to training and the second day to the test session, with an interval of 24 h. The apparatus was made of black-painted Plexiglas measuring 50 × 50 cm and was surrounded by 50-cm-high walls. Each rat was placed in the center of the arena and the distance traveled in meters was recorded during 10 min. The experiments were conducted in a sound-attenuated room under low-intensity light (12 lux); activity was recorded with a video camera positioned above the arena and monitored in an adjacent room by an observer blinded to the treatment of the animals. The open-field apparatus was cleaned after the end of each session.

Novel object recognition task. The object recognition test was carried out 24 h after the test session (second day) in the open-field apparatus, as previously described by our group (e.g. Costa et al., 2008a,b; Botton et al., 2010). Rats (PND 50) first underwent a training session, in which two identical objects were placed near the two corners of one side of the chamber. Rats were placed individually into the open field facing the center of the opposite wall and allowed to explore the objects during 5 min. The test session was performed 90 min after training and two dissimilar objects were presented, a familiar one and a novel one (Dere et al., 2005; Bevins and Besheer, 2006). Exploration was defined by directing the nose to the object at a distance of at least 2 cm and/or touching the object with the nose or forepaws. Rearing onto the object was not considered as exploratory behavior. The discrimination ratio was defined as: TN/(TN + TF), [TN = time spent exploring the novel object; TF = time spent exploring the familiar object]. After the end of each session, we cleaned both the objects as well as open-field apparatus.

Elevated plus maze task. The plus maze apparatus allows a pharmacologically validated measure of anxiety in rodents (Pellow et al., 1985). It consists of two 50 × 10-cm^2 open arms, and two 50 × 10 × 50-cm^3 enclosed arms, which are elevated to 50 cm (height) with an open roof arranged in such a way that the two arms of each type are opposite to each other. Each rat was placed in the central square facing an open arm. The number of entries in each arm (when all four paws had entered the arm), and time spent in each arm were recorded for 5 min. The experiments were conducted with a low-intensity red light, 24 h after the object recognition task. The maze was thoroughly cleaned before testing another animal.