Behavioural Brain Research 303 (2016) 76–84

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Caffeine exposure during rat brain development causes memory impairment in a sex selective manner that is offset by caffeine consumption throughout life

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HIGHLIGHTS

- Behavior and synaptic proteins were modified by caffeine throughout life and during development.
- Adult female rats receiving caffeine during development displayed impaired recognition memory.
- Caffeine at highest dose throughout life attenuates anxiety-related behavior in both sexes.
- Caffeine treatments modified locomotion in a different manner according to sexes.
- Caffeine treatments change BDNF and related proteins in the hippocampus from both sexes.

ARTICLE INFO

Article history:
Received 4 November 2015
Received in revised form 7 January 2016
Accepted 9 January 2016
Available online 13 January 2016

Keywords:
Brain development
Memory
BDNF
Psychostimulant
Sex differences
Caffeine

ABSTRACT

Caffeine is the psychostimulant most consumed worldwide. In moderate doses, it affords a beneficial effect in adults and upon aging, but has a deleterious effect during brain development. We now tested if caffeine consumption by rats (0.1, 0.3, 1.0 g/L in the drinking water, only during active cycle and weekdays) during adulthood could revert the potentially negative effects of caffeine during early life. Thus, we compared caffeine intake starting 15 days before mating and lasting either up to weaning (development) or up to adulthood, on behavior and synaptic proteins in male and female rats. Recognition memory was impaired only in female rats receiving caffeine (0.3 and 1.0 g/L) during development, coincident with increased proBDNF and unchanged BDNF levels in the hippocampus. Caffeine in both treatment regimens caused hyperlocomotion only in male rats, whereas anxiety-related behavior was attenuated in both sexes by caffeine (1.0 g/L) throughout life. Both caffeine treatment regimens decreased GFAP (as an astrocyte marker) and SNAP-25 (as a nerve terminals marker) in the hippocampus from male rats. TrkB receptor was decreased in the hippocampus from both sexes and treatment regimens. These findings revealed that caffeine intake during a specific time window of brain development promotes sex-dependent behavioral outcomes related to modification in BDNF signaling. Furthermore, caffeine throughout life can overcome the deleterious effects of caffeine on recognition memory during brain development in female rats.

1. Introduction

Caffeine is the most widely consumed psychoactive drug worldwide, which acts through the antagonism of adenosine receptors to exert arousal effects [1]. A renewed interest in the central effects of caffeine emerged from observations that moderate doses of caffeine attenuate cognitive decline in aging [2–4], Alzheimer's disease [5–8] and neuropsychiatric disorders [9,10]. These beneficial effects

Abbreviations: BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; GFAP, glial fibrillar acid protein; SNAP-25, synaptosomal-associated protein 25.

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http://dx.doi.org/10.1016/j.bbr.2016.01.026
0166-4328/

of caffeine in adults have been associated with a normalization of synaptic activity by antagonism of adenosine A_{2A} receptors [11] and more recently with a control of astrocytic function [12,13]. Caffeine also exerts impact on the BDNF neuromodulation system in adulthood [2,4,14,15] and during brain development [16].

Although caffeine consumption affords general health benefits in the adult population, the safety of caffeine consumption during pregnancy and childhood remains controversial [17,18]. In animal studies, caffeine inhibited adult neurogenesis [19], altered the proliferation of neuronal precursors [20], promoted defective neural tube closures in chick and mouse embryos [21,22] and delayed GABAergic neurons migration [23]. These deleterious effects of caffeine during early brain development seem to trigger long-lasting deficits of brain function; thus, adult rodents that received caffeine during gestation and lactation through their dams, display reduced locomotor activity [24–26], anxiogenic-like profile [27] and impaired performance in learning and memory tasks [23,28,29].

Since caffeine has opposite effects on brain function during brain development (deleterious) and later in life (beneficial), we now tested if caffeine intake throughout life might offset the deleterious effects of caffeine during brain development. Furthermore, since sex differences have previously been reported for caffeine neuroprotection [8,30,31], we also probed for possible sex differences in the effects of caffeine during brain development and later in life.

2. Materials and methods

2.1. Animals

Female Wistar rats, 60–70 days of age, were mated within our colony at Federal University of Rio Grande do Sul. Animals from different ages and both sexes were maintained under 12 h light-dark-cycle (lights on at 7:00 AM), at constant temperature ($22 \pm 1\,^{\circ}C$) and with free access to food, water or caffeinated solution. All experimental procedures were designed to minimize the number of animals used and their suffering and were approved by the Committee on Ethics of Animal Experiments of the Federal University of Rio Grande do Sul (CEUA-UFRGS—Protocol number 20332).

2.2. Caffeine treatment

In order to mimic the pattern of caffeine intake by humans, caffeine (0.1, 0.3 or 1.0 g/L) was administered in the drinking water only during the dark cycle (lights off at 7:00 PM) and only on weekdays. The doses regimen corresponds to low, moderate and high caffeine intake in humans, which low and moderate intake acting selectively on adenosine receptors [1]. The treatment started 15 days before mating and lasted throughout the pregnancy cycle. Tap water was available during the light cycle (lights on at 7:00 AM). After birth, pups received caffeine or tap water from dams throughout the lactation period. Pups were weaned at postnatal day 21 (PND 21) and 3–4 rats were maintained per cage so that rats of the same litter and sex could be housed together. At PND 21, litters were separated by sex and divided into three groups: a pups that received only tap water; b pups that received caffeine up to weaning (development); c pups that received caffeine up to adulthood (throughout life). The timeline summarizes the schedule of administration and the subsequent behavioral and neurochemical analysis (Fig. 1).

2.3. Caffeine measurements

Three to four animals were separated and killed by rapid cervical dislocation and blood samples were collected as follows: a at the morning when replacing water by caffeine (7:00 AM); b at night when shifting to water again (7:00 PM). Samples were centrifuged at $4000 \times g$ for 10 min and the organic layer was lyophilized. Plasma levels of caffeine were analyzed using high-pressure liquid chromatography (HPLC). The chromatographic separation was achieved on a C18 Kinetex® ODS column (4.6 mm × 25 cm i.d., particle size 5 μm), UV detection at 280 nm. The quantification was achieved using a calibration curve and the internal standard was used to estimate the recovery rate of the extraction procedure.

2.4. Behavioral analysis

All behavioral tests were conducted in a sound-attenuated room under low-intensity light (12 lux) and recorded by means of a computer-operated tracking system (Any-maze, Stoelting, Woods Dale, IL) and ranked by two observers blind to the treatments. All procedures were carried out during the light cycle (7:00 to 12:00 AM).

2.5. Open field

The open field exposure was performed as previously described [7,14] in an apparatus made of black-painted Plexiglas, measuring 50 × 50 cm, 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.

2.6. 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 [7,14]. Rats first underwent a training session, in which two identical objects were placed near the two corners at either end 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 for 5 min. The test session was performed 90 min after training and two dissimilar objects were presented, a familiar and a novel one [32,33]. The exploration was defined by directing the nose to the object at a distance of less than 2 cm and/or touching the object with the nose or forepaws. Rearing on to object was not considered exploratory behavior. The discrimination ratio was defined as: TN/(TN + TF), [TN = time spent exploring the novel object; TF = time spent exploring familiar object].

2.7. Elevated plus maze

The elevated plus maze, a pharmacologically validated apparatus for the measurement of anxiety in rodents [34,35], was carried out as previously described [18], 24 h after the object recognition task. The maze consists of two $50 \times 10\,cm^2$ open arms, and two $50 \times 10 \times 50\,cm^3$ enclosed arms, which were elevated at 50 cm (height) with an open roof arranged in such a way that the two arms of each type were 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 the time spent in each arm were recorded for 5 min.

2.8. Western blot

Twenty-four hours after the end of behavioral tests, rats were sacrificed under anesthesia. The hippocampi were dissected out and immediately homogenized in a 5% SDS solution containing a protease and phosphatase inhibitor cocktail (Sigma, São Paulo/SP,