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Caffeine and sleep-deprivation mediated changes in open-field behaviours, stress response and antioxidant status in mice☆

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ABSTRACT

Objectives: Effects of daily caffeine consumption on open-field behaviours, serum corticosterone and brain antioxidant levels were investigated after six hours of total sleep-deprivation in prepubertal mice. We tested the hypothesis that daily caffeine consumption may significantly alter behaviour, stress and antioxidative response of prepubertal mice to an acute episode of total sleep-deprivation.

Methods: Prepubertal Swiss mice of both sexes were assigned to two main groups of 120 each (subdivided into 6 groups of 10 each, based on sex), and administered vehicle or graded oral doses of caffeine (10, 20, 40, 80 and 120 mg/kg/day) for 14 days. On day 14, a main group was subjected to 6 h of total sleep-deprivation by 'gentle-handling'. Open-field behaviours were then assessed in both groups, after which animals were euthanized, and levels of corticosterone, superoxide dismutase and glutathione peroxidase assayed.

Results: Horizontal locomotion, rearing and grooming increased significantly, compared to control, with sleep-deprived (SD) mice showing stronger caffeine-driven responses at higher doses; and SD female mice showing sustained response to caffeine, compared to respective males. Plasma corticosterone increased with increasing doses of caffeine in both non sleep-deprived (NSD) and SD mice; although SD mice had higher corticosterone levels. Sleep-deprivation and/or higher doses of caffeine were associated with derangements in brain antioxidant levels.

Conclusion: Repeated caffeine consumption and/or acute sleep-deprivation led to significant changes in pattern of open-field behaviour and stress/antioxidant response in mice. Responses seen in the study are probably due to modulatory effects of caffeine on the total body response to stressful stimuli.

1. Introduction

Caffeine is a central nervous system stimulator that belongs to the class of molecules known as xanthines [1,2]. Caffeine is found in tea, coffee, mate, guarana paste and kola nuts, and it is consumed globally, irrespective of age or social status [3]. In humans, studies continue to shed light on the behavioural effects of caffeine [4,5], especially in adults; however, there is also a noticeable increase in the consumption of caffeine and caffeine-containing products by children and adolescents [6–9]. Studies have also been conducted to evaluate caffeine safety in the young [6,7], and to determine dose/consumer-dependent influences of caffeine use on the heart, blood-pressure and general body physiology in children [8,9]; however, in comparison to adults, data on caffeine research in the young is still less available.

A major reason for deliberate caffeine consumption is to combat sleepiness. Sleepiness is defined as difficulty in maintaining alertness during the major wake period of the day, resulting in unintended lapses into drowsiness or sleep [10]. Sleepiness is a known consequence of sleep-deprivation in healthy humans; however, the quantity of sleep needed by children, adolescents and young adults is still debated [11,12]. Recent literature reveal that about 7–9 h of sleep is adequate in young adults (18–25), 8–10 h in teenagers and 9–11 h in school aged children (6–13) [13]. It is also reported that sleep-deficits are higher in adolescents compared to other age-groups, with females showing greater need for sleep than males [14]. A number of human studies have reported no difference in total sleep time all through the

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adolescence period on non-school days, and significant loss of total sleep time only during school days [15,16], suggesting psychosocial factors as a sleep-time determinant in adolescents [17]. Sleep loss, and poor quality of sleep have been associated with alterations in emotional behaviour and decreased quality of life in young adults [18], and now studies are beginning to show evidence of increasing caffeine consumption in adolescents, to help cope with sleep loss [19]. Therefore, in this group, cycles of caffeine consumption and insufficient sleep tend to succeed each other.

The existence of sex differences in the response to caffeine in young subjects have been studied in humans [8,20] and rodents [21–23], with different conclusions. Soellner et al. [24] reported improved object-discrimination in female rats, following caffeine ingestion. Elkins et al. [20], looking at effects of caffeine in prepubertal boys, concluded that memory-impairment associated with caffeine in males was due to increased vigilance and decreased reaction-time with caffeine at lower doses. Fischer and Guillet [25] reported sex-differences in caffeine effect on memory retention in neonatal rats; however Temple et al., [8] reported no sex-differences in cardiovascular response to caffeine in adolescent boys or girls. The differences in the results of these studies would suggest that in the outcomes of tasks involving caffeine administration, the influences of sex are probably task-specific. Sex-differences in drug self-administration was ruled out in the present study, by giving caffeine through gavage; since studies have reported that female rodents are more vulnerable to stimulant self administration [26,27] or drug seeking behaviour [28].

There is a dearth of information on the influence of sex on effects of caffeine on open- field behaviours in rodents or motor activity in humans; and where available, results of studies vary. Uzbay et al. [23] reported the absence of sex-differences with caffeine administration in mice, although a sex-differential response was seen with agmatine on caffeine-induced locomotor response. In an earlier study [21], we reported that male mice (more than females) showed an early locomotor response, although females showed higher basal levels of locomotion. However, the present study differs from our previous study in a few areas: age of animals used, doses of caffeine used and presence of sleep-deprivation.

Sleep-deprivation by gentle-handling is a stressor, and has been shown to be associated with elevated corticosterone [29] and alterations in antioxidant activity [30,31]. Oxidative stress occurs due to an imbalance between the production of oxidants and the strength of antioxidant defences. These imbalances can cause structural changes due to oxidation of proteins, lipids, and nucleic acids [32]. A number of studies have reported evidence of oxidative stress following acute or chronic sleep-deprivation [31–34]. An overall effect of sleep-deprivation on the body is a general sympathetic activation.

The rationale for this study was the need to answer the question: can a history of caffeine consumption affect behavioural phenotypes, stress and antioxidant response, after a single episode of total sleep deprivation? Therefore, we tested the hypothesis that background caffeine consumption may significantly alter novelty-induced behaviours, plasma corticosterone levels, superoxide dismutase and glutathione peroxidase activity in prepubertal mice (with or without sleep deprivation); and that these parameters could be influenced by sex.

2. Methods

2.1. Drugs

99.9% anhydrous caffeine (E. Merck, Darmstadt, Germany) was weighed and dissolved in distilled water to get the desired concentrations. Caffeine (10, 20, 40, 80 and 120 mg/kg) was administered orally by using a cannula. The selection of these doses was based on a previous study [22,35], although cognizance was also taken of the LD_{50} (oral) of caffeine in mice which is 127 mg/kg (male) and 137 mg/kg (female) [36].

2.2. Animals

Five-week old Swiss mice (Empire Breeders, Osogbo, Osun State, Nigeria) weighing 10–15 g at the commencement of study were used. Male and female mice were housed separately in plastic cages measuring 16×12×10 in. (10 mice in each cage). General housing is a temperature-controlled (22.5 °C ± 2.5 °C) quarters with 12 h of light. Mice had free access to food and water except during the behavioural test. All animals were fed commercial standard chow (Calories: 29% protein, 13% fat, 58% carbohydrate) from weaning. All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

2.3. Experimental methods

Two main groups comprising one hundred and twenty mice each were used (i.e. 120 males and 120 females). Each main group was further subdivided into: Sleep deprived (SD) or non sleep-deprived (NSD) of 60 each. Each subdivision has six sub-groups of 10 animals each (Table 1); which received either vehicle (distilled water) or one of five doses of caffeine (10, 20, 40, 80 and 120 mg/kg/day) for a period of 14 days. Tests were carried out after the last dose of caffeine or vehicle in the NSD groups. Mice in the sleep-deprived groups (SD) were subjected to six hours of gentle-handling after the last dose of vehicle or caffeine, and then exposed to the behavioural tests. Immediately after the behavioural tests, animals were euthanized and blood taken via cardiac puncture for estimation of plasma corticosterone, while whole brain homogenates were used for estimation of antioxidant levels.

2.3.1. Sleep-deprivation

Gentle-handling as a method of sleep-deprivation was first used in feline and rodents [37]. It has also been validated for use as a method of total sleep-deprivation in mice [38]. It is a widely accepted as a way to keep mice awake for periods of hours, while minimally disturbing ongoing activity. Gentle-handling was the method of total sleep-deprivation employed in this study. The gentle-handling protocol used lasted for 6 h, starting at 7 a.m. It involved cage-shaking, touching the animals with the hand or a soft brush, introducing novel objects into the cage and cage-tapping.

2.3.2. Behavioural test

Behavioural tests were conducted in a quiet room between the hours of 1 p.m and 3 p.m on day 14 of administration (5 animals/day). On the test days, mice were transported in their home cages to the testing room, and allowed to acclimatise for 30 min before testing. At the beginning of the test, each mouse was placed in the open-field box and its behaviour videotaped for subsequent analysis. After testing, the mouse was removed from the maze and all interior surfaces of the open-field box were cleaned thoroughly with 70% ethanol, and then wiped dry to remove any trace of odour.

Table 1
Experimental groups, time-line and treatments administered.

	Day 1–14	Day 14	
Group/Treatment	DW or Caffeine	SD(6 h) or NSD	Open-field (30 min)
Vehicle	+	+	+
10 mg/kg	+	+	+
20 mg/kg	+	+	+
40 mg/kg	+	+	+
80 mg/kg	+	+	+
120 mg/kg	+	+	+

Number of animals/group=10, Total number of animals/main group=120 (60 NSD, 60 SD), NSD: non sleep-deprived, SD: sleep-deprived, DW: distilled water