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장단기 고용량 카페인 투여가 청소년기 동물모델의 행동에 미치는 영향

박종민 · 김윤주 · 김하은 · 김연정
경희대학교 간호과학대학

Influence of Short- and Long-term High-dose Caffeine Administration on Behavior in an Animal Model of Adolescence

Park, Jong-Min · Kim, Yoonju · Kim, Haeun · Kim, Youn-Jung
College of Nursing Science, Kyung Hee University, Seoul, Korea

Purpose: Caffeine is the most widely consumed psychostimulant of the methylxanthine class. Among adolescents, high-dose of caffeine consumption has increased rapidly over the last few decades due to the introduction of energy drinks. However, little is known about the time-dependent effect of high doses of caffeine consumption in adolescents. The present study aims to examine the short- and long-term influence of high-dose caffeine on behavior of adolescence. **Methods:** The animals were divided into three groups: a "vehicle" group, which was injected with 1 ml of phosphate-buffered saline for 14 days; a "Day 1" group, which was injected with caffeine (30 mg/kg), 2 h before the behavioral tests; and a "Day 14" group, which was infused with caffeine for 14 days. An open-field test, a Y-maze test, and a passive avoidance test were conducted to assess the rats'activity levels, anxiety, and cognitive function. **Results:** High-dose caffeine had similar effects in short-and long-term treatment groups. It increased the level of locomotor activity and anxiety-like behavior, as evidenced by the increase in the number of movements and incidences of rearing and grooming in the caffeine-treated groups. No significant differences were observed between the groups in the Y-maze test. However, in the passive avoidance test, the escape latency in the caffeine-treated group was decreased significantly, indicating impaired memory acquisition. **Conclusion:** These results indicate that high-dose caffeine in adolescents may increase locomotor activity and anxiety-like behavior and impair learning and memory, irrespective of the duration of administration. The findings will be valuable for both evidence-based education and clinical practice.

Key Words: *Caffeine; Adolescent; Locomotion; Anxiety; Memory*
국문주요어: 카페인, 청소년, 활동, 불안, 기억

Introduction

Caffeine is an alkaloid, identified structurally as methylxanthine, which is found in drinks such as coffee and tea[1]. It is a widely used and easily accessible psychostimulant that stimulates the autonomic nervous system and acts as a nonselective adenosine receptor antagonist that

Corresponding author: **Kim, Youn-Jung**
College of Nuing Science, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul, 02447, Korea
Tel: +82-2-961-0311 Fax: +82-2-961-9398 E-mail: yj129@khu.ac.kr

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maintains arousal, boosts attention, and enhances exercise ability [2]. It can be used in primary treatment for apnea of prematurity [3]. In addition, chronic caffeine administration halts age-related cognitive decline in middle-aged rats. Caffeine also prevents Alzheimer's disease by reducing the production of β-amyloid [4,5]. However, excessive intake of caffeine can produce syndromes such as "caffeinism," where subjects experience symptoms and behavioral changes that include nervousness, anxiety, insomnia, headaches, and palpitations [6]. These adverse effects can be more significant in adolescents than in adults [6].

Adolescence is a key period of major physiological, psychological, and social development in the brain. Although there is no change in the total volume of the brain during this period, the prefrontal cortex, the limbic system, and white matter all mature at this time [7]. The mean amount of caffeine consumption did not increase among children and adolescents from 1999 to 2010, however, there has been a surge in the sale of energy drinks since 2009 as soda intake decreases, and these drinks contain a large proportion of caffeine[8]. Due to the introduction of energy drinks, children and adolescents were exposed to higher caffeinated beverages.

One can of energy drink contains between 70 and 200 mg caffeine. The recommended daily caffeine intake that does not produce an adverse effect is 100 mg or 2.5 mg/kg per day for both children and adolescents[9]. Consuming one or two cans of energy drinks exceeds the recommended daily dose. Increased consumption of energy drinks has been associated with the exploration of better cognitive performance in college students through altering their circadian rhythms [10] and improving the speed and accuracy of their working and episodic memory [11]. Conversely, according to a recent report from the American Academy of Pediatrics, energy drinks have a potentially harmful effect on children [12]. In developmental stages, high-dose caffeine can have an adverse effect on neuronal migration and the wiring of brain circuits [13]. There has been little research on high-dose caffeine consumption in adolescence [14]. Moreover, most studies have not focused on the short- and long-term effects of high-dose caffeine consumption on adolescents.

The present study aims to examine the short- and long-term influence of high-dose caffeine on locomotor activity, anxiety-like behavior, and cognitive function of adolescence.

Methods

Animals

Male Sprague-Dawley rats (Daehan bio, Chungcheongbuk-do, Republic of Korea) weighing 80 ± 10 g (postnatal day 21) were assigned to three different groups by a nonparticipating researcher. Animals were housed under conditions of controlled temperature (25°C ± 2°C), humidity (50%–55%), and light (12-hour light–dark cycles), with access to food and water ad libitum. In the laboratory experimental design, the animals were divided at random into three groups: vehicle (n = 8), Day 1 (n = 8), and Day 14 (n = 8). This study was approved by the K University Animal Care and Use Committee. All the experimental procedures were performed in accordance with the animal care guidelines of the National Institute of Health and the Korean Academy of Medical Sciences.

Drug

Moderate to high doses of caffeine (approximately 100–400 mg, 2.5–10 mg/kg/day) are known to produce symptoms of nervousness, jitteriness, and fidgetiness in children and adolescents [15]. However, experiments using an animal model should consider the pharmacokinetic differences between animals and humans. In our study, the dose we administered was higher than what one would administer to a human because the half-life of caffeine in the human body is approximately 2.5–4.5 hr before it is decomposed by enzymes within the liver, whereas the half-life of caffeine in rats is known to be 0.7–1.2 hr [16]. The most active and damaged cognitive function was observed in rats that received 30 mg/kg of caffeine via intraperitoneal injection [17,18]. Pharmacokinetic studies of subjects who consumed caffeine either through oral ingestion or intravenous administration have confirmed that similar blood levels of caffeine are present in both cases [19]. So, we administered the 30 mg/kg of caffeine (Enzo Life Sciences, Inc., Farmingdale, NY, USA) via intraperitoneal injection at the same time (14:00). The vehicle group was injected with 1 ml of phosphate-buffered saline (PBS). In the other groups—Day 1 and Day 14— we administered the caffeine (30 mg/kg) dissolved in 1 ml of PBS over different periods of time, depending on the group, 2 h prior to the behavioral test. Caffeine administration for 13 days in rats was regarded as equivalent to one year in humans [20]. Thus, a period of 14 days can be interpreted as long-term administration [21,22]. To prevent the rats from experiencing caffeine withdrawal symptoms, we administered the caffeine until the final day of the experi-

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ment. We carried out three kinds of behavioral tests over 2 days: an open-field test (OFT), a Y-maze test, and a passive avoidance test. There were 3 h intervals between the behavior tests for rest and to allow for adaptation.

Open field test

The OFT was employed to assess the rats' sensorimotor function, including the general activity level and the gross locomotor level. In rodents, the activity of grooming is particularly sensitive to various kinds of stress and stimulation; consequently, we used it to represent the animals' anxiety level [23]. To observe the mobility of the rats, a wooden box (77×77×25 cm) was divided into 16 equal squares delineated by lines. All the animals were placed in the experimental apparatus and given 1 h to adapt to their environment. A movement was recorded every time all four paws of a rat occupied a new square; the incidences of rearing and grooming for a period of 5 min were also recorded. The observer recording the data was blind to the groups and random order. After each trial, the equipment was cleaned with an ethanol solution to erase scent clues that could affect the results.

Y-maze test

Spontaneous alternation behavior was evaluated in the Y-maze utilizing the rats' natural curiosity about new environments to assess the performance of the animals' spatial working memory. The apparatus was made from wood formed into a Y shape, with three arms, designated as A, B, and C. Each arm measured 5×25×14 cm, and the fixed angle between the arms was 120°. All the rats were placed in the experimental apparatus and were allowed 1 h to adapt to the environment. Initially, the animals were placed in the C arm. Each time an animal moved all four paws into another arm for 5 min, the name of the new arm was recorded. Alternation was calculated as one point, when the animals followed a sequence of moving into different consecutive arms, for example, A→B→C or B→A→C. If an animal's spatial working memory was intact, it would enter new arms, driven by its nature to explore novel environments. The scoring formula was as follows:

Percentage of alternation (%) = (alternation/total number of arm entries - 2)×100

Passive avoidance test

The passive avoidance test is used to evaluate the fear-motivated learning and memory of rats based on their habituation of liking dark places over light places. This test used the automatic system device (Gemini, San Diego Instruments, USA). The apparatus, which measured 66×33×43 cm, was made of acryl and aluminum and consisted of two compartments, a light chamber and a dark chamber, connected via a sliding door that closed automatically when a rat moved from one chamber to another. The light chamber was an environment disliked by rats, whereas the dark chamber served as an avoidance chamber. Exactly 3 h before the test, the rats were moved into a lightless testing room to be habituated. The test was conducted over 2 days and consisted of two sessions. On the first day, in the training session, the rats were placed in the light chamber, which was then illuminated, prompting the rats to enter the other chamber. As soon as a rat entered the dark chamber, the door was closed, and an electric shock of 0.3 mA was administered for 2 s through the floor. On the second day, the test ended when the rat moved into the dark chamber from the light chamber, and no electric shock was administered. The latency time was when the rat moved into the dark chamber from the light chamber. The maximum latency time was set at 300 sec. If a rat went over this limit, the session was finished immediately. If the rat's learning and memory were intact, it remained in the light chamber longer, rather than entering the dark chamber, because it could remember the electric shock from the training session.

Statistical analysis

All the data was expressed as a standard error of the mean and was evaluated for normal distributions via the Shapiro-Wilk test. As the variables did not follow normal distribution, the Kruskal-Wallis test was used to obtain overall group comparisons. In addition, Mann-Whitney U tests were used as post-hoc tests to compare two groups at a time. Results which had normality were performed by one-way analysis of variance test, followed by Tukey's post-hoc test. All analyses were conducted using SPSS Ver. 23.0 (IBM-SPSS Inc., Chicago, Il, USA). The standard of $p<0.05$ was used as the criterion for statistical significance.

Results

Body weight

To study the effect of high-dose caffeine on body weight in adolescents, we measure body weight on the postnatal days (PNDs) 28, 35, and 42 (Fig. 1). There was no significant difference between the groups.

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Locomotor activity

We counted the number of movements and the incidence of rearing to determine how high-dose caffeine affects general activities. In the Day 1 and Day 14 groups, the number of movements increased significantly as compared to the number counted in the vehicle group ($p < .001$; Fig. 2A). In both the Day 1 and Day 14 groups, the incidence of rearing also increased significantly as compared to the incidence observed in the vehicle group ($p < .05$; Fig. 2B). These results showed that the locomotor activity was affected by high-dose caffeine and not the duration of administration.

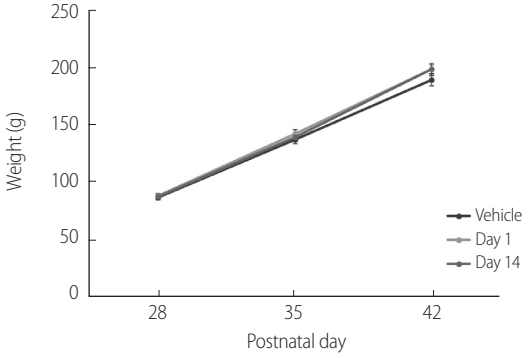

Figure 1. Body weight curve from PND 28 to PND 42. Data are expressed as mean ± S.E. PND = Postnatal day; S.E., standard errors

Anxiety-like behavior

In both the Day 1 and Day 14 groups, the incidence of grooming increased significantly as compared to the number counted in the vehicle group ($p = .028$; Fig. 2C). The rats treated with caffeine exhibited more

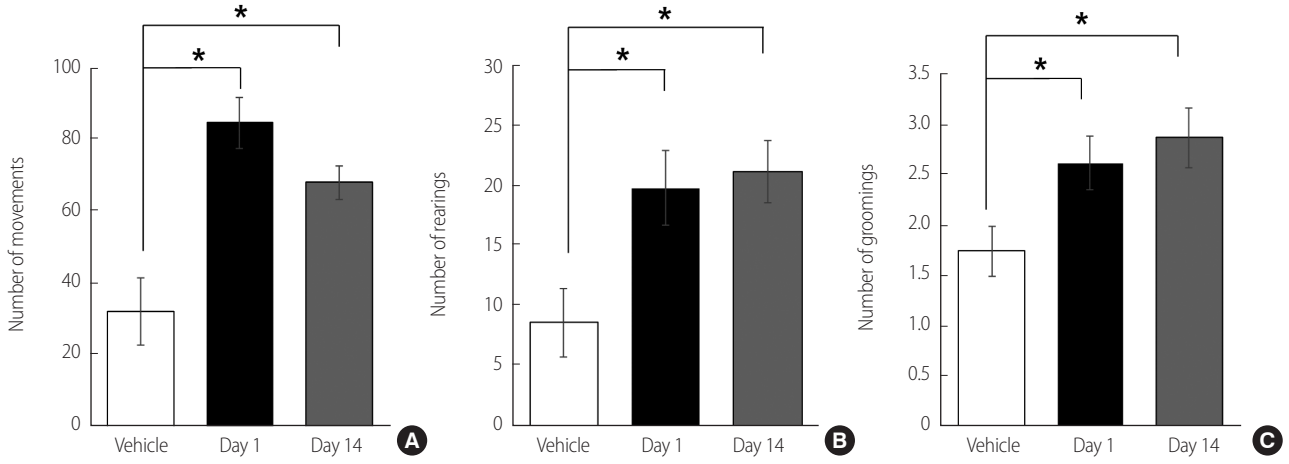

Figure 2. Locomotor activities and anxiety-like behavior in the open field test. Number of (A) movements, (B) climbing and rearing behaviors, and (C) grooming events in the open field maze. Data are expressed as mean ± S.E. (*$p < .05$ compare to the vehicle group). S.E., standard errors

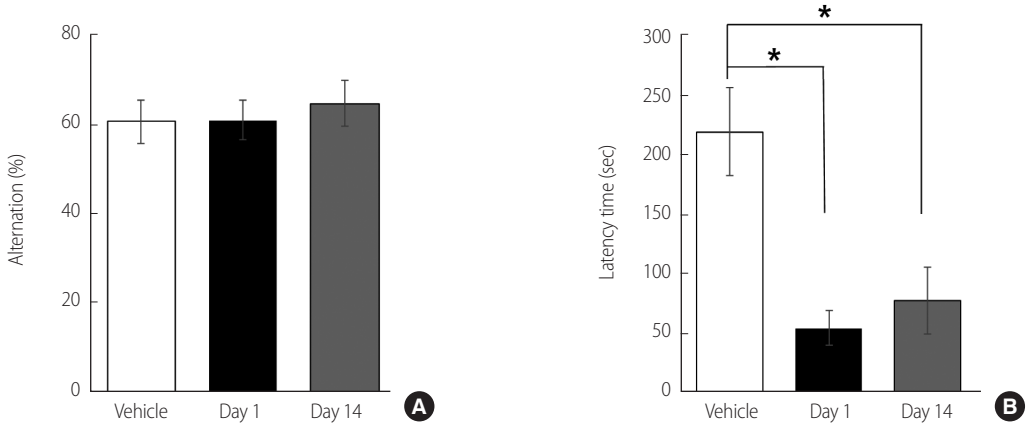

Figure 3. Memory function tests. (A) Evaluation of spatial working memory in the Y-maze test and (B) fear-motivated learning and memory in the passive avoidance test. Data are expressed as mean ± S.E. S.E., standard errors

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frequent self-grooming, which indicates anxiety-related behavior.

Memory performance

There was no significant difference between the groups in the Y-maze test, as indicated by the statistics of the vehicle group, Day 1 group, and Day 14 group (p = .802) in Fig. 3A. These results suggest that high-dose caffeine had no effect on spatial working memory, which is a type of short-term memory. However, in both Day 1 and Day 14 groups, the level of escape latency in the passive avoidance test decreased significantly as compared to that observed in the vehicle group (p = .014; Fig. 3B). The learning and memory function was also only influenced by high-dose caffeine.

Discussion

The purpose of this study was to investigate the effect of high-dose caffeine and the duration of its administration on behavior in adolescent animal models, because frequent high caffeine intake in adolescents is an emerging social problem. In all study groups, a high dose of caffeine had no effect on the animals' body weight, which increased steadily. The findings of studies in which caffeine was dissolved in water (0.1, 0.3, 1 g/ml) and administrated through oral ingestion [2,18] were consistent with the results of our research.

In the current study, high-dose caffeine had an adverse effect on various aspects of the animals' behavior. Interestingly, both short-term (Day 1) and long-term (Day 14) high-dose caffeine treatment exhibited similar effects on behavior in adolescent rats. According to these results, a single intake of high-dose caffeine in adolescents might have a negative effect on behavior. Both locomotor activity and the incidence of rearing increased in all animal groups that received a high dose of caffeine. In both adolescent and adult rats, consumption of low doses of caffeine up to 30 mg/kg, increased daily activities[24]. However, adolescence is a key period of brain development, and locomotor activity during that stage will be different from that during adulthood. The behavioral changes induced by high-dose caffeine in adolescents are greater than those in adults [2, 25].

The groups treated with a high dose of caffeine also exhibited increased anxiety-like behavior. Previous studies have validated the view that rearing is related to exploratory behavior, and an increased incidence of grooming indicates an anxiogenic effect [23,26]. Consistent with our findings, adult rats that drank caffeine dissolved in water (0.3 g/ml) for 28 days exhibited anxiety-related behavior in the OFT, social interaction test, and elevated plus-maze test [25].

In adolescents, a high dose of caffeine affects brain development, especially in relation to learning and memory. Thus, this phenomenon is worth further attention and study. Caffeine had no effect on the working memory in one study [27], but working memory errors and reference errors increased in the radial arm maze task of another study [28]. Our results did not reveal any change in spatial working memory in all groups. However, in the caffeine-treated group, there was decreased escape latency in the passive avoidance test, which indicates a degree of learning and memory impairment due to failed acquisition of memory. Memory is widely known to include acquisition, consolidation, and retrieval [29]. The results of our study suggest that caffeine may affect memory acquisition. In the passive avoidance test, high doses of caffeine (80 mg/kg/day) increased latency in reentering the dark compartment. Similarly, neonates (PND 2–6) treated with caffeine showed the same result in the step-through avoidance task at PND 35–37 [30]. However, few studies have elucidated the mechanism by which caffeine affects the memory process. Furthermore, few previous studies have focused on youth. Therefore, we recommend that further studies be undertaken to confirm the effect of caffeine on learning and memory in adolescents.

This study involves a long-term administration of high-dose caffeine, which is thought to be appropriate for animal study as it may have adverse effects on humans. An animal study also has the advantage of being able to block exogenous variables, observe the behavioral changes directly, and to generalize with a small number of groups. However, it is difficult to apply this to humans with only one study as the subjects of this study were rats. We expect more research to be conducted using various methodologies on adolescence. In addition, further research will be needed to clarify the underlying mechanism of the influence of caffeine on behavior at a preclinical level.

Conclusion

In summary, we have demonstrated that high doses of caffeine in adolescents increase locomotor activity and anxiety-like behavior and reduce functions of learning and memory, irrespective of the length of time of administration. Despite the fact that nurses are the first line of patient care and are responsible for health promotion and prevention of disease, most studies are currently performed only in the food and nu-

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trition field. Therefore, we should strive to understand the influence of high-dose caffeine and prevent adolescents from being exposed to high-caffeine beverages, such as energy drinks. This goal can only be accomplished by educating adolescents about the harmful effects of caffeine. We are hopeful that the present study will be valuable for making evidence-based practices, identified through the use of animal models, for preventive and educational nursing interventions.

CONFLICT OF INTEREST

The authors declared no conflict of interest

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