European Journal of Pharmacology 660 (2011) 363–367

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Behavioural Pharmacology

Comparison of caffeine-induced locomotor activity between adolescent and adult rats

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ARTICLE INFO

Article history:
Received 29 October 2010
Received in revised form 8 March 2011
Accepted 28 March 2011
Available online 9 April 2011

Keywords:
Caffeine
Adolescence
Locomotor activity
Psychostimulant

ABSTRACT

Caffeine is the psychostimulant drug most consumed in the world. This drug is present in food, beverages and medicines marketed for individuals of all ages. In spite of this, caffeine effects on adolescents are poorly understood. The aim of this study was to evaluate the differences on caffeine-induced locomotor stimulant or depressant effects in adolescent and adult rats. Adolescent (37–40 days old) or adult (70–74 days old) Wistar rats were tested for stimulant and depressant caffeine effects in two different experiments. The first was designed to evaluate the locomotor effect of caffeine in habituated rats. To this end, rats were previously habituated to test environment and had their locomotor activity registered following i.p. injections of vehicle or caffeine (3, 10, 30, 60 or 120 mg/kg). In the second experiment adolescent or adult rats were not habituated to the test environment and their locomotor activity was registered following i.p. injections of vehicle or caffeine (30, 60 or 120 mg/kg). In both experiments caffeine-induced a biphasic effect, with stimulation in small to moderate drug doses and no effect or locomotor depression in higher caffeine doses. Moreover, caffeine-induced locomotor stimulation was higher in adolescent than adult rats. Also, locomotor depression was only revealed in adult rats non-habituated to the test environment. These results suggest that adult and adolescent respond differently to caffeine indicating the need of more studies on the effects of caffeine in animals' models of adolescence.

1. Introduction

Caffeine is the most widely consumed psychostimulant drug in the world. Nearly all caffeine comes from dietary sources, such as beverages and food (Fredholm et al., 1999) but it is also present in supplements to increase sports performance and as a component of many combination medications marketed for the relief of headache symptoms (Shapiro, 2007; Burke, 2008). Caffeine containing drinks are consumed regularly since childhood (Temple, 2009) and children and adolescents are the fastest growing population of caffeine users with an increase of 70% in the past 30 years in the USA (Harnack et al., 1999). In adolescents, high caffeine consumption, defined as four or more caffeinated beverages per day, was associated with daily cigarette use, aggressive behavior and social problems (Martin et al., 2008). Despite the fact that caffeine is widely consumed by children and adolescents, the majority of pre-clinical studies concerning its effects is performed in adult animals.

Adolescence is a period of ontogeny when individuals exhibit age specific behavioral characteristics, such as risk taking and novelty seeking, which could predispose them to initiate drug use (Spear, 2000). Brain pathways that play a key role in reward and motor effects of psychostimulant drugs undergo maturational changes during this transitional period (Casey et al., 2008; Crews et al., 2007). It has been reported that adolescent rodents are hyposensitive to the effect of psychostimulant drugs such as amphetamine (Bolanos et al., 1998) and cocaine (Laviola et al., 1995; Marin and Planeta, 2004). Nevertheless, the literature is scarce in studies about caffeine effects during adolescence.

Caffeine effects are dose-dependent. In humans lower caffeine doses produce more favorable subjective effects than the higher doses, whereas unpleasant effects are more common at higher doses (Fredholm et al., 1999; Kaplan et al., 1997). In rats, there is a markedly biphasic effect of caffeine on locomotion. Small to moderate caffeine doses increase locomotor behavior, while higher doses tend to decrease it (Fisone et al., 2004; Garrett and Holtzman, 1994; Halldner et al., 2004). Also, the locomotor decreasing effect of higher caffeine doses is more evident when the animals are not habituated to the test environment (El Yacoubi et al., 2000a).

Given that the behavioral effects of caffeine are poorly investigated in adolescents, the aim of our study was to examine the locomotor

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doi:10.1016/j.ejphar.2011.03.052

effects of caffeine in this age period and compare it with adults. To this end, adolescent and adult rats were injected with a wide range of caffeine doses and their locomotor activity was measured in an activity box.

2. Materials and methods

2.1. Subjects

Male Wistar rats were obtained from the animal breeding facility of the Univ. Estadual Paulista — UNESP (Botucatu-SP, Brazil) just after weaning, on postnatal day (PND) 21. They were housed until the ages of behavioral tests in groups of 3–5 animals in a room maintained at 23±2 °C and a 12:12 h light/dark cycle (lights on at 7:00) with free access to food and water.

Adolescence was defined, according to Spear (2000), as the age period between PND 28–42, during which behavior discontinuities from younger to older (PND 60 forward) rats are evident and a time when growth spurt and neuronal changes mainly occur.

Body weight was 149±5 for adolescent rats and 289±10 for adults. All groups were tested in the same times during the light phase (between 9:00 and 17:00).

The experimental procedures were approved by the Ethical Committee for Use of Human or Animal Subjects of the School of Pharmaceutical Science/UNESP and the experiments were conducted according to ethics principles of the Brazilian College of Animals' Experimentation — (COBEA), in compliance with NIH Guide for Care and Use of Laboratory Animals.

2.2. Apparatus and drug

Behavioral testing was conducted in a Plexiglas activity-monitoring chamber (Columbus Instruments, Columbus, OH, USA). This chamber, measuring 44 (width)×44 (length)×20 (height) cm, includes 10 pairs of infrared photocells, which were used to measure the horizontal locomotor activity. The consecutive interruption of two beams was recorded as one unit of locomotor activity.

Caffeine anhydrous (Purifarma, São Paulo-SP, Brazil) was diluted in NaCl 0.9% and intraperitoneally (i.p.) injected at 6 mL/kg. The volume of vehicle to solubilize and inject the drug was chosen based on low caffeine solubility in water at room temperature.

2.3. Experiment 1: caffeine locomotor effect on habituated rats

Rats were tested only once when adolescent (PND 37–40) or when adult (PND 70–74). Caffeine effect on locomotor activity was evaluated following habituation of rats in the activity-monitoring chamber. The habituation to the chamber was performed to decrease spontaneous exploratory behavior of the animals allowing better observation of the stimulatory drug effect.

In the test day rats were removed from their home cages and placed in the activity-monitoring chamber for habituation for 30 min. Following this period, animals received i.p. injections of vehicle (NaCl 0.9%) or caffeine at doses of 3, 10, 30, 60 or 120 mg/kg. Immediately after injections, animals returned to the activity-monitoring chamber. Locomotor activity was recorded for 60 min after the injections (n=7–12 per group). The time period of analysis was selected in previous experiments showing that caffeine locomotor stimulation occurred mainly within 60 min.

2.4. Experiment 2: caffeine locomotor effect on non-habituated rats

Based on results of experiment 1, caffeine at doses of 30, 60 and 120 mg/kg were chosen to evaluate possible differences of the locomotor depressant drug effect between adolescents and adult rats. To this end, caffeine effect on locomotor activity was evaluated in a protocol in which control rats display high exploratory behavior. This protocol was based on previous data from our laboratory showing that non-habituated rats exhibit an intense locomotor exploratory behavior in the first 10 min of exposure to the novel environment. Also, rats were exposed to the test environment 15 min after vehicle or caffeine injection to ensure the observation of maximum drug effect during the short period of behavioral measure.

Then, in the test day adolescent or adult rats (same age described in experiment 1) were transferred to a room adjacent to the behavioral test room and were kept in their home cages for at least 1 h. Next, animals received i.p. injections of vehicle, caffeine 30, 60 or 120 mg/kg and were placed in individual cages. Fifteen minutes after injections rats were placed in the activity-monitoring chamber and their locomotor activity was recorded during a session of 10 min (n=9–13 per group).

2.5. Statistical analyses

Data were analyzed by Statistica program (StatSoft Inc, Tulsa, OK, USA). In experiment 1, locomotor activity during the 60-min session was analyzed by two-way ANOVA considering the factors age (adolescent and adult rats) and treatment (vehicle or caffeine 3, 10, 30, 60 and 120 mg/kg). In experiment 2, locomotor activity was analyzed by two-way ANOVA considering the factors age (adolescent and adult rats) and treatment (vehicle or caffeine 30, 60 and 120 mg/kg). When significant interaction between factors was detected (P<0.05) the analysis was followed by Duncan post hoc test.

3. Results

3.1. Experiment 1: caffeine locomotor effect on habituated rats

The time course of locomotor activity is shown in Fig. 1A for adolescent and in Fig. 1B for adult rats. Statistical analyses were performed locomotion counts accumulated during the 60 min session (Fig. 1C). Two-way ANOVA revealed significant differences on locomotor activity for both age [$F_{(1,85)}$=7.6; P<0.01] and treatment [$F_{(5,85)}$=25.8; P<0.001] factors. In addition, interaction between factors was detected [$F_{(5,85)}$=2.4; P<0.05]. Duncan post hoc test showed that caffeine increased locomotor activity in adolescent rats at doses 10, 30, 60 and 120 mg/kg while only the doses of 10 and 30 mg/kg increased it in adult rats (P<0.01). Also, adolescent rats showed higher locomotor activity in response to caffeine 10 and 30 mg/kg than adult rats (P<0.05) challenged with the same drug doses (Fig. 1C).

3.2. Experiment 2: caffeine locomotor effect on non-habituated rats

Two-way ANOVA revealed significant differences on locomotor activity for treatment [$F_{(3,76)}$=51.0; P<0.001] but not for age [$F_{(1,76)}$=0.8; P=0.36] factors. In addition, interaction between factors was significant [$F_{(3,76)}$=3.0; P<0.05]. Duncan post hoc test, showed that caffeine at doses 30 and 60 mg/kg increased locomotor activity in adolescent rats while only the doses of 30 mg/kg increased it in adult rats (P<0.05). Also, adult rats injected with 120 mg/kg of caffeine showed a decrease of locomotor activity (P<0.001) compared with its vehicle-injected controls, whereas no significant decrease was found in adolescent rats (Fig. 2A). Another important difference observed was a smaller locomotor activity in adolescent rats compared to adults following vehicle injections (P<0.05). This difference between the control groups could influence the observation of caffeine effect. Then, the data of locomotor activity of this experiment were expressed as percentage of vehicle injected animals of the same age and reanalyzed in the Fig. 2B.

Further two-way ANOVA on locomotor activity normalized by vehicle injected rats revealed significant differences for age [$F_{(1,76)}$=26.7;

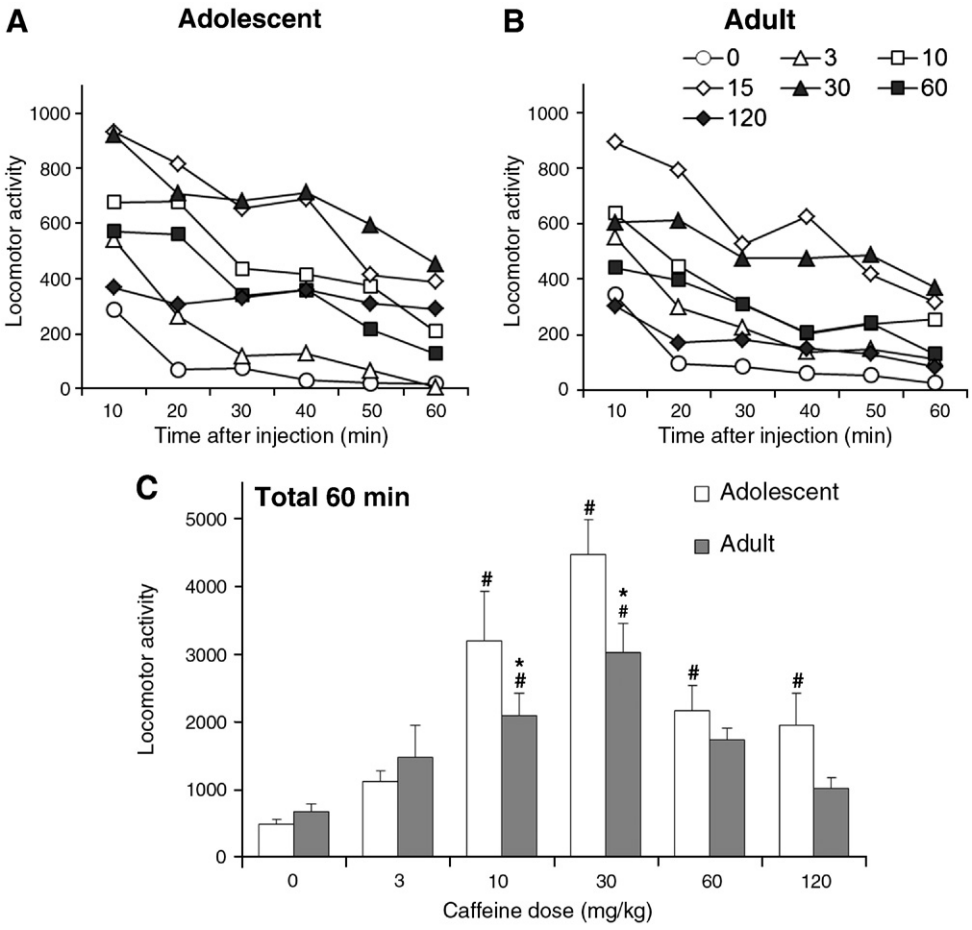

Fig. 1. Locomotor activity following caffeine administration in adolescent and adult rats habituated to the test environment. Data are expressed as the time course of the locomotor response in adolescent (A) and adult (B) rats and total locomotor activity during total 60 min session (C) ± S.E.M. (N = 7–12 animals per group). # $P<0.05$ compared to vehicle injected group with the same age; * $P<0.05$ compared to adolescent group injected with the same drug dose (Duncan post hoc test).

$P<0.001$] and treatment [$F_{(3,76)}=47.8$; $P<0.001$] factors. Interaction between factors was also significant [$F_{(3,76)}=7.1$; $P<0.001$]. Duncan post hoc test showed that caffeine at doses 30 and 60 mg/kg increased locomotor activity in adolescent rats while only the 30 mg/kg dose of caffeine increased locomotor activity in adult rats ($P<0.05$). Also, adult rats injected with 120 mg/kg of caffeine showed a decrease of locomotor activity ($P<0.01$) compared to the respective control (vehicle) group, whereas no significant decrease was found in adolescent rats (Fig. 2B). The expression of the data normalized to vehicle groups, made it possible to reveal the effect of the age factor on caffeine injected animals. Adolescent rats showed higher increase in locomotor activity in response to 30 and 60 mg/kg of caffeine in this analysis as compared to adults ($P<0.01$).

4. Discussion

We investigated the locomotor effects of caffeine in adolescent and adult rats. Our results showed that caffeine induce higher stimulation of locomotor activity in adolescent than adult rats when the animals were habituated to the test environment. Also, results from rats non-habituated to the test environment corroborated the higher psychomotor stimulation in adolescents and evidenced caffeine-induced depression of locomotor activity in adult but not adolescent rats.

The biphasic effect of caffeine on locomotor activity is already known in rodents. Small to moderate caffeine doses increase locomotor behavior while higher doses do not change or decrease it in adult animals (Garrett and Holtzman, 1994; Halldner et al., 2004; Karcz-Kubicha et al., 2003). Our data in rats habituated to the test environment (experiment 1) corroborated these findings. Moreover, our results also showed differences in the adolescent response to caffeine as compared to adult animals. Both adolescent and adult rats showed an inverted U-shaped dose–effect curve with stimulant caffeine effect beginning at 10 mg/kg and peaking at 30 mg/kg. Higher caffeine doses (60 and 120 mg/kg) were still stimulant in adolescent but not in adult rats. The adolescent to adult differences were significant at small to moderate doses of caffeine (10 to 30 mg/kg).

El Yacoubi et al. (2000a) showed that the locomotor depressant effect of higher doses of caffeine is more evident when animals are not habituated to the test environment. Since in our first experiment higher caffeine doses (60 and 120 mg/kg) were still stimulant in adolescent but not in adult rats, we tested the effect of high doses of caffeine in the period of intense exploratory behavior in rats non-habituated to the test environment. This second experiment evidenced the decrease of locomotor activity induced by 120 mg/kg of caffeine in adult but not adolescent rats. However, control (vehicle-injected) adults displayed higher locomotor counts than adolescent rats. As this observation could bias the interpretation of the results obtained in this experiment the data were normalized to vehicle injected groups at the same age and expressed as percentage. This new analysis (Fig. 2B) highlighted the larger caffeine-induced locomotor stimulation at doses 30 and 60 mg/kg in adolescent rats as well as the locomotor depression following injection of 120 mg/kg of caffeine in adult but not in adolescent rats.

Locomotor stimulation in response to other psychostimulants, such as amphetamine and cocaine, has been reported to be smaller in adolescent than adult rats and mice (Adriani and Laviola, 2000; Bolanos

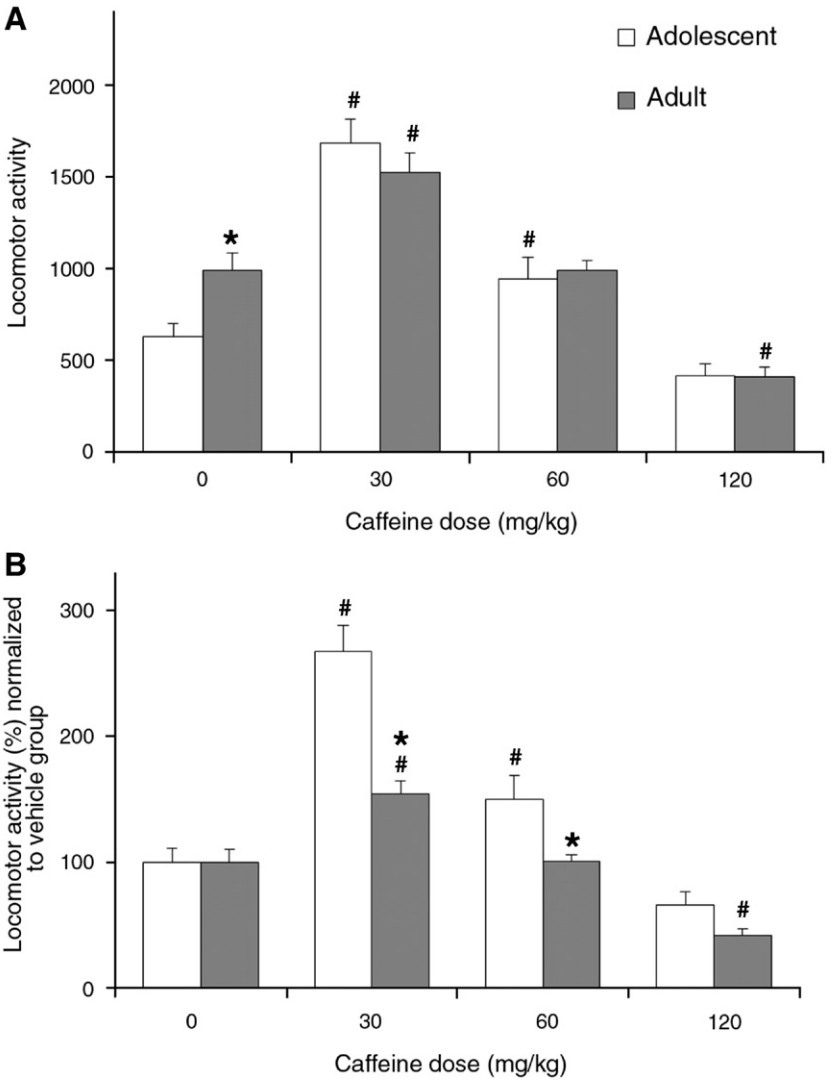

Fig. 2. Locomotor activity following caffeine administration in adolescent and adult rats non-habituated to test environment. Data are expressed as absolute values of locomotor activity (A) and percentage of the respective vehicle injected group (B). Bars represent locomotor activity during 10 min session ± S.E.M. (N = 9–13 animals per group). # $P<0.05$ compared to vehicle injected group with the same age; * $P<0.01$ compared to adolescent group injected with the same drug dose (Duncan post hoc test).

et al., 1998; Laviola et al., 1995). On the other hand, nicotine-induced locomotor stimulation is higher in adolescent than adult rats (Collins and Izenwasser, 2004; Cruz et al., 2005). Nevertheless, in spite of the large consumption of caffeine during adolescence the literature investigating caffeine psychomotor effects at this age period is scarce.

Administration of other psychostimulants such as amphetamine and cocaine also generate an inverted U-shaped dose–effect curve with locomotor activity being substituted by stereotyped behavior at high drug doses (Nordquist et al., 2008; Ushijima et al., 1995). Nevertheless, a stereotyped behavior has not been detected in response to caffeine administration (Antoniou et al., 1998). Then, behavioral alterations other than stereotypy are supposed to influence locomotor depressant effect on high caffeine doses. Administration of caffeine doses from 25 to 100 mg/kg in rats or mice induces anxiety related behaviors in tests such as elevated plus-maze and light/dark box (Bhattacharya et al., 1997; Jain et al., 2005; El Yacoubi et al., 2000b). Moreover, caffeine doses from 30 to 120 mg/kg are reported to impair motor coordination in mice on the holeboard test (Meyer and Caston, 2005). Then, heightened anxiety or motor incoordination could be responsible for the more pronounced depressant effect of caffeine on adult rats. Investigation of anxiety related behaviors and motor coordination between adolescent and adult treated with caffeine should be an issue for future studies.

Some studies showed that administration of caffeine and other methylxanthines induces a state of behavioral excitation closely resembling the characteristic withdrawal syndrome precipitated by naloxone in morphine-dependent rats (Butt et al., 1979). This phenomenon has been termed quasi-morphine withdrawal syndrome and is characterized by behaviors such as jumps, facial rubbing, paw fluttering, wet dog shakes, teeth chattering, genital grooming and body tremors among others (Collier et al., 1974; Bilbao et al., 2006). We cannot rule out the influence of these behaviors on the biphasic effects of caffeine, decreasing the time that the animal spends with locomotor activity at high drug doses. However, such quasi-morphine syndrome was not quantified in our study.

Caffeine's effects on locomotor activity are known to be due to blockade of adenosine A_1 and A_{2A} receptors (Fisone et al., 2004). Antagonism of A_{2A} receptors is clearly related to stimulant properties of caffeine while the action of A_1 receptor antagonism on motor activation or its participation on caffeine motor depression is still a matter of debate (Karcz-Kubicha et al., 2003; Svenningsson et al., 1997). An elucidating study performed by El Yacoubi et al. (2000a) strengthened the A_{2A} participation on the stimulant effects of caffeine. These authors demonstrated that A_1 antagonism mediates psychomotor depression induced by high doses of caffeine or at least counteracts the psychostimulation induced by caffeine action on A_{2A} receptors. Corroborating these findings, a study on knock-out mice showed that adenosine A_{2A} receptors

but not A_1 receptors are necessary for caffeine-induced motor stimulation and that caffeine stimulant effect is facilitated in mice lacking A_1 receptors (Halldner et al., 2004). Our results showed a higher caffeine-induced locomotor stimulation in adolescent rats and higher locomotor depression in adult rats. Then, we can speculate that caffeine action on A_{2A} receptors is larger during adolescence or caffeine action on A_1 receptors is smaller during this age period. High caffeine doses also act on less specific cellular targets other than adenosine antagonism. These mechanisms include the inhibition of phosphodiesterase enzyme, blockade of $GABA_A$ receptors or mobilization of calcium from intracellular stores (Fisone et al., 2004). These targets could also be related to the adolescent to adult differences on caffeine actions. In addition, current evidence suggests that the psychostimulant and reinforcing effects of caffeine may be the result of increased activity of dopaminergic neurotransmission, perhaps via adenosine–dopamine interactions (Powell et al., 2001; Ferré, 2008). Thus, dopaminergic neurotransmission differences between adolescent and adult rats could also be related to higher caffeine-induced locomotor stimulation in adolescent rats and higher locomotor depression in adult rats.

Caffeine dose of 10 mg/kg administered to rats correspond to about 2 to 3 cups of coffee in human weighing 70 kg (Fredholm et al., 1999). Then, caffeine doses which showed more sensitivity to locomotor stimulation in adolescent rats (10 and 30 mg/kg) are easily consumed by humans.

In humans, caffeine effects are also dose-dependent. Lower caffeine doses produce more favorable subjective effects than higher doses, whereas unpleasant effects are more common at higher doses (Fredholm et al., 1999; Kaplan et al., 1997). The observation in our study that adolescent rats exhibited high sensitivity to the stimulant effect of caffeine on small to moderate doses and less sensitivity to motor depressant doses of caffeine suggests that adolescents can consume higher amounts of this substance than adults due to lesser unpleasant effects at this age period. This fact can be problematic because it has been demonstrated that high caffeine consumption in adolescent humans was associated to aggressive behavior, attention deficit/hyperactivity disorder, daily cigarette use and social problems (Martin et al., 2008). Despite the fact that the safety of caffeine use among children and adolescents are poorly understood, some caffeine-containing beverages are marketed directly to children (Bramstedt, 2007). In addition, adolescents and children are the population with the fastest growing caffeine use (Harnack et al., 1999).

In conclusion, both adolescent and adult rats show biphasic locomotor effect of caffeine but locomotor stimulation is higher during adolescence and locomotor depression is more evident during adulthood. Thus, adolescence to adulthood differences on locomotor effects of caffeine should be considered when products such as food, beverages and medicines containing caffeine are marketed. This fact may be more important when high amounts of caffeine are consumed.

Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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