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Acute and adaptive motor responses to caffeine in adolescent and adult rats

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ABSTRACT

Caffeine is a psychostimulant with intake through foods or beverages tending to increase from childhood through adolescence. The goals of the present study were to examine the effects of caffeine on young adolescent Long–Evans rats and to compare the motor-behavioral responses of adolescent and adult rats to acute and chronic caffeine. Adolescent rats had a biphasic dose–response to caffeine comparable to that reported for adult rats. The magnitude of the motor response to a challenge dose of caffeine (30 mg/kg, ip) was similar between adolescent and adult rats. Administration of caffeine in the drinking water (1 mg/ml) for a period of 2 weeks led to overall consumption of caffeine which was not significantly different between adolescents and adults when normalized to body mass. There were no impacts of caffeinated drinking water on volume of fluid consumed nor weight gain in either age group compared to age matched controls drinking non-caffeinated tap water. Following this period of caffeine consumption, return to regular drinking water (caffeine withdrawal) led to a significant decrease in baseline movement compared to caffeine-naïve rats. This effect inversion was observed for adolescents but not adults. In addition, the response of the adolescents to the challenge dose of caffeine (30 mg/kg, ip) was reduced significantly after chronic caffeine consumption and withdrawal. This apparent tolerance to the caffeine challenge dose was not seen with the adults. Thus, the developing brain of these adolescents may show similar sensitivity to adults in acute caffeine exposure but greater responsiveness to adaptive changes associated with chronic caffeine consumption.

1. Introduction

Caffeine is a psychostimulant that works by blocking adenosine A_1 and A_{2A} receptors in the brain (reviewed by Daly and Fredholm, 1998; Ferre, 2008; 2010). Rodents have provided a useful model system wherein the locomotor-activating effects of caffeine have been studied. Motor activity increases as a response to relatively low doses of caffeine (maximal around 30 mg/kg in rats) and decreases at higher doses (Daly and Fredholm, 1998; Powell and Holtzman, 1998; Karcz-Kubicha et al., 2003). Recent receptor knock-out studies have added support to the view that A_{2A} receptors are required for the locomotor-activating effects of caffeine (reviewed by Chen et al., 2010). Tolerance to these effects of a caffeine challenge can develop in rats during chronic caffeine consumption (Holtzman, 1983; Holtzman and Finn, 1988; Svenningsson et al., 1999; Karcz-Kubicha et al., 2003). Several lines of evidence have associated tolerance with compensatory changes in A_1 receptors including increases in the number of receptors (Johansson et al., 1993; Jacobson et al., 1996; Karcz-Kubicha et al., 2003) and biochemical sensitization of the receptors to agonists (Ramkumar et al., 1988). The latter may contribute to symptoms associated with caffeine withdrawal after chronic consumption. These symptoms include sluggishness and headaches in humans and the equivalent "sluggishness" may be seen in some rodent models as a decrease in motor activity during withdrawal (Finn and Holtzman, 1986; Griffiths and Woodson, 1988; Holtzman and Finn, 1988; Johansson et al., 1993). This change from motor activation by caffeine to decreased motor activity after chronic consumption and withdrawal has been termed "effect inversion" (Fredholm et al., 1999; Jacobson et al., 1996). The psychostimulatory effects of caffeine also lead to pronounced sleep disturbances in rats, comparable in several respects to its well known disruption of human sleep patterns (Paterson et al., 2009). Locomotor-activating doses of caffeine induce a distinct pattern of activity in the arousal-promoting system of the rat brain (Deurveilher et al., 2006).

The availability of this stimulant to children and adolescents is a source of growing concern (for comprehensive review see Temple, 2009). What is frequently regarded as the greater sensitivity of children to caffeine has been attributed largely to differences in dose (smaller body mass per beverage consumed) rather than a true difference in sensitivity (Nehlig et al., 1992; Leviton, 1992). However, in contrast to the numbers of studies addressing acute sensitivity to caffeine, there have been relatively few studies directed at regular caffeine use among children and adolescents (Temple, 2009). Caffeine intake in the human population increases from childhood through adolescence (Knight et al., 2004) and children and young adolescents are rapidly growing populations of caffeine users (Harnack et al., 1999; Frary et al., 2005). Young adolescents show marked disturbances in sleep with caffeine intake (Pollak and Bright, 2003) and regular caffeine consumers among both children (Heatherley et al., 2006) and adolescents (Bernstein et al., 2002; Oberstar et al., 2002) show withdrawal symptoms regarded as

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potential signs of physical dependence (Ogawa and Ueki, 2007). It was noted that withdrawal symptoms appeared consistently with modest levels of caffeine intake (Heatherley et al., 2006). The association of caffeine intake and altered sleep patterns led investigators to suggest that the availability of caffeine to teenagers (e.g., in school) should be limited (Pollak and Bright, 2003).

The shortage of information regarding effects of chronic caffeine and adaptive processes associated with tolerance and dependence in the developing brain of children and adolescents can be addressed with an animal model. Rats undergo developmental changes in the brain during adolescence with many parallels to human brain development during the comparable stage (Spear 2000; Crews et al., 2007). Thus, adolescent rats are valuable in exploring interactions of drugs with the developing brain Moreover, the locomotor stimulatory effect of a growing number of other drugs are differentially affected in adolescent rats (Brandon et al., 2001; Collins and Izenwasser, 2004; Faraday et al., 2003; Laviola et al., 1995; Schochet et al., 2004; White et al., 2008). The goals of the present study were to examine the effects of caffeine on young adolescent Long–Evans rats and to compare the motor-behavioral responses of adolescent and adult rats to acute and chronic caffeine.

2. Materials and methods

2.1. Rat model

Male Long–Evans rats were obtained from Charles River Breeding Labs (Raleigh NC) and housed in our facility under conditions of controlled temperature and humidity and a simulated 12 hr light/dark cycle. Rats were given free access to rat chow and water and were housed in randomly-assigned pairs. Because sexual maturation begins and ends over a range of postnatal ages from P28 to P60 (Spear, 2000), we chose to test rats as adolescents at the very beginning of this range (P28). In chronic studies, rats began consuming caffeine at the same age (P28) and continued for 2 weeks into the normal adolescent period. Rats in the range of P65–95 were selected for testing of young adults and an identical 2 week administration period was used in chronic testing. All procedures involving the rats were reviewed and approved by the Institutional Animal Care and Use Committee at Monmouth University according the Public Health Service Guidelines for Care and Use of Laboratory Animals.

2.2. Caffeine injections and motor activity

Fourteen caffeine-naïve adolescent rats and nine caffeine-naïve adult rats were included in the initial acute phase of testing. Activity chambers (Med Associates, Columbus OH) were housed within sound attenuating enclosures and were equipped with a grid of photobeams allowing automated processing and computer analysis of motor behavior. Similar to the design of published studies with adult rats (Powell and Holtzman, 1998; Karcz-Kubicha et al., 2003), all rats were first given an acclimatization period in the activity chambers. In this study, 10 min was chosen for this period since by the 10th minute, exploratory movements decreased to zero in more than half of the animals tested and to levels less than 10% of the total movement in the remaining rats in both age groups. The amount of movement during the acclimatization period was recorded as baseline exploratory movement in the novel environment of the activity chamber and used later for comparison with rats during withdrawal from chronic caffeine. Following this period of acclimatization, rats were injected with either saline (vehicle) or caffeine (30 mg/kg, ip). Caffeine was purchased from Sigma-Aldrich (St. Louis MO). Previous work by a number of groups (e.g., Powell and Holtzman, 1998; Karcz-Kubicha et al., 2003) established this dose as consistently producing maximum activation of motor behavior in adult rats. To validate use of this dose as the challenge for adolescent rats, dose responses were performed with the adolescents using the cumulative dosing procedure of Powell and Holtzman (1998). Following injection, each rat was returned to its home cage for a 10–15 minute interval (again based on Powell and Holtzman, 1998; Karcz-Kubicha et al., 2003) before proceeding with activity measurements. Motor activity was then recorded for a 10 minute test period in the activity chambers. In between trials, activity chambers were cleaned with lemon-scented Clorox wipes, wiped with paper towels and fan-ventilated for 5 min to provide sanitation and a consistent chamber odor.

2.3. Chronic caffeine and motor activity

Seventeen P28 rats and fourteen adults (P65–95) were given free access to 1 mg/ml caffeine (Sigma Aldrich, St. Louis MO) in their drinking water for a period of 2 weeks (Powell et al., 2001). Additional rats at each age were given drinking water without caffeine and served as age-matched controls. After chronic consumption and following 24 or 48 h of withdrawal, baseline exploratory movements were determined in a 10 minute trial without pre-acclimatization to assess effect inversion by comparison with baseline movement of caffeine-naïve animals. Rats were then challenged with saline (vehicle) or caffeine (30 mg/kg, ip) as in the acute phase of testing. We saw no difference between 24 and 48 h of withdrawal and so data from these time points was combined.

2.4. Data analysis

Results are presented as mean ± SEM. All statistical analyses were performed using ProStat software (Poly Software International, Pearl River NY). Differences in motor responses were analyzed by two-factor ANOVA with repetition. Differences in water and caffeine consumption were also evaluated by two-factor ANOVA with repetition. Where needed, Tukey's post test was used for multiple comparisons. Pair-wise comparisons of weights and caffeine consumption were evaluated by student's t test. In all cases, significance was set at $p<0.05$.

3. Results

3.1. Acute testing of caffeine-naïve rats

Following acclimatization of adolescent rats to the test chamber and then injection with saline vehicle or caffeine (30 mg/kg, ip), motor behavior was characterized for each minute of a 10 minute test period. The saline-injected control rats showed an initial burst of activity upon reintroduction to the test chamber and then activity diminished to near zero (Fig. 1). This pattern was identical when rats were reintroduced to the chamber after acclimatization but without injection (data not shown). Rats injected with caffeine showed an even higher initial burst of activity when reintroduced to the chamber and then continued moving at a reasonably constant level throughout the test period (Fig. 1). Thus, the motor stimulatory effect of caffeine was apparent at the earliest time point and persisted throughout the test period. The activity over the 10 minute test period was summed for the remaining studies.

Adolescent rats responded to different doses of caffeine with the biphasic pattern typical of adults (Fig. 2). Movement increased up to doses of 20–30 mg/kg and then decreased at higher doses. In side-by-side tests, adolescents and adults were acclimated to the test chambers and then challenged with vehicle (saline) or caffeine (30 mg/kg) to compare the magnitude of the motor response to caffeine (Fig. 3). Two-factor ANOVA (treatment group x developmental stage with repetition) indicated a highly significant main effect of the acute caffeine challenge [$F(1,38)=78.847$, $p<0.001$]. There was no main effect of developmental stage [$F(1,38)=0.031$, $p=0.862$] and no significant interaction effect [$F(1,38)=1.447$, $p=0.236$]. Thus with the same acute challenge, caffeine-naïve adolescents responded

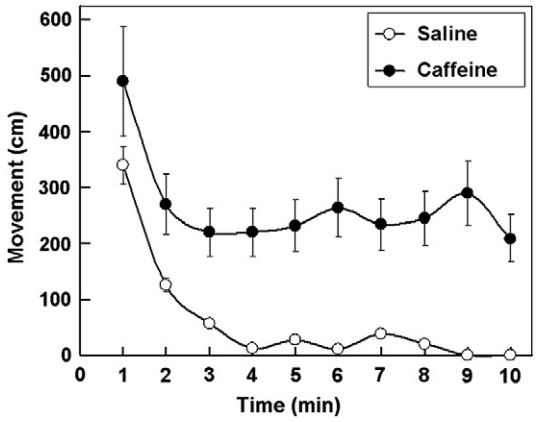

Fig. 1. Time course of adolescent rat responses to caffeine. Rats were first acclimated to the activity chamber and then tested following injection with either saline (vehicle) or caffeine (30 mg/kg, ip). Movement (cm) was determined for each minute of the 10 minute test period. The results are mean ± SEM, N = 6.

similarly to caffeine-naïve adults, with both test groups showing significant motor activation.

3.2. Chronic caffeine administration

To rule out potential concern about dehydration resulting from under-consumption of liquid due to the presence of caffeine in the drinking water, volume of liquid consumed per day was evaluated in a side-by-side comparison of a subgroup of caffeine consumers with a group of age-matched controls drinking regular (non-caffeinated) tap water. For the adolescents, the group consuming caffeinated water averaged 124 ± 6 ml/day/kg body weight and those consuming regular tap water averaged 120 ± 7 ml/day/kg body weight. For the adults, the group consuming caffeinated water averaged 119 ± 16 ml/day/kg body weight and those consuming regular tap water averaged 107 ± 12 ml/day/kg body weight. Two-factor ANOVA (treatment x developmental stage with repetition) indicated there was no significant main effect of treatment [$F(1,22) = 0.451$, $p = 0.509$], no significant main effect of developmental stage [$F(1,22) = 1.232$, $p = 0.279$] and no interaction effect [$F(1,22) = 0.579$, $p = 0.817$]. Thus, caffeine consumers were not taking in less fluid than age matched controls and there was no significant difference between adolescents and adults.

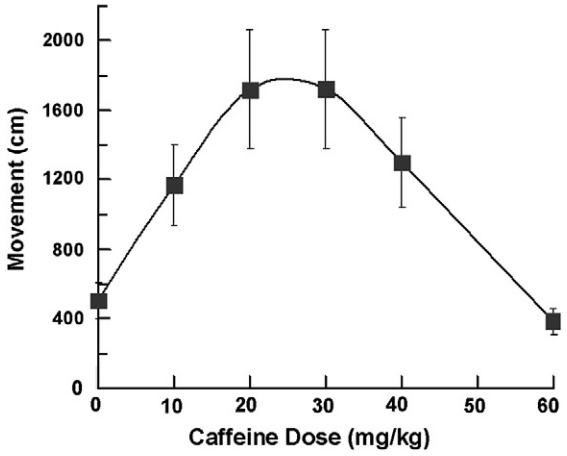

Fig. 2. Adolescent rats have a biphasic dose response to caffeine. Rats were first acclimated to the test chamber and then tested after being injected (ip) with either saline (vehicle) or caffeine. Cumulative dosing was used to obtain doses of caffeine from 10 to 60 mg/kg. Total movement (cm) during the 10 minute test period was determined. A biphasic response was apparent with doses of caffeine up to 30 mg/kg resulting in motor activation and higher doses resulting in decreased motor behavior. The results are mean ± SEM, N = 3–6.

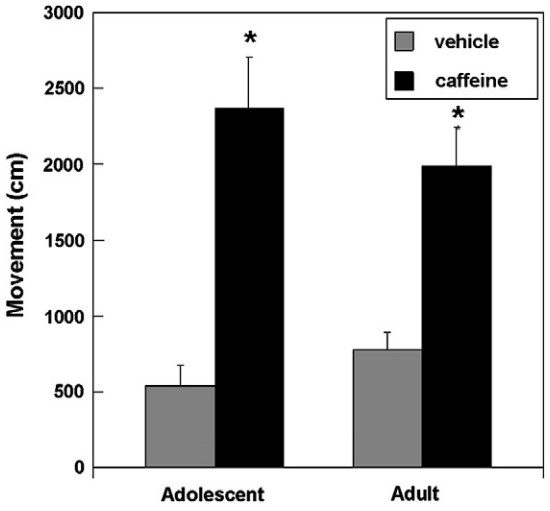

Fig. 3. Motor responses of adolescent rats to a challenge dose of caffeine (30 mg/kg) were comparable to the responses of adults. Rats were either early adolescent (P28) or young adult (P60–90) at the time of testing. The rats were first acclimated to the test chamber and then tested after being injected with either saline (vehicle) or caffeine (30 mg/kg, ip). The results are mean ± SEM (N = 14 adolescents, 9 adults) for total motor activity (cm) during a 10 min trial. Differences between adolescents and adults were not significant. *Compared to vehicle, movement was significantly greater when rats were injected with caffeine ($p < 0.005$) and this was seen with both adolescents and adults.

As a further gage of health during the chronic administration of caffeine in the drinking water, overall weight gain was evaluated for these groups. The average weight of the adolescents at the start of the chronic phase was 90 ± 2 g. Following 2 weeks of caffeine consumption, the average weight of the adolescents consuming caffeine was 178 ± 8 g and the average weight of those consuming regular tap water was 165 ± 10 g. This difference was not significant ($p > 0.05$). The average weight of the adults at the start of the chronic phase was 283 ± 16 g. Following 2 weeks of caffeine consumption, the average weight of the adults consuming caffeine was 351 ± 16 g and the average weight of those consuming regular tap water was 353 ± 8 g. This difference was also not significant ($p > 0.05$). Thus, there was no impact of caffeine consumption on weight gain during the chronic administration period for either adolescents or adults.

Average consumption of caffeine for all rats over the entire administration period was 139 ± 5 mg caffeine/day/kg body weight for the adolescents and 115 ± 15 mg caffeine/day/kg body weight for the adults. This difference was not statistically significant ($p > 0.05$).

3.3. Testing of rats chronically administered caffeine

Baseline testing of naïve rats (without the pre-acclimatization period) provided a measure of exploratory movement in the novel environment of the chamber. This was compared to corresponding baseline movement of rats following chronic consumption of caffeine and subsequent withdrawal (return to normal tap water) to assess the impact of withdrawal on initial exploratory movement. This comparison was made for adult and adolescent rats (Fig. 4). Two-factor ANOVA (treatment group x developmental stage with repetition) indicated a significant main effect of caffeine withdrawal on baseline movement [$F(1,58) = 9.046$, $p = 0.004$], a significant main effect of developmental stage [$F(1,58) = 12.819$, $p = 0.001$], and a significant interaction effect [$F(1,58) = 4.426$, $p = 0.040$]. Post tests confirmed that adolescent rats moved significantly less following chronic caffeine administration and withdrawal than did caffeine-naïve adolescent rats ($p = 0.003$). This was not the case for the adults where movement following caffeine withdrawal was not significantly different from that of caffeine-naïve adults ($p = 0.967$). Different cohorts of adult rats were examined up to 72 h following withdrawal with no change in motor behavior consistent with an altered response due to

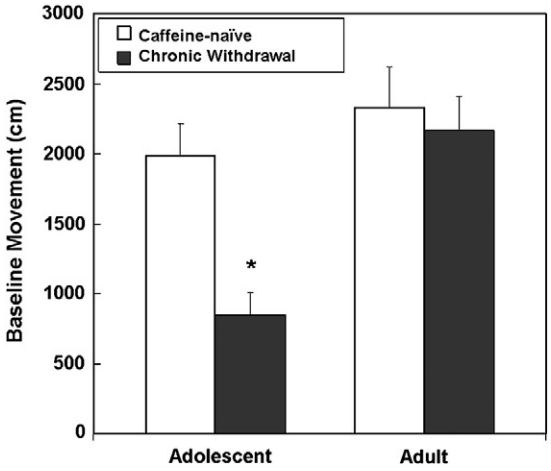

Fig. 4. Non-acclimated initial exploratory behavior was depressed following chronic caffeine consumption and withdrawal in adolescent but not adult rats. The adolescent 'chronic withdrawal' group began consuming caffeinated drinking water (1 mg/ml) at P28 and was tested after 2 weeks of caffeine consumption, followed by 24–48 h of withdrawal (return to regular drinking water). Caffeine-naïve adolescents were age-matched at the time of testing. The adult 'chronic withdrawal' group began consuming caffeinated drinking water (1 mg/ml) at P60–90 and was tested after 2 weeks of caffeine consumption followed by 24–48 h of withdrawal (return to regular drinking water). Naïve rats in the adult group were age-matched at the time of testing. In contrast to the work presented in Figs. 1–3, these rats were not acclimated to the chambers before testing and thus it was initial exploratory motor activity (cm) that was determined for the 10 minute trial period. *Movement of adolescents in the chronic withdrawal group was significantly less than the caffeine-naïve adolescents ($p=0.003$) and significantly less than the adult chronic withdrawal group ($p=0.001$). There was no significant difference between the two adult treatment groups ($p=0.967$) nor was there a significant difference between caffeine-naïve adolescents and adults ($p=0.724$).

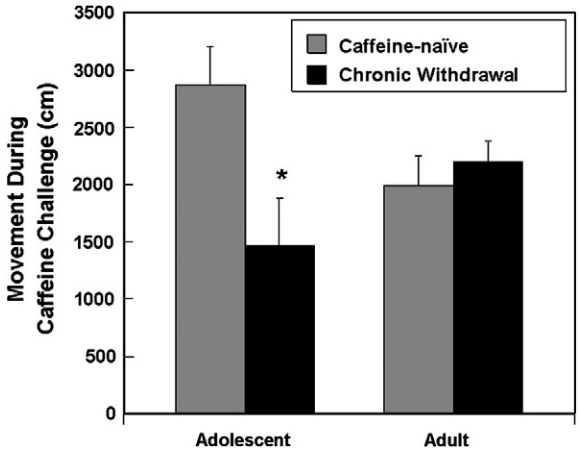

Fig. 5. Comparison of caffeine challenge on caffeine-naïve and caffeine-withdrawing rats at two developmental stages. Similar to the experimental procedure for Fig. 3, the rats were acclimated to the test chambers and then injected with caffeine at the challenge dose (30 mg/kg, ip) with total motor activity (cm) determined subsequently in a 10 min trial. The adolescent 'chronic' group began consuming caffeinated drinking water (1 mg/ml) at P28 and was tested after 2 weeks of caffeine consumption, followed by 24–48 h of withdrawal (return to regular drinking water). Caffeine-naïve adolescents were age-matched at the time of testing. The adult 'chronic withdrawal' group began consuming caffeinated drinking water (1 mg/ml) at P60–90 and was tested after 2 weeks of caffeine consumption followed by 24–48 h of withdrawal (return to regular drinking water). Naïve rats in the adult group were age-matched at the time of testing. The results for saline injections were not significantly different among these groups and only results for the caffeine challenge are shown. *The adolescent chronic caffeine (plus withdrawal) group had a significantly lower motor response to caffeine challenge than caffeine-naïve adolescents ($p=0.001$). There was no significant difference between the two adult treatment groups ($p=0.223$).

withdrawal. Post tests also showed that movement of adolescents in the chronic caffeine/withdrawal group was significantly less than the adult chronic withdrawal group ($p=0.001$) but there was no significant difference between caffeine-naïve adolescents and adults ($p=0.724$). Thus, baseline motor behavior was depressed during caffeine withdrawal in the adolescent caffeine consumers but not adults.

If this decreased exploratory movement following withdrawal was truly the result of some adaptive changes in the brain during caffeine consumption, then these rats might also show tolerance to the caffeine challenge dose. This was the case and again exposed a difference between adolescents and adults. Following the acclimatization period during which the exploratory motor behavior was measured and assessed above, rats were injected with either saline vehicle or the challenge dose of caffeine (30 mg/kg, ip) as in the acute studies. There were no significant differences among age-matched caffeine-naïve rats and rats following withdrawal from chronic caffeine in response to vehicle (data not shown). This reflects the decrease in exploratory movement in the control groups beyond the acclimatization period which thus appeared to mask any difference in the caffeine-withdrawing groups. However, it facilitated comparisons of the caffeine challenge within treatment groups for each developmental stage (Fig. 5). Analysis of the motor responses of the adolescent rats showed a significant main effect of the caffeine challenge [$F(1,20)=44.799$, $p<0.001$]. There was also a significant main effect of treatment group [$F(1,20)=16.311$, $p=0.001$] and a significant interaction effect between treatment group and caffeine challenge [$F(1,20)=15.871$, $p=0.001$]. Post tests indicated that adolescent rats injected with caffeine following chronic caffeine consumption and withdrawal showed significantly less movement in response to caffeine when compared to caffeine-naïve adolescents ($p=0.005$). Although a full dose response to caffeine was not determined for these rats, decreased response to the challenge dose after chronic caffeine consumption is consistent with development of tolerance in the adolescents. The combination of decreased baseline exploratory movement (Fig. 4) and decreased responsiveness to caffeine (Fig. 5) indicated significant adaptive responses in the adolescent rats.

Decreased responsiveness to the caffeine challenge was not observed with the adults under identical treatment conditions (Fig. 5). For adult rats, two-factor ANOVA (caffeine challenge x treatment group) showed a significant main effect of the caffeine challenge [$F(1,22)=22.025$, $p<0.001$]. There was no significant effect of treatment group [$F(1,22)=1.502$, $p=0.233$] and no interaction effect [$F(1,22)=1.862$, $p=0.186$]. Adults injected with caffeine after chronic treatment and withdrawal showed the same level of motor activation seen in caffeine-naïve animals injected with caffeine. Thus, there is no apparent tolerance to the effect of a caffeine challenge in the adults after this regimen of chronic caffeine consumption and withdrawal.

4. Discussion

This study was distinct in its focus on the developing brain of the adolescent, with side-by-side comparison of adolescent and adult rats and with both acute and chronic caffeine treatments. Overall, adolescent rats showed similar responses to the initial acute challenge doses of caffeine but showed greater signs of tolerance and dependence than adults following 2 weeks of regular caffeine consumption. As reviewed by Spear (2000), it is generally accepted that rats can move into the characteristic adolescent period as early as P28 and complete the transition as late as P60. Thus, naïve rats were challenged acutely with caffeine at the perceived earliest entry point to adolescence (P28) and then subsequent to chronic caffeine intake that continued well into the normal adolescent period. Adolescent rats responded to caffeine with a biphasic dose response as has been well-established in multiple laboratories for adult rats (Daly and Fredholm, 1998; Powell and Holtzmann, 1998; Karcz-Kubicha et al., 2003). The challenge dose for acute testing of caffeine-naïve rats was based on this does response and

chosen to be consistent with several previous studies showing that activation of motor behavior was maximal in adults at 30 mg/kg caffeine (Daly and Fredholm, 1998; Powell and Holtzman, 1998; Karcz-Kubicha et al., 2003). At this challenge dose, motor activation occurred similarly in adult and adolescent rats with no significant difference in the magnitude of response. The motor activation of the adolescent rats to caffeine extends the results of an early study that showed juvenile LE rats responded to a somewhat lower challenge dose of caffeine (20 mg/kg) with a disruption in pinning and other juvenile behaviors (Holloway and Thor, 1983). Movement was assessed as position changes between quadrants of an activity chamber and was greater for juveniles (P24) than for P44 adolescents or P84 adults. Interestingly P54 rats were more active in response to caffeine than P44 so quadrant entries did not provide a simple relationship between caffeine response and age. We did some preliminary comparisons with older adolescents (P45) and both younger (P60) and older (P90) adult rats and saw no obvious differences in the response to a 30 mg/kg challenge dose (unpublished results). Reviews of the literature have suggested that children may not be more sensitive generally than adults to caffeine and that perceived differences are due more to lower individual body weights and thus higher doses, than to a true difference in sensitivity (Nehlig et al., 1992; Leviton, 1992). Given that the rat brain progresses through comparable stages of development (reviewed in Spear, 2000; Crews et al., 2007), our results support the view that the adolescent and adult brain may have similar sensitivity to caffeine, with comparable dose response (normalized to body mass) and overall magnitude of response to caffeine when measuring motor behavior as the outcome. Children and young adolescents are rapidly growing populations of caffeine users (Harnack et al., 1999; Frary et al., 2005). Although surveys indicate that consumption tends to be below levels causing concern (Castellanos and Rapoport, 2002; Knight et al., 2004), human caffeine consumption clearly increases through adolescence (Knight et al., 2004), a time during which the developing brain is seen as especially sensitive to modification by drugs (reviewed in Crews et al., 2007; Temple, 2009). Thus, children are still considered an 'at risk' subpopulation as far as health and caffeine consumption (Nawrot et al., 2003; Temple, 2009) and withdrawal symptoms develop upon regular use (Heatherley et al., 2006).

Effects of withdrawal from chronic caffeine consumption were not reported previously for adolescent rats. For chronic caffeine testing, caffeine was included in the drinking water as an established method for obtaining caffeine-tolerant adult rats (Holtzman and Finn, 1988; Jacobson et al., 1996; Johansson et al., 1993; Powell and Holtzman, 1998; Powell et al., 2001; Svenningsson et al., 1999; Karcz-Kubicha et al., 2003). Rats began drinking caffeinated water at P28 and were tested after 2 weeks. Adults were also tested after 2 weeks of caffeine consumption. It was important to confirm that weight gain and overall fluid consumption were comparable to age-matched rats drinking regular tap water. There was no significant difference in either of these parameters measured between age-matched control and caffeine-consuming adolescents. This was also demonstrated for the adult rats. Caffeine consumption to the point of testing was not significantly different for the adolescents and adults when daily consumption was normalized to body weight. The level of consumption for both groups was well within the range reported for adult rats in other studies, up to 136 mg/kg/day (Powell et al., 2001). Previous work has shown that withdrawal symptoms peak 24–48 h after removing caffeine from the drinking water (Finn and Holtzman, 1986; Holtzman and Finn, 1988; Johansson et al., 1993). The important withdrawal symptom in this case is a disruption of motor behavior or effect inversion seen as a decrease in exploratory movement following caffeine withdrawal (Finn and Holtzman, 1986; Holtzman and Finn, 1988; Johansson et al., 1993). The disruption in motor behavior during withdrawal has not been seen in all cases of chronic caffeine studies with adult rats and appears sensitive to the experimental conditions (reviewed in Griffiths and Woodson, 1988; Daly and Fredholm, 1998). Side-by-side comparison of Long–Evans adolescents and adults following an identical period of caffeine consumption showed that the adolescents had a pronounced effect inversion during caffeine withdrawal. Thus, baseline exploratory movement in the chamber as a novel stimulus decreased significantly for the adolescent rats following chronic caffeine consumption and withdrawal. This was not observed in the adults even after 72 h of withdrawal. That this decrease in exploratory movement was truly the result of adaptive changes during chronic caffeine consumption was supported by additional studies in which rats were challenged with caffeine during withdrawal and the motor response was compared to that of caffeine-naïve animals. For adults, there was still a significant activation of motor behavior, with no obvious impact of withdrawal following the 2 weeks of caffeine consumption. In contrast, the adolescent rats showed much less response to caffeine than that of age-matched naïve controls. Thus, the adolescent rats showed tolerance to a caffeine challenge on top of the appearance of withdrawal-induced effect inversion. Only one period (2 weeks) of chronic caffeine consumption was tested and it cannot be ruled out that adults might show similar effects if the period of caffeine consumption is extended. Nevertheless, the side-by-side design of the present study allows us to conclude that the adolescent rats developed apparent adaptive effects under conditions that the adults did not.

Other strains of rats should be tested to determine whether the heightened responses to chronic caffeine are unique among adolescents of the Long–Evans strain. Long–Evans rats were chosen because data was available that juveniles of this strain responded behaviorally to caffeine (Holloway and Thor, 1983) and because of studies of alcohol withdrawal after chronic alcohol consumption that showed the adolescent rats are more sensitive than the adults or than adolescents of the more commonly used Sprague–Dawley strain (Chung et al., 2008). With the adenosine system among the brain targets for alcohol (Dar, 2001; El Yacoubi et al., 2001; 2003; Prediger et al., 2004), caffeine also served as a tool for us to probe the adenosine system in this rat strain. In this context, the results suggest that differences in the apparent adaptability of the adenosine system in these adolescents should be studied more in relation to the effects of ethanol.

In conclusion, tolerance and withdrawal symptoms are taken as signs of adaptive changes in the brain exposed chronically to caffeine and the present study implies that these adaptive changes may be occurring faster or to a greater extent in the still-developing adolescent brain. The effect inversion disruption of motor behavior during withdrawal is of particular interest because it may parallel the sluggishness reported in humans during caffeine withdrawal and because withdrawal symptoms have been presented as part of an argument for physical dependence (e.g., Ogawa and Ueki, 2007). Human surveys report levels of caffeine consumption in adolescents close to that of adults and increasing popularity of caffeinated beverages, including the so-called 'energy' drinks (Boyle and Castillo, 2006), among students from middle school through college (Pollak and Bright, 2003; Malinauskas et al., 2007; Miller, 2008a,b). The present study was designed to model acute and chronic caffeine effects on the adolescent brain. We conclude that the adolescent rats showed acute responses to caffeine comparable to those in adults with no evidence for a difference in acute sensitivity to caffeine. However, given the apparent adaptive responses to caffeine, the developing brain of the adolescent may be more prone both to developing tolerance to caffeine and to developing adverse withdrawal symptoms when caffeine use is discontinued.

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