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Low doses of amphetamine lead to immediate and lasting locomotor sensitization in adolescent, not adult, male rats ☆

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ABSTRACT

Although there is much evidence for age differences in behavioural responses to psychostimulants in rats, the differential, lasting impact of drug exposures has rarely been investigated using direct comparisons of adolescent and adult rats. Male rats were pre-treated with 0.5 mg/kg amphetamine or saline on either postnatal days (P) 31 and P33 or P76 and P78, and locomotor activity was measured for 1 h. Adolescent, and not adult, rats showed a significant increase in distance traveled from the first to second pre-treatment. There was no evidence of sensitization of locomotor activity in either adolescents or adults on Challenge 1 to the same dose of amphetamine when tested 12 days later on P45 (late adolescence) or on P90. Rats that were pre-treated as adolescents exhibited locomotor sensitization to 1.5 mg/kg amphetamine as adults (P60) on Challenge 2, 27 days after pre-treatment, particularly in the group that had also received amphetamine on Challenge 1 at P45. Rats that were pre-treated as adults did not show sensitization on Challenge 2. The results suggest that the rapid adaptations to drug exposures in adolescence have greater consequences than identical treatment in adulthood, and highlight the unique vulnerability of adolescents to brief, low dose drug exposure.

1. Introduction

Adolescents are less sensitive than are adults to psychostimulants at first drug exposure (Weiss et al., 1994), and they transition from drug use to dependence more rapidly than do adults (Wu and Schlenger, 2003). Moreover, risk for drug abuse in adulthood is greater in individuals who initiated first use in adolescence (Merline et al., 2004), suggesting that exposure to psychostimulants in adolescence may have unique and lasting effects on sensitivity to psychostimulants in adulthood. Elevated risk for addiction in adolescence has been attributed in part to the heightened novelty seeking of adolescents (reviewed in Doremus-Fitzwater et al., 2010). The neural pathways that mediate novelty-seeking, most notably the mesocorticolimbic dopamine system (reviewed in Bardo et al., 1996), are also sites of action of psychostimulants, indicating that extensive developmental remodelling of this circuitry may underlie the differential vulnerability of adolescents and adults to drugs of abuse (e.g., Ernst et al., 2009). The changes in mesocorticolimbic circuitry in adolescence are similar in people and in rats and include increased dopamine transporter (people: Haycock et al., 2003; rats: Moll et al., 2000) and dopamine receptor (people: Montague et al., 1999; Seeman et al., 1987; Weickert et al., 2007) (rats: Andersen, 2003; Andersen et al., 1997, 2000; Tarazi and Baldessarini, 2000; Tarazi et al., 1998) density in adolescence.

In rodents, adolescence begins shortly after weaning and can be divided into 3 stages (Tirelli et al., 2003): Early adolescence spans the period after weaning and before puberty, lasting from approximately postnatal days 21 (P21) to P34. Mid-adolescence encompasses the time shortly before and after puberty and lasts from P34 to P45, with puberty (as indicated by balanopreputial separation) occurring at approximately P42 in males (reviewed in McCormick and Mathews, 2007). Late adolescence begins on P45 and lasts until P60, when rats attain sexual maturity. As in people, adolescent rodents exhibit increased levels of novelty seeking (Stansfield et al., 2004) and altered sensitivity to psychostimulants (Spear, 2000) compared to adults, indicating that studies with rodent models of adolescence can provide valuable insight into vulnerability to drug abuse in people. Consistent with age differences in psychostimulant sensitivity in people, studies with rodents find that adolescents are less sensitive to the locomotor activating effects of acute psychostimulant treatment than are adults (e.g., Adriani and Laviola, 2000; Bolanos et al., 1998; Lanier and Isaacson, 1977; Mathews and McCormick, 2007; Mathews et al., 2010, 2009), and more sensitive to the locomotor sensitizing effects of

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repeated psychostimulant treatment compared to adults (Adriani et al., 1998; Laviola et al., 1999; Mathews and McCormick, 2007; Schramm-Sapyta et al., 2004). We have found that adolescent (~P30), and not adult, rats show a significant increase in locomotor activity in response to a second injection of amphetamine 24 h after the first injection (Mathews et al., 2009), which suggests an increased susceptibility to rapid sensitization in adolescence. In addition, these latter studies involved testing confined to the adolescent period compared to the adult period, and did not address the extent to which any differences observed after adolescent treatment persist into adulthood.

The few studies that have investigated possible lasting effects of psychostimulant treatment in adolescence have involved high doses and/or long periods of pre-treatment, and rarely involved a comparison group for which pre-treatment occurred in adulthood, which is necessary to characterize the developmental-specificity of drug effects. For example, repeated pre-treatment with a high dose of amphetamine (2.0–10 mg/kg) (Kolta et al., 1990; McPherson and Lawrence, 2005) or cocaine (10–15 mg/kg) (Marin et al., 2008; Ujike et al., 1995) in adolescence induced locomotor sensitization to a challenge dose of the drug in adulthood. Other studies have reported a sensitized response to amphetamine (Burton et al., 2010; Valvassori et al., 2007) and to cocaine (Achat-Mendes et al., 2003; Adriani et al., 2006; Brandon et al., 2001) in adulthood after chronic methylphenidate treatment in adolescence (but see Ferguson and Boctor, 2010), clearly indicating that repeated drug treatment in adolescence can induce locomotor sensitization in adulthood. Nevertheless, it is not known whether the effects described in the latter studies are unique to pre-treatment in adolescence, or if similar effects would be observed in rats pre-treated in adulthood. Studies using nicotine and methylphenidate that directly compared the effects of pre-treatment in adolescence or in adulthood suggest that lasting effects may depend on age. Whereas chronic methylphenidate treatment (2 mg/kg twice a day for 15 days) in adulthood had no effect on locomotor activity, the same pre-treatment in early adolescence (P20 to P35) reduced sensitivity to the locomotor activating effects of cocaine in adulthood (Andersen et al., 2001). In contrast, pre-treatment with nicotine (0.4 mg/kg twice a day for 7 days) in adolescence, but not in adulthood, enhanced locomotor sensitization to amphetamine 30 days later in adulthood (Collins et al., 2004). The latter studies suggest that lasting effects of chronic drug exposure may be greater in adolescents than in adults.

Here, we test the hypothesis that brief, low dose amphetamine exposures experienced in adolescence may be sufficient to increase the vulnerability to later drug exposures, and thus the present study involves a different approach than that of previous studies, which involved higher doses and/or more numerous pre-treatment injections. The treatment regimen we used [two injections of 0.5 mg/kg amphetamine, which falls in the therapeutic dose range for ADHD (Heijtz et al., 2003) and in the low to moderate dose range for enhancement of locomotor activity (Gulley et al., 2007)], was based on our previous finding that early adolescent, but not adult rats, exhibited rapid behavioural sensitization to a low dose of amphetamine after a single pre-treatment 24 h earlier (Mathews et al., 2009). Here, our goal was to determine whether such rapid sensitization in adolescent rats is temporary or would the effects of such a treatment regimen be observed after much longer intervals. The expression of behavioural sensitization after pre-treatment was examined at two different time points, once in later adolescence (12 days after pre-treatment) and again in adulthood (27 days after pre-treatment). Others have found that adolescent pre-treatment with amphetamine altered locomotor sensitization to amphetamine only in adulthood and not in adolescence (P37) (Santos et al., 2009), indicating that the expression of sensitization may depend on age. We have found that early (P30) and not late (P45) adolescent rats develop rapid sensitization to a second injection of 0.5 mg/kg of amphetamine 24 h after the first injection (Mathews et al., 2009), indicating that susceptibility to sensitization at this dose is reduced in late adolescence. Thus, the first challenge day for the adolescent pre-treatment group occurred in late adolescence (P45) to determine whether late adolescent rats would express sensitization at this dose when amphetamine pre-treatment occurred in early adolescence (P31, P33). The second amphetamine challenge occurred 15 days after the first challenge, when the rats were adults (P60). The second challenge involved a higher dose of amphetamine (1.5 mg/kg) to improve the likelihood of detecting pre-treatment effects 27 days after pre-treatment, as the use of high challenge doses facilitates the expression of sensitization when a low pre-treatment dose is used (Kuczenski and Segal, 2001). Lastly, to test for the developmental specificity of the pre-treatment regimen, a group of rats underwent pre-treatment in adulthood, and was tested for behavioural sensitization after the same intervals (12 and 27 days) as those rats pre-treated in adolescence. Behavioural sensitization to amphetamine in the 0.5–0.6 mg/kg dose range has been found in adult rodents after lengthy pre-treatments with several repeated injections (e.g., Hall et al., 2008; Kelsey and Grabarek, 1999). We predicted that the use of a short pre-treatment regimen that is known to have different effects on sensitization of adolescent and adult rats in the short-term (Mathews et al., 2009) would also reveal age differences in long-lasting sensitization.

2. Method

2.1. Animals

Male Long Evans rats were purchased from Charles River (St. Constant, QC, Canada) and arrived at the colony on either postnatal day 22 (P22; N = 34) or P60 (N = 34). Rats were housed in pairs and maintained on a 12 h light–dark cycle with lights on at 0800 h. Use of animals was approved by the Brock University Animal Care and Use Committee and followed the Canadian Council on Animal Care and National Institutes of Health guidelines.

2.2. Locomotor activity testing

Locomotor testing was conducted in four white open top melamine arenas (58 cm × 58 cm × 58 cm) under indirect red light illumination to reduce anxiety associated with bright lighting. On P30 or P75, rats received an intra-peritoneal injection of saline and were immediately placed into the test arena for 1 h of habituation. The pre-treatment phase began the next day and rats were randomly assigned to receive 0.5 mg/kg of amphetamine (n = 16 at each age) or saline (n = 18 at each age) immediately before placement into the locomotor test arenas for 1 h on each of two pre-treatment days, 48 h apart. Locomotor activity during the test sessions was recorded with a Sony digital video camera mounted from the ceiling and connected to the Smart tracking system (Smart; Panlab; Spain) that measured horizontal distance traveled. The first challenge day (Challenge 1) took place 12 days after pre-treatment on either P45 or P90 (see Table 1 for the experimental design).

For Challenge 1, rats in each drug pre-treatment group were assigned to receive either saline or 0.5 mg/kg of amphetamine. The second challenge (Challenge 2) took place another 15 days later when all rats were adult (either P60 or P105). Rats from each age at time of pre-treatment group were further divided into five groups: Rats that received saline during pre-treatment and saline on the first challenge day received either saline (SSSS group) or 1.5 mg/kg amphetamine (SSSA group) for Challenge 2, and rats that received saline during the pre-treatment phase and 0.5 mg/kg amphetamine on the first challenge day received 1.5 mg/kg amphetamine (SSAA) on Challenge 2. Rats that were treated with 0.5 mg/kg amphetamine during pre-treatment and saline on challenge 1 received 1.5 mg/kg amphetamine

Table 1
Experimental design.

Adolescent ages (days)	30	31 // 33 // 45 // 60		
	N = 34	Saline (**S**) n = 18 (SS)	**S** n = 12 (SSS) **A** n = 6 (SSA)	**S** n = 6 (SSSS) **A** n = 6 (SSSA) **A** n = 6 (SSAA)
		Amphetamine (**A**) n = 16 (AA)	**S** n = 8 (AAS) **A** n = 8 (AAA)	**A** n = 6 (AASA) **A** n = 6 (AAAA)
Phases of the experiment	Habituation	Induction (0.5 mg/kg)	Challenge 1 (0.5 mg/kg)	Challenge 2 (1.5 mg/kg)
	N = 34	Saline (**S**) n = 18 (SS)	**S** n = 12 (SSS) **A** n = 6 (SSA)	**S** n = 6 (SSSS) **A** n = 6 (SSSA) **A** n = 6 (SSAA)
		Amphetamine (**A**) n = 16 (AA)	**S** n = 8 (AAS) **A** n = 8 (AAA)	**A** n = 6 (AASA) **A** n = 6 (AAAA)
Adult ages (days)	75	76 // 78 // 90 // 105		

(AASA) on Challenge 2. The final group of rats received amphetamine at all time points: 0.5 mg/kg during pre-treatment and on Challenge 1 and 1.5 mg/kg on Challenge 2 (AAAA) (see Table 1 for the experimental design). Each rat was always tested in the same arena. All testing occurred between 0900 h and 1700 h and time of testing was counterbalanced across groups.

2.3. Statistics

Analyses consisted of mixed-factor ANOVA for pre-treatment days and between-groups ANOVA for Challenges 1 and 2. Follow-up analyses for within-group comparisons were conducted using paired-samples t-test, and for between group comparisons, follow-up analyses consisted of Fisher's least significant difference (LSD) test. Alpha level for statistical significance was set at $p<0.05$, two-tailed, however, tests of a priori hypotheses with alpha levels of $p<0.10$ two-tailed are noted.

3. Results

3.1. Locomotor activity in the pre-treatment phase

A Pre-treatment Day (Pre-treatment 1, Pre-treatment 2) × Pre-treatment drug group (SS, AA) × Age (P30, P75) ANOVA on distance traveled found a significant Pre-treatment Day × Pre-treatment drug group interaction ($F_{1,64} = 12.30$, $p = 0.001$) and a near significant Pre-treatment Day × Age × Pre-treatment drug group interaction ($F_{1,64} = 3.75$, $p = 0.057$). Follow up analyses were conducted by age to test the hypothesis that activity would increase from the first to the second pre-treatment in adolescent, but not in adult rats. For adolescent rats, a Pre-treatment Day × Pre-treatment Group ANOVA revealed a significant interaction ($F_{1,32} = 10.11$, $p<0.01$): For rats treated with saline, distance traveled decreased from the first to the second pre-treatment ($p = 0.01$) and for rats treated with amphetamine, distance traveled increased from first to second amphetamine pre-treatment ($p = 0.04$). In adulthood, there was no change in distance traveled for either saline or amphetamine treated rats (see Fig. 1).

For saline treated rats, adolescents were significantly less active than were adults during the first ($p = 0.05$) and second ($p<0.0001$) days of pre-treatment, whereas the age difference between adolescent and adult rats after amphetamine treatment approached significance only during the first day of pre-treatment ($p = 0.065$; adolescent < adult) (see Fig. 1).

3.2. Locomotor activity in Challenge 1

An Age × Pre-treatment Drug × Challenge 1 Drug ANOVA on distance traveled on Challenge 1 revealed a main effect of Age ($F_{1,60} = 11.43$, $p = 0.001$; adolescent < than adult) and a main effect of Challenge 1 Drug ($F_{1,60} = 122.83$, $p<0.0001$), with amphetamine treated rats more active than saline treated rats irrespective of pre-treatment drug (main effect of Pre-treatment drug, $p = 0.09$). There were no significant interactions among factors (all $p>0.14$). This pattern of results did not change when the analyses were conducted for each age group separately (see Fig. 2).

3.3. Locomotor activity in Challenge 2

Because Challenge 2 did not involve a balanced design, the data for Challenge 2 were analyzed with two different approaches. The first approach considered the five Challenge 2 groups (SSSS, SSSA, SSAA, ASAA, and AAAA) as levels of one-factor. For rats pre-treated in adolescence ($F_{4,29} = 15.44$, $p<0.0001$), all rats that received amphetamine for Challenge 2 were more active than rats that received saline (all $p<0.0001$). History of amphetamine treatment also had a significant effect on locomotor activity after an amphetamine challenge, such that rats that received amphetamine during pre-treatment and Challenge 1 (AAAA) were more active than both saline pre-treatment groups, whether or not they had amphetamine on Challenge 1 (SSSA, $p = 0.04$; SSAA, $p = 0.02$). The higher activity of

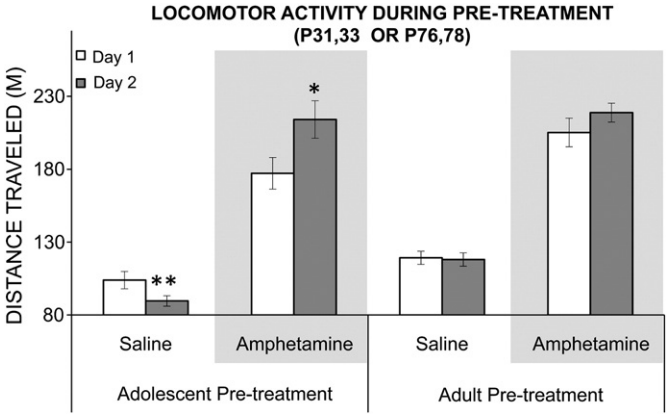

Fig. 1. Mean (±SEM) distance traveled during the two days of pre-treatment with saline or 0.5 mg/kg of amphetamine in adolescent or adult rats. *$p = 0.04$ compared to activity on first pre-treatment day in amphetamine-treated adolescents; **$p = 0.01$ compared to activity in on first pre-treatment day in saline-treated adolescents.

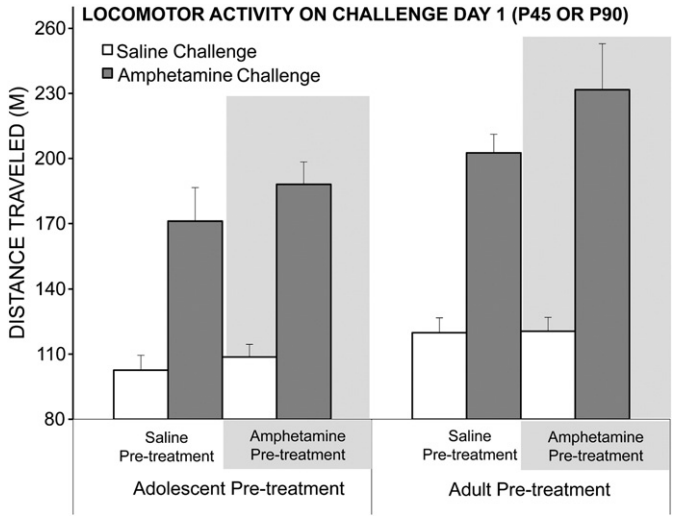

Fig. 2. Mean (±SEM) distance traveled after treatment with saline or 0.5 mg/kg of amphetamine on Challenge 1, 12 days after pre-treatment. Locomotor activity after amphetamine is shown in shaded bars. Main effect of age ($p=0.001$), of Challenge 1 drug treatment ($p<0.0001$), and of Pre-treatment drug ($p=0.09$); no interaction of the factors (all $p>0.20$).

AAAA rats than AASA rats missed significance ($p=0.07$). For adult pre-treated rats ($F_{4,29}=10.33$, $p<0.0001$), rats in all four groups that received amphetamine for Challenge 2 were more active than the group that received saline (all $p<0.0001$), but no other group difference was significant (see Fig. 3).

The second approach was to have a balanced design for statistical analysis by removing the group treated with saline throughout the experiment (SSSS). A Pre-treatment drug group × Challenge 1 drug group ANOVA of distance traveled in Challenge 2 for rats pre-treated in adolescence found that the higher locomotor activity in rats pre-treated with amphetamine compared to rats pre-treated with saline missed statistical significance ($F_{1,24}=3.59$, $p=0.07$). No group difference approached significance for rats pre-treated as adults (see Fig. 3).

To address the possibility that adult rats did not express locomotor sensitization because of an enhanced expression of stereotypy on the second challenge day, locomotor activity over 5 min time blocks is shown for adult rats that were repeatedly treated with amphetamine (AAAA) and for the rats that were receiving amphetamine for the first time (SSSA) in Fig. 4.

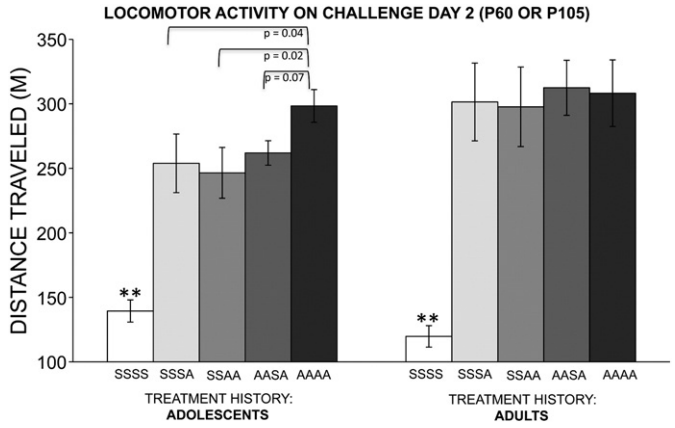

Fig. 3. Mean (±SEM) distance traveled on Challenge 2. **Within age group, rats given saline on challenge 2 (SSSS; white bar) were lower than each of the other groups, each of which received 1.5 mg/kg of amphetamine (grey and black bars, all $p<0.0001$). Differences among the amphetamine-treated groups are indicated on the figure.

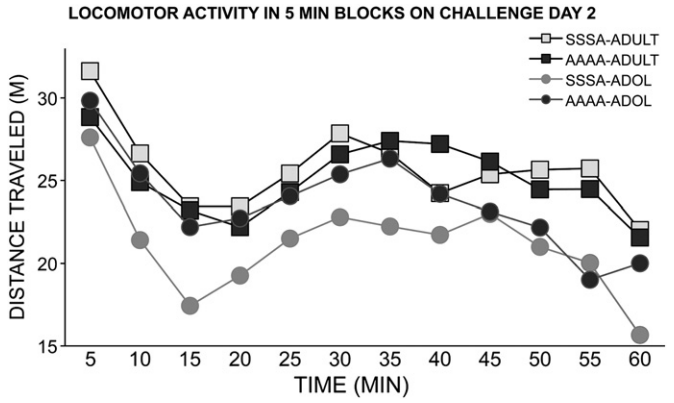

Fig. 4. Distance traveled on Challenge 2 in 5 min blocks illustrates the same pattern and degree of activity to amphetamine over time irrespective of whether pre-treated with amphetamine or with saline in adults, but nor adolescents.

4. Discussion

Consistent with our hypothesis, a brief treatment regimen with low doses of amphetamine in early adolescence led to a lasting change in locomotor activity to subsequent exposures, highlighting the heightened sensitivity of adolescents compared to adults to drug-induced behavioural plasticity. First, a low dose of amphetamine in adolescence, not in adulthood, increased the locomotor activating effects of a second injection of amphetamine given 48 h later. Second, the rapid sensitization observed during the two days of pre-treatment in adolescence was associated with long-lasting locomotor sensitization to amphetamine, whereas pre-treatment was without effect on later responses to amphetamine in adults. Third, the enhanced sensitization observed on the second challenge day in adulthood can be attributed primarily to a combinatorial effect of amphetamine treatment during early and late adolescence. That the same treatment regimen in adult rats had no lasting effect on the locomotor activating effects of amphetamine is consistent with adolescence as a unique period of sensitivity to the enduring effects of psychostimulants. Each of these findings and their implications are discussed in greater detail in the following section.

4.1. Locomotor activity during pre-treatment

Consistent with previous reports of hyporesponsivity to an acute exposure to psychostimulants in adolescence (e.g., Adriani and Laviola, 2000; Bolanos et al., 1998; Lanier and Isaacson, 1977; Mathews et al., 2010), we found a trend for reduced locomotor activity in adolescent compared to adult rats to the first dose of amphetamine during the pre-treatment phase. In addition, only adolescent rats exhibited a significant increase in the locomotor activating effects of amphetamine on the second pre-treatment day, confirming our previous report of rapid amphetamine-induced behavioural plasticity in adolescence (Mathews et al., 2009). The increased activity in adolescents on the second test day eliminated the age differences that were observed for the first amphetamine treatment, indicating that the initial hyporesponsiveness cannot be attributed to age differences in amphetamine pharmacokinetics or to reduced locomotor capacity in adolescents than in adults. Other studies have found that pharmacokinetic factors do not account for age differences in locomotor activity, in that the brain levels of amphetamine, cocaine, or methamphetamine did not explain age differences in locomotor activity (Frantz et al., 2006; Spear and Brake, 1983; Zombeck et al., 2009). Furthermore, we have found adolescent and adult rats to differ in locomotor activity after a range of acute doses of amphetamine administered via cannulae directly into the nucleus accumbens (Mathews and McCormick, 2009).

The increase in activity found in the present study to a second treatment of amphetamine cannot be attributed to a nonspecific increase in exploratory activity in adolescence, because adolescent saline-treated rats traveled less on the second than on the first day of pre-treatment. We have argued that the rapid change in the locomotor response to amphetamine in adolescence may reflect greater plasticity in the mesolimbic circuitry in adolescents than in adults (Mathews et al., 2009), which is consistent with the report of a lower release of striatal dopamine in adolescent than in adult rats after acute treatment of amphetamine, and a higher release in adolescent rats than in adult rats after repeated treatment of amphetamine (Laviola et al., 2001). One potential problem in the interpretation of age effects in the present experiment is that different lengths of acclimation to the colony in adolescent (8 days) and adult (15 days) rats may have resulted in age-specific effects of transportation stress on locomotor activity. Nevertheless, it is unlikely that transportation stress accounts for age differences during the pre-treatment phase, as we have found the same result of rapid behavioural sensitization to a second dose of amphetamine in adolescents and not in adults in rats that were reared in our colony and were thus not exposed to the stress of transportation (Mathews et al., 2009).

4.2. Locomotor activity on Challenge day 1

Adolescent and adult pre-treated rats were tested for locomotor sensitization to 0.5 mg/kg of amphetamine 12 days after pre-treatment (Challenge 1) when the adolescent group was post-pubertal (P45). Locomotor sensitization was not observed in either adolescent or adult rats at this time point, although activity appeared to be slightly higher in rats of both age groups that were pre-treated with amphetamine compared to the age-matched groups pre-treated with saline. In addition, adolescent rats were less active than were adults on Challenge 1, irrespective of drug treatment. We previously found that, in contrast to P30 rats for which adult-like activity was evident after a second treatment of amphetamine, P45 rats remained hyporesponsive (Mathews et al., 2009), indicating that there are significant developmental shifts in drug responses within the time span conventionally considered adolescence. Early and late adolescent rats differ on various behavioural and neural parameters, including conditioned place preference (Badanich et al., 2006; Brenhouse et al., 2008), cocaine-induced locomotor activity (Badanich et al., 2008), cocaine-induced dopamine release in the nucleus accumbens (Badanich et al., 2006), tyrosine hydroxylase immunoreactivity in the caudate nucleus (Mathews et al., 2009) and dopamine receptor expression throughout the mesocorticolimbic dopamine system (Andersen et al., 1997, 2000). Thus, lower locomotor activity in P45 rats compared to adult rats is likely a reflection of a developmental shift in neural regions that regulate the locomotor activating effects of psychostimulants.

The lack of expression of locomotor sensitization in rats that were challenged with amphetamine in late adolescence is consistent with a lack of sensitization during adolescence reported by others. For example, cross-sensitization to amphetamine after 7 days of nicotine pre-treatment in early adolescence was not found if the challenge test also occurred in adolescence, but it was found if the challenge test occurred in adulthood (Santos et al., 2009). Similarly, effects of MDMA pre-treatment in adolescence on locomotor sensitization to cocaine increased with longer delays between pre-treatment and challenge day (Achat-Mendes et al., 2003). Twice daily pre-treatment with 0.5 mg/kg of amphetamine from P22 to P34 also failed to produce sensitization in rats when the test for sensitization occurred in late adolescence (~P48) (Heijtz et al., 2003), but this study did not involve additional testing in adulthood. Another explanation for the lack of sensitization on Challenge 1 may be that the dose of amphetamine used on the first challenge day was not sufficiently high to reveal sensitization, as the use of low doses of amphetamine for pre-treatment and challenge sessions may compromise the ability to detect sensitization (Kuczenski and Segal, 2001).

4.3. Locomotor activity on Challenge day 2

Adolescent and adult pre-treatment groups were challenged with 1.5 mg/kg of amphetamine on either P60 or P105, 27 days after pre-treatment. At this time point, locomotor sensitization was observed only in rats that were pre-treated with amphetamine in adolescence, indicating that enhanced plasticity observed in adolescence was associated with effects on locomotor activity that persisted into adulthood. Although rapid sensitization to amphetamine during the pre-treatment phase was observed in early adolescent rats, the expression of lasting sensitization (i.e., P60, Challenge 2) also required exposure to amphetamine in late adolescence (P45, Challenge 1). These data suggest that enhanced sensitization in adulthood involves a combinatorial effect of amphetamine treatment at the early and the late stage of adolescence and that amphetamine treatment at either stage alone is not sufficient for enhancing locomotor sensitization in adulthood. We cannot rule out the possibility that increased locomotor activity in amphetamine pre-treated adolescent rats at least in part reflects an increase in conditioned locomotion because we did not include an amphetamine pre-treated group that was challenged with saline during the final test session. Nevertheless, we did not find any evidence of conditioned locomotion in amphetamine pre-treated rats that were given saline during the first challenge session. Moreover, we did not find evidence of conditioned locomotion in adult rats that were tested 9 days after pre-treatment with 5 injections of 1.0 mg/kg of amphetamine in late adolescence (Mathews et al., 2008), which supports our contention that sensitization observed in the present experiment is a reflection of amphetamine-specific effects on locomotor activity.

Most studies of the long-lasting effects of psychostimulant treatment in adolescence have used methylphenidate (Achat-Mendes et al., 2003; Brandon et al., 2001; Burton et al., 2010), and those that have examined the lasting effects of amphetamine (Kolta et al., 1990; McPherson and Lawrence, 2005) and cocaine (Marin et al., 2008; Ujike et al., 1995) have used high doses (2–10 mg/kg of amphetamine) and prolonged pre-treatment periods. Here, we show that three days of low-dose amphetamine in adolescence are sufficient for inducing sensitization in adulthood. This is an important point to consider because many studies have reported age differences in the locomotor activating effects of acute psychostimulant treatment (Badanich et al., 2008; Bolanos et al., 1998; Lanier and Isaacson, 1977; Mathews and McCormick, 2007; Mathews et al., 2010, 2009), but the age-specific impact of acute psychostimulant treatment on subsequent drug responses has not been investigated thoroughly. Moreover, there is a lack of studies that directly compare the effects of amphetamine pre-treatment in adolescence and in adulthood. A crucial advantage of including an adult pre-treatment group is the ability to draw conclusions regarding effects that are unique to the developmental period at which the treatment occurred. Evidence for adolescence as a unique period of sensitivity during which exposure to various environmental stimuli can alter vulnerability to drugs of abuse is growing. Previous work from our lab has shown that exposure to a mild chronic social stressor throughout adolescence, but not in adulthood, increases locomotor sensitization to amphetamine in adulthood (Mathews et al., 2008; McCormick et al., 2005). Results of the present study extend these findings by demonstrating that three exposures to a relatively low dose of amphetamine administered during adolescence can also produce lasting effects on subsequent responses to amphetamine in adulthood. These data do not suggest that sensitization does not develop in adulthood. In fact, even a single pre-treatment with a high dose of 5.0 mg/kg amphetamine has been shown to produce locomotor sensitization

that increased with longer periods of withdrawal in adult rats (Vanderschuren and Kalivas, 2000). Instead, our results highlight the differential sensitivity of adolescents than adults for developing sensitization, even when exposure involves few treatments at low doses. One possible explanation for the lack of sensitization in adult rats is that activity in adults is replaced by stereotypy, which would cause a reduction in locomotor activity. However, this possibility is unlikely, given that the pattern of locomotor activity in 5 min intervals over the test session on the second challenge day did not differ for adult rats that had repeatedly received amphetamine compared to the rats that were receiving amphetamine for the first time.

One limitation of developmental comparisons such as in our study is that controlling for the time interval between pre-treatment and challenge results in a difference in age at time of challenge over and above the manipulation of age at time of pre-treatment: Even though all rats were adults for the final test day (Challenge 2), there is nonetheless a 45 day age discrepancy that confounds direct comparisons of locomotor sensitization because of differences in basal activity between young (P60) and older (P105) adults. For this reason, it is critical that conclusions regarding age-specific pre-treatment effects on locomotor sensitization are limited to comparisons of age-matched controls (within pre-treatment age groups).

5. Conclusion

Comparable to age differences in drug effects in people (Weiss et al., 1994), adolescent and adult rats differ in sensitivity to initial treatment with amphetamine. Acute amphetamine treatment produced adaptations that increased sensitivity to subsequent amphetamine treatment more readily in adolescent rats than in adults, indicating that very few low-dose amphetamine exposures in adolescence can have lasting consequences even though identical pre-treatment in adulthood has no detectable effect. Enhanced sensitization after adolescent pre-treatment in adulthood suggests that adolescence, particularly the early period, may represent a unique window of vulnerability to psychostimulants.

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