Pharmacology, Biochemistry and Behavior 95 (2010) 63–71

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Individual differences in activity predict locomotor activity and conditioned place preference to amphetamine in both adolescent and adult rats

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ARTICLE INFO

Article history:
Received 9 October 2009
Received in revised form 12 November 2009
Accepted 7 December 2009
Available online 13 December 2009

Keywords:
Adolescence
Amphetamine
Novelty seeking
Forced novelty
Locomotion
Activity
Conditioned place preference
Individual differences

ABSTRACT

Individual and developmental differences in novelty seeking have been implicated in differential sensitivity to psychostimulants in rodents, but findings are mixed. The extent to which age differences in activity in a novel arena depended on test duration was examined by comparing adolescent and adult rats after 5 and after 60 min of testing (session 1). Rats were tested again after amphetamine or saline administration 24 h later (session 2) to examine whether activity in a novel arena predicts sensitivity to locomotor-activating effects of amphetamine. Data from two experiments were used to examine consistency of the findings. Only activity in 60 min sessions produced a consistent age difference (adolescent<adult) and predicted activity after amphetamine in session 2. Session 1 activity also predicted saline activity in session 2, indicating that individual differences in activity is a stable trait. A third data set was used to determine whether general (saline) and amphetamine-induced activity predicted magnitude of conditioned place preference (CPP) in late-adolescent and adult rats. Age was not a significant predictor, but CPP was positively associated with amphetamine activity and negatively associated with saline activity. Thus, in contrast to enhanced psychostimulant sensitivity in high novelty-seekers, rats higher in general activity are less sensitive to amphetamine conditioned place preference.

1. Introduction

In people, the propensity for novelty seeking is associated with increased engagement in risky behaviour (Wills et al., 1999). Individual differences in this trait have received much attention because of the strong association between novelty seeking and drug abuse, both of which are elevated in human adolescents (Arnett, 1992). In animal models, novelty seeking can be quantified on the basis of preference for novel objects or a novel space, which are referred to as free-choice tests of novelty seeking. Nevertheless, for tests of drug sensitivity, novelty seeking is more often defined using a forced novelty paradigm in which rats are classified as high or low responders on the basis of locomotor activity in a novel test arena. Adult rats that are more active in a novel environment (HR) typically exhibit enhanced locomotor activity in response to psychostimulants compared to less active rats (LR) (Bevins et al., 1997; Hooks et al., 1992), and the enhanced activity is taken as an index of greater sensitivity to psychostimulants in HR rats. Nevertheless, a common problem in studies of locomotor activity is the failure to examine whether the effects observed are specific to psychostimulant treatment (Quertemont et al., 2004). When similar differences after saline treatment are found, the conclusion that more active rats are more sensitive to psychostimulants is problematic, and the conclusion instead should be that individual differences in activity after psychostimulants are a reflection of individual differences in trait activity/arousal (Quertemont et al., 2004). Consistent with this argument is the finding that rats that are more active in a novel arena show the same level of activity over repeated testing (up to 10 sessions) (Carey and DePalma, 2003), and thus activity in a novel arena is a stable trait, predicting activity to repeated exposures to the arena.

Baseline arousal, or trait activity, is an important consideration in understanding age differences in the effects of psychostimulants, particularly when adolescents and adults differ in activity in novel arenas. Nevertheless, some studies using forced novelty tests report enhanced locomotor activity in a novel arena in adolescence (e.g., Philipot and Wecker, 2008; Stansfield and Kirstein, 2005) and others report enhanced activity in adulthood (e.g., Adriani and Laviola, 2000; Badanich et al., 2008; Mathews et al., 2009; Wooters et al., 2006). In a recent review of the literature, Philipot and Wecker (2008) concluded that adolescents tend to be more active than adults in a forced novelty test when test duration is approximately 5 min long, but less active when test duration is longer than 30 min, likely because adolescents habituate to novelty more rapidly than adults. Given the importance of novelty seeking for evaluating addiction risk in people, it is important to test directly whether the different direction of age differences in forced novelty seeking is due to test duration. One can

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doi:10.1016/j.pbb.2009.12.007

then ask which measure best predicts psychostimulant sensitivity, and whether any relationship observed is different for adolescents than for adults.

The categorization of rats as HR and LR based on locomotor activity is often used to investigate sensitivity to rewarding and discriminative stimulus properties of psychostimulants, but the results have not been consistent. Evidence for an enhanced susceptibility to the rewarding effects of psychostimulants in HR rats is based largely on findings of greater self-administration (Klebaur et al., 2001; Piazza et al., 1989; Pierre and Vezina, 1997). Individual differences in conditioned place preference (CPP) are less conclusive, with some studies finding no association (Dietz et al., 2007; Erb and Parker, 1994; Gong et al., 1996), and others finding a negative association (Brabant et al., 2005; Shimosato and Watanabe, 2003) between activity in a novel arena and magnitude of psychostimulant CPP. In addition, the locomotor response to acute amphetamine or cocaine has not been found to predict CPP magnitude (Brabant et al., 2005; Erb and Parker, 1994), though increased locomotor response to psychostimulants is thought to be an index of their reinforcing effects (Wise and Bozarth, 1987). Finally, there is disagreement regarding differences in sensitivity to the discriminative effects of amphetamine, with some studies finding that LR are more sensitive (Exner and Clark, 1993) and others finding that HR are more sensitive (Bevins et al., 1997) to discriminative stimulus effects of amphetamine.

Here, we first tested the hypothesis put forth by Philipot and Wecker (2008) that age differences in novelty-induced locomotion vary with test duration by comparing age differences in locomotor activity in the first 5 min in a novel arena to age differences in activity over 60 min of the acclimation session to a novel arena in the same animals using a forced novelty paradigm. We also calculated the percentage of time spent in the centre and in corners in shorter versus longer periods of observation as measures of free-choice exploration. These measures are typically used as indices of exploratory or of anxiety-like behaviour, and some evidence suggests that adolescents spend more time in corners (Lanier and Isaacson, 1977) and less time exploring the centre of an open field (Hefner and Holmes, 2007) compared to adults. We utilized data from two separate experiments to test whether any relationship observed between locomotor measures and their ability to predict drug-induced locomotor activity in adolescents and adults are replicable. The main questions we address is whether predictors of individual differences in activity after psychostimulants also account for age differences in the locomotor-activating effects of amphetamine or is the relationship between amount and /or pattern of activity in a novel arena and amphetamine-induced activity age-specific. In addition, we used a third data set to investigate whether individual differences in locomotor activity are relevant for predicting amphetamine CPP, and in the same way for adolescents as for adults.

2. Materials and methods

2.1. Animals

The measures used to address the specific questions regarding age differences in activity during habituation and locomotor response to amphetamine were obtained from two separate experiments (Experiment 1a: $N=76$; Experiment 1b, $N=36$). To investigate predictors of amphetamine CPP (referred to here as Experiment 2: $N=80$), we re-analyzed data from adolescent and adult males from a previously published experiment for which there were no age differences in CPP (Mathews et al., 2008). Thus, the data presented here involved a total of 192 male Long Evans rats (Charles River, St. Constant, QC) that were obtained at either 22 or 65 ± 5 days of age, and pair-housed in plastic cages (46 cm × 24 cm × 20 cm). Rats were kept on a standard 12:12 h light–dark cycle (lights on at 8 am) with food and water freely available. All procedures were in accordance with the National Institutes of Health (NIH) and the Canadian Council of Animal Care (CCAC) guidelines, and were approved by the Brock University Animal Care Committee.

2.2. Experiment 1: Locomotor activity testing

Rats in both Experiments 1a and 1b were tested in one of four white open-top melamine arenas (58 cm × 58 cm × 58 cm) illuminated indirectly by red light to attenuate anxiety related to bright illumination, and with constant white noise in the background. Test sessions were between 0900 and 1700 h, and test arenas were cleaned with 50% ethanol after each session. Testing began on PND 30 or PND 75. For session 1 (acclimation), rats in both experiments were given an intra-peritoneal injection of saline and were immediately placed into the novel test arena for 1 h of acclimation. Locomotor activity was recorded with a Sony digital video camera mounted from the ceiling and connected to a computer tracking system (Smart; Panlab, Spain) that measured distance traveled in centimeters, as well as the percentage of time spent in the corners and in the centre of the test arena (12 cm away from any wall). Session 2 was 24 h after the acclimation session for rats in both experiments, but rats in Experiment 1a received i.p. injections of saline or 0.5 mg/kg of amphetamine (random assignment to injection group) and rats in Experiment 1b received saline or either 0.5 or 1.5 mg/kg of amphetamine (random assignment to injection group) immediately before placement into the test arena. These doses were chosen based on our previous report of dose-specific age differences in locomotor activity (Mathews et al., 2009). Locomotor activity in session 2 was recorded for 1 h in Experiment 1a and for 30 min in Experiment 1b.

2.3. Experiment 2: Conditioned place preference

Eighty male rats underwent an unbiased conditioned place preference (CPP) procedure adapted from Sellings and Clarke (2003) as previously described during either late adolescence ($n=40$; PND 45–52) or as adults ($n=40$; PND 69–76). In brief, the apparatus consisted of white-walled open-top melamine arenas [58.1 cm (length) × 28.8 cm (width) × 53.0 cm (height)]. Grid and bar floor-tiles were used as tactile cues and were designed to fit two tiles per box [28.5 cm (length) × 28.5 cm (width) × 5.5 cm (height)]. Two identical tiles (grid or bar) were placed in the box during conditioning sessions, and a grid and a bar tile was placed in the box for CPP testing. The grid texture was constructed of wire mesh (1 × 1 cm squares) and the bar texture was constructed of plastic bars (1.2 cm diameters spaced 1.5 cm apart). Both bars and grid were mounted on square frames, and the floor underneath the tiles was covered with scent-saturated bedding. Tests in a separate group of rats found no preference for one cue over the other (i.e., rats spent approximately 50% of the time on each tactile cue). Six separate CPP boxes were used, with an individual rat tested always in the same box. The boxes were indirectly illuminated by red light.

One day before the start of testing (P45 or P69), rats were injected with saline and placed in the CPP apparatus without tiles (bedding on floor) for 30 min of acclimation. Locomotor activity was recorded using the same tracking system as in Experiment 1. The conditioning phase consisted of six daily 30 min sessions beginning on PND 46 or PND 70. Amphetamine (0.5 or 1.0 mg/kg) and saline were administered on alternating days for a total of three treatments with saline and three treatments with amphetamine. For half of each group, the first injection was saline and for the other half it was amphetamine. After injection, rats were placed into a box with two identical tiles (i.e., 2 bar or 2 grid tiles), such that one type of tile (bar or grid) was always associated with amphetamine and the other with saline for each rat. All components of conditioning were fully counterbalanced across rats (e.g., which tactile cue was associated with amphetamine; location of tile in box). On the day after the final conditioning session

Table 1
Age differences in distance traveled and the percentage of time spent in corners and in the centre during 5 and 60 min of testing in a novel arena.

Behavioural measure	Time point	Experiment 1a		Experiment 1b	
		PND 30	PND 75	PND 30	PND 75
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Distance traveled (cm)	5 min	2521.12* ± 51.53	2341.74 ± 54.52	2538.02 ± 73.16	2561 ± 75.25
	60 min	11,327.29 ± 379.84	13,666.99 ± 434.35	13,233.58* ± 592.62	15,123.58 ± 512.82
% Time in centre	5 min	5.37 ± 0.71	3.82 ± 0.49	9.76 ± 0.95	7.87 ± 1.07
	60 min	2.97 ± 0.28	3.52 ± 0.36	4.87* ± 0.60	7.09 ± 0.83
% Time in corners	5 min	59.61 ± 1.37	59.86 ± 1.48	51.83 ± 2.01	52.78 ± 2.03
	60 min	78.29* ± 1.81	71.32 ± 1.94	73.52* ± 1.98	64.63 ± 2.93

* $p<0.05$, adolescent vs adult.

(P52 or P76), rats were placed into the CPP apparatus for 10 min with both tiles in a drug-free state. The percentage of time spent on amphetamine-paired side was used to measure CPP for amphetamine. Testing took place between 1000 h and 1600 h.

2.4. Statistics

For Experiment 1, analyses of locomotor activity and the percentage of time spent in the centre and corners during acclimation (session 1) were conducted using independent samples *t*-test for 5 and 60 min test intervals. Because testing spanned over an 8 h period, preliminary analyses investigated time of day effects on activity within and between groups using either time of day as a factor (median split) or as a covariate. In no instance was time of day a significant factor, and time of day is not included in the reported analyses. Analyses of locomotor activity during the test session (session 2) were conducted using a 2-way ANOVA with Age (P30, P75) and Drug (Amphetamine, Saline) as independent variables. The data for session 1 in Experiment 1a were lost due to a tracking problem for 8 adolescent rats, but all data were available for session 2. Thus, 8 rats were excluded from the analysis of behaviour during the acclimation session, but not from analyses during the test session. Pearson correlations were calculated for each condition separately to determine whether activity and measures of exploration (% time in centre and in corners) in the first 5 min and over the full 60 min of session 1 would predict activity after amphetamine administration and saline administration in session 2 at both ages.

For Experiment 2, total locomotor activity across saline and across amphetamine conditioning sessions was calculated for use as predictors of amphetamine CPP (% time spent on amphetamine-paired cue). We conducted multivariate regression analyses on CPP with age, distance after amphetamine and distance after saline included as predictors. Separate analyses were conducted for each dose. Alpha level for statistical significance was $p<0.05$; however, we also report *p* values less than 0.10, two-tailed.

3. Results

3.1. Experiment 1

3.1.1. Age differences in activity and exploration during acclimation to a novel arena

In Experiment 1a, compared to adults, adolescent rats were more active in the first 5 min [$t(66)=2.35$; $p=0.02$] and less active during 60 min [$t(66)=-3.94$; $p<0.0001$] of acclimation to a novel arena (session 1). Adolescents and adults did not differ at either time point for the percentage of time spent in the centre, although adolescents spent a smaller percentage of time in corners than did adults during 60 min of session 1 [$t(66)=2.56$; $p=0.01$] (see Table 1).

In Experiment 1b, adolescent and adult rats did not differ in the first 5 min of acclimation to a novel arena, but adolescents were less active than adults when 60 min of the session was considered [$t(34)=-2.41$; $p=0.02$] (see Table 1). Adolescents spent a lower percentage of time in the centre [$t(34)=-2.16$; $p=0.04$] and a greater percentage of time in corners [$t(34)=2.51$; $p=0.02$) compared to adults only during 60 min of session 1 (see Table 1). Overall, the results for Experiment 1b were similar to those for Experiment 1a (see Table 1).

3.1.2. Session 2: Locomotor activity after amphetamine or saline

In Experiment 1a, amphetamine-treated rats traveled more than saline-treated rats during 60 min of session 2 [$F(1,72)=143.79$; $p<0.0001$], but the greater distance traveled by adults compared to adolescents was not significant [$F(1,72)=3.15$; $p=0.08$] (see Fig. 1).

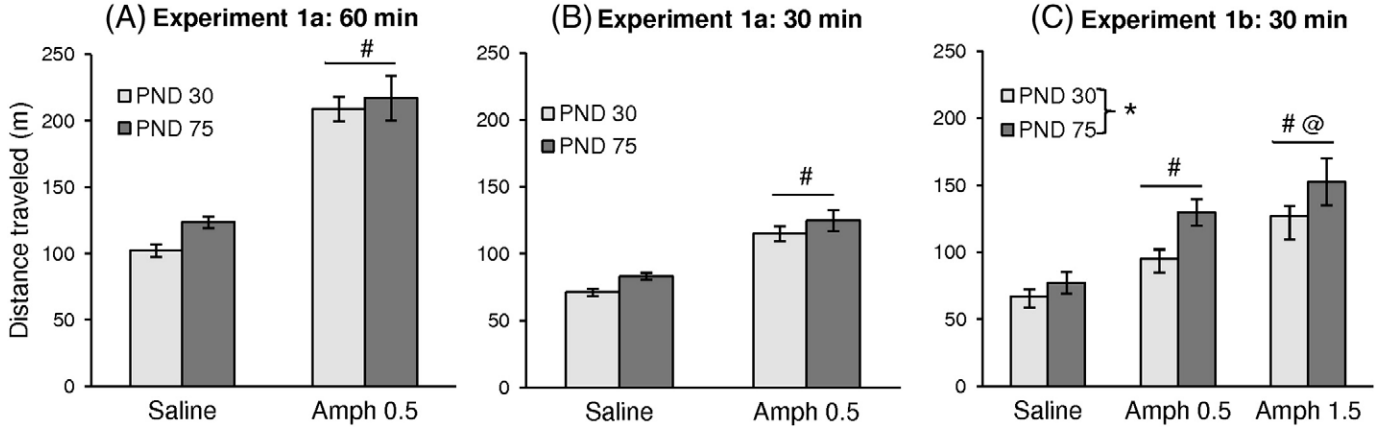

Fig. 1. Mean (±SEM) distance traveled after saline and amphetamine treatment in session 2 for (A) rats in Experiment 1a for which session 2 was 60 min and (C) for rats in Experiment 1b for which session 2 was 30 min. In (B) distance traveled for the first 30 min of session 2 in Experiment 1a is shown for comparison with Experiment 1b. *higher activity in PND 75 than PND 30, $p<0.05$; #higher activity compared to saline groups, $p<0.05$; @higher activity compared to 0.5 mg/kg groups, $p<0.05$.

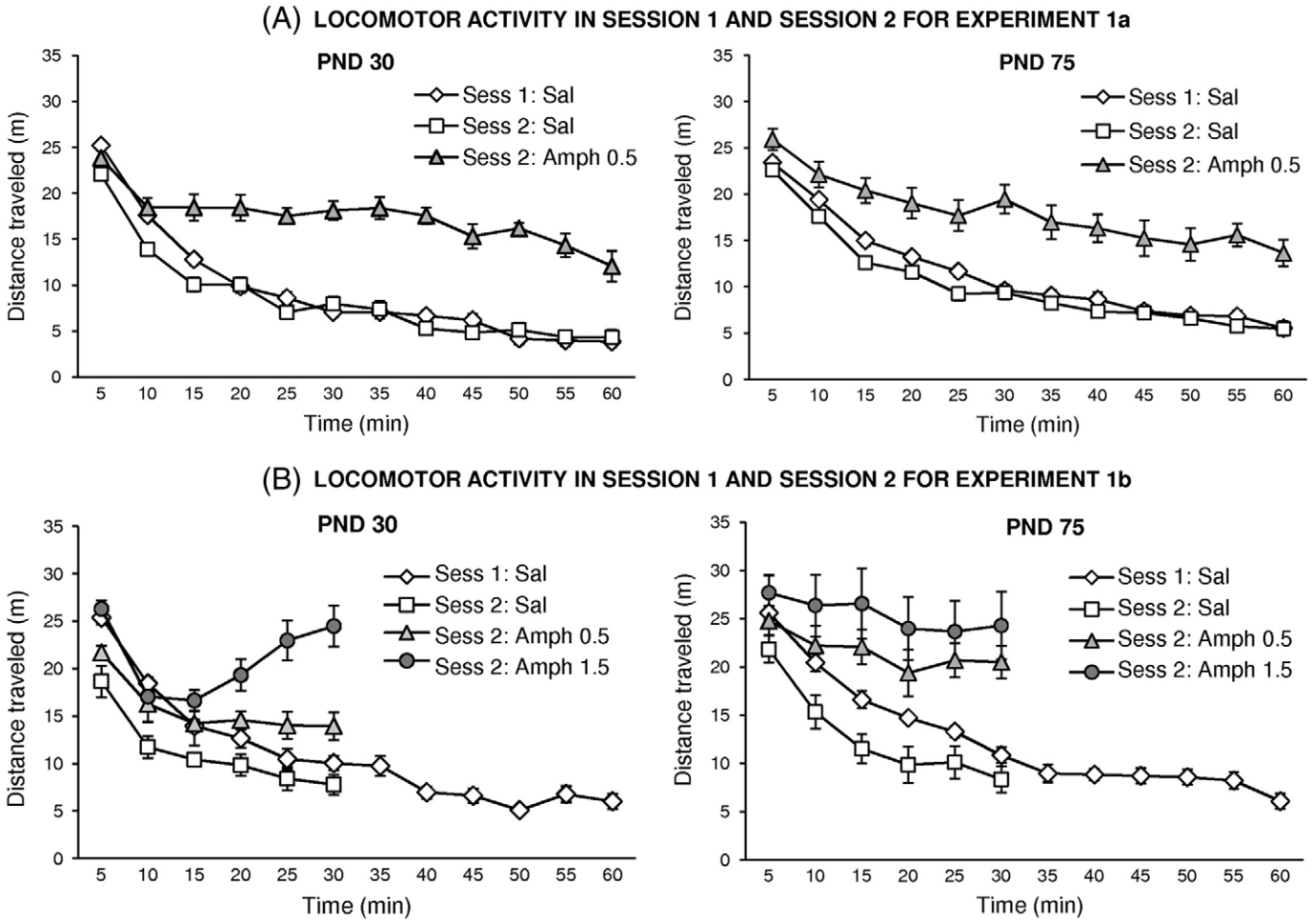

Fig. 2. Changes in distance traveled (mean, ±SEM) over the duration of session 1 and session 2 in 5 min intervals after treatment with saline or amphetamine for (A) Experiment 1a and (B) Experiment 1b.

In Experiment 1b, the distance traveled during session 2 increased according to dose [$F(2,30)=23.10$; $p<0.0001$; Post hoc t-tests indicated $0<0.5<1.5$, all $p\leq0.01$), and adults traveled more than adolescents [$F(2,30)=8.34$; $p=0.007$) (see Fig. 1). Distance traveled in 5 min intervals for all treatment groups and for both sessions is shown in Fig. 2.

3.1.3. Predicting locomotor activity in response to amphetamine

Pearson correlations were calculated to determine whether locomotor activity and the percentage of time spent in the centre and corners during acclimation (session 1) were associated with locomotor activity in session 2. Results of these analyses are presented in Table 2. Briefly, for both age groups, both experiments, and irrespective of treatment with amphetamine or with saline, the most consistent predictor of activity in session 2 was locomotor activity during 60 min of acclimation in session 1 (see Table 2 and Figs. 3 and 4). In contrast, neither percentage of time spent in the centre or corners, nor any measure in the first 5 min of acclimation consistently predicted activity in session 2 (see Table 2). There were no significant differences in the slopes of the relationship of activity in session 1 and session 2 between treatment groups within age or by age within treatment groups (all $p>0.05$).

3.2. Experiment 2: Predicting conditioned place preference (CPP)

Results of Experiment 1 showed that individual differences in activity were stable from session 1 to session 2 irrespective of drug treatment and age group. To confirm that individual differences are stable, we calculated Pearson r coefficients for activity over the three saline and the three amphetamine conditioning sessions. For the 0.5 mg/kg dose, activity during any one saline session was positively associated with activity during the other saline sessions (all $r\geq0.61$; all $p<0.0001$), and activity during any one amphetamine session was positively associated with activity during the other amphetamine sessions (all $r\geq0.60$; all $p<0.0001$). Similar observations were found for the 1.0 mg/kg dose, whereby activity during any one saline session was positively associated with activity in the other saline sessions (all $r\geq0.49$; all $p\leq0.001$), and activity during any one amphetamine session was positively associated with activity during the other amphetamine sessions (all $r\geq0.87$; all $p<0.0001$). Thus, the summed scores of activity in the saline and in the amphetamine sessions were used for regression analysis to predict CPP. In addition, one adult rat was identified as an outlier at the 0.5 mg/kg dose, based on a CPP score 3 SD below the mean. Therefore, all reported analyses are conducted with this rat removed.

To evaluate whether activity after saline and/or amphetamine predicted CPP and whether this relationship depended on age, we conducted a multiple regression with age, activity in saline sessions, and activity in amphetamine sessions as predictors. For the 0.5 mg/kg dose, the overall model accounted for 47% of the variance ($R^2=0.47$; $F(3,35)=10.40$; $p<0.0001$) in CPP. Age was not a significant predictor of CPP magnitude. CPP was negatively associated with activity in saline sessions (partial $r=-0.63$; $t=-4.78$; $p<0.0001$) and positively associated with activity in amphetamine sessions (partial $r=0.61$, $t=4.59$; $p<0.0001$) at the 0.5 mg/kg dose (see Fig. 5 for partial regression plots). Zero-order correlations are shown for

Table 2
Association between measures in session 1 and locomotor activity (distance traveled) in session 2 for Experiments 1a and 1b.

				Measure in 5 min or in 60 min of session 1					
				Locomotor activity		Exploration / anxiety			
				Distance traveled		% Time in centre		% Time in corners	
				5 min	60 min	5 min	60 min	5 min	60 min
Distance traveled in session 2	Experiment 1a 60 min	PND 30	Saline $n = 20$	$r = -0.17$ $p = 0.47$	**$r = 0.51$ $p = 0.02$**	$r = -0.17$ $p = 0.47$	$r = 0.28$ $p = 0.23$	$r = 0.20$ $p = 0.39$	$r = -0.22$ $p = 0.35$
			0.5 AMPH $n = 10$	$r = 0.41$ $p = 0.24$	**$r = 0.61$ $p = 0.06$**	$r = 0.26$ $p = 0.47$	**$r = 0.63$ $p = 0.05$**	$r = -0.28$ $p = 0.43$	$r = 0.20$ $p = 0.39$
		PND 75	Saline $n = 24$	$r = 0.15$ $p = 0.50$	**$r = 0.60$ $p = 0.002$**	$r = 0.21$ $p = 0.33$	$r = 0.31$ $p = 0.14$	$r = -0.23$ $p = 0.27$	$r = -0.25$ $p = 0.24$
			0.5 AMPH $n = 14$	$r = 0.11$ $p = 0.71$	**$r = 0.64$ $p = 0.01$**	$r = 0.08$ $p = 0.79$	$r = 0.09$ $p = 0.76$	$r = 0.04$ $p = 0.89$	$r = -0.35$ $p = 0.22$
	Experiment 1a 30 min	PND 30	Saline	$r = -0.11$ $p = 0.65$	**$r = 0.41$ $p = 0.07$**	$r = 0.01$ $p = 0.95$	$r = 0.29$ $p = 0.21$	$r = 0.02$ $p = 0.94$	$r = -0.24$ $p = 0.32$
			0.5 AMPH	$r = 0.39$ $p = 0.27$	$r = 0.32$ $p = 0.37$	**$r = 0.58$ $p = 0.08$**	$r = 0.34$ $p = 0.33$	$r = -0.45$ $p = 0.19$	$r = -0.04$ $p = 0.90$
		PND 75	Saline	$r = 0.21$ $p = 0.32$	**$r = 0.56$ $p = 0.004$**	$r = 0.31$ $p = 0.14$	$r = 0.23$ $p = 0.27$	$r = -0.20$ $p = 0.34$	$r = -0.23$ $p = 0.42$
			0.5 AMPH	$r = 0.12$ $p = 0.69$	**$r = 0.54$ $p = 0.05$**	$r = 0.08$ $p = 0.79$	$r = 0.02$ $p = 0.94$	$r = 0.15$ $p = 0.61$	$r = -0.12$ $p = 0.58$
	Experiment 1b 30 min	PND 30	Saline ($n = 6$)	$r = 0.25$ $p = 0.63$	$r = 0.69$ $p = 0.13$	$r = -0.27$ $p = 0.60$	$r = 0.42$ $p = 0.40$	$r = 0.15$ $p = 0.77$	$r = -0.06$ $p = 0.91$
			0.5 AMPH ($n = 6$)	**$r = 0.75$ $p = 0.09$**	**$r = 0.85$ $p = 0.03$**	$r = 0.45$ $p = 0.37$	$r = 0.50$ $p = 0.31$	**$r = -0.87$ $p = 0.03$**	$r = -0.43$ $p = 0.39$
			1.5 AMPH ($n = 6$)	$r = 0.17$ $p = 0.75$	**$r = 0.81$ $p = 0.05$**	$r = 0.72$ $p = 0.11$	$r = -0.49$ $p = 0.33$	$r = 0.16$ $p = 0.76$	$r = -0.55$ $p = 0.26$
		PND 75	Saline ($n = 6$)	**$r = 0.84$ $p = 0.04$**	**$r = 0.97$ $p = 0.002$**	**$r = 0.83$ $p = 0.04$**	**$r = 0.95$ $p = 0.003$**	**$r = -0.78$ $p = 0.07$**	**$r = -0.89$ $p = 0.02$**
			0.5 AMPH ($n = 6$)	$r = 0.49$ $p = 0.32$	**$r = 0.76$ $p = 0.08$**	$r = 0.03$ $p = 0.96$	**$r = 0.82$ $p = 0.05$**	**$r = -0.76$ $p = 0.08$**	**$r = -0.88$ $p = 0.02$**
			1.5 AMPH ($n = 6$)	$r = 0.31$ $p = 0.55$	$r = 0.43$ $p = 0.39$	$r = 0.59$ $p = 0.22$	$r = -0.67$ $p = 0.90$	$r = -0.52$ $p = 0.29$	$r = 0.16$ $p = 0.76$

Note: For Experiment 1a, correlations for measures in session 1 with distance traveled in session 2 are provided for both 60 min of session 2 and for 30 min of session 2 (shaded in grey) for purpose of comparisons with data in Experiment 1b for which session 2 was 30 min only. Correlations at $p<0.10$ are in larger bold font.

comparison in Table 3. The regression analysis of the 1.0 mg/kg amphetamine group data found no significant predictor of CPP.

As a test of the robustness of the relationship between activity in the conditioning sessions and 0.5 mg/kg amphetamine, regression analyses were conducted for each age group separately and for the three pairs of saline/amphetamine conditioning sessions separately. The overall regression model was significant each time (R^2 ranged from 0.25 to 0.55; all $p<0.02$), with the partial r values for activity in saline sessions always showing a significant negative association with CPP and the partial r values for amphetamine always showing a significant positive association with CPP.

4. Discussion

4.1. Age differences in locomotor activity and exploration of a novel test arena

The present results from two separate investigations in Experiment 1 provide partial support of Philipot and Wecker's (2008) conclusion that adolescents have higher locomotor activity than adults only in a short 5 min test, which they argued is a better reflection of novelty seeking than is activity in longer test sessions. Adolescent rats in the present study were more active than adults in the first 5 min of acclimation in Experiment 1a and not different from adults in Experiment 1b, indicating that activity in the first 5 min may not provide a reliable measure of age differences. In contrast, adolescent rats were less active than adults in 60 min tests in both Experiments 1a and 1b, which may better reflect age differences in general activity than in reactivity to novelty. According to Philipot and Wecker (2008), activity in a 60 min session involves both reactivity to novelty and habituation to novelty. Our data support this latter point in that activity dropped markedly after the first 5 min in both adolescent and adult rats. Thus, the age difference in activity during 60 min was in the opposite direction than that of the 5 min measure because of a more rapid habituation in the adolescents than in the adults. That our rats had received saline injections prior to habituation is unlikely to account for the pattern of age differences, given the consistency of our results with those reviewed by Philipot and Wecker (2008). Although we do not have no-injection controls here, when we compared activity in a 15 min habituation session in saline-injected and non-injected 45 days old ($n = 44$), there was no significant difference between the groups ($p>0.4$ at each 5 min interval and the total; unpublished observations).

Age differences in the percentage of time spent in the centre and in corners paralleled age differences in locomotor activity, and rats of both ages spent more time in corners and less time in the centre as activity decreased over the 60 min session in a novel arena. Others also have reported that adolescents spend more time in corners and less time in the centre than do adults (Hefner and Holmes, 2007; Lanier and Isaacson, 1977; Lynn and Brown, 2009), and these differences have been interpreted as age differences in exploratory behaviour (Hefner and Holmes, 2007; Lynn and Brown, 2009).

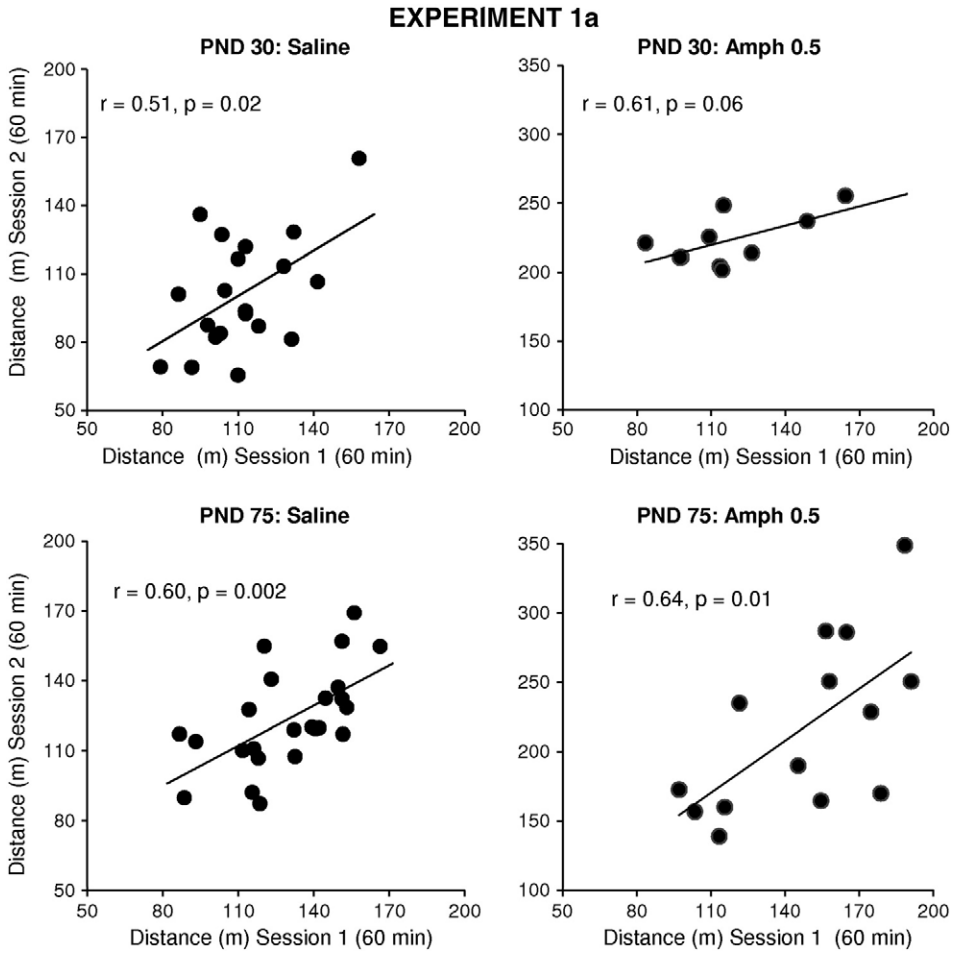

Fig. 3. The association between distance traveled in session 1 and distance traveled in session 2 for each Age and Drug Treatment group in Experiment 1b. Pearson *r* coefficient and *p* value are displayed for each scatter plot.

Increased time in corners is also thought to contribute to the reduced activity in adolescent rats (Lanier and Isaacson, 1977; Philipot and Wecker, 2008). Our results indicate that both adult and adolescent rats spend more time in corners as activity decreases, and that time in corners increases more in adolescent rats because activity also decreases to a greater extent. Reduced activity in adolescent rats during a 20 min session was also found in a circular arena in which time in corners is not a factor (e.g., Badanich et al., 2008), suggesting that test duration rather than shape of the apparatus may have a larger impact on age differences in activity. Thus, our finding of differences between adolescents and adults in the percent of time spent in corners and in the centre is more likely to be an indication of age differences in the rate of habituation to the test arena than an indication of age differences in either anxiety, exploration or novelty seeking. In addition, neither of these measures consistently predicted activity in session 2 in either adolescents or adults, which suggests that time in the centre and in corners may not be relevant for assessing sensitivity to psychostimulants at either age.

4.2. Activity in a novel arena as a predictor of activity after amphetamine

The only measure in session 1 in a novel arena that was associated consistently with activity after amphetamine in session 2 was 60 min of activity, a measure considered to reflect a stable trait of general activity (as described above). In forced novelty-paradigms, individual differences in activity in a novel arena are used as an index of individual differences in novelty seeking and the association between activity in a novel arena and activity in response to psychostimulants is taken as evidence that novelty seeking is associated with greater psychostimulant sensitivity. Nevertheless, 5 min of activity in a novel arena, which is considered to be a measure of novelty seeking (as described above), was not associated with activity after amphetamine. Furthermore, to conclude that rats showing high levels of activity in a novel arena are more sensitive to the locomotor-activating effects of amphetamine would require a significant leftward shift in the slope of the relationship between activity in session 1 and session 2 after amphetamine compared to the slope of the relationship between activity in session 1 and session 2 after saline (Quertemont et al., 2004). The only group for which the amphetamine-treated slope appeared to differ (though did not statistically) was the adolescent 0.5 mg amphetamine group, for which there was an apparent rightward shift, which would be interpreted as a reduced sensitivity to amphetamine in more active rats. In every comparison, the slopes were the same for both saline- and amphetamine-treated rats, suggesting a constant effect of amphetamine in each rat irrespective of age and baseline activity.

In sum, despite age differences in locomotor activity after saline and after amphetamine, there was no evidence of an age difference in the relationship between activity in session 1 and in session 2, with individual differences in neither age group explaining amphetamine-specific activity.

4.3. Predicting conditioned place preference (CPP) for amphetamine

Individual differences in activity in a novel arena also have been investigated as predictors of other effects of amphetamine than its

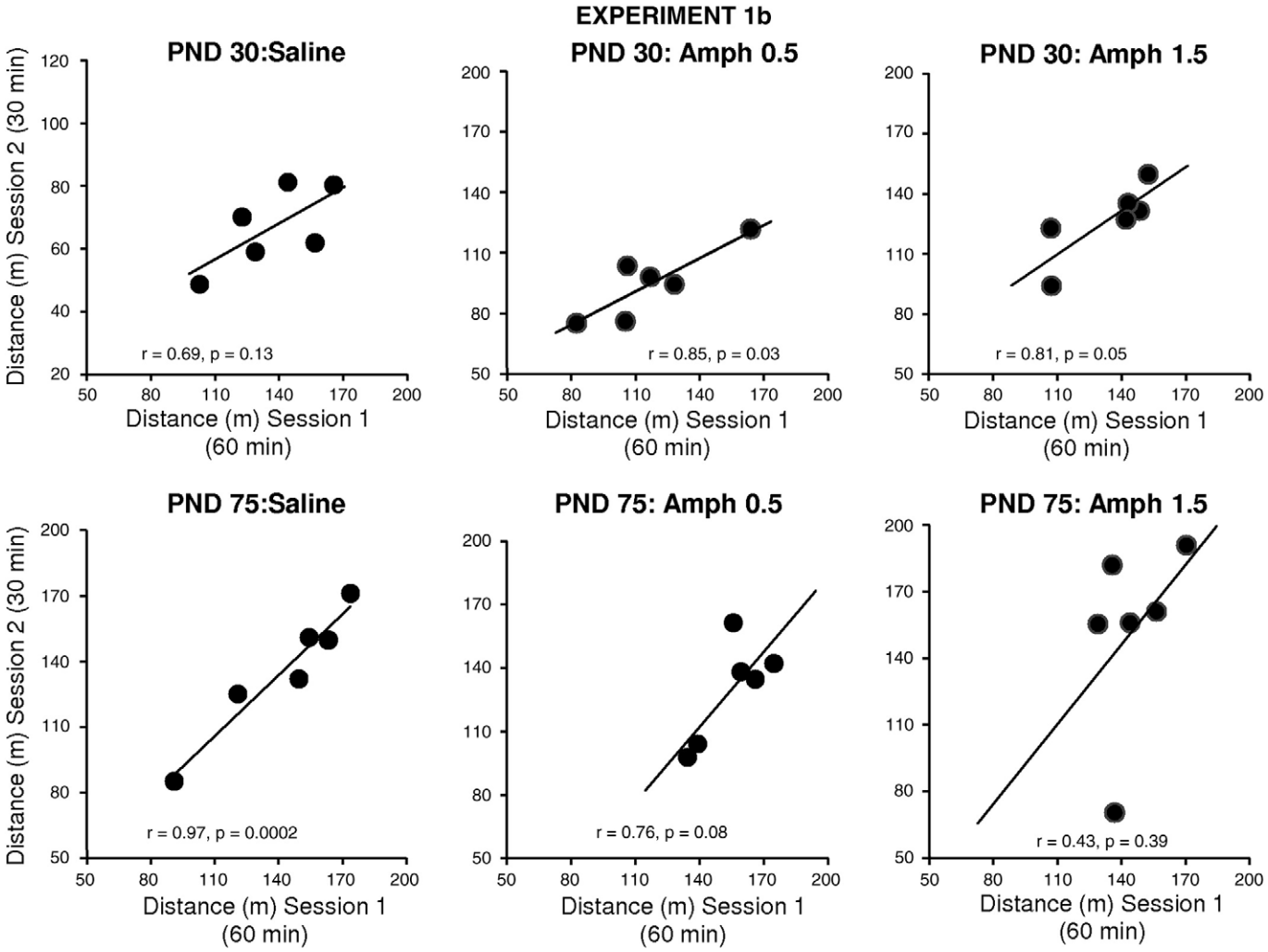

Fig. 4. The association between distance traveled in session 1 and distance traveled in session 2 for each Age and Drug Treatment group in Experiment 1b. Pearson *r* coefficient and *p* value are displayed for each scatter plot.

locomotor-stimulating effect (for rev, see Blanchard et al., 2009; Kabbaj, 2006). There is evidence that rats that are more active in a session in a novel arena exhibit enhanced self-administration of psychostimulants compared to less active rats (Cain et al., 2008; Piazza et al., 1989; Pierre and Vezina, 1997). There is much less evidence of an association between individual differences in locomotor activity and psychostimulant-induced CPP, with some studies finding no association (Dietz et al., 2007; Erb and Parker, 1994; Gong et al., 1996; Kosten and Miserendino, 1998) and some recent studies with mice finding a negative association between activity and CPP (Brabant et al., 2005; Shimosato and Watanabe, 2003). In addition, there is limited evidence for an association between psychostimulant-induced locomotor activity and CPP, with some studies failing to find an association (Brabant et al., 2005; Erb and Parker, 1994; Seymour and Wagner, 2008) and one study reporting that rats with a lower locomotor response to cocaine had greater CPP magnitude than more active rats, but only when cocaine was administered intravenously and not intra-peritoneally (Allen et al., 2007). We found that the ability to predict amphetamine-induced CPP is improved when activity in both saline and amphetamine sessions was considered and their shared variance was controlled using regression analysis. Further, age was not a significant predictor, with activity in saline and amphetamine sessions predicting CPP at both ages.

When controlling for shared variance, activity during saline sessions was negatively associated with amphetamine CPP and activity during amphetamine sessions was positively associated with amphetamine CPP at the 0.5 mg/kg dose. Thus, although activity after saline and activity after amphetamine are highly and positively associated, they are unique predictors of the magnitude of amphetamine CPP. That the relationship between locomotor effects of amphetamine and CPP-related effects of amphetamine are positively associated is not surprising, considering the reliance on closely related neural structures (Sellings and Clarke, 2003). Nevertheless, our finding of a negative relationship between CPP and saline counters the hypothesis that more active rats in a novel arena are more sensitive to psychostimulants (Blanchard et al., 2009). Our finding in rats with amphetamine is consistent with reports of a negative association between activity in a novel arena and the magnitude of cocaine CPP in mice (Brabant et al., 2005; Shimosato and Watanabe, 2003). In contrast, when novelty seeking is defined as a free-choice preference for novelty, the relationship between novelty seeking and amphetamine CPP is positive (Robinet et al., 1998). The apparent discrepancy with our findings of a negative association may be because activity in a novel arena in longer test sessions is not a measure of novelty seeking: When both activity in a novel arena and free-choice preference for novelty were investigated in the same individuals, they were not associated (Pelloux et al., 2004). Further, we observed the same relationship between saline activity and CPP irrespective of number of previous exposures to the arena, indicating that individual differences in activity are not a novelty-related measure but a general activity measure. The latter point is consistent with the evidence of different relationships observed when using

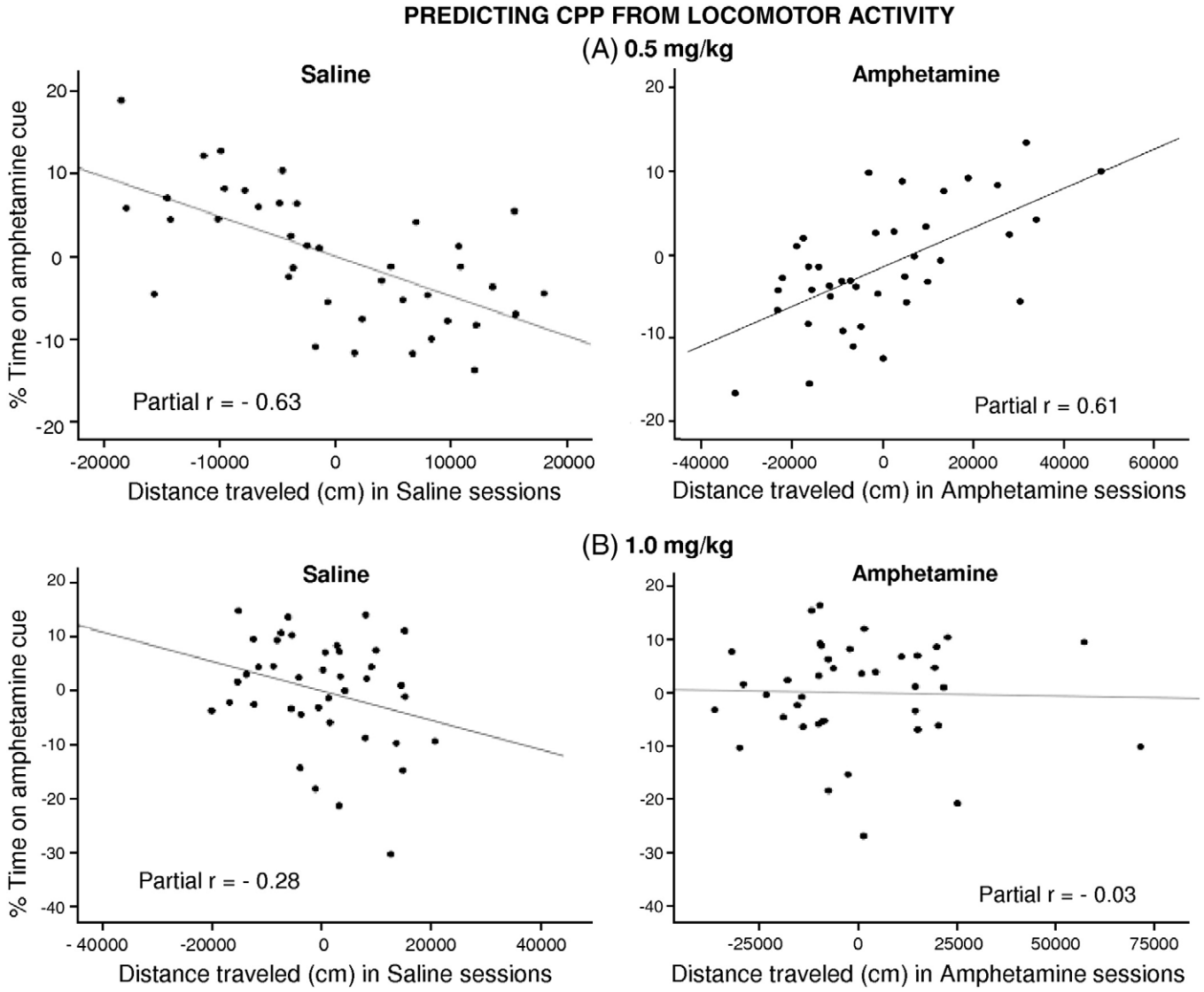

Fig. 5. Partial regression plot for activity in saline conditioning sessions (left) and in amphetamine conditioning sessions (right) as predictors of conditioned place preference. Partial *r* value is displayed for each scatter plot.

5 min of activity in a novel arena than when using 60 min of activity in the arena, with the first 5 min considered to be the better measure of reactivity to novelty (Philipot and Wecker, 2008).

Previous reports of no association between activity and magnitude of amphetamine CPP may be due to the dose used (Erb and Parker, 1994), as the relationship between activity in amphetamine and saline sessions and CPP was found only for the 0.5 mg/kg dose and not for the 1 mg/kg dose. Others have also found that individual differences in the behavioural effects of amphetamine are reduced at higher doses, likely due to ceiling effects (Brabant et al., 2005; Cain et al., 2008; Hooks et al., 1992; Kabbaj, 2006). Finally, in contrast to previous studies that have typically investigated the locomotor response to acute psychostimulant treatment and CPP (Allen et al., 2007; Brabant et al., 2005; Erb and Parker, 1994), we found that more variance in CPP magnitude was accounted for when multiple test sessions were considered together.

Table 3
Pearson *r* coefficient and *p* value for the association between distance traveled during amphetamine conditioning sessions or during saline conditioning sessions with magnitude of amphetamine CPP at the 0.5 and 1.0 mg/kg dose.

	0.5 mg/kg		1.0 mg/kg	
	Saline	AMPH	Saline	AMPH
Adolescents	***r* = −0.44**	*r* = 0.30	*r* = 0.27	*r* = −0.01
	***p* = 0.05**	*p* = 0.20	*p* = 0.24	*p* = 0.96
Adults	*r* = −0.34	***r* = 0.48**	*r* = −0.34	*r* = −0.27
	p = 0.16	***p* = 0.04**	*p* = 0.15	*p* = 0.25

Significant correlations ($p<0.05$) are in bold.

4.4. Conclusion

The results of the present experiments offer some resolution to inconsistencies between studies of age and of individual differences in novelty seeking and psychostimulant sensitivity. First, we showed that the direction of age differences in activity in a novel arena depends on the time frame measured, supporting Philipot and Weckers' (2008) conclusion that 5 min sessions are a measure of novelty seeking whereas longer sessions are a measure of general/trait activity, and thus explains why some studies have not found higher novelty seeking in adolescents. Although general trait activity (60 min), and not novelty activity (5 min), was associated with the locomotor-activating effects of amphetamine, the relationship cannot be described as greater trait activity predicting sensitivity to amphetamine because the same relationship was found between a

first and second session in saline-treated rats. Nevertheless, individual differences in general activity and amphetamine-induced activity predict CPP uniquely, and differently. Further, we showed that individual differences in trait activity are already established in adolescence, even though overall levels of activity are not yet 'adult-like'. Overall, individual differences in activity are associated with altered sensitivity to amphetamine CPP, but age differences in this measure may not account for the differential vulnerability to psychostimulants in adolescents than in adults.

Acknowledgements

We thank Harm Kelly for assistance in some of the data collection. This study is supported by the Natural Sciences and Engineering Research Council, the Canadian Foundation for Innovation, and the Ontario Innovation Trust (CMM) and an NSERC PGS fellowship (IZM).

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