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Individual differences in initial low-dose cocaine-induced locomotor activity and locomotor sensitization in adult outbred female Sprague–Dawley rats

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ABSTRACT

Sex and individual differences are important considerations when studying cocaine responsiveness. We have previously shown that male Sprague–Dawley (S–D) rats can be classified as low or high cocaine responders (LCRs or HCRs, respectively) based on their locomotor activity following a single dose of cocaine (10 mg/kg, i.p.). Further, this distinction was found to predict dopamine transporter function, cocaine-induced locomotor sensitization, cocaine conditioned place preference and motivation to self-administer cocaine. Here we investigated whether or not individual differences in cocaine-induced locomotor activity and locomotor sensitization exist in female S–D rats. Female rats exhibited a broad range of locomotor activation following either a 5 or 10 mg/kg cocaine injection, allowing for classification as LCRs or HCRs. When administered over 7 days, both doses induced locomotor sensitization in female LCRs/HCRs. However, the magnitude of effects produced by 5 mg/kg cocaine in female LCRs/HCRs was more comparable to that produced by 10 mg/kg in male LCRs/HCRs, both of which, interestingly, developed sensitization in this study. These findings suggest that female S–D rats, like male S–D rats, can be classified as LCRs/HCRs and highlight the importance of accounting for dose when studying sex and individual differences to the effects of cocaine.

1. Introduction

Gender/sex differences to the effects of cocaine exist in both humans and experimental animals. For example, female cocaine users report greater cocaine cue reactivity than males in a laboratory setting (Robbins et al., 1999), as well as greater feelings of nervousness and less intense feelings of euphoria and dysphoria than males following cocaine use (Kosten et al., 1996; Lukas et al., 1996; Lynch et al., 2002). Furthermore, female rats exhibit higher responses to the same dose of cocaine than male rats on a number of behavioral measures including: locomotor activity, rearing and other stereotyped behaviors (Festa et al., 2004; Sell et al., 2000; Walker et al., 2001); and they develop greater cocaine-induced behavioral sensitization than male rats (Hu and Becker, 2003). Research also has shown sex differences in the rewarding and reinforcing effects of cocaine. For example, compared to male rats, female rats more readily develop cocaine conditioned place preference (CPP) (Russo et al., 2003), more rapidly acquire cocaine self-administration (Lynch and Carroll, 1999; Lynch, 2006), display greater levels of reinstatement of cocaine self-administration (Lynch and Carroll, 2000; Lynch 2006), and exhibit higher break-points on a progressive ratio (PR) schedule of cocaine reinforcement (Roberts et al., 1989).

Along with sex differences, the study of individual differences is also an important consideration for cocaine research. A well-characterized model of individual differences is low and high responders to novelty (LRs or HRs, respectively) that classifies outbred rats based on their locomotor response to an inescapable novel open-field environment (Piazza et al., 1989; Stead et al., 2006). While results of acute psychomotor stimulant-induced locomotor activity in male LRs and HRs have been variable (Piazza et al., 1989; Pierre and Vezina, 1997; Carey et al., 2003; Quertemont et al., 2004), female HRs were found to exhibit greater cocaine-induced locomotor activity than female LRs (Sell et al., 2005). Further, LR or HR classification, in both male and female rats, has been shown to predict psychomotor stimulant reinforcement under particular conditions (Piazza et al., 1989; Pierre and Vezina, 1997; Klebaur et al., 2001).

Previously, we have reported that the magnitude of acute cocaine-induced activation in male rats, which is not related to their novelty response, predicts differential individual responsiveness to repeated cocaine administration. Specifically, adult outbred male Sprague–Dawley (S–D) rats can be classified as either low or high cocaine responders (LCRs or HCRs, respectively) based on the median split of their open-field locomotor activity during the first 30 min after a single relatively low dose of cocaine (10 mg/kg, i.p.; Mandt et al., in press; Allen et al., 2007; Briegleb et al., 2004; Gulley et al., 2003; Sabeti et al.,

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2002, 2003). Furthermore, the differences in initial locomotor responsiveness are related to cocaine's ability to inhibit the dopamine transporter (DAT; HCRs>LCRs), an interaction critical for cocaine's activating and rewarding effects (Sabeti et al., 2002, 2003; Chen et al., 2006). Interestingly, with repeated low-dose cocaine administration, LCRs and HCRs also differ in cocaine-induced locomotor sensitization and CPP. LCRs, but not HCRs, developed cocaine-induced locomotor sensitization, paralleled by an increase in the ability of cocaine to inhibit in vivo DAT function (Sabeti et al., 2003). LCRs also exhibited greater responses than HCRs to the rewarding effects of cocaine (Allen et al., 2007). In addition, LCRs were recently found to exhibit greater break-points than HCRs on a PR schedule of cocaine reinforcement (Mandt et al., in press).

Thus far, all of our work characterizing LCRs and HCRs has been done with male rats and whether or not these differences exist in female S–D rats is unknown. If they do, this would allow selective breeding for stable phenotypic LCR and HCR lines, as has been accomplished with LRs and HRs (Stead et al., 2006). Furthermore, female LCRs/HCRs could also be a useful experimental model for studying cocaine-abuse susceptibility. The purpose of the present study was to determine the range of individual locomotor activity responses in adult female S–D rats induced by low doses of cocaine and the relationship between this initial cocaine-induced locomotor activity and the development of locomotor sensitization.

2. Materials and methods

2.1. Animals

Twenty-one outbred female S–D rats were birthed from pregnant female rats purchased from Harlan Industries (Indianapolis, IN) and an additional 37 female and 24 male S–D rats were purchased directly from Harlan Industries. At the start of the study female rats weighed between 240–300 g, and male rats weighed between 300–340 g. Rats were housed individually on a 12-h light/dark cycle (lights on at 0700 h) with *ad libitum* access to food and water at the University of Colorado Denver (UCD)—Downtown Campus. Rats were housed in the behavioral testing room for one week before testing began and for the duration of the experiment. All behavioral testing was conducted during the light phase of the light/dark cycle. All animal care and use procedures were in strict accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the UCD-Downtown Campus Institutional Animal Care and Use Committee.

2.2. Behavioral testing

Locomotor activity was recorded using CPP chambers (Med Associates, St. Albans, VT) containing 15 photobeams spaced approximately 5 cm apart throughout the chamber. A computer program (MedPC-IV) was designed to record photobeam breaks and log them into 1-min bins. Locomotor activity was defined as horizontal movement resulting in consecutive photobeam breaks summed into 10-min intervals. Non-consecutive beam breaks, which may be more likely to monitor stereotypy, were not analyzed.

For each session, rats were placed in the CPP chambers and allowed to habituate for 60 min, during which time baseline locomotor activity was recorded. Locomotor novelty response was assessed over the first 60 min on the first day of testing. After 60 min, rats were removed, injected i.p. (1 ml/kg) with either vehicle (0.9% saline) or (–) cocaine hydrochloride (generous gift from the National Institute on Drug Abuse), and then returned to the recording chamber for an additional 60 min. Locomotor activity over the first 30 min after cocaine injection was summed, and the median split was used to determine LCRs and HCRs.

For the sensitization paradigm, rats were injected once-daily with either vehicle (saline 1 ml/kg; female control group, $n=8$; male control group, $n=8$) or cocaine (female: 5 mg/kg, $n=18$ and 10 mg/kg, $n=16$; male: 10 mg/kg, $n=16$) for 7 consecutive days (days 1–7), followed by 6 days with no treatment and a final injection on day 14. Cocaine-induced locomotor activity on day 1 was used to classify rats as LCRs/HCRs. Locomotor activity following injection of vehicle (control rats) or cocaine (LCRs/HCRs) was recorded on days 1, 3, 5, 7 and 14. Following the injection on days 2, 4 and 6, rats were immediately returned to their home cage. Four cohorts of rats were tested each day according to a Latin Squares design with test order (i.e. first, second, third, or fourth) as the determining variable to control for potential time of day effects on cocaine responsiveness. Sensitization was defined as an increase in cocaine-induced locomotor activity on each day of the repeated treatment relative to cocaine-induced locomotor activity on day 1.

A cocaine dose–response curve was collected in another group of female S–D rats ($n=16$). Locomotor activation following administration of vehicle and three doses of cocaine (in testing order: vehicle, 5, 1 and 20 mg/kg) was measured on separate days, 24 h apart, using the procedure described above.

Two male rats were excluded from the analysis. One rat was excluded due to health concerns on day 1; and the other rat was excluded because its locomotor activity score became the median, preventing classification as LCR or HCR by median split.

2.3. Data analysis

All statistical analyses were conducted using SPSS, version 16.0. Kolmogorov–Smirnov normality testing confirmed that initial (day 1) cocaine-induced locomotor activity data for both female and male rats

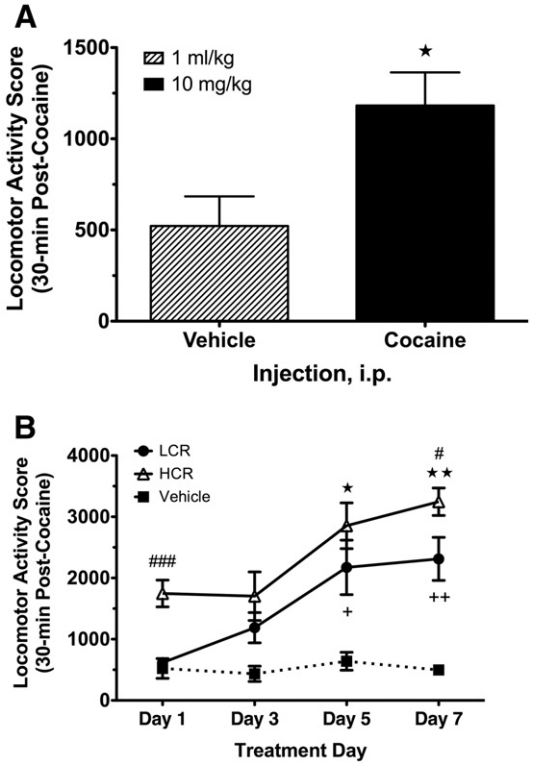

Fig. 1. Individual differences in locomotor activity and locomotor sensitization induced by 10 mg/kg cocaine in female S–D rats. Vehicle (saline, 1 ml/kg) or cocaine (10 mg/kg) was injected i.p. Mean values ± SEM for the sum of locomotor activity over the first 30-min post-injection are shown. A Vehicle ($n=8$) and cocaine-induced locomotor activity in the entire group of female rats ($n=16$), $^{*}p<0.05$. B Cocaine-induced locomotor activity on days 1, 3, 5 and 7 of the seven-day, once-daily treatment are shown as mean values ± SEM for rats classified as LCRs ($n=8$) and HCRs ($n=8$); $^{\#}p<0.05$, $^{\#\#\#}p<0.001$, LCR vs. HCR, for between-group analyses;. $^{*}p<0.05$, $^{**}p<0.01$, HCR; $^{+}p<0.05$, $^{++}p<0.01$, LCR, for within-group analyses. Each day of repeated treatment was compared to day 1.

were normally distributed. Independent samples *t*-test (vehicle vs. 10 mg/kg cocaine; Fig. 1) and one-way repeated-measures ANOVA (cocaine dose–response curve; Fig. 2) were used to compare acute vehicle and cocaine-induced locomotor activity. Two-way RMANOVA (with session as the repeated measure) was used in all sensitization analyses, with simple one-way RMANOVA or ANOVA to explore interaction effects. Post-hoc pairwise comparisons were conducted according to the Least Significant Difference (LSD) method. One control group was used for both sensitization analyses in female rats. When the assumption of sphericity was violated for a particular repeated-measures analysis, as revealed by Mauchly's test statistic, tests of significance were based on the more conservative Huynh–Feldt corrected degrees of freedom. The symbol [a] indicates Huynh–Feldt corrected values throughout the text. Data are presented as mean ± standard error of the mean (SEM), throughout.

3. Results

3.1. Individual differences in initial cocaine responsiveness and cocaine-induced locomotor sensitization in female S–D rats: 10 mg/kg cocaine

Our previous studies used 10 mg/kg cocaine to classify male S–D rats as LCRs/HCRs and to assess cocaine sensitization. Consequently, this same dose of cocaine was used first to explore potential individual differences in cocaine-induced locomotor activity and sensitization in female S–D rats. Administration of 10 mg/kg cocaine (i.p.) resulted in a significant increase in locomotor activity in female S–D rats, compared to vehicle-treated controls (Fig. 1A). Female rats ($n=16$) exhibited a five-fold range of locomotor activity scores (542–3014) during the first 30 min following the initial injection of cocaine. A median split of the group's cocaine-induced locomotor activity scores (median = 881) allowed us to classify rats below the median as LCRs and above the median as HCRs. Similar to our previous findings with male LCRs and HCRs (Briegleb et al., 2004; Gulley et al., 2003), female LCRs and HCRs did not differ significantly in their locomotor responses during the first 60 min in a novel environment (data not shown). In contrast, HCRs exhibited significantly greater cocaine-induced locomotor activity than LCRs on the first day of testing (Fig. 1B, day 1). Initial cocaine-induced locomotor activity for LCRs and HCRs was 618 ± 50 and 1747 ± 218, respectively.

Both LCRs and HCRs developed locomotor sensitization with repeated administration of 10 mg/kg cocaine (Fig. 1B). Analysis with two-way RMANOVA revealed significant main effects for session [$F(3, 63)=13.35$, $p<0.001$], classification [$F(2, 21)=39.54$, $p<0.001$] and a session x classification interaction [$F(6, 63)=3.19$, $p<0.01$]. HCRs exhibited significantly greater cocaine-induced activity than LCRs on days 1 ($p<0.001$) and 7 ($p<0.05$) of repeated cocaine treatment and significantly greater locomotor activity than vehicle-treated rats on all test days. LCRs were not significantly different from vehicle-treated rats on days 1 or 3, but their cocaine-induced locomotor activity increased over days such that it was significantly greater than controls on days 5 ($p<0.01$) and 7 ($p<0.001$) of repeated cocaine treatment. Within classification analysis with one-way RMANOVA revealed significant effects of session for both LCRs [$F(3, 21)=6.06$, $p<0.01$] and HCRs [$F(3, 21)=8.03$, $p<0.01$]. Compared to their initial cocaine-induced locomotor activity, LCRs exhibited a strong trend for increased cocaine-induced activity on day 3 ($p=0.051$) and significantly greater activity on days 5 ($p<0.05$) and 7 ($p<0.01$). Compared to day 1, HCRs did not exhibit increased cocaine-induced locomotor activity on day 3, but did exhibit significantly increased activity on days 5 ($p<0.05$) and 7 ($p<0.01$). Vehicle-treated control rats' locomotor activity did not differ on any of the test days.

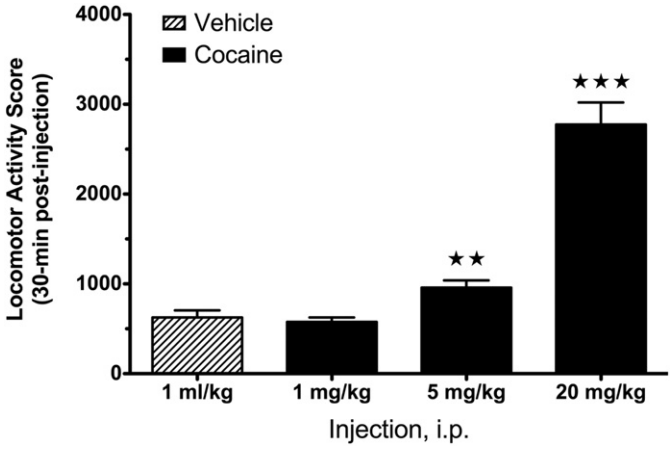

Fig. 2. Cocaine dose–response in female S–D rats. Rats ($n=16$) were administered vehicle (saline, 1 ml/kg) and 5, 1, and 20 mg/kg cocaine, respectively, over four separate sessions. Sessions were separated by 24 h. Mean values ± SEM for the sum of locomotor activity over the first 30-min post-injection are shown; $^{**}p<0.01$, $^{***}p<0.001$).

LCRs/HCRs were also tested for locomotor sensitization with a cocaine challenge (10 mg/kg, i.p.) on day 14, following a 6-day withdrawal, to determine if changes observed on day 7 were persistent. Both LCRs and HCRs exhibited sustained elevations in the magnitude of cocaine-induced locomotor activation on this day. While there was no further increase in HCRs cocaine-induced locomotor activity from day 7 (mean = 3245 ± 225) to day 14 (mean = 3621 ± 530), LCRs exhibited a significant increase in cocaine-induced locomotor activity during this time (day 7 mean = 2312 ± 352; day 14 mean = 3562 ± 229; paired samples *t*-test, $p<0.05$).

3.2. Cocaine dose–response in female S–D rats

The cocaine dose–response relationship was explored in another group of female S–D rats to determine a) if a lower dose of cocaine could be used to classify female rats as LCRs/HCRs and b) any ceiling effects for cocaine-induced locomotor activity in HCRs (Fig. 2). Analysis with one-way RMANOVA revealed a significant main effect of dose [$^{a}F(1.4, 20.5)=69.73$, $p<0.001$]. Cocaine-induced locomotor activity (30-min) was significantly greater than vehicle after 5 and 20 mg/kg cocaine, but not after 1 mg/kg.

3.3. Individual differences in initial cocaine responsiveness and cocaine-induced locomotor sensitization in female S–D rats: 5 mg/kg cocaine

The 5 mg/kg i.p. cocaine dose was chosen to further assess individual differences in initial cocaine-induced locomotor activity and cocaine-induced sensitization in another group of female rats ($n=18$). Rats exhibited a four-fold range of locomotor activity scores (313–2261) during the first 30-min following the initial injection of cocaine and were classified as LCRs/HCRs by median split (median = 730; individual data from LCRs/HCRs on day 1 are shown in Fig. 3A). Again, LCRs and HCRs did not differ in their response to a novel environment (data not shown). Initial cocaine-induced locomotor activity scores for LCRs and HCRs were 539 ± 42 and 1326 ± 206, respectively. Analysis with two-way RMANOVA revealed main effects for session [$^{a}F(2.6, 60.8)=8.77$, $p<0.001$], classification [$^{a}F(2, 23)=11.33$, $p<0.001$] and a session x classification interaction [$^{a}F(5.3, 60.8)=2.90$, $p<0.05$].

HCRs exhibited significantly greater cocaine-induced locomotor activity than LCRs on day 1 ($p<0.01$), but not day 7 of testing, and significantly greater locomotor activity than vehicle-treated control rats on all test days. LCRs were not significantly different from control rats on day 1, but increased their cocaine-induced activity over days such that they exhibited significantly greater locomotor activity than controls on days 3 ($p<0.05$), 5 ($p<0.05$) and 7 ($p<0.01$) of repeated cocaine treatment. Analysis with one-way RMANOVA revealed significant effects of session for both LCRs [$^{a}F(2.1, 17.0)=7.04$, $p<0.01$] and HCRs [$F(3, 24)=4.57$, $p<0.05$]. Compared to their initial

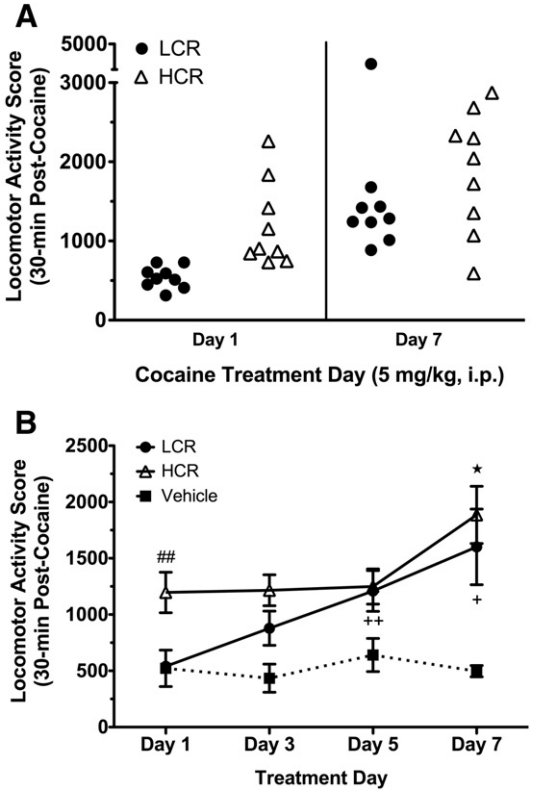

Fig. 3. Individual differences in locomotor activity and locomotor sensitization induced by 5 mg/kg cocaine in female S–D rats. A Comparison of individual locomotor activity scores for LCRs (n=9) and HCRs (n=9) on days 1 and 7 of the repeated cocaine treatement. B Cocaine-induced locomotor activity on days 1, 3, 5, and 7 of the seven-day, once-daily treatment are shown as mean values±SEM. The control group (vehicle) is the same as presented in Fig. 1. B; $^{\#\#}p<0.01$, LCR vs. HCR, for between-group analyses. $^{*}p<0.05$, HCR; $^{+}p<0.05$, $^{++}p<0.01$, LCR, for within-group analyses. Each day of repeated treatment was compared to day 1.

cocaine-induced locomotor activity, LCRs exhibited a strong trend for increased cocaine-induced activity on day 3 (p=0.051) and significantly greater activity on days 5 ($p<0.01$) and 7 ($p<0.05$). Compared to day 1, HCRs did not exhibit significantly different cocaine-induced locomotor activity on days 3 or 5, but did exhibit significantly increased activity on day 7 ($p<0.05$).

As with the 10 mg/kg cocaine experiment, the increased magnitude of cocaine's effects remained on day 14, following a 6-day withdrawal. However, with this lower drug dose, neither LCRs (day 7 mean=1607±306; day 14 mean=1822±300) nor HCRs (day 7 mean=1956±234; day 14 mean=2166±250) showed a significant increase in the magnitude of cocaine's effects on day 14.

3.4. Individual differences in initial cocaine responsiveness and cocaine-induced locomotor sensitization in male S–D rats: 10 mg/kg cocaine

Male S–D rats (n=14) exhibited a wide range of locomotor activity scores (94–1895) during the first 30-min following the initial injection of cocaine (10 mg/kg, i.p.), allowing them to be classified as LCRs/HCRs by median split (median=636; individual data from LCRs/HCRs on day 1 are shown in Fig. 4A). Neither LCRs nor HCRs differed in their response to a novel environment (data not shown). Initial cocaine-induced locomotor activity scores for LCRs and HCRs were 390±60 and 972±153, respectively. Analysis with two-way RMANOVA revealed main effects for session [$^{a}F(2.6, 19.4)=19.06$, $p<0.001$], classification [$F(2, 19)=35.48$, $p<0.001$] and a session x classification interaction [$^{a}F(5.2, 49.4)=5.85$, $p<0.001$].

HCRs exhibited significantly greater cocaine-induced locomotor activity than LCRs on days 1 ($p<0.05$) and 5 ($p<0.05$), but not day 7 of testing, and significantly greater locomotor activity than vehicle-treated control rats on all test days. LCRs were not significantly different from control rats on days 1 or 3, but increased activity over days such that they exhibited significantly greater locomotor activity on days 5 ($p<0.001$) and 7 ($p<0.001$) of repeated cocaine treatment. Analysis with one-way RMANOVA revealed significant effects of session for both LCRs [$F(3, 18)=23.15$, $p<0.001$] and HCRs [$F(3, 18)=5.29$, $p<0.01$]. Compared to their initial cocaine-induced locomotor activity, LCRs exhibited significantly increased cocaine-induced activity on days 3 ($p<0.01$), 5 ($p<0.001$) and 7 ($p<0.001$). Compared to day 1, HCRs did not exhibit significantly different cocaine-induced locomotor activity on day 3, but did exhibit significantly increased activity on days 5 ($p<0.05$) and 7 ($p<0.05$). Vehicle-treated control rats' locomotor activity did not differ on any of the test days.

As seen in the female LCR/HCR experiments, the increased magnitude of cocaine's effects remained in both the male LCRs and HCRs on day 14, following a 6-day withdrawal. However, similar only to the 5 mg/kg female experiment, neither LCRs (day 7 mean=1712±183; day 14 mean=2184±277) nor HCRs (day 7 mean=1929±179; day 14 mean=2468±274) showed a significant increase in the magnitude of cocaine's effects on day 14.

Locomotor activity of the female rats, treated with 5 or 10 mg/kg cocaine, was compared to that of the male rats treated with 10 mg/kg cocaine over the 7-day repeated treatment. For female rats treated with 10 mg/kg cocaine, analysis with two-way RMANOVA revealed significant main effects of session [$F(3, 78)=25.04$, $p<0.001$] and sex [$F(1, 26)=24.83$, $p<0.001$], but not a session x sex interaction. In contrast, for the female rats treated with 5 mg/kg cocaine, this analysis revealed a significant effect of session [$^{a}F(2.64, 73.96)=27.11$, $p<0.001$], but neither a significant effect of sex nor a session×sex interaction.

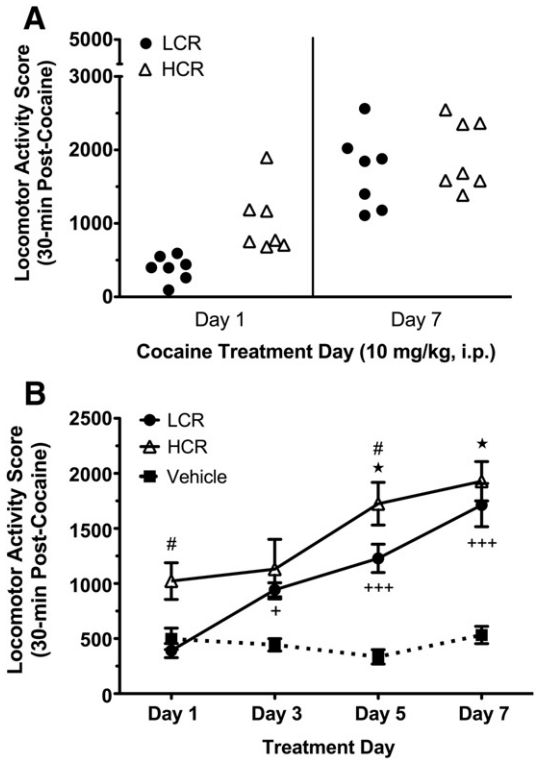

Fig. 4. Individual differences in cocaine-induced locomotor activity and locomotor sensitization induced by 10 mg/kg cocaine in male S–D rats. A Comparison of individual locomotor activity scores for LCRs (n=7) and HCRs (n=7) on days 1 and 7 of the repeated cocaine treatement. B Cocaine-induced locomotor activity on days 1, 3, 5 and 7 of the seven-day, once-daily treatment are shown as mean values±SEM; $^{\#}p<0.05$, LCR vs. HCR, for between-group analyses; $^{*}p<0.05$, HCR; $^{+}p<0.05$, $^{+++}p<0.001$, LCR, for within-group analyses. Each day of repeated treatment was compared to day 1.

4. Discussion

This study exploring individual differences in cocaine-induced activation in adult outbred female S–D rats is an important extension of our previous findings in male rats. Our current results, similar to those in male rats (Fig. 4A; Mandt et al., in press; Allen et al., 2007; Briegleb et al., 2004; Gulley et al., 2003; Sabeti et al., 2002, 2003), indicate that individual differences to the initial locomotor-stimulating effects of low-dose cocaine (5 and 10 mg/kg, i.p.) exist in female rats. However, the magnitude of locomotor activation induced by 5 mg/kg cocaine in female rats was more comparable to that induced by 10 mg/kg in male rats. In addition, cocaine-induced locomotor activity was significantly different between LCRs and HCRs on day 1, but not day 7, in female rats treated with 5 mg/kg, but not 10 mg/kg, cocaine; a finding similar to present and previous results for male LCR/HCR rats treated with 10 mg/kg cocaine. Female rats classified as HCRs, as well as LCRs, developed locomotor sensitization to cocaine at both doses tested, whereas male HCR rats typically have not (Mandt et al., in press; Allen et al., 2007; Sabeti et al., 2003). Interestingly, however, in the present study male LCRs and HCRs both developed locomotor sensitization. Thus, our findings suggest that it is more appropriate to use different doses of cocaine to study female (5 mg/kg) vs. male (10 mg/kg) LCR/HCR rats, because 10 mg/kg cocaine may not differentiate female rats in a similar manner. Importantly, our results also suggest that it will be feasible for future studies to selectively breed LCR and HCR rat lines for the investigation of cocaine-abuse susceptibility.

Female rats have been shown to be more sensitive to cocaine's activating effects than male rats (Festa et al., 2004; Sell et al., 2000; Walker et al., 2001). We initially classified and tested female LCR and HCR rats with a 10 mg/kg dose of cocaine because this is the dose that we have studied most often in male LCRs/HCRs (Mandt et al., in press; Allen et al., 2007; Briegleb et al., 2004; Gulley et al., 2003; Sabeti et al., 2002, 2003). However, when we tested 5 mg/kg cocaine in a second experiment, total locomotor activity scores for female LCRs/HCRs were more similar to values reported previously for 10 mg/kg cocaine-induced male LCR/HCR activity in the CPP boxes (Mandt et al., in press; Allen et al., 2007) and not significantly different from males in the present study that received 10 mg/kg cocaine. In contrast, total locomotor activity scores for female rats treated with 10 mg/kg cocaine were significantly greater than those of male rats, suggesting the lower dose of cocaine is functionally more comparable in female rats.

With 10 mg/kg cocaine, female LCRs and HCRs both developed cocaine-induced locomotor sensitization by day 5. However, only LCRs exhibited a further increase in the magnitude of the effect induced by this dose following a 6-day withdrawal (day 14). Both of these observations are unique from previous findings with male LCRs/HCRs (Sabeti et al., 2003). However, this differential result on day 14 in female LCRs and HCRs could have been due to a ceiling effect in HCRs, as a higher dose of cocaine (20 mg/kg) in the dose–response experiment produced locomotor activity similar to the 10 mg/kg cocaine, day 14 challenge in LCRs/HCRs. Further, neither female LCRs/HCRs treated with 5 mg/kg cocaine nor male LCRs/HCRs treated with 10 mg/kg cocaine in the present study exhibited greater locomotor activity on day 14.

On days 3 and 5 of repeated treatment with 5 mg/kg cocaine, female LCRs and HCRs looked similar to male LCRs and HCRs in previous studies, with LCRs, but not HCRs, developing locomotor sensitization (Mandt et al., in press; Allen et al., 2007; Sabeti et al., 2003). By day 7, however, female HCRs also began to develop locomotor sensitization. Further, male HCRs in this study also developed sensitization following repeated treatment with 10 mg/kg cocaine, a finding not observed previously (Allen et al., 2007; Sabeti et al., 2003), including in a recent study that used an identical treatment regimen and CPP chambers (Mandt et al., in press). However, in one previous study using CPP chambers (Allen et al., 2007), male HCRs showed a trend ($p=0.09$) toward increased activity on day 7 of repeated 10 mg/kg cocaine administration. On the other hand, we also note that the group of male HCRs used here consisted of fewer rats with cocaine-induced locomotor activity scores >1000 than we typically see (e.g. Mandt et al., in press). Regardless, the important finding presented here is that individual differences in initial cocaine-induced locomotor activity and locomotor sensitization exist in female rats and that a lower cocaine dose appears more appropriate for comparison with males. Together, these results suggest that both female and male LCRs more readily develop cocaine-induced locomotor sensitization than HCRs, although HCRs can also develop sensitization under some conditions.

Several biochemical measures have been found to correlate with the behavioral differences measured in male LCRs and HCRs. For example, initial cocaine-induced locomotor activity is positively correlated with cocaine's inhibition of DA clearance by the DAT in vivo (Sabeti et al., 2002) and is also predictive of [^{3}H]DA uptake measured ex vivo in striatal synaptosomes 30 min after cocaine (HCRs>LCRs; Briegleb et al., 2004). Sex differences in cocaine's effects on brain dopaminergic systems have also been reported. Cocaine induces significantly more DA efflux in the striatum of female rats than of male rats (Walker et al., 2006). Furthermore, estrogen potentiates cocaine's effects by acting directly within the striatum (Becker, 1999; Becker et al., 2001), and it is unknown if this effect might differentially affect female LCRs and HCRs. Thus, it will be interesting to determine in the future if biochemical differences, like those found in male LCRs and HCRs, also exist in female LCRs and HCRs administered 5 mg/kg cocaine.

Estrous cycle stage has been shown to affect the locomotor-stimulating effects of cocaine (Festa et al., 2004; Sell et al., 2000; Walker et al., 2001). Our aim in this initial study was to determine if individual differences were detected in the activating and sensitizing effects of cocaine in female outbred S–D rats. It will be key for future studies to examine potential differences in the effects of the estrous cycle on these individual differences.

It is also important to note that rats classified as LCRs/HCRs are not the same as LR/HR rats classified by their response to novelty. LR or HR classification can also predict the locomotor effects of psychomotor stimulants; however, these findings have been variable. Male rats classified as HRs exhibit greater amphetamine-induced locomotor activity than LRs under certain conditions (Piazza et al., 1989), but other studies found no differences in amphetamine-induced locomotor activity between LRs and HRs (Pierre and Vezina, 1997) or greater cocaine-induced locomotor activity in LRs than HRs (Carey et al., 2003; but see Quertemont et al., 2004). There have been fewer studies examining the LR/HR model in female rats. While female HRs were previously reported to exhibit greater cocaine-induced locomotor activity than LRs (Sell et al., 2005), in the present study LR/HR classification did not predict either acute response to cocaine or cocaine-induced locomotor sensitization (data not shown).

LR or HR classification has also been shown to predict the reinforcing effects of psychomotor stimulants. Male HRs, compared to LRs, more readily acquire amphetamine self-administration (Piazza et al., 1989) and display greater responding for low-dose amphetamine following amphetamine pre-exposure (Pierre and Vezina, 1997). Likewise, both male and female HRs self-administered more amphetamine than LRs on a fixed ratio schedule of reinforcement (Klebaur et al., 2001). However, novelty response also predicts the rate of acquisition of an operant response for food as well as cocaine, suggesting that HRs may simply be quicker learners and not uniquely sensitive to the reinforcing effects of cocaine (Mitchell et al., 2005). Further, individual differences in response to inescapable novelty did not predict conditioned rewarding effects of either cocaine or amphetamine in male rats (Gong et al., 1996; Erb and Parker, 1994) and have not been reported to predict responding for cocaine on a PR schedule of reinforcement. In contrast, LCRs were recently shown to exhibit increased cocaine place conditioning and increased breakpoints on a PR schedule of cocaine reinforcement, as compared to

HCRs (Allen et al., 2007; Mandt et al., in press). These latter findings suggest that LCRs may be more likely than HCRs to associate an environment with the rewarding effects of cocaine and may be more motivated to respond for cocaine. Female rats have also been shown to be more sensitive than male rats to the conditioned reinforcing effects of cocaine (Russo et al., 2003). Therefore, it will be important to determine if differences in the rewarding/reinforcing effects of cocaine exist in female LCRs and HCRs.

Recently, studies of individual differences and their effects on drug abuse have been conducted in experimental animals selectively bred for specific behavioral traits. As already mentioned, selectively bred lines of LR and HR rats have been generated (Stead et al., 2006). In addition, rats have also been bred based on their acquisition of an avoidance test (Roman high- and low-avoidance; RHA and LHA, respectively); these lines also differ in their response to drugs of abuse (Giorgi et al., 2007; Corda et al., 2005). Further, mice have been selectively bred for low or high methamphetamine-induced behavioral activation (Kamens et al., 2005). Selected lines such as these facilitate identification of underlying genetic factors that contribute to individual differences in drug abuse susceptibility. The present results in female rats, together with our present and previous findings in male rats, support the prospect of selective breeding studies for the LCR/HCR phenotype. Overall, our findings help to highlight the importance of taking into consideration both sex and individual differences when studying the neurobehavioral effects of cocaine and present the opportunity to investigate the underlying genetics of these differences.

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