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Individual Differences in Cocaine- and Amphetamine-Induced Activation of Male Sprague–Dawley Rats: Contribution of the Dopamine Transporter

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Previously we found that outbred male Sprague–Dawley rats can be classified as either low or high cocaine responders (LCRs or HCRs, respectively), based on their open-field locomotor response to acute cocaine (COC; 10 mg/kg, i.p.). Here, we extended this analysis to amphetamine (AMPH; 0.5, 1, and 5 mg/kg, i.p.) and found that the individual differences in behavioral activation were not as pronounced as with COC. This was confirmed with observational analysis of behaviors. Differences in drug-induced activation could involve differential dopamine transporter (DAT) function/trafficking. To address this possibility, we measured [^{3}H]DA uptake into dorsal striatal synaptosomes prepared from rats injected 30 min earlier with saline, COC, or AMPH to determine DAT activity, and radioligand binding to determine the total number of DATs. Striatal [^{3}H]DA uptake in COC-treated HCRs was significantly higher than in LCRs. Furthermore, regardless of LCR/HCR classification, uptake in individual COC-treated rats was significantly correlated with their locomotor behavior in the 30 min after drug administration. In contrast, AMPH-treated rats did not differ in uptake, nor were uptake and locomotor activity correlated. DAT number did not differ between LCRs or HCRs, or between AMPH-treated rats. In addition, when individual differences in COC-induced behavior were no longer detected in LCRs and HCRs 1 week after initial classification, uptake was also similar. Together, these results suggest that a difference in expression of functional DATs on the cell surface contributes to the individual differences observed in COC-induced, but not AMPH-induced, behavioral activation of rats.
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INTRODUCTION

The abuse potential of the psychomotor stimulant cocaine (COC) is related to its ability to bind to the dopamine transporter (DAT) and prevent reuptake of released dopamine (DA) (see Ritz *et al*, 1987; Kuhar *et al*, 1991). Low doses of COC generally produce locomotor activation. However, considerable variability (eg different magnitudes) in the behavioral responsiveness of individual animals is often observed. These individual differences have been observed both in outbred and inbred strains of mice (Ruth *et al*, 1988; George and Ritz, 1990; Henricks *et al*, 1997; Marley *et al*, 1998; Rocha *et al*, 1998). Likewise, variability in COC-induced behaviors has been observed in rats, predominantly in the propensity of rats, often initially classified as either low or high responders to novelty, to self-administer drug (Glick *et al*, 1994; Piazza *et al*, 2000), or to become behaviorally sensitized (Hooks *et al*, 1991a; Hooks *et al*, 1992; Djano and Martin-Iverson, 2000; Chefer *et al*, 2003). Differences in initial responsiveness of humans to cocaine also reflect both genetic and environmental influences and are one factor that determines whether or not an individual will become addicted to cocaine (Haertzen *et al*, 1983; Davidson *et al*, 1993).

d-Amphetamine (AMPH) is another psychomotor stimulant that interacts with the DAT. However, unlike COC, AMPH is a substrate for the DAT, thereby entering the nerve terminal, disrupting DA storage in synaptic vesicles, and releasing DA by reversing the DAT (Sulzer and Rayport, 1990; Seiden *et al*, 1993; Sulzer *et al*, 1995; Floor and Meng, 1996). Individual differences in AMPH-induced behaviors have also been reported and, like COC, particularly in rats previously classified by their response to novelty (Segal and Schuckit, 1983; Segal and Kuczenski, 1987; Piazza *et al*, 1989; Hooks *et al*, 1991b; Exner and Clark, 1993; Bevins *et al*, 1997; Cools *et al*, 1997; DeSousa *et al*, 2000; Klebaur *et al*, 2001).

Recently, our laboratory reported that outbred, male Sprague–Dawley rats exhibit a wide range of behavioral

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responses when they are given an acute injection of COC (10 mg/kg, i.p) in an open-field environment. We found that the median distance traveled in the 30 min following injection can be used to classify rats as either low or high COC responders (LCRs or HCRs, respectively) (Sabeti *et al*, 2002; Gulley *et al*, 2003). This behavioral variability, which is not due to differences in COC pharmacokinetics (Gulley *et al*, 2003), appears to be related to individual differences in the function of the DAT in the dorsal striatum (dSTR) and nucleus accumbens (NAc; Sabeti *et al*, 2002). Specifically, by measuring clearance of exogenous DA in the dSTR and NAc, which has been shown to reflect the number of functional DA uptake sites (Cass *et al*, 1992; Hebert and Gerhardt, 1999), we demonstrated that the magnitude of COC-induced changes in clearance and locomotor activation of individual rats are strongly correlated (Sabeti *et al*, 2002). The functional differences in DAT are not due to differences between LCRs and HCRs in NAc total DAT number or affinity, or DAT affinity for COC (Gulley *et al*, 2003). The source of this functional difference may instead be due to differences in either basal or COC-induced changes in cell surface expression of DAT.

It is well documented that psychomotor stimulants that are DAT substrates—such as AMPH, methamphetamine, and methylenedioxymethamphetamine—induce rapid downregulation of DAT cell surface expression *in vitro* or *ex vivo* (Kokoshka *et al*, 1998; Fleckenstein *et al*, 2000; Saunders *et al*, 2000; Hansen *et al*, 2001; Gulley *et al*, 2002; Gulley and Zahniser, 2003). Typically, COC has been shown to have the opposite effect on DAT, increasing its cell surface expression (Daws *et al*, 2002; Little *et al*, 2002; but see Chi and Reith, 2003). Therefore, the first aim of the present study was to examine [^{3}H]DA uptake into synaptosomes prepared from dSTR of animals pretreated with an acute injection of 10 mg/kg COC 30 min prior to killing. This measure of DAT function, when combined with radioligand binding assays to determine the total number of DATs, has been used previously as an indirect measure of DAT cell surface expression (Pristupa *et al*, 1998; Kokoshka *et al*, 1998). We hypothesized that differences in the locomotor response to COC could be due, at least in part, to differences in basal or COC-induced changes in DAT cell surface expression in the dSTR.

A second aim was to examine whether, similar to COC, AMPH produces considerable individual variability in locomotor activation of outbred, male Sprague–Dawley rats. This was particularly interesting to assess given the opposite regulation of DAT surface expression induced by AMPH and COC (see above). Thus, we also tested the extent to which differences in DAT function and/or expression in the dSTR might mediate AMPH-induced behavioral variability.

MATERIALS AND METHODS

Animals

Outbred, male Sprague–Dawley rats (200–300 g) were obtained from Charles River Laboratory (Sasco, Omaha, NE) and housed on a 12-h light/dark cycle with *ad libitum* food and water. All animal use procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Colorado Health Sciences Center.

Behavioral Characterization

On the day of behavioral characterization, rats were removed from the animal colony, housed individually, and allowed 1–2 h to habituate to the testing room. They were then placed in an open-field apparatus consisting of white laminate flooring and a clear acrylic box ($16'' \times 16'' \times 15''$), which was surrounded by a photobeam frame (San Diego Instruments, San Diego, CA). The apparatus was enclosed in a sound-attenuating box ($24'' \times 24'' \times 24''$) and illuminated by a white light bulb (4 W). Rats were given 90 min to habituate to the apparatus, at which time they were removed and injected (i.p.) with either saline ($n = 30$), 10 mg/kg (−)COC HCl ($n = 82$), or AMPH sulfate (0.5, 1, or 5 mg/kg; $n = 24$, 56 or 12, respectively). They were returned to the apparatus for either 30 min (behavioral analysis followed by either [^{3}H]DA uptake or radioligand binding measurements) or 60 min (behavioral analysis only). A video camera connected to a VCR was used to record the rats' behavior for the 30 min before and the 30–60 min following injection. During testing, locomotor behavior was recorded as consecutive, horizontal photo beam breaks and subsequently converted to distance traveled (cm) per 5-min interval. The distance traveled in the 30 min postinjection was used to classify COC-treated rats as LCRs (whose locomotion fell below the median distance traveled) or HCRs (above the median). AMPH-treated rats were also classified based on the median, and are described as ABs (AMPH response below median) or AAs (above median).

For a subset of AMPH-treated animals (0.5 mg/kg, $n = 14$; 1 mg/kg, $n = 18$; 5 mg/kg, $n = 12$), videotapes were observed by an individual unaware of the behavioral classification of the rats. Behaviors, scored in 1-min intervals for the 30 min before and 60 min following injection, included the following: (1) *nonmovement*, defined as inactivity or sleeping; (2) *grooming*, defined as movements directed against self that typically included forepaw movements over the body, scratching, licking, and face washing; (3) *head movement/sniffing*, defined as movements of the head and/or sniffing that occurred in the absence of locomotion; (4) *exploring*, defined as locomotion around the apparatus that was continuous or occurred in repeated bouts and typically included head movements and sniffing; (5) *stereotypy*, defined as highly focused, repetitive head movements and sniffing, head bobs, and/or side-to-side head sways; and (6) *rearing*, defined as lifting both forepaws off the floor and ending when at least one forepaw returned to the floor. If a behavior was present for at least 10 s during the 1-min interval, it was scored as present (1 = present, 0 = absent). If no single behavior persisted for 10 s, the behavior expressed during the majority of the 1-min interval was scored as present. For rearing, each individual occurrence was tabulated.

Based upon [^{3}H]DA uptake results in the rats treated acutely with COC and previous behavioral results 1 week after COC treatment (Gulley *et al*, 2003), a follow-up [^{3}H]DA uptake experiment was conducted in a separate group of saline- and COC-treated rats 1 week after their

initial classification (see below). These rats were treated either twice with saline—on days 1 and 8 ($n=8$)—or twice with 10 mg/kg COC—once on day 1 when they were classified as either LCRs or HCRs and once 1 week later (day 8; $n=16$). After the initial treatment and classification, these rats were housed in the room in which the behavioral experiments took place on a 12-h light/dark cycle with *ad libitum* food and water. On day 8 following the 90-min habituation period in the same open-field apparatus in which day 1 behavior was tested, the behavior of the rats given COC on day 1 was assessed for 30 min following COC injection. All of the rats were then killed for [^{3}H]DA uptake measurements.

Synaptosome Preparation

At 30 min after injection with saline ($n=18$), 10 mg/kg COC ($n=19$ for day 1 uptake; $n=16$ for day 8), or AMPH (1 mg/kg, $n=16$; 0.5 mg/kg, $n=8$), rats were decapitated and their dSTR were dissected on an ice-cold glass dish. The tissue was weighed, homogenized in a 0.32 M sucrose buffer (pH 7.4) using a Teflon pestle/glass homogenizer (Wheaton #358003; Millville, NJ) and centrifuged at 1000*g* for 12 min at 4°C. The resulting pellet was discarded, and the supernatant was centrifuged at 16 000*g* for 12 min at 4°C to isolate the synaptosomes in a P2 pellet. The pellet was then used immediately for [^{3}H]DA uptake assays or stored at −80°C ($\leqslant$7 days) until radioligand binding assays were performed (see below).

[^{3}H]DA Uptake

Uptake was determined using a modified version of the method described by Fleckenstein *et al* (1997). The P2 pellet was resuspended by gentle aspiration at a dilution of 15 mg/ml of original tissue weight in a modified Kreb's assay buffer (in mM: 126 NaCl, 4.8 KCl, 1.3 $CaCl_2$, 16 $NaPO_4$, 1.4 $MgSO_4$, 11 dextrose, 1 ascorbic acid; pH 7.4) that contained 1 μM pargyline. The re-suspended synaptosomes were then incubated with 0.5 nM [^{3}H]DA for 3 min at 37°C. Nonspecific uptake was defined with 1 mM COC. Except for this 3-min incubation, the synaptosomes were kept at 4°C in order to minimize trafficking of DAT. Incubation with [^{3}H]DA was terminated by rapid vacuum filtration through 0.05% polyethylenimine-soaked filter paper (GF/B; Brandel, Gaithersburg, MD) and washed three times with 5 ml of ice-cold sucrose buffer (0.32 M). Radioactivity was quantified 24 h later by liquid scintillation spectroscopy. Protein levels in the resuspended pellet were quantified using the Bradford protein assay (Bradford, 1976) with bovine serum albumin as the standard.

DAT Binding

Prior to radioligand binding, P2 pellets were resuspended at a dilution of 10 mg/ml of wet tissue weight in buffer (in mM: 0.32 sucrose, 30 NaH_2PO_4, 15 Na_2HPO_4; pH 7.4) by homogenization with a Polytron for 3 s. The synaptosomal membranes were incubated for 1 h in an ice bath with 5 nM [^{3}H]WIN 35 428 and increasing concentrations of unlabeled WIN 35 428 (0.3–3 μM). Nonspecific binding was defined with 30 μM benztropine. Incubation was terminated as described above, except that the wash buffer was an ice-cold sodium phosphate solution (in mM: 30 NaH_2PO_4, 15 Na_2HPO_4; pH 7.4). Radioactivity was quantified and protein levels were determined as described above for the uptake assays.

Statistical Analyses

Data in the text are presented as mean ± SD, except where noted. Data in the table and figures are presented as mean ± SEM. The median of all treated animals for each drug and dose was used to separate animals into their respective categories (LCR or HCR for COC; AB or AA for AMPH).

For analysis of drug effects on locomotion, the distance traveled in centimeter was averaged into 15-min bins and compared using a one-way ANOVA and Tukey *post hoc* test (SigmaStat 3.0; SPSS, Inc., Chicago, IL). Frequency distributions of locomotor activity were analyzed using the Kolmogorov–Smirnov test and a kurtosis analysis. Differences between groups were analyzed using a mixed, two-factor ANOVA (group × time, with time as the repeated measure) followed by pairwise comparisons of specific time points using the Tukey *post hoc* test. For behavioral observation categories, 1-min incidence scores were summed into 15-min bins and transformed into a frequency score by dividing the cumulative score by 15. This allowed the fraction of the 15-min interval to be calculated (eg 0.4 means the behavior was present in 40% of the 1-min intervals in a 15-min bin). The exception was rearing, which was tabulated as the total number of occurrences during a 1-min interval summed into 15-min bins. Drug effects were analyzed using a mixed two-factor ANOVA (group × time, with time as the repeated measure) followed by pairwise comparisons of specific time points using the Tukey *post hoc* test.

Specific [^{3}H]DA uptake levels in the dSTR of LCRs/HCRs or ABs/AAs were expressed as a percent of uptake in saline-treated rats tested on the same day. Group differences (eg LCRs *vs* HCRs or ABs *vs* AAs) were analyzed using one-way ANOVA. In addition, linear regression was used to evaluate the relationship between specific [^{3}H]DA uptake and locomotor activation during the 30-min period following COC or AMPH injection. Note that due to technical problems with the assay, data from a [^{3}H]DA uptake experiment performed on rats treated on days 1 and 8 with saline ($n=1$) and COC ($n=2$) were excluded from the analysis.

Radioligand binding data were analyzed with nonlinear curve fitting for one- and two-site binding models to obtain affinity values (IC_{50} and K_i; GraphPad Prism, GraphPad Software, Inc., San Diego, CA). The total number of binding sites (B_{max}) was determined using the formula described by DeBlasi *et al* (1989). Differences in [^{3}H]WIN 35 428 binding parameters were analyzed by two-way ANOVA (group × drug concentration), while differences in K_i and B_{max} values were analyzed with Student's *t*-tests.

Drugs

(−)-COC HCl, AMPH sulfate and WIN 35 428 were obtained from the National Institute on Drug Abuse (RTI

International, Research Triangle Park, NC). [^{3}H]WIN 35 428 and [^{3}H]DA were purchased from Perkin–Elmer (Shelton, CT). All other chemicals were purchased from Sigma/RBI (St Louis, MO) or Fisher (Pittsburgh, PA).

RESULTS

Behavior of Rats Treated with COC

The open-field locomotor activity of COC-treated, outbred male Sprague–Dawley rats is shown in Figure 1. Before drug administration, all animals responded to the novel open-field apparatus with increased locomotion that diminished by 45 min, at which time most animals moved very little until after injection. At 90 min, rats were injected with 10 mg/kg COC ($n=82$). This resulted in increased locomotor activity over baseline, with rats traveling 9200 ± 3940 cm (mean ± SD) in the 30 min following drug injection. Animals whose behavior was below or above the median, 9430 cm/30 min, were designated as either LCRs or HCRs, respectively. Both LCRs and HCRs had a similar response to novelty (Figure 1b; the first 60 min of the time course). Following COC administration, the average distance traveled by LCRs was 5940 ± 1880 cm/30 min and by HCRs was 12 500 ± 2450 cm/30 min.

Even though the distribution of COC-induced locomotor activity in the individual rats was within the range of normality (Figure 1a; $D=0.068$, $p>0.05$; Kolmogorov–Smirnov test), the scatter about the mean appeared somewhat greater than that of an ideal unimodal curve. In confirmation of this observation, kurtosis analysis revealed that the COC distribution was somewhat flat (kurtosis $=-0.72$; zero represents an ideal bell-shaped distribution). In addition to being broader, the frequency distribution of the COC-treated rats also had bimodal characteristics (Figure 1a).

[^{3}H]DA Uptake and DAT Binding in COC-Treated Rats

At the time of maximal behavioral activation (30 min after injection of 10 mg/kg COC), a subgroup of rats was killed, and dSTR synaptosomes were prepared in order to assess whether specific [^{3}H]DA uptake differed between LCRs (mean distance traveled: 6170 ± 2220 cm/30 min, $n=10$) and HCRs (12 200 ± 2780, $n=9$). Owing to day-to-day variability in the absolute values of uptake (range = 1.56–3.94 pmol/mg protein/3 min, across 10 experiments in saline-treated rats), data for each of the drug-treated rats were normalized as a percent of the uptake for a control animal treated with saline and assayed on the same day (see Materials and Methods). Specific [^{3}H]DA uptake was significantly different between LCRs and HCRs (Figure 2a $F_{1,17}=16.4$, $p<0.001$), with HCRs displaying 32% more uptake than LCRs 30 min after the COC treatment. Note that

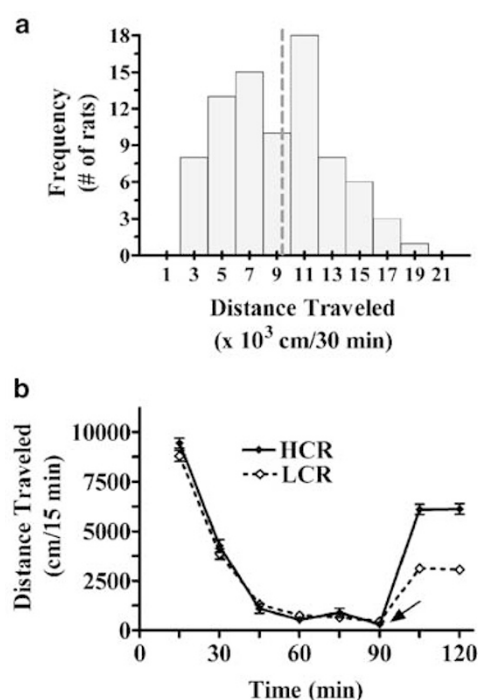

Figure 1 Open-field locomotor activity of male Sprague–Dawley rats before and after i.p. injection of 10 mg/kg COC. (a) Frequency distribution of COC-induced locomotor activity. On the *x*-axis, distance traveled by the rats in the 30 min following COC was separated into 2000-cm bins. Rats whose locomotor activity was below the median (dashed line; 9430 cm/30 min) were classified as LCRs, while those above it were classified as HCRs ($n=82$ rats). (b) Time courses for the locomotor activity of LCRs and HCRs (mean ± SEM) in the 90 min before and 30 min after COC injection (arrow).

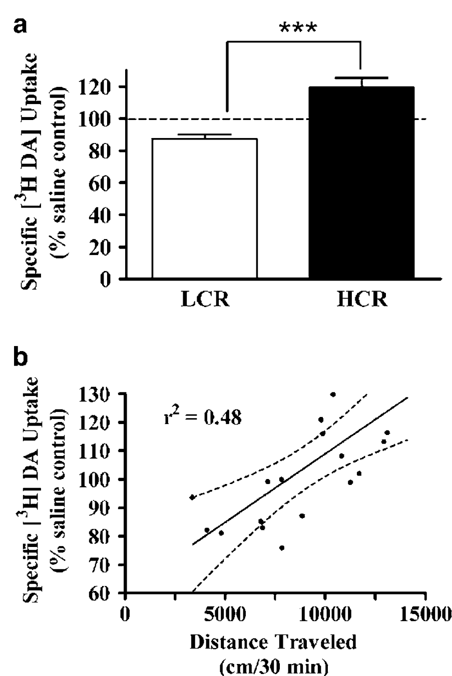

Figure 2 Specific [^{3}H]DA uptake into dSTR synaptosomes, prepared from LCRs and HCRs 30 min after treatment with 10 mg/kg COC, and correlation with COC-induced locomotor activity. (a) Data are presented as percent of uptake in dSTR synaptosomes prepared from saline-treated rats assayed in the same experiment (see Materials and Methods; $n=10$ saline, 9 LCRs, 10 HCRs; ***$p<0.001$). (b) [^{3}H]DA uptake in the individual rats, regardless of their LCR/HCR classification, was significantly correlated with their locomotor activity in the 30 min following COC injection ($r^2=0.48$, $p<0.01$). The linear regression fit (—) and 95% confidence intervals (– – –) are shown in the figure.

Table 1 Affinity (K_i) and Total Number (B_{max}) of DAT Binding Sites Measured with [^{3}H]WIN 35 428 in Synaptosomal Membranes Prepared from the dSTR

Group	Treatment	n	K_i (nM)	B_{max} (pmol/mg protein)
Control	Saline	9	7.8±0.69	2.4±0.13
LCR	COC	8	8.0±0.67	2.3±0.23
HCR	COC	8	9.8±1.4	2.4±0.25
AB	AMPH	7	7.9±1.6	1.8±0.23
AA	AMPH	6	8.1±1.1	2.2±0.32

Rats were pretreated for 30 min with saline, 10 mg/kg COC and classified as LCRs/HCRs, or 1 mg/kg AMPH and classified as ABs/AAs. Data are presented as mean ± SEM. There were no significant group differences between saline- and drug-pretreated animals in K_i (COC: $F_{2,22} = 1.43$, $p > 0.05$; AMPH: $F_{2,19} = 0.04$, $p > 0.05$) or B_{max} (COC: $F_{2,22} = 0.12$, $p > 0.05$; AMPH: $F_{2,19} = 1.97$, $p > 0.05$).

the mean (±SEM) values (non-normalized) of uptake in LCRs (2.10±0.15 pmol/mg protein/3 min) and HCRs (2.91±0.41 pmol/mg protein/3 min) were also below and above, respectively, the mean uptake measured in saline-treated rats (2.45±0.24 pmol/mg protein/3 min), but that these differences were not statistically significant ($F_{2,26} = 1.80$, $p > 0.05$). Additionally, regardless of the LCR/HCR designation, specific [^{3}H]DA uptake was significantly correlated with distance traveled in the 30 min following COC injection, with rats that were more highly activated demonstrating greater uptake (Figure 2b).

Specific [^{3}H]WIN 35 428 binding was used to measure the total number of DAT binding sites (B_{max}) in synaptosomal membranes prepared from the dSTR of rats pretreated in a manner identical to the [^{3}H]DA uptake experiments, namely for 30 min with either saline or 10 mg/kg COC. Analysis of indirect saturation curves for [^{3}H]WIN 35 428 binding revealed no differences in either the affinity (K_i) or B_{max} of dSTR DATs between saline-pretreated controls, LCRs, or HCRs (Table 1).

COC-Induced Behavior and [^{3}H]DA Uptake 1 Week after Initial LCR/HCR Classification

Previous results demonstrated that when COC was administered to the rats 1 week after their initial injection and classification as LCRs or HCRs, the differences in COC-induced locomotor activity were no longer apparent (Gulley *et al*, 2003). This observation provided a means to further test the relationship between the behavioral and DAT functional differences found after the initial COC administration in LCRs and HCRs. Therefore, rats were injected with 10 mg/kg COC on day 1 and classified as either LCRs (mean distance traveled: 5840±1780 cm/30 min, $n = 8$) or HCRs (12800±2630, $n = 6$). After 1 week, on day 8, 10 mg/kg COC was again administered to these rats, and locomotor activity was measured (Figure 3a). Baseline locomotor activity on day 8 prior to COC injection was significantly elevated between 30 and 90 min in all rats compared to day 1. However, after the COC injection, the activity of LCRs and HCRs did not differ. Specific [^{3}H]DA uptake was assessed in dSTR synaptosomes prepared

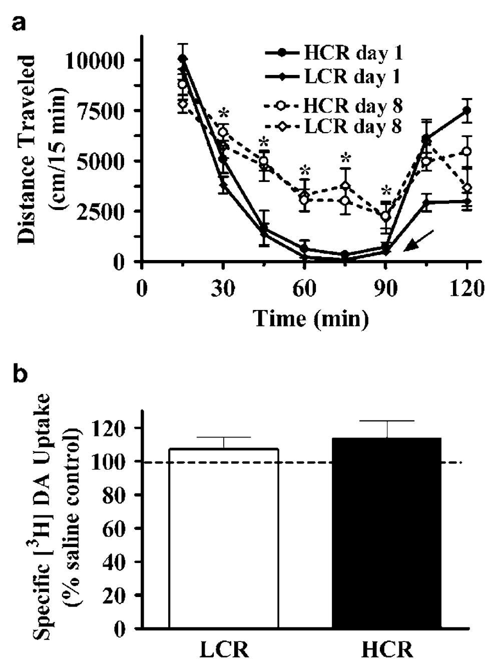

Figure 3 Locomotor activity and specific [^{3}H]DA uptake into dSTR synaptosomes of LCRs and HCRs treated with 10 mg/kg COC 1 week after their initial behavioral classification with COC. (a) Time courses for the locomotor activity of LCRs ($n = 8$) and HCRs ($n = 7$) in the 90 min before and 30 min after COC injection (arrow) on days 1 and 8 (*$p < 0.001$, comparison with day 1 within both groups). (b) No difference between LCRs and HCRs in [^{3}H]DA uptake assessed 30 min after COC on day 8 ($F_{1,13} = 0.32$; $p > 0.05$).

30 min after COC-pretreatment on day 8 and, similar to locomotor activity, did not differ significantly between LCRs and HCRs (Figure 3b). As with our uptake experiments performed on day 1 (see above), data from COC-treated rats were normalized as a percent of the uptake for saline-treated control rat tested on the same day. However, the non-normalized mean (±SEM) values also revealed that uptake was similar across all groups (saline: 2.25±0.21 pmol/mg protein/3 min; LCR: 2.34±0.20 pmol/mg protein/3 min; HCR = 2.48±0.29 pmol/mg protein/3 min). Thus, on the first day, when LCRs and HCRs displayed considerable differences in COC-induced locomotor activity, HCRs displayed ~32% greater uptake than LCRs; 1 week later, when LCRs and HCRs behaved similarly, HCRs only had ~7% greater uptake.

Behavior of Rats Treated with AMPH

The open-field locomotor activity of saline- and 1 mg/kg AMPH-treated rats is shown in Figure 4. All of the rats exhibited similar behavior during the first 90 min in the open field (Figure 4b). After this period, rats were injected with either saline ($n = 13$) or 1 mg/kg AMPH ($n = 56$; Figure 4b). Saline increased locomotor activity only during the first 15 min following injection; by 30 min, saline-treated rats moved very little. In contrast, AMPH increased locomotor activity over baseline, with rats traveling 13 400±3190 cm (mean±SD) in the 30 min following injection. The frequency distribution of the locomotor activity during this time, although normal (D = 0.093,

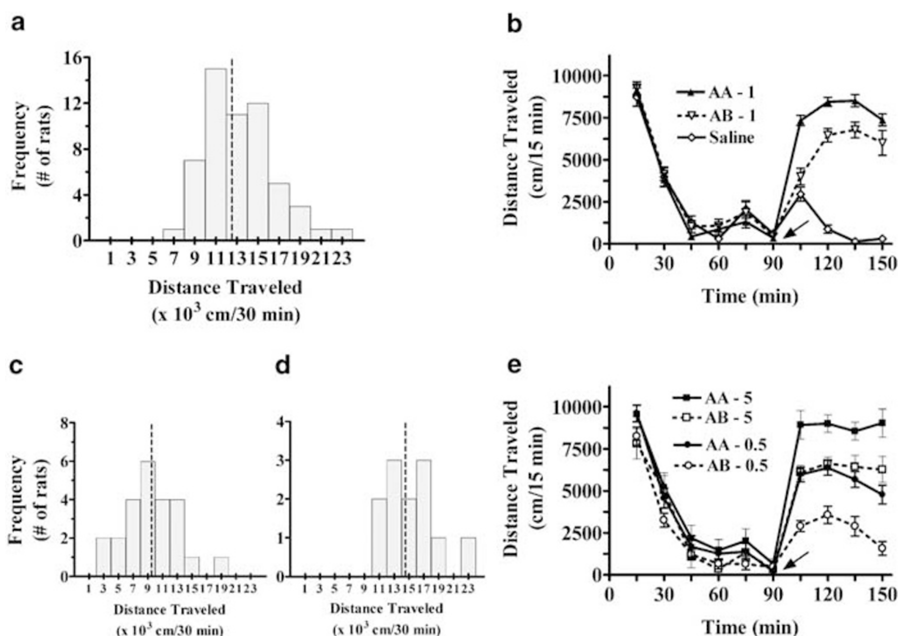

Figure 4 Open-field locomotor activity of male Sprague–Dawley rats before and after i.p. injection of either saline or AMPH. (a), (c), (d) Frequency distributions of AMPH-induced locomotor activity induced by 1 mg/kg (a; $n = 56$ rats), 0.5 mg/kg (c; $n = 24$ rats), and 5 mg/kg AMPH (d; $n = 12$ rats). Rats whose locomotor activity was below the median (dashed line) were classified as ABs, while those above it were classified as AAs. (b, e: Time courses for the locomotor activity of ABs and AAs in the 90 min before and 30 min after injection (arrow) of either saline or AMPH (b: 1 mg/kg; d: 0.5 and 5 mg/kg).

$p > 0.05$), was more peaked than expected for an ideal bell-shaped distribution (kurtosis = 0.49; Figure 4a). While individual differences in AMPH-induced locomotor behavior were apparent, they were not as pronounced as with COC and were less suggestive of two behavioral subgroups. However, in order to examine individual differences in the same way as with COC, we divided the AMPH-treated rats into two groups based on the median AMPH-induced activity (12 700 cm/30 min) for further behavioral assessment, as well as for uptake and radioligand binding experiments. Rats whose distance traveled in the 30 min after AMPH administration was below the median were classified as ABs, while those with AMPH-induced activity above the median were classified as AAs. The mean distance traveled in the 30 min after AMPH injection was $10\,800 \pm 1300$ cm/30 min for ABs and $15\,300 \pm 1810$ for AAs (Figure 4b).

Observational analysis of the behavior of a subset of rats ($n = 18$) in the 60 min following injection with 1 mg/kg AMPH revealed, compared to baseline, decreases in nonmovement and increases in head movement/sniffing, exploring, and rearing (Figure 5). Grooming and stereotypy were not differ from baseline (data not shown). No statistically significant differences were observed between ABs and AAs in any of the behavioral categories, with the exception of exploring (Figure 5). Specifically, AMPH induced greater exploratory behavior in AAs than ABs only in the first 15 min following AMPH treatment. While head movement and sniffing behaviors were slightly elevated in ABs over AAs, these were not significantly different.

Since locomotor activation induced by 1 mg/kg AMPH was less suggestive of two subpopulations of rats, we tested two additional doses of AMPH (0.5 and 5 mg/kg) in separate groups of rats. As shown in Figure 4c and d, the frequency distributions for both doses were approximately normal (0.5 mg/kg: $D = 0.079$, $p > 0.05$; 5 mg/kg: $D = 0.160$, $p > 0.05$). Furthermore, both distributions were peaked and lacked prominent bimodal characteristics (kurtosis = 0.51 and 1.23 for 0.5 and 5 mg/kg, respectively). Regardless of this relative homogeneity of responses, we split the animals into ABs and AAs based on the median (0.5 mg/kg: 9660 cm/30 min; 5 mg/kg: 14 600 cm/30 min) in order to test for potential individual differences (Figure 4e). The mean distance traveled after injection of 0.5 mg/kg AMPH was 6794 ± 2032 cm/30 min for ABs and $12\,300 \pm 2700$ cm/30 min for AAs; following 5 mg/kg AMPH, the mean distance traveled was $12\,700 \pm 1310$ and $17\,900 \pm 2950$ for ABs and AAs, respectively.

Observational analysis revealed that after injection of 0.5 mg/kg AMPH, compared to baseline, nonmovement was reduced whereas head movement/sniffing and exploring were increased in both ABs and AAs (Figures 6a–c). Rearing was increased relative to baseline only in AAs (Figure 6d). Neither group displayed increases in grooming or stereotypy relative to baseline (data not shown). ABs and AAs differed in two of the scored behaviors. Specifically, AAs exhibited significantly more exploring during the first 30 min after injection (Figure 6c). Rearing was also greater in AAs compared to ABs, although this difference was significant only during the 15-min interval concluding 30 min after injection (Figure 6d).

Compared to the effects of the lower doses, 5 mg/kg AMPH produced a unique behavioral profile that was characterized by the emergence of repetitive, stereotyped behaviors. For example, normal head movement/sniffing was increased relative to baseline in ABs and AAs only during the first 15-min period following injection (Figure 7a). Exploring and rearing were also significantly

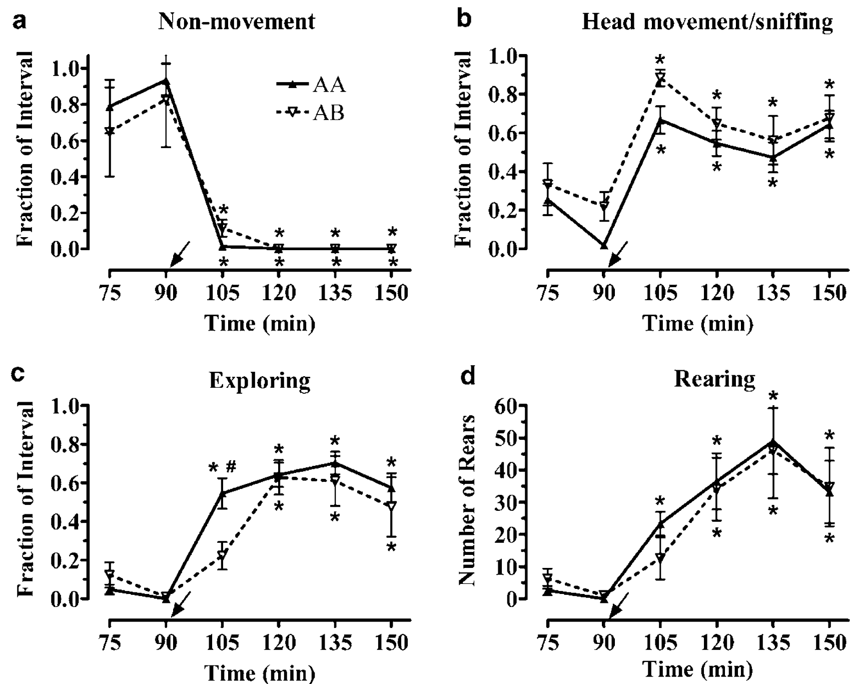

Figure 5 Observational analysis revealed minimal behavioral differences induced by 1 mg/kg AMPH in ABs and AAs. Behaviors exhibited by the rats ($n = 18$) during the 30 min preceding and 60 min following injection (arrow; see Figure 4 for time course of the entire experiment) included nonmovement (a), head movement/sniffing (b), exploring (c), and rearing (d; see Materials and Methods). Data (mean ± SEM) are presented as the fraction of 15-min intervals that the rats displayed the specific behavior (a–c) or as the total number of rears in each interval (d). Only exploring in the first 15 min after injection differed significantly between ABs and AAs ($^{\#}p < 0.05$). $^{*}p < 0.05$, within-group comparison to the 15-min period before injection (ie time = 90 min).

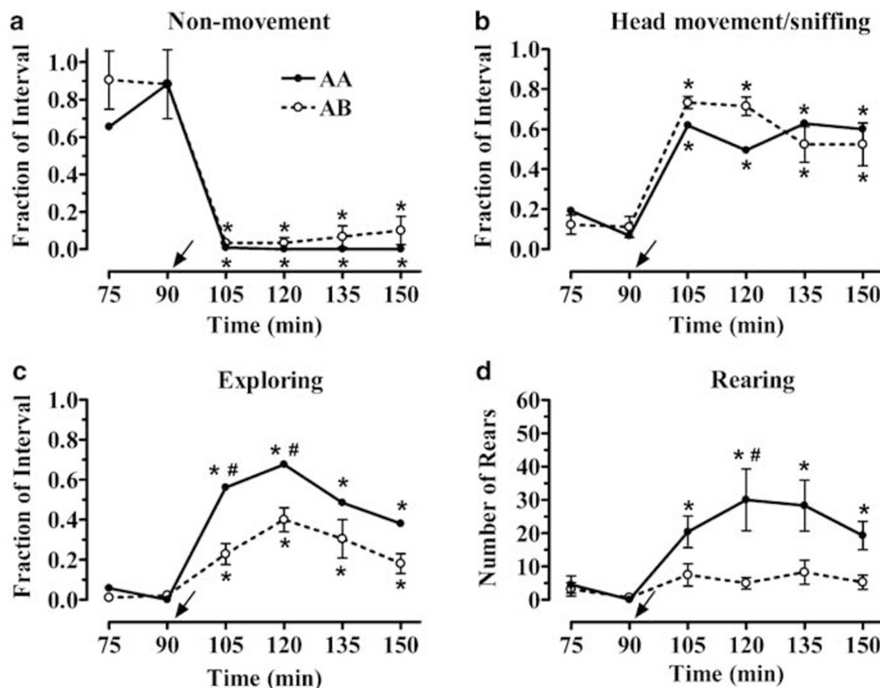

Figure 6 Observational analysis revealed some behavioral differences induced by 0.5 mg/kg AMPH in ABs and AAs. Behaviors exhibited by the rats ($n = 14$) are presented as in Figure 5. Exploring (c) differed significantly between ABs and AAs in the first 30 min after injection ($^{\#}p < 0.05$). Rearing (d) also differed significantly between ABs and AAs from 15 to 30 min after injection ($^{\#}p < 0.05$). $^{*}p < 0.05$, within-group comparison to the 15 min period before injection (ie time = 90 min).

elevated in both groups relative to baseline (Figure 7b and c). However, in rats classified as AAs, these increases were no longer significantly different from baseline beyond the 30-min period following AMPH injection. Instead, behavior in AA rats was characterized primarily by repetitive, stereotyped movements. These typically included focused

sniffing and head movements that occurred in the absence of locomotion (Figure 7d). AB rats, in contrast, displayed high levels of exploring and rearing for the entire postdrug session. Although significant increases in focused stereotypy did not occur until 45 min after AMPH injection, the exploratory and rearing behavior exhibited by AB rats was clearly repetitive in nature. These rats typically traversed the perimeter of the open-field arena in either a clockwise or counterclockwise direction, stopping frequently to rear multiple times before continuing their locomotor pattern.

[^{3}H]DA Uptake and DAT Binding in Rats Treated with AMPH

In order to compare results with those we found in rats given COC, we measured DAT function in a subset of animals given AMPH 30 min before killing. Since the behavioral effects of 5 mg/kg AMPH were characterized primarily by stereotyped responses that were not seen with 10 mg/kg COC, we measured specific [^{3}H]DA uptake only in rats given 1 or 0.5 mg/kg AMPH. At the time of near maximal behavioral activation (30 min after injection of 1 mg/kg AMPH), rats were killed and dSTR synaptosomes were prepared to assess whether specific [^{3}H]DA uptake differed between ABs (mean distance traveled: 10 800 $\pm$ 1260 cm/30 min, $n=7$) or AAs (14 800 $\pm$ 1770, $n=9$). In contrast with COC pretreatment, no difference in uptake was found between ABs (mean $\pm$ SEM: 98.1 $\pm$ 2.33% of saline-treated controls) and AAs (104 $\pm$ 6.07%) following the 30-min AMPH pretreatment. This was also evident by inspection of the non-normalized mean ($\pm$SEM) uptake values for each group (saline: 2.24 $\pm$ 0.11 pmol/mg protein/3 min; ABs: 2.26 $\pm$ 0.09 pmol/mg protein/3 min; AAs: 2.19 $\pm$ 0.13 pmol/mg protein/3 min). Furthermore, there was no correlation between uptake and locomotor activity in the AMPH-pretreated rats ($r^2=0.10$, $p>0.05$). Similar to LCRs and HCRs, no differences existed in the affinity or number of dSTR DAT binding sites among saline-pretreated controls, ABs, or AAs (Table 1).

Specific [^{3}H]DA uptake was also assessed in a subset of rats treated with 0.5 mg/kg AMPH 30 min before killing to determine whether it differed between ABs (mean distance traveled: 6280 $\pm$ 965 cm/30 min, $n=3$) or AAs (12 000 $\pm$ 755, $n=5$). As seen with 1 mg/kg AMPH, no difference in uptake was found between ABs (mean $\pm$ SEM: 97.6 $\pm$ 5.44% of saline-treated controls) and AAs (98.5 $\pm$ 11.0%). The non-normalized mean uptake values were also similar (saline: 2.13 $\pm$ 0.37 pmol/mg protein/3 min; ABs: 2.03 $\pm$ 0.06 pmol/mg protein/3 min; AAs: 1.94 $\pm$ 0.20 pmol/mg protein/3 min), and uptake and locomotor activity were not correlated ($r^2=0.04$, $p>0.05$).

DISCUSSION

Previously, we found that an acute injection of 10 mg/kg COC in outbred male Sprague–Dawley rats results in variable locomotor responsiveness suggestive of two subpopulations, LCRs and HCRs (Sabeti *et al*, 2002; Gulley *et al*, 2003). LCRs and HCRs also displayed differences in other behaviors such as nonmovement, head movement/sniffing,

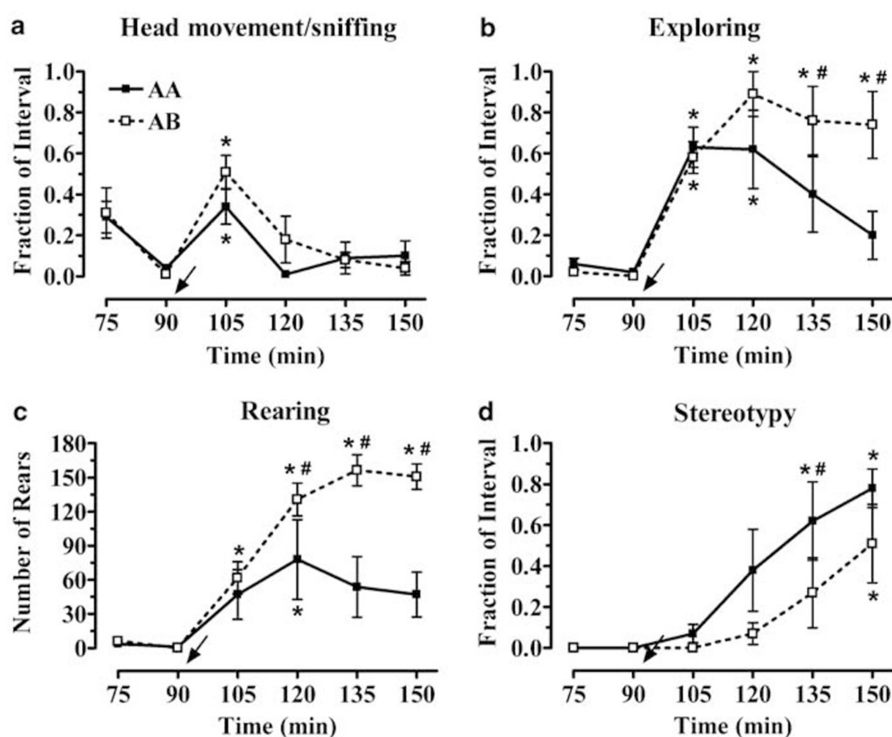

Figure 7 Compared to the lower doses of AMPH, the 5 mg/kg dose produced a unique behavioral profile characterized by repetitive, stereotyped movements. Behaviors exhibited by the rats ($n=12$) are presented as in Figure 5. Following injection, neither group spent time in periods of nonmovement and grooming was not increased significantly above baseline in either group (data not shown). *$p<0.05$, within-group comparison to the 15 min period before injection (ie time = 90 min); #$p<0.05$, AAs and ABs compared in the same time interval.

and rearing. This behavioral variability is correlated with individual differences in DAT function (Sabeti *et al*, 2002). Here, we again observed differential behavioral responsiveness consistent with two subpopulations in COC-pretreated rats. We then examined levels of DAT functional expression as a possible mechanism responsible for the behavioral variability, as well as extended this analysis to AMPH-pretreated rats.

Numerous mechanisms could explain individual differences in COC-induced behaviors. Several studies using inbred strains of mice have reported pharmacokinetic differences that could account for differential COC-induced locomotor activation (Benuck *et al*, 1987; Reith *et al*, 1987; Wiener and Reith, 1990; Jones *et al*, 1993). Others have found increased number and affinity of DATs in the NAc of high, compared to low, novelty responders (Chefer *et al*, 2003). However, we have demonstrated that these mechanisms are unlikely to explain the differential responsiveness of LCRs and HCRs to COC (Gulley *et al*, 2003; Table 1).

The fact that we found no differences in DAT B_{max} values, however, does not obviate the possibility that the functional pool of DATs at the cell surface may differ. [^{3}H]WIN 35 428 binding to DAT in crudely purified membranes, as used here and by Gulley *et al* (2003), measures total cellular transporters and would not detect differences in cell surface DATs between LCRs and HCRs. Thus, it is possible that differences in COC-induced cell surface expression of DAT could explain the differential COC-induced locomotor activation in LCRs and HCRs. In fact, it has been reported that COC rapidly alters trafficking and upregulates surface-expressed DAT in cell expression systems (Daws *et al*, 2002; Little *et al*, 2002) and brain (Daws *et al*, 2002; but see Chi and Reith, 2003). HCRs, for example, might have sufficient DATs to take up DA released under basal conditions, but not when even a relatively low percentage of their DATs were inhibited by a low dose of COC. The increased extracellular DA would, in turn, stimulate locomotor activity. If this were the case, then the COC-induced increase in DATs in HCRs might reflect a secondary compensatory change to reduce the high levels of extracellular DA. In contrast, LCRs could have ‘spare DATs’ in their dSTR, relative to DA release; then COC blockade of a relatively low percentage of DATs might induce only a slight increase in their extracellular DA concentrations and locomotor activity. Alternatively, COC could induce greater increases in dendritic DATs in HCRs than LCRs. This would locally reduce DA concentrations and thereby decrease autoinhibition of DA neuronal firing, which might result in sufficiently increased DA release at the terminals to elevate locomotor activity to a greater extent in HCRs.

We tested the potential for differences in COC-induced trafficking of the DAT in LCRs and HCRs by measuring DAT function using [^{3}H]DA uptake assays. A difference in specific [^{3}H]DA uptake with no change in the number of [^{3}H]WIN 35 428 binding sites is an indirect measure of differences in DAT cell surface expression (Kokoshka *et al*, 1998; Pristupa *et al*, 1998). Indeed, while we found no differences in DAT B_{max} in dSTR of LCRs and HCRs at the time of maximal COC activation, HCRs accumulated 32% more [^{3}H]DA in dSTR than LCRs. It is unlikely that residual drug in the synaptosomes confounded our results because K_i values between COC- and saline-treated rats were similar. Also, residual COC would have reduced, rather than increased [^{3}H]DA uptake, compared to controls. Indeed, Fleckenstein *et al* (1997) reported that the amount of drug in ‘unwashed’ synaptosomes (P2 pellets) was minimal and that uptake in P2 pellets and washed P2 pellets was similar.

Thus, our uptake experiments in COC-treated rats suggest that HCRs have greater cell surface expression of DAT. Importantly, specific [^{3}H]DA uptake showed a significant correlation with COC-induced locomotor activity in all of the rats, regardless of the LCR/HCR classification. Specifically, the rats that locomoted more after COC injection had greater [^{3}H]DA uptake in dSTR, whereas those that locomoted less had lower uptake. Furthermore, 1 week after initial COC treatment and LCR/HCR classification, LCRs and HCRs exhibited not only similar COC-induced locomotor activation but also similar [^{3}H]DA uptake. Therefore, when LCRs and HCRs displayed considerable differences in COC-induced locomotor activity, their [^{3}H]DA uptake differed significantly. However, when the behavioral distinction between LCRs and HCRs was no longer apparent, neither was the difference in [^{3}H]DA uptake.

Although we do not know the mechanism(s) underlying the loss of the LCR/HCR phenotypes 1 week after the initial COC administration, potential candidates include expression of behavioral sensitization in the LCRs and/or altered DA release and DAT regulation in the HCRs. Previously, we observed a loss of LCR/HCR behavioral differences after retest with COC (Gulley *et al*, 2003) because both LCRs and HCRs showed little COC-induced activation. In the present study, relative to day 1, COC-induced activation of HCRs was still reduced but COC-induced activation of LCRs appeared to be enhanced. However, this latter observation was due in large part to the elevated baseline activity prior to injection on day 8. We noted similar anticipatory behavior in our previous study, but it occurred only in HCRs and was of a relatively lesser magnitude, compared to their behavior on day 1. It is unclear why preinjection behavior was more robust in the present study, but it may indicate that the LCRs and HCRs tested here were more responsive to environmental cues and thereby developed stronger conditioned drug-related responses.

Differences in basal levels of DAT expression could also explain the LCR/HCR phenotypes since chronically higher basal DA release might result in upregulated DAT expression and greater COC-induced increases in DA. However, to confirm this, LCRs would have to be distinguished from HCRs in the absence of drug. Unfortunately, since their responses to novelty are identical (Gulley *et al*, 2003; Figure 1), we have found no way so far to classify the rats, other than to administer COC. This fact also made it impossible for us to use data collected from saline-treated rats as a test for consistent, basal difference in DA uptake among Sprague–Dawley rats, which ultimately would have been classified as LCRs or HCRs if given COC. The alternative of administering COC to classify the rats and then waiting a relatively long period so that their DA systems returned to baseline is also problematic because even a single COC treatment induces changes in DA systems lasting longer than a month (Robinson *et al*, 1982; Peris and Zahniser, 1987, 1989).

Unlike the initial pretreatment with 10 mg/kg COC, pretreatment with 1 mg/kg AMPH did not produce the same degree of individual behavioral variability. This is particularly apparent when the frequency distributions for locomotor activity induced by COC and AMPH are compared. The distribution of COC-treated rats had a broader spread and bimodal characteristics, compared with that of AMPH-treated rats, which had a more peaked distribution that lacked bimodal characteristics. Furthermore, observational analysis of rats treated with 1 mg/kg AMPH revealed no differences between ABs and AAs in behaviors other than exploring. Individual differences in AMPH-induced behaviors observed here could be less pronounced than those reported by others (eg Segal and Kuczenski, 1987) because of differences in the selection and classification of animals. We included all of the animals tested by separating them based on the median split, whereas some reports of individual differences have examined only the extremes of the distribution.

Since 1 mg/kg AMPH produced less prominent individual differences but slightly greater locomotor activation compared to 10 mg/kg COC, we also tested lower and higher doses of AMPH. Like 1 mg/kg, both 0.5 and 5 mg/kg AMPH resulted in peaked, unimodal-like distributions that did not strongly support the existence of two subgroups. After 0.5 mg/kg AMPH, however, ABs and AAs showed somewhat greater differences in AMPH-induced locomotor activity, compared to rats classified with 1 mg/kg AMPH. Observational analysis also revealed some differences between ABs and AAs in AMPH-induced exploring and rearing. However, individual differences were still not as great with AMPH as with COC (cf. Figures 1, 4–6; Sabeti *et al* 2002; Gulley *et al*, 2003). Whereas injections of 0.5 and 1 mg/kg AMPH produced behavioral effects that were similar, but of a different magnitude, to those produced by 10 mg/kg COC, rats injected with 5 mg/kg AMPH exhibited unique patterns of drug-induced behavior. Specifically, they displayed high levels of behavioral activation that were characterized by prolonged periods of repetitive, stereotyped movements. In some cases, stereotyped behavior was manifested as focused head movement and sniffing, whereas in other cases rats circled the chamber in one direction stopping for frequent bouts of rearing behavior. Similar effects induced by this dose of AMPH have been reported previously (eg Rebec *et al*, 1997) and are mediated by a number of factors including DA receptor sensitivity (Segal and Kuczenski, 1999), non-DA mechanisms (Joyce and Iversen, 1984), and subregions of both the dSTR and NAc (Kelly *et al*, 1975; Kelley *et al*, 1988). Owing to our emphasis on 'low-dose' locomotor activating effects of COC and AMPH, we did not analyze the effects of 5 mg/kg AMPH on DAT function.

In contrast to COC-treated rats, specific [^{3}H]DA uptake measured in rats given saline, 0.5 or 1 mg/kg AMPH did not differ either between ABs and AAs, or from saline. Likewise, regardless of AB/AA classification, AMPH-induced locomotor activity and [^{3}H]DA uptake were not correlated. The lack of AMPH-induced regulation of DAT activity was surprising since AMPH exposure has been shown to downregulate DAT surface expression in cells (Saunders *et al*, 2000). However, only a dose of 10 mg/kg has been reported to produce downregulation measured *ex vivo* (Fleckenstein *et al*, 1997, 1999). The lower AMPH doses in the present study (0.5 and 1 mg/kg), which were used because they induced locomotor activation similar to that seen with 10 mg/kg COC without inducing stereotypic behaviors, may be too low to induce detectable changes in [^{3}H]DA uptake and/or DAT surface expression. Alternatively, it is possible that the lack of difference in DAT function with either dose of AMPH reflects the less distinct AB/AA behavioral separation. In any case, two potentially relevant neurochemical differences that could explain the different results with COC and AMPH are that (1) the two drugs rapidly regulate DAT in an opposing manner and (2) compared to COC, the ability of AMPH to increase extracellular DA is much less dependent on neuronal activity.

While [^{3}H]WIN 35 428 binding combined with [^{3}H]DA uptake can indirectly measure cell surface expression of DAT, it will be important in the future to use a direct method to confirm COC-induced differences in DAT trafficking in LCRs and HCRs. For example, cell surface DAT has been crosslinked with biotin in dSTR synaptosomes and quantified with Western blot analysis (Chi and Reith, 2003; Salvatore *et al*, 2003). In addition, it will be important to examine cell surface expression of DAT in the NAc, where we initially saw both basal differences between LCRs and HCRs in *in vivo* DA clearance and greater COC-induced changes in DA clearance in HCRs than LCRs (Sabeti *et al*, 2002). However, NAc studies will be challenging. Recent studies suggest locomotor activation is associated with the shell, but not the core (Sellings and Clarke, 2003). Currently, we can measure [^{3}H]DA uptake only in synaptosomes prepared from the entire NAc because of its small size.

In summary, whereas 10 mg/kg COC induced considerable individual variability in locomotor activation suggestive of two subpopulations, the variability observed with AMPH (1, 0.5, and 5 mg/kg) was not as pronounced. The behavioral variation that did exist with AMPH cannot be explained by differences in cell surface expression of DAT in dSTR, as ABs and AAs displayed no differences in total DAT number, affinity, or [^{3}H]DA uptake. Interestingly, this was not the case for COC-treated rats. While LCRs and HCRs displayed no differences in total DAT number or affinity, HCRs had greater [^{3}H]DA uptake in dSTR than LCRs. Our results suggest a difference in cell surface expression of DAT that is responsible, at least in part, for the observed variability in COC-induced behavioral activation of male Sprague–Dawley rats.

The clinical implications of our findings are unknown. However, LCRs, but not HCRs, express locomotor sensitization with repeated cocaine administration (Sabeti *et al*, 2003). Furthermore, differential initial responsiveness of humans to COC is related to abuse potential (Haertzen *et al*, 1983; Davidson *et al*, 1993). Imaging studies have shown that the magnitude of DAT blockade in brain is one of the important parameters contributing to the cocaine 'high' (Volkow *et al*, 1999). A lower number of transporters would be more readily blocked by a given dose of COC. Thus, the number of functional DATs undoubtedly plays an important role in cocaine reinforcement and addiction in humans.

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