Low or High Cocaine Responding Rats Differ in Striatal Extracellular Dopamine Levels and Dopamine Transporter Number

Anna M. Nelson, Gaynor A. Larson, and Nancy R. Zahniser

Department of Pharmacology (A.M.N., G.A.L., N.R.Z.) and Neuroscience Program (N.R.Z.) University of Colorado Denver, Aurora, Colorado

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ABSTRACT

Both humans and animals exhibit marked individual differences in cocaine responsiveness. By using the median split of cocaine-induced locomotor activity, we have classified outbred male Sprague-Dawley rats as either low or high cocaine responders (LCRs or HCRs, respectively). LCR/HCR classification predicts differences in cocaine inhibition of striatal dopamine (DA) transporters (DATs), cocaine-induced locomotor sensitization, cocaine-conditioned place preference, and motivation to self-administer cocaine. In this study, we used in vivo microdialysis to investigate whether the differential cocaine inhibition of DATs in LCRs and HCRs is translated into differential extracellular DA levels. Paralleling their locomotor profiles, LCRs and HCRs had similar basal extracellular DA levels in dorsal striatum (dSTR) and nucleus accumbens (NAc); after acute cocaine injection (10 mg/kg i.p.), HCRs showed greater cocaine-induced increases in DA than LCRs, with more pro-

nounced differences in NAc. After repeated cocaine injection, LCRs and HCRs no longer differed in cocaine-induced locomotor activity or extracellular DA. To further explore the differential susceptibility of LCR/HCR DATs to cocaine, we used in vitro [³H]-carbomethoxy-3-(4-fluorophenyl)tropane ([³H]WIN 35,428) binding and quantitative autoradiography to measure the number of DAT binding sites and cocaine's affinity for them. After acute cocaine administration, HCRs had fewer DAT binding sites in dSTR and NAc shell, compared to LCRs. No LCR/HCR differences were observed in DAT number after repeated cocaine injection or in cocaine's affinity. Our findings suggest that levels of striatal extracellular DA and DATs both make important contributions to initial differences in cocaine activation, which in LCRs/HCRs predict differential cocaine reward and reinforcement.

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Individuals differ in their responsiveness to the abused stimulant drug cocaine (Lambert et al., 2006; Goldstein et al., 2008). An estimated 33.7 million Americans have used cocaine at least once in their lifetime, and an estimated 10 to 15% of these users will become addicted (Wagner and Anthony, 2002; SAMHSA, 2006). Understanding why individuals differ in cocaine responsiveness and how these differences influence susceptibility to drug addiction is critical to addressing this public health problem.

Much like the variability seen in humans, rodents also exhibit differential responsiveness to stimulants (Piazza et al., 1989; Hooks et al., 1991; Sabeti et al., 2002; Homberg et al., 2004; Giorgi et al., 2005). To study individual differences in initial cocaine responsiveness, we have classified adult outbred male Sprague-Dawley rats as either low or high cocaine responders (LCRs or HCRs, respectively) based on the median split of open-field locomotor activity induced by an acute injection of a relatively low dose of cocaine (10 mg/kg

ABBREVIATIONS: LCR, low cocaine responder; HCR, high cocaine responder; DA, dopamine; DAT, DA transporter; NAc, nucleus accumbens; dSTR, dorsal striatum; [³H]WIN 35,428, [³H]-carbomethoxy-3-(4-fluorophenyl)tropane; QAR, quantitative autoradiography; SN, substantia nigra; VTA, ventral tegmental area; RMANOVA, repeated measures analysis of variance.

i.p.) (Sabeti et al., 2002, 2003; Gulley et al., 2003; Allen et al., 2007; Mandt et al., 2008). Brain cocaine levels and competing stereotyped behaviors do not explain the differential responsiveness of LCRs and HCRs to cocaine (Sabeti et al., 2002, 2003; Gulley et al., 2003). It is noteworthy that compared with HCRs, LCR classification predicts greater cocaine-induced locomotor sensitization (Sabeti et al., 2003; Allen et al., 2007), greater sensitivity to the rewarding properties of cocaine as measured by intravenously conditioned place preference (Allen et al., 2007), and greater motivation to self-administer cocaine (Mandt et al., 2008). An animal model of individual differences in initial response to an addictive drug is of particular interest because low initial response to ethanol in humans is associated with the later development of alcohol use disorder (Schuckit, 1994).

Cocaine produces its activating and rewarding effects primarily by binding to the neuronal dopamine (DA) transporter (DAT) (Ritz et al., 1987; Chen et al., 2006), resulting in increased extracellular DA levels in DA-rich brain regions, including nucleus accumbens (NAc) and dorsal striatum (dSTR) (Carboni et al., 1989; Kalivas and Duffy, 1990; Cass et al., 1992; Kuczenski and Segal, 1992). Not only do LCRs and HCRs exhibit differences in cocaine's rewarding and reinforcing effects, but also their individual locomotor activity profiles are correlated with cocaine's inhibition of striatal DAT function. In particular, after acute cocaine administration, HCRs show greater reductions than LCRs in in vivo DAT-mediated clearance of exogenous DA (i.e., greater cocaine inhibition of DATs), and this LCR/HCR difference is more pronounced in NAc than in dSTR (Sabeti et al., 2002). However, with repeated cocaine administration, LCRs develop locomotor sensitization and cocaine becomes an effective inhibitor of NAc DA clearance, thus eliminating these LCR/HCR differences (Sabeti et al., 2003). In the present study, our first goal was to determine whether the differential LCR/HCR DAT inhibition is translated into downstream differences in levels of endogenous extracellular DA in dSTR and NAc, before and/or after both acute and repeated cocaine administrations. To this end, we used in vivo microdialysis concomitantly with open-field locomotor activity measurements.

In humans, individual differences in DAT number and function have been observed both in healthy controls and cocaine users and may contribute to variable cocaine responsiveness (Little et al., 1993; Mash et al., 2002; Drgon et al., 2006). Furthermore, cocaine's apparent functional potency, but not its absolute binding affinity, depends on DAT number (Chen and Reith, 2007). Low-dose cocaine (10 mg/kg i.p.) appears to be less effective in LCRs than HCRs, both in terms of inhibiting exogenous DA clearance and stimulating locomotor activity (Sabeti et al., 2002; Gulley et al., 2003). Although Gulley et al. (2003) previously demonstrated no significant differences in cocaine's binding affinity for DATs between LCRs and HCRs. This assessment was completed in NAc-membrane homogenates prepared 7 days after an acute cocaine injection (Gulley et al., 2003). However, this time point does not coincide with any of our previous LCR/HCR measurements. Thus, in this study, we used in vitro [³H]WIN 35,428 binding assays and quantitative autoradiography (QAR) to investigate whether total striatal DAT binding site number and/or cocaine's binding affinity for striatal DATs also differ in LCRs and HCRs at the time points studied behaviorally.

Materials and Methods

Animals. Outbred male Sprague-Dawley rats (180–200 g; Charles Rivers Laboratories, Inc., Wilmington, MA) were used in microdialysis ($n = 76$) and binding/QAR experiments ($n = 48$). Rats were maintained on a 12-h light/dark cycle (0600–1800) and housed in ventilated microisolator cages with water and rat chow ad libitum. The vivarium and this research program operate in accordance with the Public Health Service *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication 85-23, revised in 1985), the Animal Welfare Act, and other applicable federal, state, and local laws.

Drugs. (–)-Cocaine hydrochloride (2β-carbomethoxy-3β-benzoyloxy tropane hydrochloride) and WIN 35,428 (2-carbomethoxy-3-(4-fluorophenyl)tropane) were gifts from the National Institute on Drug Abuse (RTI International, Research Triangle Park, NC). In all cases, 0.9% sterile saline was injected at 1 ml/kg i.p. and cocaine (dissolved in saline) was injected at 10 mg/kg i.p. [³H]WIN 35,428 was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). All other chemicals were purchased from Thermo Fisher Scientific (Waltham, MA) or Sigma-Aldrich (St. Louis, MO).

Microdialysis Experiments. *Surgery.* Under ketamine [2-(2-chlorophenyl)-2-(methylamino)cyclohexanone; 100 mg/kg i.m.] and xylazine [N-(2,6-dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine hydrochloride; 10 mg/kg i.m.] anesthesia, rats were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). A 2-mm diameter hole was drilled in the skull for a guide cannula (CMA Microdialysis, North Chelmsford, MA), which was lowered by using the following coordinates: NAc (6° angle from vertical, 1.6 mm anterior, and 2.4 mm lateral to bregma, 6.0 mm below dura) or dSTR (6° angle from vertical, 1.2 mm anterior, and 3.0 mm lateral to bregma, 3.5 mm below dura). Contralaterally, a second 2-mm diameter hole was drilled above the dSTR (above coordinates), through which a second guide cannula was lowered; it served solely as an attachment point for the stainless steel tether. Both cannulae and two anchor screws were secured with dental acrylic resin. Bacitracin was applied, and the incision was sutured shut. Rats were weighed daily and observed for signs of infection. One rat with >10% decrease in body weight was euthanized.

Acute cocaine, LCR/HCR classification, and microdialysis. Between 4 and 6 days postsurgery, animals were transported to the behavioral testing room (day 0). Animals were allowed a 30-min room habituation, during which time microdialysis probes (CMA 12 Elite) were primed before insertion by perfusion at 10 μl/min (WPI, Stoelting Company, Wood Dale, IL) for 10 min with artificial cerebrospinal fluid (145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl₂, 1 mM MgCl₂, 2 mM NaH₂PO₄, pH 7.4). Dialysis probes were inserted into the guide cannulae, and rats were placed in open-field chambers (San Diego Instruments, San Diego, CA). Locomotor activity and LCR/HCR classification protocols were adapted from previously published methods (Sabeti et al., 2002, 2003). In brief, locomotor activity was measured in chambers that each consisted of a clear acrylic box (16 × 16 × 15 inches) fitted with a photobeam frame (San Diego Instruments) surrounded by a sound-attenuating box equipped with a counter-weighted lever arm fitted to a stainless steel one-channel liquid swivel (Instech Laboratories, Plymouth Meeting, PA). After attaching the animal's microdialysis probe to the tether, it was placed into the activity chamber. Initial locomotor activity was recorded during the first 2 h in the chamber (evening of day 0), the first 60 min of which were summed to determine initial response to novelty (Sabeti et al., 2002, 2003; Gulley et al., 2003). Probes were perfused overnight at 0.3 μl/min with artificial cerebrospinal fluid. On the next day (day 1), the flow rate was first increased to 2 μl/min for 2 h for probe equilibration. Then, locomotor activity was recorded for 2 h before and after either saline or cocaine injection. Concomitantly, dialysate fractions (40 μl) were collected every 20 min into 10 μl of HCl (0.05 M). Samples remained on dry ice until storage at –80°C. Locomotor activity was recorded as consecutive, horizontal

beam breaks, converted to distance traveled (cm/1 min), and summed in 10-min bins.

Repeated cocaine and microdialysis. A separate group of animals was used for the repeated microdialysis experiments ($n = 4$ saline; $n = 14$ cocaine); all rats from this cohort were randomly divided into subgroups each containing up to four animals, because we were limited by the number of open-field activity/microdialysis chambers available for testing each day. For the first two subgroups ($n = 2$ saline, $n = 6$ cocaine), we adapted the protocol of Sabeti et al. (2003). On days 1 to 7, rats received once-daily saline or cocaine. Animals had a 160-min testing room habituation, after which locomotor activity was recorded for 90 min before (novelty/baseline response) and 60 min after injection. Only locomotor activity was measured on days 1, 3, and 5. On even days, animals received injections in their home cage in the testing room. On day 6, at least 5 h postinjection, microdialysis probes were placed into guide cannulae, and on day 7 both behavior and microdialysis measures were completed as described for the acute experiment. After observing a notable drop in cocaine-induced activity between days 5 and 7 in the first two subgroups ($n = 6$), the remaining rats ($n = 2$ saline, $n = 8$ cocaine) were treated for 5 days with once-daily injections. Only locomotor activity was measured on days 1 and 3; both activity measures and microdialysis samples were collected on day 5. Thus, within the repeated cocaine microdialysis experiment, rats received either 5 or 7 once-daily injections, with microdialysis sampling occurring on the final injection day. All animals used in these repeated cocaine microdialysis experiments were combined for data analyses and presentation, because there were no cocaine-induced behavioral differences between groups tested on day 5 or 7 [$t(12) = 0.77$, $p = 0.46$].

DA determinations. DA levels in the dialysate samples were measured by using high-pressure liquid chromatography with electrochemical detection. The high-pressure liquid chromatography system consisted of a model 118 pump (Beckman Coulter, Inc., Fullerton, CA), an ESA Coulochem III EC detector with a model 5011A dual-detector analytical cell (guard cell = 300 mV, E1 = -100 mV, E2 = 220 mV), and a microbore column (80×4.6 mm; $3 \mu\text{m}$) (ESA Analytical, Chelmsford, MA). Mobile phase (150 mM NaH_2PO_4 , 4.76 mM citric acid, 50 μM EDTA, 2.5 mM SDS, 10% methanol, 17% acetonitrile, pH 5.6) was run at a flow rate of 0.9 ml/min. The retention times of DA standards (0.1–4 nM) were used to identify DA peaks. Chromatogram peak heights, relative to each day's standard curve, were used to calculate DA concentrations.

Histology. After the microdialysis experiments, rats were anesthetized with urethane (1.5 g/kg i.p.) and sacrificed. Brains were removed, placed in 10% formalin for ≥ 24 h, and sectioned with a microtome (100 μm). Slide-mounted sections were stained with cresyl violet. Only data from animals with histologically confirmed probe placements were included.

Binding/QAR Experiments. *Locomotor activity.* Locomotor activity measurements were completed as described above with the following exceptions. Two groups of rats were used: 1) acute rats were sacrificed ~ 35 min after a single injection of saline ($n = 8$) or cocaine ($n = 16$), and 2) repeated rats were given seven once-daily injections of saline ($n = 8$) or cocaine ($n = 16$) and sacrificed ~ 35 min postinjection on day 7. This time point for sacrifice (~ 35 min) was selected such that the full 30 min of postinjection locomotor activity could be collected before sacrificing the animals. On days 1, 3, and 5, after activity measurements, repeated animals were returned to the colony room 30 min postinjection.

Radioligand binding assays and QAR. After sacrifice, brains were removed, frozen in dry ice, and stored at -80°C . Coronal brain sections (15 μm) were cut with a cryostat. Duplicate sections were thaw mounted onto slides and stored at -80°C . Sections included either 1) dSTR and NAc or 2) substantia nigra (SN) and ventral tegmental area (VTA). Optimal binding conditions, previously established in our laboratory for the cocaine congener [^3H]WIN 35,428 (4 nM), were used (Hebert et al., 1999). Nonspecific and total binding were determined with and without 30 μM benzotropine [(1R,5R)-3-

[di(phenyl)methoxy]-8-methyl-8-azabicyclo[3.2.1]octane], respectively (Hebert et al., 1999). For indirect saturation curves, concentrations of unlabeled WIN 35,428 ranging from 0.3 nM to 1 μM were used. For cocaine competition curves, concentrations of cocaine ranging from 3 nM to 10 μM were used. Slides and tritium-labeled standards (GE Healthcare, Chalfont St. Giles, UK) were exposed to film (Kodak BioMax-MR-1 Autoradiograph Film; PerkinElmer Life and Analytical Sciences) for either 12 (dSTR/NAc) or 16 (VTA/SN) weeks. After development of the films (Kodak X-OMAT1000A processor), each brain region of interest was outlined using the atlas of Paxinos and Watson (2007). The radioligand labeling intensity was measured using an MCID M4 Image Analysis System (Imaging Research, Inc., St. Catharines, ON, Canada). By using a standard curve generated for each film from the digitized autoradiograms of the tritium-labeled standards, relative optical density of the samples was converted to bound radioligand (nanocuries per milligram).

Data and Statistical Analysis. *General.* Throughout the article, data are expressed as mean $\pm$ S.E.M. For all analyses, n = number of rats and significance was set at $p < 0.05$.

Locomotor activity. All locomotor activity data were analyzed with two-way (time $\times$ group) repeated measures analysis of variance (RMANOVA) (SPSS 16.0 software; SPSS Inc., Chicago, IL). Significant main effects were further analyzed with one-way ANOVA (group) or RMANOVA (time). When Mauchly's test of the assumption of sphericity failed, significance was tested by using the more conservative Huynh-Feldt corrected degrees of freedom, indicated by an asterisk (*).

DA levels. To compare extracellular DA levels, two-way (time $\times$ group) RMANOVAs were used, followed by ANOVAs and least significant difference post hoc comparisons. Linear regressions and the resulting Pearson r values (GraphPad Prism; GraphPad Software, Inc., San Diego, CA) were used to compare relationships between cocaine-induced locomotor activity (first 30 min; period of peak behavioral activation) and extracellular DA (40 min; samples including peak activation).

Radioligand binding/QAR. For all binding studies, competition curves were statistically better fit by nonlinear regression curve algorithms for one-site, as opposed to two-site, binding (Hill coefficients ranged from 0.87 to 1.10; GraphPad Prism). For [^3H]WIN 35,428 indirect saturation experiments, B_{max} values were estimated by using the relationship $B_{\text{max}} = B_0 \times \text{IC}_{50}/[*\text{D}]$, where B_{max} = maximal number of binding sites, B_0 = specific binding of [^3H]WIN 35,428 without unlabeled WIN 35,428, IC_{50} = 50% inhibitory concentration of WIN 35,428, and $[*\text{D}]$ = concentration of [^3H]WIN 35,428 (DeBlasi et al., 1989). IC_{50} values were converted to affinities (K_d values) by using $K_d = \text{IC}_{50} - [*D]$ (DeBlasi et al., 1989). For cocaine competition experiments, IC_{50} values were determined from curve fitting; affinities (K_i values) were calculated by using the relationship $K_i = \text{IC}_{50}/(1 + [*D]/K_d)$, where K_d = affinity of [^3H]WIN 35,428 (Cheng and Prusoff, 1973). One-way ANOVAs with least significant difference post hoc comparisons were used for statistical analysis of the binding parameters.

Results

Classification of LCRs/HCRs during in Vivo Microdialysis Sampling in dSTR. In the first experiment, we verified that rats with surgically implanted microdialysis guide cannulae exhibited characteristically variable magnitudes of acute cocaine-induced locomotor activity during in vivo microdialysis. As such, they were readily classified as either LCRs or HCRs based on the median split of the distance traveled during the first 30 min after injection of 10 mg/kg (i.p.) cocaine (Fig. 1, a and b). None of the rats subsequently defined as either LCRs or HCRs, or saline-treated rats (Saline), differed in activity during the first 60 min in the inescapable novel environment (LCR = 1858 ± 180 cm/60

Fig. 1. Open-field locomotor activity and extracellular DA in dSTR of LCRs, HCRs, and saline-treated controls. **a**, time course of locomotor activity during in vivo microdialysis sampling before and after injection (arrow) of either saline or cocaine (10 mg/kg i.p.). Mean $\pm$ S.E.M., $n = 13$ (LCR), 13 (HCR), and 8 (Saline). +++, $p < 0.001$, HCR versus LCR; ###, $p < 0.001$, HCR versus Saline. **b**, summed baseline (30 min preinjection) and postinjection (30 min) locomotor activity for the following: saline-treated ($n = 8$) and cocaine-treated ($n = 26$) rats, subsequently defined as LCRs ($n = 13$, white bar) or HCRs ($n = 13$, black bar) (**a**). Line represents cocaine group median; bars are means $\pm$ S.E.M. **c**, extracellular DA levels before and after either saline or cocaine injection (arrow) expressed as a percentage of each animal's respective baseline value (no baseline differences: LCR = 1.57 ± 0.06 nM, HCR = 1.54 ± 0.10 nM, Saline = 1.32 ± 0.16 nM). Mean $\pm$ S.E.M., $n = 13$ (LCR), 11 (HCR), and 8 (Saline). Two HCRs with clogged outlets were excluded because no microdialysis samples could be collected. +, $p < 0.05$, HCR versus LCR; ##, $p < 0.001$, HCR versus Saline. **d**, positive correlation between cocaine-induced locomotor activity (30 min) in all rats treated with cocaine and extracellular DA levels (40 min; $r = 0.44$, $p = 0.03$). Linear regression fit (solid line) and median split of LCRs/HCRs (dotted line) are shown.

min; HCR = 1726 ± 315 cm/60 min; Saline = 2373 ± 285 cm/60 min; $p = 0.27$). In contrast, HCRs exhibited greater cocaine-induced locomotor activity compared with LCRs and saline-treated rats (LCR = 689 ± 91 cm/30 min; HCR = 2880 ± 384 cm/30 min; Saline = 354 ± 78 cm/30 min; Fig. 1, **a** and **b**). Two-way RMANOVA revealed significant main effects for time [$*F_{(2,9,85.3)} = 26.73$, $p < 0.001$], group [$*F_{(2,29)} = 16.47$, $p < 0.001$], and a time by group interaction [$*F_{(5,9,85.3)} = 10.41$, $p < 0.001$]. One-way ANOVA with post hoc analysis revealed significant differences at each 10-min bin during the first 60 min postinjection between LCR and HCR ($p < 0.001$), HCR, and Saline ($p < 0.001$), but not between LCR and Saline. Therefore, LCR/HCR rats undergoing in vivo microdialysis exhibited the expected locomotor activity profiles, in concordance with those published previously (Sabeti et al., 2002, 2003; Gully et al., 2003; Allen et al., 2007).

Cocaine-Induced Extracellular DA in dSTR of LCRs and HCRs. Concurrent with locomotor activity measurements (Fig. 1, **a** and **b**), we collected 40- μ l microdialysis samples every 20 min from dSTR to determine whether endogenous extracellular DA levels in this brain region of LCRs and HCRs paralleled their unique activity profiles. No differences in baseline extracellular DA levels in dSTR were ob-

served between groups [$*F_{(2,29)} = 0.31$, $p = 0.74$] (LCR = 1.57 ± 0.06 nM, HCR = 1.54 ± 0.10 nM, or Saline = 1.32 ± 0.16 nM; Fig. 1c). Therefore, extracellular DA levels for each rat were calculated as a percentage of their baseline level (average of the six baseline samples). Two-way RMANOVA revealed significant main effects for time [$*F_{(2,9,84.6)} = 7.0$, $p < 0.001$], group [$*F_{(2,29)} = 3.45$, $p < 0.05$], and a time by group interaction [$*F_{(5,84,84.6)} = 4.23$, $p = 0.001$]. Extracellular DA levels in dSTR of HCRs peaked at ~260% above baseline, whereas those in LCRs peaked at ~180% above baseline; there was no increase in extracellular DA in dSTR of saline-injected rats (Fig. 1c). One-way ANOVA with post hoc analysis of group at each time point revealed significant differences between LCR and HCR at $t = 160$ min ($p = 0.03$, 40 min after cocaine injection; and a trend at $t = 140$, $p = 0.07$), between HCR and Saline at $t = 140$ and 160 ($p = 0.001$), and between LCR and Saline at $t = 140$ ($p = 0.04$). There was a striking similarity between the time course of cocaine-induced changes in locomotor activity and extracellular DA in dSTR of LCRs and HCRs (Fig. 1, **a** and **c**). Irrespective of LCR/HCR classification, there was also a significant, positive correlation between cocaine-induced locomotor activity and extracellular DA levels in dSTR ($r = 0.44$, $p = 0.03$; Fig. 1d). These data show that a single injection of

cocaine resulted in greater increases in dSTR extracellular DA in HCRs, compared with LCRs, and that this difference paralleled their respective locomotor-activity profiles.

Cocaine-Induced Locomotor Activity and Extracellular DA in NAc. Cocaine's ability to inhibit DAT activity acutely is greater in HCRs than LCRs, and this difference is more pronounced in NAc than in dSTR (Sabeti et al., 2002). Therefore, we next measured endogenous levels of NAc extracellular DA in a second group of rats. As in the dSTR microdialysis experiment, similar locomotor activity profiles were observed in this cohort of rats such that there were no differences in response to the inescapable novel environment (LCRs, 2372 ± 743 cm/60 min; HCRs, 3135 ± 683 cm/60 min; Saline, 1644 ± 499 cm/60 min; $p = 0.78$), and clear individual variability in cocaine responsiveness was observed, again allowing ready classification of LCRs and HCRs (Fig. 2, a and b). Figure 2a shows the time course of locomotor activity during in vivo microdialysis sampling before and after either saline or cocaine. After cocaine, HCRs exhibited greater locomotor activity than LCRs, and their locomotor activity was also greater than saline-treated rats (LCR = 293 ± 68 cm/30

min; HCR = 2152 ± 470 cm/30 min; Saline = 310 ± 106 cm/30 min; Fig. 2b). Two-way RMANOVA revealed significant main effects for time [$F_{(6,2,73.8)} = 16.5$, $p < 0.001$], group [$F_{(2,12)} = 28.0$, $p < 0.001$], and time by group interaction [$F_{(12,3,73.8)} = 6.86$, $p < 0.001$]. One-way ANOVA with post hoc analysis revealed significant differences at each 10-min bin during the first 60 min postinjection between LCR and HCR ($p < 0.001$), HCR and Saline ($p < 0.01$), but not LCR and Saline.

Analysis of NAc dialysate samples again revealed no between group differences in baseline extracellular DA levels [$F_{(2,12)} = 1.96$, $p = 0.18$] (LCR = 0.74 ± 0.18 nM, HCR = 0.78 ± 0.14 nM, Saline = 0.47 ± 0.17 nM; Fig. 2c). However, as expected, overall baseline DA levels in NAc were ~50% lower than those measured in dSTR. It is interesting to note that cocaine-induced NAc DA levels in HCRs peaked at ~500% above baseline, compared to ~160% above baseline in LCRs, and remained elevated above those in LCRs or saline-treated rats for 60 min. Analysis of extracellular DA levels in NAc as a percentage baseline revealed significant main effects for time [$F_{(4,2,50.5)} = 34.6$, $p < 0.001$], group [$F_{(2,12)} =$

Fig. 2. Open-field locomotor activity and extracellular DA in NAc of LCRs, HCRs, and saline-treated controls. **a**, time course of locomotor activity during in vivo microdialysis sampling before and after injection (arrow) of either saline or cocaine (10 mg/kg i.p.). Mean $\pm$ S.E.M., $n = 6$ (LCR), 6 (HCR), and 4 (Saline). +++, $p < 0.001$, HCR versus LCR; ###, $p < 0.001$, HCR versus Saline. **b**, summed baseline (30 min preinjection) and postinjection (30 min) locomotor activity for the following: saline-treated ($n = 4$) and cocaine-treated ($n = 12$) rats, subsequently defined as LCRs ($n = 6$, white bar) or HCRs ($n = 6$, black bar) (**a**). Line represents cocaine group median; bars are means $\pm$ S.E.M. **c**, extracellular DA levels before and after either saline or cocaine injection (arrow) expressed as a percentage of each animal's respective baseline value (no baseline differences: LCR = 0.74 ± 0.18 nM, HCR = 0.78 ± 0.14 nM, Saline = 0.47 ± 0.17 nM). Mean $\pm$ S.E.M., $n = 6$ (LCR), 5 (HCR), and 4 (Saline). One HCR was excluded based on probe placement. +++, $p < 0.001$, HCR versus LCR; ###, $p < 0.001$, HCR versus Saline. **d**, positive correlation in all rats treated with cocaine between cocaine-induced locomotor activity (30 min) and extracellular DA levels (40 min; $r = 0.89$, $p < 0.001$). Linear regression fit (solid line) and median split of LCRs/HCRs (dotted line) are shown.

24.7, $p < 0.001$], and a time by group interaction [$*F_{(8,4,50.5)} = 20.2$, $p < 0.001$]. Post hoc analyses revealed significant differences at $t = 140, 160$, and 180 min between LCR and HCR ($p < 0.001$) and between HCR and Saline ($p < 0.001$), but not between LCR and Saline. Furthermore, there was a strong, positive correlation between cocaine-induced extracellular DA levels in NAc and locomotor activity in all of the cocaine-treated rats ($r = 0.89$, $p < 0.001$; Fig. 2d).

Repeated Cocaine-Induced Locomotor Activity and Extracellular DA in NAc. To investigate whether differences in cocaine-induced locomotor activity and/or extracellular DA in NAc would still be present between LCRs and HCRs after repeated cocaine, a third group of animals was tested. Locomotor activity was measured every other day and microdialysis samples were collected on the final day of treatment, both pre- and postinjection. There were no differences in preinjection locomotor activity data on any of the days that locomotor activity data were collected, including on the final day during microdialysis sampling (data not shown). For this group of animals, locomotor activity was measured on day 1 without tethering the animals in the activity chambers overnight, to replicate most closely our previous repeated cocaine-induced sensitization protocol (Sabeti et al., 2003). In addition, the cocaine-induced activity levels observed in our acute microdialysis experiments with tethered rats (Figs. 1b and

2b) did not differ significantly from rats that were not tethered (e.g., Fig. 4, a and b). Activity profiles on day 1 in the repeated cocaine group (Fig. 3a) demonstrated wide variability in magnitudes of cocaine-induced locomotor activity, with no differences in response to novelty (LCR, 3433 ± 447 cm/60 min; HCR, 3956 ± 466 cm/60 min; Saline, 3356 ± 435 cm/60 min), consistent with our previous observations. Two-way RMANOVA revealed a main effect of group [$*F_{(2,14)} = 15.82$, $p < 0.001$] and a time by group interaction [$*F_{(3,2,22.5)} = 3.74$, $p = 0.024$]. On day 1 LCRs and HCRs differed in cocaine-induced activity, with HCRs exhibiting 6-fold greater cocaine-induced activity than LCRs ($p < 0.001$; Fig. 3b).

By day 3 of repeated cocaine, however, cocaine-induced locomotor activity in LCRs had increased such that it no longer differed from HCRs, and both were significantly greater than Saline (Fig. 3b). Thus, LCRs developed locomotor sensitization by day 3 of this repeated cocaine protocol. On the day of microdialysis sampling (see under *Materials and Methods*), there was an unanticipated overall decrease in cocaine-induced locomotor activity below the levels seen on day 1 (HCRs) or day 3 (LCRs and HCRs; Fig. 3b). Nonetheless, cocaine-induced locomotor activity did not differ between LCRs and HCRs (Fig. 3b). It is noteworthy that also on this day, despite the overall lower cocaine-induced activity, the activity in both LCRs and HCRs was significantly greater

Fig. 3. Locomotor sensitization in LCRs and extracellular DA in NAc of LCRs and HCRs after once-daily repeated cocaine (10 mg/kg i.p.). a, day 1 baseline (30 min preinjection) and postinjection (30 min) locomotor activity for all saline-treated ($n = 4$) and cocaine-treated ($n = 14$) rats, subsequently defined as LCRs ($n = 7$, white bar) or HCRs ($n = 7$, black bar). Line represents cocaine group median; bars are means $\pm$ S.E.M. b, locomotor activity for the rats shown in a during the 60-min postcocaine or -saline period on days 1, 3, and the day of microdialysis (either day 5 or 7). After day 1, LCRs exhibited locomotor sensitization and no longer differed from HCRs. Mean $\pm$ S.E.M. +++, $p < 0.001$, HCR versus LCR; ###, $p < 0.001$ and ##, $p < 0.01$, HCR versus Saline; *, $p < 0.05$ LCR versus Saline; &&, $p < 0.01$ cocaine versus Saline. c, extracellular DA levels before and after either saline or cocaine injection (arrow) expressed as a percentage of each animal's respective baseline value (no baseline differences: LCR = 0.72 ± 0.45 nM, HCR = 0.63 ± 0.27 nM, Saline = 0.86 ± 0.12 nM). Mean $\pm$ S.E.M., $n = 7$ (HCR), 6 (LCR), and 4 (Saline). One LCR with a clogged outlet was excluded because no microdialysis samples could be collected. d, positive correlation on the day of microdialysis between cocaine-induced locomotor activity (30 min) and extracellular DA levels (40 min; $r = 0.72$, $p = 0.006$). Linear regression fit shown by solid line.

than activity in saline-injected rats [Fig. 3b; $t(14.8) = -2.88$, $p < 0.01$, corrected for unequal variances].

On the day of microdialysis sampling after repeated cocaine or saline, baseline extracellular DA levels in NAc did not differ between groups—LCR, HCR, or Saline [$F_{(2,14)} = 0.605$, $p = 0.56$] (Fig. 3c; LCR = 0.72 ± 0.45 nM, HCR = 0.63 ± 0.27 nM, Saline = 0.86 ± 0.12 nM)—and did not differ from the baseline values measured in the acute NAc microdialysis experiment. As measured by RMANOVA, there was not a significant effect of time by day [$*F_{(4.13,107.3)} = 0.34$, $p = 0.86$], time by group [$*F_{(8.25,107.3)} = 0.63$, $p = 0.76$], or a time by group by day interaction [$*F_{(8.25,107.3)} = 1.08$, $p = 0.39$]. In addition, after repeated cocaine administration, no group differences were observed in extracellular DA levels in NAc between LCRs and HCRs [$*F_{(2,14)} = 2.88$, $p = 0.09$], with peak increases over baseline of 350 and 250%, respectively (Fig. 3c). However, it is noteworthy that, as seen with the behavioral analyses, an overall significant effect of cocaine was present with respect to saline-injected animals [$t(12.7) = -3.46$, $p = 0.004$]. Moreover, after repeated cocaine administration and irrespective of LCR/HCR classification, cocaine-induced extracellular DA levels were still positively correlated with locomotor activity on the day of microdialysis ($r = 0.72$, $p < 0.01$; Fig. 3d). Therefore, repeated cocaine, compared to acute cocaine, resulted in markedly different profiles for cocaine-induced locomotor activity and extracellular DA, such that after repeated cocaine LCR/HCR differences in cocaine-induced activity and NAc extracellular DA levels were no longer detected, paralleling the lack of LCR/HCR differences reported in cocaine's inhibition of DAT in NAc after repeated cocaine administration (Sabeti et al., 2003).

Locomotor Activity and Sensitization in Rats Used in DAT Binding Experiments. Two additional groups of outbred male Sprague-Dawley rats were used to investigate whether LCRs and HCRs differ in total DAT binding site number (B_{max}) and/or cocaine's affinity (K_i) for DAT: group 1) "Acute" animals received a single injection of saline or cocaine, and group 2) "Repeated" animals received 7 days of once-daily saline or cocaine. No differences were observed in baseline locomotor activity between LCRs, HCRs, and saline-treated rats, in either the Acute or Repeated groups (Fig. 4, a and b, respectively). Wide individual variability was again seen in the magnitudes of the cocaine-induced locomotor activity in the first 30 min postcocaine on treatment day 1 in both Acute (LCR = 1174 ± 267 cm/30 min; HCR = 2543 ± 118 cm/30 min; Saline = 529 ± 79 cm/30 min) and Repeated (LCR = 733 ± 79 cm/30 min; HCR = 2393 ± 395 cm/30 min; Saline = 789 ± 51 cm/30 min) groups (Fig. 4, a and b, respectively), such that rats were readily classified as LCRs and HCRs. Although ANOVAs revealed no significant differences in either baseline or cocaine-induced activity between the Acute and Repeated groups, the group median (used to classify LCRs/HCRs) was approximately 2-fold higher in the Acute group (2067 cm/30 min) compared to the Repeated group (1141 cm/30 min). This activity primarily affected the LCR group medians [1174 ± 267 cm/30 min (Fig. 4a); 733 ± 79 cm/30 min (Fig. 4b)]. Because locomotor activity levels vary between groups of rats, possibly due to vendor rearing/handling stress differences and/or genetic variability, we and others (Marinelli, 2005) have classified individual differences within each group of rats tested and observed consistent

Fig. 4. Cocaine-induced locomotor activity in rats used in DAT binding/QAR experiments. Day 1 baseline (30 min preinjection) and postinjection (30 min) locomotor activity for all saline-treated ($n = 8$) and cocaine-treated ($n = 16$; 10 mg/kg i.p.) rats, subsequently defined as LCRs ($n = 8$, white bars) or HCRs ($n = 8$, black bars) in the Acute group (a) that received a single injection and the Repeated group (b) that subsequently received six additional days of once-daily cocaine. Lines represent cocaine group medians; bars are means $\pm$ S.E.M. +++, $p < 0.001$ and ++, $p < 0.01$, HCR versus LCR. c, Saline- or cocaine-induced locomotor activity (30-min postinjection period) on days 1, 3, 5, and 7 in Repeated group (b). LCRs developed locomotor sensitization by day 3 of the repeated cocaine treatment. Mean $\pm$ S.E.M. +++, $p < 0.001$, HCR versus LCR; ###, $p < 0.001$, ##, $p < 0.01$, and #, $p < 0.05$, HCR versus Saline; ***, $p < 0.001$ and **, $p < 0.01$, LCR versus Saline.

differences (Sabeti et al., 2002, 2003; Allen et al., 2007; Mandt et al., 2008).

Repeated cocaine administration resulted in the develop-

ment of sensitized cocaine-induced locomotor activity in LCRs, but not HCRs, such that on days 3, 5, and 7 activity no longer differed between the two cocaine-treated groups (Fig. 4c). These LCR/HCR repeated cocaine-induced locomotor activity profiles (Fig. 4c) mirrored those of previous LCR/HCR studies (Sabeti et al., 2003; Allen et al., 2007; Mandt et al., 2008). Two-way RMANOVA revealed main effects of time [$F_{(3,54)} = 3.49, p = 0.02$], group [$F_{(2,18)} = 24.93, p < 0.001$], and time by group interaction [$F_{(6,54)} = 4.95, p < 0.001$]. One-way RMANOVA revealed a main effect of time for LCRs [$F_{(3,18)} = 12.71, p < 0.001$] but not HCRs [$F_{(3,18)} = 0.671, p = 0.58$]; post hoc analysis revealed significant differences for LCRs between day 1 and days 3, 5, and 7 ($p = 0.009, p = 0.002$, and $p < 0.001$, respectively). Thus, the locomotor activity of HCRs differed significantly from that of saline-treated rats on all treatment days, but only on day 1 was the activity of LCRs significantly lower than that of HCRs (Fig. 4c).

[³H]WIN 35,428-Labeled DAT Binding Sites. By using brain sections prepared from both the forebrain (dSTR and NAc) and midbrain (VTA and SN) regions of the rats shown in Fig. 4, in vitro [³H]WIN 35,428 binding assays followed by QAR were conducted. From these analyses, the [³H]WIN 35,428 binding affinity (K_d) for DAT, total number of extracellular and intracellular DAT binding sites (B_{max}), and cocaine affinity (K_i) for DAT (see next paragraph) were determined in LCRs, HCRs, and saline-treated rats ~35 min after the acute or the final repeated injection of either cocaine or saline. It should be noted that the Acute (day 1) determinations probably reflect basal DAT binding parameters, because it is unlikely that total DAT number could be regulated by cocaine, via protein synthesis or degradation, within 35 min postinjection.

[³H]WIN 35,428 affinities calculated in the brain striatal regions (dSTR, NAc core, NAc shell) of the Acute (day 1) and Repeated (day 7) groups are shown in Table 1. ANOVAs revealed no differences for either the Acute or Repeated groups (Table 1; day 1: dSTR = [$F_{(2,21)} = 1.85, p = 0.18$], NAc Core = [$F_{(2,20)} = 1.72, p = 0.20$], NAc Shell = [$F_{(2,20)} = 1.46,$

$p = 0.25$]; day 7: dSTR = [$F_{(2,20)} = 1.45, p = 0.25$], NAc Core = [$F_{(2,20)} = 0.63, p = 0.54$], NAc Shell = [$F_{(2,20)} = 1.28, p = 0.30$]). The overall IC_{50} and K_d values were 28.1 ± 0.79 and 24.0 ± 0.79 nM, respectively. Likewise, VTA and SN also revealed no differences (Table 2; day 1: VTA = [$F_{(2,19)} = 0.59, p = 0.57$], SN = [$F_{(2,17)} = 0.41, p = 0.67$]; day 7: VTA = [$F_{(2,19)} = 0.21, p = 0.81$], SN = [$F_{(2,19)} = 0.17, p = 0.84$]). The overall IC_{50} and K_d values in the midbrain regions were 26.4 ± 2.15 and 22.1 ± 2.15 nM, respectively.

[³H]WIN 35,428 B_{max} values were calculated for each treatment group and brain region on day 1 (Acute). Because two different groups of rats were used for the Acute and Repeated experiments, one-way ANOVAs were used to compare each treatment group separately. On day 1, ANOVAs revealed that LCRs had significantly greater B_{max} values, compared to HCRs, in dSTR and NAc shell ($p < 0.05$), with a strong trend for a similar difference in the NAc core ($p = 0.053$, Figs. 5, a–c). In addition, we used correlations to assess the relationship between striatal DAT number and cocaine-induced locomotor activity. On day 1, there was a strong trend for an inverse relationship (i.e., negative correlation) between DAT B_{max} and cocaine-induced activity in NAc core ($p = 0.054$; Fig. 5e). Although there appeared to be similar inverse relationships in the other two forebrain regions, no correlations were observed in dSTR ($p = 0.10$) or NAc shell ($p = 0.083$; Fig. 5, d and f, respectively). In the VTA and SN, the lower B_{max} values were more variable, and no statistically significant LCR/HCR differences on day 1 were revealed by ANOVAs for either VTA [$F_{(2,19)} = 1.05, p = 0.37$] or SN [$F_{(2,17)} = 1.05, p = 0.37$] (Fig. 6, a and b). However, on day 1, when compared with saline controls, LCRs had consistently higher B_{max} values than HCRs in all brain regions: LCRs: dSTR = 150% of saline, NAc Core = 147%, NAc Shell = 150%, VTA = 135%, SN = 140%; HCRs: dSTR = 109%, NAc Core = 113%, NAc Shell = 95%, VTA = 91%, SN = 94% (Figs. 5, a–c, and 6, a and b).

[³H]WIN 35,428 B_{max} values were also calculated for each treatment group and brain region on day 7 (Repeated). It is interesting to note that after repeated saline or cocaine treatment, no significant differences were observed in DAT B_{max} values between treatment groups in any brain region (Fig. 7, a–c: dSTR = [$F_{(2,20)} = 0.83, p = 0.45$], NAc Core = [$F_{(2,20)} = 0.24, p = 0.79$], NAc Shell = [$F_{(2,20)} = 0.98, p = 0.39$]; Fig. 6, c and d: VTA = [$F_{(2,19)} = 0.13, p = 0.88$], SN = [$F_{(2,19)} = 0.43,$

TABLE 1

[³H]WIN 35,428 binding affinity for DATs in dSTR, NAc core, and NAc shell in LCRs, HCRs, and saline-treated rats 35 min after treatment on days 1 (Acute) and 7 (Repeated)

See Figs. 5 and 7. Mean $\pm$ S.E.M.

	Region	Group (n)	log IC_{50}	log K_d
log (mol/l)				
Day 1 (Acute)	dSTR	HCR (8)	-7.60 $\pm$ 0.07	-7.69 $\pm$ 0.09
		LCR (8)	-7.48 $\pm$ 0.04	-7.54 $\pm$ 0.04
		Saline (8)	-7.61 $\pm$ 0.04	-7.69 $\pm$ 0.05
	NAc Core	HCR (7)	-7.64 $\pm$ 0.07	-7.73 $\pm$ 0.08
		LCR (8)	-7.55 $\pm$ 0.04	-7.63 $\pm$ 0.05
		Saline (8)	-7.67 $\pm$ 0.03	-7.76 $\pm$ 0.04
	NAc Shell	HCR (7)	-7.67 $\pm$ 0.08	-7.77 $\pm$ 0.09
		LCR (8)	-7.52 $\pm$ 0.06	-7.59 $\pm$ 0.06
		Saline (8)	-7.63 $\pm$ 0.04	-7.71 $\pm$ 0.05
Day 7 (Repeated)	dSTR	HCR (7)	-7.60 $\pm$ 0.05	-7.68 $\pm$ 0.06
		LCR (8)	-7.48 $\pm$ 0.04	-7.55 $\pm$ 0.05
		Saline (8)	-7.53 $\pm$ 0.04	-7.59 $\pm$ 0.04
	NAc Core	HCR (7)	-7.68 $\pm$ 0.06	-7.73 $\pm$ 0.08
		LCR (8)	-7.55 $\pm$ 0.05	-7.61 $\pm$ 0.05
		Saline (8)	-7.59 $\pm$ 0.04	-7.63 $\pm$ 0.05
	NAc Shell	HCR (7)	-7.63 $\pm$ 0.07	-7.72 $\pm$ 0.08
		LCR (8)	-7.50 $\pm$ 0.05	-7.56 $\pm$ 0.06
		Saline (8)	-7.56 $\pm$ 0.04	-7.63 $\pm$ 0.04

TABLE 2

[³H]WIN 35,428 binding affinity for DATs in VTA and SN in LCRs, HCRs, and saline-treated rats 35 min after treatment on days 1 (Acute) and 7 (Repeated)

See Fig. 6. Mean $\pm$ S.E.M.

	Region	Group (n)	log IC_{50}	log K_d
log (mol/l)				
Day 1 (Acute)	VTA	HCR (8)	-7.69 $\pm$ 0.08	-7.82 $\pm$ 0.11
		LCR (7)	-7.63 $\pm$ 0.11	-7.74 $\pm$ 0.14
		Saline (7)	-7.68 $\pm$ 0.04	-7.78 $\pm$ 0.05
	SN	HCR (7)	-7.58 $\pm$ 0.07	-7.66 $\pm$ 0.08
		LCR (6)	-7.61 $\pm$ 0.14	-7.73 $\pm$ 0.18
		Saline (7)	-7.63 $\pm$ 0.04	-7.72 $\pm$ 0.05
Day 7 (Repeated)	VTA	HCR (6)	-7.75 $\pm$ 0.21	-7.64 $\pm$ 0.12
		LCR (8)	-7.57 $\pm$ 0.05	-7.65 $\pm$ 0.06
		Saline (8)	-7.57 $\pm$ 0.09	-7.67 $\pm$ 0.11
	SN	HCR (6)	-7.83 $\pm$ 0.32	-7.49 $\pm$ 0.14
		LCR (8)	-7.57 $\pm$ 0.10	-7.53 $\pm$ 0.14
		Saline (8)	-7.55 $\pm$ 0.14	-7.63 $\pm$ 0.20

Fig. 5. Greater number of DAT binding sites ($B_{\max}$) in dSTR and NAc of LCRs than HCRs in the Acute (day 1) cocaine treatment group. After measuring saline- or cocaine-induced locomotor activity (Fig. 4a), rats were sacrificed ~35 min postinjection. $B_{\max}$ values were determined from in vitro [3 H]WIN 35,428 binding assays and QAR analysis in dSTR (a and d), NAc Core (b and e), and NAc Shell (c and f). a–c, results in LCRs, HCRs, and saline-treated rats. Affinity values from these analyses and sample sizes (n) are shown in Table 1. Mean $\pm$ S.E.M. *, $p < 0.05$ LCR versus HCR and LCR versus Saline. d–f, correlations between cocaine-induced locomotor activity and striatal DAT $B_{\max}$ values for all Acute cocaine-treated rats [$n = 16$ (d), $n = 15$ (e and f)].

$p = 0.67$)). In addition, day 7 DAT $B_{\max}$ values did not correlate with cocaine-induced locomotor activity in any of these brain regions (Fig. 7, d–f). Overall (i.e., collapsing across cocaine- and saline-treated rats), $B_{\max}$ values differed between each brain region examined such that dSTR > NAc core > NAc shell > VTA > SN (Figs. 5, 6, and 7). The differences observed in the striatal DAT $B_{\max}$ between LCRs and HCRs on day 1 suggest that baseline differences in striatal DAT number exist and may contribute to acute cocaine's differential inhibition of DATs, which we measure as the downstream readout of cocaine-induced locomotor activity. Likewise, the similar number of striatal DATs found in LCRs and HCRs after repeated cocaine administration is consistent with their comparable cocaine-induced locomotor activation.

Cocaine Binding Affinity for DATs in LCRs and HCRs. To rule out differences in cocaine's affinity for DATs as one potential source of the individual differences in cocaine responsiveness between LCRs and HCRs, cocaine competition binding experiments were conducted, using striatal sections (dSTR/NAc) prepared from the Acute and Repeated animals, and followed by QAR analysis. It is noteworthy that no differences were found in cocaine's binding affinity for DATs between LCRs, HCRs, and saline-treated rats (Table 3). By collapsing these values across groups (i.e., combining cocaine- and saline-treated rats), 2-fold, statistically significant differences emerged between striatal regions. The lowest affinity was found on day 1 in dSTR ($K_i = 2.17 \pm 0.15$ μ M), and the highest affinity was found on day 7 in NAc shell ($K_i = 1.01 \pm 0.09$ μ M).

Fig. 6. No differences in DAT B_{max} values in VTA (a and c) and SN (b and d) of LCRs, HCRs, and saline-treated rats after Acute (day 1; a and b) or Repeated (day 7; c and d) treatment. After measuring saline- or cocaine-induced locomotor activity on day 7 (Fig. 4c), rats were sacrificed ~35 min postinjection. B_{max} values were determined as in Fig. 5. Affinity values from these analyses and sample sizes (n) are shown in Table 2. Mean $\pm$ S.E.M.

Discussion

Our results significantly strengthen the link between striatal DA/DATs and individual differences in acute responsiveness to low-dose cocaine in the LCR/HCR rat model. After acute cocaine, LCRs exhibited markedly lower increases in locomotor activity and striatal extracellular DA levels, compared to HCRs, as well as higher numbers of striatal [3 H]WIN 35,428-labeled DATs. LCR/HCR differences in cocaine-induced extracellular DA were more pronounced in NAc than in dSTR, and each rat's cocaine-induced locomotor activity and DA levels were positively correlated. However, after repeated cocaine administration, the cocaine-induced locomotor activity, extracellular DA levels, and DAT B_{max} values no longer differed between LCRs and HCRs. Taken together, our results suggest that initial LCR/HCR DAT differences help to explain both the differential acute cocaine inhibition of DATs and locomotor activation. Furthermore, our results support the conclusion that with repeated cocaine administration, the LCR phenotype exhibits greater striatal DAT/DA plasticity and is predictive of locomotor sensitization, as well as enhanced cocaine-conditioned place preference, and motivation to self-administer cocaine (Sabeti et al., 2003; Allen et al., 2007; Mandt et al., 2008).

As predicted by the lack of LCR/HCR baseline locomotor activity differences, no group differences were seen in baseline extracellular DA in either dSTR or NAc. Likewise, baseline extracellular DA levels did not differ between animals that sensitized to repeated cocaine administration and those that did not (Kalivas and Duffy, 1990). There was also good correspondence between both the time course and magnitude

of cocaine-induced activity and striatal extracellular DA levels in LCRs and HCRs. Correlations between these measures revealed that after acutely administered cocaine, extracellular DA levels account for between 20% (dSTR) and 80% (NAc) of the variability in cocaine-induced locomotor activity. The greater strength of this relationship in NAc was predicted by the more pronounced LCR/HCR differences in cocaine inhibition of DAT-mediated clearance of locally applied DA in NAc, compared to dSTR (Sabeti et al., 2002). In addition, others have demonstrated both the overall importance of NAc in cocaine-induced locomotor activity (Delfs et al., 1990) and extracellular DA levels (Carboni et al., 1989; Kuczenski and Segal, 1992). A regional difference in cocaine affinity for DAT seems to be an unlikely explanation (Cass et al., 1992). Unexpectedly, in this study we observed that the binding affinity of cocaine was significantly higher in NAc than dSTR; however, the functional relevance of a 2-fold affinity difference is debatable and requires further confirmation.

Our results are also novel in that shortly after (~35 min) acute cocaine, we found LCR/HCR differences in striatal DAT binding site number. Because total DAT numbers would not be expected to change via protein synthesis or degradation so quickly after drug administration, the 33% higher B_{max} in LCRs probably reflects a basal LCR/HCR difference. Cocaine's apparent potency depends on DAT number in an inverse relationship, such that lower potency reflects increased DAT expression (Chen and Reith, 2007). This relationship could help to explain part of the differential effects of low-dose cocaine in LCRs and HCRs. For example, lower apparent cocaine potency in LCRs is consistent with minimal

Fig. 7. No differences in striatal DAT B_{max} values between LCRs and HCRs on day 7 in the Repeated cocaine treatment group. B_{max} values were determined as in Fig. 5 in dSTR (a and d), NAc Core (b and e), and NAc Shell (c and f). a–c, results in LCRs, HCRs, and saline-treated rats. Affinity values from these analyses and sample sizes (n) are shown in Table 1. Mean $\pm$ S.E.M. d–f, correlations between cocaine-induced locomotor activity and striatal DAT B_{max} values for all Repeated cocaine-treated rats ($n = 15$ for d–f).

cocaine inhibition of DAT-mediated DA clearance and lower cocaine-induced activity in LCRs (Sabeti et al., 2002). This idea is strengthened by considering the following points. First, LCRs and HCRs received the same dose of cocaine (10 mg/kg, i.p.), resulting in similar brain drug levels (Gulley et al., 2003). Second, this dose occupies the same percentage of LCR/HCR striatal DATs (i.e., we found no LCR/HCR differences in cocaine's binding affinity for DAT). Third, LCRs have a higher striatal DAT B_{max} than HCRs; thus, we would expect a greater absolute number of LCR DATs to be unoccupied after low-dose cocaine. This "reserve" population of active DATs would efficiently clear extracellular DA in the presence of cocaine. Our microdialysis studies support this model, because LCRs had relatively low levels of cocaine-induced extracellular DA and, presumably, nominal stimulation of DA receptors and activity. However, without zero net flux microdialysis we cannot rule out the potential contribution of LCR/HCR differences in DA release. It is noteworthy that without reserve DATs, HCRs would clear cocaine-induced extracellular DA less well, and the resulting

elevated levels of extracellular DA (verified via microdialysis) would produce greater stimulation of DA receptors and activity. Although a full cocaine dose-response has not been determined in LCRs and HCRs, increasing the dose of cocaine to 20 mg/kg (i.p.) results in greater activation in LCRs, and thereby less pronounced LCR/HCR distinction (Gulley et al., 2003).

LCRs are more likely than HCRs to develop locomotor sensitization after repeated cocaine (10 mg/kg i.p.) administration, thus eliminating LCR/HCR differences in cocaine-induced locomotor activity (Sabeti et al., 2003; Allen et al., 2007; Mandt et al., 2008). Our results confirmed these observations. After repeated cocaine administration, extracellular DA in rat dSTR/NAc has been reported to increase, decrease, or remain the same (Kalivas and Duffy, 1990; Pettit et al., 1990; Segal and Kuczenski, 1992; Zhang et al., 2001). Some of this interexperiment variability may depend on protocol differences; however, the assumption that outbred male Sprague-Dawley rats are homogeneous may also obscure important differences revealed when initial individual variance

TABLE 3

Cocaine binding affinity for DATs in dSTR, NAc core, and NAc shell in LCRs, HCRs, and saline-treated rats 35 min after treatment on days 1 (Acute) and 7 (Repeated)

Mean $\pm$ S.E.M.

	Region	Group (n)	log K_i
			log (mol/l)
Day 1 (Acute)	dSTR	HCR (7)	-5.71 ± 0.08
		LCR (7)	-5.73 ± 0.06
		Saline (8)	-5.64 ± 0.04
	NAc Core	HCR (7)	-5.83 ± 0.08
		LCR (7)	-5.80 ± 0.06
		Saline (8)	-5.74 ± 0.05
	NAc Shell	HCR (7)	-5.89 ± 0.11
		LCR (7)	-5.90 ± 0.08
		Saline (8)	-5.81 ± 0.05
Day 7 (Repeated)	dSTR	HCR (6)	-5.89 ± 0.06
		LCR (7)	-5.91 ± 0.05
		Saline (8)	-5.84 ± 0.07
	NAc Core	HCR (6)	-5.92 ± 0.07
		LCR (7)	-6.00 ± 0.04
		Saline (8)	-5.90 ± 0.06
	NAc Shell	HCR (6)	-6.05 ± 0.07
		LCR (7)	-6.02 ± 0.07
		Saline (8)	-6.02 ± 0.06

is considered. By comparing our acute and repeated cocaine microdialysis results, we found that LCRs exhibited an increase above baseline in peak NAc extracellular DA from 160 to 350%, whereas HCRs exhibited a decrease from 500 to 280%, resulting in similar DA levels in LCRs and HCRs.

However, we were surprised to find that both LCRs and HCRs showed a marked decrease in repeated cocaine-induced locomotor activity during microdialysis sampling. We had not previously observed such a decrease (Sabeti et al., 2003; Allen et al., 2007; Mandt et al., 2008). Therefore, we believe that this apparent tolerance is due to the repeated and then prolonged overnight test chamber exposure specific to microdialysis and may not be mediated by changes in DA function. Drug dose and testing environment exposure duration can influence both cocaine-induced locomotor sensitization (Todtenkopf and Carlezon, 2006) and associated increases in extracellular DA (Duvauchelle et al., 2000). Thus, the relatively low dose of cocaine used, repeated 60-min post-cocaine chamber exposure, and overnight chamber exposure for microdialysis may have all contributed to the low levels of activation/sensitization. However, changing these variables would have prevented us from comparing our results with those published previously or from obtaining reliable baseline DA levels. In addition, we have previously demonstrated that this dose of cocaine (10 mg/kg i.p.) does not result in stereotyped behaviors that explain the differential LCR/HCR locomotor activation after either acute (Gulley et al., 2003) or repeated cocaine (Sabeti et al., 2003). Nonetheless, the behavior profiles of LCRs were markedly different on day 1 and the day of microdialysis, and these results were reflected by parallel differences in extracellular DA.

Although many studies have not detected changes in total DAT number after repeated cocaine (Zahniser and Doolen, 2001) administration, our results showed that after 7 days of once-daily cocaine the DAT B_{max} values in dSTR and NAc of the LCRs, but not HCRs, were changed with respect to saline controls. Thus, LCR/HCR differences in striatal DAT number were no longer observed after repeated cocaine administration. Total DAT number could be regulated readily within

this time scale; the physiologic half-life of DAT has been estimated to be ~ 2 days in rat brain (Kimmel et al., 2000). It is noteworthy that no group differences in cocaine binding affinity for DAT were detected. Therefore, changes in overall DAT number, but not cocaine's binding affinity for DAT, may be one contributing mechanism underlying our observations that locomotor activity and extracellular DA levels no longer differed between LCRs and HCRs after repeated cocaine administration.

Other mechanisms, potentially mediated by neurotransmitters other than DA, may contribute to the disappearance of LCR/HCR differences in striatal DAT number, extracellular DA levels, or locomotor activity after repeated cocaine. Glutamate plays an important role in repeated cocaine-induced behaviors (Kalivas, 2009) and thus may contribute to repeated cocaine-induced changes in LCR/HCR behavior. In addition, serotonin 5-HT_{2A/2C} receptors play a role in cocaine-induced activity, extracellular DA levels, and cocaine reinforcement (Bubar and Cunningham, 2006). By using Long-Evans rats, another group recently demonstrated that LCRs are more sensitive than HCRs to cocaine's interceptive effects, in part due to 5-HT_{2A/2C} receptors (Klein and Gulley, 2009). Therefore, glutamate, its receptors, and 5-HT_{2A/2C} receptors would be of interest for future investigation of potential "driving forces" for the paradox of why LCRs do not exhibit initial cocaine-induced locomotor activity but display marked changes in repeated cocaine-induced behaviors.

The results presented here have begun unraveling the neurochemical underpinnings of the LCR/HCR model, in which animals exhibiting low initial cocaine activation (LCRs) more readily than HCRs exhibit cocaine-induced locomotor sensitization, place-conditioned reward, and greater motivation to self-administer cocaine (Sabeti et al., 2003; Allen et al., 2007; Mandt et al., 2008). Our findings suggest that both striatal DAT number and extracellular DA can play an important role in initial individual differences in cocaine activation and support further investigation into what triggers repeated cocaine-induced changes in these parameters and the predictive value of the LCR/HCR phenotypes for cocaine-addictive behaviors.

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Address correspondence to: Anna M. Nelson, University of Colorado Denver, Anschutz Medical Campus, Department of Pharmacology, Mail Stop 8303, RC1-North, 12800 E. 19th Ave., Aurora, CO 80045. E-mail: anna.nelson@ucdenver.edu