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Acute Cocaine Differentially Alters Accumbens and Striatal Dopamine Clearance in Low and High Cocaine Locomotor Responders: Behavioral and Electrochemical Recordings in Freely Moving Rats

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ABSTRACT

Behavioral responses of rodents to cocaine are characterized by marked individual variability. Here, outbred male Sprague-Dawley rats were profiled based on concomitant recording of behavioral and electrochemical responses. Rats were categorized as either low or high cocaine responders (LCRs or HCRs, respectively) based on their differential locomotor responsiveness to an acute, low-dose injection of cocaine (10 mg/kg i.p.). LCRs and HCRs also differed in other cocaine-induced behaviors. The role of the dopamine transporter (DAT) in mediating the behavioral differences in cocaine responsiveness in LCRs and HCRs was investigated by high-speed chronoamperometric recording of exogenous dopamine (DA) clearance signals in nucleus accumbens (NAc) and dorsal striatum (dSTR). Higher volumes of DA were required in NAc of HCRs, than of LCRs, to produce equivalent peak DA signal amplitude (A_{max}) responses. In HCRs, systemic cocaine administration evoked an immediate and prolonged 2-fold augmentation in A_{max} in both brain regions, coincident with locomotor activation. The cocaine-induced decrease in the efficiency of DA clearance (k) in NAc of HCRs was more immediate and prolonged than in dSTR, where the transient decrease coincided with maximal stereotypic behavior. In contrast, in LCRs, A_{max} was not altered by cocaine, and decay rate constant (k) was transiently attenuated only in dSTR. Correlation analyses of individual responses revealed that cocaine-induced changes in DA clearance signal parameters accounted for 20 to 40% of the variation in behavioral responsiveness to cocaine. Overall, our findings emphasize the importance of characterizing individual responses to understand more fully the range of functional consequences resulting from DAT inhibition.

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Psychomotor stimulants such as cocaine bind to the dopamine (DA) transporter (DAT), thereby inhibiting the machinery primarily responsible for the clearance of DA from the extracellular space and termination of DA neurotransmission (Ritz et al., 1987). The consequences of DAT inhibition in vivo have been investigated using either microdialysis or voltammetry. These studies have demonstrated that both local and systemic administrations of psychomotor stimulants elicit an increase in extracellular DA concentration and prolongs the time course of DA clearance within rat nucleus accumbens (NAc) and dorsal striatum (dSTR) (Nomikos et al., 1990; Kuczenski and Segal, 1992; Cass et al., 1993b; Zahniser et al., 1999). It has been postulated that this spatial and temporal augmentation in DA signaling following reuptake blockade mediates the behavioral activating and addictive properties of stimulants that target DAT (Kuhar et al., 1991).

A common spectrum of behavioral effects, including locomotor activation, stereotyped behaviors, and self-administration, is produced by DAT inhibitors. Nonetheless, individual differences have been observed in the behavioral responsiveness to acute or repeated administration of either cocaine (Hooks et al., 1991a; Cass et al., 1993a; Djano and Martin-Iverson, 2000; Piazza et al., 2000) or d-amphetamine (Segal and Schuckit, 1983; Segal and Kuczenski, 1987; Piazza et al., 1989; Hooks et al., 1991a; Cools et al., 1997; Cools and Gingras, 1998). Furthermore, the individual heterogeneity in behavioral responsiveness occurs over a wide range of doses (Segal and Schuckit, 1983; Hooks et al., 1991b; Piazza et al.,

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ABBREVIATIONS: DA, dopamine; DAT, dopamine transporter; dSTR, dorsal striatum; NAc, nucleus accumbens; LCR, low cocaine responder; HCR, high cocaine responder; A_{max}, peak signal amplitude; k, decay rate constant; ANOVA, analysis of variance; KS, Kolmogorov-Smirnov.

2000; but see Cools et al., 1997). At the neurochemical level, microdialysis has been used to detect differences among rats in basal, as well as in *d*-amphetamine- and cocaine-stimulated, extracellular DA concentrations (Bradberry et al., 1991; Hooks et al., 1992). It has been suggested that such individual variations in resting DA levels in terminal field areas may reflect between-subject differences in firing rates of DA neurons originating in the ventral tegmental area and substantia nigra pars compacta (Marinelli and White, 2000). However, little is known about individual differences in DA reuptake/uptake, which is critical for regulating dopaminergic tone. Using in vivo chronoamperotometry to measure the clearance of exogenous DA largely by uptake in dSTR of anesthetized rats, we found a wide range of changes in peak DA signal amplitudes (A_{max}) in response to local application of cocaine (Zahniser et al., 1999). However, we were unable to determine the relationship of such variability in DAT function with behavior since the rats were anesthetized for the electrochemical recordings. Nonetheless, the reported diversity in the behavioral and neurochemical effects of cocaine in individual animals suggests the possibility that differential responsiveness to DAT inhibition may contribute to important functional differences.

Previously, we developed technology to couple high-speed chronoamperometric recordings with exogenous DA microejections so that DAT-mediated clearance of DA could be measured simultaneously with behavior in freely moving rats (Gerhardt et al., 1999). The kinetics of DAT function in freely moving rats can be specifically evaluated with this methodology by examining A_{max} values evoked by local ejections of exogenous DA and the rate constants for DA clearance (k; Sabeti et al., 2000). Specifically, we and others have previously demonstrated that A_{max} values, measured in response to DA with voltammetry, reflect the number of functional reuptake/uptake sites (for discussion see Cass et al., 1992; Hebert and Gerhardt, 1999). Furthermore, the usefulness of the k parameter in quantifying changes in the clearance efficiency of DA at low concentrations has been demonstrated (Sabeti et al., 2000; also see *Materials and Methods*).

We hypothesized that differential cocaine-induced changes in the kinetics of DAT function within NAc and dSTR, regions essential for mediating motor activation and stereotyped behaviors, respectively, would be functionally relevant to specific behaviors evoked by acute systemic cocaine administration. We tested a single low dose of cocaine (10 mg/kg i.p.) that promotes enhanced locomotion and elevates dialysate DA levels in both NAc and dSTR of freely moving rats (Kuczenski et al., 1991). Specifically, our aims were: 1) to characterize the baseline kinetics of DA clearance in NAc and dSTR of drug-naive, freely moving rats; 2) to compare the time courses for the behavioral activation and altered kinetics of DA clearance induced by cocaine; and 3) to investigate whether the magnitude of changes in behavioral responsiveness and kinetics of DA clearance were correlated in individual rats following DAT inhibition.

Materials and Methods

Animals. Outbred male Sprague-Dawley rats, 1 to 3 months of age on the day of surgery [average age 2.2 ± 0.2 months, low cocaine responder (LCR) group, $n = 17$; or 1.9 ± 0.3 months, high cocaine responder (HCR) group, $n = 15$; see Fig. 2B for group categorization criterion), were obtained from Charles River Laboratory (Sasco, Omaha, NE). Rats were housed no more than six per cage with a 12-h light/dark cycle and unrestricted access to food and water. One to 2 days before surgery, rats were handled for 10 to 15 min each day to reduce handling stress during injector tubing insertions on the experimental days. Following surgery, rats were housed individually. All animal care procedures were in accordance with the National Institutes of Health *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.

Surgical Implantation of Electrode/Microcannulae Assemblies and Testing of Placement. Recording electrode/microcannulae assemblies were constructed using a single 30-μm-diameter carbon-fiber (Textron Systems, Wilmington, MA), which was sealed inside fused silica tubing (Polymicro Technologies, Phoenix, AZ) according to procedures previously described in detail (Gerhardt et al., 1999). The exposed tip of the carbon-fiber (200–300 μm in length) was coated with Nafion (5% solution; Aldrich Chemical Co., Milwaukee, WI; 5–6 coats baked at 120°C for 5 min per coat) to provide DA over ascorbic acid selectivity ≥1000:1. Microelectrodes displayed linear chronoamperometric responses to 1 to 6 μM DA challenges during in vitro calibrations in 0.1 M phosphate-buffered saline (pH 7.4). To allow for delivery of DA adjacent to the microelectrode, each microelectrode was wax-assembled onto two stainless steel guide microcannulae, creating a tripod-like geometric configuration. Injectors were fabricated from fused silica tubing (40 μm i.d. × 150 μm o.d.) and, once inserted through the guide, extended to the exposed tip of the carbon-fiber, with a center to center distance of 250 to 350 μm from the microelectrode. A miniature Ag/AgCl reference microelectrode was prepared by plating a Teflon-coated Ag wire (0.011-inch diameter; A-M Systems, Carlsborg, WA) for 1 h in a 1 M HCl solution saturated with NaCl. The reference microelectrode was soaked in 3 M NaCl for 1 h before implantation. Prior to implantation, leads from the carbon-fiber recording microelectrode and the Ag/AgCl reference microelectrode were soldered to a four-pin modular telephone connector.

Anesthesia was induced by an initial dose of chloral hydrate (4% solution; 200 mg/kg i.p.) followed by two supplemental doses (100 mg/kg i.p.) administered at 15- to 20-min intervals. Anesthesia was maintained with additional 100 mg/kg i.p. injections as required. The electrode/microcannulae assembly was stereotaxically implanted into the core of the NAc (anterior-posterior: +1.2–1.5 mm from bregma; medial-lateral: 2.2 mm left from the midline; dorsal-ventral: 6.5–7.5 mm below surface), or into the dSTR (same coordinates as above, with the exception of dorsal-ventral: 4.5–5.5 mm below surface) of anesthetized rats. The reference microelectrode was implanted into the posterior cerebral cortex. To verify that the microelectrode was situated in close proximity to DA neurons on which DATs are localized, pulses of KCl (120 mM in 29 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4; 300–800 nl) were infused through the injectors to stimulate DA release (Gerhardt et al., 1986). Once an appropriate placement in a DAT-rich site was verified, dummy injectors were inserted through the guide microcannulae to prevent obstruction. The connecting wires and all connections were encased in heat shrink tubing. A plastic cap protected the entire assembly, which was anchored to five small screws in the skull and embedded in dental cement and a thick layer of 5-min epoxy.

Experimental Protocol. Three to 5 days after surgery, each rat was transferred from its home cage to an open field activity apparatus (San Diego Instruments, San Diego, CA), which consisted of a clear acrylic box (16 × 16 × 15 inches) fitted with a photo beam frame (eight beams per dimension) near the base. The activity apparatus was enclosed in a Faraday cage (2 × 2 × 2 feet). Room lights were kept on throughout the experiment. The rat was connected to a miniature potentiostat headstage/tether linked to an IVEC-10/FAST-12 electrochemical recording system (Quanteon, LLC, Lexington, KY; Gerhardt et al., 1999). Simultaneous behavioral and in vivo chronoamperometric recordings were obtained according to the ex-

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perimental timeline described next and shown in Fig. 1. Rats were acclimated to the activity apparatus for a total of 60 min before recording "baseline" behavioral and electrochemical activity. During this period, rats were handled momentarily while the injector tubing was inserted through the guide microcannulae in preparation for repeated ejections of exogenous DA. A stable background oxidation signal was established in the absence of exogenous DA and set to zero. Subsequently, finite volumes (150–1500 nl) of exogenous DA (200 μM in saline and 100 μM ascorbic acid, pH 7.4 adjusted with sodium hydroxide) were ejected at the recording site at 5-min intervals, using a microprocessor-controlled syringe-pump (infusion rate 1.01 μl/s; Stoelting Co., Wood Dale, IL), as described elsewhere (Gerhardt et al., 1999). A single ejection volume of exogenous DA was selected for each rat so that the A_{max} values ranged from 0.3 to 1.5 μM. Once selected, the ejection volume was kept constant throughout the remainder of the recording session. We have previously demonstrated that ejection volumes of DA within these ranges result in localized and transient increases in DA that have no measurable effects on behavior (Gerhardt et al., 1999). Furthermore, based on previous measurements of clearance velocity in dSTR, such ejection volumes deliver subsaturating quantities of DA to DAT-rich sites (Zahniser et al., 1999; Sabeti et al., 2000, 2001). "Baseline" measurements of behavior and DA clearance signals consisted of 30 min of recording immediately prior to the i.p. administration of saline (1 ml/kg) or cocaine (10 mg/kg). Behavioral and electrochemical data continued to be collected for an additional 60 min.

Behavioral Data Acquisition and Analysis. Behavior was measured by both automated and observational methods. "Locomotor activity" in the open field was quantified using the automated consecutive horizontal photo beam interruptions converted to distance traveled (centimeters) per unit time. Observational behavioral categories, rated by an observer blind to the LCR/HCR identity of the rats, were defined as follows. "Quadrant crossings" were movements of at least two limbs into a new quadrant of the activity apparatus.

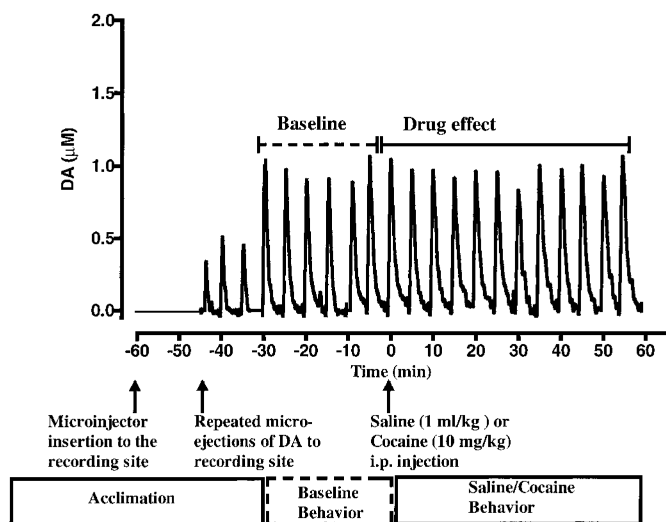

Fig. 1. Experimental timeline for concomitant recordings of behavior and DA clearance signals in freely moving rats. A representative electrochemical signal trace is shown above the timeline. For the first 60 min the rat was allowed to acclimate to the open field activity apparatus. After ~30 min, an injector was inserted through the microcannulae guide in preparation for local applications of DA into the NAc or dSTR. Within the next 15 min (prior to DA application), a stable background chronoamperometric signal was established and set to zero. Next, an ejection volume of DA was selected so that signals with A_{max} values of 0.3 to 1.5 μM were obtained. Once an ejection volume was established, it was held constant and used to induce DA signals every 5 min throughout the remainder of the experiment. Note the reproducibility of these signals in the saline-treated trace shown. Behavior and DA signals were recorded for the 30 min preceding (baseline responses) and the 60 min following (drug effect) an i.p. injection of either saline or cocaine.

"Freezing" was defined by an absence of locomotion across quadrants, minimal activity confined to a quadrant, and standing on all four limbs in an alert position. "Sleep" was defined by the rat lying down, sometimes curled up with its eyes closed. "Grooming" was movements directed against self, forepaws over body, including scratching, licking, body gnawing, and face washes. No distinction was made between repetitive versus random grooming patterns. "Head/limb stereotypies" were repetitive head movements, including head bobs and side-to-side head sways, or back and forth repetitive forelimb movements directed at the environment and confined to a small area. "Rearing" was scored when both forepaws were lifted and then at least one forepaw was placed back onto the floor. No distinction was made as to the event occurring either centrally within the activity apparatus or peripherally against the walls of the activity box. With the exception of rearing events, which were summed within each 15-min interval, each observationally rated behavior was quantified using a binary scale modified from Fray et al. (1980). Briefly, the incidence of a particular behavior was recorded, with 0 indicating the absence and 1 indicating the presence of a behavior for more than 10 s during each 1-min interval. Incidence scores in each behavioral category were summed within each 15-min interval and transformed into a frequency score by dividing the cumulative score by 15. This value thus reflected the fraction of time during each 15-min interval in which a behavior was present.

Electrochemical Data Acquisition and Analysis. High-speed chronoamperometric recordings of oxidation currents were obtained by applying continuous 100-ms square-wave potential pulses (0.0 to + 0.55V versus Ag/AgCl reference) at 5 Hz to the carbon-fiber microelectrode. The DA-evoked oxidation currents were converted online to micromolar concentration changes based on the in vitro calibrations. For recordings in freely moving rats, all DA signal traces were digitally filtered (Microsoft Excel software; Microsoft, Redmond, WA) using a Fourier low-pass digital filter (cut-off frequency: 0.03 Hz) to eliminate high frequency spike artifacts, which occurred more frequently when the rat was behaviorally activated. DA clearance parameters were derived from the resultant smoothed DA signal trace. To determine the kinetics of exogenous DA clearance by uptake, the decay segment of each DA signal trace following the peak DA signal amplitude was fitted to a single monoexponential decay function:

$$A(t) = A_{max} * e^{-k(t-t_0)}$$

where A is the amplitude of the DA signal (micromolar concentration) at any time t (seconds) following the peak signal amplitude (A_{max}), and k is the first-order rate constant for DA clearance (s^{-1}). A_{max} was fixed to the experimentally determined value, and fitting was performed from the time at which A equaled approximately 80% of A_{max} (t_0). Based on the Michaelis-Menten kinetic model of reuptake [$v = V_{max} * S/(K_m + S)$; where v is the velocity of uptake, V_{max} is the maximum velocity, S is the extracellular DA concentration, and K_m is the DA concentration at half-maximal velocity], at $S << K_m$, k reflects the V_{max}/K_m ratio or efficiency of DA clearance. R^2 values for the monoexponential curve fits of the digitally filtered experimental data ranged from 0.8999 to 0.9966. Data are expressed as percentage of predrug "baseline", where "baseline" A_{max} and k represent the mean values from five to six reproducible DA clearance signals recorded immediately preceding i.p. saline or cocaine administration.

Statistical Analysis. Data are expressed as mean values ± standard error of the mean (S.E.M.), except where otherwise noted. Two-way analyses of variance (ANOVAs), with time as the repeated measure, were performed to analyze the effects of group and time on the behavioral and electrochemical measures. For the automated locomotor and electrochemical responses, this analysis was followed by Bonferroni's multiple t test comparisons to the saline response at each time bin. For observational behaviors, Student-Newman-Keuls comparisons were performed among treatment groups collapsed

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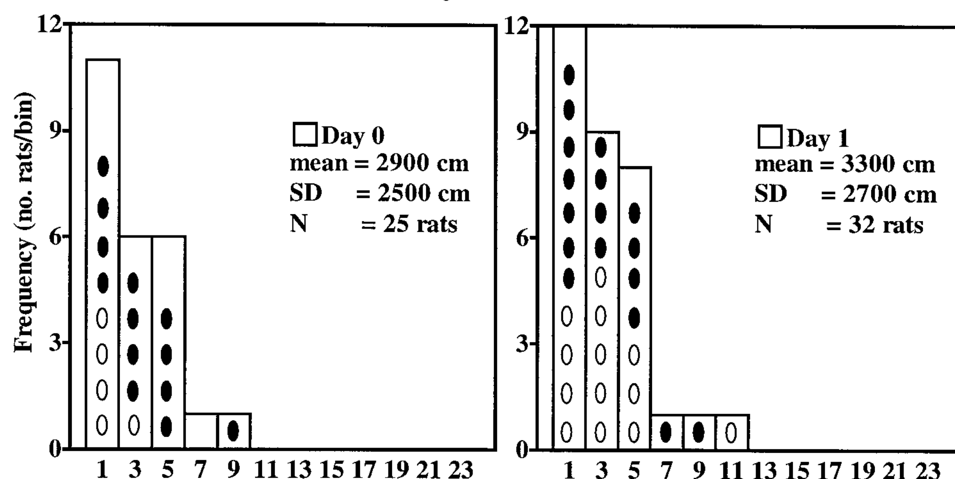
across time, in addition to pair-wise comparisons at each level of time. Pearson correlation analysis was used to evaluate the relationships between cocaine-induced changes in locomotor activity and electrochemical parameters in individual rats. Statistical procedures were performed using either SigmaStat (Jandel Scientific Software Corporation, San Rafael, CA) or Prism (GraphPad Software, San Diego, CA) software. A level of $p < 0.05$ was considered to be statistically significant.

Drugs. (−)-Cocaine HCl was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC). Dopamine and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Results

Identification and Behavioral Profile of LCRs and HCRs. All outbred male Sprague-Dawley rats in this study were chronically instrumented with electrochemical electrode/microcannulae assemblies in either NAc or dSTR. Three to 5 days postsurgery, locomotor responsiveness was tested on two consecutive days (days 0 and 1). Following measurement of baseline locomotor activity on day 0, 25 rats received a saline injection (1 ml/kg i.p.), whereas on day 1 these 25 rats, plus an additional 7 rats, received an acute injection of cocaine (10 mg/kg i.p.). The frequency distributions of the baseline, saline-induced, and cocaine-induced locomotor activity were compared (Fig. 2). Based on the Kolmogorov-Smirnov (KS) test for normality, the distributions of baseline activity on both days were unimodal (KS distance = 0.1466 and 0.1117, $p > 0.1$ for days 0 and 1, respectively). Furthermore, the mean baseline activity did not change significantly from days 0 and 1, despite the additional 7 rats (Fig. 2A). Likewise, locomotor responses to saline and cocaine were both normally distributed (KS distance = 0.1526 and 0.1098, $p > 0.1$, respectively). In contrast to saline, however, cocaine produced a rightward shift in the mean of the distribution of locomotor responses (Fig. 2B). In addition, the scatter about the mean was approximately 3-fold greater for the cocaine- than the saline-induced activity, resulting in a flatter than normal distribution for the cocaine-induced locomotor responses (kurtosis = 0.71 and 0.18 for saline and cocaine responses, respectively). Based on this greater distribution of cocaine-induced locomotor responses, two groups of cocaine responders, namely LCRs and HCRs, were defined using the median cocaine-induced locomotor activity of 7692 cm/30 min as the split criterion (Fig. 2B, right panel).



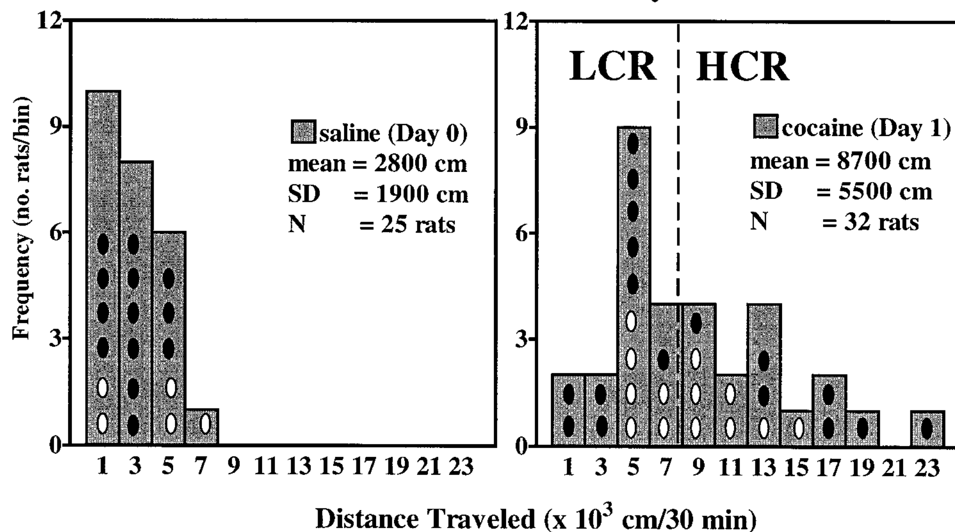


Fig. 2. Identification of LCR and HCR rats based on frequency distributions of locomotor activity following i.p. cocaine administration. See Fig. 1 for experimental protocol. Locomotor activity was the cumulated distance traveled (centimeters) in the 30 min preceding (baseline; A) and the 30 min following (B) injection of either saline (1 ml/kg i.p.) or cocaine (10 mg/kg). Histograms show the number of rats on each treatment day (day 0, left panels; day 1, right panels) that traveled within a specified range in increments of 2000 cm/30 min. Thus, the first bin, centered at 1000 cm/30 min, represents rats scoring between 0 and 2000 cm/30 min. Note the variability in cocaine-induced locomotor activity. The dashed vertical line represents the median cocaine-induced locomotor activity, which served as the selection criterion for subsequent groupings of rats as either LCRs or HCRs. All rats had electrode/microcannulae assemblies chronically implanted in either NAc or dSTR. Filled and open circles within histogram bars represent the number of rats from which simultaneous electrochemical recordings were obtained in NAc (see Fig. 5) or dSTR (see Fig. 6), respectively.

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LCRs and HCRs did not differ significantly in their baseline or saline-induced locomotor responses on day 0 (data not shown). However, because of the relatively long 60-min period of acclimation preceding baseline locomotor activity recording, we examined whether LCRs and HCRs differed in locomotor responses during a time period more immediate to their exposure to the activity chamber. Thus, locomotor activity was analyzed during the 30 min preceding baseline measurements. On day 0, during this period in which the activity chamber was more novel, HCRs displayed a nearly 3-fold significantly greater spontaneous locomotor activity than did LCRs (LCRs: 1899 ± 488 cm/30 min, n = 16; HCRs: 5008 ± 1543, n = 9; $p < 0.05$). However, these differences in spontaneous locomotor activity were no longer apparent on day 1 (LCRs: 3122 ± 582 cm/30 min, n = 17; HCRs: 3790 ± 898, n = 15).

In addition to the automated recording of locomotor activity, several other behaviors, including quadrant crossing, rearing, head/limb stereotypies, grooming, freezing, and sleeping, were quantified (Fig. 3). This allowed a more complete profiling of the LCR and HCR behavioral phenotypes. We also wanted to know whether the diminished locomotor response to acute cocaine in LCRs was a consequence of increased stereotypic behaviors and/or whether the augmented locomotor response to cocaine in HCRs was at the expense of other behaviors. Because there were no significant differences among LCRs and HCRs in the various behavioral responses to saline on day 0, these data were collapsed and constitute the saline response. Differences in behavior among the three groups (saline-treated rats, cocaine-treated LCRs, and cocaine-treated HCRs) and across time were assessed by two-way repeated measures ANOVAs. Significant group dif-

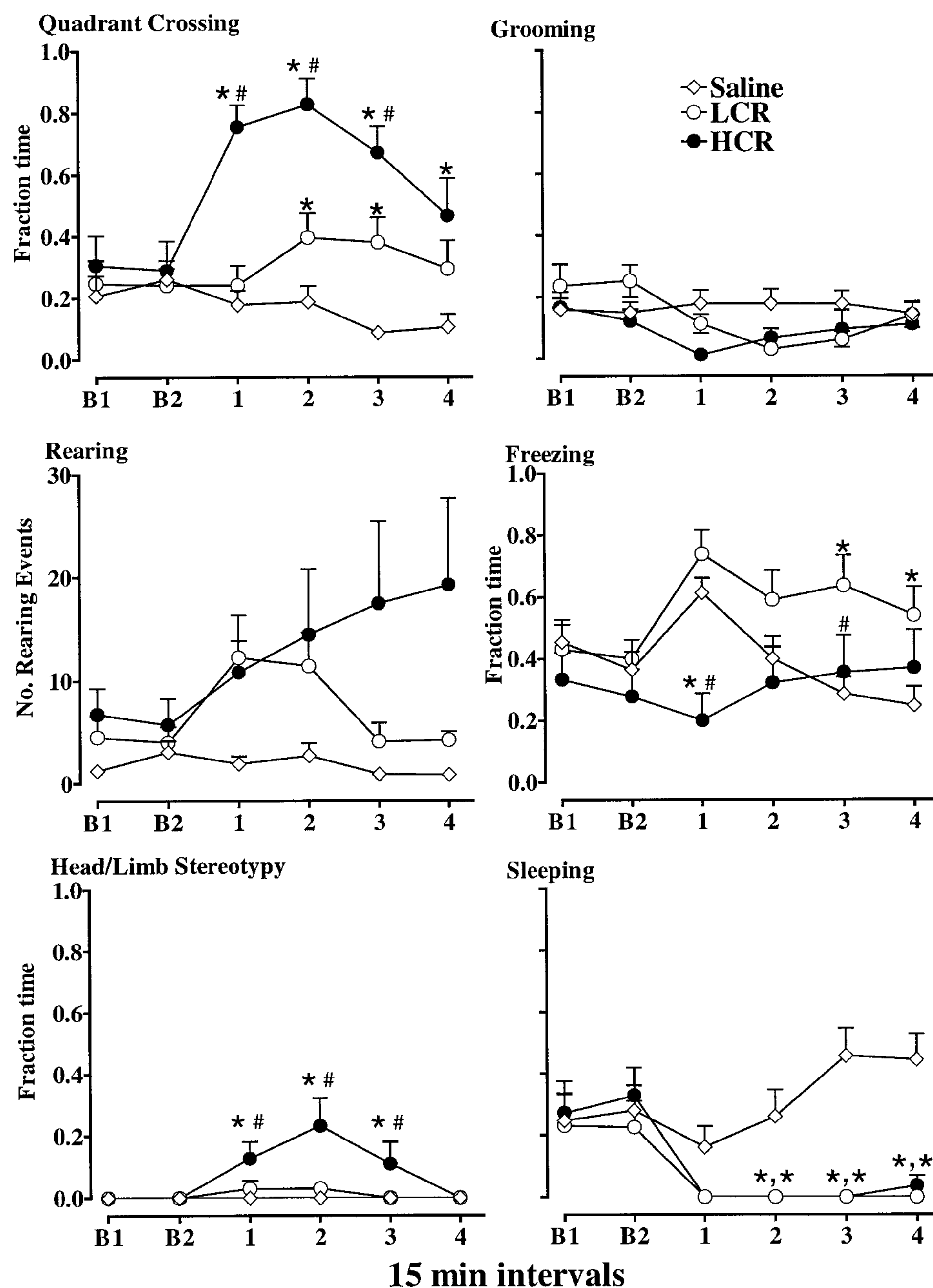

Fig. 3. Differential patterns of behavioral activation induced by cocaine in LCR and HCR rats. Behaviors (clockwise from top left panel: quadrant crossing, grooming, freezing, sleeping, head/limb stereotypy, and rearing) were scored at 15-min intervals (see *Materials and Methods*) for 30 min of baseline (B1 and B2) and for 60 min after an i.p. injection of either saline (1 ml/kg) or cocaine (10 mg/kg). Scores for each behavior were transformed into the fraction of time the particular behavior was observed during each 15-min interval, with the exception of rearing which was expressed as total number of rears per interval. Since there were no significant differences between LCRs and HCRs in any of the behavioral responses to saline (data not shown), these data were collapsed together and constitute the saline treatment group. Data are shown as mean ± S.E.M. (n = 21, saline; n = 13, LCRs; n = 12, HCRs). Significant group and time differences were revealed by two-way repeated measures ANOVAs (see *Results*). Significant differences indicated reflect post hoc Student-Newman-Keuls pairwise comparisons: ⋆, $p < 0.05$ versus time-matched saline response; #, $p < 0.05$ versus time-matched cocaine response in LCRs.

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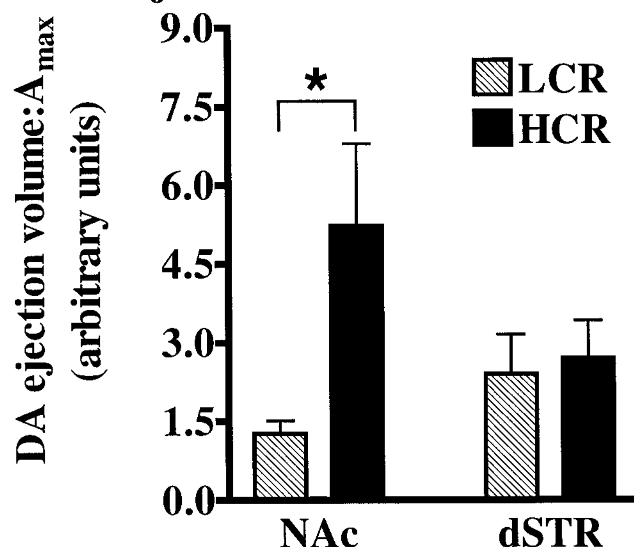
ferences were revealed in quadrant crossing ($F_{2,199}$ = 13.1, $p < 0.001$), rearing ($F_{2,199}$ = 9.352, $p < 0.001$), head/limb stereotypy ($F_{2,199}$ = 7.231, $p < 0.01$), freezing ($F_{2,199}$ = 3.485, $p < 0.05$), and sleeping ($F_{2,199}$ = 6.999, $p < 0.01$). There was also a significant effect of time on quadrant crossing ($F_{5,199}$ = 6.015, $p < 0.001$), head/limb stereotypy ($F_{5,199}$ = 6.689, $p < 0.001$), freezing ($F_{5,199}$ = 3.615, $p < 0.01$), and sleep ($F_{5,199}$ = 5.405, $p < 0.001$). Finally, group differences depended on the level of time as reflected by a significant interaction between these two main effects on quadrant crossing ($F_{10,199}$ = 5.777, $p < 0.001$), rearing ($F_{10,199}$ = 2.082, $p < 0.05$), head/limb stereotypy ($F_{10,199}$ = 4.385, $p < 0.001$), freezing ($F_{10,199}$ = 1.998, $p < 0.05$), and sleep ($F_{10,199}$ = 3.315, $p < 0.001$). To isolate specifically where these differences occurred, pairwise multiple comparisons were carried out on each behavioral measure using the Student-Newman-Keuls test. All baseline behaviors among the three groups were similar. However, with cocaine, HCRs were immediately engaged in quadrant crossings to a greater extent than LCRs and saline-treated rats ($p < 0.05$). During the first 45 min, this behavior dominated over other behaviors in HCRs, the incidence being approximately 80% of the time. The incidence of quadrant crossings decreased at later time points as HCRs became engaged in behaviors confined to small areas, such as rearing. Additionally, during the first 45 min after cocaine, head/limb stereotypy in HCRs was observed maximally 20% of the time, whereas this behavior was absent in LCRs and saline-treated rats. Nonetheless, cocaine-induced behavioral activation in LCRs was evidenced by a significant increase in the number of rearing events, compared with the saline-treated rats. Furthermore, although both LCRs and saline-treated rats displayed an initial freezing response to the injection, freezing behavior in LCRs following cocaine dominated across all levels of time compared with both saline-treated rats and HCRs ($p < 0.05$). Thus, in contrast to the saline-treated rats, which showed increased incidence of sleep over time, LCRs and HCRs were both behaviorally activated by cocaine across the 60 min after drug injection, albeit with distinctly different behavioral profiles.

Baseline DA Clearance Signal Parameters in LCRs and HCRs. Of the 32 rats tested on day 1 for initial behavioral responsiveness to cocaine, 17 had working electrode/microcannulae assemblies in NAc (n = 10, LCRs; n = 7, HCRs), and 13 had assemblies in dSTR (n = 6, LCRs; n = 7, HCRs). The baseline DA clearance parameters immediately prior to the injection of cocaine were compared in both brain regions of LCR and HCR rats (Fig. 4). DA ejection volumes to the recording site were adjusted initially to achieve reproducible baseline A_{max} responses that ranged from 0.3 to 1.5 μM. A wide range of ejection volumes (150–1500 nl; 30–300 pmol) was required across all rats to evoke signals with these amplitudes. Nonetheless, the mean baseline A_{max} values were similar (NAc, 0.8 ± 0.1 μM for LCRs and 0.6 ± 0.1 for HCRs; dSTR, 0.6 ± 0.1 for LCRs and 0.7 ± 0.1 for HCRs). In NAc of LCRs nearly 2-fold lower ejection volumes of DA were required to achieve A_{max} responses comparable with dSTR (Fig. 4A), whereas the opposite trend was observed in HCRs. More importantly, in NAc, 5-fold greater ejection volumes of DA were required in HCRs to elicit baseline A_{max} responses equivalent to those in LCRs. This group difference was not observed in dSTR where LCRs and HCRs required similar volumes of DA to elicit equivalent baseline A_{max} responses. Interestingly, the baseline efficiency of DA clearance (k or V_{max}/K_m ratio) was not significantly different between LCRs and HCRs in either NAc or dSTR (Fig. 4B).



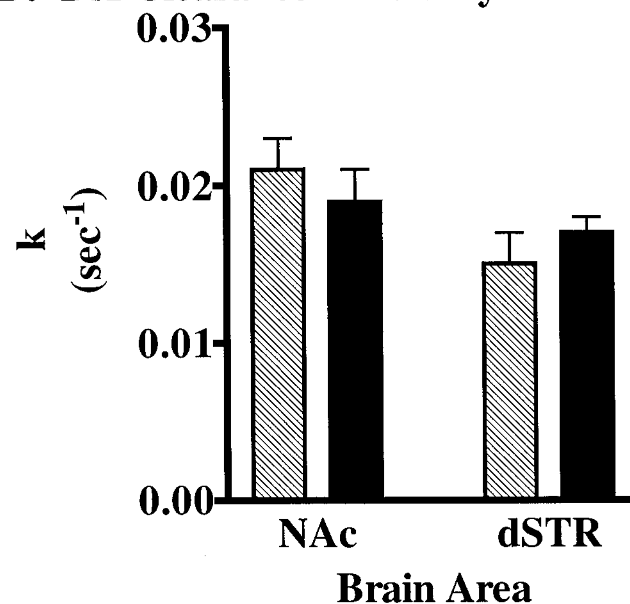


Fig. 4. Baseline DA clearance signal parameters in LCR and HCR rats. Baseline DA signals were induced at 5-min intervals prior to i.p. cocaine administration by application of exogenous DA (200 μM) into either NAc or dSTR (see Fig. 1). Data represent the mean ± S.E.M. of five to six reproducible signals in each rat (NAc: n = 10 LCRs; n = 7 HCRs; dSTR: n = 6 LCRs; n = 7 HCRs) A, the DA ejection volume in each rat was normalized by its baseline A_{max} value. The mean baseline A_{max} values were similar (see *Results*). Thus, this analysis revealed that higher volumes of DA were required in NAc of HCRs to obtain baseline A_{max} values similar to those in LCRs. B, the DA clearance efficiency reflects the baseline k parameter (V_{max}/K_m ratio) of the DA signal and was derived from monoexponential curve fitting of the decay segment of each baseline DA signal trace (see *Materials and Methods*). Two-way ANOVA followed by Bonferroni's multiple t test comparisons. ⋆, $p < 0.05$ versus LCR in NAc.

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Differential Behavioral Responsiveness to Cocaine in LCRs and HCRs Is Associated with Differential Inhibition of DA Clearance by Cocaine in Both NAc and dSTR. Consistent with the divergent behavioral responses of LCRs and HCRs to an acute i.p. injection of cocaine, their DA clearance signals were also differentially modulated following this treatment. We first compared the time course of changes in locomotor activity with changes in the DA clearance signal parameters in the group of LCR and HCR rats instrumented in NAc (filled circles in Fig. 2). Baseline behavior in these rats was stable and comparable on both days 0 and 1 (Fig. 5A, left panel). Similarly, during the baseline period, A_{max} responses to repeated microejections of DA were relatively stable in all rats, as were the k values for DA clearance (Fig. 5, B and C, respectively, left panels). The locomotor activity in LCRs following i.p. cocaine administration on day 1 was similar to baseline activity and to the saline response over the entire 60 min postinjection (Fig. 5A, left panel). In contrast, the locomotor activity in HCRs following cocaine was immediately and robustly increased above baseline activity, but returned to baseline by 60 min postinjection. Consistent with the uniform, low levels of locomotor activity in LCRs, their electrochemical signal parameters remained unchanged (within 20% of one another) over the 60 min following cocaine, as was also the case following saline (Fig. 5, B and C, left panels). On the other hand, both the A_{max} and k parameters were altered in HCRs following cocaine. The increase in A_{max} was consistent with the time course of their locomotor activation, whereas the decrease in k was more transient.

The cumulative effect of saline or cocaine on locomotor activity and the mean effects on the A_{max} and k values are summarized for the first two 15-min intervals following injection in the histograms in Fig. 5 (right panels). During the initial 15 min postinjection, cocaine significantly increased locomotor activity in HCRs by 4-fold versus LCRs, and by 6-fold versus saline-treated rats (Fig. 5A, right panel). Locomotor activity remained significantly augmented in HCRs

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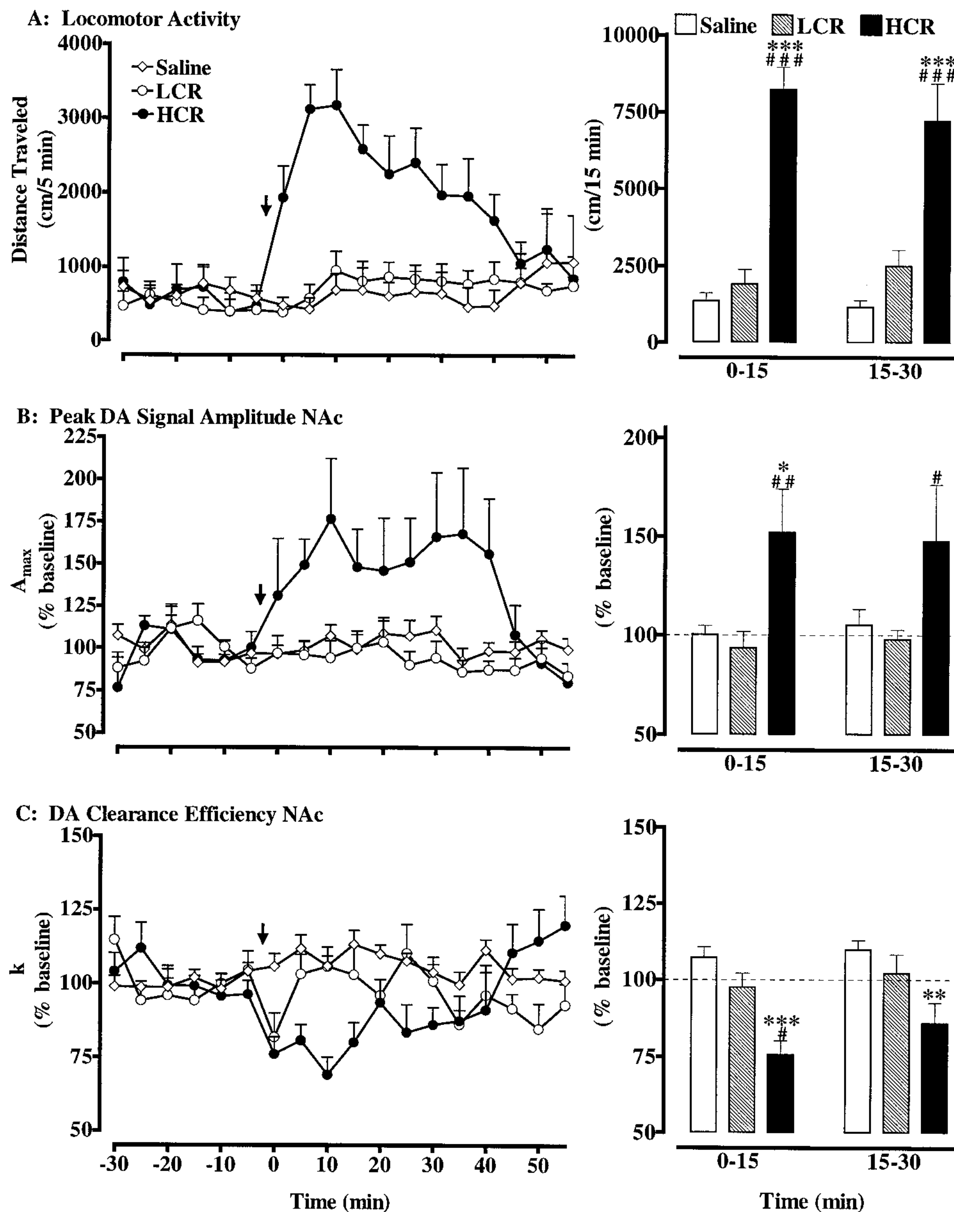

Fig. 5. Time courses for saline- and cocaine-induced changes in locomotor activity (A) and DA signal A_{max} (B) and k (C) parameters in NAc. All three measures were recorded concurrently in freely moving rats at 5-min intervals for 30 min before (baseline) and 60 min after an i.p. injection (arrow) of either saline (1 ml/kg) or cocaine (10 mg/kg). See Fig. 1 for experimental protocol. The time courses for treatment effects on locomotor activity and DA signal parameters are shown for the 5-min recording intervals (left panels) and summarized across two 15-min post-treatment bins (right panels). Data are shown as mean ± S.E.M. (n = 13, saline; n = 10, LCRs; n = 7, HCRs). Repeated two-way measures ANOVAs revealed significant overall group differences: locomotor activity ($F_{2,27}$ = 54.724, $p < 0.001$), A_{max} ($F_{2,27}$ = 5.593, $p < 0.01$), and k ($F_{2,27}$ = 10.207, $p < 0.001$). The significant differences indicated reflect Bonferroni's multiple t test comparisons: *, $p < 0.05$, **, $p < 0.01$, and ***, $p < 0.001$ versus saline; and #, $p < 0.05$, ##, $p < 0.01$, and ###, $p < 0.001$ versus LCRs at that time interval.

during the next 15-min period, as well, whereas activity in LCRs was similar to that of the saline-treated controls during both periods. Parallel with locomotor activation in HCRs, the A_{max} responses to exogenous DA were significantly increased by 52 ± 22% during the initial 15 min versus both LCRs and saline-treated controls (Fig. 5B, right panel) and remained significantly augmented by 48 ± 28% during the next 15-min period versus LCRs. Additionally, k was significantly attenuated in HCRs by 25 ± 5% during the initial 15 min versus both LCRs and saline-treated controls (Fig. 5C, right panel) and remained significantly attenuated by 15 ± 5% during the next 15-min period versus saline-treated controls.

Similar time course comparisons between the cocaine-induced changes in locomotor activation and DA clearance signal parameters were made in rats with electrochemical assemblies implanted in dSTR (open circles in Fig. 2). Baseline behavior and electrochemical responses to repeated microejections of DA were stable and comparable among the groups on days 0 and 1 (Fig. 6, left panels). Similar to NAc, the time course of locomotor activation in HCRs following i.p. cocaine administration more closely paralleled that of the increase in A_{max} responses in dSTR than the change in k. Specifically, during the initial 15 min following cocaine, the locomotor activity of HCRs significantly increased by 2-fold versus LCRs and nearly 5-fold versus saline-treated rats (Fig. 6A, right panel). Locomotor activity remained significantly elevated in HCRs during the next 15-min period versus both LCRs and saline controls, whereas activity in LCRs was similar to that of the saline-treated rats during both time periods. Concomitant with locomotor activation in HCRs, A_{max} responses were significantly increased by 52 ± 25% during the initial 15 min following cocaine versus LCRs and remained significantly elevated by 74 ± 31% during the next 15-min period versus both LCRs and saline-treated controls (Fig. 6B, right panel). In contrast, A_{max} responses in LCRs were not altered. Interestingly, in LCRs the k parameter was attenuated transiently, but significantly, by 28 ± 10% during

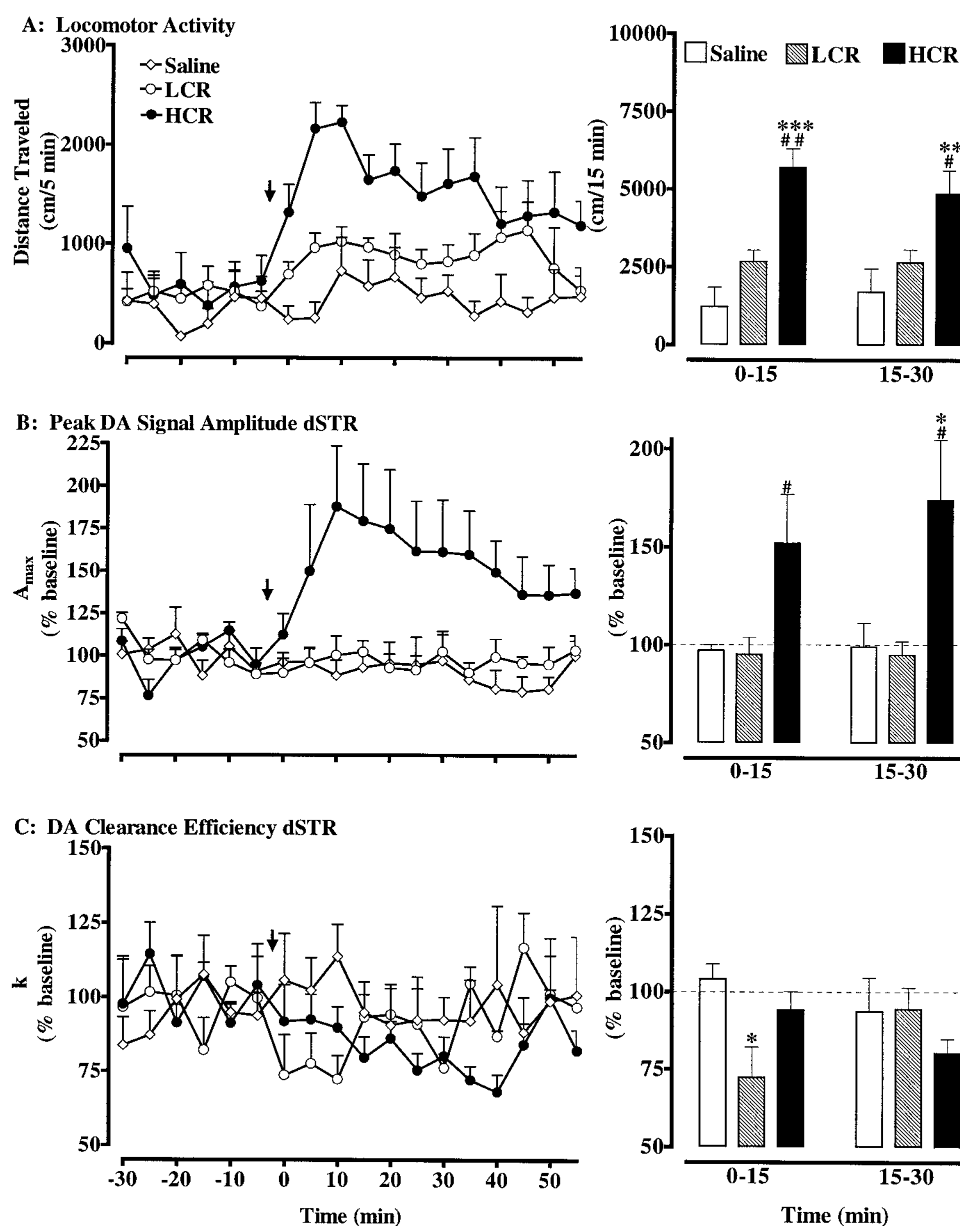

Fig. 6. Time courses for saline- and cocaine-induced changes in locomotor activity (A) and DA signal A_{max} (B) and k (C) parameters in dSTR. Experiments were conducted similarly to those in Fig. 5. The time courses for treatment effects on locomotor activity and DA signal parameters are shown for the 5-min recording intervals (left panels) and summarized across two 15-min post-treatment bins (right panels). Data are shown as mean ± S.E.M. (n = 5, saline; n = 6, LCRs; and n = 7, HCRs). Repeated two-way measures ANOVAs revealed significant overall group differences in measures of locomotor activity ($F_{2,15}$ = 15.623, $p < 0.001$) and A_{max} ($F_{2,15}$ = 4.142, $p < 0.05$) and a significant group × time interaction for k ($F_{2,15}$ = 9.286, $p < 0.01$). The significant differences indicated reflect Bonferroni's multiple t test comparisons. *, $p < 0.05$, **, $p < 0.01$, and ***, $p < 0.001$ versus saline; and #, $p < 0.05$ and ##, $p < 0.01$ versus LCRs at that time interval.

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the initial 15 min following cocaine versus saline-treated controls, whereas in HCRs there was a trend for k to be attenuated by 20 ± 5% during the second 15-min period (Fig. 6C, right panel). This decrease in k in HCRs was significant compared with the baseline value but was not significantly different from LCRs or saline-treated controls. Nevertheless, this decrease coincided with the period during which the incident of head/limb stereotypy peaked at 20 to 30% of the time in HCRs (Fig. 3).

The most convincing evidence supporting a significant relationship between cocaine-induced changes in behavior and DA clearance came from Pearson correlation analysis of the responses of the individual rats. The relationships between the locomotor responsiveness of each rat and changes in their A_{max} and k parameters were examined during the initial 30 min after i.p. cocaine administration (Fig. 7, A and B, respectively). Rats with electrochemical assemblies in both NAc and dSTR were initially included in these analyses. The magnitude of the cocaine-induced locomotor activation was positively and highly significantly correlated with the cocaine-induced increase in A_{max} responses ($r = 0.6378$; $p < 0.0001$). The magnitude of locomotor activation was also positively and significantly correlated with the cocaine-induced inhibition in k ($r = 0.4172$; $p < 0.01$). Additionally, when the k values from rats with assemblies in dSTR were not included, a stronger relationship was demonstrated between cocaine-induced locomotor activation and inhibition in k in NAc ($r = 0.5564$; $p < 0.001$).

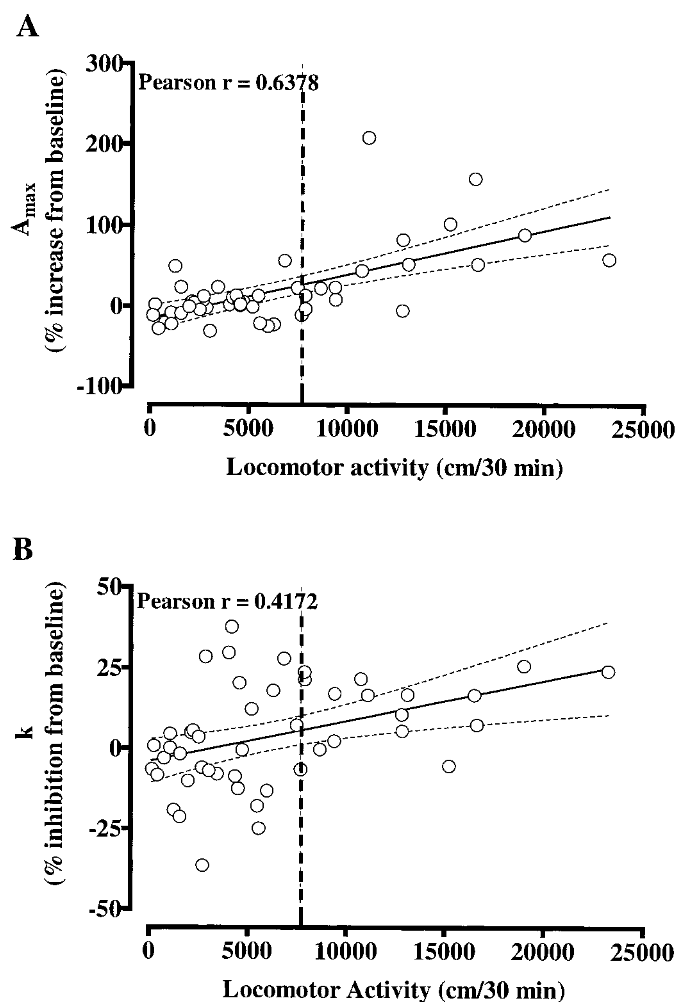

Fig. 7. Individual differences in locomotor responsiveness to acute i.p. cocaine administration correlate with cocaine-induced changes in DA clearance signal parameters. Locomotor activity for each rat is displayed as total distance traveled during the 30-min interval following cocaine injection (10 mg/kg). A_{max} (A) and k (B) parameters in each rat were averaged across the same time interval and expressed as percentage change from baseline. Dashed lines represent the 95% confidence interval of the linear regression fit (solid line). The Pearson correlation coefficients were significant in both cases. Bold vertical dashed lines represent the median cocaine locomotor score used to define LCRs and HCRs.

Discussion

It is well established that the behavioral responsiveness of individual rats to psychomotor stimulants can vary widely (Segal and Kuczenski, 1987; Piazza et al., 1989, 2000; Hooks et al., 1991a,b; Cass et al., 1993a; Cools et al., 1997; Djano and Martin-Iverson, 2000). Our results extend this idea by showing that individual differences in behavioral responsiveness of outbred male Sprague-Dawley rats to a low dose of cocaine (10 mg/kg) were associated with the extent of cocaine-induced DAT inhibition in both NAc and dSTR. Specifically, the magnitudes of cocaine-induced increases in locomotor activity and the DA clearance signal A_{max} responses were highly correlated. Initially, two distinct cocaine behavioral phenotypes, LCRs and HCRs, were defined and found to exhibit baseline differences in DAT function in NAc. Furthermore, whereas LCRs showed no cocaine-induced A_{max} increases and HCRs showed marked increases, the efficiencies of DA clearance, or k parameters, were differentially inhibited by cocaine depending on brain region and time after treatment.

Several groups have identified traits predictive of low and high responsiveness to cocaine. Rats with higher spontaneous locomotor activity in a novel environment exhibit enhanced locomotor or self-administration responses to cocaine, compared with rats with lower locomotor responsiveness to novelty (Hooks et al., 1991a,b; Marinelli and White, 2000). Additionally, behavioral responses to cocaine have been predicted based on the endocrine responses of individual rats in a novel environment (Marinelli et al., 1997). In contrast, our LCR/HCR classification was based upon the cocaine-induced locomotor response in an open field to which the animal had been acclimated. Nevertheless, we did observe significantly higher spontaneous locomotor activity in HCRs than in LCRs on day 0 during the initial exposure to the open field. Interestingly, novelty seeking has been associated with specific D_4 and D_2 DA receptor alleles (Lusher et al., 2001; Ratsma et al., 2001), and D_2 receptor signaling can regulate DAT activity (Dickinson et al., 1999; Mayfield and Zahniser, 2001; but see Prasad and Amara, 2001). Thus, it will be of interest to determine whether D_2 receptor-mediated regulation following DAT blockade contributes to differential cocaine-induced changes in behavior and DA clearance in LCRs versus HCRs.

Differences in cocaine pharmacokinetics could be a simple explanation for the differential behavioral responsiveness of LCRs and HCRs. However, we argue against this explanation for several reasons. First, individual differences in behavioral responses to psychomotor stimulants have been observed across a wide range of doses, supportive of individual differences in maximal response magnitudes (Segal and Schuckit, 1983; Hooks et al., 1991b; Piazza et al., 2000; but

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see Cools et al., 1997). Second, although cocaine-induced locomotor activity and head/limb stereotypy were markedly higher in HCRs than in LCRs, not all cocaine-induced behaviors were higher. Rearing was increased to a similar extent in both groups. Third, differential induction of competing behaviors by cocaine, such as the freezing response, likely precluded increased locomotor activity in LCRs. Lastly, we previously reported that variability in cocaine levels in striata of male Sprague-Dawley rats following this dose was 20 to 25% (Cass and Zahniser, 1993) and, thus, unlikely to account for the marked behavioral differences to cocaine observed here.

When DAT was governed by first-order kinetics ($A_{max} \leq 1.5\ \mu M$), exogenous DA was cleared with equal efficiency in both LCRs and HCRs (Fig. 4B). Furthermore, in similar drug-naive rats, we previously found that under zero-order kinetics ($A_{max} > 10\ \mu M$), the between-subject variation in the V_{max} for DA clearance in dSTR was ≤15% (Sabeti et al., 2001). Together these findings suggest minimal heterogeneity among rats in baseline DAT activity and/or cell surface expression, particularly in dSTR. On the other hand, the greater ejection volumes of DA required in NAc of naive HCRs, as compared with LCRs, is consistent with more DA uptake. However, it remains to be investigated whether higher baseline DAT activity and/or number in NAc are an important trait marker for initial behavioral responsiveness to cocaine. Individual differences in behavioral responsiveness to cocaine have also been associated with differences in basal firing rates and bursting activity of DA neurons in the ventral tegmental area and to a lesser extent in the substantia nigra (Marinelli and White, 2000). Recently, DA release in somatodendritic regions has been shown to occur via reversal of the DAT (Falkenburger et al., 2001), providing a more direct link between cocaine inhibition of DAT and DA neuronal firing rates.

Cocaine-induced behavioral activation in HCRs was accompanied by augmented A_{max} responses to exogenous DA in both NAc and dSTR, nearly 2-fold above values in LCRs, suggestive of a greater inhibition of DAT in HCRs by cocaine. However, the fact that we have observed little variability in cocaine binding affinities in NAc and dSTR of male Sprague-Dawley rats (Cass et al., 1992, 1993a) is inconsistent with a greater cocaine occupancy of DAT in HCRs. Furthermore, because cocaine is a competitive DAT inhibitor in both NAc and dSTR (Jones et al., 1995; Wu et al., 2001; but see McElvain and Schenk, 1992), the apparent enhanced inhibition was unexpected in NAc of HCRs where greater volumes of DA were locally applied. The requirement for more DA suggests a greater number of cell surface DATs in NAc of HCRs, relative to LCRs. However, fewer DATs and lower rates of DA release and uptake have most often been suggested to underlie higher sensitivity to cocaine (Cass et al., 1993a; Wu et al., 2001). Thus, future studies are required to resolve these apparent discrepancies.

Similar to its effect in NAc of HCRs, cocaine immediately attenuated the efficiency of DA clearance in dSTR of LCRs, suggesting that the effective dose of cocaine at DAT sites was similar in both groups. However, unlike its effects in HCRs, the cocaine-induced decrease in k in LCRs was not accompanied by an increase in the A_{max}. Although the efficiency of DAT function would be expected to decrease following competitive cocaine inhibition, a rapid compensatory up-regulation of cell surface expression of functional DATs in response to cocaine would preclude any measurable increase in the A_{max} of the DA signal. Interestingly, LCRs exhibited cocaine-induced freezing, which would be consistent with an acute DA-depleted state arising from a potential cocaine-induced increase in uptake capacity. Increased cell surface expression of DAT has been demonstrated in DAT-expressing cell lines following cocaine exposure (Daws et al., 2002; Little et al., 2002). Acute administration of cocaine has also been shown to alter measures of DAT activity in vivo, suggestive of increases in cell surface levels or activation of preexisting functional DATs (Daws et al., 2002). On the other hand, the marked increase in the A_{max} following cocaine in HCRs is consistent with rapid inhibition of DAT and suggestive of an absence of such a nongenomic up-regulation of DAT in these rats. Clearly, the precise mechanisms by which DAT may be rapidly regulated in vivo in response to acute inhibition by cocaine remain to be established. Nonetheless, it is possible that individual variability in DAT regulation may have functional consequences resulting in differential initial behavioral responsiveness to cocaine.

Locomotor activation and augmentation in the A_{max} responses in both NAc and dSTR in individual rats were highly correlated during the initial 30 min after cocaine. There was also a significant, but less strong, correlation between locomotor activation and inhibition of DA clearance efficiency. Overall, these analyses revealed that cocaine-induced changes in the DA clearance signals accounted for 20 to 40% of the variation in the behavioral responsiveness to cocaine in LCRs and HCRs. Additional contributions could come from DA receptor activation resulting from the increased extracellular DA concentrations, neurotransmitters downstream from DA such as GABA, and/or cocaine-induced increases in both noradrenergic and serotonergic neurotransmission. The cause-and-effect relationship between cocaine-evoked changes in DA clearance signals and behavioral activation could not be resolved temporally. However, the neurochemical modulations occur in the absence of behavioral activation because similar cocaine-induced changes in DA clearance have been observed in anesthetized rats (Cass et al., 1992; Zahniser et al., 1999). Furthermore, although behavior can influence DA neurotransmission (Di Ciano et al., 1998), physiological DA concentrations do not contribute directly to the exogenous DA clearance responses measured here.

In conclusion, we have shown that changes in NAc and dSTR DAT function in freely moving rats following an acute cocaine injection correlate well with individual differences in behavioral responsiveness. If the initial behavioral response to psychomotor stimulants is indeed predictive of vulnerability to addiction (Koob and Le Moal, 2000), then our results suggest that intrinsic differences in DAT inhibition may reflect different addiction phenotypes. Although this hypothesis remains to be tested, the results presented here underscore the importance of considering individual neurochemical responses in understanding differential behavioral responsiveness.

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