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Individual Differences in Cocaine-Induced Locomotor Sensitization in Low and High Cocaine Locomotor Responding Rats Are Associated with Differential Inhibition of Dopamine Clearance in Nucleus Accumbens[1]

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Running Title Page

Individual Differences in Cocaine-Induced Sensitization

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Number of Text Pages	41
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Number of References	41
Number of Words-Abstract	249
Introduction	742
Discussion	1565

ABBREVIATIONS: A_{max}, peak signal amplitude; DA, dopamine; DAT, dopamine transporter; HCR, high cocaine responder; k, decay rate constant; LCR, low cocaine responder; NAc, nucleus accumbens; SD, standard deviation; VTA, ventral tegmental area

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ABSTRACT

Behavioral sensitization to cocaine reflects neuroadaptive changes that intensify drug effects. However, repeated cocaine administration does not induce behavioral sensitization in all male Sprague-Dawley rats. Since cocaine inhibits the dopamine transporter (DAT), we investigated whether altered DAT function contributes to these individual differences. Freely-moving rats had electrochemical microelectrode/microcannulae assemblies chronically implanted in the nucleus accumbens (NAc) so that exogenous DA clearance signals were recorded simultaneous with behavior. The peak DA signal amplitude (A_{max}) and efficiency of clearance (k) were utilized as indices of in vivo DAT function. Low and high cocaine responders (LCRs and HCRs, respectively) were identified based on their locomotor responsiveness to an initial injection of cocaine (10 mg/kg, i.p). Consistent with DAT inhibition, cocaine elevated A_{max} and reduced k in HCRs, but not in LCRs. The same dose of cocaine was administered for 6 additional days and after a 7-day withdrawal. Baseline behavioral and DA clearance indices were unaltered by repeated cocaine or following withdrawal. Only LCRs expressed cocaine-induced sensitized locomotor activation, and this was accompanied by cocaine-induced elevations in A_{max} and reductions in k. These sensitized responses to cocaine persisted in LCRs after withdrawal. In contrast, neither locomotor nor electrochemical responses were altered by repeated saline administration or a saline challenge following repeated cocaine administration, suggesting that conditioning did not significantly contribute. Our results suggest that increased DAT inhibition by cocaine is associated with locomotor sensitization and that DAT serves as a common substrate for mediating both the initial and sensitized locomotor responsiveness to cocaine.

Repeated administration of psychomotor stimulants, such as cocaine and amphetamine, has been utilized as an experimental paradigm to model the progression of behavioral and neurochemical changes leading to compulsive drug use in addicts. Repeated stimulant administration often results in behavioral sensitization, a progressive increase in responsiveness to drug. Sensitization in rats is manifested as augmented locomotor activity and stereotypic behaviors, as well as self-administration and drug-seeking behaviors (Robinson and Berridge, 1993; Covington and Miczek, 2001; De Vries et al., 2001; Vezina et al., 2002). Thus, it is important to understand the neurobiological changes induced by repeated stimulant administration because they likely contribute to intensification of drug motivation and/or craving that promote relapse in addicts (Robinson and Berridge, 1993).

Cocaine potentiates dopamine (DA) signaling in mesocorticolimbic reward pathways by inhibiting uptake of DA by the DA transporter (DAT). With repeated administration, the responsiveness of DA systems to stimulants is enhanced in behaviorally sensitized rats (Vanderschuren and Kalivas, 2000). Transient increases in somatodendritic DA release and spontaneous firing activity of DA neurons in the ventral tegmental area (VTA) have been associated with the induction of behavioral sensitization, whereas long-lasting alterations within DA neuronal terminal fields have been associated with its expression (Vanderschuren and Kalivas, 2000; Nestler, 2001; Everitt and Wolf, 2002). Initially, reduced sensitivity of inhibitory DA D_2-autoreceptors may result in the potentiated cocaine-induced increases in extracellular DA concentrations in nucleus accumbens (NAc; see Vanderschuren and Kalivas, 2000). However, the D_2-autoreceptor changes appear to be transient, whereas the ability of a cocaine challenge to produce a greater augmentation in NAc DA levels persists. This suggests that other mechanisms must underlie the long-lasting changes in DA neuronal function.

Since cocaine blocks DAT, repeated cocaine may regulate DAT expression/activity. However, whether it does, is controversial (Izenwasser and Cox, 1990; Ng et al., 1991; Cass et al., 1993a; Masserano et al., 1994; Meiergerd et al., 1994; Zahniser et al., 1995; Kuhar and Pilotte, 1996; Zahniser and Doolen, 2001; Chefer and Shippenberg, 2002). Discrepancies may result from differences in strains/individuals, drug administration/withdrawal paradigms, and/or assays used. We have combined high-speed chronoamperometry with local applications of DA to measure dynamic changes in extracellular DA concentrations following acute and repeated cocaine administration (Cass et al., 1993a; Zahniser et al., 1999; Sabeti et al., 2002b). Using this approach in striatum of drug-naïve rats, we have demonstrated that the decay or clearance of locally-applied DA primarily reflects in vivo DAT activity and that diffusion makes a more minor contribution to cessation of the DA signals (Cass et al., 1993b; Sabeti et al., 2002a & b). Furthermore, we observed cocaine-induced changes consistent with behavioral sensitization: greater cocaine-induced inhibition of exogenous DA clearance in NAc of anesthetized rats withdrawn from repeated cocaine administration, compared to rats given the same dose acutely (Cass et al., 1993a). This finding was consistent with reports of decreased DAT binding sites (Pilotte et al., 1994; Wilson et al., 1994; Boulay et al., 1996; but see Cass et al., 1993a; Letchworth et al., 1999) and increased cocaine potency (Lee et al., 1998) following repeated cocaine treatment. However, since the rats were anesthetized for the clearance measurements (Cass et al., 1993a), it was impossible to relate cocaine-induced regulation of DAT function with individual behavioral changes. This relationship was further complicated by the fact that not all male Sprague-Dawley rats exhibit behavioral sensitization with repeated cocaine administration (Cass et al., 1993a).

Greater locomotor activity in a novel environment predicts enhanced vulnerability to behavioral sensitization induced by repeated amphetamine (Bardo et al., 1996; Piazza and Le Moal, 1996; Cools and Gingras, 1998), although this relationship may not generalize to cocaine (Djano and Martin-Iverson, 2000; Sutton et al., 2000). High novelty responding rats also exhibit higher DA release (Bardo et al., 1996; Piazza and Le Moal, 1996; Cools and Gingras, 1998) and firing rates of VTA DA neurons (Marinelli and White, 2000). We have found that greater acute inhibition of DA clearance is correlated with high initial locomotor responsiveness to cocaine (Sabeti et al., 2002b). However, it is unclear if this association extends to cocaine-induced locomotor sensitization.

To determine if long-lasting adaptations in DAT function contribute to cocaine-induced locomotor sensitization, we simultaneously recorded behavior and DA clearance in NAc of freely-moving rats over a two week period. We then (1) compared the time-course of changes in behavior and DA clearance and (2) correlated individual variations in the magnitude of sensitization with initial cocaine-induced locomotor activation and with basal and cocaine-induced changes in DA clearance.

Materials and Methods

Animals. Outbred male Sprague-Dawley rats were obtained from Charles Rivers Laboratory (Sasco, Omaha, NE). Prior to surgeries, rats were housed no more than six per cage with a 12-hour light/dark cycle and unrestricted access to food and water. To habituate rats to handling and the insertion of injector tubing on experimental days, rats were handled for one to two days before surgery and prior to each recording session. Following surgery, rats were housed individually. All animal care procedures followed the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of Colorado Health Sciences Center.

Stereotaxic implantation of recording microelectrode/microcannulae assemblies. Procedures for the construction and implantation of recording microelectrode/microcannulae assemblies have been previously described in detail (Gerhardt et al., 1999; Sabeti et al., 2002a & b). Briefly, microelectrodes were fabricated from a single carbon fiber (fiber diameter: 30 µm; exposed length: 150-300 µm; Textron Systems, Wilmington, MA) that was coated with Nafion (5% solution, Aldrich Chemical Co., Milwaukee, WI). They were calibrated in vitro as previously described. Microelectrodes displayed a DA over ascorbic acid selectivity of $\geq$1000:1, and linear responses to DA (1-6 µM) in vitro. Each microelectrode was assembled onto two stainless steel guide microcannulae, which permitted the delivery of KCl or DA from injectors that were inserted before each recording session. Injectors were fabricated from fused silica tubing (40 µm I.D. x 150 µm O.D.; Polymicro Technologies, Phoenix, AZ). Insertion of the injector through the guide positioned the tip of the injector precisely within 250-350 µm from the exposed tip of the carbon fiber microelectrode.

For implantation of the microelectrode/microcannulae assembly, rats were deeply anesthetized with chloral hydrate, as previously described (Sabeti et al., 2002b). The assembly was then lowered stereotaxically into the core of the NAc (anterior-posterior: +1.2-1.5mm from bregma; medial-lateral: 2.2mm left from the midline; dorsal-ventral: 6.5-7.5 mm below surface; see Sabeti et al., 2002b). A Ag/AgCl reference microelectrode (0.011 inch diameter; A-M Systems, Carlsborg, WA) was implanted into the posterior cerebral cortex. Leads from the recording and reference microelectrodes were soldered to a 4-pin modular telephone connector and encased in heat shrink tubing (flexible polyolefin; 1/16 in. expanded; 1/32 in. recovered; JT&T Products, San Jose, CA). To ensure that the recording microelectrode was situated at a site densely innervated by DA terminals, as opposed to the anterior commissure, KCl (120 mM in 29 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4; 300 – 800 nl) was infused through the injector to stimulate DA release, which was measured using high speed chronoamperametry (see below; Hebert and Gerhardt, 1998; Sabeti et al., 2002a & b). Once an appropriate recording microelectrode placement was verified, the entire assembly was cemented in place using dental cement, a thick layer of Quick Set Epoxy (Duro, Loctite Corporation, Rocky Hill, CT), and 5 small screws in the skull as anchors. Except during recording sessions, dummy injectors were inserted through the guide microcannulae to prevent obstruction.

Treatment protocol and timeline of simultaneous behavioral and electrochemical recordings. Three to five days after implantation of the microelectrode/microcannulae assembly, each rat was transferred from its home cage to an open field activity apparatus (16 in x 16 in x 15 in; San Diego Instruments, San Diego, CA; enclosed inside a 2 ft x 2 ft x 2 ft Faraday cage). On this day (Day 0), initial behavioral and electrochemical responses in the activity apparatus and to a saline injection (1 ml/kg, i.p.) were examined in all of the rats. Subsequently, rats were divided into two groups (Table 1). On Days 1-6, once daily injections of either saline (Control Group) or (-)cocaine HCl (10 mg/kg, i.p.; Experimental Group) were administered. On Day 7, both groups were injected with cocaine. Rats in the Control Group continued to be injected with cocaine for 5 more days and then were injected with saline on the seventh day. Following a 7-day withdrawal in the Experimental Group, cocaine was administered on Day 15. All injections were given in the activity apparatus. The data on Days 0 and 1 from rats in the Experimental Group have been previously presented (Sabeti et al., 2002b) and are included in the mean values reported here.

On recording days (Days 0, 1, 3, 5, 7 and 15; Table 1), rats were acclimated to the activity apparatus for 1 hour. During this period, rats were handled momentarily while new injectors were inserted through the guide microcannulae in preparation for the repeated application of DA into NAc. Next, 'baseline' measurements of behavior and DA clearance signals were recorded for 30 min immediately prior to the i.p. injection of either saline or cocaine. Thus, rats had acclimated to the activity apparatus for a total of 1.5 hrs prior to the saline or cocaine injection. Following injection, data were collected for an additional 60 min. Room lights were on throughout the experiment. Injectors were removed at the end of each recording session, dummy injectors were reinserted and rats were returned to their home cages. On all other treatment days, rats were immediately transferred to their home cages following injection.

Only rats with electrochemical assembly localizations in NAc were included in the results reported here. Placement was verified at the end of the experiment by visual inspection when removing the assembly from the brain of euthanized rats. In a limited number of rats, the location of the assembly was further confirmed by microscopic examination of coronal sections stained with cresyl violet (Sabeti et al., 2002a).

Behavioral data acquisition. Automated recordings of locomotor activity were obtained in the open field apparatus using a single photo beam frame (8 beams per dimension) near the base of the apparatus. Consecutive beam interruptions were converted to distance traveled (cm) per 5-min period. Head/limb stereotypy was defined as repetitive head movements, including head bobs and side-to-side head sways, or back and forth repetitive forelimb movements. The frequency of stereotypy was determined by observation and expressed as the fraction of time during each 15-min interval in which the behavior was exhibited, as previously described in detail (Sabeti et al., 2002b). Rearing was the cumulative number of times within each 15-min interval during which both forepaws were lifted and then at least one forepaw was placed back onto the floor.

High-speed chronoamperometry in freely-moving rats. Upon transfer to the activity apparatus, the rat was connected to a miniature potentiostat headstage/tether (RAT HAT; Quanteon, L.L.C., Lexington, KY) via a four-pin telephone connector mounted on the rat's head. This allowed the rat free movement within the activity apparatus (Gerhardt et al., 1999; Sabeti et al., 2002a & b). The headstage/tether was linked to an IVEC-10/FAST-12 electrochemical recording system (Quanteon, LLC, Lexington, KY). Continuous 100 ms square-wave potential pulses (0.0 to +0.55 V vs. Ag/AgCl reference) were applied at 5 Hz to the recording microelectrode. A stable background oxidation signal was established in the absence of exogenous DA and set to zero. Subsequently, exogenous DA (40-300 pmol in saline containing 100 μM ascorbic acid, pH 7.4 adjusted with sodium hydroxide) was applied at 5-min intervals into NAc, using a microprocessor-controlled syringe-pump (1.01 μL/sec; Stoelting Co., Wood

Dale, IL; Gerhardt et al., 1999; Sabeti et al., 2002a & b). During each recording session, the DA ejection volume was initially adjusted for each rat to evoke baseline peak DA signal amplitude (A_{max}) responses within a range of 0.3 µM – 1.2 µM. Once 3 reproducible (i.e., within 20%) A_{max} responses were elicited by the same ejection volume, DA was applied once every 5 min at this constant amount throughout the remainder of the recording session for that day.

High frequency spike artifacts in the DA signals were digitally filtered (cut-off frequency > 0.028 Hz), as previously described in detail (Sabeti et al., 2002a). A_{max} responses were determined from the peak of the DA signal amplitudes using in vitro electrode calibration data to convert oxidation currents, averaged over 1-sec epochs, to µM concentrations above the background. The efficiency of DA clearance was determined by fitting the decay segment of each DA signal to a single monoexponential decay function [$A(t) = A_{max} * e^{-k(t-t_0)}$; where A is the amplitude of the DA signal (µM) at any time t (sec) following A_{max}; t_0 is the time at which A had decayed to approximately 80% of A_{max}; and k is the first-order decay rate constant (sec^{-1}); Sabeti et al., 2002a]. R^2 values for the exponential curve fits to the smoothed data ranged from 0.8999 to 0.9966. At the low pmol amounts of DA applied here, k reflects the V_{max}/K_m ratio, or efficiency of DA clearance, according to the Michaelis-Menten kinetic model of uptake (Sabeti et al., 2002a).

Statistical Analysis. Data are expressed as mean values ± standard error of the mean (SEM). Statistical analyses, including one and two-way analysis of variance (ANOVA) and Pearson correlation analysis, 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.

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

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Results

Individual differences in cocaine-induced behavioral sensitization are associated with initial locomotor responsiveness to cocaine. Previously we demonstrated that outbred male Sprague-Dawley rats can be effectively divided into two subgroups of cocaine responders, namely low or high cocaine responders (LCRs or HCRs, respectively), using the median split of locomotor activity during the initial 30 min following an acute i.p. injection of 10 mg/kg cocaine (Sabeti et al., 2002b). In the follow-up longitudinal cocaine study reported here, a subset of rats from this previously characterized population – the group that had electrochemical microelectrode/microcannulae assemblies implanted chronically in NAc – continued to receive daily cocaine treatment. Thus, these rats received an injection of saline (1 ml/kg, i.p.) on Day 0 and daily injections of cocaine (10 mg/kg, i.p.) on Days 1-6 (Experimental group, Table 1). Rats were subsequently challenged with an i.p. injection of the same dose of cocaine following a 24-hr withdrawal (i.e., Day 7) and a 7-day withdrawal (i.e., Day 15) from the daily cocaine treatment.

We hypothesized that individual variability in initial responsiveness to cocaine might influence whether or not robust sensitized responses in behavioral activation and DA clearance inhibition were induced by the repeated cocaine treatment. To test this hypothesis, this subset of rats was re-profiled as either LCRs or HCRs. In this group, the median split of the distance traveled after the initial cocaine injection on Day 1 was 7200 cm/30 min, resulting in 10 LCRs with a mean activity of 3800 ± 888 cm/30 min and 7 HCRs with a mean activity of 18100 ± 2910 (Fig. 1). As we previously observed in the larger population (Sabeti et al., 2002b), there was no correspondence between baseline activity in the 30 min preceding the cocaine injection and the cocaine-induced activity on Day 1 (Fig. 1).

Before examining relationships between cocaine-induced alterations in behavior and DA clearance, first the time courses of the locomotor responses were compared within the two groups across all of the recording days (Figs. 2A and B, left panels, LCRs and HCRs, respectively). All rats were fully acclimated to the activity apparatus prior to initiating the daily cocaine treatment. This was demonstrated on Day 0 by the low levels of baseline activity in both LCRs and HCRs during the 30 min before and the 60 min after injection of saline. It should also be noted that these low levels of activity were observed in both groups despite the local applications of DA into NAc every 5 min. Furthermore, baseline locomotor activity did not change significantly over the 7 days of cocaine treatment or following the 7-day withdrawal in either LCRs or HCRs.

Cocaine-stimulated locomotor activity was analyzed over the 60 min following drug injection on Days 1, 3, 5, 7 and 15 using a two-way ANOVA, with repeated measures on both factors ($\text{Days}_{1,3,5,7,15}$ x $\text{Time}_{0\text{-}60\text{min}}$). An overall significant effect of days was observed for cocaine-stimulated activity in LCRs (Fig. 2A, left panel; $F_{4,402} = 7.102$, $p < 0.001$), but not in HCRs (Fig. 2B, left panel). Further analysis showed that sensitized locomotor responses to cocaine (versus Day 1) were expressed in LCRs only after Day 3 of daily cocaine treatment and that they persisted in response to a cocaine challenge on Day 15 following the 7-day withdrawal (Fig. 2A, left panel). The maximal effect of cocaine on locomotor activity was summarized for the two groups by averaging responses during the first 30 min after cocaine injection during the induction (i.e., Days 1-3) and expression (i.e., Days 5-7) of locomotor sensitization. These effects were compared to the saline-induced locomotor response over the same time interval on Day 0 and to the cocaine-induced response on Day 15 (Fig. 2, right panels). This analysis showed that during induction of sensitization in LCRs, cocaine-stimulated locomotor activity was not significantly different from saline-induced activity (Fig. 2A, right panel). However, by Days 5-7 cocaine-stimulated activity was significantly potentiated by 400% above the cocaine-stimulated response during induction. Following the 7-day withdrawal from the repeated treatment in LCRs, the cocaine-stimulated locomotor activity remained significantly augmented on Day 15 versus Days 1-3 and the saline-induced response. Interestingly, in HCRs cocaine-stimulated locomotor activity on Days 1-3 and Days 5-7 did not differ significantly (Fig. 2B, right panel) but was similar in magnitude to the sensitized responses in LCRs (Fig. 2A, right panel). The 7-day withdrawal from repeated cocaine produced no further augmentation in locomotor responses of HCRs to cocaine (Fig. 2B, right panel).

To determine whether cocaine-induced locomotor sensitization in individual rats was correlated with initial locomotor responsiveness to cocaine, the magnitudes of locomotor sensitization in LCRs on Day 7 were plotted against their cocaine-induced locomotor activity on Day 1 (Fig. 3). The magnitude of sensitization was defined as the ratio of each animal's Day 7: Day 1 cocaine-stimulated locomotor activity during the first 30 min following injection. Thus, a ratio in the range of 0-1 indicated a lack of cocaine-induced behavioral sensitization, whereas progressively higher values > 1 were indicative of increasing levels of sensitization. In LCRs the magnitude of locomotor sensitization was robust, ranging from 2.1 to 21-fold increases over the initial cocaine-stimulated locomotor activity; and there was a significant inverse correlation between the magnitudes of initial locomotor responsiveness to cocaine and locomotor sensitization (Pearson $r = -0.6832$, $p < 0.05$; Fig. 3). Because locomotor sensitization was either absent or relatively modest in HCRs, with ratios varying from 0.3 to 1.7, only data from LCRs were included in the correlational analysis shown in Fig. 3. However, independent of the LCR/HCR classification, a significant inverse relationship was observed between the magnitudes of initial locomotor responsiveness to cocaine and locomotor sensitization in all of the rats studied (Pearson r = -0.5905, $p < 0.05$; n = 17).

We also examined whether cocaine-induced sensitization was manifested in other behaviors, in particular those which may have competed for the expression of locomotor sensitization. Therefore, the effects of the repeated cocaine treatment were examined on cocaine-stimulated head/limb stereotypy and rearing behaviors (Fig. 4). In LCRs, cocaine-induced head/limb stereotypy appeared to increase progressively with repeated treatment and this persisted following withdrawal; however, a statistically significant effect of days was not found (Fig. 4A, left panel). Cocaine-induced rearing responses in LCRs were increased on all days tested; therefore, there was not a significant effect of days (Fig. 4A, right panel). In contrast, although head/limb stereotypy was displayed in HCRs to the same extent across all cocaine treatment days, cocaine-induced rearing responses were progressively and significantly augmented by the repeated cocaine treatments ($\text{Days}_{1\text{-}3,5\text{-}7,15}$: $F_{2,72} = 13.7$, $p < 0.0001$; Fig. 4B). Thus, HCRs did exhibit behavioral sensitization of rearing, but not locomotor, responses.

Individual differences in cocaine-induced locomotor sensitization are associated with differential cocaine-induced modulations in DA clearance in NAc. Simultaneous with the behavioral measurements, changes in DA clearance in NAc of all the rats were continuously monitored by high-speed chronoamperometry on Days 0, 1, 3, 5 and 7 (Fig. 5). Additionally, the effects of the 7-day withdrawal and challenge by cocaine were evaluated on DA clearance in a subset of these rats whose microelectrode/microcannulae assemblies remained patent on Day 15 (n = 7, LCRs; n = 4, HCRs). A constant amount of DA (40-300 pmol; subsaturating relative to the maximal clearance rates measured in vivo; Sabeti et al., 2002a) was applied at the recording site every 5 min. These DA applications evoked reproducible oxidation signals that were meaned

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over a 30 min recording interval to obtain the pre-drug baseline value on each recording day (Fig. 5). Changes over baseline in the A_{max} and k parameters of the DA signal were utilized as indices of altered DAT function (Fig. 5). On each recording day amounts of DA locally applied were initially adjusted for each rat so that similar baseline A_{max} responses were achieved. Thus, mean baseline A_{max} values did not differ between LCRs and HCRs; these averaged 0.8 ± 0.4 μM and 0.7 ± 0.1, respectively, over the two-week period in which rats were treated repeatedly and then withdrawn from cocaine. The amounts of DA applied relative to A_{max} values at baseline were averaged across Days 1 and 3 and across Days 5 and 7 to correspond to the induction and expression phases of locomotor sensitization, respectively, in the LCRs (see above). Initially (i.e., Days 1-3) HCRs required 3-fold significantly greater amounts of DA than LCRs to elicit similar A_{max} responses (Fig. 6A). This finding is in agreement with our previous observations on Day 1 in the same LCR and HCR rats (Sabeti et al., 2002b). Furthermore, although DA applications in LCRs did not differ significantly across the repeated treatment days or after withdrawal, in HCRs significantly lower amounts were necessary on Days 5-7 than on Days 1-3 to achieve comparable A_{max} responses (Fig. 6A). Despite the differences in the amounts of DA applied, pre-drug baseline k values ($0.016 – 0.023\ sec^{-1}$) were not significantly different between LCRs and HCRs across the repeated cocaine treatment days and following withdrawal (Fig. 6B). This finding is in agreement with our observation that k is independent of applied DA within the relatively narrow range of DA applied here, whereas higher amounts of exogenous DA modulate the k for DA clearance as expected by active uptake kinetics (Sabeti et al., 2002a). Furthermore, pre-drug baseline k values were not significantly correlated with initial locomotor responsiveness to cocaine in individual rats ($p = 0.673$).

In contrast to the modest changes in baseline DA clearance efficiency, robust alterations in cocaine-induced inhibition of DA clearance in NAc closely paralleled the time course of locomotor sensitization induced by the repeated cocaine administration. For example, in LCRs there was an overall significant effect of days on the cocaine-induced increases in A_{max} (Fig. 7A, left panel; $F_{4,314} = 4.101$, $p < 0.05$), as revealed by a two-way ANOVA ($Days_{1,3,5,7,15}$ x $Time_{0-60min}$; time as the only repeated measure). This effect was not observed in HCRs (Fig. 7B, left panel), which also did not exhibit locomotor sensitization to cocaine (Fig. 2B). The effects of cocaine on A_{max} were summarized in LCRs and HCRs over the first 30 min after injection to correspond to the maximal effects of cocaine on locomotor activity (Fig. 2) and compared to the effect of saline on Day 0 (Fig. 7, right panels). Specifically, on Days 1-3 cocaine did not significantly alter A_{max} responses in LCRs, as compared to saline (Fig 7A, right panel). However, during the expression of locomotor sensitization on Days 5-7 in LCRs, cocaine significantly potentiated the A_{max} response by 46 ± 12%, as compared to both Days 1-3 and saline. A_{max} responses were potentiated to 31 ± 11% by cocaine on Day 15 following the 7-day withdrawal, although this effect was not significantly different compared to either Days 1-3 or saline. As with locomotor activation, in HCRs there was no overall significant effect of days on cocaine-induced increases in A_{max} (Fig. 7B right panel). In contrast to LCRs, cocaine administration on Days 1-3 significantly potentiated A_{max} by 51 ± 18%, as compared with saline. Although there was also a trend for A_{max} to be increased by cocaine on Days 5-7 (33 ± 11%) and Day 15 (23 ± 19%), these effects did not reach statistical significance versus the saline response.

Similar to cocaine-induced increases in A_{max} values in LCRs, an overall significant effect of days was observed on cocaine-induced reduction in the k for DA clearance in LCRs (Fig. 8A, left panel; $F_{4,304} = 3.396$, $p < 0.05$). Specifically, on Days 1-3 during the induction of locomotor sensitization, k was not significantly altered by cocaine, as compared with saline on Day 0 (Fig. 8A, right panel). However, during the expression of locomotor sensitization on Days 5-7 in LCRs, cocaine significantly attenuated k by 24 ± 5%, versus Days 1-3 and saline. This greater effect of cocaine on k persisted on Day 15 following the 7-day withdrawal from repeated cocaine treatment. Interestingly, in HCRs the cocaine-induced reductions in k on Days 1-3 and Days 5-7 did not differ significantly from each other but were of a similar magnitude as the sensitized responses in LCRs (Fig. 8B, right panel). The cocaine-induced reduction in k persisted, but did not change in magnitude, following the 7-day withdrawal from repeated cocaine (Fig. 8B, right panel). These results for cocaine-induced changes in the efficiency of DA clearance in LCRs versus HCRs are in agreement with both the cocaine-induced changes in locomotor activity and the DA clearance signal A_{max} responses.

Conditioning does not contribute significantly to the sensitized behavioral and electrochemical responses to cocaine measured here. To control for the passage of time and any potential conditioned responses to the repeated injections of cocaine and/or local applications of DA, another group of rats with electrochemical assemblies chronically implanted in NAc received repeated saline injections for 7 days prior to an acute cocaine challenge injection (Control Group, Table 1). Furthermore, this same group subsequently received cocaine injections on five additional days, followed by a saline challenge injection on the seventh day (Table 1). Data collected in the Experimental Group on the Day 7 cocaine challenge were averaged across the LCR and HCR responses and reanalyzed for significant differences from the Control Group. These comparisons are summarized in Fig. 9. Baseline locomotor activity during the 30 min immediately preceding either the cocaine or saline challenge injections did not differ significantly between the treatment groups, confirming that differences in locomotor responsiveness to the challenges were not due to differences in levels of baseline activity per se. The cocaine challenge on Day 7 significantly increased locomotor activity above baseline in both the saline-pretreated Control Group and cocaine-pretreated Experimental Group. However, this increase was significantly greater (by 80%) in the cocaine-pretreated, as compared to saline-pretreated, rats. This result confirmed that, independent of the LCR/HCR classification, the repeated cocaine regimen used here was effective in inducing locomotor sensitization. In contrast with the cocaine challenge following repeated saline treatment in the Control Group, the saline challenge following repeated cocaine treatment in these same rats did not increase locomotor activity above baseline. Thus, there was no apparent conditioned locomotor response to this particular repeated cocaine regimen.

Electrochemical responses in the Control and Experimental Groups were analyzed in a similar manner to the behavioral responses (Fig. 10). On average A_{max} responses were increased by 42 ± 6% above baseline following the cocaine challenge in the cocaine-pretreated Experimental Group (Fig. 10A). This effect was significantly greater, but only by 25%, when compared to the effect of the cocaine challenge in the saline-pretreated Control Group (Fig. 10A). Importantly, the potentiation of A_{max} responses in cocaine-pretreated rats was expressed only following a cocaine, but not a saline, challenge injection (Fig. 10A). Also consistent with DAT inhibition, k was decreased by 24 ± 3% below baseline following the cocaine challenge in the cocaine-pretreated Experimental Group (Fig. 10B). This was a significant reduction compared to the Control Group, with respect to the effect of both the cocaine challenge following repeated saline administration and the saline challenge following repeated cocaine administration (Fig. 10B). Overall, there was good concordance between the challenge-induced changes in locomotor activity and DA clearance parameters.

Discussion

Whether DAT expression/activity is up- or down-regulated by repeated cocaine administration remains controversial (Zahniser et al., 1995; Kuhar and Pilotte, 1996; Zahniser and Doolen, 2001). Furthermore, the exact relationship between cocaine-induced adaptations in DAT function and changes in behavior has remained elusive. Previously, we found that DA clearance in NAc of anesthetized rats was more sensitive to cocaine inhibition after withdrawal from repeated cocaine administration (Cass et al., 1993a). Here, by monitoring the time course of cocaine-induced changes in behavior concomitantly with inhibition kinetics of DA clearance, we demonstrate the essential role of greater inhibition of DAT function to locomotor sensitization induced by repeated cocaine administration. Specifically, we found that the potential for expression of locomotor sensitization (1) can be predicted by the animal's initial locomotor responsiveness to an acute cocaine injection and (2) is confined to a subgroup of rats (LCRs) in which the efficiency to clear exogenous DA in NAc in the presence of cocaine diminishes with repeated administration.

Previously, we identified two distinct populations of cocaine responders using the median split of initial locomotor responses to acute low dose cocaine in male Sprague Dawley rats (Sabeti et al., 2002b). Subsequently, we have confirmed a trend for two components in this distribution (LCR component: mean 5130 cm/30 min; SD = 2140; proportion = 49%; HCR component: mean = 12370; SD = 4950; proportion = 51%; n = 32; p = 0.07; NOCOM program; Ott, 1979). Likewise, the distribution of A_{max} responses to acute cocaine in this larger population was bimodal (LCR component: mean 98% baseline; proportion = 58%; HCR component: mean 170% baseline; proportion = 42%; $p < 0.01$). Here, using a subset of these rats, we observed that differences in the initial locomotor responsiveness to cocaine accounted for nearly 47% of the variability in the magnitude of locomotor sensitization expressed by LCRs (Fig. 3). Furthermore, the LCR/HCR classification was predictive of the potential of individual rats to express cocaine-induced locomotor sensitization. LCRs exhibited minimal locomotor activation to the initial injection of cocaine but locomotor sensitization with repeated cocaine, whereas HCRs exhibited significant initial locomotor responsiveness to cocaine but failed to express locomotor sensitization with repeated administration. However, not all cocaine-induced behaviors were augmented in LCRs. Furthermore, HCRs exhibited sensitized cocaine-induced rearing responses. These observations, together with the finding that brain levels of cocaine are consistently increased in all male Sprague-Dawley rats receiving repeated i.p. injections of cocaine (Cass and Zahniser, 1993), suggest that pharmacokinetic differences can not satisfactorily explain the LCR versus HCR behavioral differences. It is also unlikely that increased rearing or head/limb stereotypy explained the lack of locomotor sensitization in HCRs because stereotypies were exhibited by LCRs to a similar extent on Days 5-7 and did not preclude locomotor sensitization in these rats. However, we cannot rule out the possibility that a ceiling in locomotor activation precluded sensitization in HCRs because only the 10 mg/kg dose of cocaine was tested here. Dose-response studies would address whether HCRs are able to increase activity further and whether repeated cocaine administration shifts the dose-response relationship in LCRs, making cocaine a more potent or efficacious inhibitor of DAT. In any case, conditioned locomotor responses to the repeated injections were not apparent with our treatment paradigm, supporting earlier findings that the development of conditioned responses is not necessary for the expression of behavioral sensitization (Fraioli et al., 1999).

Greater activation in response to novelty, rather than to acute stimulant administration, has been used more often to predict enhanced vulnerability to psychostimulant-induced sensitization and self-administration (see Introduction). We observed the opposite relationship: HCRs exhibit higher spontaneous locomotor activity than LCRs during their initial exposure to the activity apparatus (i.e., Day 0; Sabeti et al., 2002b) but did not express locomotor sensitization with repeated cocaine administration (this study). This lack of correspondence in expression of sensitization between our cocaine responders and the low and high novelty responders defined in the literature may reflect differences in experimental conditions. For instance, our rats underwent extensive handling and habituation to the testing environment prior to drug administration—factors previously documented to modulate drug responsiveness (Cools and Gingras, 1998; Fraioli et al., 1999; Tuinstra and Cools, 2000). Specifically, habituation can decrease the behavioral and NAc DA sensitivity to stimulants in high novelty responders and increase sensitivity in low novelty responders (Cools and Gingras, 1998; Tuinstra and Cools, 2000). These investigators have hypothesized that individual differences in the regulation of DA neurotransmission by neuroendocrine and/or noradrenergic systems underlie this reversal in sensitivity under various experimental conditions. On the other hand, the discrepancy may reflect differences underlying responsiveness to novelty and cocaine (Djano and Martin-Iverson, 2000; Sutton et al., 2000). The finding that unique provisional quantitative trait loci exist for novelty- versus cocaine-induced initial locomotor activity and sensitization (Phillips et al., 1998) further supports our hypothesis that HCRs may be phenotypically distinct from the high responders to novelty. Studies using self-administration and/or conditioned place preference paradigms are needed to define the relationship of the LCR/HCR phenotypes and cocaine reinforcement.

The temporal association between changes in behavior and DAT function in response to repeated cocaine (Figs. 2, 7 and 8) provides strong evidence that cocaine-induced regulation of DAT in NAc plays a critical role in the expression of locomotor sensitization to cocaine. This association reflected multiple recordings of exogenous DA clearance signals across the 7 days of repeated cocaine in the same individual rats. For example, initially on Days 1-3, when locomotor sensitization was not yet expressed in LCRs, A_{max} and k parameters in NAc were not modulated by cocaine. The expression of cocaine-induced locomotor sensitization in LCRs on Days 5-7 was, however, accompanied by potentiated A_{max} and reduced k, consistent with higher levels of extracellular DA and sensitized DA-related behaviors. Importantly, the sensitized effects of cocaine on behavior and DA clearance parameters in LCRs persisted after a 7-day withdrawal from repeated cocaine treatment, consistent with the long-lasting nature of sensitization. The absence of such regulation in HCRs and the Control Group ruled out a more general effect of the repeated DA applications on increased DAT sensitivity to cocaine. Overall, our findings are in agreement with reports of enhanced cocaine-induced inhibition of DA uptake following repeated cocaine administration (Izenwasser and Cox, 1990; Cass et al, 1993a; Lee et al., 1998; but see Ng et al., 1991; Masserano et al., 1994; Meiergerd et al., 1994; Chefer and Shippenberg, 2002).

The finding that cocaine-induced inhibition of DA clearance in LCRs was expressed only after 3 days of repeated cocaine administration strongly suggests that protein synthesis and/or recruitment of other systems was/were required for the long-term alterations in DAT sensitivity to cocaine in LCRs. On the other hand, LCR/HCR differences in the acute cocaine response may reflect intrinsic variations in more rapid, nongenomic mechanisms of DAT regulation in response to acute inhibition (i.e., cell surface trafficking; Daws et al., 2002; Little et al., 2002). Also, although the NAc is sufficient for mediating the initial locomotor response to acute cocaine (Delfs et al., 1990), a number of long-lasting neuroadaptations in NAc, as well as other DA projection sites, are necessary for the expression of stimulant-induced locomotor sensitization (Vanderschuren and Kalivas, 2000; Nestler, 2001; Everitt and Wolf, 2002). Whether and how glutamatergic and/or GABAergic systems is/are involved in the LCR/HCR differences in sensitization remains to be investigated. Furthermore, future studies will address whether sensitization in cocaine-induced rearing, as opposed to locomotor activity, in HCRs reflects alterations in DAT sensitivity to cocaine inhibition in the dorsal striatum, which plays an important role in stereotypic behaviors.

On Day 1 greater amounts of exogenous DA were applied in NAc of HCRs than LCRs to achieve similar A_{max} responses (Sabeti et al., 2002b; present study). Here, we demonstrated that this group difference was surmounted after Day 3, suggesting that the initial difference likely reflected higher baseline DA clearance capacity in NAc of HCRs than LCRs, rather than variability in injector locations. Although this is discordant with the observed higher initial sensitivity to cocaine inhibition, future kinetic experiments will address whether lower DAT affinity for DA might explain this apparent discrepancy. In contrast, in dorsal striatum equivalent amounts of DA were required on Day 1 to achieve similar A_{max} responses in LCRs and HCRs (Sabeti et al., 2002b). This suggests that, by itself, differences in amounts of DA applied are unlikely to explain the absence of altered DAT sensitivity to cocaine inhibition over time, as observed here in HCRs. Two possible explanations for the decreased amounts of DA needed in NAc of HCRs over time are fewer functional uptake sites secondary to tissue damage and/or regulation resulting in reduced basal DAT function. Since the amount of DA needed in LCRs remained constant across time, the latter is the more likely explanation. Chefer and Shippenberg (2002) have reported such changes in behaviorally sensitized rats, namely a reduction in basal DAT function with no changes in cocaine-induced DAT inhibition. Interestingly, behavioral sensitization in this study was assessed by rating repetitive/stereotypic behaviors. Together, these findings underscore the importance of assessing sensitization by both locomotor activity and stereotypy in order to understand the relevance of DAT regulation to changes in behavioral responsiveness. Furthermore, our results, along with previous reports, support the hypothesis that cocaine-induced adaptations in DAT in the NAc are necessary for the expression of locomotor sensitization. Therefore, LCR/HCR rats may be useful models for further study of differential phenotypes for initial sensitivity and sensitization to cocaine.

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Footnotes

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Figure Legends

Figure 1. Baseline and cocaine-stimulated locomotor activity of individual rats in the Experimental Group (Table 1) on Day 1 reveals differential initial responsiveness to cocaine. Horizontal lines in the scatter plots represent the median responses during the 30 min preceding (baseline) and the 30 min following injection of cocaine (10 mg/kg, i.p.). Subsequently, individual rats were identified as either LCRs (open circles) or HCRs (filled circles) based on the median split of locomotor responsiveness to the acute cocaine injection. The bar graphs illustrate the mean values ± SEM of the cocaine-stimulated activity in LCRs and HCRs and demonstrate the effectiveness of the median-split procedure for sub-dividing the rats into two groups of cocaine responders.

Figure 2. Differences in induction, expression and persistence of cocaine-induced locomotor sensitization in LCRs **(A)** and HCRs **(B)**. Baseline, saline- or cocaine-induced locomotor activity was recorded in the rats characterized as LCRs or HCRs in Fig. 1 (Experimental Group, Table 1). Saline-induced activity was recorded on Day 0. Cocaine-induced activity was recorded on Days 1, 3, 5, 7 during the 7-day regimen of once-daily cocaine administration and following a 7-day withdrawal (Day 15). **Left panels**: Time courses of baseline and saline- or cocaine-induced locomotor activity during the 5-min recording intervals. Arrows indicate the time at which saline (1 ml/kg, i.p.) or cocaine (10 mg/kg, i.p.) was injected. Two-way ANOVAs, with both ‘Treatment Day’ and ‘Time’ as the repeated measures, were used to analyze the activity during the 60 min following cocaine injection (see Results). **Right panels**: Comparison of peak cocaine-stimulated locomotor responses. Activity was averaged during the first 30 min post-treatment. Since no significant differences existed in peak responses between Days 1 and 3 or between

Days 5 and 7, these data sets were collapsed to represent the induction and expression phases of locomotor sensitization, respectively. Data are mean values ± SEM (n = 10, LCRs; and n = 7, HCRs). Significant differences reflect post-hoc Bonferroni's multiple t-test comparisons. ***p < 0.001 versus Days 1-3 cocaine-induced response; and #p < 0.05, ##p < 0.01, ###p < 0.001 versus Day 0 saline-induced response.

Figure 3. The magnitude of cocaine-induced locomotor sensitization in individual LCR rats correlates inversely with their initial locomotor responsiveness to an acute cocaine injection. 'Sensitization score' is the ratio of Day7:Day1 activity induced in the first 30 min after cocaine injection (see Fig. 2A). The linear regression fit (—) and the 95% confidence interval (--) are shown. A sensitization score of 1 (horizontal dashed line) would indicate a lack of locomotor sensitization, whereas scores above 1 indicate increasing magnitudes of locomotor sensitization.

Figure 4. Time course of cocaine-induced changes in stereotypic behaviors and rearing during repeated cocaine administration and following withdrawal in LCRs **(A)** and HCRs **(B)**. The head/limb stereotypies and rearing were scored by observation (see Methods) in the same rats and during the same periods as the locomotor activity presented in Fig. 2. Behaviors were summed for 15-min intervals during the 30 min of baseline (B1 and B2) and 60 min of saline- or cocaine-induced responses (1-4). Data are mean values ± SEM for LCR (n = 10) and HCR (n = 7) rats. For statistical analysis, cocaine-induced behaviors were averaged for Days 1 and 3 and for Days 5 and 7 and compared to Day 15, following a 7-day withdrawal. A two-way ANOVA (Treatment $\text{Days}_{1\text{-}3,5\text{-}7,15}$ X $\text{Time}_{1,2,3,4}$) revealed a significant effect of days on rearing in HCRs ($F_{2,72} = 13.7$, $p < 0.0001$).

JPET Fast Forward. Published on January 21, 2003 as DOI: 10.1124/jpet.102.047258

Figure 5. Representative time course of the effects of a challenge injection of cocaine (10 mg/kg, i.p., arrow) on high-speed chronoamperometric recordings of exogenous DA signals in NAc of a rat that had been treated with cocaine (10 mg/kg/day; i.p.) for 6 days prior to this cocaine challenge on Day 7. Oxidation currents were evoked by local application of DA (80 pmol) at the recording site at 5-min intervals, averaged across 1-sec bins and converted to concentrations based on in vitro electrode calibration. Note the reproducibility of the DA signals during the 30 min of pre-drug baseline recording and the transient increase following administration of cocaine. **Inset:** Two representative signals evoked by local applications of DA (arrow) in this rat are shown on an expanded time scale to illustrate the increase above baseline in maximal signal amplitude, A_{max}, and the decrease in the first-order decay rate constant, k, 10 min after the cocaine challenge. See Methods for details. Increased A_{max} and decreased k values are indicative of reduced DAT function.

Figure 6. Baseline DA clearance parameters in NAc of LCRs and HCRs across repeated cocaine treatment days and following a 7-day withdrawal. Data are mean values ± SEM of 5-6 reproducible pre-drug DA signals in LCRs (open bars; n = 10, Days 1-3 and Days 5-7; n = 7, Day 15) and HCRs (closed bars; n = 7, Days 1-3 and Days 5-7; n = 4, Day 15). Data were collapsed across Days 1 and 3 and across Days 5 and 7 to correspond to the induction and expression phases of locomotor sensitization, respectively. **(A)** The DA ejection volumes are indicated in arbitrary units relative to the baseline A_{max} responses evoked in each individual rat. **(B)** The baseline first-order decay rate constant, or k, reflects the efficiency of exogenous DA clearance in the absence

of cocaine. Mean A_{max} responses were similar in all rats (0.6-0.8 μM). *p < 0.05 versus the time-matched value in LCRs; and #p < 0.05 versus Day 1-3 value.

Figure 7. Time course comparisons of baseline and cocaine-induced changes in DA signal A_{max} during repeated cocaine administration and following withdrawal in LCRs **(A)** and HCRs **(B)**. Electrochemical data were recorded simultaneously with behavior in the same rats shown in Figs. 2 and 4 (n = 10, LCRs; n = 7, HCRs), with the exception of Day 15 in which electrochemical data were obtained from a subset of these same animals (n = 7, LCRs; n = 4, HCRs). Data from Days 1 and 3, and likewise from Days 5 and 7, were collapsed to correspond to the induction and expression phases of locomotor sensitization, respectively (see Fig. 2A). Arrows indicate time of the i.p. injections of saline (1 ml/kg) or cocaine (10 mg/kg). **Left panels**: The time courses for repeated treatment effects on baseline and cocaine-induced changes in A_{max} are shown for the 5-min recording intervals. Two-way ANOVAs, with time as the only repeated measure, were performed on cocaine-induced changes in A_{max} across 0-60 min (see Results). **Right panels**: Bar graphs summarize the peak effects on A_{max} during the first 30 min post-treatment. Significant differences reflect post-hoc Bonferroni's multiple t-test comparisons. **p < 0.01 versus the Days 1-3 cocaine-induced response; and #p < 0.05, ##p < 0.01 versus the Day 0 saline-induced response.

Figure 8. Time course comparisons of baseline and cocaine-induced changes in DA signal k during repeated cocaine administration and following withdrawal in LCR **(A)** and HCR **(B)** rats. See Fig. 7 for experimental details and groups. Arrows indicate time of the i.p. injections of saline (1 ml/kg) or cocaine (10 mg/kg). **Left panels**: The time courses for repeated treatment

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effects on baseline and cocaine-induced changes in k are shown for the 5-min recording intervals. Two-way ANOVAs, with time as the only repeated measures, were performed on cocaine-induced changes in k across 0-60 min (see Results). **Right panels**: Bar graphs summarize the peak effects on k during the first 30 min post-treatment. Significant differences reflect post-hoc Bonferroni's multiple t-test comparisons. **p < 0.01 versus the averaged Days 1-3 cocaine-induced effect; and #p < 0.05, ##p < 0.01, ###p < 0.001 versus the Day 0 saline-induced effect.

Figure 9. Only male Sprague-Dawley rats treated repeatedly with cocaine exhibit locomotor sensitization to a subsequent cocaine challenge. The effects of repeated treatment with either saline (S) or cocaine (C) on baseline and saline or cocaine challenge-induced locomotor activity are shown. The first and third pairs of bars represent the Control Group and the second pair of bars represents the Experimental Group (Table 1). Locomotor activity is the cumulative distance traveled (cm) in the 30 min immediately preceding (baseline) and the 30 min following the cocaine or saline challenge injection. Data are mean values ± SEM. A two-way ANOVA revealed a significant effect of treatment ($F_{2,68} = 18.16$, $p < 0.001$) and time (baseline versus challenge; $F_{1,68} = 21.03$, $p < 0.001$). Significant effects indicated reflect post-hoc Bonferroni's multiple t-test comparisons. +p < 0.05, +++p < 0.001 versus the respective baseline; **p < 0.01 versus the daily saline with a cocaine challenge; and ###p < 0.001 versus the daily cocaine with a saline challenge.

Figure 10. Only rats treated repeatedly with cocaine exhibit inhibition of DA clearance to a subsequent cocaine challenge. The effects of repeated treatment with either saline (S) or cocaine

(C) on cocaine- or saline-induced changes in DA signal A_{max} **(A)** and k **(B)** parameters in NAc are shown. Data are mean values ± SEM for the same rats in which locomotor responses were recorded simultaneously and reported in Fig. 9. For each rat, the DA clearance signal parameters were averaged across the first 30 min following the ‘challenge’ injection of either cocaine (10 mg/kg) or saline (1 ml/kg) and expressed relative to its ‘baseline’ value (average of 5-6 signal parameters immediately preceding the challenge injection). A one-way ANOVA revealed a significant effect of treatment on A_{max} ($F_{2,36} = 11.042$, $p < 0.001$) and k ($F_{2,36} = 27.941$, $p < 0.001$). Significant differences indicated reflect post-hoc Bonferroni’s multiple t-tests comparisons. *$p < 0.05$, **$p < 0.01$ versus the daily saline with a cocaine challenge; and #$p < 0.05$, ###$p < 0.001$ versus the daily cocaine with a saline challenge.

Table 1: Treatment Groups

	Days			
Groups	*0*	*1-6*	*7*	*15*
Experimental (n = 17)	Saline	Cocaine	Cocaine	Cocaine
Control (n = 10)	Saline	Saline	Cocaine	--
Control (n = 10)	--	Cocaine	Saline	--

Injections (i.p.) of either saline (1 ml/kg/day) or cocaine (10 mg/kg/day) were administered to rats chronically instrumented with microelectrode/microcannulae assemblies in NAc in the activity apparatus on the days indicated. Behavioral and electrochemical responses were obtained in the freely-moving rats, on Days 0, 1, 3, 5 and 7. Additionally, responses were recorded on Day 15, following a 7-day withdrawal in the Experimental Group only. In the Control Group, the same rats were used in the two sequentially conducted control experiments.

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Figure 1

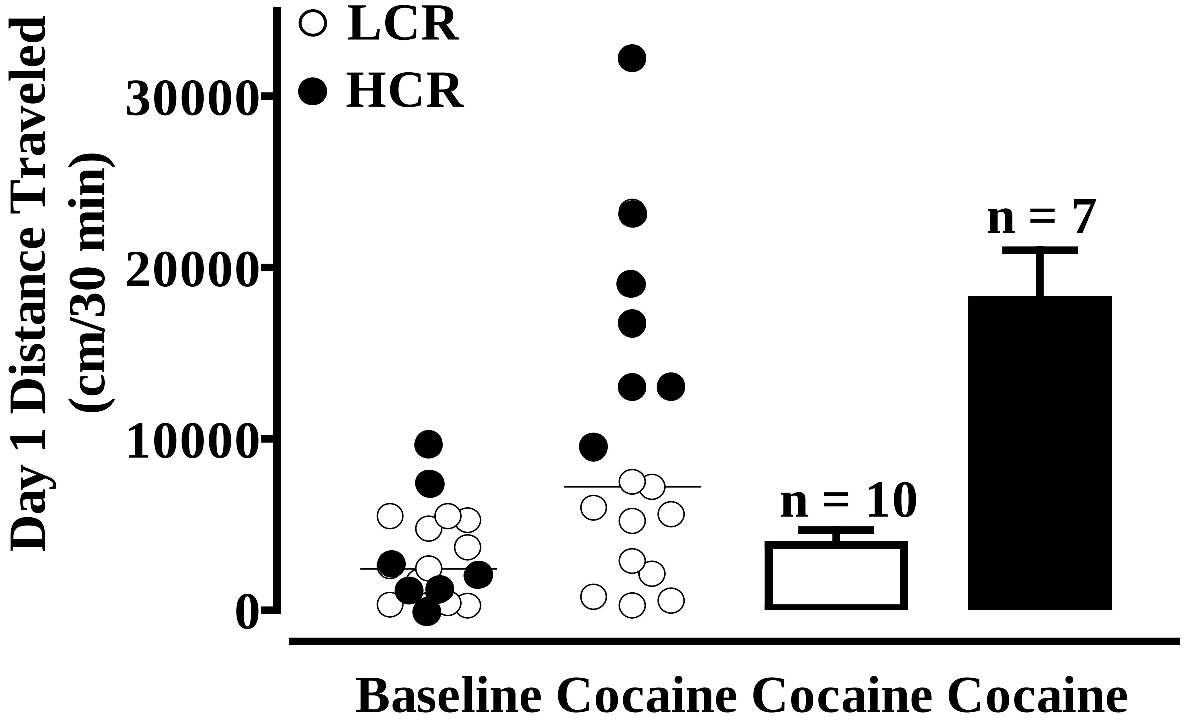


Figure 2

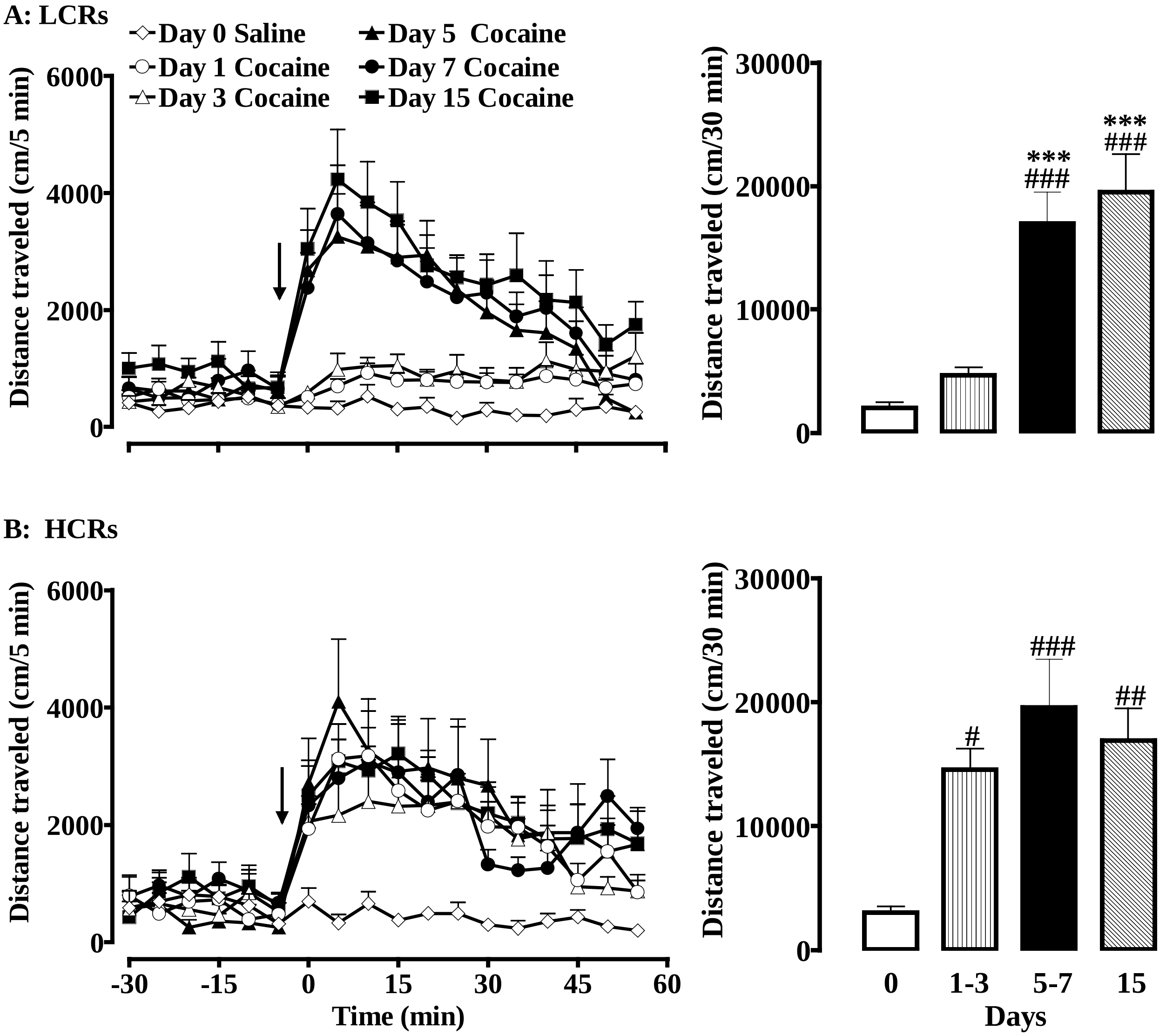
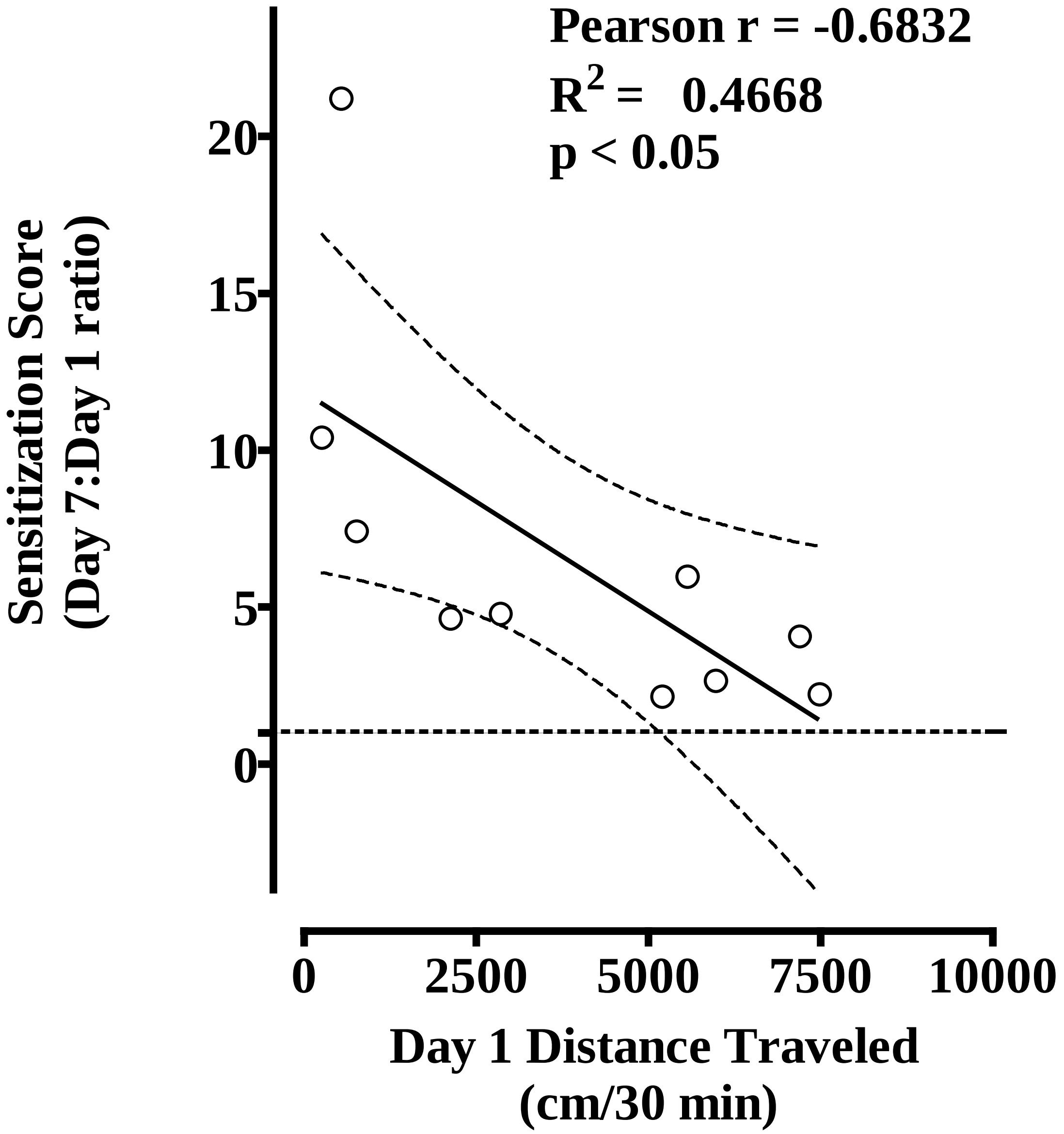

A: LCRs
Day 0 Saline
Day 1 Cocaine
Day 3 Cocaine
Day 5 Cocaine
Day 7 Cocaine
Day 15 Cocaine
Distance traveled (cm/5 min)
0
2000
4000
6000
Distance traveled (cm/30 min)
0
10000
20000
30000
***
###
***
###
B: HCRs
Distance traveled (cm/5 min)
0
2000
4000
6000
-30
-15
0
15
30
45
60
Time (min)
Distance traveled (cm/30 min)
0
10000
20000
30000
#
###
##
0
1-3
5-7
15
Days

Figure 3


Pearson r = -0.6832
R^2 = 0.4668
$p < 0.05$
Sensitization Score
(Day 7:Day 1 ratio)
20
15
10
5
0
0
2500
5000
7500
10000
Day 1 Distance Traveled
(cm/30 min)

JPET Fast Forward. Published on January 21, 2003 as DOI: 10.1124/jpet.102.047258

Figure 4

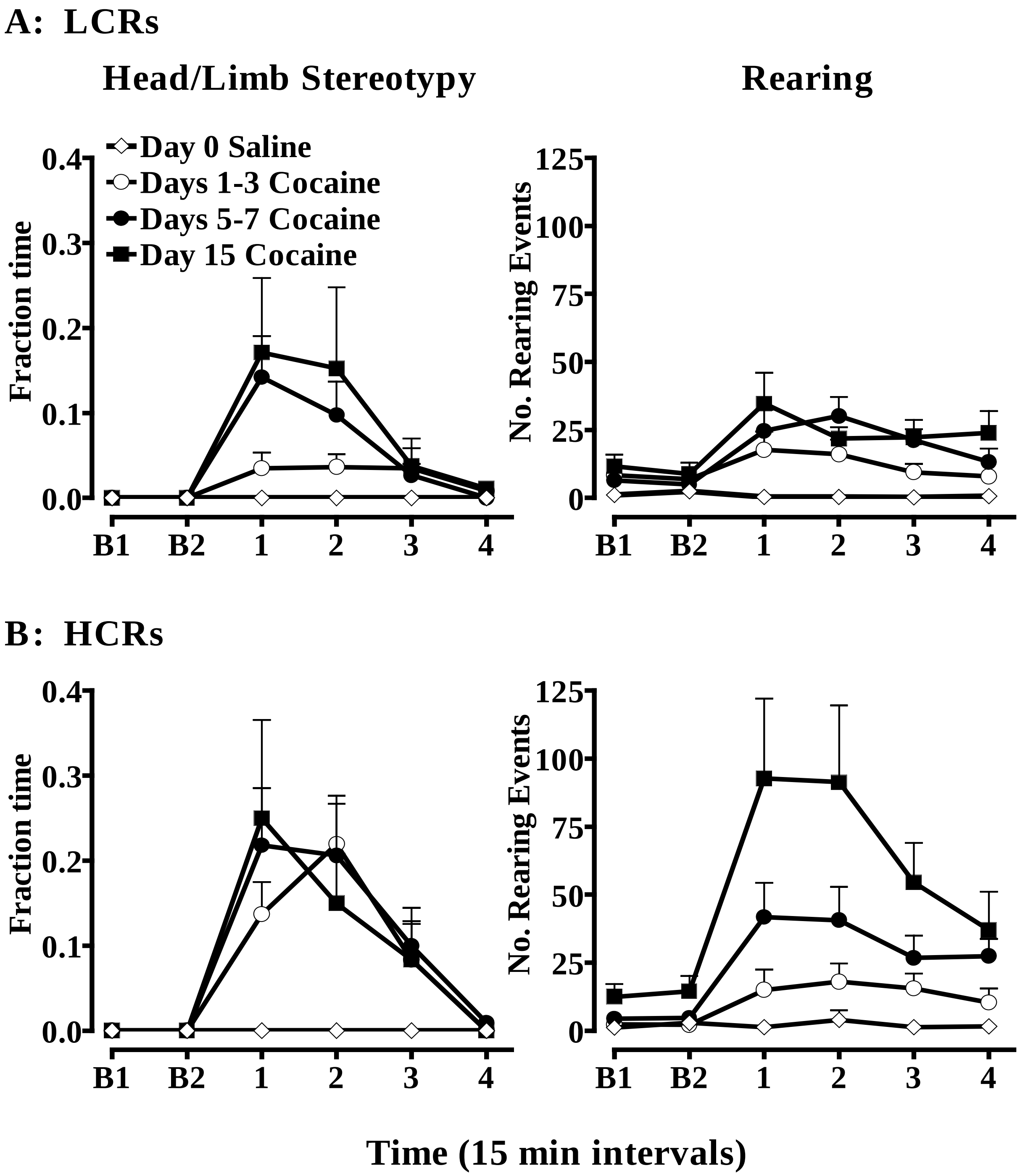


Figure 5

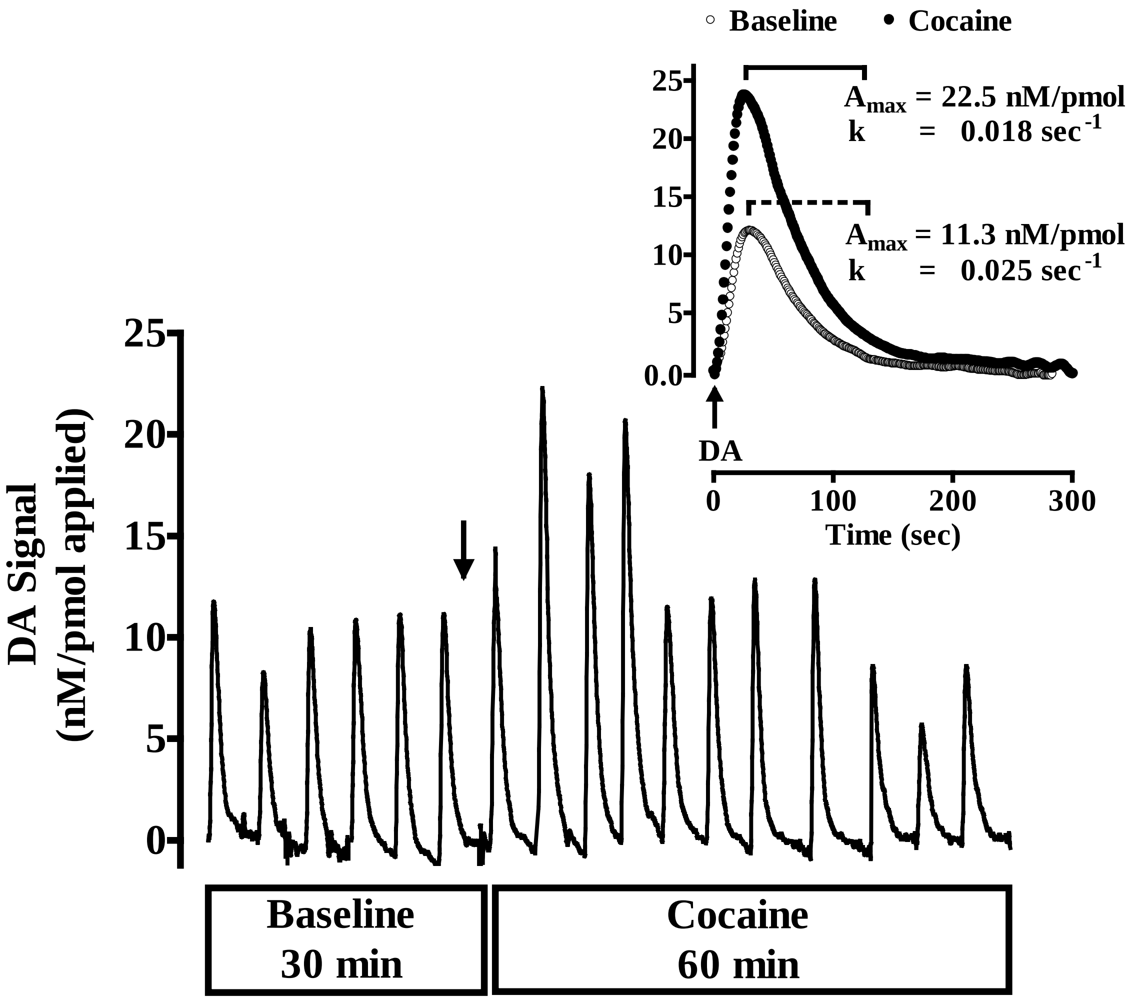

○ Baseline
● Cocaine
25
20
15
10
5
0.0
A_{max} = 22.5 nM/pmol
k = 0.018 sec^{-1}
A_{max} = 11.3 nM/pmol
k = 0.025 sec^{-1}
DA
0
100
200
300
Time (sec)
DA Signal
(nM/pmol applied)
25
20
15
10
5
0
Baseline
30 min
Cocaine
60 min

Figure 6

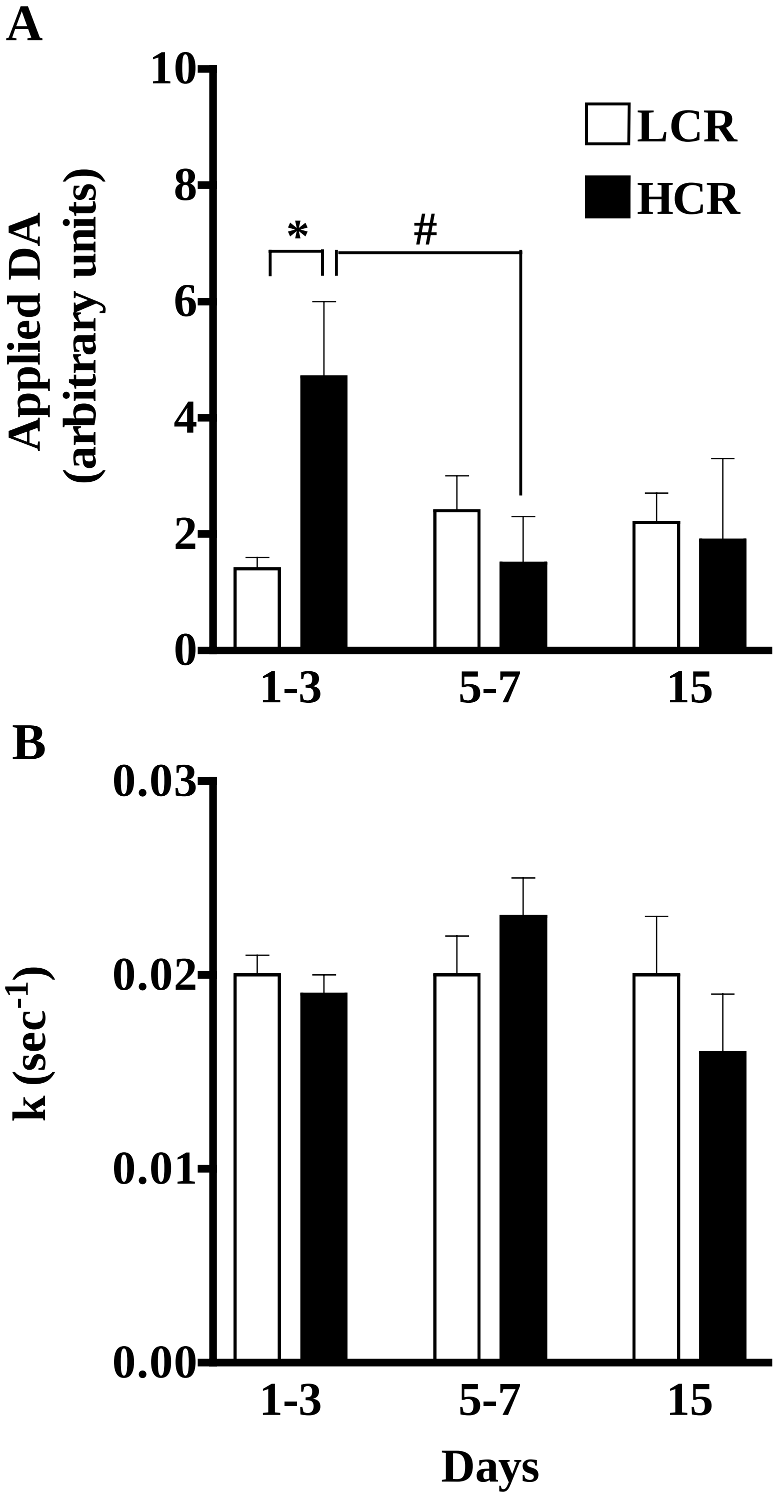

A
Applied DA
(arbitrary units)
10
8
6
4
2
0
LCR
HCR
*
#
1-3
5-7
15
B
k (sec^{-1})
0.03
0.02
0.01
0.00
1-3
5-7
15
Days

Figure 7

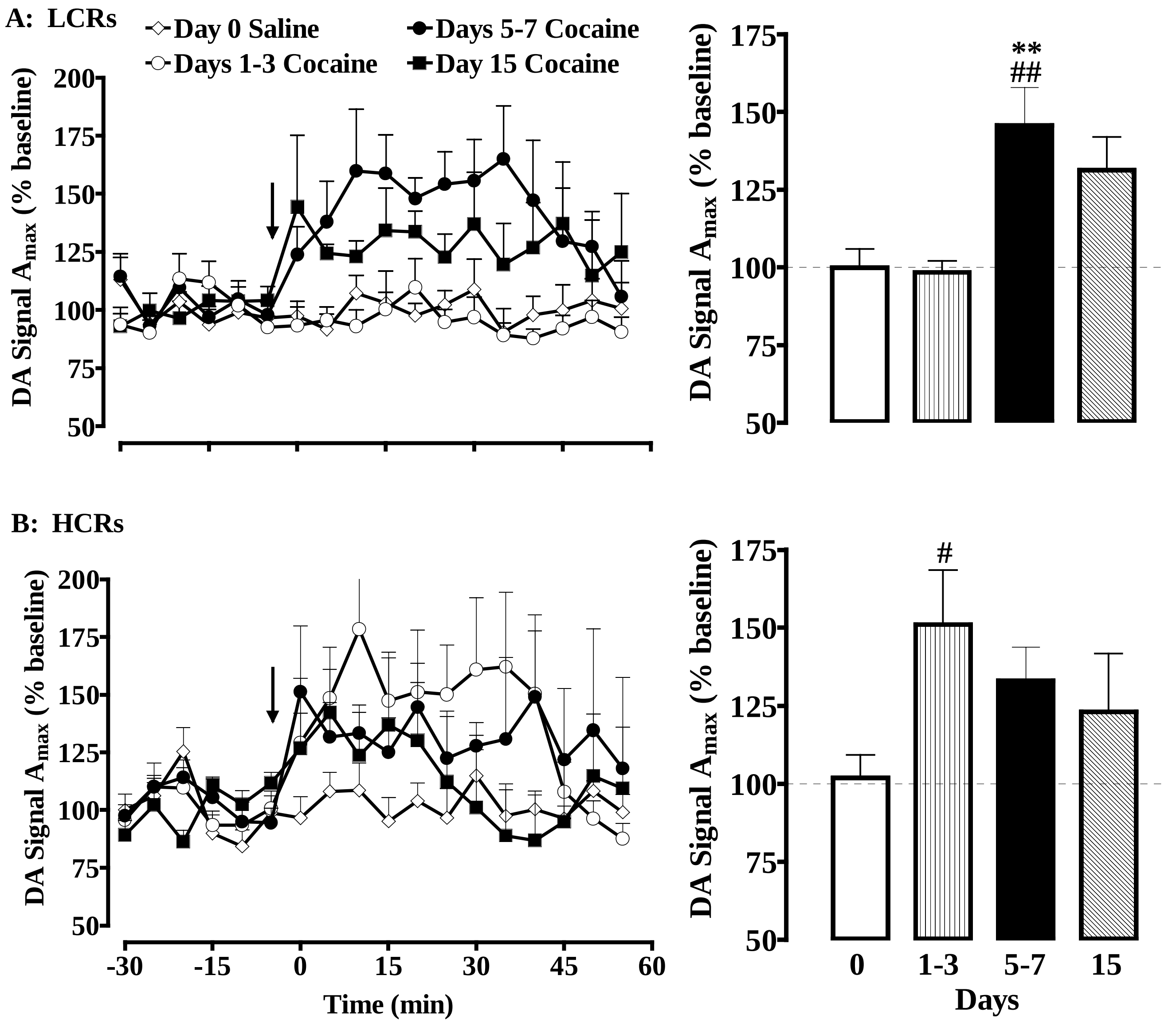
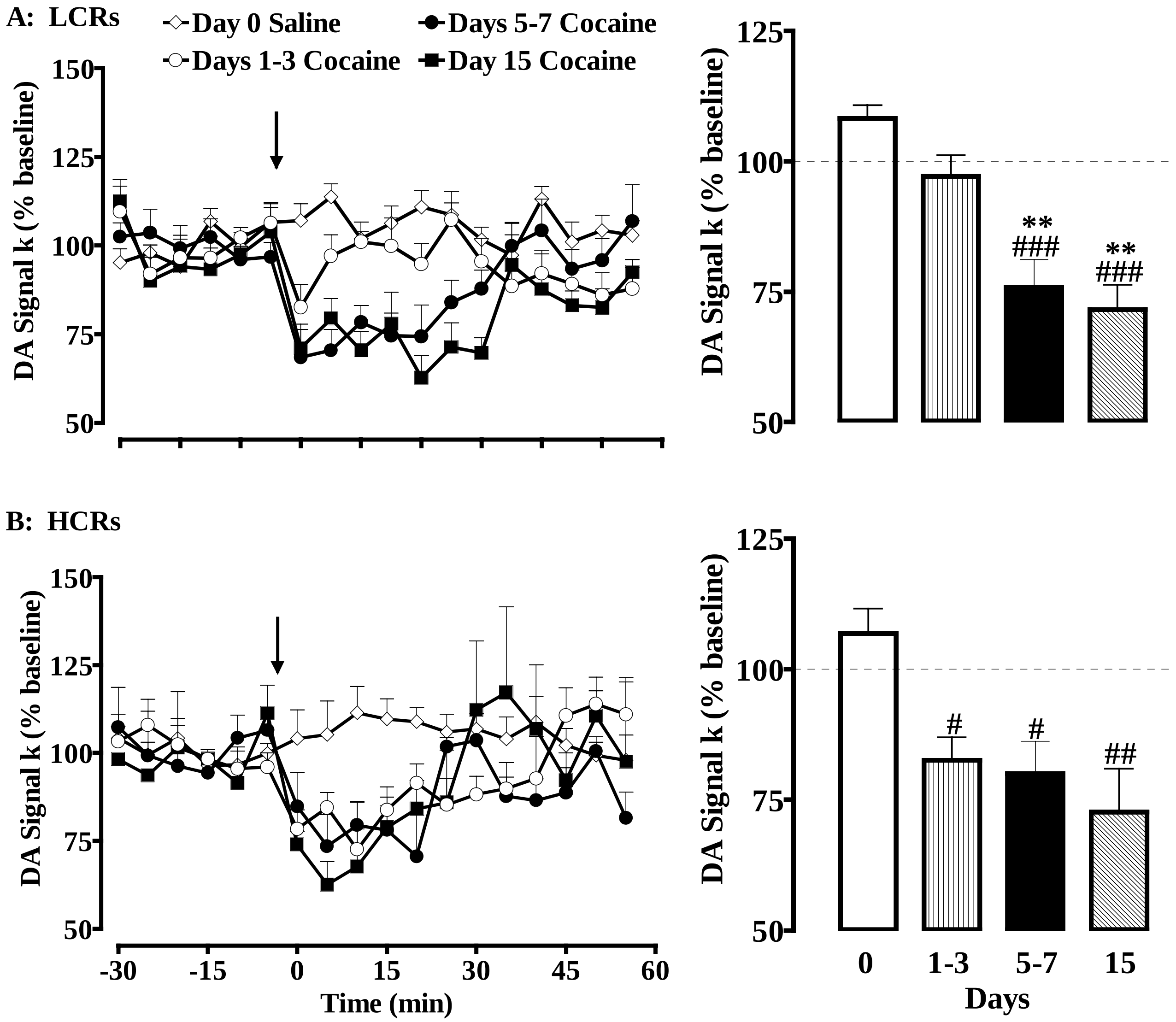

A: LCRs
Day 0 Saline
Days 1-3 Cocaine
Days 5-7 Cocaine
Day 15 Cocaine
DA Signal A_{max} (% baseline)
200
175
150
125
100
75
50
**
##
B: HCRs
#
-30
-15
0
15
30
45
60
Time (min)
0
1-3
5-7
15
Days

Figure 8


A: LCRs
Day 0 Saline
Days 1-3 Cocaine
Days 5-7 Cocaine
Day 15 Cocaine
DA Signal k (% baseline)
150
125
100
75
50
**
###
**
###
B: HCRs
DA Signal k (% baseline)
150
125
100
75
50
-30
-15
0
15
30
45
60
Time (min)
125
100
75
50
#
#
##
0
1-3
5-7
15
Days

JPET Fast Forward. Published on January 21, 2003 as DOI: 10.1124/jpet.102.047258

Figure 9

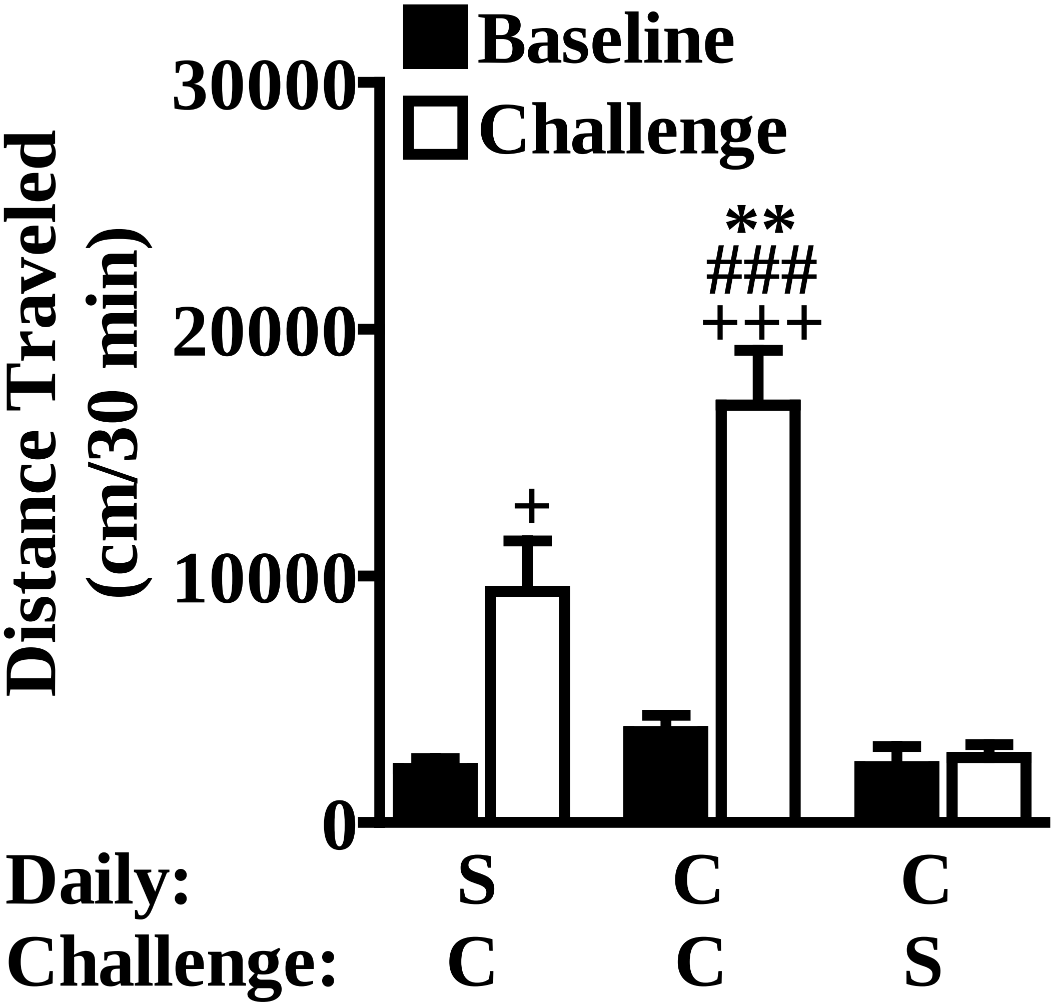
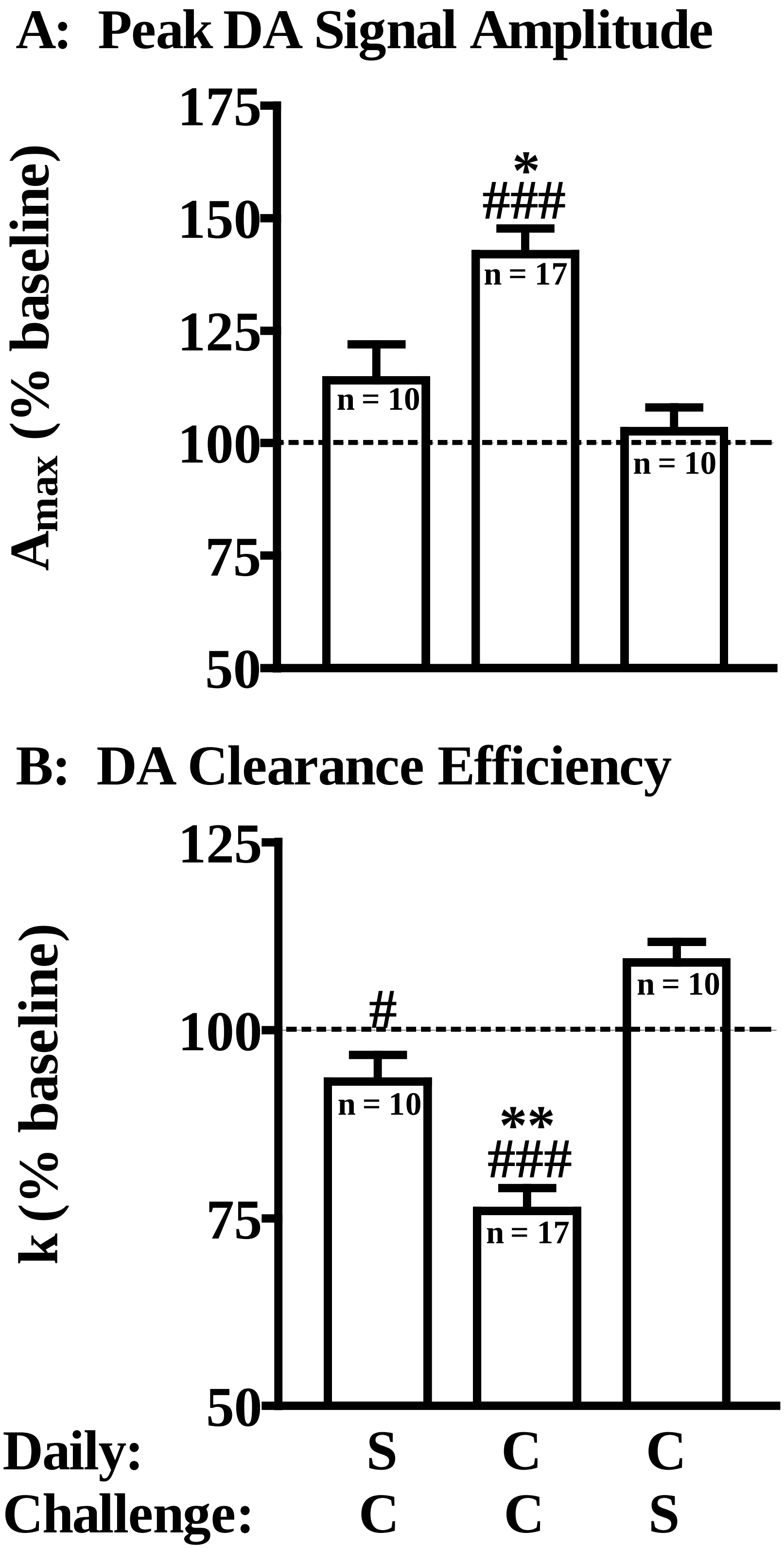


Figure 10


A: Peak DA Signal Amplitude
A_{max} (% baseline)
175
150
125
100
75
50
n = 10
n = 17
n = 10
*
###
B: DA Clearance Efficiency
k (% baseline)
125
100
75
50
n = 10
n = 17
n = 10
#
**
###
Daily: S C C
Challenge: C C S