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Distinct Effects of Enriched Environment on Dopamine Clearance in Nucleus Accumbens Shell and Core Following Systemic Nicotine Administration

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Abstract

Environmental enrichment during development may reduce drug abuse liability by modulating dopamine transporter (DAT) function. Nucleus accumbens (NAc) shell and core respond differentially to regulate the rewarding properties and locomotor stimulant effects of psychostimulants. The current study evaluated dopamine (DA) clearance (CL_{DA}) in the NAc shell and core using in vivo voltammetry in rats raised in an enriched condition (EC) or an impoverished condition (IC) and determined the effect of nicotine (0.4 mg/kg) on CL_{DA} . Baseline CL_{DA} in NAc shell and core was not different between EC and IC rats. In the saline control group, CL_{DA} in NAc shell was greater across time in IC when compared with EC rats, whereas CL_{DA} in NAc core was greater in EC rats when compared with IC rats. Consistent with these findings, opposite effects of enrichment on DA clearance in shell and core were obtained following acute nicotine administration. In NAc shell, nicotine increased CL_{DA} in EC rats, but not in IC rats. Conversely, in NAc core, nicotine increased CL_{DA} in IC rats, but not in EC rats. The current results demonstrate that environmental enrichment differentially regulates the response to nicotine in NAc shell and core via alterations in DAT function, which may explain how environmental enrichment reduces the behavioral response to nicotine.

Keywords

dopamine transporter; nicotine; nucleus accumbens; environmental enrichment; dopamine clearance; in vivo voltammetry

INTRODUCTION

The effects of nicotine on the mesolimbic dopamine (DA) system contribute to nicotine-induced behavioral changes (Kita et al., 1992; Laviolette and van der Kooy, 2004; Mansvelder et al., 2002; Panagis et al., 1996). Nicotine increases DA release in nucleus accumbens (NAc) through activation of nicotinic acetylcholine receptors (nAChRs) in the ventral tegmental area (Clarke and Pert, 1985; Laviolette and van der Kooy, 2004; Picciotto et al., 1998). The NAc is divided into shell and core subregions, which have distinct roles in

reward and sensitization (Boye et al., 2001; Kelley et al., 1997; Maldonado-Irizarry and Kelley, 1995; Parkinson et al., 1999; Weiner et al., 1996). NAc shell is responsive to the rewarding effects of nicotine, whereas NAc core is responsive to the aversive effects (Sellings et al., 2008). This finding suggests that DA neurotransmission in NAc may enable oppositional motivational processes. Previous studies have shown that acute nicotine administration preferentially increases extracellular DA in NAc shell relative to core (Iyaniwura et al., 2001; Nisell et al., 1997). In contrast, repeated nicotine increases extracellular DA in core, but not in shell, which is associated with nicotine-mediated locomotor sensitization (Balfour et al., 1998; Benwell and Balfour, 1992; Cadoni and Di Chiara, 2000). Furthermore, intravenous nicotine self-administration is associated with long-term increases in DA in NAc shell relative to core (Lecca et al., 2006). Thus, DA in NAc shell and core plays distinct roles in the complex behavioral response to nicotine.

The DA transporter (DAT), a primary pharmacological target for psychostimulant drugs, transports extracellular DA into the presynaptic terminals and thereby regulates extracellular DA concentration (Gainetdinov and Caron, 2003). In contrast to stimulant drugs, such as cocaine and amphetamine, nicotine increases DAT function through an indirect mechanism via nAChR activation (Hart and Ksir, 1996). In vivo voltammetry studies have shown that acute nicotine enhances DAT function by measuring DA clearance rate of exogenously applied DA in NAc (Hart and Ksir, 1996) and in medial prefrontal cortex (mPFC) and striatum (Middleton et al., 2004). Nicotine-induced enhancement of DA clearance is mediated by nAChR activation (Middleton et al., 2004). nAChR activation also augments amphetamine-stimulated DA release from rat prefrontal cortex slices (Drew et al., 2000). Nicotine-induced enhancement of DA clearance may represent a compensatory response to the nicotine-stimulated increase in extracellular DA concentration.

Environmental enrichment during development may reduce drug abuse liability by modulating DAT function. For example, rats raised in an enriched condition (EC) have a reduced propensity to acquire amphetamine self-administration when compared with rats raised in an impoverished condition (IC; Bardo et al., 2001; Bowling and Bardo, 1994; Bowling et al., 1993). EC rats also exhibit reduced sensitization in response to repeated nicotine stimulation when compared with IC rats (Green et al., 2003). EC rats show a decreased maximal velocity of [³H]DA uptake and reduced DAT cell surface expression in mPFC when compared with IC rats (Zhu et al., 2004). In vivo DA clearance in mPFC was lower in EC than in IC rats following saline injection, and importantly, nicotine increased DA clearance in EC, but not in IC rats (Zhu et al., 2007). As the NAc is a critical brain region in the behavioral activating, rewarding and aversive effects of nicotine, the purpose of this study was to determine whether rearing environment differentially alters basal and nicotine-mediated DA clearance in NAc shell and core.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (21 days of age), obtained from Harlan Laboratories (Indianapolis, IN), were housed with free access to food and water in the Division of Laboratory Animal Resources at the University of Kentucky. Rats were maintained on a 12-h light:dark cycle (lights on at 07:00 h). All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Kentucky and performed in accordance with the 1996 version of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drugs

S(-)-Nicotine ditartrate (nicotine), desipramine hydrochloride, 3-hydroxytyramine hydrochloride (DA), and sodium phosphate dibasic were purchased from Sigma/RBI (Natick, MA). Sodium phosphate monobasic, sodium chloride, ascorbic acid, and urethane were purchased from Fisher Scientific. (Pittsburgh, PA). Nafion-perfluorinated ion-exchange resin was purchased from Sigma-Aldrich Chemical (Milwaukee, WI). Dental wax and acrylic were purchased from Patterson Dental Supply (Louisville, KY), and sticky wax was purchased from KEPP (Romulus, MI).

Environmental conditions

On arrival, rats were randomly assigned to either EC or IC rearing conditions. EC and IC conditions were those described previously (Wooters et al., 2011). Briefly, EC rats were group housed (10–15 per cage) in a metal cage (120 cm length × 60 cm width × 45 cm height). Fourteen hard nonchewable plastic objects were placed randomly in the cage. Each day, seven of the 14 objects were replaced with seven new objects, and the remaining seven objects were rearranged to provide a novel spatial configuration. EC rats were handled each day. In contrast, IC rats were housed individually in wire mesh hanging cages (25 cm length × 18 cm width × 17 cm height) with solid metal sides and wire mesh floor. IC rats were neither handled nor exposed to any objects except food and water. Rats were maintained in these environments until 53 days of age and throughout all neurochemical experiments.

In vivo electrochemical measurements

To assess dynamic changes in DAT function, DA clearance from the extracellular space was determined following local ejection of DA into either NAc shell or core in anesthetized EC and IC rats using our published methods (Zhu et al., 2007). Electrochemical recording electrodes (Quanteon LLC, Lexington, KY) consisted of a single Nafion-coated carbon fiber (30 μ m diameter) sealed in a glass capillary (4 mm outer diameter, 0.80 mm wall diameter), with a 150- to 200- μ m length exposed. Electrodes were calibrated in vitro in 0.1 M phosphate buffered saline (pH 7.4) at 22°C and showed a linear response to DA (2–8 μ M) with correlation coefficients ranging from 0.993 to 1.000. Selectivity for DA over ascorbic acid was 3840 ± 398 to 1 ($n = 48$). Electrode sensitivity for DA was evaluated intermittently before and after experiments and was demonstrated to be stable as reported previously. Nicotine (0.01–1.0 mM) did not alter electrode sensitivity to DA, as reported previously (Ksir et al., 1995; Zhu et al., 2007). Each electrode was attached to a single-barrel micropipette (tip diameter, 10–15 μ m) with sticky wax such that electrode and micropipette tips were positioned 250–300 μ m apart. Immediately prior to conducting the experiment, micropipettes were filled with a nonsaturating DA concentration (200 μ M in 154 mM NaCl and 100 μ M ascorbic acid, pH 7.4), which provided linear kinetics of DA uptake (Zahniser et al., 1999).

Rats were anesthetized with urethane (1.25–1.5 g/kg, i.p.) and placed into a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Body temperature was maintained at 37°C by a heating pad coupled to a rectal thermometer (Harvard Apparatus, Holliston, MA). A longitudinal incision (2 cm) was made in the scalp, the skin was retracted, and the skull and dura over the frontal cortex were removed. A small hole was drilled in the skull overlying the posterior cortex for placement of two Ag/AgCl reference electrodes, which were secured with dental acrylic. An electrode/micropipette assembly was lowered into NAc shell (1.2–1.7 mm anterior to bregma, 0.6–0.8 mm lateral from midline, and 6.0–8.0 mm below the surface of the cortex) or core (1.5–1.7 mm anterior to bregma, 2.2 mm lateral from midline, and 6.5–8.0 mm below the surface of the cortex).

DA (200 μM) was pressure ejected (30 psi, 0.1–2.5 s) from the micropipette at 5-min intervals using a Picospritzer II (General Valve Corporation, Fairfield, NJ). Ejection volume (250 nl/mm) applied was measured using a reticule with a millimeter (mm) scale in an eyepiece fitted to a stereomicroscope. Average volumes of locally applied DA solution into shell and core from EC and IC rats are provided in Table I. High-speed chronoamperometric electrochemical measurements of oxidation and reduction currents were made at 5 Hz and averaged to 1 Hz using an IVEC 10 system (Medical Systems Corporation, Greenvale, NY). Oxidation potential (+0.55 V) was applied for 100 ms (versus the Ag/AgCl electrode), and then the reduction potential of 0.0 V was applied for 100 ms.

Stable baseline was defined as three signals with maximal amplitudes that did not differ by $\pm 10\%$ following a constant ejection volume of DA solution. Once stable baseline signals were obtained, two additional baseline DA signals were recorded, followed by nicotine (0.4 mg/kg, s.c., dose expressed as salt weight) or saline (1 ml/kg, s.c.) injection. For the subsequent 55-min recording period, the same volume and amount of DA were ejected locally every 5 min, and DA signal response was determined after each ejection. Nicotine dose was chosen based on previous reports showing effects on CL_{DA} in mPFC and striatum in EC and IC rats (Zhu et al., 2007).

Electrochemical data acquisition and analysis

Electrochemical data acquisition and analysis were performed as described previously (Zhu et al., 2007). Three electrochemical signal parameters were measured from DA oxidation currents. Maximal signal amplitude (A_{max}) was defined as the maximal height of the DA oxidation signal, which reflects maximal extracellular DA concentration following local application of exogenous DA and expressed as concentration (μM). T_{80} was defined as the time required for the DA signal to decay by 80% from its maximal value (A_{max}). DA clearance (CL_{DA}) is a pharmacokinetic parameter, which provides a measure of efficiency of DAT to remove DA from the extracellular space. CL_{DA} was defined as the amount (pmol) of DA ejected divided by the area under the curve of the DA signal (AUC_{DA}), which reflects DA concentration across time. AUC_{DA} was determined by fitting the decay segment of each DA signal trace following the maximal DA signal amplitude to the following equation:

$$\sum_{i=0}^n (y_i + y_{i+1}/2) \times (t_{i+1} - t_i)$$

where y is DA signal amplitude (μM) at any time (t , s) following DA ejection, and i is the initial point at which DA signal amplitude moves above baseline. Volume in the AUC term refers to volume into which the entire amount of ejected DA distributes, that is, not volume at the probe site. The voltammetric response reflects a direct measurement of DA concentration at the electrode, which is influenced by the volume of distribution into which the DA distributes. AUC_{DA} was determined from 0 to 160 s for both NAc subregions. Therefore, CL_{DA} (L/s) for each DA signal was derived from the amount (pmol) of DA ejected divided by AUC_{DA} (pmol/L $\times$ s) for each DA signal as described previously (Zhu et al., 2007).

Verification of electrode placement

Immediately after each recording session, a small amount of current passed through the electrode to facilitate localization of the electrode placement on sectioning of the brain and subsequent histological determination. Brains were immersed for 5 min in 2-methylbutane, stored at -80°C , and subsequently sectioned and stained with cresyl violet to determine

electrode placement. Thin coronal sections (25 μm) were prepared from frozen whole brain between rostral and caudal borders of NAc (0.5–2.8 mm anterior to bregma). The sections were mounted on microscope slides. Probe placements were evaluated on stained and unstained sections by observations of tissue displacement and electrolytic marks from the recording electrodes under a light microscope.

Statistical analyses

Data are presented as mean $\pm$ SEM; n represents the number of rats. Data are presented as follow: (1) absolute values for parameters (A_{max} , T_{80} , and CL_{DA}) at baseline, that is, the three reproducible DA signals obtained prior to nicotine or saline injection; (2) time course for each parameter across the 55-min period after nicotine or saline injection, expressed as a percentage of baseline; and (3) average value for each parameter across the 55-min period after nicotine or saline injection, expressed as percentage of baseline.

For each baseline parameter (A_{max} , T_{80} , and CL_{DA}), separate two-factor ANOVAs with rearing condition (EC and IC) and brain region (shell and core) as between-group factors were performed, followed by simple effects comparisons. To analyze the effects of nicotine on A_{max} , T_{80} , and CL_{DA} in shell and core, separate three-way mixed factor ANOVAs with rearing condition (EC and IC) and treatment (nicotine and saline) as between-group factors and time (5-min intervals across the 55-min period) as a within-subjects factor were performed. Significant three-way interactions were followed by separate two-way ANOVAs: (1) with rearing condition as a between-group factor and time as a within-subjects factor, and (2) with treatment as a between-group factor and time as a within-subject factor. Significant two-way interactions in the latter analyses were followed by one-way ANOVAs at each time point across the 55-min period following injection to determine differences between rearing conditions or between treatment groups. In addition, to determine the onset of the effect of nicotine, repeated-measures one-way ANOVAs compared the time points after injection with baseline within a group. Finally, to analyze the DA clearance parameters averaged across the 55-min period after nicotine or saline injection, two-way ANOVAs were performed with rearing condition and treatment as between-group factors. To determine the between-group differences, significant interactions were followed by post hoc Student's t -tests using Bonferroni's correction. Statistical analyses were performed using SPSS (standard version 14.0; SPSS, Chicago, IL). Differences were considered significant when $P < 0.05$.

RESULTS

Histological assessment of electrode placement in NAc shell and core

Distribution of electrode placements in the shell and core are shown in Figure 1. Localization of the electrodes was between +1.0 and +2.2 mm anterior to bregma. In addition, 24 of 37 electrodes were confirmed histologically to be within the shell, whereas 24 of 27 electrodes were confirmed as located within the core. Only data from rats with confirmed electrode placements in shell or core were used for data analyses.

Effect of differential rearing environment on DA clearance parameters in NAc shell following nicotine or saline administration

Prior to systemic injection of nicotine or saline, no differences between EC and IC rats in amount of DA applied or values for A_{max} , T_{80} , and CL_{DA} in NAc shell were observed ($P > 0.05$; Table I). As DA clearance parameters in shell were not different between EC and IC rats, data for each parameter obtained across the 55-min session after nicotine or saline injection are expressed as a percentage of baseline to facilitate comparisons between saline

control EC and IC rats, as well as comparisons between nicotine- and saline-injected groups (Fig. 2).

Separate three-way ANOVAs were performed to determine the effects of nicotine on DA clearance parameters ($A_{\max}$, T_{80} , and CL_{DA}) across the 55-min session in NAc shell in EC and IC rats (Fig. 2). The analysis of $A_{\max}$ values revealed a three-way interaction of rearing condition $\times$ treatment $\times$ time [$F_{(12, 240)} = 3.82$, $P < 0.05$; Fig. 2A]. Subsequent two-way ANOVA determined the differences between the groups in $A_{\max}$ values across the 55-min recording sessions. For the EC and IC saline control groups, analysis of $A_{\max}$ data revealed a rearing condition $\times$ time interaction [$F_{(1, 20)} = 5.76$, $P < 0.05$; Fig. 2A]. $A_{\max}$ values were greater in EC rats when compared with IC rats in the saline control groups from 25 to 55 min ($P < 0.05$, Bonferroni t -test). Consistent with these data, the mean $A_{\max}$ across the 55-min recording period was greater in the EC saline control group when compared with the IC saline control group (Fig. 2D). One-way ANOVAs on $A_{\max}$ values at each time point after saline injection revealed that $A_{\max}$ values in both EC and IC rats were decreased from 20 to 55 min when compared with respective baseline $A_{\max}$ values ($P < 0.05$). In the saline control EC and IC rats, $A_{\max}$ decreased to 21% and 37%, respectively, at 55 min (Fig. 2A).

Regarding the effect of nicotine in EC rats, two-way ANOVA on the $A_{\max}$ data revealed a nicotine treatment $\times$ time interaction [$F_{(1, 20)} = 7.0$, $P < 0.05$; Fig. 2A]. $A_{\max}$ values following nicotine were decreased from 25 to 55 min when compared with the respective saline control EC group ($P < 0.05$). Following nicotine injection, mean $A_{\max}$ across the 55-min period was decreased to 15% when compared with the saline control group ($P < 0.05$; Fig. 2D). In contrast, analysis of the $A_{\max}$ data for the IC group revealed no significant main effect of nicotine treatment or interaction of nicotine treatment $\times$ time, indicating that nicotine did not alter $A_{\max}$ in IC rats (Figs. 2A and 2D). Regarding the analysis of T_{80} for NAc shell, no significant main effects or interactions of rearing condition, treatment, and time were found (Fig. 2B).

Three-way ANOVA of CL_{DA} data from NAc shell revealed a three-way interaction of rearing condition $\times$ treatment $\times$ time [$F_{(12, 240)} = 1.84$, $P < 0.05$; Fig. 2C]. Subsequent two-way ANOVAs explored between-group differences in CL_{DA} in NAc shell across the 55-min session. Following saline, a rearing condition $\times$ time interaction was found [$F_{(1, 20)} = 5.61$, $P < 0.05$]. CL_{DA} was slower in shell from EC rats when compared with IC rats from 25 to 55 min after saline injection ($P < 0.05$). For the IC group injected with saline, CL_{DA} increased from 25 to 55 min with a maximal increase of 93% above baseline at 55 min. In contrast, no increase in CL_{DA} in NAc shell was observed following saline injection in EC rats (Fig. 2C). After saline injection, the mean CL_{DA} averaged across the 55-min recording period was decreased to 33% in EC rats when compared with IC rats (Fig. 2F).

Two-way ANOVAs determined the effect of nicotine on CL_{DA} in NAc shell of EC and IC rats. For EC rats, a treatment $\times$ time interaction was found [$F_{(1, 20)} = 6.49$, $P < 0.05$; Fig. 2C]. CL_{DA} in the nicotine-treated EC rats was increased from 15 to 55 min, with a maximal 163% increase at 55 min when compared with the saline control group ($P < 0.05$; Fig. 2C). In contrast, regarding CL_{DA} in NAc shell from IC rats, no significant treatment $\times$ time interaction was found, indicating that nicotine does not alter CL_{DA} in IC rats (Figs. 2C and 2F).

Effect of differential rearing environment on DA clearance parameters in NAc core following nicotine or saline administration

As shown in Table I, prior to systemic injection of nicotine or saline, no differences between EC and IC rats were observed in absolute values for baseline $A_{\max}$, T_{80} , and CL_{DA} in NAc core. To facilitate comparisons between the saline control EC and IC rats, as well as

comparisons between nicotine treatment and saline control within either EC or IC groups, data for each parameter obtained across the 55-min session following nicotine or saline administration are expressed as a percent of baseline (Fig. 3).

Separate three-way ANOVAs were performed to determine the effect of nicotine on DA clearance parameters ($A_{\max}$, T_{80} , and CL_{DA}) across the 55-min session in NAc core in EC and IC rats (Fig. 3). The analysis of $A_{\max}$ values from EC and IC rats revealed a three-way interaction of rearing condition $\times$ treatment $\times$ time [$F_{(12, 240)} = 3.31$, $P < 0.05$; Fig. 3A]. To further analyze the significant three-way interaction, separate two-way ANOVAs were conducted. In saline control groups, a rearing condition $\times$ time interaction was obtained [$F_{(1, 20)} = 4.48$, $P < 0.05$; Fig. 3A]. $A_{\max}$ values from NAc core were lower in EC rats when compared with IC rats from 25 to 55 min ($P < 0.05$). Mean $A_{\max}$ across the 55-min recording period was lower after saline injection in EC rats than in IC rats (Fig. 3D). One-way ANOVAs at each time point revealed that $A_{\max}$ values in EC rats were decreased from 20 to 55 min when compared with the respective baseline $A_{\max}$ values ($P < 0.05$). The maximal decrease in $A_{\max}$ values in the EC saline control group was 36% at 55 min (Fig. 3A).

Separate two-way ANOVAs determined the effect of nicotine on $A_{\max}$ values in the EC and IC rats. In EC rats, the analysis of $A_{\max}$ data revealed no significant interaction of treatment $\times$ time, indicating that nicotine did not alter $A_{\max}$ in this group (Figs. 3A and 3D). In contrast, a significant treatment $\times$ time interaction was found in IC rats [$F_{(1, 20)} = 16.7$, $P < 0.05$; Fig. 3A], indicating that nicotine decreased $A_{\max}$ in NAc core in IC rats when compared with the respective saline control group. Additionally, one-way ANOVAs revealed that $A_{\max}$ values in the nicotine-treated IC group were decreased from 15 to 55 min when compared with the IC saline control group ($P < 0.05$). Furthermore, the mean $A_{\max}$ value obtained across the 55-min period was decreased to 30% ($P < 0.05$) in the nicotine-treated IC group relative to the IC saline control group (Fig. 3D). Separate three-way ANOVAs on T_{80} values obtained in NAc core of EC and IC rats revealed no main effects or interactions of rearing condition, treatment, and time (Fig. 3B).

A three-way interaction of rearing condition $\times$ treatment $\times$ time was found following the analysis of CL_{DA} data obtained from NAc core [$F_{(12, 240)} = 1.93$, $P < 0.05$; Fig. 3C]. Subsequent two-way ANOVAs explored between-group differences in CL_{DA} across the 55-min session. Following saline injection, a rearing condition $\times$ time interaction was found [$F_{(1, 20)} = 4.30$, $P < 0.05$]. CL_{DA} was faster in EC rats than IC rats from 35 to 55 min ($P < 0.05$). CL_{DA} was increased at 25–55 min with a maximal increase of 188% above baseline at 55 min in the saline control EC group; however, no increase in CL_{DA} was observed in the saline control IC group (Fig. 3C). Across the 55-min recording period, mean CL_{DA} value was faster in the EC saline control when compared with the IC saline control (Fig. 3F).

Separate two-way ANOVAs determined the differences in CL_{DA} between groups administered with nicotine and saline. For the EC rats, the analysis of CL_{DA} data revealed no interaction of treatment $\times$ time, indicating that nicotine did not alter CL_{DA} in EC rats (Fig. 3C). In contrast, a significant interaction of treatment $\times$ time was found for IC rats [$F_{(1, 20)} = 25.1$, $P < 0.05$], indicating that nicotine increases CL_{DA} when compared with the saline control IC group (Fig. 3C). CL_{DA} in the nicotine-treated IC group was increased from 15 to 55 min with a maximal 147% increase at 55 min ($P < 0.05$; Fig. 3C) and a 101% increase in the mean CL_{DA} in the IC rats across the 55-min period when compared with saline control IC rats (Fig. 3F).

DISCUSSION

The current findings demonstrate that rearing environment differentially regulates DAT function (CL_{DA}) in NAc shell and core under both control conditions (i.e., after a saline injection) and following nicotine administration. In NAc shell, DA clearance was decreased in EC rats relative to IC rats under control conditions, whereas clearance was increased in NAc core in EC relative to IC rats under control conditions. In addition, differential effects of nicotine on CL_{DA} were also observed in NAc core and shell in EC and IC rats. Specifically, following acute nicotine, EC rats showed enhanced DA clearance in shell, whereas IC rats showed enhanced clearance in core. Therefore, relative to the isolation rearing condition, environmental enrichment has opposite and regionally specific effects on DAT function under control conditions and in response to acute nicotine, with nicotine enhancing DAT function in NAc shell and reducing DAT function in NAc core. These differences in DA clearance between EC and IC rats may play a role in the observed enrichment-induced increases in locomotor activity in response to acute nicotine as reported previously (Coolon and Cain, 2009).

In this study, *in vivo* voltammetry was used to evaluate the dynamic activity of DAT through assessing extracellular DA clearance (Cass and Gerhardt, 1995; Cass et al., 1993a; Middleton et al., 2004; Zhu et al., 2007). Notably, enrichment-induced changes were reflected mostly in changes in CL_{DA} , a pharmacokinetic parameter providing a measure of the efficiency of DAT to remove DA from the extracellular space. In contrast, T_{80} was not altered by enrichment. T_{80} most closely approximates the pharmacokinetic parameter half-life ($t_{1/2}$), that is, the time required for DA concentration to decline by 50%. T_{80} and $t_{1/2}$ are parameters dependent on both clearance and volume into which DA distributes ($t_{1/2} = 0.693 \times V/CL_{DA}$). The larger the volume into which DA distributes following ejection, the longer the $t_{1/2}$ and T_{80} . Effective volume into which DA distributes increases as the distance from the probe to the DA ejection site increases. Consequently, $t_{1/2}$ and T_{80} are affected by relative placement of the ejection pipette and detector electrode. The $t_{1/2}$ of the DA to be eliminated is also not the most direct measure of DAT efficiency, because of its dual dependence on two processes, elimination and distribution.

Furthermore, DA concentration measured as a voltammetric response is directly proportional to the DA concentration at the electrode; however, this DA concentration is influenced by the volume into which the DA distributes. When the amount of DA ejected is not saturating DAT (under linear first-order conditions), clearance (CL_{DA}) is a constant value, and variations in amount of DA ejected are reflected in AUC, but not in the value for CL_{DA} . Using the current model, the concentration of DA ejected has been shown previously to not be saturating (Zahniser et al., 1999). The changes in CL_{DA} in response to repeated DA application are also not due to an impediment of DA diffusion to the electrode, because this would be reflected as an increase in T_{80} , and this was not the case. A_{max} can also be influenced by ejection processes and is not considered the best pharmacokinetic parameter to define the differences in DAT function. A_{max} represents a single instantaneous concentration at a fixed time point and not a direct measure of DAT function. The amount of DA ejected can vary from experiment to experiment, which can affect AUC and A_{max} ; however, under linear conditions, the amount of DA ejected will not alter clearance as CL_{DA} is independent of concentration, dose, and volume of DA ejected. Rather, CL_{DA} represents a more accurate estimate of DA clearance, that is, transport of DA via DAT.

Previous studies have demonstrated that the density of DAT in the shell is different from that in the core (David et al., 1998; Jones et al., 1996). Using autoradiography, the density of specific [3H]mazindol binding in the core was found to be twofold greater than that in the shell; and using *in vivo* voltammetry, the core was shown to exhibit about threefold faster

DA clearance than the shell (Jones et al., 1996). In the current study, a trend for a difference in DA clearance (CL_{DA}) between accumbens core and shell was found (Table I), in which CL_{DA} values from both EC and IC groups were greater in the core than that in the shell. Thus, these findings suggest that functional expression of DA transporters in subregions of accumbens is consistent with transporter density measured using radioligand binding.

Under control conditions, following saline injection and across the 55-min session of repeated DA application, CL_{DA} in shell was slower (36%) in EC rats than in IC rats, whereas CL_{DA} in core was faster (40%) in EC rats when compared with IC rats. The current observations of altered responsiveness of NAc subregions to differential rearing are consistent with our previous findings, that is, slower CL_{DA} in mPFC across the 55-min session in control EC when compared with control IC rats (Zhu et al., 2007). Thus, these results suggest that under control conditions, enrichment differentially regulates DAT function in subregions of the NAc. It should be noted that CL_{DA} was measured throughout each 55-min session as a response to repeated local application of DA. Locally applied substrates have been shown to decrease DAT function in cell systems and various brain regions expressing DAT (Gulley and Zahniser, 2003; Gulley et al., 2002; Middleton et al., 2004; Saunders et al., 2000). However, the potential influence of repeated DA application on the enrichment-induced differential response observed herein is mitigated by the fact that both EC and IC control groups were treated identically, that is, DA was applied repeatedly in both NAc shell and core across the 55-min session under each of the respective rearing conditions. Thus, repeated DA application does not explain the differential DAT function in NAc shell and core between EC rats and IC rats.

Several studies have reported that applications of DA at 5-min intervals into brain result in relatively stable DA signals across a 60-min recording period (Cass et al., 1993b; Gulley et al., 2002; Middleton et al., 2004; Zahniser et al., 1999). However, in the current study, CL_{DA} changed across time in the saline control animals. One explanation for the apparent discrepancy between the current study and the previous findings could be the treatment of the animals. In the current study, male Sprague-Dawley rats were housed in different rearing conditions from 21 days of age until experiments were performed at 53–70 days of age, whereas standard-housed adult male Sprague-Dawley rats were used in the previous studies. Rearing condition has been reported to alter inherent DA levels, DAT function, and basal locomotor activity (Stairs and Bardo, 2009). Alterations in CL_{DA} across time in the saline control animals were not due to deterioration of carbon fiber electrodes, because the electrodes used were calibrated before and after the experiment, and importantly, no significant decline in sensitivity or selectivity was found. Thus, changes in DA clearance across time in the shell and core in the saline control groups reflect the impact of environmental enrichment, which differentially regulates DAT function in the subregions of NAc.

DAT trafficking is thought to be a major determinant of DAT activity. Our previous studies show that enrichment diminishes DA clearance in mPFC (Zhu et al., 2007), which is the result of a decrease in DAT cell surface expression without a change in total DAT density (Zhu et al., 2005). One possible explanation for the current findings is that enrichment resulted in differential levels of DAT cell surface expression, which in turn was responsible for the differential CL_{DA} in shell and core. For example, when compared with IC rats, the slower DA clearance in NAc shell in EC rats may be the outcome of a decrease in cell surface DAT expression. Thus, enrichment-induced modulation of DAT cellular localization may mediate the observed enrichment-induced differential DA clearance in NAc shell and core.

With respect to the effect of acute nicotine, CL_{DA} in NAc shell in EC rats was increased (161% of EC saline control), whereas nicotine had no effect on CL_{DA} in the NAc shell in IC rats. In contrast, acute nicotine increased CL_{DA} (188% of IC saline control) in NAc core in IC rats, whereas nicotine had no effect on CL_{DA} in NAc core in EC rats. Nicotine gradually enhanced DA clearance across the 55-min session when compared with the respective saline controls. Again, it should be noted that following both acute nicotine and saline administration, CL_{DA} was measured as a response to repeated local application of DA into the respective accumbal subregions. Although repeated application of DA may have had an effect on transporter function on its own, all of the groups underwent this procedure, mitigating this as an explanation for the effects of nicotine, which were observed.

Our previous study in standard-housed rats demonstrated that nicotine dose dependently increased DA clearance, which was completely blocked by pretreatment with mecamylamine, a nonselective nicotinic receptor antagonist, indicating a nicotinic receptor-dependent mechanism (Middleton et al., 2004). As acute nicotine has been shown to increase extracellular DA levels in NAc shell, but not in the core (Nisell et al., 1997), the effect of nicotine to increase CL_{DA} in the shell of EC rats may represent a compensatory response to the nicotine-induced increase in extracellular DA in NAc shell of EC rats. Nicotine induced an increase in CL_{DA} in the shell in EC rats. In addition, nicotine also increased CL_{DA} in the core of IC rats. The lack of effect of nicotine on CL_{DA} in the shell of IC rats and the core of EC rats cannot be explained by a reduction in sensitivity to the effect of nicotine on modulation of DAT function. Therefore, the differential regulation of DA clearance in subregions of NAc in response to nicotine is more likely due to enrichment-induced alterations of DAT function.

Enrichment-induced differences in CL_{DA} in response to acute nicotine may underlie the acute effects of nicotine on locomotor activity. At a dose similar to that used in the current study, nicotine produced hyperactivity in EC rats, but had no effect on activity in IC rats when compared with their respective saline controls (Coolon and Cain, 2009). Based on the currently observed effects of nicotine on DAT function, a greater extracellular DA concentration would be expected in NAc shell in IC rats and in NAc core in EC rats. Thus, the effect of nicotine in NAc core to increase extracellular DA may be associated with the enrichment-induced increase in locomotor activity in response to acute nicotine. However, a limitation of the current study is that only a single dose of nicotine was investigated.

Enrichment-induced alterations in DAT function may also underlie the finding that EC rats have reduced psychostimulant self-administration and are more readily extinguished when compared with IC rats (Stairs and Bardo, 2009). The nicotine-induced increase in DAT function in NAc shell would be expected to reduce extracellular DA concentrations as well as the reinforcing effects of nicotine in EC relative to IC rats. Furthermore, nicotine-induced increases in extracellular DA within NAc core are associated with aversive effects of nicotine (Sellings et al., 2008). Therefore, the lack of effect of nicotine on DAT function in NAc core in EC rats may result in greater extracellular DA concentrations and increased aversive effects of nicotine in comparison with IC rats, thereby leading to a reduction in the reinforcing and psychostimulant effects of nicotine. Thus, enrichment effects on DAT function in NAc shell and core in EC rats may serve to protect against nicotine abuse liability.

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Fig. 1.

Neuroanatomical localization of electrodes. Open triangles represent electrodes in NAc shell ($n = 12$ /rearing condition) and open circles represent electrodes in NAc core ($n = 12$ /rearing condition) for in vivo voltammetry experiments in EC (left) and IC (right) rats. Brain slices were sampled at four anterior–posterior levels. Numbers are distances (in millimeters) anterior to bregma according to Paxinos and Watson (1986). Abbreviations: Ca, anterior commissure; cc, corpus callosum; CPU, nucleus caudatus putamen; Sh, NAc shell; Co, NAc core.

Fig. 2.

Effect of acute nicotine on A_{max} (Panels A and D), T_{80} (Panels B and E), and CL_{DA} (Panels C and F) in NAc shell in EC and IC rats. Arrow indicates administration of nicotine (0.4 mg/kg, s.c.) or saline. Panels A–C show the time course (5-min recording intervals) of three parameters (A_{max} , T_{80} , and CL_{DA}) expressed as the mean $\pm$ SEM as a percentage of the respective baseline values following nicotine (EC-Nic and IC-Nic) or saline (EC-Sal and IC-Sal) injection. Mean absolute baseline values for each parameter are provided in Table I. Panels D–F show the mean $\pm$ SEM values as a percentage of baseline for the three parameters across the 55-min recording period after nicotine or saline injection ($n = 5-7$ rats per group); *different from IC-Sal, $P < 0.05$; #different from EC-Nic, $P < 0.05$.

Fig. 3.

Effect of nicotine administration on A_{max} (Panels A and D), T_{80} (Panels B and E), and CL_{DA} (Panels C and F) in NAc core in EC and IC rats. The arrow indicates administration of nicotine (0.4 mg/kg, s.c.) or saline. Panels A–C show the time course (5-min recording intervals) of A_{max} , T_{80} , and CL_{DA} expressed as the mean $\pm$ SEM as a percentage of the respective baseline values in nicotine-treated EC and IC groups (EC-Nic and IC-Nic) and EC and IC rats injected with saline (EC-Sal and IC-Sal). Mean absolute baseline values for each parameter are provided in Table I. Panels D–F show the mean $\pm$ SEM as a percentage of baseline for A_{max} , T_{80} , and CL_{DA} across the 55-min recording period after nicotine or saline injection ($n = 5-7$ rats per group); *different from IC-Sal, $P < 0.05$; #different from IC-Nic, $P < 0.05$.

TABLE I

Baseline DA clearance parameters in NAc shell and core of EC and IC rats

		DA applied (pmol)	DA applied (nl)	A _{max} (μM)	T ₈₀ (s)	CL _{DA} (10 ⁻⁸ L/s)
Shell	EC (n = 12)	12 ± 1.0	60 ± 6.2	2.0 ± 0.2	27 ± 2.8	7.2 ± 1.2
	IC (n = 12)	13 ± 1.9	65 ± 5.5	2.5 ± 0.4	29 ± 5.6	6.6 ± 0.8
Core	EC (n = 12)	13 ± 1.1	65 ± 6.2	2.7 ± 0.3	43 ± 6.8	9.6 ± 1.9
	IC (n = 12)	14 ± 2.2	70 ± 5.7	3.1 ± 0.4	47 ± 9.4	7.1 ± 2.4

Exogenous DA (200 μM) was pressure ejected at 5-min intervals. Data represent the reproducible baseline DA signals recorded prior to subcutaneous injection of nicotine or saline and are expressed as mean ± SEM absolute values for each parameter (n for each group is shown in parentheses).