Published in final edited form as:

Addict Biol. 2014 March ; 19(2): 145–155. doi:10.1111/j.1369-1600.2012.00456.x.

Methylphenidate and Cocaine Self-Administration Produce Distinct Dopamine Terminal Alterations

Erin S. Calipari, Mark J. Ferris, James R. Melchior, Kristel Bermejo¹, Ali Salahpour¹, David C. S. Roberts, and Sara R. Jones

Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston Salem, North Carolina 27157

¹Department of Pharmacology, University of Toronto, Toronto, Canada

Abstract

Methylphenidate (MPH) is a commonly abused psychostimulant prescribed for the treatment of attention deficit hyperactivity disorder. MPH has a mechanism of action similar to cocaine (COC) and is commonly characterized as a dopamine transporter (DAT) blocker. While there has been extensive work aimed at understanding dopamine (DA) nerve terminal changes following COC self-administration, very little is known about the effects of MPH self-administration on the DA system. We used fast scan cyclic voltammetry in nucleus accumbens core slices from animals with a five-day self-administration history of 40 injections/day of either MPH (0.56 mg/kg) or COC (1.5 mg/kg) to explore alterations in baseline DA release and uptake kinetics as well as alterations in the interaction of each compound with the DAT. Although MPH and COC have similar behavioral effects, the consequences of self-administration on DA system parameters were found to be divergent. We show that COC self-administration reduced DAT levels and maximal rates of DA uptake, as well as reducing electrically stimulated release, suggesting decreased DA terminal function. In contrast, MPH self-administration increased DAT levels, DA uptake rates, and DA release, suggesting enhanced terminal function, which was supported by findings of increased metabolite/DA tissue content ratios. Tyrosine hydroxylase mRNA, protein and phosphorylation levels were also assessed in both groups. Additionally, COC self-administration reduced COC-induced DAT inhibition, while MPH self-administration increased MPH-induced DAT inhibition, suggesting opposite pharmacodynamic effects of these two drugs. These findings suggest that the factors governing DA system adaptations are more complicated than simple DA uptake blockade.

Keywords

Cocaine; Dopamine; Dopamine Transporter; Methylphenidate; Nucleus Accumbens; Self-administration

Corresponding author: Sara R. Jones, Ph.D. Department of Physiology and Pharmacology Wake Forest School of Medicine Winston Salem, NC 27157 Phone: 336-716-8533 Fax: 336-716-8501 srjones@wakehealth.edu.

Author contributions ESC, MJF, SRJ, and DCSR were responsible for the study concept and design. JRM and ESC contributed to the self-administration experiments. ESC, MJF, and JRM contributed to the acquisition of voltammetric data. ESC and KB performed the western blot analysis. KB performed all polymerase chain reaction experiments. ESC, MJF, and AS assisted with data analysis and interpretation of findings. ESC drafted the manuscript. MJF, AS, SRJ, and DCSR provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved the final version for publication.

Introduction

Methylphenidate (MPH), the active ingredient in medications (Ritalin®, Methylin®, Focalin®) used in the treatment of attention deficit/hyperactivity disorder (ADHD) and narcolepsy, is prone to diversion for non-medical use. MPH abuse in non-ADHD individuals has become increasingly prevalent through oral, intranasal or intravenous (i.v.) routes of administration (Teter et al., 2006; Sherman et al, 1987). The drug is used for cognitive enhancement and to “get high”, causing effects indistinguishable from cocaine (COC) when taken i.v. or intranasally (Morton and Stockton, 2000; Sherman et al, 1987; Teter et al., 2006; Rush and Baker, 2001). In 2008, 4.8 million people reported abusing MPH in the past year, compared to 5.3 million for COC, suggesting that both of these drugs pose a significant threat to public health (SAMHSA, National Survey on Drug Use and Health, 2008).

From a behavioral standpoint, MPH and COC share a very similar preclinical profile. In rats, the discriminative stimulus effects of MPH and COC are nearly identical as seen by MPH’s ability to fully substitute for COC (Li et al., 2006). Several i.v. self-administration studies in rats have demonstrated that COC and MPH have similar reinforcing effects. Both compounds maintain high rates of operant responding, and extended access to MPH results in an escalation of intake similar to COC self-administration (Ahmed & Koob, 1998; Marusich et al, 2010). With regard to reinforcing efficacy, doses of MPH or COC at the peak of their respective progressive ratio dose response curves engender similar break-points, indicating that the motivation to take each drug is comparable (Marusich et al., 2010; Roberts et al., 2007; Calipari et al. unpublished).

Like many psychostimulants, COC and MPH exert their rewarding and reinforcing properties primarily via their ability to bind to dopamine transporters (DATs) and inhibit dopamine (DA) uptake, elevating DA in the nucleus accumbens (DiChiara and Imperato, 1988; Woods and Meyer, 1991; Gerasimov et al., 2000). For example, a triple mutation in the second transmembrane domain of the DAT, which reduces affinity for MPH and COC, also abolishes conditioned place preference (Chen et al, 2005, 2006; Tilley et al, 2008a, b), highlighting the role of DAT inhibition in their rewarding properties. Acute inhibition of DA uptake by psychostimulants consistently results in enhanced DA signaling, but repeated or prolonged exposure to such drugs can lead to compensatory changes in presynaptic DA terminal function as well as alterations in the pharmacological actions of the drugs themselves (Biederman and Spender, 2002; Bouffard, 2003; Cadet et al., 2009).

The neurobiological effects of MPH self-administration on the DA system remain to be elucidated; however, COC effects on the DA system have been well characterized and we hypothesized that MPH, a DAT blocker with a similar mechanism of action, would produce similar changes. In previous studies, COC self-administration resulted in lower basal levels of DA, decreased stimulated DA release and modestly decreased maximal rate of uptake (V_{max}) (Mateo et al., 2005; Ferris et al., 2011). In addition to baseline DA system alterations, COC self-administration also resulted in a decreased potency of COC in the nucleus accumbens, producing a marked tolerance to its DA-elevating and DAT-inhibiting effects (Hurd et al., 1989; Mateo et al., 2005; Ferris et al., 2011). These data, taken together, suggest an overall hypodopaminergic and less responsive DA state following COC self-administration.

Because MPH is increasingly becoming a drug of abuse (Marusich et al, 2009, 2010; Burton et al., 2010; Botly et al, 2008), but the neurochemical alterations following contingent administration have yet to be characterized, we chose to compare the behavioral and neurochemical effects of MPH self-administration with self-administration of an extensively

studied DA uptake blocker, COC. The purpose of this study was to systematically evaluate the neurochemical consequences of MPH and COC self-administration by evaluating baseline presynaptic DA system parameters such as release and uptake rate, as well as the ability of COC and MPH to inhibit the reuptake of endogenous DA. Surprisingly, MPH produced a distinctly different pattern of results from COC, suggesting that MPH does not behave as a prototypical dopamine uptake blocker. The present results suggest that the factors governing DA system adaptations are more complicated than simple DA uptake blockade.

Materials and Methods

Animals

Male, Sprague-Dawley rats (375–400 g; Harlan Laboratories, Frederick, MD) were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine.

Self-Administration

Rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), implanted with chronic indwelling jugular catheters, and trained for i.v. self-administration as previously described (Roberts and Goeders 1989). Following surgery, animals were singly housed, and all self-administration sessions took place in the home cage. Each animal was maintained on a reverse light cycle (3:00 am lights off; 3:00 pm lights on), and all self-administration procedures occurred during the active/dark cycle. Sessions were six hours in length and were terminated at the end of the six hours or after 40 injections of drug. Animals self-administered either COC (1.5mg/kg/inj over 4 sec) or MPH (0.56 mg/kg/inj over 4 sec) on a fixed-ratio 1 schedule of administration. Concurrent with the start of each injection, the lever retracted and a stimulus light was activated for 20 seconds to signal a time-out period. Under these conditions, animals acquired a stable pattern of COC or MPH self-administration within 1 to 5 days. For self-administering animals, acquisition (Day 1) was counted when the animal reached 35 or more responses. There was no significant difference between groups in the injections received before acquisition criteria was met. Following acquisition, the animals were given access to 40 injections per day for a period of 5 consecutive days for both the COC and MPH self-administration experiments.

Fixed ratio self-administration allows animals to titrate drug intake by spacing injections to maintain a preferred brain concentration of drug (Norman & Tsibulski, 2006). We compared doses of MPH and COC that were behaviorally equivalent. Due to the significant difference in half-life of MPH, which is approximately two times longer than COC (Huff and Davies, 2002; Weikop et al, 2004), we compared 0.56 mg/kg MPH and 1.5 mg/kg COC, doses which produce maximal responding on a progressive ratio schedule of reinforcement (Richardson and Roberts 1996; Marusich et al., 2010). To confirm behavioral equivalence we also measured the time to complete session, inter-dose interval and rate of intake.

Control animals were naïve rats housed under the same reverse light-dark light cycle for at least one week prior to neurochemical analysis.

Fast Scan Cyclic Voltammetry in brain slices

Animals were sacrificed 24 hours after commencement of the last session, during the dark phase of the light cycle. 400 μ m thick coronal brain sections containing the nucleus accumbens core were cut using a vibrating tissue slicer. A carbon fiber electrode was placed

approximately 75 μm below the surface of the slice in close proximity to a bipolar stimulating electrode in the nucleus accumbens core. DA release was evoked by a single electrical pulse (300 μA , 4 msec, monophasic) applied every 5 minutes. Extracellular DA was recorded using fast-scan cyclic voltammetry (FSCV) by applying a triangular waveform (-0.4 to $+1.2$ to -0.4 V vs. silver/silver chloride, 400 V/sec) to the electrode every 100 msec. Once the extracellular DA response was stable for three consecutive stimulations, individual brain slices were treated with either COC or MPH (0.3–30 μM), with increasing concentrations cumulatively added after stable responses were recorded, approximately every 45 minutes.

Western Blot Hybridization

Tissue sections were dissected 24 hours after commencement of the last self-administration session, and homogenized in ice-cold lysis buffer (10mM Tris, 2% SDS, 1mM EDTA) and spun in a centrifuge at 13,000g for 20 minutes to remove cellular debris. Bicinchoninic acid (BCA) protein assays (Thermo Scientific, Rockford, IL) were used to determine the protein content of each sample. Proteins were separated on 10% reducing SDS-PAGE gels (Invitrogen, Carlsbad, CA) and transferred to hydrophobic polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). For estimation of the molecular weight of the protein bands, Precision Plus protein standards (10–250 kDa, Bio-Rad) were run in parallel with the samples on gels. Nonspecific binding was blocked by incubation with blocking buffer (5% non-fat milk in tris-buffered saline with tween-20) for 30 minutes before incubation with primary antibodies: rabbit anti-DAT antibody (Millipore, Temecula, CA; 1:5,000 dilution), rabbit anti-phosphorylated tyrosine hydroxylase (TH) Ser19, Ser31, Ser40 (PhosphoSolutions, Aurora, CO; 1:2000) and mouse anti-TH (Millipore; 1:5000) at 4°C overnight. Membranes were then incubated with either secondary goat anti-rabbit HRP-conjugated antibody (Millipore; 1:10,000, Rockland, Gilbertsville, PA; 1:5000) or donkey anti-mouse antibody (Invitrogen; 1:5000). Immunoreactive products were visualized by either chemiluminescence (Pico chemiluminescent substrate, Thermo Scientific) or by infrared detection using the Odyssey LI-COR Infrared Imaging System and quantified using Image J (NIH). Protein loading was visualized by incubation with either a monoclonal antibody to β -actin (Abcam, Cambridge, MA; 1:10,000) or mouse anti-GAPDH (Sigma; 1:6000).

Tissue Content

The striatum was dissected, snap-frozen, and samples (10–30 mg/sample wet weight) were homogenized in 250 μL of 0.1 M HClO_4 and analyzed for protein concentration by the BCA method (Thermo Scientific). Extracts were centrifuged and the supernatants removed and analyzed for DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using high performance liquid chromatography (HPLC) coupled to electrochemical detection at +220 mV (ESA Inc., Chelmsford, MA) and separated on a Luna 100 $\times$ 3.0 mm C_{18} 3 μm HPLC column (Phenomenex, Torrance, CA). The mobile phase consisted of 50 mM citric acid, 90 mM sodium dihydrogen phosphate, 1.7–2.0 mM 1-octanesulfonic acid, 50 μM ethylenediaminetetracetic acid, 10–12 % acetonitrile and 0.3 % triethylamine in a volume of 1 L (pH 3.0). Analytes were quantified using PowerChrom software (eDAQ Inc, Colorado Spring, CO) and a calibration curve.

Quantitative Polymerase Chain Reaction (PCR)

To obtain RNA from tissues, snap-frozen ventral tegmental area (VTA) tissue sections were homogenized in cold TRI-Reagent (BioShop Canada Inc) as described previously (Hu et al., 2009). Optical density readings at 260/280 nm were used to determine the quality and concentration of the resuspended RNA. 1 μg of RNA was used for reverse-transcriptase PCR (RT-PCR) to obtain cDNA using SuperScript III Reverse Transcriptase (Invitrogen)

following the manufacturer's instructions. Relative quantification of gene targets was obtained using the GoTaq® qPCR Master Mix (Promega, Fitchburg, Wisconsin) on the Applied Biosystems 7500 Real-Time PCR System. Phosphoglycerate kinase 1 (PGK1) was used as the reference gene (forward primer: ACCAAAGGATCAAGGCTGCTGTC; reverse primer: GACGGCCCAGGTGGCTCATA). Dopamine transporter (DAT) (forward primer: CCTGGTTCTACGGCGTCCAGC; reverse primer: GCCGCCAGTACAGGTTGGGT) and TH (forward primer: TTGAAGGAGCGGACTGGCTTCCA; reverse primer: TGGCCAGAAAATCACGGGCGG) were used as the target genes. Target genes were relatively quantified using the $\Delta\Delta C_t$ method, and are represented as % control normalized to PGK-1.

Data Analysis

DA current was converted to concentration by calibration with 3 μ M DA at the end of each experiment. For all analyses of FSCV data, DEMON voltammetry software was used (Yorgason et al., 2011). To evaluate the effects of MPH and COC self-administration on baseline DA system kinetics, evoked levels of DA were modeled using Michaelis-Menten kinetics, as a balance between release and uptake (Yorgason et al., 2011). For COC and MPH dose-response curves, apparent K_m , a measure of apparent affinity of DA for the DAT, was used to determine changes in ability of the psychostimulants to inhibit DA uptake in the nucleus accumbens core relative to baseline.

Statistics

Graph Pad Prism (version 4, La Jolla, CA) was used to statistically analyze data sets and create graphs. Baseline voltammetry, western blot hybridization, and PCR data were compared across groups using a two-tailed Student's t-test. Data obtained after perfusion of COC or MPH was subjected to a two-way analysis of variance with experimental group and concentration of COC or MPH as the factors. When significant interactions or main effects were obtained ($p < .05$), differences between groups were tested using Bonferroni post hoc tests. Behavioral data were subjected to a two-way analysis of variance with experimental group and hours to complete self-administration session as the factors.

Results

COC and MPH intake increases over time

Each self-administration session was six hours in length and consisted of 40 injections per session. Time to complete 40 injections of COC ($n = 8$) significantly decreased over the five sessions ($F(4,7) = 8.858$, $p < 0.01$,) (Fig. 1A, Top). In addition, the inter-infusion interval was also significantly decreased across sessions, demonstrating an escalation in rate of intake over sessions ($F(4,7) = 8.180$, $p < 0.01$).

MPH self-administration resulted in nearly identical changes in behavior. MPH self-administration ($n = 11$) engendered an increase in rate of lever pressing over self-administration sessions ($F(4,10) = 7.956$, $p < 0.01$) (Fig. 1A, Bottom). The same trend was observed with inter-infusion interval, demonstrating that the rate of intake also escalates across MPH self-administration sessions ($F(4,10) = 7.041$, $p < 0.01$).

Thus, the effects of MPH ($n = 11$) and COC ($n = 8$) self-administration on behavioral responding for drug were not significantly different as the two compounds produced the same inter-dose intervals and the same escalation (decreases in time to complete sessions) over days (Fig. 1B).

Opposite effect of MPH and COC self-administration on baseline DA system kinetics

Baseline DA system kinetics were measured using FSCV and DAT levels were determined using western blot hybridization. COC self-administration ($n=11$) engendered a decrease in electrically stimulated DA release as compared to naïve control animals ($n=22$) ($t_{31} = 2.348$, $p < 0.05$, Fig. 2A, Center; Fig. 2B). Also, after COC self-administration there was a significant decrease in maximal rate of DA uptake ($t_{30} = 2.719$, $p < 0.05$) (Fig. 2A, Center; Fig. 2C). This decrease in maximal rate of uptake was accompanied by a decrease in DAT density in the COC group ($n=5$) compared to controls ($n=3$), as measured by western blot hybridization ($t_6 = 2.182$, $p < 0.05$) (Fig. 3A, Center; Fig. 3B). Relative expression levels of DAT mRNA as measured by quantitative PCR in the VTA ($n = 12$) were not significantly different from controls ($n = 18$).

Conversely, MPH self-administration resulted in increases in all DA system measurements. Stimulated DA release in the MPH group ($n = 11$) was increased compared to controls ($n = 22$) ($t_{31} = 2.076$, $p < 0.05$) (Fig. 2A, Right; Fig. 2B). Maximal rate of DA uptake was also significantly increased in MPH ($n = 7$) versus control animals ($n = 22$) ($t_{24} = 2.719$, $p < 0.05$) (Fig. 2A, Right; Fig. 2B). Total DAT levels were significantly increased following MPH self-administration ($n = 4$) compared to controls ($n = 5$) ($t_7 = 3.532$, $p < 0.01$) (Fig. 3A, Right; Fig. 3B). In addition, DAT mRNA levels were assessed in the VTA. MPH group levels ($n = 18$) were not significantly different than control animals ($n = 18$), suggesting that the increases in protein expression are not due to increased synthesis of new protein.

Differential Effects of COC and MPH SA on Tissue Content of DA and Metabolites

Post mortem tissue content of DA and its metabolites DOPAC and HVA were assessed in the striatum following COC and MPH SA (Fig. 4A). COC SA ($n = 5$) had no effect on DA or any of its metabolites as compared to controls ($n = 6$) (Fig. 4A). MPH SA ($n = 9$) resulted in a significant decrease in DA tissue content as compared to control animals ($n = 6$) ($t_{13} = 3.598$, $p < 0.01$) (Fig. 4A). While DA tissue content was significantly decreased, there was no significant difference between control and MPH SA in the metabolites HVA or DOPAC (Fig. 4A).

Metabolite to monoamine ratios were assessed in the striatum to determine changes in functional activity of the DA neurons in this region (Fig. 4B). COC SA ($n = 5$) did not produce significant differences in either DOPAC/DA or HVA/DA ratios as compared to controls ($n = 6$) (Fig. 4B). MPH SA ($n = 9$) resulted in a significant increase in the DOPAC/DA ratio as compared to control animals ($n = 6$) ($t_{13} = 2.852$, $p < 0.01$), and although not significant, there was a trend towards an increased HVA/DA ratio ($t_{13} = 2.144$, $p = 0.0515$) (Fig. 4B).

No effect of MPH or COC SA on Tyrosine Hydroxylase Protein, mRNA, or Phosphorylation Levels

To determine if changes in stimulated DA release could be due to differences in TH, mRNA levels of TH were assessed in the VTA of COC and MPH groups using quantitative PCR. COC SA ($n = 18$) resulted in a significant increase in TH mRNA levels as compared to controls ($n = 12$) ($t_{28} = 5.704$, $p < 0.0001$) (Supp. Fig. 1A). MPH SA ($n=18$) also significantly increased levels of TH mRNA in the VTA versus controls ($n = 18$) ($t_{34} = 2.480$, $p < 0.05$) (Supp. Fig. 1A), although the levels were higher in COC ($n = 12$) than MPH groups ($n = 18$) ($t_{28} = 4.475$, $p < 0.0001$) (Supp. Fig. 1A). However, this difference was not reflected in protein levels (see below; Supp. Fig. 1 B, C, D), highlighting the well known complex translational regulation of this protein (Tank et al., 2008).

TH protein levels as well as two phosphorylation sites were quantified via western blot hybridization (Supp. Fig. 1B). COC SA (n = 5) did not change total TH levels or phosphorylation at either serine 40 (THp Ser40) or 19 (THp Ser19) in the striatum (Supp Fig. 1B, left; 1C) or VTA (Supp. Fig. 1B right; 1D) as compared to controls (n = 6). Serine 31 was not quantifiable in any samples. Similarly, MPH SA (n = 7) also resulted in no changes in TH, THp Ser40, or THp Ser19, in either the striatum (Supp. Fig. 1B, left; 1C) or the VTA (Supp. Fig. 1B; 1D) as compared to controls (n = 6).

MPH self-administration resulted in increased MPH potency, while COC potency remained unchanged

Cumulative dose-response curves of COC and MPH were collected to determine changes in the compounds' ability to inhibit DA uptake following MPH self-administration. The Michaelis-Menten measure of DA affinity for the transporter, apparent K_m , was used to measure changes in the ability of a drug to inhibit DA uptake relative to baseline. Following MPH self-administration (n = 4), there was a significant main effect of MPH treatment as compared to controls (n = 4) ($F(4,1) = 23.74$, $p < 0.001$), with significant increases in the maximal apparent K_m of MPH self-administration groups at $30\mu\text{M}$ ($p < 0.001$) (Fig. 5D). MPH self-administration resulted in a leftward shift in the dose-response curve, indicating that MPH potency is increased following MPH self-administration.

Cumulative COC dose response curves were performed to determine if there was a shift in the potency of COC following MPH self-administration. There was no change in the dose response curve for COC (n = 3) as compared to controls (n = 3), indicating that COC's ability to inhibit DA uptake in the nucleus accumbens core remained unchanged after MPH self-administration (Fig. 5E).

COC self-administration resulted in decreased COC potency, while MPH potency remained unchanged

COC self-administration resulted in an overall group effect ($F(4,1) = 13.85$, $p < 0.01$) and a rightward shift in the dose response curve for COC (n = 3) as compared to controls (n = 4), signifying a significant decrease in COC-induced DA uptake inhibition (Fig. 6E). Bonferroni analysis demonstrated a significant effect at $30\mu\text{M}$ ($p < 0.001$).

Conversely, following 5 days of COC self-administration, there was no significant change in MPH's DA uptake-inhibiting effects (Fig. 6D), indicating that MPH (n = 5) potency remained unaltered as compared to controls (n = 3) following repeated administration of COC.

Discussion

MPH and COC are both DAT inhibitors and produce similar acute DA-elevating effects. Despite the close similarities in self-administration behavior, the current study demonstrates that MPH and COC intake can produce strikingly different effects on DA system kinetics and drug potency. Consistent with previous studies, 5 days of high-dose COC self-administration resulted in decreased DAT density, maximal rate of uptake (V_{max}), and stimulated DA release (Mateo et al., 2005; Ferris et al., 2011). In contrast, MPH self-administration increased DAT density, V_{max} , and stimulated DA release. In addition to robust differences in baseline DA nerve-terminal kinetics, COC self-administration resulted in a decreased potency of COC to inhibit DA uptake, with no change in potency of MPH. Conversely, MPH self-administration resulted in increased potency of MPH and no change in COC potency. Overall, COC self-administration seems to result in sub-sensitive DA terminals while MPH self-administration produces super-sensitive terminals. These

profound differences in the neurobiological effects of two DA uptake inhibitors provide evidence that the DA-elevating effects of psychostimulants are not solely, or even primarily, responsible for altered DA system function after repeated exposure.

The goal of this study was to compare DA system alterations that occurred following 5 days of COC or MPH self-administration. One challenge was to select comparable doses of these two drugs with different time courses and binding affinities. The doses we chose for MPH (0.56 mg/kg) and COC (1.5 mg/kg) are at the peak of the respective PR dose response curves, indicating that these are the maximally reinforcing dose of each drug. The selected doses of these two drugs produced similar inter-injection intervals within a session and produced escalation in rates of intake across sessions, indicating that these doses are behaviorally equivalent. Therefore, although there are differences in half-life and potency, we were able to equate the behavioral responses to these drugs in order to accurately compare the neurochemical consequences of MPH and COC self-administration.

Despite almost identical behavioral profiles, baseline changes in maximal DA uptake rate ($V_{\max}$) and stimulated DA release following MPH and COC self-administration were found to be opposite. COC self-administration resulted in a decreased $V_{\max}$ and decreased stimulated release, effects that have been observed previously with the same paradigm (Ferris et al., 2011). DAT density was found to be decreased following COC self-administration, suggesting that the observed decrease in uptake rate was due to reduced numbers of DA transporters. The alterations in DAT density following self-administration are most likely due to differences in protein processing as there were no observed changes in mRNA levels in COC or MPH self-administration groups. In the literature, there are reports of increased, decreased and unaltered DA uptake rates following COC self-administration (Ferris et al., 2011; Ramamoorthy et al., 2010; Mateo et al., 2005; Wilson and Kish, 1996), and clearly, different self-administration paradigms produce different neurobiological effects, emphasizing the need for equating doses as well as injection frequency, timing and duration. Self-administration of MPH resulted in an increase in $V_{\max}$ and DAT density as well as an increase in stimulated DA release in the nucleus accumbens core, demonstrating that two drugs which cause identical self-administration behavior are capable of causing opposite neurobiological adaptations.

To address the differences in stimulated DA release between COC and MPH groups, various markers of presynaptic function were evaluated. DA tissue content was found to be decreased in the MPH group, with corresponding increases in metabolite/DA ratios, indicating increased functional activity, consistent with the increased DA release in this group. No changes were found in the COC group. A number of factors could be responsible for the observed difference between COC and MPH, for example, MPH has been shown to redistribute vesicular DA pools (Volz et al., 2008) and may alter both release and tissue content directly. Also, MPH has a longer half-life of uptake inhibition than COC, which in combination with the increased release and ability to mobilize DA vesicles, could lead to a reduced ability to refill vesicular stores and explain the difference in tissue content between the two groups. These findings further emphasize the differential impact of these two psychostimulant blockers on the DA system.

We initially postulated that the MPH-induced DA release and tissue content changes might be explained by alterations in TH, the rate-limiting enzyme in DA synthesis, but no differences were found in protein or phosphorylation levels, indicating that TH is most likely not driving the changes. There were, however, increases in TH mRNA levels in both self-administration groups, indicating that either post-translational processing or protein degradation may be altered.

With regard to the interaction of stimulants with the DAT, previous work found reduced pharmacological effects of COC in the nucleus accumbens after high-dose COC self-administration (Hurd et al., 1989; Mateo et al., 2005; Ferris et al., 2011). In order to determine if the effects of COC self-administration were unique to cocaine or common to all psychostimulants, Ferris et al. (2011) determined that the effects of amphetamine (AMPH) on DA uptake in nucleus accumbens core slices remained unchanged following COC self-administration. The same study also showed that self-administration of AMPH had no effect on either COC or AMPH potency in slices, suggesting that COC self-administration produced COC-specific pharmacological changes. We hypothesized that AMPH did not alter the pharmacology of the DAT because it was recognized as a substrate/releaser, while COC, a pure uptake blocker, induced allosteric modifications resulting in altered pharmacology. Next, it was reasonable to test whether self-administration of a structurally dissimilar uptake inhibitor, MPH, would also produce altered DAT pharmacology. The current results demonstrate that MPH self-administration produces opposite effects from COC self-administration, with increased MPH potency on slices but no change in COC effects. Thus, it appears that DAT pharmacology is altered according to individual drug interactions with the DAT and not according to drug class, such as blocker or releaser.

While MPH and COC are both classified as uptake blockers, they have different chemical structures, which may account for the disparate neurochemical changes that occur following self-administration. MPH is structurally related to amphetamine, while COC is a tropane (Uhl et al., 2002; Volkow et al., 2002; Heal et al., 2009; Han & Gu 2006). MPH, although typically categorized as a DAT blocker, has unique properties that are distinct from both releasers and blockers. Studies using cell culture and striatal tissue preparations have shown that MPH exhibits binding properties of both blockers and releasers (Dar et al., 2005; Waymunt et al., 1999). MPH is not a DAT substrate, as it is not transported into cells (Sonders et al., 1997); however, some studies have shown that MPH has the ability to promote reverse-transport of DA through the DAT at very high doses (Sproson et al., 2001; Russell et al., 1998; Heal et al., 1996). It is clear that DAT inhibition-induced increases in extracellular DA levels do not explain the distinct adaptations produced by the two drugs studied here, and we hypothesize that drug interactions with specific regions of the DAT may explain the differences observed.

Changes in DAT density and $V_{\max}$ do not explain the potency changes found with MPH and COC. With regard to COC self-administration, in the present study we show decreases in DAT protein levels and $V_{\max}$ for DA uptake after COC self-administration. However, in previous studies with COC self-administration, $V_{\max}$ was either increased or unchanged, with the same reduction in potency for DA uptake inhibition in slices (Mateo et al., 2005; Ferris et al., 2011). With regard to MPH self-administration, to our knowledge this is the first study examining neurochemical changes and thus we cannot compare to other regimens; however, the Michaelis-Menten based kinetic model used to fit the DA release and uptake profiles takes into account any changes in baseline uptake rate using the pre-drug electrically stimulated DA overflow curves, and this allows the effects of uptake inhibition to be separated from changes in maximal uptake rate. This indicates that the maximal DA uptake rate ($V_{\max}$), which is correlated with DA transporter number, cannot account for the observed alterations in DA uptake inhibition by both COC and MPH. Thus, these effects are most likely due to allosteric alterations in the DA transporter, making it more or less sensitive to inhibition by specific drugs.

These DA system alterations induced by self-administration of COC or MPH have important implications for the subsequent abuse or co-abuse of other psychostimulants. MPH and COC are dependent upon their actions at the DAT for their reinforcing effects, and changes that occur to the DA system would be expected to affect the reinforcing potency or efficacy of

other psychostimulant drugs (Chen et al, 2005, 2006; Tilley et al, 2008 a, b). Because psychostimulants that are DA releasers rely on DATs to have their reverse-transport effects, the present results from MPH self-administering animals would predict greater behavioral potency. Consistent with this prediction, previous studies have already demonstrated that MPH self-administration, as well as increasing DAT levels by transgenic overexpression, results in increased AMPH-induced locomotor activity (Burton et al., 2010; Salahpour et al., 2008; Yang et al., 2003). In addition, epidemiological studies have provided evidence that individuals who abuse MPH are more likely to abuse other substances and to have problems with drug use (Barrett et al, 2005; Teter et al, 2006; Wilens et al, 2008). Given the widespread abuse of MPH and the potential risk for greater stimulant effects of other drugs, further studies of the consequences of MPH self-administration will be important.

Supplementary Material

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Acknowledgments

This work was funded by NIH grants R01DA024095, RO1DA03016 (SRJ), R01DA014030 (DCSR), T32DA007246 (MJF), Institutional NRSA T32DA007246 and Individual NRSA F31 DA031533 (ESC), and a Canadian Institutes of Health Research (CIHR) grant (AS).

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Figure 1.

Escalation in rate of cocaine (COC) and methylphenidate (MPH) self-administration. **(A)** Representative self-administration plots from individual animals; each tick mark represents an infusion that was obtained. Five sessions with a maximum of 40 injections of either COC (1.5 mg/kg/inj) or MPH (0.56 mg/kg/inj) resulted in significant increases in rate of intake in over sessions. **(B)** The increase in rate of intake of was not significantly different between COC (●) and MPH (●).

Figure 2.

Baseline dopamine (DA) system kinetics following methylphenidate (MPH) and cocaine (COC) self-administration. **(A)** Representative traces of electrically-evoked DA signals in nucleus accumbens core slices from control, MPH self-administration or COC self-administration animals. Traces show decreased maximal rate of uptake (rate of return to baseline) and DA release (peak height max) following COC self-administration and increased uptake and release following MPH self-administration. **Insets:** Background-subtracted cyclic voltammograms indicate signal is DA. **(B)** Grouped data showing that stimulated DA release is reduced after COC self-administration and increased after MPH self-administration. **(C)** Grouped data showing that the maximal rate of DA uptake was decreased after COC self-administration and increased after MPH self-administration. $*p < 0.05$ versus control animals.

Figure 3.

Western blot hybridization for the dopamine transporter (DAT) after cocaine (COC) or methylphenidate (MPH) self-administration. **(A)** Representative photographs of Western blots on tissue from the nucleus accumbens core region of control, COC self-administration, and MPH self-administration groups. **(B)** COC self-administration reduced DAT levels while MPH self-administration increased DAT levels in the nucleus accumbens. Protein expression levels were determined as the ratio of DAT to the level of β -actin and are reported as percent control values. * $p < 0.05$ versus control group.

Figure 4.

Tissue content of dopamine (DA) and metabolites (**A**) in the striatum of cocaine (COC) and methylphenidate (MPH) self-administration groups. The MPH group had significantly lower DA tissue content levels with no differences in metabolite levels. Tissue content levels of DA and metabolites were unaffected by COC self-administration. (**B**) Metabolite/monoamine ratios, a measure of functional activity, in MPH and COC groups as compared to controls. COC self-administration did not affect ratios, while MPH increased DOPAC/DA ratio. * $p < 0.05$ versus control group.

Figure 5.

Significantly greater dopamine (DA) uptake inhibition by methylphenidate (MPH), but not cocaine (COC), after MPH self-administration. The effect of COC and MPH applied to nucleus accumbens core slices on representative electrically-evoked (one pulse) DA signals is shown in (A) Pre-drug (B) 10 μ M MPH and (C) 10 μ M COC from control and MPH self-administering rats. Representative plots are shown as percent peak height; control and MPH self-administration animals are overlaid. MPH-induced DA uptake inhibition is present in naive animals but significantly increased in MPH self-administering rats as depicted by a longer time course of the signal to return to baseline; there is no effect of MPH self-administration on COC uptake inhibition. Group data showed that MPH inhibited DA uptake to a greater extent (D), and that COC uptake is unaffected (E) following 5 days of MPH self-administration (●) compared with control rats (●), with uptake inhibition measured as apparent K_m (***) $p < .001$, app., apparent).

Figure 6.

Significant reduction in dopamine (DA) uptake inhibition by cocaine (COC), but not methylphenidate (MPH), after COC self-administration. The effect of COC and MPH on representative electrically evoked (one pulse) DA signals in nucleus accumbens core slices is shown. (A) Pre-drug (B) 10 μ M MPH and (C) 10 μ M COC from control and cocaine self-administering rats. Representative plots are represented as percent peak height; control and COC self-administration animals are overlaid. COC-induced DA uptake inhibition is significantly reduced in cocaine self-administering rats as evidenced by a shorter time course of the signal's return to baseline; there is no effect of COC self-administration on MPH uptake inhibition. Group data showed that MPH uptake is unaffected (D) but DA uptake inhibition by COC is reduced (E) following 5 days of COC self-administration (●) compared with control rats (●), measured as apparent K_m (***) ($p < .001$, app., apparent).