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Sensitized Nucleus Accumbens Dopamine Terminal Responses to Methylphenidate and Dopamine Transporter Releasers after Intermittent-Access Self-Administration

Erin S. Calipari¹ and Sara R. Jones¹

¹Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC 27157

Abstract

Long-access methylphenidate (MPH) self-administration has been shown to produce enhanced amphetamine potency at the dopamine transporter and concomitant changes in reinforcing efficacy, suggesting that MPH abuse may change the dopamine system in a way that promotes future drug abuse. While long-access self-administration paradigms have translational validity for cocaine, it may not be as relevant a model of MPH abuse, as it has been suggested that people often take MPH intermittently. Although previous work outlined the neurochemical and behavioral consequences of long-access MPH self-administration, it was not clear whether intermittent access (6 h session; 5min access/30min) would result in similar changes. For cocaine, long-access self-administration resulted in tolerance to cocaine's effects on dopamine and behavior while intermittent-access resulted in sensitization. Here we assessed the neurochemical consequences of intermittent-access MPH self-administration on dopamine terminal function. We found increased maximal rates of uptake, increased stimulated release, and subsensitive D2-like autoreceptors. Consistent with previous work using extended-access MPH paradigms, the potencies of amphetamine and MPH, but not cocaine, were increased, demonstrating that unlike cocaine, MPH effects were not altered by the pattern of intake. Although the potency results suggest that MPH may share properties with releasers, dopamine release was increased following acute application of MPH, similar to cocaine, and in contrast to the release decreasing effects of amphetamine. Taken together, these data demonstrate that MPH exhibits properties of both blockers and releasers, and that the compensatory changes produced by MPH self-administration may increase the abuse liability of amphetamines, independent of the pattern of administration.

Keywords

Voltammetry; Self-administration; Cocaine; Methylphenidate; Amphetamine; Rat

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Corresponding Author: Sara R. Jones, Ph.D., Department of Physiology and Pharmacology, Wake Forest School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157, Tel: 336.716.8533, Fax: 336.716.8501, srjones@wakehealth.edu.

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1. Introduction:

Previous work has shown that methylphenidate (MPH) self-administration in rodents enhances the potency and reinforcing efficacy of amphetamine-related drugs, suggesting that MPH abuse may change the dopamine system in a way that promotes future drug abuse (Calipari et al., 2013a,b). MPH, the active compound in Ritalin, is commonly used off-label orally as a “study drug” (Teter et al., 2006), and in college students it has also been reported to be used to stay awake longer to party on weekends (Hall et al., 2005; Prudhomme-White et al., 2006; Teter et al., 2003). MPH is also taken intranasally or intravenously (IV) in larger doses, to “get high” (Gautschi & Zellweger, 2006; Levine et al., 1986; Parran & Jasinski, 1991; Sherman et al., 1987; Shaw et al., 2008; Teter et al., 2003); however, currently there is limited information on these patterns or routes of administration in pre-clinical models (Marusich et al., 2010; Calipari et al., a,b). When taken via the same route of administration, the subjective effects of MPH are indistinguishable from cocaine or amphetamine, two commonly abused and highly addictive drugs (Rosen et al., 1985; Silverman & Ho, 1980). Although the subjective effects of MPH and other stimulants are similar, recent work has shown that the compensatory changes induced by repeated administration of MPH are divergent from other stimulants of the same type, making it difficult to predict the neurochemical consequences of MPH abuse based on literature on compounds such as cocaine, the prototypical dopamine transporter blocker (Calipari et al., 2013a,d,e; Ferris et al., 2011, 2012, 2013a,b). For example, while a five-day history of cocaine self-administration selectively reduced cocaine, but not MPH, potency at the DAT, MPH self-administration selectively increased MPH potency (Calipari et al., 2013b). Additionally, although long-access MPH self-administration did not alter blocker potency (cocaine, nomifensine), it enhanced both the potency and reinforcing efficacy of releasers (amphetamine, methamphetamine) (Calipari et al., 2013a), suggesting that these compounds may have an increased abuse liability following repeated MPH exposure.

Long-access self-administration of cocaine in rodents results in escalation of intake over sessions, a phenomenon that has been documented in humans and is considered essential to the addiction process (Ahmed et al., 2002, 2003; Ahmed and Koob, 2005; Dackis and O'Brien, 2001). However, while self-administration studies using long-access paradigms have high translational validity for stimulants such as cocaine and amphetamine, which have been reported to be taken in multiple day binges (Dackis and O'Brien, 2001; Koob and Le Moal, 2001), such paradigms may not model MPH abuse as accurately. MPH abuse in humans has been suggested to occur intermittently, in single large doses many hours or days apart (Hall et al., 2005; Prudhomme-White, Becker-Blease, & Grace-Bishop, 2006; Teter et al., 2003). Thus, it is important to determine if the same neurochemical changes occur following a more clinically relevant pattern of voluntary MPH intake. Additionally, although previous work has determined the consequences of long-access MPH self-administration on the dopamine system, recent work has shown that the pattern of self-administration (intermittent versus continuous) can play an integral role in determining the neuroadaptations that occur following stimulant exposure. For example, intermittent versus continuous patterns of cocaine or self-administration resulted in opposite adaptations, characterized by the development of sensitization and tolerance to cocaine's effects on the

dopamine system, respectively. Hence, we aimed to determine how an intermittent paradigm of MPH administration influences the compensatory changes that occur within the dopamine system as compared to previous work following long-access conditions.

2. Methods:

2. 1. Animals

Male Sprague-Dawley rats (375-400 g; Harlan Laboratories, Frederick, Maryland) were used for all self-administration experiments. Rats were maintained on a 12:12 hour reverse light/dark cycle (3:00 am lights off; 3:00 pm lights on) with food and water *ad libitum*. All animals were maintained according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) 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. All efforts were made to minimize suffering, reduce the number of animals, and to utilize alternatives to *in vivo* techniques.

2. 2. Self-Administration

Rats were anesthetized and implanted with chronic indwelling jugular catheters and trained for i.v. self-administration as previously described (Calipari et al., 2013c). Following surgery, animals were singly housed, and all self-administration sessions took place in the home cage during the active/dark cycle (9:00 am-3:00 pm). After a 2-day recovery period, animals underwent a training paradigm within which animals were given access on a fixed ratio one (FR1) schedule to a MPH-paired lever, which, upon responding, initiated an intravenous injection of MPH (0.56 mg/kg, infused over 4 s). After each response/infusion, the lever was retracted and a stimulus light was illuminated for a 20 second timeout period, during which the animal had no access to the lever, and thus could not respond for drug. During training, sessions were terminated after a maximum of 20 infusions or 6 hours, whichever occurred first. An animal was considered to have acquired upon responding for 20 injections for two consecutive days and a stable pattern infusion intervals was present. Following training, animals were assigned intermittent access after which the dopaminergic alterations that resulted were assessed.

2. 3. Controls

Controls were animals housed in the same room, on the same light cycle, with similar handling conditions to the animals that performed self-administration. In previous publications we have confirmed with a number of measures that the surgery, novelty of being present in the self-administration chambers, and/or limited drug self-administration, does not influence the results (Calipari et al, 2013c; Calipari et al. 2013e).

2. 4. Intermittent Access Group

Animals were given access to MPH on an intermittent schedule of administration described previously (Calipari et al., 2013c). During each 6h session animals had access to MPH for 12 five minute trials separated by 25-minute timeout periods. Within each five-minute session, there were no timeouts other than during each infusion, and the animal could press

the lever on an FR1 schedule to receive a 1-sec infusion of MPH (0.140 mg/kg/inf). Upon responding a stimulus light illuminated concurrently with the 1-sec infusion of drug.

2. 5. In Vitro Voltammetry

Voltammetry experiments were conducted during the dark phase of the light cycle 18 hours after commencement of the final self-administration session. Following the completion of the self-administration paradigms animals were deeply anesthetized with isoflurane and rapidly decapitated. The brain was rapidly removed and the tissue was immediately immersed in ice-cold oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), CaCl_2 (2.4), MgCl_2 (1.2), NaHCO_3 (25), glucose (11), L-ascorbic acid (0.4) and pH was adjusted to 7.4. A vibrating tissue slicer was used to prepare 400 μm thick coronal brain sections containing the NAc. Once sliced, the tissue was transferred to the testing chambers containing bath aCSF (32°C), which flowed at 1 ml/min. After a 30-min equilibration period, a cylindrical carbon fiber microelectrode (100-200 M length, 7 M radius) and a bipolar stimulating electrode were placed into the core of the NAc. The nucleus accumbens (NAc) was selected because of the important role in the reinforcing and rewarding actions of cocaine. Further, our previous research has concentrated on plasticity of dopamine transporters in the core and demonstrated that these alterations occur due to self-administration history of a number of drugs (Calipari et al., 2013a,b,c,d,e; Ferris et al., 2011, 2012, 2013a,b). Fast scan cyclic voltammetry was used to characterize baseline dopamine system kinetics, D2-like autoreceptor activity, and the ability of psychostimulants to inhibit dopamine uptake. Endogenous dopamine release was evoked by a single electrical pulse (300 A, 4 msec, monophasic) applied to the tissue every 5 minutes. Extracellular dopamine was recorded by applying a triangular waveform (-0.4 to $+1.2$ to -0.4V vs Ag/AgCl, 400 V/s). Once the extracellular dopamine response was stable for three consecutive stimulations, cocaine (0.3-30 mol/L), MPH (0.3-30 mol/L) and (amphetamine 0.1-10 mol/L) were applied cumulatively to the brain slice to determine the effects of cocaine self-administration on drug-induced uptake inhibition. In order to determine D2-like autoreceptor sensitivity following these self-administration paradigms, we also ran dose-response curves for quinpirole (0.01-1 mol/L), a D2-like receptor agonist, and reversed the effects with sulpiride (2 mol/L), a D2-like antagonist.

2. 5. 1. Data Analysis—For all analysis of FSCV data Demon Voltammetry and Analysis software was used (Yorgason et al., 2011). To evaluate the effects of MPH self-administration on dopamine system kinetics, evoked levels of dopamine were modeled using Michaelis-Menten kinetics as described previously (Calipari et al., 2012). Immediately following the completion of each concentration-response curve, recording electrodes were calibrated by recording their response (in electrical current; nA) to a known concentration of dopamine in aCSF (3 M) using a flow-injection system. This value was then used to convert electrical current to dopamine concentration. For cocaine, MPH, and amphetamine dose-response curves, K_m , a measure of apparent affinity for the dopamine transporter, was used to determine changes in ability of the psychostimulants to inhibit dopamine uptake in the NAc relative to baseline. Because quinpirole is a D2-like agonist it concentration-dependently reduces the peak height of the dopamine signal. As a result we can assess the functional sensitivity of D2-like autoreceptors by determining if there is a shift in the

concentration-response curve for quinpirole. All curves for D2-like function are expressed as percent baseline dopamine release.

2. 5. 2. Calculating K_i values—As described by Jones et al. (1995), inhibition constants (K_i) were determined by plotting the linear concentration-effect profiles and determining the slope of the linear regression. The K_i was calculated by the equation K_m/slope . K_i values are reported in μM and are a measure of the drug concentration that is necessary to produce 50% uptake inhibition.

2. 6. Statistics

Graph Pad Prism (version 5, La Jolla, CA, USA) was used to statistically analyze data sets and create graphs. Pre-drug measures of stimulated dopamine release, dopamine uptake, and K_i values were compared using a two-tailed Student's t-test. Release data and data obtained after perfusion of cocaine, MPH, amphetamine, or quinpirole were subjected to a two-way analysis of variance (ANOVA) with experimental group and concentration of drug as the factors. Behavioral (self-administration) data was analyzed using a one-way ANOVA and regression analysis. When main effects were obtained ($P < 0.05$), differences between groups were tested using a Bonferroni *post hoc* tests.

3. Results

3. 1. Intermittent-access MPH self-administration did not result in escalation

Long-access self-administration of MPH has been previously been shown to result in escalation of intake over sessions (Marusich et al., 2010; Calipari et al., 2013a,b). One-way ANOVA revealed no main effect of self-administration session on intake, demonstrating that intermittent self-administration of MPH resulted in stable intake over sessions (Fig 1 A). Regression analysis indicated that the slope of intake over sessions is not significantly different from zero indicating that escalation does not occur in these animals with this model (Fig 1 B).

3. 2. Intermittent-access MPH self-administration resulted in increased stimulated dopamine release and $V_{\max}$ at baseline

Pre-synaptic dopamine system kinetics were assessed using FSCV in MPH self-administration and control groups. MPH self-administration resulted in an increase in maximal rate of uptake (Fig 2A) and stimulated dopamine release (Fig 2B). A two-tailed Student's t-test revealed a significant increase in stimulated dopamine release following intermittent MPH self-administration ($t_{36} = 2.191$, $p < 0.05$). In addition, a two-tailed Student's t-test also revealed a significant increase in $V_{\max}$ following MPH self-administration ($t_{33} = 2.919$, $p < 0.01$; Fig 2C).

3. 3. D2-like autoreceptors were subsensitive following MPH self-administration

In order to determine if the differential dopamine kinetic effects could be due to differences in D2-like autoreceptor activity, we assessed the sensitivity of the dopamine system to quinpirole, a D2-like autoreceptor agonist. ANOVA revealed a main effect of self-administration ($F_{1, 32} = 5.422$, $p < 0.05$) and a significant self-administration versus dose

interaction ($F_{4, 32} = 5.437$, $p < 0.01$) (Fig 3). Bonferroni post-hoc analysis revealed that quinpirole was significantly less effective at reducing dopamine release at the 10 nM ($p < 0.05$) and 30 nM ($p < 0.01$) doses following MPH self-administration, indicating that D2-like autoreceptor activity is reduced. The subsensitive D2-like autoreceptors that are present following MPH self-administration could explain, at least in part, the increase in stimulated dopamine release.

3. 4. Intermittent-access MPH self-administration resulted in increased MPH potency

To investigate how intermittent MPH self-administration affected MPH potency, we ran cumulative dose-response curves for MPH. Using FSCV in brain slices, MPH-induced dopamine uptake inhibition was recorded over a five-point dose-response curve for the compound. MPH was found to be more effective at inhibiting dopamine uptake inhibition following MPH self-administration (Fig 4A). ANOVA revealed a significant main effect of self-administration ($F_{1, 40} = 5.316$, $p < 0.05$; Fig 4B) and a significant interaction of MPH self-administration on the dose-response curve for MPH ($F_{4, 40} = 5.387$, $p < 0.01$; Fig 4B). Bonferroni post hoc analysis revealed that MPH-induced uptake inhibition was augmented at the 10 M ($p < 0.05$) and 30 M ($p < 0.001$) doses following MPH self-administration. A linear dose-response curve was plotted to calculate the K_i of MPH in control and self-administration animals (Fig 4C). The slopes of the regression lines of the linear dose-response curve for MPH-induced uptake inhibition were significantly different ($p < 0.01$; Fig 4D), indicating that MPH was more potent following MPH self-administration.

The K_i is the dose of drug at which 50% uptake inhibition is achieved, and is calculated from the slope of the linear dose-response curve for the compound. A Student's t-test revealed that MPH potency as measured by K_i was increased ($t_{10} = 2.698$, $p < 0.05$; Fig 4D), with a lower concentration of MPH resulting in 50% uptake inhibition following MPH self-administration as compared to controls.

3. 5. Amphetamine potency was increased following intermittent-access MPH self-administration

Because MPH is structurally similar to the releaser amphetamine, but functionally similar to the blocker cocaine, we assessed the effects of MPH self-administration on amphetamine and cocaine potencies to determine if the neurochemical are conferred to compounds of a similar structural or functional class. If the effects are structure specific amphetamine potency should be similar to the effects of MPH self-administration on MPH potency. Amphetamine was found to be more effective at inhibiting dopamine uptake inhibition following MPH self-administration (Fig 5A), an effect that was similar to that of MPH. ANOVA revealed a significant main effect of self-administration ($F_{1, 32} = 6.607$, $p < 0.05$; Fig 5B) and a significant interaction of MPH self-administration on the dose-response curve for amphetamine ($F_{4, 32} = 2.744$, $p < 0.05$; Fig 5B). Bonferroni post hoc analysis revealed that amphetamine-induced uptake inhibition was augmented at the 10 M dose ($p < 0.05$) following MPH self-administration. In addition, the linear dose-response curve was plotted to calculate the K_i of amphetamine in control and self-administration animals (Fig 5C). The slopes of the regression lines of the linear dose-response curve for amphetamine-induced

uptake inhibition were significantly different ($p < 0.05$), indicating that amphetamine was more potent following MPH self-administration.

A Student's t-test revealed that MPH potency as measured by K_i was increased ($t_7 = 2.7$, $p < 0.05$; Fig 5D) as well, with a lower concentration of amphetamine resulting in 50% uptake inhibition following MPH self-administration as compared to controls. Because amphetamine effects are similar to MPH following MPH self-administration it is likely that these effects are specific to the structure of these compounds rather than their function as blocker or releaser.

3. 6. Cocaine potency was unchanged following intermittent-access MPH self-administration

Because amphetamine and MPH behaved similarly after MPH self-administration, we hypothesized that these effects were due to the structure of the compound; however, to confirm this, we used cocaine, a compound structurally dissimilar from MPH, but functionally the same. Cocaine was found to be equivalently effective at inhibiting dopamine uptake inhibition following MPH self-administration (Fig 6A), an effect that was dissimilar to that of MPH. There was no significant effect of MPH self-administration on the dose-response curve for cocaine (Fig 6B).

In addition, the linear dose-response curve was plotted to calculate the K_i of amphetamine in control and self-administration animals (Fig 6C). The slopes of the regression lines of the linear dose-response curve for cocaine-induced uptake inhibition were not significantly different. In addition, cocaine potency as measured by K_i was unchanged (Fig 6D).

3. 7. Stimulated dopamine release profiles for MPH, cocaine, and amphetamine were differentially affected by MPH self-administration

To determine how dopamine release was affected by a history of MPH self-administration we measured the release effects of MPH, amphetamine, and cocaine across a dose-response curve for each of these drugs. First, we assessed the release profile of MPH to determine how it was affected by a prior MPH self-administration history. MPH exhibits an inverted "U" shaped profile that is indicative of a dopamine transporter blocker (Fig 7A). Curves are expressed as percent peak height at baseline, and peak height is a measure of both release and uptake as uptake occurs continuously in the presence of dopamine. Because of this, blockers result in inverted-u shaped dose-response curves, where they increase peak height at the early doses, due to their uptake-inhibition affects. At the higher doses, the peak height of the signal is reduced. In addition, ANOVA revealed a significant main effect of MPH self-administration ($F_{1, 33} = 6.929$, $p < 0.05$), indicating that MPH-induced dopamine release was increased as compared to control animals.

To determine if the release effects were common to all drugs or specific to a certain class/structure, cocaine was also run. As with MPH, cocaine exhibited an inverted "U" shaped release profile indicative of blockers (Fig 7B). Although the release profile was similar to MPH, the release effects of cocaine were unaffected by MPH self-administration. This gives further support to the idea that the effects of MPH self-administration on subsequent

psychostimulant effects are driven by the structure of the compounds and are not specific to function as blocker or releaser.

To further characterize the release effects of MPH self-administration, the release profile over a dose-response curve for amphetamine was assessed. Amphetamine has a different profile due to its mechanism of action as a dopamine releaser. Because amphetamine is releasing dopamine at all times, independent of stimulated release, it dose-dependently depletes dopamine releasable pools terminals leading to decreased dopamine release over the dose-response curve (Fig 7C). ANOVA revealed a significant main effect of MPH self-administration on amphetamine release ($F_{1, 28} = 13.96$, $p < 0.01$). Bonferroni post hoc analysis revealed that amphetamine release was significantly reduced at the 300nM dose ($p < 0.001$). The increase in amphetamine-induced dopamine reduction indicates an increase in the potency of amphetamine to release dopamine in these animals. In addition, the fact that both MPH and amphetamine are more potent in their dopamine releasing effects, although it is through different release profiles, gives further support to the idea that the effects of MPH self-administration on subsequent psychostimulant effects are driven by structure.

4. Discussion

The present study found that intermittent MPH self-administration resulted in dopamine system changes that may increase the abuse liability of some psychostimulants. With regard to dopamine signaling in the absence of drug, we found increased maximal rates of uptake and increased stimulated dopamine release as well as subsensitive D2-like autoreceptors. Further, the potencies of amphetamine and MPH were increased, as indicated by decreased K_i values for uptake inhibition, while cocaine effects were unchanged. Amphetamine and MPH effects on the peak height of evoked dopamine signals were also augmented, although in opposite directions, wherein MPH-induced elevations and amphetamine-induced reductions in peak height were enhanced after intermittent MPH self-administration. Additionally, MPH did not lead to reductions in release due to depletion of dopamine vesicles, confirming that MPH is not a classical releaser, even though it is similar to releasers with regard to potency changes at the dopamine transporter. The enhanced potency of MPH and releasers is consistent with previous work showing enhanced amphetamine- and MPH-seeking behaviors following extended-access MPH administration (Calipari 2013b). The similarities between dopamine system adaptations induced by extended-access protocols and the current intermittent study further highlight the unique properties of MPH, as cocaine exhibited opposite effects when self-administered intermittently versus continuously (Calipari et al., 2013a,b,c). Taken together, these results suggest that MPH abuse, regardless of pattern of intake, may result in increased abuse potential for amphetamine-like drugs.

The NAc core region was examined because of its strong dopamine innervation as well as its critical role in aspects of drug addiction, including learning of drug-cue associations and drug seeking behaviors (Willuhn et al, 2010). Alterations in dopamine signaling in this region have also been implicated in the learning and execution of goal-oriented behaviors in the absence of drugs (Berridge 2007; Yawata et al., 2012; Wassum et al., 2013). We show an increase in evoked dopamine release following MPH self-administration, which could

lead to increased associations between drugs and their rewarding / reinforcing effects (Flagel et al., 2011; Wanant et al., 2009). This increase in evoked dopamine release is consistent with the decrease in D2-like autoreceptor activity found in the current study. It has been suggested that there is no dopamine “tone”, or unstimulated extracellular dopamine levels, in slice preparations; therefore, during single pulse stimulations it is unlikely that D2-like autoreceptors actively regulate dopamine release (Kennedy et al., 1992). However, because D2-like autoreceptors function to reduce dopamine synthesis as well as release, it is possible that their reduced activity leads to increased dopamine synthesis via increased tyrosine hydroxylase activity (Håkansson et al., 2004). Indeed, following MPH self-administration tyrosine hydroxylase expression is increased (Calipari et al., 2013a). It is important to note that, although there are increases in evoked dopamine release following intermittent MPH self-administration, uptake rates are also increased, which could be a compensatory response to the increased dopamine release. The increase in uptake rates is most likely caused by an increase in dopamine transporter levels (Salahpour et al., 2008; Ferris et al., 2013b), which has been shown previously following MPH self-administration (Calipari et al., 2013a). Increases in dopamine transporter levels are particularly important in this case, as increasing dopamine transporter levels by transgenic over-expression has been shown to increase the behavioral and neurochemical potency of releasers such as amphetamine (Salahpour et al., 2008; Calipari et al., 2013a), highlighting the idea that these changes may increase the susceptibility of individuals to abuse/addiction of psychostimulants.

In addition to changes in evoked release in the absence of drugs, stimulant-enhanced release was also altered following MPH self-administration. MPH self-administration enhanced the effects of MPH and amphetamine on dopamine release, but not cocaine. However, unlike amphetamine, MPH did not cause reductions in evoked release due to depletion of vesicles, suggesting that MPH is not a classical dopamine releaser. At high concentrations, amphetamine leads to depletion of vesicles in dopamine terminals, which is experimentally observed, using voltammetric techniques, as reduced stimulated dopamine release (Jones et al., 1998), an effect that is enhanced by intermittent MPH self-administration. Although the effects of MPH self-administration on MPH potency at the dopamine transporter are similar to those found with dopamine releasers, MPH exhibits the release profile of a blocker, which is characterized by an inverted “U” shaped release curve with increased stimulated dopamine release at low concentrations (above pre-drug) which return to pre-drug levels at higher doses, likely because of extended D2 autoreceptor activation. This demonstrates that MPH is not a pure dopamine releaser, but rather exhibits properties of both releasers and blockers. This is in line with previous work showing that although MPH is not transported into cells, and thus cannot release dopamine from vesicles, it binds to the dopamine transporter in a way that is similar to releasers (Sonders et al., 1997; Dar et al., 2005; Waymunt et al., 1999).

Following methylphenidate self-administration, cross-sensitization occurs only for amphetamine-like compounds, and not cocaine, a stimulant of the same pharmacological class. Although it is not a novel concept that exposure to one stimulant may increase the abuse potential for other stimulants (Shuman et al., 2012; Rosine et al., 2009; Valvassori et al., 2007; Tronci et al., 2006; Yang et al., 2003), this paper is particularly relevant when considering previous data on cross sensitization of stimulants. While many groups have

previously shown that stimulants do, indeed, produce cross sensitization and cross-tolerance (Shuman et al., 2012; Rosine et al., 2009; Valvassori et al., 2007; Tronci et al., 2006; Yang et al., 2003; Ferris et al., 2012, 2013a; Calipari et al., 2013c), here we expand and clarify this idea. First, until recently sensitization and cross-sensitization have been phenomena primarily attributed to experimenter-delivered stimulant administration protocols, while self-administration of stimulants has repeatedly been shown to result in tolerance (Hurd et al., 1989; Calipari et al., 2013b,c,d; Ferris et al., 2011, 2012, 2013a,b). Therefore, this is one of the first reports of stimulant self-administration resulting in the sensitization of the neurochemical effects of a drug. Second, we show that not only does cross-sensitization not occur for all stimulants, but it may not even occur for stimulants of the same blocker or releaser class. Therefore the idea of cross-sensitization as a general process needs to be refined and made more specific for individual drugs.

A potential mechanism for the increased potency and enhanced effects on dopamine release in the presence of amphetamine and MPH is via increased dopamine transporter levels. Previous work demonstrated that similar changes following extended access MPH self-administration were due to elevated dopamine transporter levels, and here we observe the same neurochemical effects, suggesting that the current changes may occur via the same mechanism (Calipari et al., 2013a,b). The current study showed that intermittent-access MPH self-administration resulted in increased uptake rates, suggesting that dopamine transporter levels are likely to be elevated as well (Ferris et al., 2013b). Further, it has been shown that elevations in dopamine transporter levels alone are capable of enhancing both the behavioral and neurochemical effects of amphetamine and MPH, but not cocaine (Calipari et al., 2013a; Salahpour et al., 2008). Although this is the first study to show enhanced MPH-induced dopamine release following MPH self-administration, it is possible that dopamine transporter levels influence these effects as well. MPH has been shown to redistribute vesicles from storage pools into the readily releasable pool (Chadchankar et al., 2012; Volz et al., 2008). This could be a potential mechanism for its release effects and it is possible that these effects could be dependent on MPH binding to the dopamine transporter. Additionally, the increases in dopamine transporter levels may also explain the enhanced effects of amphetamine on evoked dopamine release following MPH self-administration. Increased uptake / dopamine transporter levels may function to allow increased concentrations of amphetamine into the cell, resulting in enhanced vesicular dopamine depletion.

It is also possible that the augmented uptake inhibition effects that occur at the DAT for MPH following MPH self-administration are driving the increased peak height of evoked dopamine signals. The peak height of evoked dopamine release is dictated by the opposing processes of release and uptake (Wightman et al., 1990; Yorgason et al., 2011), which are occurring simultaneously; therefore, when uptake inhibition occurs it can manifest as increases in the peak height of evoked dopamine, especially at lower concentrations. Indeed, we do observe increases in the peak height of evoked dopamine release at the lower concentrations of MPH (0.3, 1 μ M). Thus it is possible that the increased uptake inhibition effect of MPH is contributing to the enhanced evoked release effects of the compound, in addition to potential direct effects of MPH on dopamine release. It should be noted that both MPH and amphetamine exhibit enhanced uptake inhibition following MPH self-

administration, but amphetamine's increased potency leads to greater decreases in the evoked dopamine peak height, presumably through an increased rate of entry into the cells due to the higher uptake rate and subsequent interactions with exocytotic vesicles.

These neurochemical changes have important behavioral implications, because stimulant effects on behavior, such as reward and reinforcement, are primarily mediated by the ability of the drug to inhibit the dopamine transporter and elevate dopamine levels (Ritz et al., 1989; Roberts et al., 1977). Further, changes in the NAc core have been shown to be directly associated with changes in reinforcement-related behaviors, where increased accumbal dopamine responses to cues or drugs result in increased reinforcing efficacy / drug-seeking behaviors (Calipari et al., 2013b; Saunders et al., 2013; Graf et al., 2013; Holmes and Fam, 2013). This suggests that intermittent administration of MPH may lead to an increase in reward and reinforcement-related behaviors for amphetamines and other releasers, which could increase the abuse liability/addiction potential of these compounds. Although in the current study, we did not measure postsynaptic changes, which could compensate for the enhanced potency of MPH and releasers, previous work on long-access MPH self-administration would suggest that the changes specifically at the DAT are predictive of self-administration behavior and reinforcing efficacy as measured by both progressive ratio and behavioral economic approaches (Calipari et al., 2013a). Calipari et al., 2013a demonstrated that long-access MPH self-administration resulted in the same changes in DAT function and psychostimulant potency and showed that these changes were positively correlated with reinforcing efficacy. This is of particular importance, given the high and increasing levels of MPH abuse in the adult and adolescent populations in recent years (Imbert et al., 2013; Frauger et al., 2013; Sembower et al., 2013; Zosel et al., 2013), as well as the co-abuse of MPH with other drugs such as amphetamines (Teter et al., 2006).

A broader implication of findings such as these can help to elucidate the mechanisms by which stimulants alter the effects of other stimulant effects to promote addictive-like behaviors. Knowing what components of a compound (structure, function) are responsible for specific neurochemical adaptations that occur following repeated use of a stimulant is important because it allows for the determination of the consequences/abuse potential of different treatment compounds or new drugs that are developed for either treatment or abuse. Additionally, knowing the structure/function relationship of the neurochemical changes that occur after the administration of a compound could allow for the development of novel and specialized treatment options. Thus, understanding the factors that are responsible for the expression of changes in stimulant potency at the DAT can allow for the prediction of the neurochemical effects of other compounds, or the development of novel therapeutics that could be used to treat addiction.

5. Conclusions

Taken together, this work highlights the idea that MPH is a unique compound, both in its acute effects as well as the compensatory effects that occur following self-administration. Acutely, MPH exhibits the uptake-inhibition profile of a releaser but the release profile of a blocker, suggesting that MPH is part of a unique class of drugs that does not fit into either the releaser or blocker category. Further, the compensatory effects that occur following

MPH self-administration are not dependent on the temporal profile of MPH intake, an effect that is different from previous work with cocaine self-administration. Thus MPH, when taken in larger amounts than prescribed and via any pattern of self-administration, could result in an increased susceptibility to abuse of / addiction to dopamine releaser compounds such as amphetamine, methamphetamine, and designer amphetamines such as a “bath salts”.

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Highlights

- Intermittent-access (IntA) methylphenidate (MPH) self-administration was assessed
- IntA increased dopamine uptake and release, and reduced D2 autoreceptor function
- Amphetamine and MPH, but not cocaine, potencies were increased following IntA
- MPH exhibits properties of both blockers and releasers
- MPH self-administration may increase the abuse liability of amphetamines

Figure 1. Animals self-administer methylphenidate (MPH) at high rates when given intermittent access

Rats were given five minute unlimited access followed by a 25 minute time-out period to induce an intermittent pattern of self-administration within each six hour sessions. (A) Representative data showing the pattern of self-administration over the 14 sessions. Tick marks represent earned infusions. (B) Group data plotting total infusions (0.140 mg/kg/inf) in each session over the 14 total sessions. The slope of the regression line (red) is not significant indicating that animals do not escalate their intake.

Figure 2. Intermittent methylphenidate (MPH) self-administration leads to increased stimulated dopamine (DA) release and uptake

Baseline dopamine system kinetics assessed by fast scan cyclic voltammetry. (A) Representative plot of dopamine release and uptake at baseline in control and MPH self-administration groups. Data is normalized to peak height to highlight differences in uptake between groups. Color plots which show dopamine (green) over time as measured in current (z-axis). (B) Group data showing that stimulated release is elevated following intermittent MPH self-administration. (C) Group data demonstrating that maximal rate of dopamine uptake (V_{max}) is elevated at baseline following MPH self-administration. IntA, intermittent access; *, $p < 0.05$; **, $p < 0.01$.

Figure 3. Intermittent methylphenidate (MPH) self-administration causes D2-like autoreceptor subsensitivity

Quinpirole dose-response curve represented as percent of baseline dopamine peak height.

Intermittent MPH self-administration resulted in subsensitive D2-like autoreceptors as compared to controls. IntA, intermittent access; *, $p < 0.05$ vs control.

Figure 4. Sensitization of the neurochemical effects of methylphenidate (MPH)

Fast scan cyclic voltammetry was used to assess changes in the potency of MPH at the dopamine transporter following MPH self-administration. (A) Representative data showing MPH-induced uptake inhibition at the 10 μ M dose. Traces demonstrate that MPH is more effective at inhibiting uptake, as demonstrated by the increased time to return to baseline. Data was normalized to peak height to highlight differences in uptake across groups. Color plots which show dopamine (green) over time as measured in current (z-axis). (B) Group data showing that MPH self-administration results in increased potency of MPH over a dose-response curve. (C) K_i values across groups were calculated based off of the linear dose-response curve for MPH in each group. (D) Bar graph of K_i values for cocaine in control and MPH self-administration groups. K_i values are a measure of the concentration of drug at which 50% inhibition is achieved. IntA, intermittent access; *, p < 0.05 vs control; **, p < 0.01 vs control.

Figure 5. Enhanced amphetamine potency following methylphenidate (MPH) self-administration
Fast scan cyclic voltammetry was used to assess changes in the potency of amphetamine following MPH self-administration. (A) Representative data showing amphetamine-induced uptake inhibition at the 1 μM dose. Amphetamine is more effective at inhibiting uptake, as demonstrated by the increased time to return to baseline in the traces. Data was normalized to peak height to highlight differences in uptake across groups. Color plots which show dopamine (green) over time as measured in current (z-axis). (B) Group data showing that MPH self-administration results in increased potency of amphetamine. (C) K_i values across groups were calculated based off of the linear dose-response curve for amphetamine in each group. (D) Bar graph of K_i values for amphetamine in control and MPH self-administration groups. IntA, intermittent access; *, $p < 0.05$ vs control.

Figure 6. Cocaine potency is unaffected by a history of methylphenidate (MPH) self-administration

Fast scan cyclic voltammetry was used to assess changes in the potency of MPH at the dopamine transporter following MPH self-administration. (A) Representative data showing cocaine (10 μM)-induced uptake inhibition. Data was normalized to peak height. Color plots which show dopamine (green) over time as measured in current (z-axis). (B) Group data showing no change in cocaine potency following MPH self-administration. (C) K_i values across groups were calculated based off of the linear dose-response curve for cocaine in each group. (D) Bar graph of K_i values for cocaine in control and MPH self-administration groups. K_i values are a measure of the concentration of drug at which 50% inhibition is achieved. IntA, intermittent access; *, $p < 0.05$ vs control.

Figure 7. Stimulated dopamine release profile of cocaine, methylphenidate (MPH), and amphetamine following intermittent MPH self-administration

Stimulated release was determined by the peak height of the signal over the dose-response curve for each drug. Data was then reported as percent pre-drug dopamine peak height. (A) Amphetamine dose-response curve demonstrating that evoked release in the presence of amphetamine is reduced following MPH self-administration. The profile of releasers is characterized by decreased exocytotic dopamine release, due to vesicular depletion, as doses are increased. (B) MPH dose-response curve that indicates that dopamine release in the presence of MPH is augmented following MPH self-administration. The release profile of

MPH indicates that it is a dopamine transporter blocker. The profile of dopamine transporter blockers is characterized by an inverted “U” shaped curve with increased dopamine at lower concentrations and reduced dopamine at higher concentrations. (C) Cocaine dose-response curve that indicates that dopamine release is unchanged following MPH self-administration. IntA, intermittent access; *, $p < 0.05$ vs control; ***, $p < 0.001$ vs control.