HHS Public Access
Author manuscript
Nat Commun. Author manuscript; available in PMC 2014 May 12.

Published in final edited form as:
Nat Commun. 2013 ; 4: 2720. doi:10.1038/ncomms3720.

Methylphenidate amplifies the potency and reinforcing effects of amphetamines by increasing dopamine transporter expression

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Abstract

Methylphenidate (MPH) is commonly diverted for recreational use, but the neurobiological consequences of exposure to MPH at high, abused doses are not well defined. Here we show that MPH self-administration in rats increases dopamine transporter (DAT) levels and enhances the potency of MPH and amphetamine on dopamine responses and drug seeking behaviors, without altering cocaine effects. Genetic over-expression of the DAT in mice mimics these effects, confirming that MPH self-administration-induced increases in DAT levels are sufficient to induce the changes. Further, this work outlines a basic mechanism by which increases in DAT levels, regardless of how they occur, are capable of increasing the rewarding and reinforcing effects of select psychostimulant drugs, and suggests that individuals with elevated DAT levels, such as ADHD sufferers, may be more susceptible to the addictive effects of amphetamine-like drugs.

Introduction

Recent epidemiological studies show that rates of illicit drug use have declined in recent years, while the abuse of prescription drugs, including stimulant medications such as Ritalin, are on the rise[1]. Methylphenidate (MPH), the active compound in Ritalin, is prescribed for attention deficit/hyperactivity disorder (ADHD) and narcolepsy, and is commonly used off-label, with up to 17% of college students reporting abuse of MPH for its cognitive enhancing or euphoric effects[2]. MPH is taken orally, intranasally or intravenously, and is one of the prescribed drugs most diverted onto the illicit market[3, 4]. MPH abuse occurred at rates comparable to cocaine abuse in the United States in 2008[5]. However, while there are

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Competing financial interests: The authors have no competing financial interest.

Author Contributions: E.S.C. designed and performed experiments, analyzed data, and wrote the paper; M.J.F. designed experiments, analyzed data and wrote the paper; A.S. and M.G.C. created the transgenic mouse line and gave technical support and conceptual advice; and S.R.J. designed experiments and wrote the paper.

numerous studies outlining the neurochemical consequences of therapeutic MPH use, there is a paucity of data outlining the neurobiological adaptations that occur during MPH abuse.

When taken via the same route of administration, the subjective effects of MPH are indistinguishable from cocaine or amphetamine (AMPH), two commonly abused and highly addictive drugs[6, 7]. In addition, the behavioral effects of MPH and other psychostimulants are similar. For example, MPH resembles both cocaine and AMPH in extended access self-administration experiments which result in escalation of intake over sessions, a change thought to model the switch from abuse to addiction[8, 9, 10, 11]. Because of the similar subjective and behavioral profile of MPH compared to highly addictive drugs, it is critically important to ascertain the neurochemical consequences of abuse of MPH.

MPH, AMPH, and cocaine exert their rewarding and reinforcing effects by inhibiting the dopamine transporter (DAT) and elevating synaptic dopamine levels[12]. Additionally, changes in potency at the DAT are predictive of changes in the rewarding effects of these compounds in place preference paradigms[13]. In order to elucidate the consequences of MPH self-administration on the abuse/addiction potential of psychostimulants, we determined the changes in blocker and releaser potency at the DAT as well as concomitant changes in motivation and drug seeking behaviors for AMPH, MPH, and cocaine.

MPH self-administration resulted in increased maximal rates of dopamine uptake mediated by increased DAT levels. Previous work has shown that fluctuations in DAT levels can alter the potency of psychostimulants[14], although the specific drugs affected and the direction of potency shifts remain equivocal. Here we proposed that MPH self-administration-induced elevations in DAT levels would result in enhanced potency and reinforcing efficacy of releasers and MPH. To test this hypothesis we used transgenic DAT over-expressing mice (DAT-tg) which have elevated levels of, native, non-drug altered DATs. Using these mice, we elucidated a basic mechanism whereby increased DAT levels drive enhanced potency and motivation to obtain MPH and releaser drugs such as AMPH, while leaving the effects of blockers such as cocaine unchanged. The robust enhancement in the potency of releasers, but not blockers, following increases in DAT levels in DAT-tg mice is particularly important as it suggests that individual variations in DAT levels in the human population (e.g. ADHD sufferers[15], PTSD[16], early life stress[17], repeated stressors[18]) may predict susceptibility to abuse of compounds such as MPH, AMPH and methamphetamine.

Results

MPH self-administration increases MPH reinforcement

To determine the neurochemical consequences of MPH administration, we allowed rats six-hour access to the most reinforcing dose of MPH (0.56 mg/kg/infusion[11]), on a fixed ratio one (FR1) schedule, with a maximum of 40 injections for five consecutive days (n = 11). This resulted in escalation of first-hour intake (one-way ANOVA; $F_{(4, 10)} = 20.00$, $p < 0.001$) as well as overall rate of intake (one-way ANOVA; $F_{(4, 10)} = 7.956$, $p < 0.001$; Fig 1 A, B). On an FR1 schedule it is impossible to differentiate whether increased intake is due to tolerance or increased motivation to take drug, so we measured reinforcing efficacy using a progressive ratio (PR) paradigm[19]. We found an increase in breakpoint for MPH (Student's t-test; $t_{12} = 2.068$, $p < 0.05$; Fig 1 C) following MPH self-administration (n = 7 per group).

MPH increases MPH potency and MPH-induced dopamine overflow

The reinforcing and rewarding effects of many drugs of abuse are related to their ability to elevate dopamine in the nucleus accumbens (NAc) [20]. While the NAc shell is involved in the acute rewarding effects of drugs and acquisition of self-administration, the NAc core plays a critical role in cue-reward learning as well as continued responding for drugs after repeated administration as in the current paradigm[21, 22]. We found dopamine uptake in the NAc core to be supersensitive to MPH inhibition following MPH self-administration (two-way ANOVA; $F_{(1, 9)} = 25.11$, $p < 0.05$; Fig 1 D, E) (n = 5 control, 6 MPH self-administration), which is consistent with our microdialysis data demonstrating increased MPH-induced dopamine overflow in the NAc (two-way ANOVA; $F_{(1, 5)} = 20.80$, $p < 0.001$; Supplementary Fig S1A) (n = 4 control, 5 MPH self-administration) The proposed mechanism for augmented MPH potency is increased DAT levels, which has been suggested to modulate the potency of dopamine releasers[23]. Indeed, after MPH self-administration (n = 31 per group), the maximal rate of dopamine uptake was increased (V_{max}; Student's t-test; $t_{60} = 2.434$, $p < 0.01$) (Fig 1 G, H) as was the peak amplitude of stimulated dopamine release (Fig 1 H; Student's t-test; $t_{24} = 2.719$, $p < 0.01$). We showed previously that the increase in V_{max} after MPH was due to increased total DAT levels[24].

MPH self-administration increases dopamine releaser potency

To further understand the impact of MPH self-administration on psychostimulant potencies, we used voltammetry and microdialysis to examine the ability of a number of DAT blockers and dopamine releasers to enhance dopamine signals in the NAc core. We found that MPH self-administration had no effect on blocker potency, but enhanced the potency of releasers. Thus, the ability of the blockers cocaine (Fig 2 A, C; n = 5 control, 6 MPH self-administration) and nomifensine (Fig 2 B, D; n = 5 per group) to inhibit dopamine uptake was unaffected by MPH self-administration. In contrast, the 7potency of AMPH (two-way ANOVA; $F_{(1, 4)} = 76.81$, $p < 0.001$; Fig 2 E, G) (n = 5 per group) and methamphetamine (two-way ANOVA; $F_{(1, 10)} = 33.51$, $p < 0.001$; Fig 2 F, H) (n = 7 control, 5 MPH self-administration) was increased.

The increase in drug potency for dopamine uptake inhibition for MPH and releasers following MPH self-administration was accompanied by increases in drug-induced dopamine overflow as measured using *in vivo* microdialysis. AMPH (two-way ANOVA; $F_{(1, 5)} = 11.51$, $p < 0.0001$; n = 5 control, 6 MPH self-administration; Supplementary Fig S1B), but not cocaine (n = 6 per group; Supplementary Fig S1C), resulted in increased drug-induced dopamine overflow in the NAc. This confirms that shifts in the ability of releasers to inhibit dopamine uptake corresponds to shifts in the ability of releaser to augment extracellular dopamine levels.

MPH increases releaser reinforcement and drug-seeking

Given the enhanced potency of dopamine releasers, it is possible that these compounds would have increased abuse liability following MPH self-administration. To this end, motivation to take drugs was measured using a “threshold” procedure[25]. The threshold procedure uses a within-session dose-response curve, with doses decreasing every 10–15 min, which determines the price (in the form of lever pressing) an animal is willing to pay to receive drug. The maximal price an animal is willing to pay (P_{max}) is a measure of the reinforcing efficacy of a compound. After a history of MPH self-administration, there was increased lever responding (two-way ANOVA; $F_{(1, 13)} = 35.16$, $p < 0.001$; Fig 3A, right) and P_{max} (Student’s t-test; $t_{14} = 2.196$, $p < 0.05$; Fig 3A, left) for MPH (n = 7 control, 8 MPH self-administration). Consistent with the neurochemical data, there was also increased responding (two-way ANOVA, $F_{(1, 11)} = 51.60$, $p < 0.001$; Fig 3B, right) and P_{max} (Student’s t-test, $t_{10} = 3.136$, $p < 0.01$; Fig 3B, left) for AMPH (n = 7 control, 6 MPH self-administration), but not cocaine (Fig 3C; n = 5 control, 6 MPH self-administration). This shows that the increased potency of MPH and AMPH in the NAc is associated with increased drug-seeking behavior.

Increased releaser and MPH potency in DAT-tg mice

We hypothesized that the increase in releaser and MPH potency was due to the increase in DAT levels and corresponding increase in V_{max} that occurred following MPH self-administration. To test this hypothesis we used DAT over-expressing mice (DAT-tg) [23], which have increased levels of native, non-drug altered DATs. As expected, DAT-tg (n = 16 WT, 18 DAT-tg) animals exhibited increased V_{max} (Student’s t-test; $t_{31} = 3.787$, $p < 0.001$; Fig 4A, B) as well as increased stimulated dopamine release in the NAc core (Student’s t-test; $t_{31} = 2.129$, $p < 0.05$; Fig 4A, C). We hypothesize that although MPH is categorized as a blocker, it shares some properties of releasers; thus, increases in baseline DAT levels alone would be capable of increasing the neurochemical potency of MPH. In support of our hypothesis and consistent with MPH self-administration results, we showed that increasing DAT levels was sufficient to increase the ability of MPH (two-way ANOVA; $F_{(1, 9)} = 21.44$, $p < 0.001$; n = 5 WT, 6 DAT-tg; Fig 4D), and AMPH (two-way ANOVA; $F_{(1, 9)} = 16.05$, $p < 0.001$; n = 5 WT, 7 DAT-tg; Fig 4E), but not cocaine (n = 6 WT, 5 DAT-tg; Fig 4F), to inhibit dopamine uptake. Consistent with MPH self-administering animals and voltammetry data, DAT-tg mice had increased MPH- (two-way ANOVA; $F_{(1, 5)} = 18.53$ $p < 0.001$; n = 4 per group; Supplementary Fig S1D), and AMPH- ($F_{(1, 5)} = 68.84$, $p < 0.001$; n = 3 WT, 5 DAT-tg; Supplementary Fig S1E), but not cocaine- (n = 4 per group; Supplementary Fig S1F), induced dopamine overflow in the NAc.

Finally, DAT-tg animals showed increased MPH- (two-way ANOVA; $F_{(1, 10)} = 4.093$, $p < 0.05$; n = 6 per group; Fig 4G) and AMPH- (two-way ANOVA; $F_{(1, 12)} = 9.934$, $p < 0.01$; n = 7 WT, 8 DAT-tg; Fig 4H), but not cocaine- (n = 8 WT, 6 DAT-tg; Fig 4I) induced locomotion. This further demonstrates that the shifts in neurochemical potency correspond to shifts in the behavioral activating effects of the drug.

Oral MPH administration does not alter drug potency

Varying the dose and route of administration of a drug can produce variable effects, thus we aimed to study the effects of oral, therapeutic doses on dopamine kinetics. MPH (5 mg/kg; 2xday) delivered orally for a period of 14 days (n = 15) resulted in no changes in dopamine kinetics or psychostimulant potencies (Fig 5). These data suggest that although MPH could enhance the reinforcing efficacy of MPH and releasers when taken via non-oral routes and in larger doses than prescribed, the therapeutic use of MPH does not result in any changes in psychostimulant potencies.

Discussion

Although MPH is often viewed as a safe drug with little addiction potential, emerging evidence indicates that MPH abuse is common and increasing[2, 3, 4]. We report here that a rat model of MPH abuse produces profound alterations in dopamine neurotransmission that may enhance vulnerability to addiction. First, we show that MPH self-administration results in escalation of MPH intake and enhances the neurochemical potency and reinforcing effects of MPH and dopamine releasers, but not DAT blockers. Second, increases in DAT levels are sufficient to augment the potency of releasers and MPH, but not blockers. Third, although MPH is traditionally classified as a DAT blocker, it shares some functional properties with releasers. And fourth, models of therapeutic use and abuse of MPH have completely different neurochemical consequences.

With respect to the first finding, MPH self-administration resulted in escalation of intake over sessions and resulted in enhanced potency of releasers and MPH. The increased intake over sessions was shown to be associated with an increased motivation to administer MPH, as measured by a PR schedule of reinforcement, which is consistent with previous work suggesting that escalation of cocaine intake is due to increased motivation to administer drugs[26]. Because of the fixed injection maximum per session we imposed in order to reduce variability in intake, animals cannot escalate in total intake. However, first hour intake was increased, which is a hallmark of traditional escalation[8, 9, 10, 11, 26]. This first hour escalation is consistent with work using long-access conditions showing that MPH intake does escalate at a number of doses[11]. Thus, like cocaine and AMPH, MPH intake transitions from low to high levels of intake over time.

In addition to enhanced MPH reinforcement, MPH self-administration also resulted in increased motivation to administer AMPH, a dopamine releaser, but not cocaine, a DAT blocker. The increased motivation to administer MPH and AMPH is likely driven by increased MPH and AMPH potency at the DAT and concomitant increases in extracellular dopamine. We found that increased DAT levels, observed following MPH self-administration, were sufficient to elicit the selective increase in MPH and releaser potencies. Indeed, genetic over-expression of the DAT in mice, in the absence of pharmacological intervention, recapitulated the neurochemical and behavioral alterations associated with MPH self-administration. In addition to providing a possible mechanism for the MPH self-administration-induced augmentation of the reinforcing efficacy of releasers, this demonstrates that fluctuations in DAT levels, regardless of how they occur, will change the potency of releaser compounds.

Second, we find that DAT level changes alter the potency of dopamine releasers and MPH, but not DAT blockers, despite the general consensus in the field that shifts in cell-surface DAT expression lead to inverse shifts in cocaine, but not AMPH, potency. This theory originates from two main sources. The first is that historically, cocaine elevates dopamine in the NAc shell, where DAT levels are low, to a much greater extent than in the dorsal striatum, where DAT levels are high[27, 28]. Secondly, cell culture work shows that DAT overexpression in cells results in a decrease in cocaine potency with no change in AMPH potency[14].

With respect to the first, the increased potency of cocaine in the shell relative to the caudate has been demonstrated *in vivo* with microdialysis, and there are a number of factors other than DAT numbers that could influence these results. For example, there are many differences between afferent inputs to caudate and nucleus accumbens shell, including levels of serotonin and norepinephrine innervations, which are also cocaine targets and influence presynaptic dopamine release. These differences could greatly influence the regional specificity of cocaine effects on dopamine[29]. In addition, previous work using voltammetry in freely-moving animals has shown that regional variations in the potency of cocaine are likely attributable to differences in D2 autoreceptor sensitivity in regulating dopamine release, and not differences in DAT levels[30].

With respect to discrepancies with cell culture work, one possibility is that recording endogenous dopamine fluctuations with voltammetry may produce different results than cell culture studies using exogenous [^{3}H]-DA in order to measure uptake and uptake inhibition[14]. It is possible that [^{3}H]-DA is sequestered into intracellular compartments differently than endogenous DA, and that releaser compounds interact differently with these compartments. Alternatively, it should be noted that many cell culture studies have determined the absolute effects of psychostimulants on dopamine levels, while not necessarily taking into account baseline rates of uptake in the overall effects of these drugs. This is particularly relevant since substantial work has suggested that behavioral outcomes are dependent on a change relative to baseline, not the absolute dopamine levels in isolation[31, 32, 33]. Voltammetry determines the effects of the drug while accounting for baseline uptake rates and this approach correlates well with behavioral outcomes not only in this study, but in previous work as well[23].

The DAT-dependent changes in psychostimulant potencies are particularly relevant in clinical treatment settings because disorders such as PTSD and ADHD are associated with increased DAT levels, ranging from 17–70%[15, 16, 17, 18]. These people could be more sensitive to the neurochemical, behavioral, and neurotoxic effects of prescribed AMPH and MPH. In addition, these findings may explain in part the high frequency of drug abuse in untreated ADHD sufferers[34].

Third, this work suggests that MPH is unique in the way in which it interacts with the DAT, and that the characterization of MPH solely as a prototypical DAT blocker may need revision. Indeed, although MPH is considered a DAT blocker, the increased behavioral and neurochemical potency of MPH following both MPH self-administration and DAT overexpression resembles the shift in AMPH’s effects, and not cocaine’s. Also, the acute effects of MPH following cocaine self-administration have previously been shown to be similar to releasers and not blockers. For example, Ferris et al. (2012) found that cocaine self-administration resulted in DAT tolerance to all blockers, but not releasers or MPH[35]. In addition to the evidence provided by our laboratory, others have shown that, although MPH is not a substrate for the DAT[36], it functions as a releaser at high concentrations [37, 38]. Additionally, MPH binds to the DAT in a manner that is distinct from prototypical blockers or releasers[39, 40], with significant overlap between the binding sites for both cocaine and AMPH. It is possible that although MPH is not transported into the cell, the interaction with the AMPH site results in conformational changes in the DAT which promote reverse transport in the same way as AMPH. More work is needed to clarify the molecular mechanisms unique to MPH, but our findings suggest that MPH shares some characteristics of both blockers and releasers.

Our fourth major finding was that oral administration of low-dose MPH had different neurochemical consequences than self-administration of high-dose MPH. We found that oral administration of low, therapeutic doses had no discernible neurochemical consequences on the dopamine system. This is consistent with other work showing no changes in dopamine kinetics or stimulant potencies following low-dose therapeutic administration of MPH[41, 42]. This differential effect of MPH at low and high doses may be due to the fact that high dose MPH substantially elevates dopamine levels in the NAc, whereas low-dose MPH does not [43, 44]. Additionally, changes associated with MPH abuse models may be due to their higher doses and rapid onset, compared to therapeutic administration.

In summary, this study shows that MPH self-administration elevates DAT levels and leads to enhanced MPH and dopamine releaser potency. We showed that changes in DAT levels alone were sufficient to alter the potency of psychostimulant releasers and MPH, but not blockers. Thus, MPH is likely increasing DAT levels in individuals that are abusing the drug, which may lead to increased potency for dopamine uptake inhibition, neurotoxicity, and abuse potential for releasers and MPH. Additionally, this study demonstrates that MPH potency is altered by transporter fluctuations in a manner that is not significantly different from releasers, although it is not a substrate for the DAT. Thus, we have discovered novel properties of MPH interactions with the DAT, which coincide with novel neuroadaptations following high-dose exposure.

Methods

Animals

Male Sprague-Dawley rats (375–400 g; Harlan Laboratories, Frederick, Maryland) were used for all self-administration experiments. Male, C57/Bl6 wild-type and DAT over-expressing mice were used for locomotor analysis and voltammetry. Animals were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities on a 12:12 hour light-dark cycle with food and water *ad libitum.* The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine. All animals were randomly assigned to experimental groups. There was no blinding done in the current study.

Self-Administration

Male rats (≈ PD 70) were anesthetized and implanted with chronic indwelling jugular catheters[45]. Following surgery, animals were singly housed, and all sessions took place in the home cage during the active/dark cycle (9:00 am–3:00 pm). All animals underwent a training paradigm where they were given access to a MPH paired lever on a FR1 schedule of reinforcement. Training sessions were terminated after a maximum of 20 infusions or six hours, whichever occurred first. Acquisition occurred when an animal responded for 20 injections for two consecutive days. Typically, animals acquired a stable pattern of MPH self-administration within 1 to 5 days. A small percentage of animals did not acquire self-administration behavior, but this is typical of self-administration studies. We removed non-acquiring animals from this study. Control animals for PR and threshold self-administration experiments underwent the training paradigm, and then were immediately switched to PR or threshold tests of reinforcing efficacy. We have confirmed that the control group (2 days, 20 MPH injections) is not neurochemically different from naïve controls.

Treatment animals underwent five consecutive days of MPH self-administration. Sessions were six hours long and were terminated after the animal responded for 40 injections of drug or at the end of the session. The dose of 0.56 mg/kg/injection MPH was chosen because it was the dose that produced maximal responding on a dose response curve measuring reinforcing efficacy. It is important to note that this method is different from previously described extended-access paradigms as animals do not escalate in total intake as an injection limit (40 inj per day) was set in order to eliminate variability in total consumption of MPH.

Progressive Ratio Procedure

Response requirements were increased through the following ratio progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, etc. Breakpoints were defined as the number of completed ratios before 1 h elapsed without completion of the next ratio. Data from the MPH self-administration group was compared to a control group (2 days, 20 MPH injections). Differences between groups cannot be due to differences in task learning or experience, as there was no difference in the cocaine threshold task, indicating that there was not an overall increase in task performance with longer experience.

Threshold Procedure

In order to determine the reinforcing efficacy of psychostimulants, subjects underwent a within-session threshold procedure [46]. Rats were given access to a descending series of 12 unit doses of cocaine, MPH, or AMPH (421, 237, 133, 75, 41, 24, 13, 7.5, 4.1, 2.4, 1.3, and 0 μg/injection) on an FR1 schedule during consecutive 10 or 15-min bins within a daily session. Because MPH and AMPH are not metabolized as rapidly as cocaine, each bin was 15 minutes for those compounds, while it was 10 min for cocaine. The lever was not retracted at any time during the session. Doses were manipulated by holding the concentration constant and adjusting the pump duration. We switched from a PR procedure because the threshold procedure can assess both drug taking (in the early bins in the session where the dose is high) and drug seeking (within the latter bins where the dose is low, and the final bin where animals are responding for saline).

Oral Methylphenidate Administration

MPH was administered using an oral dosing procedure in which subjects are trained, in less than 3 days, to voluntarily consume MPH in a 5 ml volume of 10% sucrose. This procedure results in a pharmacokinetic profile of MPH distribution close to that observed in humans. MPH was administered twice daily (7:00 a.m. and 2:00 p.m.) at a dose of 5 mg/kg for two weeks (14 days). This dosing regimen takes into account the fact that MPH metabolism is more rapid in rats than in humans and yields blood serum levels near the upper end of the therapeutic range observed in children (5–40 ng/ml).

Ex Vivo Voltammetry

Fast scan cyclic voltammetry in brain slices was used to characterize dopamine system kinetics, as well as the ability of psychostimulants to inhibit dopamine uptake in the NAc. *Ex Vivo* voltammetry was chosen because it allows for the accurate assessment of psychostimulant potency by running within-subject concentration response curves for each drug. Voltammetry experiments were conducted during the dark phase of the light cycle, beginning 18 hours after commencement of the final self-administration or oral MPH session. A carbon fiber electrode and a stimulating electrode were placed on the surface of the slice in close proximity to each other in the core of the NAc. Endogenous dopamine release was evoked by a single electrical pulse (300 μA, 4 msec, monophasic) applied to the tissue every 5 minutes. Extracellular dopamine was recorded by applying a triangular waveform (−0.4 V to +1.2 V to −0.4 V). Once the extracellular dopamine response was stable for three consecutive stimulations, blockers (cocaine, nomifensine), MPH, and releasers (AMPH and methamphetamine) were applied to the brain slice. Dopamine current was converted to concentration by electrode calibration with 3 μM dopamine at the end of each experiment.

Demon Voltammetry and Analysis software was used for analysis[47]. To evaluate the effects of drugs, evoked levels of dopamine were modeled using Michaelis–Menten kinetics, as a balance between release and uptake[48]. Michaelis–Menten modeling provides parameters that describe the amount of dopamine released following stimulation, the maximal rate of dopamine uptake (V_{max}), and inhibition of the ability of dopamine to bind to the DAT, or apparent K_m. For pre-drug modeling, we followed standard voltammetric modeling procedures by setting the apparent K_m parameter to 160 nM based on the affinity of dopamine for the DAT, whereas baseline V_{max} values were allowed to vary as the baseline measure of the rate of dopamine uptake. Following drug application, apparent K_m was allowed to vary to account for changes in drug-induced dopamine uptake inhibition while the respective V_{max} value determined for that subject at baseline was held constant. The *apparent* K_m parameter models the amount of dopamine uptake inhibition following a particular dose of drug.

Locomotor Analysis

Locomotor activity was assessed in automated locomotor activity monitors (MedAssociates). Mice were placed into the activity monitor chamber (20 cm × 20 cm) for 60 min, then injected with saline or the drug in 0.1 ml total volume, returned to the chamber, and monitored for 60 min after injection. Locomotor activity was measured as horizontal distance covered as a percent of each animal's saline baseline. Dose response curves were run for each drug.

Microdialysis

Animals were anesthetized with ketamine/xylazine, placed in a stereotaxic frame, and concentric microdialysis probes (2 mm (rat) or 1mm (mouse) membrane length; cut off 6000 Daltons; CMA-11, CMA/Microdialysis, Solna, Sweden) were implanted into the NAc core in both rats and mice the day before recording[49]. Appropriate placement of probes was verified by histological examination after the experiments. For rats, microdialysis surgery was performed the day following the final session of MPH self-administration. The day following surgery, probes were perfused with artificial CSF at 1.0 μl/min. Samples were collected every 20 min and analyzed for dopamine by high-performance liquid chromatography (HPLC, Bionalytical Systems, Mt. Vernon, IN). Once stable baselines were established, the inlet line was switched to a syringe containing the same artificial CSF with AMPH (10μM), MPH (30μM), or cocaine (30μM).

HPLC

A 2 × 50 mm (3 μm particle) reverse-phase column (Luna, Phenomenex, Torrance, CA) was used and the applied potential was +650 mV as referenced to an Ag/AgCl electrode. The mobile phase (75 mM NaH_2PO_4, 1.7 mM 1-octanesulfonic acid sodium salt, 100 μL/L triethylamine, 25 μM EDTA, 10% acetonitrile *v*/*v*, pH=3.0) was pumped at a rate of 170 μL/min, with a detection limit for dopamine of 10 pM. Dopamine quantification was achieved by comparing dialysate samples with dopamine standards of known concentration.

Statistics

Graph Pad Prism (version 5; La Jolla, CA) was used to analyze data sets and compose graphs. Baseline voltammetry, PR, and P_{max} data were compared across groups using a two-tailed Student's t-test. Data obtained from MPH self-administration/DAT-tg animals were subjected to a two-way analysis of variance (ANOVA) with experimental group and concentration of drug as the factors. TH and locomotor data were subjected to a two-way ANOVA with experimental group and dose as the factors. When significant interactions or main effects were obtained ($p < .05$), differences between groups were tested using Bonferroni post hoc tests. Parametric statistics were used for all analysis. Data met the assumptions of normality and the variance between groups was similar for all experiments. Power analyses were utilized for every measure to determine the number of animals needed to detect small to medium sized effects, with expected effect sizes and standard deviations based on previous experiments performed in the laboratory and from published manuscripts. Exclusion of data points only occurred if the points were significant outliers as determined by statistical analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by NIH grants R01 DA021325, R01 DA030161, R01 DA014030 (SRJ), P50 DA06634 (SRJ & MJF), K99 DA031791 (MJF), 5R37-MH-073853(MGC), T32 DA007246 and F31 DA031533 (ESC).

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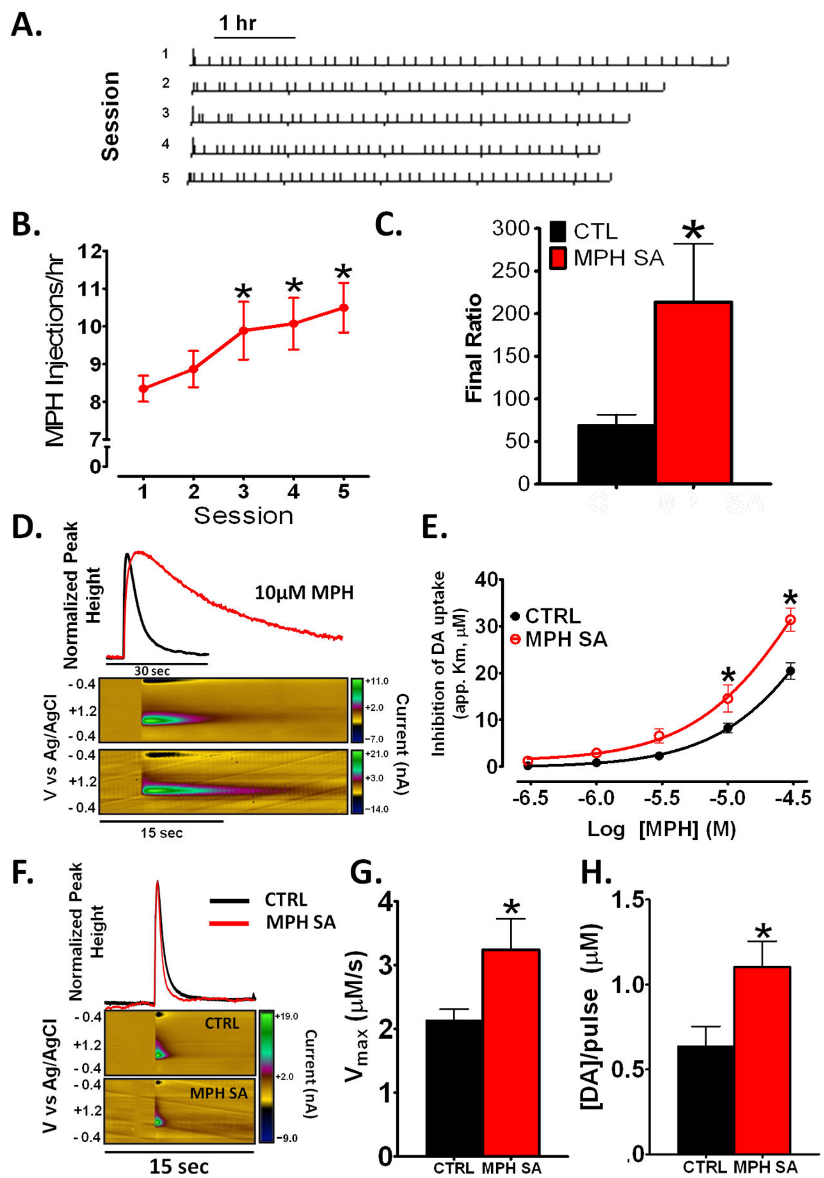

Figure 1. MPH self-administration increases dopamine uptake and MPH potency

A, Self-administration event record from a representative animal. Ticks marks represent injections earned. **B**, Rate of responding for MPH on a fixed-ratio 1 schedule is increased over sessions. Session 1 refers to the first session following acquisition. *$p < 0.05$ vs. session 1, n = 11 per group, one-way ANOVA. **C**, MPH self-administration (red) increased the final ratio during a progressive ratio schedule of reinforcement for MPH as compared to minimally trained control animals (CTRL; black). *$p < 0.05$ vs. control, n = 7 per group, Student's t-test, unpaired. **D**, **E**, MPH self-administration resulted in an increased ability of MPH to inhibit the dopamine transporter. *$p < 0.05$ vs. control, n = 5 control, 6 MPH self-administration, two-way ANOVA. **F–H**, MPH self-administration increased the maximal rate of dopamine uptake (V_{max}; *$p < 0.05$ vs. control, n = 31 per group, Student's t-test, unpaired) and stimulated dopamine release ([DA]/pulse) (*$p < 0.05$ vs. control, Student's t-test, unpaired). Error bars are reported as mean ± S.E.M.; MPH, methylphenidate; SA, self-administration; CTRL, control

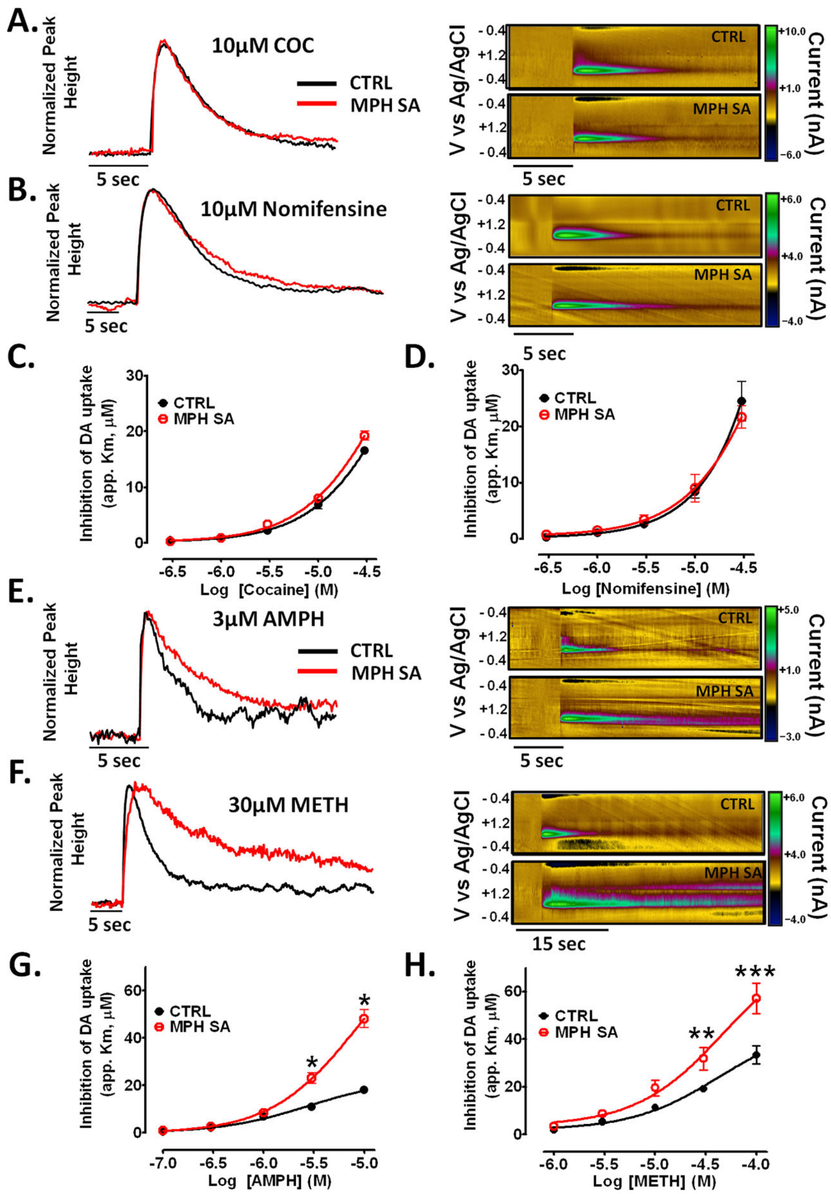

Figure 2. MPH self-administration increases the potency of releasers

A, **B**, Representative traces showing cocaine- and nomifensine- induced uptake inhibition at the dopamine transporter, respectively. Color plots show the concentration of dopamine (green) over time. The ability of the blockers, **C**, cocaine (n = 5 control, 6 MPH self-administration), and **D**, nomifensine (n = 5 per group), to inhibit the dopamine transporter following MPH self-administration was unchanged. **E**, **F**, Representative traces showing AMPH- and methamphetamine- induced uptake inhibition, respectively. Color plots show the concentration of dopamine (green) over time. The ability of the releasers, **G**, amphetamine (*p < 0.05 vs. control, n = 5 per group, two-way ANOVA) and, **H**, methamphetamine (** p < 0.01, *** p < 0.001 vs. control, n = 7 control, 5 MPH self-administration, two-way ANOVA) to inhibit the DAT following MPH self-administration is enhanced. Error bars are reported as mean ± S.E.M.; SA, self-administration; METH, methamphetamine; COC, cocaine; MPH, methylphenidate; AMPH, amphetamine; DAT, dopamine transporter

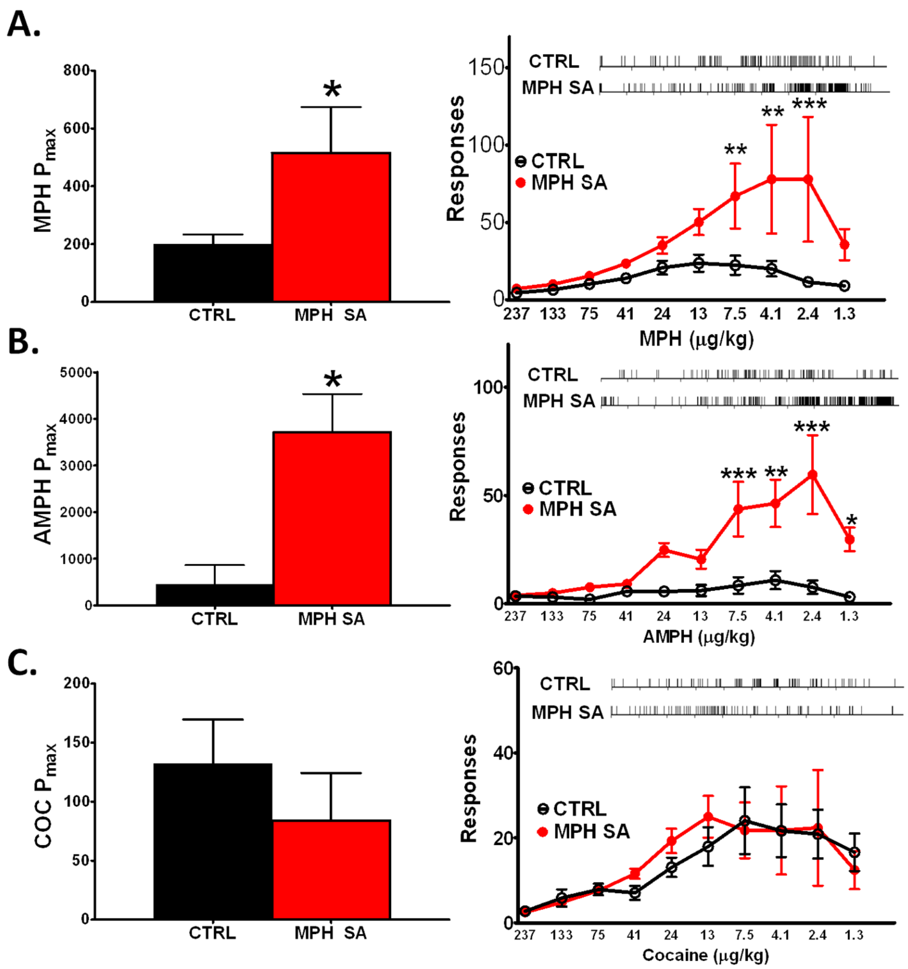

Figure 3. MPH self-administration increases the motivation to administer releasers and MPH

Panels **A–C** show significantly higher maximal price paid (P_{max}; **Left**) to administer both MPH (*p < 0.05 vs. control, n = 7 control, 8 MPH self-administration, Student's t-test, unpaired; **A**, left) and AMPH (*p < 0.05 vs. control, n = 7 control, 6 MPH self-administration, Student's t-test, unpaired; **B**, left), but not cocaine (n = 5 control, 6 MPH self-administration; **C**, ledt) following MPH self-administration. **(Right)** The motivation to administer drug for MPH (**p < 0.01, ***p < 0.001 vs. control, n = 7 control, 8 MPH self-administration, two-way ANOVA; **A**, right) and AMPH (*p < 0.05, **p < 0.01, ***p < 0.001 vs. control, n = 7 control, 6 MPH self-administration, two-way ANOVA; **B**, right), but not cocaine (n = 5 control, 6 MPH self-administration; **C**, right) was enhanced following MPH self-administration. Error bars are reported as mean ± S.E.M.; SA, self-administration; COC, cocaine; CTRL, control; MPH, methylphenidate; AMPH, amphetamine

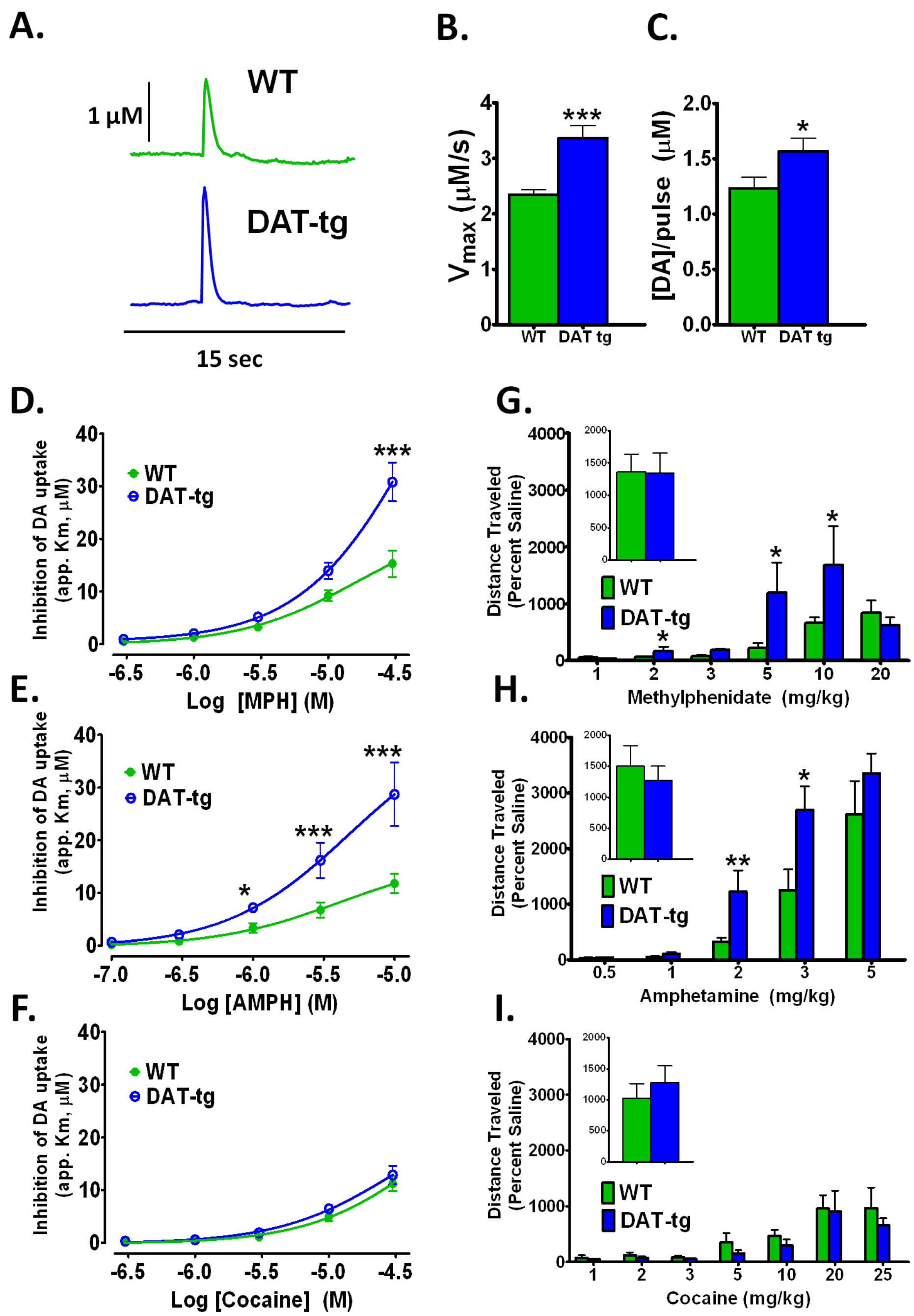

Figure 4. Over-expression of DAT increases the behavioral and neurochemical potency of MPH and releasers

A–C, Rate of dopamine uptake (V_{max}; *** $p < 0.001$ vs. WT, n = 16 WT, 18 DAT-tg, Student's t-test, unpaired) and stimulated dopamine release ([DA]/pulse; * $p < 0.05$ vs. WT, n = 16 WT, 18 DAT-tg, Student's t-test, unpaired) are increased in DAT over-expressing mice (DAT-tg; blue) relative to wild-type (WT; green). **D–F**, Grouped data showing enhanced MPH- (*** $p < 0.001$ vs. control, n = 5 WT, 6 DAT-tg, two-way ANOVA) and AMPH- (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control, n = 5 WT, 7 DAT-tg, two-way ANOVA), but not cocaine - (6 WT, 5 DAT-t), induced dopamine uptake inhibition in the nucleus accumbens core of DAT-tg mice as compared to WT. **G–I**, Locomotor activating effects of MPH (*$p < 0.05$ vs. control, n = 6 per group, two-way ANOVA) and AMPH (*$p < 0.05$, **$p < 0.01$ vs. control, n = 7 WT, 8 DAT-tg, two-way ANOVA), but not cocaine (n = 8 WT, 6 DAT-tg), were enhanced in DAT-tg mice. Error bars are mean ± S.E.M.; MPH, methylphenidate; AMPH, amphetamine; V_{max}, maximal rate of dopamine uptake; WT, wild-type

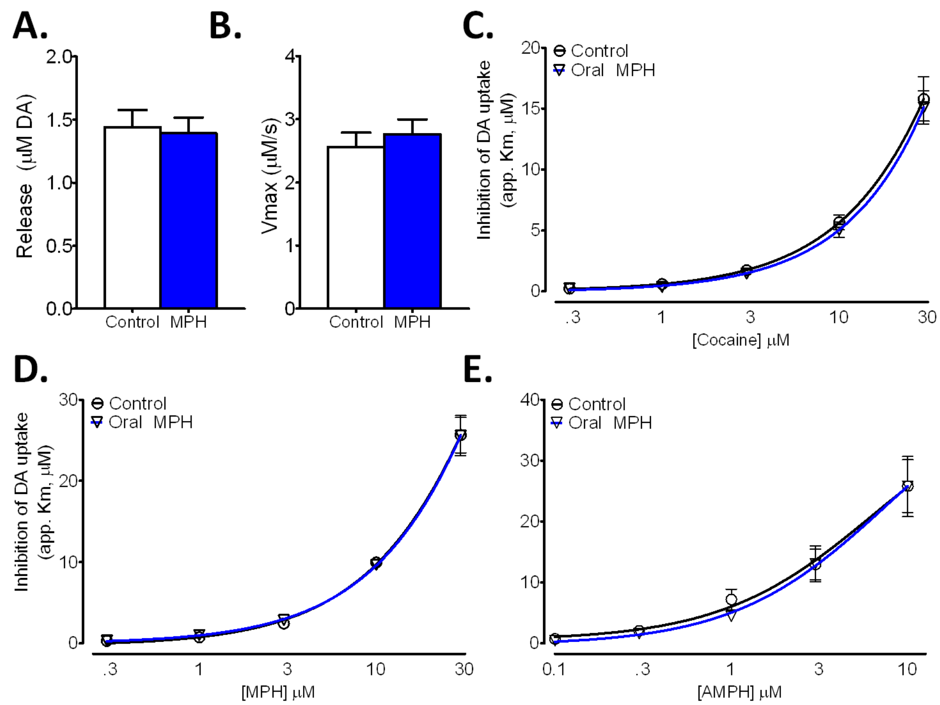

Figure 5. Oral MPH administration does not affect dopamine kinetics or the potency of psychostimulants

A, **B**, Grouped data from animals treated with oral MPH (5mg/kg, 2x daily, 14 days; n = 15 per group; blue) as compared to controls (n = 15 per group; white), demonstrating no difference in dopamine system kinetics. Panels **C**–**E** show the potency of cocaine, MPH, and AMPH in controls (black) and animals with a history of oral MPH self-administration (blue). Error bars are reported as mean ± S.E.M.; MPH, methylphenidate; AMPH, amphetamine