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ARTICLE

The abuse-related effects of pyrrolidine-containing cathinones are related to their potency and selectivity to inhibit the dopamine transporter

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Synthetic cathinones are common constituents of abused "bath salts" preparations and represent a large family of structurally related compounds that function as cocaine-like inhibitors or amphetamine-like substrates of dopamine (DAT), norepinephrine (NET), and serotonin (SERT) transporters. Preclinical evidence suggests that some cathinones (e.g., MDPV and α-PVP) are more effective reinforcers than prototypical stimulant drugs of abuse, such as cocaine or methamphetamine. Although the reinforcing potency of these cathinones is related to their potency to inhibit DAT, less is known about the pharmacological determinants of their unusually high reinforcing effectiveness. To this end, we tested the hypothesis that reinforcing effectiveness of cathinone stimulants is positively correlated with their selectivity for DAT relative to SERT. Uptake inhibition assays in rat brain synaptosomes were used to directly compare the potency of MDPV, MDPBP, MDPPP, α-PVP, α-PPP, and cocaine at DAT, NET, and SERT, whereas intravenous self-administration in rats was used to quantify relative reinforcing effectiveness of the drugs using progressive ratio (PR) and behavioral economic procedures. All cathinones were more potent at DAT than NET or SERT, with a rank order for selectivity at DAT over SERT of α-PVP > α-PPP > MDPV > MDPBP > MDPPP > cocaine. These synthetic cathinones were more effective reinforcers than cocaine, and the measures of reinforcing effectiveness determined by PR and demand curve analyses were highly correlated with selectivity for DAT over SERT. Together, these studies provide strong and convergent evidence that the abuse potential of stimulant drugs is mediated by uptake inhibition at DAT, with activity at SERT serving as a negative modulator of reinforcing effectiveness.

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INTRODUCTION

The abuse of stimulant drugs is a serious worldwide public health concern, with recent estimates suggesting 75 million people use cocaine, amphetamine-type stimulants, or "ecstasy" [1]. In recent times, the stimulant drug problem has been exacerbated by the emergence of new psychoactive substances, which are alternatives to more traditional illicit drugs of abuse. The United Nations Office on Drugs and Crime has identified >700 chemically distinct new psychoactive substances since 2009, over a third of which are classified as stimulants [1]. Synthetic cathinones comprise a large family of structurally related compounds that function as either cocaine-like inhibitors or amphetamine-like substrates at plasma membrane transporters for dopamine, norepinephrine, and serotonin (DAT, NET, and SERT, respectively) (e.g., refs. [2–7]). Synthetic cathinones are common constituents of abused "bath salts" preparations and account for approximately 20% of all new psychoactive substances found in non-medical (i.e., recreational) drug markets [1]. Although "bath salts" preparations were originally marketed as legal alternatives to illicit stimulants, high rates of abuse and toxicity led the US Drug Enforcement Administration (DEA) to place three of the most commonly detected synthetic cathinones (3,4-methylenedioxypyrovalerone [MDPV], 4-methylmethcathinone [mephedrone], and 3,4-methylenedioxymethcathinone [methylone]) under emergency Schedule I control in 2011 [8]. Subsequent to this initial regulation, a large number of structurally similar synthetic cathinones (e.g., 3,4-methylenedioxy-α-pyrrolidinobutiophenone [MDPBP], 3,4-methylenedioxy-α-pyrrolidinopropiophenone [MDPPP], α-pyrrolidinopentiophenone [α-PVP], and α-pyrrolidinopropiophenone [α-PPP]) have emerged in the recreational drug markets worldwide. The US DEA now lists 13 synthetic cathinones (including MDPV and α-PVP) as Schedule I compounds [9], yet synthetic cathinones remain a largely unregulated and understudied family of drugs of abuse.

Consistent with in vitro studies suggesting that synthetic cathinones primarily interact with monoamine transporters (e.g., refs. [2–7]), a growing number of synthetic cathinones have been reported to increase locomotor activity, produce cocaine-, methamphetamine-, or 3,4-methylenedioxymethamphetamine-like discriminative stimulus effects, and maintain intravenous self-administration (e.g., refs. [10–19]). For instance, we have recently shown that rats will acquire self-administration of the pyrrolidine-containing cathinones MDPV, MDPBP, MDPPP, α-PVP,

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and α-PPP at rates comparable to those observed with cocaine and that the potencies of these drugs to maintain responding under a fixed ratio (FR) 5 schedule of reinforcement are highly correlated with their potencies to inhibit uptake at DAT and NET but not SERT [13, 16]. These findings are consistent with reports that the potency of cocaine-like drugs to function as reinforcers positively correlates with in vitro binding at DAT (e.g., refs. [20, 21]), but potency to inhibit DAT is not sufficient to predict reinforcing effectiveness (i.e., maximum reinforcing effect) or abuse potential. Instead, it has been hypothesized that the relative ratio of activity at DAT compared to activity at SERT determines the reinforcing effectiveness of stimulant drugs. To date, the strongest support for this hypothesis has been provided by studies demonstrating analogs of cocaine or amphetamine that are less selective for DAT over SERT (i.e., have more serotonergic activity) are also less effective reinforcers (e.g., refs. [22–24]). While it is logical to suppose that drugs which are more selective than cocaine or amphetamine for DAT over SERT would also function as more effective reinforcers than cocaine or amphetamine, there has been little direct evidence to support this hypothesis. In fact, although Thomsen and colleagues [25] provided clear evidence that DAT is necessary for cocaine to function as a reinforcer, they also showed that eliminating the serotonergic activity of cocaine, by knocking out SERT, failed to alter the reinforcing effectiveness of cocaine. Thus, while the addition of SERT activity appears to negatively modulate the reinforcing effectiveness of drugs that interact with monoamine transporters, it is unclear whether the contrary is true for drugs that are more selective for DAT over SERT than drugs such as cocaine.

To this point, we have recently shown that two synthetic cathinones (MDPV and α-PVP) purported to be highly selective for DAT and NET relative to SERT [5, 26–28] are capable of maintaining ~3-fold greater levels of responding under a progressive ratio (PR) schedule than less selective stimulants such as cocaine and methamphetamine [13, 14]. In addition to suggesting that MDPV, α-PVP, and other pyrrolidine-containing cathinones might have an unusually high potential for abuse, these data also suggest that pyrrolidine-containing synthetic cathinones (e.g., MDPBP, MDPPP, and α-PPP) could provide tools to evaluate the relative contributions of uptake inhibition at DAT, NET, and SERT to the reinforcing effectiveness of stimulant drugs. Accordingly, the current studies aimed to directly compare the pharmacology of MDPV, MDPBP, MDPPP, α-PVP, α-PPP, and cocaine using (1) uptake inhibition assays in rat brain synaptosomes to determine potency at DAT, NET, and SERT; and (2) intravenous self-administration in rats to quantify their relative reinforcing effectiveness using a PR schedule of reinforcement (which increased response requirements within a session), as well as behavioral economic analyses of responding maintained under a FR schedule of reinforcement (with response requirements increased across sessions).

METHODS

Subjects

Male Sprague-Dawley rats (275–300 g upon arrival) were purchased from Envigo (Indianapolis, IN, USA) and maintained in a temperature- and humidity-controlled room. Rats were singly housed, maintained on a 10-/14-h dark/light cycle, and provided with ad libitum access to water and Purina rat chow. All experiments were conducted during the light cycle (0600–2000) with sessions for individual subjects initiated at approximately the same time each day. These studies were carried out in accordance with the Institutional Animal Care and Use Committees of the University of Texas Health Science Center at San Antonio or the Intramural Research Program of the National Institute on Drug Abuse and the Eighth Edition of the Guide for Care and Use of Laboratory Animals [29].

In vitro uptake assays in synaptosomes

Rats ($n = 16$) were euthanized by CO_2 narcosis and their brains were processed to produce synaptosomes as previously described [3, 30, 31]. Neurotransmitter uptake by DAT, NET, or SERT was assessed using 5 nM [^{3}H]dopamine, 10 nM [^{3}H]norepinephrine or 5 nM [^{3}H]serotonin, respectively. For DAT, the selectivity of assays was established by surgical isolation of the caudate putamen, a region that is so enriched in DAT that measurable uptake of [^{3}H]dopamine by NET or SERT does not occur. For SERT, the selectivity of the assay was established by adding GBR 12935 (50 nM) and nomifensine (100 nM) to block uptake of [^{3}H]serotonin by DAT and NET, respectively. For NET, the selectivity of the assay was established by adding GBR 12935 (50 nM) to block uptake of [^{3}H]norepinepherine by DAT; [^{3}H]norepinepherine has low affinity for SERT, and therefore selective blockade of SERT is not required. The potent non-selective uptake inhibitor indatraline (1 μM) was used to define non-specific binding at DAT, NET, and SERT. Tissue suspension (100 μl) was added to tubes containing the test drug, [^{3}H]neurotransmitter, and 900 μl Krebs-phosphate assay buffer (126 mM NaCl, 2.4 mM KCl, 0.83 nM $CaCl_2$, 0.8 nM $MgCl_2$, 0.5 nM KH_2PO_4, 0.5 mM Na_2SO_4, 11.1 mM glucose, 0.05 nM pargyline, 1 mg/ml bovine serum albumin, and 1 mg/ml ascorbic acid, pH 7.4) to begin the uptake inhibition assay. Rapid vacuum filtration through Whatman GF/B filters terminated the assay, and liquid scintillation counting was used to quantify the retained radioactivity.

Surgical procedures

Rats were anesthetized with 2% isoflurane and indwelling catheters were surgically implanted in the left femoral vein as previously described [13–16]. Following the surgery, catheters were flushed with 0.5 ml heparinized saline (100 U/ml) via a vascular access port that had been attached to the catheter and secured between the scapulae. Penicillin G (60,000 U/rat) was subcutaneously administered to prevent infection. Rats were provided at least 5 days to recover from the surgery during which time catheters were flushed daily with 0.5 ml heparinized saline.

Self-administration

Daily self-administration sessions were conducted using operant conditioning chambers situated within noise-reduction cubicles (Med Associates, St Albans, VT, USA). Each chamber was equipped with two response levers located on one wall, sets of three LEDs (one red, yellow, and green) located above each lever, and a white house light located at the top center of the wall opposite the levers. A variable speed syringe pump delivered drug infusions through Tygon® tubing that was attached to a fluid swivel held in place by a counterbalance arm and connected to the animal by a spring tether.

A catheter-loading infusion was delivered 0.5 min before the start of each session, with the start of the session and drug availability signaled by illumination of the yellow LED above the active lever (right or left; counterbalanced). Completion of the response requirement resulted in infusion of drug (0.1 ml/kg over ~1 s), illumination of the three LED lights above the active lever as well as the houselight, and the initiation of a 5-s timeout. Responses made during timeouts or on the inactive lever were recorded but had no scheduled consequence. Throughout the study, catheters were flushed daily with 0.2 ml saline before operant sessions, and 0.5 ml heparinized saline after operant sessions to verify and maintain catheter patency. If backpressure was noted during catheter flushing, catheters were further tested with an infusion of methohexital (3 mg/kg) that produces a rapid loss of righting reflex in rats with functional catheters. If a catheter failed a patency test, a second surgery was performed to place a catheter in the right femoral vein.

Four groups of 12 rats were first allowed to acquire responding for MDPBP (0.1 mg/kg/inf), MDPPP (0.32 mg/kg/inf), α-PVP (0.032

mg/kg/inf), or α-PPP (0.32 mg/kg/inf) under an FR1:TO 5-s schedule of reinforcement across 10 consecutive daily sessions, 90 min in length. Subsequently, the response requirement was increased to an FR5, and rats were allowed to self-administer the same dose/drug for at least 10 sessions and until stability criteria (infusions varied by no more than 20% of the mean for 3 consecutive session with no increasing or decreasing trend) were met. Once responding stabilized under the FR5:TO 5-s schedule, dose substitution was used to generate full dose–response curves for their assigned drug. This portion of the studies (acquisition and FR5 dose–response curves) has been reported elsewhere [16].

Upon completion of the FR5 dose–response curves, all rats were transitioned to PR schedule of reinforcement, under which the response requirement incremented with each successive infusion according to the following equation: response requirement = [5e^(infusion number × 0.2)] − 5 . The duration of operant sessions was not capped but ended when an infusion was not earned within 45 min. Each group of 12 rats was used to generate full dose–response curves for their respective drugs (i.e., MDPBP, MDPPP, α-PVP, or α-PPP) after which they were also used to generate full dose–response curves for both MDPV and cocaine (counterbalanced). The first dose of MDPBP (0.032 mg/kg/inf), MDPPP (0.32 mg/kg/inf), α-PVP (0.032 mg/kg/inf), or α-PPP (0.32 mg/kg/inf) to be evaluated corresponded to the dose that was available during the initial acquisition of responding [16], with the remaining doses evaluated in a random order. Each dose was evaluated until the stability criterion (±2 infusions from the previous session) was met; all doses of a particular drug were evaluated before moving to the next drug.

A subset of these rats ($n = 11$) were subsequently used to generate demand curves for MDPV (0.032 and 0.1 mg/kg/inf), MDPBP (0.032 and 0.1 mg/kg/inf), MDPPP (0.32 and 1.0 mg/kg/inf), α-PVP (0.032 and 0.1 mg/kg/inf), α-PPP (0.32 and 1.0 mg/kg/inf), and cocaine (0.32 and 1.0 mg/kg/inf) in daily 120-min sessions. For these studies, response requirements incremented across sessions according to the following series: 3, 10, 18, 32, 56, 100, 178, etc., and until zero reinforcers were earned for two consecutive sessions. Each ratio was in place for at least 2 sessions, and until the stability criterion was met (2 consecutive sessions where the number of infusions varied by <15% of the mean for those two sessions). Although dose and drug order were randomized across rats, both doses of a single drug were assessed before moving to another drug. Doses were selected based on their relative position on the descending limb of a FR5 dose–response curve [13, 16, 32].

Drugs

MDPV, MDPBP, MDPPP, α-PVP, and α-PPP were synthesized as racemic HCl salts by Agnieszka Sulima and Kenner Rice at the Drug Design and Synthesis Section of the Molecular Targets and Medications Branch on the Intramural Research Programs of the National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism (Bethesda, MD). Cocaine hydrochloride was provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD). All drugs were dissolved in sterile saline and administered intravenously at 0.1 ml/kg body weight.

Data analysis

Concentration–response data for uptake inhibition at DAT, NET, and SERT are presented as the percentage of control [^{3}H] neurotransmitter uptake determined in the absence of any drug (mean ± SEM). The potency to inhibit uptake by 50% (IC_{50} values) was estimated by non-linear regression analysis. Because the largest concentration of α-PPP tested failed to inhibit uptake at SERT to a level that allowed for non-linear regression to be performed, the IC_{50} for α-PPP at SERT was approximated by assuming the next larger concentration of α-PPP would have begun to produce a concentration-dependent inhibition of serotonin uptake and that the resulting SERT inhibition curve would be parallel to those observed for DAT and NET inhibition (see Fig. 1 and Table 1).

Data obtained under the PR schedule of reinforcement are presented as the mean ± SEM of the number of infusions obtained at each unit dose. For each subject, the greatest number of infusions earned for each drug (i.e., E_{max}) was identified, with mean ± SEM E_{max} values serving as a dose-independent measure of relative reinforcing effectiveness. Measures of reinforcing potency (i.e., dose that maintains half-maximal responding; ED_{50} ± 95% confidence intervals [CIs]) were also determined for individual subjects by normalizing dose–response curves to E_{max} (i.e., 100%) and using a linear regression of the data spanning the 20–80% effect levels to estimate the dose necessary to produce a 50% effect for individual subjects. ED_{50}s for two drugs were considered statistically different if their 95% CIs did not overlap. One-way repeated-measures analysis of variance (ANOVA) with post hoc Tukey's tests were used to determine if measures of reinforcing effectiveness derived from the PR study (E_{max}) differed among the three drugs evaluated for each group (cocaine, MDPV, and test drug). One-way ANOVA with post hoc Holm–Sidak tests was also used to determine (1) if the reinforcement history impacted the reinforcing effectiveness of cocaine or MDPV across groups; and (2) if E_{max} values differed among all six drugs evaluated in the current study.

Data from the demand curve analyses are graphically presented as proportional consumption (mean number of infusions earned [Q]/mean number of infusions earned when the FR was set to 3 [Q_0]) plotted as a function of standardized price (the FR value × Q_0) and fit according to equation 6 from Hursh and Silberberg [33]:

$$\log Q = \log Q_0 + 1.76(e^{\wedge}(-\alpha Q_0 C) - 1)$$

where C is the cost of each reinforcer (i.e., the FR) and α determines the rate of decline in proportional consumption as the standardized price changes. Thus α serves as a quantitative measure of the value (i.e., reinforcing effectiveness) of each drug and can be used to calculate the essential value (α^{-1}) of a commodity. Data from both doses of each drug were evaluated separately and when combined; however, because a single curve could be fit to the data when both doses were combined, only these values are reported. Essential values (α^{-1}) were obtained for individual subjects, with non-overlapping 95% CIs used to determine whether the reinforcing effects of the drugs differed significantly (Table 2). Although statistical analyses were performed on individual subject data, group means were used to prepare graphical representations of the data.

Pearson's correlation analyses were performed to determine relationships among various end points including: (1) potency (ED_{50}) of each drug to maintain responding under a PR schedule of reinforcement; (2) reinforcing effectiveness (E_{max}) of each drug under a PR schedule of reinforcement; (3) reinforcing effectiveness (α^{-1}) of each drug as determined using demand curve analyses; (4) potency (IC_{50}) for uptake inhibition at DAT, NET, or SERT; and (5) selectivity for inhibiting uptake at DAT relative to SERT (i.e., DAT/SERT ratio = ([DAT IC_{50}]$^{-1}$/[SERT IC_{50}]$^{-1}$), where larger values reflect greater selectivity for DAT) (Fig. 3). Prism 7 software (GraphPad Software Inc., La Jolla, CA, USA) was used to conduct statistical analyses and plot figures.

RESULTS

Figure 1a shows the chemical structures of MDPV, MDPBP, MDPPP, α-PVP, cocaine, and α-PPP, and Fig. 1b shows the concentration–response curves to inhibit uptake of [^{3}H]dopamine, [^{3}H]norepinephrine, and [^{3}H]serotonin at DAT, NET, and SERT, respectively. Although each of the synthetic cathinones containing a methylenedioxy moiety (i.e., MDPV, MDPBP, and MDPPP)

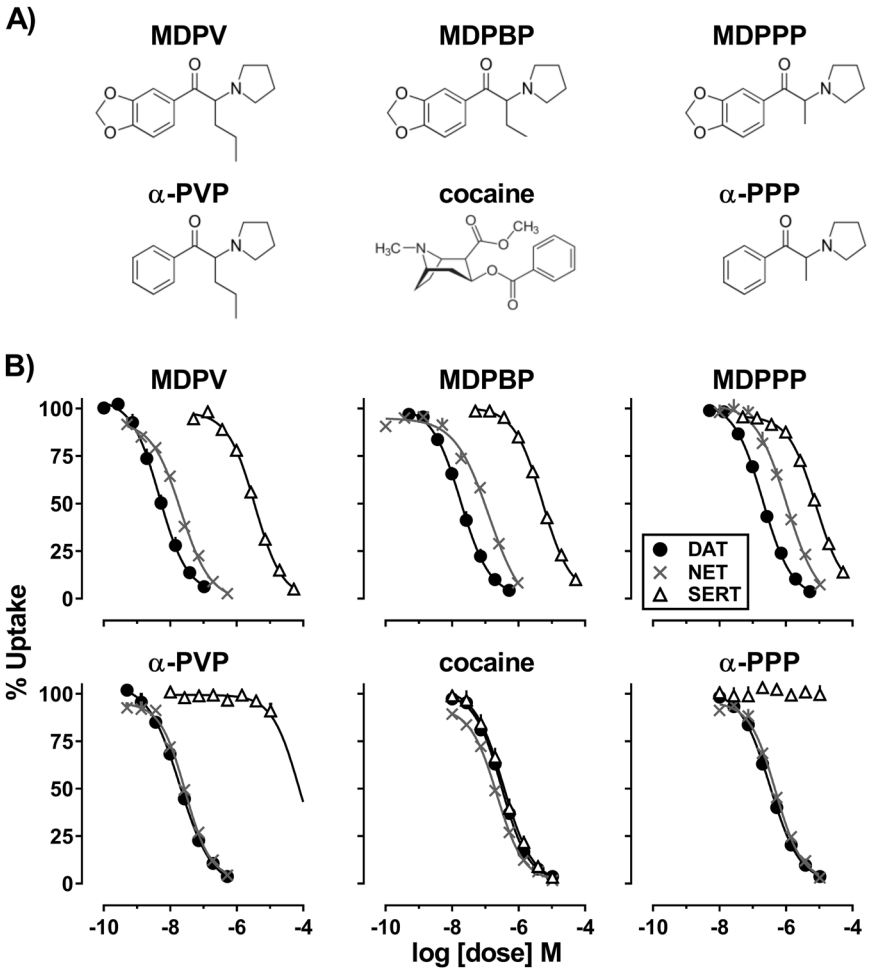

Fig. 1 **a** Chemical structures of MDPV, MDPBP, MDPPP, α-PVP, cocaine, and α-PPP, and **b** concentration–response curves for these drugs inhibit uptake of 5 nM [^{3}H]dopamine, 10 nM [^{3}H]norepinephrine, and 5 nM [^{3}H]serotonin at DAT (filled circles), NET (Xs), and SERT (open triangles), respectively. Data represent the mean ± SEM percentage of [^{3}H]transmitter uptake for $n = 3$ experiments

Table 1. Effects of test drugs on monoamine transporter inhibition in rat brain synaptosomes

Drug	Monoamine transporter IC_{50} (μM ± 95% CI)			DAT/SERT ratio[a]
	DAT	NET	SERT	
MDPV	0.0047 (0.003, 0.006)	0.021 (0.017, 0.025)	3.51 (2.9, 4.2)	751.6
MDPBP	0.018 (0.014, 0.023)	0.130 (0.074, 0.23)	5.10 (4.1, 6.4)	281.2
MDPPP	0.200 (0.16, 0.25)	1.05 (0.63, 1.76)	7.92 (5.4, 11.6)	39.6
α-PVP	0.019 (0.015, 0.025)	0.028 (0.023, 0.034)	74.47 (64.1, 86.8)	3819.4
α-PPP	0.332 (0.24, 0.46)	0.486 (0.40, 0.60)	349.9 (257.6, 475.3)[b]	1054.4[b]
Cocaine	0.305 (0.24, 0.40)	0.222 (0.19, 0.26)	0.30 (0.20, 0.40)	1.15

[a]DAT/SERT ratio = $[DAT\ IC_{50}]^{-1}/[SERT\ IC_{50}]^{-1}$ [b]estimated SERT IC50 values assuming a parallel inhibition would have occurred begining with the next largest concentration

were ~5-fold more potent at inhibiting uptake at DAT than at NET, selectivity for DAT over SERT varied with MDPV, MDPBP, and MDPPP being ~750-, ~280, and ~40-fold more potent at inhibiting uptake at DAT than at SERT, respectively (Fig. 1b and Table 1). Similar to MDPV, MDPBP, and MDPPP, α-PVP and α-PPP were most potent at inhibiting uptake at DAT; however, the absence of the methylenedioxy moiety significantly reduced their potencies to inhibit SERT, resulting in significantly greater selectivities for DAT over SERT, with α-PVP and α-PPP being ~3800- and ~1050-fold more potent at inhibiting uptake at DAT than at SERT, respectively (Fig. 1b and Table 1). Unlike the methylenedioxy-containing cathinones, which exhibited modest selectivity for DAT over NET, α-PVP and α-PPP were equipotent at inhibiting uptake at DAT and NET. Cocaine was roughly equipotent at inhibiting uptake at DAT, NET, and SERT (Fig. 1b and Table 1). Although the length of the α-alkyl carbon chain affected potency to inhibit uptake at DAT, NET, and SERT (i.e., longer chain length conferred greater potency; MDPV > MDPBP > MDPPP and α-PVP > α-PPP), this structural feature impacted potency at DAT and NET to a greater degree than potency at SERT. The presence of the methylenedioxy moiety

Table 2. Behavioral data for intravenous self-administration of the test drugs in male Sprague-Dawley rats

Drug	Progressive ratio		Demand curves	R^2
	ED_{50} (mg/kg/inf ± 95% CI)	E_{max} (infusions ± SEM)	α (±95% CI)	
MDPV	0.017 (0.016, 0.019)	25.3 ± 0.5	0.76×10^{-5} (0.71×10^{-5}, 0.81×10^{-5})	0.85
MDPBP	0.033 (0.021, 0.049)	23.0 ± 1.0	0.92×10^{-5} (0.88×10^{-5}, 0.95×10^{-5})	0.95
MDPPP	0.20 (0.12, 0.30)	22.8 ± 1.0	0.93×10^{-5} (0.89×10^{-5}, 0.98×10^{-5})	0.92
α-PVP	0.030 (0.024, 0.037)	29.2 ± 0.5	0.63×10^{-5} (0.60×10^{-5}, 0.66×10^{-5})	0.90
α-PPP	0.11 (0.065, 0.16)	25.3 ± 0.8	0.96×10^{-5} (0.93×10^{-5}, 1.00×10^{-5})	0.93
Cocaine	0.17 (0.15, 0.19)	20.9 ± 0.4	1.99×10^{-5} (1.90×10^{-5}, 2.09×10^{-5})	0.92

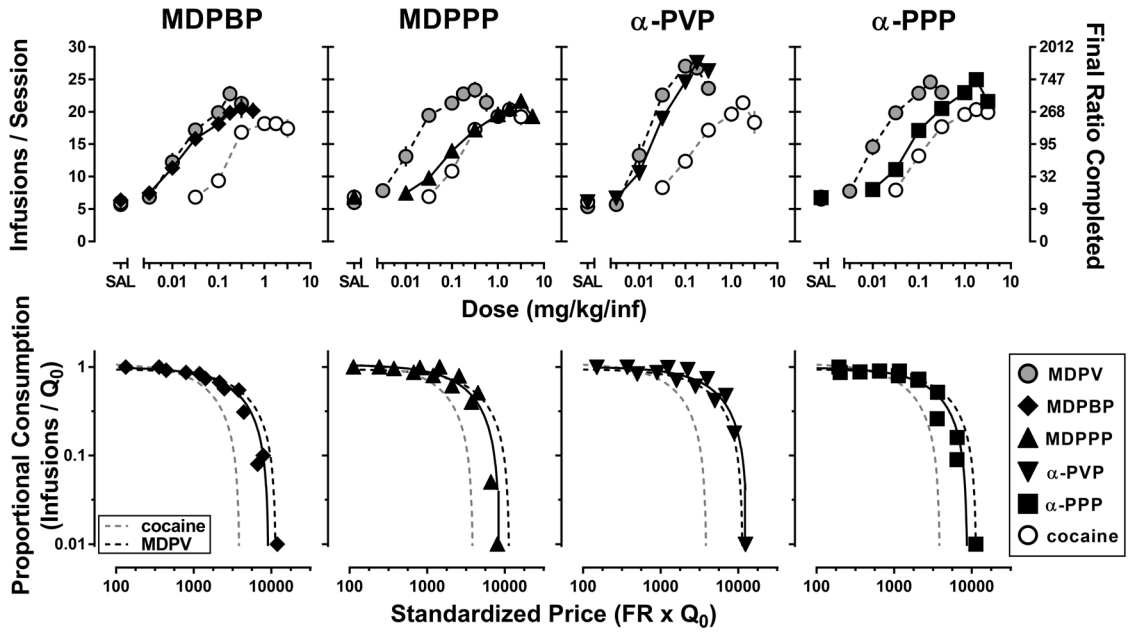

Fig. 2 Self-administration data for MDPV (gray circles [top]; black dashed lines [bottom]), cocaine (open circles [top]; gray dashed lines [bottom]), MDPBP (diamonds), MDPPP (upward triangles), α-PVP (downward triangles), and α-PPP (squares). Top row: Dose–response curves obtained under a PR schedule of reinforcement ($n = 12$ per panel), with data expressed as the mean ± SEM number of infusions earned as a function of the dose available for infusion. Abscissa: SAL represents data obtained when saline was available for infusion, whereas dose refers to the unit dose of each drug available for infusion expressed as mg/kg/infusion on a log scale. Left ordinate: total infusions obtained during the session. Right ordinate: final ratio completed during the session. Bottom row: Demand curve analyses of self-administration data obtained during 2-h sessions in which the FR response requirement was increased across sessions ($n = 11$). Abscissa: Standardized price of each drug presented on a log scale. Left ordinate: proportional consumption expressed on a log scale

also increased potency to inhibit SERT, with MDPV being ~20-fold more potent than α-PVP and MDPPP being ~40-fold more potent than α-PPP at inhibiting uptake at SERT. Rank order selectivity for uptake inhibition at DAT relative to SERT is α-PVP (~3800) > α-PPP (~1050) > MDPV (~750) > MDPBP (~280) > MDPPP (~40) > cocaine (~1) (Table 1).

Self-administration data are shown in Fig. 2, with dose–response curves generated under the PR schedule of reinforcement shown in the top row and demand curves shown in the bottom row. Dose-dependent increases in responding under the PR schedule were observed for each of the cathinones and cocaine, with a rank order reinforcing potency of MDPV > α-PVP ≈ MDPBP > α-PPP ≈ MDPPP ≈ cocaine (Table 2; ED_{50}). Differences were also observed among the cathinones with respect to the maximal number of infusions obtained (Table 2; E_{max}), with a rank order reinforcing effectiveness of α-PVP > MDPV ≈ α-PPP > MDPBP ≈ MDPPP > cocaine. Within-group analysis of E_{max} values by one-way repeated-measures ANOVA revealed main effects of drug for all groups of rats (MDPBP: $F[2,22] = 16.34$; $p < 0.0001$; MDPPP: $F[2,22] = 12.23$; $p < 0.001$; α-PVP: $F[2,22] = 51.3$; $p < 0.0001$; α-PPP: $F[2,22] = 18.38$; $p < 0.0001$), with post hoc tests indicating that MDPV, MDPBP, α-PVP, and α-PPP each maintained significantly greater levels of responding than cocaine ($p < 0.001$ for all), whereas the amount of responding maintained by MDPPP was not significantly different than cocaine. Although one-way ANOVA failed to identify a main effect of reinforcement history (i.e., acquisition of responding and FR5 dose–response curve for MDPBP, MDPPP, α-PVP, or α-PPP; [16]) on the reinforcing effectiveness of cocaine, a significant effect of reinforcement history was observed for MDPV ($F[3,44] = 5.28$; $p < 0.01$), an effect that appeared to be driven by MDPV maintaining significantly greater levels of responding in rats with a history of α-PVP self-administration relative to those with a history of MDPBP ($p < 0.01$) or MDPPP ($p < 0.05$) self-administration. Reinforcement history did not impact the potency of MDPV or cocaine to function as a reinforcer.

Results from the demand curve analyses of FR responding for MDPV, MDPBP, MDPPP, α-PVP, α-PVP, and cocaine are shown in Fig. 2 (bottom row). Because these data were collected in the same group of rats, the data for MDPV (black dashed line) and

cocaine (gray dashed line) are represented in each panel. For each drug, the data generated for both doses was able to be fit by a single curve, the R^2 of which are shown in Table 2, suggesting demand did not differ between the two doses evaluated for each drug. Comparisons of the elasticity coefficients (Table 2; *a*) from these best-fit curves indicate a rank order reinforcing effectiveness of α-PVP > MDPV > α-PPP ≈ MDPBP ≈ MDPPP > cocaine.

The results of correlation analyses investigating relationships between the effects of the drugs obtained from in vitro uptake inhibition assays (potency and selectivity) and the self-administration assays (potency and effectiveness) are shown in Fig. 3. The potencies (ED_{50}s) of the drugs to reinforce responding under a PR schedule of reinforcement were positively correlated with their potencies to inhibit uptake at DAT ($R^2 = 0.91$; $p < 0.01$) and NET ($R^2 = 0.81$; $p < 0.05$) but not potency to inhibit uptake at SERT ($R^2 = 0.01$) or selectivity for DAT relative to SERT ($R^2 = 0.47$) (Fig. 3, top row). With regard to measures of reinforcing effectiveness obtained from the PR schedule (E_{max}), there were weak negative correlations with potencies to inhibit uptake at DAT ($R^2 = 0.21$) and NET ($R^2 = 0.34$), a weak positive correlation with potencies to inhibit uptake at SERT ($R^2 = 0.52$), and a significant positive correlation between E_{max} values and selectivities for DAT relative to SERT ($R^2 = 0.78$; $p < 0.05$) (Fig. 3, middle row). Similarly, when *a* values obtained from the demand curve analyses are converted to essential values (a^{-1}), weak negative correlations are observed with potencies to inhibit uptake at DAT ($R^2 = 0.47$) and NET ($R^2 = 0.33$), a weak positive correlation is observed with potencies to inhibit uptake at SERT ($R^2 = 0.34$), and a significant positive correlation between measures of essential value and selectivities for DAT relative to SERT ($R^2 = 0.82$; $p < 0.05$) was observed (Fig. 3, bottom row).

DISCUSSION

Since 2009, >700 new psychoactive substances have appeared in recreational drug markets worldwide, with synthetic cathinones accounting for 1 in 5 or ~140 of these compounds [1]. Similar to other illicit stimulant drugs of abuse, synthetic cathinones interact with monoamine transporters (i.e., DAT, NET, and SERT), where they function as either cocaine-like inhibitors of monoamine uptake or amphetamine-like substrates for monoamine transporters. Importantly, the reinforcing effects (and abuse liability) of both inhibitors and substrates of monoamine transporters have been hypothesized to be related to their interactions at DAT. Although in vitro studies have indicated that novel synthetic cathinones vary in their potency and selectivity for DAT (e.g., refs. [2–7]), the abuse liability of many of these cathinones remain unknown. We have recently reported that a series of MDPV-like synthetic cathinones that contain a pyrrolidine ring (i.e., MDPBP, MDPPP, α-PVP, and α-PPP) all function as reinforcers in rats [16]. The current studies confirm and extend these findings by directly comparing the potency and effectiveness of MDPV, MDPBP, MDPPP, α-PVP, α-PPP, and cocaine to inhibit uptake at DAT, NET, and SERT and to function as a reinforcer in rats. There were three main findings: (1) each of the

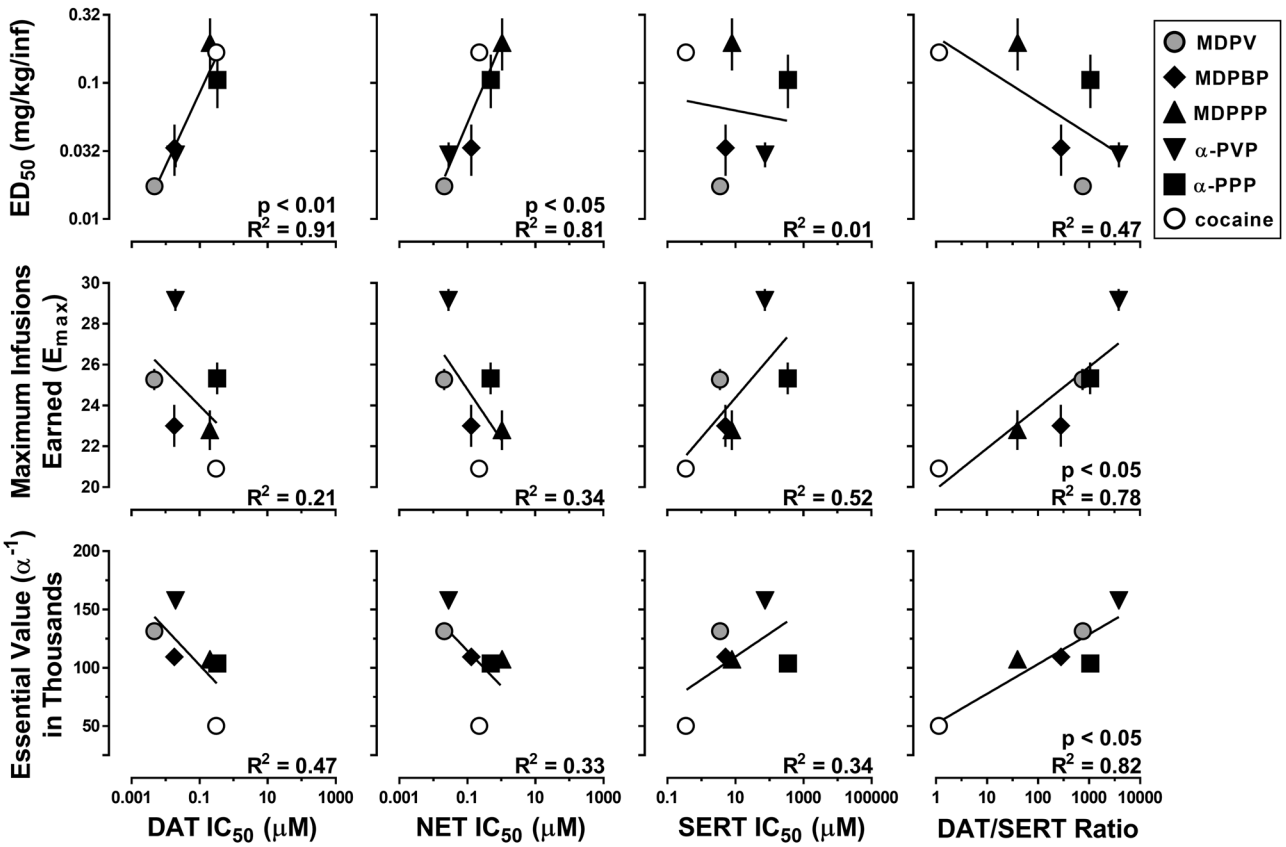

Fig. 3 Correlational analyses of the relationships between reinforcing effects and monoamine transporter activity for MDPV (gray circles), cocaine (white circles), MDPBP (diamonds), MDPPP (upward triangles), α-PVP (downward triangles), and α-PPP (squares). Abscissa: Top row: mean ± SEM reinforcing potency (mg/kg/inf) determined from dose–response curves generated under a PR schedule of reinforcement. Middle row: mean ± SEM reinforcing effectiveness (E_{max}) determined from dose–response curves generated under a PR schedule of reinforcement. Bottom row: mean ± SEM reinforcing effectiveness (essential value) as determined from demand curve analyses of self-administration under a FR schedule of reinforcement. Ordinate: Left column: potency to inhibit [^{3}H]dopamine uptake at DAT. Center column: potency to inhibit [^{3}H]norepinephrine uptake at NET. Third column: potency to inhibit [^{3}H]serotonin uptake at SERT. Right column: selectivity for uptake inhibition at DAT relative to SERT

synthetic cathinones were more potent at inhibiting DAT than NET or SERT, with a rank order for selectivity at DAT over SERT of α-PVP > α-PPP > MDPV > MDPBP > MDPPP > cocaine; (2) each of the synthetic cathinones were more effective reinforcers than cocaine as evidenced by their capacity to maintain responding at larger ratios, regardless of whether the response requirement was increased within (PR) or across sessions (behavioral economic demand curve analyses); and (3) that the reinforcing potency of these drugs was positively correlated with their potency to inhibit uptake at DAT, whereas their reinforcing effectiveness was positively correlated with their selectivity to inhibit uptake at DAT relative to SERT. Together, these findings not only add to a literature demonstrating that synthetic cathinones can be highly effective reinforcers but also provide strong evidence that the reinforcing effectiveness of monoamine uptake inhibitors is primarily mediated by their capacity to inhibit uptake (or stimulate release) at DAT, with activity at SERT serving to negatively modulate their reinforcing effectiveness.

The in vitro functional profiles obtained in the current study are consistent with previous reports [5, 27, 28] and confirm that MDPV, MDPBP, MDPPP, α-PVP, and α-PPP all function as cocaine-like inhibitors of monoamine uptake at DAT, NET, and SERT. Importantly, within this series of compounds, potency to inhibit uptake at DAT was primarily influenced by length of the α-alkyl side chain (e.g., MDPV > MDPBP > MDPPP), whereas the presence of a methylenedioxy moiety conferred increased potency to inhibit uptake at SERT (e.g., MDPV > α-PVP). Although potency to inhibit uptake at NET was primarily influenced by the length of the α-alkyl side chain, the absence of the methylenedioxy moiety also resulted in modest increases in potency to inhibit uptake at NET. Consistent with our previous work showing that the reinforcing potency of pyrrolidine-containing cathinones (MDPBP, MDPPP, α-PVP, and α-PPP) was related to their potency to inhibit DAT and NET [16], the potency of the five cathinone analogs and cocaine to maintain responding under a PR schedule of reinforcement was correlated with their potency to inhibit uptake at DAT ($p < 0.01$, $R^2 = 0.91$) and to a lesser degree at NET ($p < 0.05$, $R^2 = 0.81$). Although the presence of a methylenedioxy moiety increased potency to inhibit uptake at SERT, these actions were not related to their reinforcing potency. Together, these findings suggests that reinforcing potency (i.e., ED_{50} values) of drugs that interact with monoamine transporters is primarily determined by their capacity to inhibit uptake (or stimulate release) at DAT, as has been proposed by others (e.g., refs. [20, 21, 24]). Despite consistent evidence linking the reinforcing effects of stimulants to their actions at DAT, the present studies show that potency to inhibit uptake at DAT is insufficient to explain differences in reinforcing effectiveness (i.e., E_{max} and essential value), at least among the six drugs that were examined here.

Instead, it has been hypothesized that the relative reinforcing effectiveness of drugs that interact with monoamine transporters is influenced by not only their actions at DAT but also at SERT [22, 24, 34, 35]. Indeed, consistent evidence from studies using analogs of cocaine and amphetamine with increased SERT activity, or mixtures of amphetamine and fenfluramine, suggests that reducing selectivity for DAT relative to SERT results in drugs that are less effective reinforcers than cocaine or amphetamine [22, 24, 36]. Similar conclusions were drawn from a recent study that found a positive correlation between the selectivity of methcathinone analogs to stimulate release at DAT relative to SERT and their effectiveness to facilitate intracranial self-stimulation [37]. Collectively, these studies provide convergent evidence that drugs with greater selectivity for DAT relative to SERT would be predicted to function as more effective reinforcers; however, it is important to note that the most selective compounds to support this hypothesis were only 300-fold selective for DAT over SERT [22, 24, 36, 37]. By directly comparing the reinforcing effectiveness of a series of six monoamine uptake inhibitors that vary in their selectivity for DAT over SERT from ~1-fold (cocaine) to over 3800-fold (α-PVP), the current study extends this general line of inquiry in important ways and provides strong evidence that selectivity for DAT relative to SERT is a primary determinant of the abuse liability of synthetic cathinones and other drugs that interact with monoamine transporters.

Reinforcing effectiveness is typically assessed by systematically increasing the response requirements until the subject fails to earn a reinforcer. The current study used two different procedures to quantify the relative reinforcing effectiveness of the five cathinones and cocaine, one that increased the response requirement within a single session (i.e., a PR schedule of reinforcement), and another that increased the response requirement across multiple sessions (i.e., demand curve analyses). In the PR studies, the rank order for number of infusions earned (E_{max}) was α-PVP > α-PPP ≈ MDPV > MDPBP ≈ MDPPP ≥ cocaine, which positively correlated with the selectivity of the drugs to inhibit uptake at DAT relative to SERT ($p < 0.05$, $R^2 = 0.78$). However, it is worth noting that the MDPV and cocaine E_{max} values used in these correlations were based on the total group ($n = 48$) mean and that the E_{max} for MDPV varied as a function of reinforcement history (i.e., it was greater in rats that had self-administered α-PVP and α-PPP). In an attempt to account for the influence of drug history, correlations between selectivity for DAT over SERT and relative reinforcing effectiveness were also performed using data that were normalized to E_{max} of MDPV for individual subjects (i.e., E_{max}[test drug]/E_{max}[MDPV]), which resulted in an even stronger correlation between reinforcing effectiveness and selectivity for DAT over SERT ($p < 0.001$, $R^2 = 0.995$; Figure S1). Although these findings provide strong support for the hypothesis that selectivity for DAT over SERT is indeed an important determinant of reinforcing effectiveness, a variety of factors have been shown to influence responding under PR schedules of reinforcement, including drug accumulation, pharmacokinetic properties of the drug(s), and schedule parameters [22, 38–40], all of which can complicate conclusions about reinforcing effectiveness.

In an attempt to control for these potential confounds, demand curve analyses were also used to quantify the relative reinforcing effectiveness (essential value; α^{-1}) of each of the six drugs in a single group of rats with similar drug histories. By increasing the response requirement across rather than within sessions, it is possible to minimize the influence of drug intake occurring early in the session on responding later in the session (i.e., when PR assessments of E_{max} are made). Although there were slight differences in the rank order of essential value (α^{-1}) (α-PVP > MDPV > α-PPP ≈ MDPBP ≈ MDPPP > cocaine) and E_{max}, these two measures of relative reinforcing effectiveness were not only positively correlated with each other ($p < 0.05$, $R^2 = 0.79$; Figure S2) but a similar positive correlation was also observed between the behavioral economic measures of reinforcing effectiveness and selectivity to inhibit uptake at DAT relative to SERT ($p < 0.05$, $R^2 = 0.82$).

Although the present data are consistent with the hypothesis that selectivity for DAT over SERT is a primary determinant of the reinforcing effectiveness of stimulant drugs, they do not rule out a potential contribution of adrenergic systems to the effectiveness of these drugs to function as reinforcers. Despite the fact that significant correlations were observed between reinforcing potency and potency to inhibit NET, this was most likely due to the fact that the six drugs evaluated in the present study are not particularly selective for DAT relative to NET (0.7–7-fold selective), rather than a role of norepinephrine in stimulant self-administration. Indeed, the inability of desipramine or nisoxetine, two selective uptake inhibitors of NET, to modify cocaine self-administration [41, 42] suggests that inhibition of NET is not a primary determinant of the reinforcing effects of cocaine. Nevertheless, further studies are needed to rule out a potential contribution of NET to the reinforcing effects of MDPV and other pyrrolidine-containing synthetic cathinones.

The rate at which new psychoactive substances, including synthetic cathinones, are appearing on the world drug market has created an ever-evolving threat to public health and unique challenges to regulatory organizations. The present study confirms our previous findings that MDPV is a ~10-fold more potent and ~3-fold more effective reinforcer than cocaine in rats [13] and extended this work to include a direct comparison of five synthetic cathinones and cocaine with regard to their potency to inhibit uptake at DAT, NET, and SERT, as well as their relative reinforcing effects in rats. These studies showed that: (1) each of the pyrrolidine-containing cathinones were more potent inhibitors of DAT and NET than SERT, with functional selectivities at DAT relative to SERT ranging from ~40- to ~3800-fold; (2) each of the synthetic cathinones functioned as a more effective reinforcer than cocaine as evidenced by their maintaining higher levels of responding (E_{max}) under a PR schedule of reinforcement and significantly greater measures of demand (essential value) across a range of doses and FR values; and (3) that the reinforcing potency of these drugs was positively correlated with their potency to inhibit uptake at DAT, whereas their reinforcing effectiveness was positively correlated with their selectivity to inhibit uptake at DAT relative to SERT. Together, these findings provide new and important information about the abuse liability of a series of synthetic cathinones identified in "bath salts" preparations and provide strong evidence in support of the hypothesis that the reinforcing effects of drugs that interact with monoamine transporters are primarily mediated by their actions at DAT, with activity at SERT serving to inhibit/dampen their effectiveness to function as a reinforcer.

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ADDITIONAL INFORMATION

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