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Abuse-related neurochemical and behavioral effects of cathinone and 4-methylcathinone stereoisomers in rats

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Abstract

Cathinone and many of its analogs produce behavioral effects by promoting transporter-mediated release of the monoamine neurotransmitters dopamine, norepinephrine and/or serotonin. Stereoselectivity is one determinant of neurochemical and behavioral effects of cathinone analogs. This study compared effectiveness of the *S*(–) and *R*(+) enantiomers of cathinone and 4-methylcathinone to produce in vitro monoamine release and in vivo abuse-related behavioral effects in rats. For neurochemical studies, drug effects were evaluated on monoamine release through dopamine, norepinephrine, and serotonin transporters (DAT, NET and SERT, respectively) in rat brain synaptosomes. For behavioral studies, drug effects were evaluated on responding for electrical brain stimulation in an intracranial self-stimulation (ICSS) procedure. The cathinone enantiomers differed in potency [*S*(–) > *R*(+)], but both enantiomers were >50-fold selective at promoting monoamine release through DAT vs. SERT, and both enantiomers produced ICSS facilitation. The 4-methylcathinone enantiomers also differed in potency [*S*(–) > *R*(+)] however, in neurochemical studies, the decrease in potency from *S*(–) to *R*(+) 4-methylcathinone was less for DAT than for SERT, and as a result, DAT vs. SERT selectivity was greater for *R*(+) than for *S*(–) 4-methylcathinone (4.1- vs. 1.2-fold). Moreover, in behavioral studies, *S*(–) 4-methylcathinone produced only ICSS depression, whereas *R*(+) 4-methylcathinone produced ICSS facilitation. This study provides further evidence for stereoselectivity in neurochemical and behavioral actions of cathinone analogs. More importantly, stereoselective 4-methylcathinone effects on ICSS illustrate

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Author contributions

Hutsell completed ICSS studies and data analyses and, with Negus and Banks, assumed primary responsibility for compiling and writing the paper. All other authors contributed to manuscript preparation and review of initial drafts. In addition, Glennon and Vekariya synthesized compounds for study (*S*(–) 4-methylcathinone and *R*(+) 4-methylcathinone). Partilla and Baumann completed in vitro release assays and provided methods and results for in vitro studies. Banks and Negus aided in experimental design.

Conflict of interest

The authors declare no conflict of interest.

the potential for diametrically opposite effects of enantiomers in a preclinical behavioral assay of abuse potential.

Keywords

Cathinone; 4-Methylcathinone; Stereoselectivity; Intracranial self-stimulation; Rat; Drug abuse

1. Introduction

4-Methyl-*N*-methylcathinone (mephedrone) is a para-methyl methcathinone analog that has appeared in Europe and the United States as a common component of designer drug formulations known by names such as “bath salts” or “research chemicals”. Concern over abuse of these compounds has stimulated research to investigate mechanisms that underlie their abuse liability, and this work has led to three general conclusions. First, like its parent compound methcathinone, and like more commonly abused stimulants such as methamphetamine, mephedrone functions as a substrate at dopamine (DA), norepinephrine (NE) and serotonin (5HT) transporters (DAT, NET and SERT, respectively) and promotes activity-independent neuronal release of DA, NE and 5HT both in vitro and in vivo (Rothman et al., 2001; Baumann et al., 2012). Second, the abuse potential of monoamine releasers appears to depend in part on their selectivity to promote release via DAT vs. SERT, such that DAT-selective compounds possess higher abuse potential than non-selective or SERT-selective compounds (Wee et al., 2005; Bauer et al., 2013; Negus and Miller, 2014). Consistent with this general correlation, racemic mephedrone is a relatively non-selective substrate at DAT and SERT in comparison to more DAT-selective compounds such as methcathinone and methamphetamine (Rothman et al., 2001; Baumann et al., 2012; Cozzi et al., 2013), and relative to DAT-selective releasers, mephedrone produces more variable reinforcing effects across subjects in assays of self-administration (Aarde et al., 2013; Motbey et al., 2013; Creehan, Vandewater, and Taffe 2015), and weaker evidence for abuse-related effects in assays of intracranial self-stimulation (ICSS) (Bonano et al., 2014; Robinson et al., 2012).

Finally, a growing body of literature suggests that stereochemistry is a determinant of abuse-related effects of monoamine releasers. Specifically, methcathinone, methamphetamine, and many of their analogs possess a single chiral carbon atom (the α carbon; Figure 1), and the *S* enantiomer of these compounds is typically more potent and/or effective than the *R* enantiomer to produce abuse-related behavioral effects in assays of drug self-administration, drug discrimination or ICSS (Balster and Schuster, 1973; Bauer et al., 2013; Glennon et al., 1984; Johanson and Schuster, 1981; Yanagita, 1986) and to promote DA release via DAT (Rothman et al., 2001, 2003). However, recent studies suggest a potentially more nuanced role for stereochemistry in abuse-related effects of mephedrone. Specifically, the *R*(+) enantiomer of mephedrone is more effective than the *S*(-) enantiomer to produce locomotor activation, conditioned place preference, and facilitation of ICSS in rats (Gregg et al., 2015). Neurochemical evidence suggests that this apparent inversion of stereochemistry results from an unusual stereoselectivity not only in potency, but also in selectivity as a substrate at DAT vs. SERT. Thus, *R*(+) mephedrone is slightly more potent than its *S*(-) enantiomer in

promoting monoamine release via DAT but much less potent at SERT. As a result, the *R*(+) enantiomer displays a 50-fold greater selectivity than the *S*(-) enantiomer to promote monoamine release via DAT vs. SERT, and this stereoselectivity in neurochemical effects contributed to stereoselectivity in expression of abuse-related behavioral effects.

The purpose of the present study was to extend this evaluation of stereochemistry as a determinant of cathinone analog effects by comparing enantiomers of cathinone and 4-methylcathinone (Figure 1). 4-methylcathinone is a metabolite of mephedrone formed by hepatic biotransformation (Pedersen et al., 2013; Martínez-Clemente et al., 2013) and is reportedly present in certain designer drug formulations available on the internet (Musshoff et al., 2012). Here, the enantiomers of cathinone and 4-methylcathinone were compared in both (1) an in vitro assay of transporter-mediated monoamine release and (2) an in vivo assay of ICSS in rats identical to the assays used previously to assess stereoselectivity of neurochemical and behavioral effects of mephedrone (Baumann et al., 2012; Bonano et al., 2015; Gregg et al., 2015). We hypothesized that cathinone would display the more common profile of monoamine releaser stereoselectivity, with higher potency for the *S*(-) than *R*(+) enantiomer to produce both monoamine release via DAT and abuse-related facilitation of ICSS. Conversely, we hypothesized that, as with mephedrone, the *R*(+) enantiomer of 4-methylcathinone would display higher DAT vs. SERT selectivity and greater abuse-related effects in ICSS than the *S*(-) enantiomer.

2. Experimental procedures

2.1. In vitro assay of monoamine release

2.1.1. Subjects—Adult male Sprague-Dawley rats (Harlan, Frederick, MD, USA) weighing 250–350 g were housed three per cage with free access to food and water and maintained on a 12-h light/dark cycle with lights on from 7:00 a.m. to 7:00 p.m. Animal facilities were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, and procedures were carried out in accordance with the Institutional Animal Care and Use Committee and the National Institutes of Health guidelines on care and use of animal subjects in research (National Research Council, 2011).

2.1.2. Procedure—Potencies and selectivities of *S*(-)cathinone, *R*(+)cathinone, *S*(-) 4-methylcathinone, and *R*(+)4-methylcathinone to evoke monoamine release via rat monoamine transporters (SERT, NET, and DAT) were determined in rat brain synaptosomes as previously described (Baumann et al., 2012). Rats were euthanized with CO₂, decapitated, and brains were rapidly removed and dissected on ice. Synaptosomes were prepared from striatum for DAT assays, whereas synaptosomes were prepared from whole brain minus striatum and cerebellum for the NET and SERT assays. [³H]1-Methyl-4-phenylpyridinium ([³H]MPP+) (9 nM) was used as the radiolabeled substrate for DAT and NET, whereas [³H]5-HT (5 nM) was used as a substrate for SERT. All buffers used in the release assays contained 1 μM reserpine to block vesicular uptake of substrates. The selectivity of assays was optimized for a single transporter by including unlabeled compounds (nomifensine and GBR12935 for SERT; GBR12935 and citalopram for NET; citalopram and desipramine for DAT) to prevent the uptake of [³H]MPP+ or [³H]5-HT by

competing transporters. Synaptosomes were preloaded with radiolabeled substrate in Krebs-phosphate buffer for 1 h (steady state). Assays were initiated by adding 850 μ L of preloaded synaptosomes to 150 μ L of test drug. Assays were terminated by vacuum filtration, and retained radioactivity was quantified by liquid scintillation counting.

2.1.3. Data analysis—Effects of test drug concentrations were expressed as % Maximum Release, with maximum release (i.e., 100% $E_{\max}$) defined as the release produced by 10 nM tyramine for DAT and NET assay conditions, and 100 nM tyramine for SERT assay conditions. These doses of tyramine evoke the efflux of all “releasable” tritium from synaptosomes as determined previously (Baumann et al., 2012). EC_{50} values were determined using nonlinear least-squares curve fitting (GraphPad Prism, San Diego, CA). Transporter selectivities were calculated as ratios of EC_{50} values to promote release of labeled monoamine via DAT vs. NET (EC_{50} at NET/ EC_{50} at DAT) and for DAT vs. SERT (EC_{50} at SERT/ EC_{50} at DAT), such that larger ratios indicate higher DAT selectivity.

2.2. In vivo assay of intracranial self-stimulation

2.2.1. Subjects—Eight adult male Sprague-Dawley rats (Harlan, Frederick, MD, USA) weighing 398–444 g at the time of surgery were individually housed and maintained on a 12-h light/dark cycle with lights on from 6:00 a.m. to 6:00 p.m. Rats had free access to food and water outside of experimental sessions. Animal facilities were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, and procedures were carried out in accordance with the Institutional Animal Care and Use Committee and the National Institutes of Health guidelines on care and use of animal subjects in research (National Research Council, 2011).

2.2.2. Surgery—Rats were anesthetized with isoflurane (2.5–3% in oxygen; Webster Veterinary, Phoenix, AZ, USA) until unresponsive to toe pinch prior to implantation of stainless steel bipolar electrodes (Plastics One, Roanoke, VA, USA). The cathode of each electrode was 0.25 mm in diameter and covered with polyamide insulation except at the flattened tip, and the anode was 0.125 mm in diameter and uninsulated. The cathode was stereotactically implanted into the left medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior to the bregma, 1.7 mm lateral to midsagittal suture, 8.8 mm ventral to the skull). Three screws were placed in the skull, and the anode was wrapped around one screw to serve as the ground. The skull screws and electrode were secured to the skull with dental acrylic. Ketoprofen (5 mg/kg) was used for postoperative analgesia immediately and 24 h after surgery. Rats were allowed 7 recovery days prior to ICSS training.

2.2.3. Apparatus—Experimental sessions were conducted in sound-attenuating boxes containing modular acrylic and metal test chambers equipped with a response lever, three stimulation lights (red, yellow, and green) above the lever, a 2-W house light, and an ICSS stimulator (Med Associates, St. Albans, VT, USA). Electrodes were connected to the stimulator via bipolar cables routed through a swivel commutator (Model SL2C, Plastics One, Roanoke, VA, USA). Control of experimental events and acquisition of data were

accomplished with a computer operated by Med-PC IV software and connected to test chambers by an interface system (Med Associates).

2.2.4. Training procedure—Following initial shaping of lever pressing, rats were trained under a fixed-ratio 1 (FR 1) schedule of brain stimulation reinforcement using a previously described “frequency-rate” procedure (Gregg et al., 2015; Negus and Miller, 2014). Each lever press resulted in the delivery of a 0.5-s train of square wave cathodal pulses (0.1 ms per pulse) and illumination of the stimulus lights above the lever. Stimulation intensity and frequency were initially set at 150 μ A and 2.1 log Hz, respectively, during 30–60-min training sessions. Stimulation intensity was then adjusted on an individual basis to the lowest value that sustained ICSS rates >30 stimulations/min. This intensity (110–260 μ A across rats) was subsequently held constant for the remainder of the study, and frequency manipulations were introduced. Sessions involving frequency manipulations consisted of three sequential 10-min components. During each component, a descending series of ten frequencies (2.2–1.75 log Hz in 0.05 log increments) was presented, with each stimulation frequency available for a 60-s trial. Each frequency trial consisted of a 10-s timeout, during which five response-independent “priming” stimulations were delivered at the stimulation frequency available during that trial, followed by a 50-s “response” period, during which responding produced electrical stimulation under a FR 1 schedule. Training continued until rats responded at reinforcer rates greater than 50% of the maximal control rate (see below) for the first three to six frequency trials of each component over a period of at least three consecutive training days.

2.2.5. Testing procedure—Test sessions consisted of three sequential “baseline” components followed by a 30-min time-out period and then by two sequential “test” components. A single test drug dose was administered by intraperitoneal (i.p.) injection at the beginning of the time-out period. Test sessions were conducted on Tuesdays and Fridays, and training sessions were conducted on all other weekdays. Dose ranges were based on previous research with monoamine releasers (Bauer et al., 2013; Bonano et al. 2014) and initial empirical results. Dose-effect functions for *S*(–)cathinone (0.1–1.0 mg/kg), *R*(+)cathinone (0.32–10.0 mg/kg), *S*(–)4-methylcathinone (0.32–3.2 mg/kg), and *R*(+)4-methylcathinone (1.0–3.2 mg/kg) were determined in groups of five to six rats. The testing order for vehicle and drug doses was varied across subjects using a Latin-square design, and higher doses *R*(+)4-methylcathinone were not tested due to limited compound availability. All studies with one compound were completed before initiation of testing with another compound. Test sessions were conducted on Tuesdays and Fridays, and training sessions were conducted on all other weekdays. Two rats lost their headcaps after completion of testing with 4-methylcathinone enantiomers and were replaced prior to testing with cathinone enantiomers.

2.2.6. Data analysis—The primary dependent measure was reinforcer rate in stimulations per minute during each frequency trial. Raw reinforcer rates from each trial were converted to percent maximum control rate (% MCR), with maximum control rate defined as the mean of the maximal rates obtained at any frequency during the second and third baseline components for that day in that rat. Thus, %MCR values for each trial were calculated as

(reinforcement rate during a frequency trial $\div$ MCR) $\times 100$. For each test session, data from the second and third baseline components were averaged to yield a baseline frequency–rate curve, and data from test components were averaged to generate test frequency–rate curves. Baseline and test curves were then averaged across rats to yield mean baseline and test curves for each manipulation. Group mean frequency–rate curves were analyzed by repeated measures two-way ANOVA with ICSS frequency and drug dose as factors. A significant ANOVA was followed by the Holm-Sidak multiple comparisons post-hoc test. The criterion for statistical significance was set at the 95% confidence level ($P < 0.05$). All analyses were conducted using Prism 6.0c for Mac (GraphPad Software, La Jolla, CA).

As a summary measure of drug effects, the total number of stimulations per component was also calculated and converted to percent total baseline stimulations per component. Test data were normalized to individual baseline data expressed as percent baseline total stimulations per component = (mean total stimulations per test component) / (mean total stimulations per baseline component) $\times 100$. Data were then averaged across rats for each drug dose. Maximal increases in this measure of ICSS produced by the two enantiomers of cathinone were compared by *t*-test, and as above, the criterion for significance was $P < 0.05$. Maximal effects of 4-methylcathinone enantiomers were not compared in this way, because enantiomers produced qualitatively different effects.

2.3. Drugs

S(–)cathinone HCl and *R*(+)cathinone HCl were provided by the National Institute on Drug Abuse (Bethesda, MD) Drug Supply Program. The known *S*(–)4-methylcathinone HCl was synthesized according to a published procedure (Osorio-Olivares et al., 2003). Spectral data were consistent with the proposed structure; its melting point (220–225 °C) and optical rotation ($[\alpha]_D^{28} = -36.7^\circ$, *c* 1, MeOH) were slightly higher than previously reported (mp=192–193 °C; $[\alpha]_D^{28} = -32^\circ$). The previously unreported *R*(+)4-methylcathinone HCl was prepared in a similar manner by acid hydrolysis of its corresponding trifluoroacetamide (mp=220–225 °C; $[\alpha]_D^{28} = +34^\circ$, *c* 1 MeOH) and analyzed (Atlantic Microlabs, Norcross, GA, USA) within 0.4% of theory for C, H, and N. [³H]5-HT (specific activity=30 Ci mmol^{–1}) was purchased from Perkin Elmer (Shelton, CT, USA). [³H] MPP⁺ (specific activity=85 Ci mmol^{–1}) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). All other chemicals and reagents were acquired from Sigma-Aldrich (St. Louis, MO, USA). For the ICSS studies, compounds were dissolved in sterile saline and administered i.p at a volume of 1 mL/kg, and drug doses are expressed as the salt forms listed above.

3. Results

3.1. In vitro assay of monoamine release

Figure 2 shows dose–effect curves for cathinone and 4-methylcathinone enantiomers to evoke monoamine release via DAT, NET and SERT in rat brain synaptosomes. EC₅₀ values for each compound at each transporter are shown in Table 1. *S*(–)cathinone was more potent than *R*(+)cathinone to promote monoamine release via all three transporters; however,

selectivities of cathinone enantiomers were similar in that both enantiomers displayed highest potencies at NET and DAT and >54-fold lower potency at SERT. The highest concentration of *R*(+)cathinone (10 μ M) did not stimulate 5HT release, and, as a result, a precise DAT vs. SERT selectivity for *R*(+) cathinone was not calculated. As with cathinone, the *S*(-) enantiomer of 4-methylcathinone was more potent than the *R*(+) enantiomer at all three transporters; however, the potency loss from the *S*(-) to the *R*(+) enantiomer was smaller at DAT than at SERT, and as a result, the *R*(+) enantiomer displayed greater DAT vs. SERT selectivity (4.1 fold) than the *S*(-) enantiomer (1.2 fold).

3.2. In vivo assay of intracranial self-stimulation

For the eight rats in ICSS experiments, the mean $\pm$ SEM baseline MCR was 54.5 $\pm$ 5.7 stimulations per trial, and the mean $\pm$ SEM number of total baseline stimulations was 261.7 $\pm$ 49.9 stimulations per component. After vehicle treatment, brain-stimulation reinforcement maintained a frequency-dependent increase in ICSS rates (Figures 3a, b, and 4a, b), and frequency-rate curves after vehicle treatment were similar to baseline performance before vehicle treatment (data not shown). Figure 3 shows effects of *S*(-) cathinone (0.32–10 mg/kg) and *R*(+)cathinone (0.1–1.0 mg/kg). *S*(-)cathinone (frequency $\times$ dose: $F(27,135)=2.76$, $P<0.0001$; statistically significant difference at doses 0.32 mg/kg) was approximately 10-fold more potent than *R*(+)cathinone (frequency $\times$ dose: $F(36,180)=2.17$, $P=0.0005$; statistically significant difference at doses 3.2 mg/kg); however, both enantiomers produced qualitatively similar facilitation of low ICSS rates maintained by low brain stimulation frequencies without reducing high ICSS rates maintained by high brain stimulation frequencies (maximal ICSS facilitation by *S*(-) vs. *R*(+)cathinone, $t(5) = 2.53$, $P>0.05$).

Figure 4 shows effects of *S*(-)4-methylcathinone (0.32–3.2 mg/kg) and *R*(+)4-methylcathinone (1.0–3.2 mg/kg). As with cathinone, the *S*(-) enantiomer of 4-methylcathinone (frequency $\times$ dose: $F(27,135)=3.66$, $P<0.0001$; statistically significant effects at doses 1.0 mg/kg) was more potent than the *R*(+) enantiomer (frequency $\times$ dose: $F(18,72)=2.28$, $P=0.0073$; statistically significant effects at 3.2 mg/kg). However, in contrast to cathinone, the enantiomers of 4-methylcathinone produced qualitatively opposite effects. Specifically, *S*(-)4-methylcathinone produced a dose-dependent depression of high ICSS rates maintained by high brain stimulation frequencies with no evidence of facilitation of low ICSS rates maintained by low brain stimulation frequencies. Conversely, *R*(+)4-methylcathinone produced only dose-dependent facilitation of ICSS across the dose range tested.

4. Discussion

This study compared in vitro neurochemical and in vivo behavioral effects of the enantiomers of cathinone and 4-methylcathinone in rats. There were three main findings. First, addition of a para-methyl (i.e., 4-methyl) substituent to the cathinone scaffold produced a decrease in potency to promote monoamine release at DAT, an increase in potency to promote monoamine release at SERT, and an overall decrease in DAT vs. SERT selectivity. Second, for cathinone, the *S*(-) enantiomer displayed higher in vitro and in vivo

potency than the *R*(+) enantiomer, but both enantiomers displayed similarly high in vitro selectivity for DAT vs. SERT, and similar in vivo effectiveness to facilitate ICSS. Thus, for cathinone, there was a stereoselectivity in drug potency but not in the quality of drug effect. Third, for 4-methylcathinone, the *S*(-) enantiomer also displayed higher in vitro and in vivo potency than the *R*(+) enantiomer, but there was stereoselectivity not only in drug potency, but also in the quality of drug effect. Specifically, the *S*(-) enantiomer displayed no DAT vs. SERT selectivity in vitro and only depressed ICSS in vivo, whereas the *R*(+) enantiomer displayed modest DAT vs. SERT selectivity in vitro and facilitated ICSS in vivo.

4.1. Comparison of cathinone and 4-methylcathinone

Cathinone and methcathinone are the β -ketone derivatives of amphetamine and methamphetamine, respectively, and like their amphetamine analogs, cathinone and methcathinone are drugs of abuse in humans that produce abuse-related behavioral effects in laboratory animals and promote monoamine release via DAT and NET more potently than via SERT (Bonano et al., 2014, 2015; Glennon et al., 1987; Rothman et al., 2001; Spiller et al., 2011). Addition of the 4-methyl substituent to the cathinone scaffold reduced neurochemical selectivity at DAT vs. SERT, and this finding agrees with effects of adding the 4-methyl substituent to the amphetamine or methcathinone scaffold (Baumann et al., 2013; Blanckaert et al., 2013; Bonano et al., 2015). For example, methcathinone displays a 309-fold selectivity to promote monoamine release via DAT vs. SERT in rat brain synaptosomes, whereas 4-methylmethcathinone (mephedrone) displays only a 2.4-fold selectivity due both to reduced potency at DAT and increased potency at SERT (Bonano et al., 2015). In the present study, comparison of stereoselectivity for cathinone and 4-methylcathinone provided an opportunity to assess the impact of stereochemistry on compounds with either high (cathinone) or low (4-methylcathinone) DAT vs. SERT selectivity.

4.2. Stereoselectivity of cathinone effects

The present results are consistent with previous evidence for stereoselectivity in potency but not quality of pharmacological effects for cathinone. For example, previous studies found that both *S*(-) and *R*(+)cathinone promote DA release from rat brain striatal slices (Kalix, 1986) and substitute for *S*(+)amphetamine in rats trained to discriminate amphetamine from saline (Glennon et al., 1984), and in both of these studies, *S*(-)cathinone was more potent than *R*(+)cathinone. In addition, the present study found that both cathinone enantiomers facilitated ICSS, and drug-induced facilitation of ICSS is often interpreted as an abuse-related behavioral effect that correlates both with expression of drug reinforcement in preclinical assays of drug self-administration and with abuse potential in humans (Negus and Miller, 2014). Consistent with this interpretation of ICSS data, the higher potency of *S*(-) vs. *R*(+)cathinone to facilitate ICSS in the present study agrees with the higher potency of *S*(-) vs. *R*(+)cathinone to maintain drug self-administration in rhesus monkeys (Johanson and Schuster, 1981; Yanagita, 1986).

The present study demonstrated stereoselective effects of cathinone on potency to release monoamines via both DAT and NET, and effects at DAT are thought to underlie abuse-related behavioral effects such as facilitation of ICSS (e.g. Bauer et al., 2013). Cathinone

also appeared to produce stereoselective effects at SERT insofar as *S*(-)-cathinone promoted 5HT release whereas *R*(+)-cathinone did not at the concentrations tested. However, higher concentrations of *R*(+) cathinone may have produced 5HT release, and the magnitude of cathinone stereoselectivity for 5HT release was not determined. It is unlikely that stereoselectivity of cathinone effects at SERT influenced behavioral effects, because both cathinone enantiomers were >50-fold selective to promote monoamine release at DAT vs. SERT.

4.3. Stereoselectivity of 4-methylcathinone effects

In contrast to cathinone, the enantiomers of 4-methylcathinone produced qualitatively different effects in the behavioral assay of ICSS, such that the *R*(+) enantiomer facilitated ICSS whereas the *S*(-) enantiomer did not. The apparently opposite effects of 4-methylcathinone enantiomers may have been at least partly a product of the doses studied. Thus, higher doses of *R*(+)4-methylcathinone would likely have recruited ICSS rate-decreasing effects similar to those produced by high doses of *R*(+)mephedrone (Gregg et al., 2015); however, higher doses of *R*(+)4-methylcathinone could not be studied here due to limited drug availability. Also, it is possible that a dose of *S*(-)4-methylcathinone between 0.32 mg/kg (which produced no effect) and 0.1 mg/kg (which produced significant ICSS depression), may have produced some ICSS facilitation, although our studies with other monoamine releasers have generally shown that half-log drug increments are adequate to characterize drug effects. Nonetheless, the present results are consistent with the conclusion that the *R*(+) enantiomer of 4-methylcathinone produced stronger abuse-related effects than the *S*(-) enantiomer. This stereoselectivity in 4-methylcathinone effects on ICSS agrees with previous evidence for stereoselectivity in expression of abuse-related effects by mephedrone enantiomers. Specifically, as in the present study with 4-methylcathinone, the *R*(+) enantiomer of mephedrone is more effective than the *S*(-) enantiomer to facilitate ICSS, and also more effective than the *S*(-) enantiomer to produce conditioned place preference and locomotor activation (Gregg et al., 2015).

We have now examined 25 monoamine releasers with a >40,000-fold range of DAT-vs.-SERT selectivities in both the synaptosome release and ICSS assays used here (Bauer et al., 2013; Bonano et al., 2014; Bonano et al., 2015; Gregg et al., 2015; present study), and the correlation between DAT-vs.-SERT selectivity and ICSS facilitation for this set of compounds is strong ($r=0.84$, $P<0.0001$). Accordingly, behavioral stereoselectivity of 4-methylcathinone enantiomers to facilitate ICSS in the present study may be related to neurochemical stereoselectivity of these enantiomers to promote monoamine release at DAT vs. SERT. Thus, the *R*(+) enantiomer facilitated ICSS and displayed higher DAT vs. SERT selectivity than the *S*(-) enantiomer, which only depressed ICSS. A similar relationship between effectiveness to facilitate ICSS and selectivity to promote monoamine release at DAT vs. SERT was also observed for the enantiomers of mephedrone (i.e. greater ICSS facilitation and greater DAT vs. SERT selectivity for the *R*(+) enantiomer) (Gregg et al., 2015). Consequently, these results support both (a) the broader evidence for a correlation between DAT vs. SERT selectivity and ICSS facilitation, and (b) the potential for stereochemistry to influence both neurochemical and behavioral effects. In particular, for drugs like 4-methylcathinone and mephedrone with similar potencies to act at multiple

targets, the present results suggest that differences in stereochemistry across targets can alter the balance of drug effects mediated by those targets and produce qualitative changes in the overall effects of drugs on behavior.

Although the correlation between DAT vs. SERT selectivity and facilitation of ICSS is supported by results of this study, that correlation is not perfect, and minor deviations from predicted effects have been observed. For example, DAT vs. SERT selectivity is slightly higher for *S*(-)-4-methylcathinone (1.2) than for *S*(-)-mephedrone (0.82), but despite higher DAT vs. SERT selectivity, the former only depressed ICSS, whereas the latter produced significant though weak ICSS facilitation in addition to its ICSS depressant effects (Gregg et al., 2015; present study). At least two factors could contribute to the divergence between neurochemical selectivity and predicted behavioral effects for these compounds. First, there is some variability in both in vitro and in vivo experimental measurements, and error associated with this variability can be expected to limit resolution for distinction between drugs with similar DAT vs. SERT selectivities such as those examined here. Second, it is possible for drug effects at targets other than DAT or SERT (e.g. monoamine receptors) to contribute to behavioral effects.

The present neurochemical results also have implications for molecular mechanisms by which these compounds interact with binding sites on monoamine transporters. For example, previous modeling studies have suggested that steric volume of the 4-position substituent on the methcathinone scaffold influences interaction of these drug molecules with binding sites in DAT and SERT, such that higher volumes impair interaction with DAT but improve interaction with SERT (Sakloth et al., 2014). The present results suggest that stereoselectivity of these molecules also influences their molecular interactions with transporters. Specifically, addition of the 4-methyl substituent to cathinone reduces DAT potency and increases SERT potency less for the *R*(+) enantiomer than for the *S*(-) enantiomer.

4.4. ICSS evaluation of abuse liability

Results of this study extend previous research using ICSS to evaluate the abuse-related effects of monoamine releasers. Bauer et al. (2013) demonstrated a positive correlation between DAT-vs.-SERT selectivity and efficacy to facilitate ICSS (see also Negus and Miller, 2014). In agreement with these prior results, the in vitro pharmacological selectivity of cathinone and 4-methylcathinone enantiomers to promote release of DA vs. 5HT were positively correlated with in vivo effects to produce maximal facilitation of ICSS (Pearson $r=0.98$, $R^2=0.95$, $P=0.023$). This correlation is in agreement with a recent finding with methcathinone and para-substituted methcathinone analogs possessing a >4000-fold range of selectivities for DAT vs. SERT (Bonano et al., 2015). In addition, the regression slope was significantly different from zero (36.9 ± 5.7), but not significantly different from the regression slope reported by Bauer et al. (2013) with monoamine releasers varying over a >8000-fold range of selectivities.

Previous studies have also shown that DAT-vs.-SERT selectivity correlates with measures of reinforcing efficacy of monoamine releasers in assays of drug self-administration (Wee et al., 2005; Schindler et al., 2015), and magnitude of ICSS facilitation for monoamine

releasers also correlated with reinforcing efficacy in drug self-administration procedures (Bauer et al., 2013). Taken together, these results suggest a strong relationship between in vitro expression of DAT-vs.-SERT selectivity of monoamine releasers and behavioral expression of their abuse-related effects in assays of both ICSS and drug self-administration.

4.5. Conclusions

Taken together, these results illustrate the potential for stereoselectivity of monoamine releasers to influence not only drug potency, but also expression of abuse-related neurochemical and behavioral effects. Here we reported an inverted stereochemistry between the enantiomers of cathinone and 4-methylcathinone extending previous findings with mephedrone (Gregg et al., 2015). For cathinone, the *S*(−) enantiomer was more potent than the *R*(+) enantiomer to promote monoamine release via DAT and to produce abuse-related facilitation of ICSS. However, for 4-methylcathinone, the *R*(+) enantiomer was less potent than the *S*(−) enantiomer to promote monoamine release via DAT, but displayed greater DAT vs. SERT selectivity and produced abuse-related effects in ICSS. Despite the novel inverted stereochemistry of cathinone and 4-methylcathinone enantiomers, the expression of abuse-related ICSS facilitation was predicted by in vitro DAT vs. SERT selectivity.

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References

- Aarde SM, Angrish D, Barlow DJ, Wright MJ Jr, Vandewater SA, Creehan KM, Taffe MA. Mephedrone (4-methylmethcathinone) supports intravenous self-administration in Sprague-Dawley and Wistar rats. *Addict Biol.* 2013; 18:786–799. [PubMed: 23363010]
- Balster RL, Schuster CR. Fixed-interval schedule of cocaine reinforcement: effect of dose and infusion duration. *J Exp Anal Behav.* 1973; 20:119–129. [PubMed: 4197505]
- Bauer CT, Banks ML, Blough BE, Negus SS. Use of intracranial self-stimulation to evaluate abuse-related and abuse-limiting effects of monoamine releasers in rats. *Br J Pharmacol.* 2013; 168:850–862. [PubMed: 22978626]
- Baumann MH, Ayestas MA, Partilla JS, Sink JR, Shulgin AT, Daley PF, Cozzi NV. The designer methcathinone analogs, mephedrone and methylene, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology.* 2012; 37:1192–1203. [PubMed: 22169943]
- Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M. Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive “bath salts” products. *Neuropsychopharmacology.* 2013; 38:552–562. [PubMed: 23072836]
- Blanckaert P, van Amsterdam JGC, Brunt TM, Van den Berg JDJ, Van Durme F, Maudens K, van Bussel JCH. 4-Methyl-amphetamine: a health threat for recreational amphetamine users. *J Psychopharmacol.* 2013; 27:817–822. [PubMed: 23784740]
- Bonano JS, Glennon RA, De Felice LJ, Banks ML, Negus SS. Abuse-related and abuse-limiting effects of methcathinone and the synthetic “bath salts” cathinone analogs methylenedioxypyrovalerone

- (MDPV), methylone and mephedrone on intracranial self-stimulation in rats. *Psychopharmacology*. 2014; 231:199–207. [PubMed: 23949206]
- Bonano JS, Banks ML, Kolanos R, Sakloth F, Barnier ML, Glennon RA, Cozzi NV, Partilla JS, Baumann MH, Negus SS. Abuse-related neurochemical and behavioral effects of para-substituted methcathinone analogs in rats. I Quantitative structure-activity relationship (QSAR) analysis. *Br J Pharmacol*. 2015; 172:2433–2444. [PubMed: 25438806]
- Cozzi NV, Brandt SD, Daley PF, Partilla JS, Rothman RB, Tulzer A, Baumann MH. Pharmacological examination of trifluoromethyl ring-substituted methcathinone analogs. *Euro J Pharm*. 2013; 699:180–187.
- Creehan KM, Vandewater SA, Taffe MA. Intravenous self-administration of mephedrone, methylone and MDMA in female rats. *Neuropharmacology*. 2015; 92:90–97. [PubMed: 25600245]
- Glennon RA, Young R, Hauck AE, McKenney JD. Structure–activity studies on amphetamine analogs using drug discrimination methodology. *Pharm Biochem Behav*. 1984; 21:895–901.
- Glennon RA, Yousif M, Naiman N, Kalix P. Methcathinone: a new and potent amphetamine-like agent. *Pharm Biochem Behav*. 1987; 26:547–551.
- Gregg RA, Baumann MH, Partilla JS, Bonano JS, Vouga A, Tallarida CS, Velvadapu V, Smith GR, Peet MM, Reitz AB. Stereochemistry of mephedrone neuropharmacology: enantiomer-specific behavioural and neurochemical effects in rats. *Br J Pharmacol*. 2015; 172:883–894. [PubMed: 25255824]
- Johanson CE, Schuster CR. A comparison of the behavioral effects of l- and dl-cathinone and d-amphetamine. *J Pharmacol Exp Ther*. 1981; 219:355–362. [PubMed: 7288625]
- Kalix P. The releasing effect of the isomers of the alkaloid cathinone at central and peripheral catecholamine storage sites. *Neuropharmacol*. 1986; 25:499–501.
- Martínez-Clemente J, López-Arnau R, Carbó M, Pubill D, Camarasa J, Escubedo E. Mephedrone pharmacokinetics after intravenous and oral administration in rats: relation to pharmacodynamics. *Psychopharmacology*. 2013; 229:295–306. [PubMed: 23649883]
- Motbey CP, Clemens KJ, Apetz N, Winstock AR, Ramsey J, Li KM, McGregor IS. High levels of intravenous mephedrone (4-methylmethcathinone) self-administration in rats: neural consequences and comparison with methamphetamine. *J Psychopharmacol*. 2013; 27:823–836. [PubMed: 23739178]
- Musshoff F, Hottmann L, Hess C, Madea B. "Legal highs" from the German internet—"bath salt drugs" on the rise. *Arch Kriminol*. 2012; 232:91–103.
- Negus SS, Miller LL. Intracranial self-stimulation to evaluate abuse potential of drugs. *Pharmacol Rev*. 2014; 66:869–917. [PubMed: 24973197]
- Osorio-Olivares M, Rezende MC, Sepulveda-Boza S, Cassels BK, Baggio RF, Munoz-Acevedo JC. A two-step method for the preparation of homochiral cathinones. *Tetrahedron Asymmetry*. 2003; 14:1473–1477.
- Pedersen AJ, Reitzel LA, Johansen SS, Linnet K. In vitro metabolism studies on mephedrone and analysis of forensic cases. *Drug Test Anal*. 2013; 5:430–438. [PubMed: 22573603]
- Robinson JE, Agoglia AE, Fish EW, Krouse MC, Malanga CJ. Mephedrone (4-methylmethcathinone) and intracranial self-stimulation in C57BL/6J mice: comparison to cocaine. *Behav Brain Res*. 2012; 234:76–81. [PubMed: 22728726]
- Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, Partilla JS. Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse*. 2001; 39:32–41. [PubMed: 11071707]
- Rothman RB, Vu N, Partilla JS, Roth BL, Hufeisen SJ, Compton-Toth BA, Glennon RA. In vitro characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals selective actions as norepinephrine transporter substrates. *J Pharmacol Exp Ther*. 2003; 307:138–145. [PubMed: 12954796]
- Sakloth F, Kolanos R, Mosier PD, Bonano JS, Banks ML, Partilla JS, Glennon RA. Abuse-related neurochemical and behavioral effects of para-substituted methcathinone analogs in rats. II Focus on steric parameters, molecular modeling, and a hydrophobic interaction (HINT) analysis. *Br J Pharm*. 2014; 172:2210–2218.

- Schindler CW, Thorndike EB, Goldberg SR, Lehner KR, Cozzi NV, Brandt SD, Baumann MH. Reinforcing and neurochemical effects of the “bath salts” constituents 3, 4-methylenedioxypyrovalerone (MDPV) and 3,4-methylenedioxy-N-methylcathinone (methyline) in male rats. *Psychopharmacology*. 2015 published online of print Aug 29.
- Spiller HA, Ryan ML, Weston RG, Jansen J. Clinical experience with and analytical confirmation of “bath salts” and “legal highs” (synthetic cathinones) in the United States. *Clin Toxicol*. 2011; 49(6):499–505.
- Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, Woolverton WL. Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. *J Pharmacol Exp Ther*. 2005; 313:848–854. [PubMed: 15677348]
- Yanagita T. Intravenous self-administration of (–)-cathinone and 2-amino-1-(2,5-dimethoxy-4-methyl) phenylpropane in rhesus monkeys. *Drug Alcohol Depend*. 1986; 17:135–141. [PubMed: 3743404]

Figure 1.
Chemical structures of *S*(-) and *R*(+) enantiomers of cathinone and 4-methylcathinone tested in this study.

Figure 2. Effects of (a, b) cathinone and (c, d) 4-methylcathinone enantiomers on monoamine release via DAT, SERT, and NET in rat brain tissue. *Abscissae*: drug concentration in *M*. *Ordinates*: percent maximal monoamine release. Each point shows mean $\pm$ SD for $n=3$ experiments performed in triplicate. EC₅₀ values derived from the concentration–effect curves are shown in Table 1.

Figure 3.

Effects of cathinone enantiomers on ICSS. (a, b) *Abscissae*: frequency of electrical brain stimulation in log Hz. *Ordinates*: rate of ICSS maintained by each frequency of brain stimulation, expressed as percent maximum control rate (%MCR). (c, d) *Abscissae*: drug dose in mg/kg. *Ordinates*: rate of ICSS maintained across all brain stimulation frequencies, expressed as percent baseline number of stimulations per component. *Filled points* in (a) and (b) represent frequencies at which ICSS rates were statistically different from vehicle rates ($P < 0.05$). In (c) and (d) * indicate significant increases and # indicated significant decreases in ICSS rates relative to vehicle for at least one stimulation frequency as determined by analysis of frequency–rate curves in panels (a) and (b). All data show mean $\pm$ SEM for $n=6$ rats.

Figure 4.

Effects of 4-methylcathinone enantiomers on ICSS. (a, b) *Abcissae*: frequency of electrical brain stimulation in log Hz. *Ordinates*: rate of ICSS maintained by each frequency of brain stimulation, expressed as percent maximum control rate (%MCR). (c, d) *Abcissae*: drug dose in mg/kg. *Ordinates*: rate of ICSS maintained across all brain stimulation frequencies, expressed as percent baseline number of stimulations per component. *Filled points* in (a) and (b) represent frequencies at which ICSS rates were statistically different from vehicle rates ($P < 0.05$). In (c) and (d) * indicate significant increases and # indicated significant decreases in ICSS rates relative to vehicle for at least one stimulation frequency as determined by analysis of frequency–rate curves in panels (a) and (b). All data show mean $\pm$ SEM for $n=6$ rats.

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Table 1

EC₅₀ values in nM (±SEM) for cathinone and 4-methylcathinone enantiomers to promote monoamine release via DAT, SERT, and NET. DAT vs. SERT and DAT vs. NET selectivity was also quantified as the ratio (SERT EC₅₀÷DAT EC₅₀) and (NET EC₅₀÷DAT EC₅₀), respectively.

Drug	DAT	SERT	NET	DAT vs. SERT selectivity	DAT vs. NET selectivity
<i>S</i> (-)-cathinone	24.6±4.2	9267±3023	14.2±1.3	376	0.6
<i>R</i> (+)-cathinone	183.9±24.2	>10,000	72.0±10.2	>54	0.4
<i>S</i> (-)-4-methylcathinone	150.3±20.1	178.7±34.4	88.8±8.6	1.2	0.6
<i>R</i> (+)-4-methylcathinone	390.5±51.3	1592±465.3	114.7±12.2	4.1	0.3