Effects of methcathinone and 3-Cl-methcathinone (PAL-434) in cocaine discrimination or self-administration in rhesus monkeys

Stephen J. Kohut¹, Peter A. Fivel¹, Bruce E. Blough², Richard B. Rothman³ and Nancy K. Mello¹

¹ Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, Belmont, MA, USA
² The Research Triangle Institute, Research Triangle Park, NC, USA
³ IRP, OD, National Institute on Drug Abuse, NIH, Baltimore, MD, USA

Abstract

Monoamine releasers with varying selectivity for dopamine (DA)/norepinephrine and serotonin (5-HT) release are potential treatment medications for cocaine abuse. Although DA-selective monoamine releasers effectively reduce cocaine abuse, their clinical usefulness is limited by abuse liability. It is hypothesized that increasing 5-HT neurotransmission may reduce the abuse-related effects of DA releasers, but the optimal DA:5-HT release ratio remains to be determined. This study in rhesus monkeys compared the effects of two compounds with differing potency for 5-HT release. Methcathinone and 3-Cl-methcathinone (PAL-434) have equal potency for DA release, but PAL-434 has 10-fold higher potency for 5-HT release. In drug discrimination studies, monkeys were trained to discriminate cocaine (0.4 mg/kg i.m.) from saline in a two-key, food-reinforced procedure. In drug self-administration studies, a separate group of monkeys was trained to respond for cocaine [0.01 mg/kg/injection (inj)] and food (1 g pellets) under a second order schedule of reinforcement [FR2(VR16:S)]. When responding was stable, methcathinone (0.1–0.56 mg/kg.h i.v.) or PAL-434 (0.32–1.8 mg/kg.h i.v.) was administered chronically (one injection every 20 min for 23 h/d) for 7–10 d. In discrimination studies, both compounds dose-dependently increased cocaine-like responding but with different potencies (cocaine=methcathinone>PAL-434). Chronic treatment with methcathinone or PAL-434 dose-dependently and selectively reduced cocaine self-administration. PAL-434 was about 4-fold and methcathinone about 1.6-fold more potent at decreasing cocaine-over food-maintained responding. These data suggest that compounds with moderate selectivity for DA vs. 5-HT release (8–15-fold) may be effective for the treatment of cocaine dependence.

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Key words: Cocaine, discrimination, dopamine, self-administration, serotonin.

Introduction

Cocaine abuse is a significant public health problem that is associated with medical disorders, unemployment and high crime rates. Estimates suggest that there are up to 1.5 million current cocaine abusers in the USA alone (SAMHSA, 2011) and >14 million worldwide (UNODC, 2011). Despite the high prevalence of cocaine abuse, there is currently no Food and Drug Administration-approved medication for its treatment. One approach to treating cocaine and other drug abuse is to substitute ligands with similar mechanisms of action and effects, but with lower abuse potential. Such agonist or substitution therapies have been successful in reducing opioid and nicotine abuse (see reviews by Henningfield, 1995; Kreek, 1996; Mendelson and Mello, 1996).

The abuse-related effects of cocaine are attributed to its action as a dopamine (DA) indirect agonist (Ritz et al., 1987; Woolverton and Johnson, 1992). In humans, positron emission tomography imaging studies have demonstrated that increases in reports...
of positive subjective effects (e.g. ‘high’) after cocaine use are temporally related to increases in extracellular DA (Volkow et al., 1996, 1997). Thus, drugs that affect DA neurotransmission and attenuate the effects of cocaine, such as monoamine releasers, have been proposed as potential substitution medications for the treatment of cocaine addiction (Rothman, 1990; Rothman et al., 2006). Clinical studies have shown that d-amphetamine, a DA-selective monoamine releaser, effectively reduces cocaine abuse with good subject compliance and minimal reported side-effects (Grabowski et al., 2001; Herin et al., 2010). Preclinical studies are concordant with these clinical reports as chronic d-amphetamine administration produced a sustained and selective decrease in cocaine self-administration by rhesus monkeys (Negus and Mello, 2003a, b; Czoty et al., 2010, 2011). However, d-amphetamine and other DA-selective releasers have high abuse potential that limits their utility as pharmacotherapies for cocaine abuse.

Serotonin (5-HT)-releasing drugs have low abuse liability and can decrease the abuse-related effects of cocaine. For example, fenfluramine and other 5-HT-selective releasers lack stimulant effects and are not self-administered in rhesus monkeys (Woods and Tessel, 1974). Further, in both humans and animals, increases in 5-HT levels decrease the behavioural and neurochemical effects of cocaine (Walsh et al., 1994; Howell and Byrd, 1995; Czoty et al., 2002) and other indirect DA agonists (Bendotti et al., 1980; Baumann et al., 2000, 2011a; Wee et al., 2005). Other studies have found that various ratios (e.g. 1:3 or 1:10) of d-amphetamine or phentermine to fenfluramine show good selectivity for cocaine treatment (Glatz et al., 2002; S. J. Kohut and N. K. Mello, unpublished observations; although see Glowa et al., 1997) and that these mixtures have lower abuse liability than DA-selective agonists alone (Wee and Woolverton, 2006; see also Wee et al., 2005). Thus, compounds that have moderate selectivity for DA over 5-HT release may retain the treatment efficacy of DA-selective releasers but decrease abuse liability (Rothman et al., 2005; Negus et al., 2007; see also, Rothman et al., 2006, 2008).

We have studied the effects of chronic treatment with several compounds ranging from DA-selective to 5-HT-selective in rhesus monkeys responding for cocaine and food under a fixed ratio (FR) 2 variable ratio 16 second order [FR2/VR16:S] schedule of reinforcement (Negus and Mello, 2003a; Negus et al., 2007, 2009a, b). Those studies suggest that compounds with ≥20-fold selectivity for DA/5-HT release produce the most behaviourally selective decreases in cocaine-over food-maintained responding (see also Banks et al., 2011; Negus et al., 2007, 2009a). However, many of these compounds (e.g. d-amphetamine, phentermine, benzylpiperazine) are abused in clinical populations (Rothman et al., 2008).

The present study was designed to extend our previous research by evaluating a novel compound with moderate selectivity for DA vs. 5-HT release against the abuse-related effects of cocaine. Figure 1 and Table 1 show that 3-Cl-methcathinone (PAL-434) is the 3-chloro congener of methcathinone (MCAT) and is about 8-fold more selective for DA than for 5-HT release. This is the first assessment of a monoamine releaser with moderate selectivity for DA vs. 5-HT release across a range of cocaine doses. PAL-434 and its parent compound MCAT were first evaluated in cocaine discrimination studies to assess magnitude of cocaine-like discriminative effects, relative potency and the time-course or duration of effects in rhesus monkeys. Subsequently, the ability of chronic treatment with PAL-434 and MCAT to reduce cocaine- and food-maintained responding was examined to assess behavioural selectivity. Ideally, a treatment medication would selectively decrease cocaine-maintained behaviour but not behaviour maintained by non-drug reinforcers (e.g. food). Further, chronic treatment models clinical treatment and is important for determining if the acute effects of candidate treatment medications remain stable during repeated administration (Mello and Negus, 1996; Mello, 2005).

Method

Subjects

Cocaine-experienced adult male rhesus monkeys (Macaca mulatta; 6 and 10 kg) were studied. Monkeys received Lab Diet jumbo monkey biscuits (PMI Feeds...
Animal maintenance and research were conducted in accordance with the guidelines provided by the Institute of Laboratory Animal Resources (ILAR-NRC, 1996) and the NIH Office of Laboratory Animal Welfare. The facility is licensed by the US Department of Agriculture and the McLean Hospital Institutional Animal Care and Use Committee approved all protocols. Enrichment was provided through mirrors and toys in the home-cage, interaction with technical staff and the opportunity to manipulate their environment during operant food and drug procedures (Line, 1987).

**Drug discrimination procedures**

**Apparatus**

All monkeys were housed in a stainless steel chamber (56 × 71 × 69 cm) equipped with a custom operant response panel (28 × 28 cm) mounted on the front wall. Operant panels contained three square translucent response keys (5.1 × 5.1 cm) arranged 3.5 cm apart at the top of the operant panel. Each response key and three circular translucent stimuli (1.9 cm diameter) located in a vertical column below the centre response key could be transilluminated by red or green stimulus lights (SuperBright LEDs; Fairchild Semiconductor, USA). A pellet dispenser (model G5210; Gerbrands, USA) delivered 1-g banana-flavoured food pellets (Purina Mills Test Diet, USA) to a receptacle mounted on the front of the cage. All experimental events were controlled through custom programmed software (Med-PC) on a Hewlett-Packard (model 8100 Elite CMT PC) desktop PC located in a separate room and connected to the chambers via a Med Associates Interface (USA).

**Discrimination training**

Three, adult male monkeys were trained to discriminate cocaine (0.4 mg/kg i.m.) from saline using identical procedures to those previously reported (Negus et al., 2007, 2009a, b; Newman et al., 2010; Mello et al., 2013a).

**Discrimination testing**

The principal dependent variables were: (1) percent injection-appropriate responses for the entire cycle; (2) percent injection-appropriate responses prior to the first completed FR30; (3) response rate (responses/s). Individual performance criteria for the discrimination were: (1) at least 80% of total responses before the first completed FR30 must have been on the condition-appropriate key; (2) at least 90% of total responses in each cycle must have been completed on the stimulus-appropriate key. Training was complete when these criteria were met during each cycle for seven of eight consecutive training sessions. Testing began when monkeys met criterion levels of discrimination. Test sessions were conducted only if the individual performance criteria noted above were met during the training day immediately preceding the test day. If responding did not meet criterion level performance, training sessions were continued until criterion levels were obtained for at least two consecutive days. Test sessions were identical to training sessions except that: (1) responding on either key produced food; (2) test doses/compounds were administered using a cumulative dosing procedure. In addition to testing MCAT and PAL-434 substitution for the cocaine-discriminative stimulus, the time-course of

<table>
<thead>
<tr>
<th>Drug</th>
<th>DA</th>
<th>5-HT</th>
<th>NE</th>
<th>5-HT:DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Amphetamine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.8±3.5</td>
<td>1765±94</td>
<td>7.2±0.44</td>
<td>71</td>
</tr>
<tr>
<td>(±)-Methcathinone&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.9±3.1</td>
<td>4272±274</td>
<td>22.4±2.3</td>
<td>85</td>
</tr>
<tr>
<td>PAL-434&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.8±4.0</td>
<td>410±38</td>
<td>54.4±4.8</td>
<td>8.2</td>
</tr>
</tbody>
</table>

EC<sub>50</sub>, The concentration of the test compounds that produced half maximal effect; DA, dopamine; 5-HT, serotonin; NE, norepinephrine; PAL-434, 3-Cl-methcathinone.

<sup>a</sup>Rothman et al. (2001).

<sup>b</sup>Unpublished data. EC<sub>50</sub> values determined using methods identical to those reported by Rothman et al. (2001). The ratio of 5-HT EC<sub>50</sub>:DA EC<sub>50</sub> is also shown. Higher ratios imply greater selectivity for releasing DA. For all compounds, potency to release NE was similar to or greater than potency to release DA. Data for d-amphetamine are included for comparison with methcathinone.
substitution effects was studied by administering a single dose of MCAT or PAL-434 10 min prior to the first cycle, exactly as in training sessions. Subsequent cycles were then started at 30, 60, 100 and 300 min after injection of the test compound or until responding was predominantly saline-like (i.e. <20% cocaine-lever responding).

**Data analysis: drug discrimination**

*Substitution tests*

Responding was calculated as the percentage of total responding on the cocaine-associated lever, excluding responses during timeouts. ED50 values, defined as the dose of the test compounds that engendered 50% responding on the cocaine-associated lever, were determined using log-linear interpolation with individual subject dose-effect curves. Log ED50 values were converted to linear values for statistical tests and data presentation. Group ED50 values (with 95% confidence limits) were also determined to facilitate comparison of MCAT and PAL-434 substitution profiles with previous cocaine discrimination studies on monoamine releasers (Negus et al., 2007, 2009a).

*Time-course studies*

ET50% values were defined as the time at which the test compound dose fell below full substitution (>80% cocaine-lever responding) levels of responding. Response rates were calculated by dividing the total responses on both keys (when the cue lights were illuminated) by the total session time (s) and expressed as responses/s. The percentage of cocaine-like responding is not shown when response rate decreased to <20% of control.

**Drug self-administration**

*Apparatus*

Monkeys lived in stainless steel chambers (64×64×79 cm) equipped with a custom-designed operand response panel. A pellet dispenser (Gerbrands Model G5210, USA) and two syringe pumps (Model 981210; Harvard Apparatus, Inc., USA), one for each lumen of the double-lumen catheter, were mounted above the chamber. A food cup for food pellets, biscuits and fruit/vegetables was attached to the lower left front of the chamber. All experimental events were custom programmed on a Hewlett-Packard (model 8100 Elite CMT PC) desktop PC located in a separate room connected to a Med Associates (USA) Interface.

Double lumen Silicone® catheters (internal diameter 0.028 in, outer diameter 0.088 in; Saint Gobain Performance Plastics, USA) were surgically implanted in the internal jugular, external jugular or femoral vein under aseptic conditions. Double lumen catheters permitted i.v. cocaine self-administration concurrently with chronic i.v. saline, MCAT or PAL-434 treatment. Details of our surgical procedures have been published elsewhere (Mello et al., 2013b).

*Self-administration training*

Three monkeys were initially trained to respond for banana-flavoured food pellets (1 g) until food-maintained performance was stable (see later) and then trained to respond for cocaine (0.01 mg/kg/inj; see Mello et al., 2013b). During drug self-administration sessions, the response key was illuminated with a green light and completion of the response requirement under an FR2(VR16:S) schedule produced 0.1 ml cocaine solution over 1 s through one lumen of the double-lumen catheter. A 10 s timeout followed delivery of each drug injection or food pellet during which stimulus lights remained off and responding had no scheduled consequences. If 25 food pellets or 20 injections were delivered before the end of the 1 h session, then all stimulus lights were turned off and responding had no scheduled consequences for the remainder of that session. A monkey could earn a maximum of 100 food pellets and 80 drug or saline injections/d in four daily food and four daily cocaine self-administration sessions. Food sessions started at 11:00 hours, 15:00 hours, 19:00 hours and 06:00 hours the next morning and drug self-administration sessions began at 12:00 hours, 16:00 hours, 20:00 hours and 07:00 hours the next morning. Room lights were off during all experimental sessions. Extinction training consisted of sessions in which ‘saline’ was substituted for 0.01 mg/kg i.v. cocaine. During saline self-administration sessions, all stimulus lights operated as described earlier.

*Self-administration testing*

Training continued until monkeys met the following criteria for stable food and cocaine self-administration under the FR2(VR16:S) schedule of reinforcement: (1) three consecutive days during which the number of cocaine injections/d varied by no more than 20% of the 3-d mean with no upward or downward trend in performance; (2) the mean number of food pellets and injections delivered per day was equal to or greater than 60. Once responding was stable, saline and cocaine (0.0032-0.1 mg/kg/inj) were studied in an
irregular order for each monkey. Each dose was substituted for a minimum of 7 d and until responding was stable according to the above criteria, or for a maximum of 10 d. Following each substitution test, monkeys were returned to the maintenance dose of cocaine, 0.01 mg/kg/inj, for at least 3 d and until responding was stable to ensure reliable baseline responding prior to the subsequent substitution test. The range of cocaine doses were delivered by computer-controlled variations in pump infusion duration (Fivel, 2011).

Procedures for evaluating the effects of MCAT and PAL-434 on cocaine- and food-maintained responding were similar to those used in our previous studies of the effects of monoamine releasers on cocaine self-administration (Negus and Mello, 2003a, b; Negus et al., 2007, 2009a, b; see also Mello et al., 2013b). Saline or a dose of MCAT or PAL-434 was administered through one lumen of a double-lumen catheter every 20 min for 23 h each day (10:30 hours – 09:30 hours) for a total injection volume of 6.9 ml in 69 injections. This procedure was developed to ensure that relatively short-acting drugs would be continuously present during the test sessions (Negus and Mello, 2003a, b). Each treatment dose was studied for 7–10 d until responding was stable. Successive treatment doses were separated by an interval of saline treatment until drug- and food-maintained responding returned to baseline levels. The saline treatment interval was used to prevent any carryover effects from the preceding treatment.

Treatment with MCAT (0.1–0.56 mg/kg.h) and PAL-434 (0.32–1.8 mg/kg.h) were studied first on the maintenance dose of cocaine (0.01 mg/kg/inj) to determine which doses of MCAT and PAL-434 were most effective and selective and to monitor potential adverse effects. Subsequently, the most effective doses of MCAT and PAL-434 were tested against the cocaine dose–effect curve.

Data analysis: drug self-administration

The primary dependent variables were the total number of cocaine or saline injections and food pellets earned per day. The number of injections self-administered and rate of responding during the final 5 d of each substitution condition were averaged. Rate of responding was transformed to a percentage of saline treatment to assess the effects of a range of doses of MCAT or PAL-434 treatment on 0.01 mg/kg/inj cocaine self-administration. Changes in drug-maintained responding from saline-treatment with MCAT or PAL-434 treatment at each dose of cocaine were evaluated using a two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison post hoc. One-way ANOVA with Dunnett’s post hoc tests were used to determine which points differed from saline self-administration. All figures and statistical analyses were accomplished with GraphPad Prism 5.0c for Macintosh (GraphPad Software Inc., USA).

Drugs

Cocaine HCl and (±)-MCAT HCl were provided by the National Institute on Drug Abuse, NIH (USA). PAL-434 HCl was provided by B. Blough. All drugs were dissolved in sterile water and are expressed as the salt. Self-administered and treatment drugs were sterile-filtered with a 0.22-micron syringe-driven filter (Millipore Corporation, USA).

Results

Effects of MCAT and PAL-434 in monkeys trained to discriminate cocaine from saline.

Results are shown in Figs. 2 and 3 and Table 2. During training sessions immediately preceding dose–effect curves, monkeys responded exclusively on the saline-associated lever when saline was administered (100% saline-like responding) and almost exclusively on the cocaine-associated lever when the training dose of cocaine (0.4 mg/kg) was administered (99.93±0.07 cocaine-like responding). Mean response rate±S.E.M. was 2.46±0.55 and 3.29±0.94 responses/s during saline and cocaine training cycles, respectively.

Substitution of MCAT or PAL-434 for the cocaine discriminative stimulus

Cocaine dose-dependently increased the percentage of responses on the cocaine-associated lever (ED50=0.11 mg/kg). Figure 2 shows that similar dose-dependent increases in cocaine-appropriate responding were found with MCAT and PAL-434. MCAT was equipotent compared to cocaine in all monkeys; PAL-434 was about 3-fold less potent than cocaine in two monkeys and 8-fold less potent in the third monkey (Table 2). Cocaine, MCAT and PAL-434 did not decrease response rates at doses that engendered full substitution (Table 2 and Fig. 2).

Time-course for substitution of MCAT or PAL-434 to the discriminative stimulus effects of cocaine

The time-course of MCAT and PAL-434 substitution for cocaine was examined by administering a
dose of test compound and extending the time-out period in between drug discrimination testing cycles (i.e. 10, 30, 60, 100, 300 min; see Fig. 3). It is generally accepted that >80% cocaine-like responding is considered full (or complete) substitution of a test compound for a training drug (c.f. Sidman, 1980). Thus, the time-course profile of MCAT and PAL-434 was determined to find the time-point where 80% cocaine-lever responding was produced. The doses chosen for the time-course assessment were individually determined based on doses that produced cocaine-like responding in substitution tests. The doses that produced the highest level of responding for each individual were used for determination of ED50 values. For MCAT, 0.32 mg/kg was used for monkey R-009 and R-012 and 1.0 mg/kg was used for R-147. For PAL-434, 1.0 mg/kg was used for monkey R-009 and R-147 and 0.32 mg/kg was used for R-012. Using this analysis, both MCAT and PAL-434 had similar time-courses in two of three monkeys (R-012 and R-147) with ET50% values of 45 and 66 min for MCAT and 34.5 and 66 min for PAL-434. In the third monkey (R-009), ET50% value for MCAT was 131 min while PAL-434 was 38.46. Monkey R-009 also showed the largest difference in potency to substitute for cocaine between MCAT and PAL-434 (see Fig. 2).

Effects of chronic treatment with MCAT and PAL-434 on cocaine- and food-maintained responding

Results are shown in Figs. 4–6 and Table 3. During saline self-administration sessions with saline treatment, monkeys earned an average of 7.93±4.62 saline injections. The cocaine dose-effect curve followed an inverted-U shaped function in all monkeys during saline treatment. An intermediate dose of cocaine (0.0032 mg/kg/inj) maintained about 50% of the maximal number of injections available (41.13±5.09). Doses of 0.01 (77.93±2.07), 0.032 (78.27±1.27) and 0.1 (71.93±2.99) mg/kg/inj maintained high levels of responding in all monkeys. During saline treatment, monkeys earned 98.67±1.33 and 98.53±0.74 food pellets/d during extinction and when 0.01 mg/kg/inj cocaine, respectively, was available for self-administration. Food-maintained responding was at or near maximal levels across the entire cocaine dose-effect curve (see Fig. 5; right panels).

Effects of MCAT or PAL-434 on cocaine- and food-maintained responding when 0.01 mg/kg/inj cocaine was the available reinforcer

Initially, several doses of MCAT or PAL-434 were tested against 0.01 mg/kg/inj cocaine- and
Previous studies from this laboratory have shown that this dose of cocaine is the lowest dose that maintains high rates of responding and it is sensitive to the effects of treatment with several monoamine releasers (Negus and Mello, 2003a, b; Negus et al., 2007, 2009a). Further, rates of responding under saline treatment conditions for both reinforcers (cocaine= 1.57±0.48; food=2.22±0.24 responses/s) were not significantly different (p>0.05). Figure 4 shows the percentage of control responding when food pellets and 0.01 mg/kg/inj cocaine were available during chronic treatment with MCAT (0.1–0.56 mg/kg.h) or PAL-434 (0.32–1.8 mg/kg.h). ED50 values for each drug are shown in Table 3.

Chronic treatment with both MCAT and PAL-434 dose-dependently decreased cocaine-maintained responding. Previous studies from this laboratory have shown that this dose of cocaine is the lowest dose that maintains high rates of responding and it is sensitive to the effects of treatment with several monoamine releasers (Negus and Mello, 2003a, b; Negus et al., 2007, 2009a). Further, rates of responding under saline treatment conditions for both reinforcers (cocaine= 1.57±0.48; food=2.22±0.24 responses/s) were not significantly different (p>0.05). Figure 4 shows the percentage of control responding when food pellets and 0.01 mg/kg/inj cocaine were available during chronic treatment with MCAT (0.1–0.56 mg/kg.h) or PAL-434 (0.32–1.8 mg/kg.h). ED50 values for each drug are shown in Table 3.

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self-administration. MCAT decreased rate of cocaine self-administration to about 30% of control levels while food was decreased to about 50% of control levels at the highest dose tested (0.56 mg/kg). One subject did not decrease cocaine- or food-maintained responding to <50% of baseline even at the highest dose of MCAT tested. PAL-434 dose-dependently decreased rate of cocaine self-administration to about 14% of control levels while food remained at about 90% of control levels at the highest dose tested. Both MCAT and PAL-434 produced at least a 50% decrease in cocaine self-administration at doses that had little or no effect on food-maintained responding. MCAT was about 1.6-fold and PAL-434 about 4-fold more potent at decreasing cocaine- over food-maintained responding as determined by ED50 ratio analysis (see Table 3).

Behavioural side-effects of chronic treatment with MCAT or PAL-434

Behavioural effects of MCAT or PAL-434 were not formally monitored; however, stimulant-like effects were prominent during chronic treatment with 0.32–0.56 mg/kg MCAT. Specifically, increased whole-body locomotion, increased aggression and flushed face were prominent signs in all monkeys. Additionally, signs of behavioural toxicity such as stereotypic focused grooming of the legs and arms when animals were treated with high doses of MCAT (0.56 mg/kg) were observed. Chronic treatment with 0.56–1.8 mg/kg PAL-434 produced fewer signs of behavioural toxicity and a milder stimulant-like profile of effects. The most prominent behaviours observed were red face and reduction in appetite (defined as increased time to consumption of supplemental biscuits and/or food pellets during/after sessions). Focused grooming was not as vigorous as during treatment with MCAT, but grooming was noted during treatment with the highest dose of PAL-434 (1.8 mg/kg).

Effects of MCAT or PAL-434 on the cocaine dose–effect curve

The two highest doses of MCAT and PAL-434 that produced modest effects on food-maintained responding with few overt signs of behavioural toxicity were chosen to study against the full cocaine dose–effect curve. Figure 5 shows mean cocaine- and food-maintained responding data for the final 5 d of a 7–10 d treatment regimen with MCAT (0.1 and 0.32 mg/kg) or PAL-434 (0.56 and 1.0 mg/kg). The effects of both MCAT and PAL-434 were not consistent when the lowest, threshold dose of cocaine (0.0032 mg/kg) was available. Some monkeys showed slight increases while others showed slight decreases in cocaine self-administration. As a result, this dose was not included in statistical analyses. Chronic treatment with the lowest dose of MCAT (0.1 mg/kg) had no effect on cocaine- or food-maintained responding while the higher dose of MCAT (0.32 mg/kg) produced about a 60% decrease at 0.01 mg/kg and modest decreases (about 20–30%) at the two highest cocaine doses. Food-maintained responding did not change significantly from control except at the highest dose of cocaine and decreased to about 50% of control levels.

In contrast, PAL-434 produced a dose-dependent flattening of the cocaine dose–effect curve. At the lower dose of PAL-434 (0.56 mg/kg), self-administration was decreased by about 20–30% of control levels at the three highest cocaine doses. A higher dose of PAL-434 (1.0 mg/kg) produced a further decrease in cocaine self-administration by about 55% at 0.01 and 0.032 mg/kg and 25% at 0.1 mg/kg cocaine. Food-maintained responding during chronic treatment with PAL-434 was decreased by about 40% and 30% with the low and high doses of PAL-434 at 0.1 mg/kg cocaine, respectively, and by about 40% of control levels when 1.0 mg/kg PAL-434 was given in combination with 0.032 mg/kg cocaine.
although these decreases were not statistically significant ($p>0.05$).

**Time-course of MCAT or PAL-434 treatment effects on responding for 0.01 mg/kg/inj cocaine or food**

Figure 6 shows the time-course of effects during treatment with saline (top panel), 0.1 and 0.32 mg/kg/h MCAT (middle panels) and 0.56 and 1.0 mg/kg/h PAL-434 (bottom panels). The time-course of effects shows that treatment with the lowest dose of MCAT (0.1 mg/kg/h) had no effect on cocaine- or food-maintained responding at any treatment day. Chronic treatment with 0.32 mg/kg/h MCAT produced a sustained decrease in cocaine responding by about 60–70% of control levels on treatment day 2 until treatment day 10. Responding for cocaine recovered to 100% within 3 d after cessation of chronic MCAT treatment. Food-maintained responding decreased to about 60% of control levels at day 4 but quickly recovered to 90% at day 6 and was maintained at or near 100% for the remainder of treatment.

When 0.56 mg/kg/h PAL-434 was administered chronically, cocaine responding was decreased to...
about 55–60% of control levels on days 2–7. One animal showed minimal effects with 0.56 mg/kg.h PAL-434 treatment with a maximum decrease of about 30% on days 3–5 before recovering to 100% on days 6–10. Food-maintained responding was only minimally decreased on day 2 of treatment. The effects of treatment with a higher dose of PAL-434 decreased cocaine-maintained responding to about 50% of baseline on day 2 then to about 26 and 13% on days 3 and 4, respectively. The decrease was somewhat variable on days 6–9 with some recovery, although cocaine was still decreased to 64, 53 and 39% of baseline over the final 3 days of treatment. Food-maintained responding decreased to about 40% of control levels at day 4 but quickly recovered to 83% by day 6 and remained at maximal levels through the end of treatment.

Discussion
This is the first evaluation of the novel monoamine releaser, PAL-434, compared to its parent compound MCAT, on the abuse-related effects of cocaine in non-human primates. These compounds were examined in cocaine discrimination studies and administered as a chronic treatment in cocaine self-administration. Our major findings were that both MCAT and PAL-434 produced full substitution for cocaine in drug discrimination studies and had a similar time-course. Both MCAT and PAL-434 also decreased cocaine self-administration selectively over food-maintained responding. However, PAL-434 showed greater selectivity, a more complete suppression of cocaine self-administration (over a 30-fold range of cocaine doses) and fewer toxic side-effects.

Comparison of PAL-434 and MCAT
Despite similar efficacies as DA releasers (about 50 nM; see Table 1), PAL-434 was 3–8 fold less potent in eliciting cocaine-like discriminative stimulus effects than cocaine or MCAT. Previous studies have found MCAT to be more potent than cocaine in rats trained to discriminate either cocaine or MCAT (Glennon et al., 1987; Young and Glennon, 1993; 1998; Li et al., 2006). The reason for this discrepancy is unclear but may reflect a species difference (c.f. Ricaurte, 1989; Weerts et al., 2007). A pharmacokinetic explanation for potency differences is unlikely because PAL-434 and MCAT followed similar time-courses and both produced a rapid onset (see Fig. 3; see also Young and Glennon, 1993).
were determined using log-linear interpolation with individual subject dose–response curves. Log ED50 values were converted to linear values for statistical tests and data presentation. If responding did not decrease to below 50% for a given reinforcer an ED50 value was not calculated. Instead, a conservative estimate of ED50 was determined by assuming the next ½-log dose would eliminate responding, as we have done previously (Negus et al., 2007, 2009a, b). Confidence intervals were not determined for the group in those cases.

Table 3. ED50 values (±95% confidence intervals) for methcathinone and PAL-434 to decrease rate of responding maintained by 0.01 mg/kg/inj cocaine or food to 50% of control levels

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cocaine ED50</th>
<th>Food ED50</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Methcathinone</td>
<td>&gt;0.37</td>
<td>&gt;0.59</td>
<td>&gt;1.6</td>
</tr>
<tr>
<td>PAL-434</td>
<td>0.53 (0.37–0.67)</td>
<td>&gt;2.08</td>
<td>&gt;3.9</td>
</tr>
</tbody>
</table>

PAL-434, 3-Cl-methcathinone.

ED50 values, defined as the dose of the test compounds that reduced cocaine- or food-maintained responding by 50%, were determined using log-linear interpolation with individual subject dose–response curves. Log ED50 values were converted to linear values for statistical tests and data presentation. If responding did not decrease to below 50% for a given reinforcer an ED50 value was not calculated. Instead, a conservative estimate of ED50 was determined by assuming the next ½-log dose would eliminate responding, as we have done previously (Negus et al., 2007, 2009a, b). Confidence intervals were not determined for the group in those cases.

Our finding that PAL-434 was more effective at decreasing cocaine self-administration than MCAT with fewer side-effects has translational implications for medication development. The reduction in cocaine self-administration by MCAT is similar to the effects of other DA-selective releasers such as benzylpiperazine and 4-benzylpiperadine (Negus et al., 2009a). These compounds also produced dose-dependent and sustained decreases in cocaine self-administration but behavioural selectivity occurred over a very narrow dose range, whereas PAL-434 was effective over a 30-fold dose range. PAL-434 selectively decreased 0.01 mg/kg/inj cocaine- over food-maintained responding and shifted the cocaine self-administration dose–effect curve downward, whereas MCAT shifted the curve rightward. If DA release is the critical factor in producing selective and complete suppression of cocaine self-administration (c.f. Negus et al., 2009a), it would be predicted that MCAT and PAL-434 would be equally effective. Thus far, compounds ranging from 20–80-fold selective (i.e. benzylpiperazine, phenmetrazine, 4-benzylpiperidine, methamphetamine and PAL-353) and compounds <6.5-fold to 5-HT selective (i.e. PAL-314, PAL-287 and fenfluramine) have been studied in our procedure (see Negus et al., 2007, 2009). PAL-434 is the most non-selective monoamine releaser tested that has produced results similar to d-amphetamine on cocaine self-administration (see Negus and Mello, 2003a; Negus et al., 2007, 2009a).

Side-effects of MCAT and PAL-434

MCAT is classified as a Schedule I drug that has abuse liability in man (Tolliver, 1995) and maintains self-administration in non-human primates (Kaminski and Griffiths, 1994). In the present study, treatment with MCAT produced self-directed stereotypes at the highest dose (0.56 mg/kg.h) tested. This behavioural toxicity may be related to monoamine depletions by MCAT and related drugs reported during chronic exposure (Sparago et al., 1996). Side-effects of chronic treatment with PAL-434 were milder than those observed with MCAT and included facial flushing and reduced food intake. PAL-434 treatment decreased responding for food only when the two highest cocaine doses were available. The decreased responding for food with the combination of high doses of cocaine and PAL-434 may be due to either an additive response-disruption effect or through the combined anorectic effects of PAL-434 and cocaine. Anecdotally, it was common to find supplemental biscuits or food-pellets earned during sessions left in an animals’ food hopper after cocaine self-administration sessions. Modest reductions in responding for food may be acceptable characteristics of a treatment medication if there are few other adverse effects.

Possible mechanisms of PAL-434

PAL-434 has a similar profile of selectivity for DA to 5-HT release as a previously studied compound, PAL-314 (m-methamphetamine; see Wee et al., 2005): 8-fold compared with 6.5-fold, respectively. One study compared the effects of several amphetamine analogues, including PAL-314, on their ability to increase locomotor stimulation (ambulation) and extracellular DA and 5-HT levels in rats (Baumann et al., 2011b). The analogues tested had similar in vitro efficacy at releasing DA but differing efficacy as 5-HT releasers (ranging from 1.2–219-fold). PAL-314 administration did not significantly increase locomotor activity (i.e. forward ambulation) and this behavioural effect was correlated with lower levels of DA release in the nucleus accumbens (Baumann et al., 2011b). DA release in the nucleus accumbens was inversely related to 5-HT release; consistent with the hypothesis that increased 5-HT neurotransmission attenuates the stimulant effects of monoamine releasers (Czoty et al., 2002; Rothman et al., 2008; Baumann et al., 2011a).

The differences between PAL-434 and MCAT in the present study are also consistent with this hypothesis. MCAT has no activity as a 5-HT releaser and was equipotent to cocaine in drug discrimination studies. If DA release is attenuated by the significant serotonergic
component of PAL-434 administration as shown with PAL-314 in rats, a higher dose of the drug would be required to achieve the increases in DA necessary to reproduce the cocaine discriminative stimulus (e.g. Desai et al., 2010). However, PAL-434 did elicit full (>80%) cocaine-like responding in all monkeys at doses that did not produce behavioural disruption.

5-HT-selective releasers are generally less selective in their treatment effects; however, a low dose (0.1 mg/kg/h) of fenfluramine decreased cocaine self-administration by about 25% with no effect on food-maintained responding in rhesus monkeys (Negus et al., 2007). The 5-HT component of PAL-434 may have added to the DA releasing effect to reduce cocaine self-administration. There are other examples of combined DA/5-HT effects on cocaine self-administration. For example, acute or chronic administration of citalopram (a 5-HT reuptake inhibitor) in combination with an ED50 dose of a long-acting DA releaser (i.e. the phenyltropane cocaine analogue, RTI-336) completely eliminated cocaine self-administration (Howell et al., 2007). Similarly, combining drugs that increase extracellular levels of 5-HT either through reuptake inhibition (i.e. fluoxetine) or reverse transport (i.e. d-fenfluramine) with phentermine (a DA-selective releaser) produced a decrease in cocaine self-administration in rats (Glatz et al., 2002; though see Glowa et al., 1997). The combined effects of these drugs had a larger effect than either drug administered alone.

In addition to 5-HT-related decreases in locomotor stimulant and dopaminergic effects, several studies have shown that increasing extracellular levels of 5-HT attenuates the reinforcing efficacy of DA indirect agonists. It is generally agreed that mixed DA/5-HT drugs are less reinforcing than dopaminergic drugs (Roberts et al., 1999; Rothman et al., 2006). For example, increasing the ratio of fenfluramine (a selective 5-HT releaser) to d-amphetamine (a selective DA releaser) dose-dependently reduced the reinforcing efficacy of d-amphetamine in rhesus monkeys under a progressive ratio schedule of reinforcement (Wee and Woolverton, 2006). Similarly, in rhesus monkeys, potency of several amphetamine analogues as reinforcers was positively correlated with the ratio of in vitro potencies releasing DA/5-HT (Wee et al., 2005). These findings would predict that the reinforcing efficacy of PAL-434 would be lower than more DA-selective releasers.

**Implications for medication development**

Effective agonist therapies share some biochemical and behavioural effects with a drug of abuse and may reduce drug abuse by normalizing brain chemistry and behaviour. The opioid agonists methadone and buprenorphine are effective for the treatment of opioid dependence (Mello and Mendelson, 1995). Mello and Negus (1996) suggest that optimal medications produce sustained decreases in self-administration across a range of drug doses, with few effects on responding for non-drug reinforcers or undesirable effects. PAL-434 produced sustained and selective decreases in cocaine self-administration with few side-effects. Our results add to the growing body of literature suggesting that monoamine releasers may be effective for the treatment of cocaine abuse as agonist-like therapies (Mello, 2005; Herin et al., 2010). Drugs with moderate (approximately 8–15-fold) selectivity for DA vs. 5-HT release warrant further consideration as candidates for treatment of cocaine abuse and dependence.

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**Statement of Interest**

None.

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