Reinforcing Strength of a Novel Dopamine Transporter Ligand: Pharmacodynamic and Pharmacokinetic Mechanisms

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ABSTRACT

Drugs that block dopamine uptake often function as positive reinforcers but can differ along the dimension of strength or effectiveness as a positive reinforcer. The present study was designed to examine pharmacological mechanisms that might contribute to differences in reinforcing strength between the piperidine-based cocaine analog (+)-methyl 4β-(4-chlorophenyl)-1-methylpiperidine-3-α-carboxylate [(+)-CPCA] and cocaine. Drugs were made available to rhesus monkeys (n = 5) for i.v. self-administration under a progressive ratio schedule. Both compounds maintained responding with sigmoidal or biphasic dose-response functions (0.1–1.0 mg/kg/injection). (+)-CPCA was one-fourth as potent as cocaine and maintained fewer injections per session, at maximum. For in vitro binding in monkey brain tissue, (+)-CPCA was about one-half as potent as cocaine at the dopamine transporter (DAT), and the two compounds had similar affinities at the norepinephrine transporter. (+)-CPCA was less than 1/10 as potent as cocaine at the serotonin transporter. In ex vivo binding in rat striatum, occupancy of the DAT increased directly with dose to a maximum of approximately 80% for both compounds, and (+)-CPCA was about one-fourth as potent as cocaine. Ex vivo DAT occupancy was significantly higher for cocaine than (+)-CPCA at 2 min after injection but similar at other times. Thus, the primary differences between these compounds were in serotonin transporter affinity and the kinetics of DAT binding. These results suggest that (+)-CPCA is a weaker positive reinforcer than cocaine because it has a slower onset of action over the first few minutes after i.v. injection.

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Drugs that bind dopamine transporters (DATs) and block DA uptake often function as positive reinforcers (Ritz et al., 1987; Bergman et al., 1989). Cocaine is an excellent example of such a compound. There are, however, a number of compounds that block DA uptake but seem to differ from cocaine along the dimension of maximum reinforcing effect, or strength as a positive reinforcer. GBR 12909, for example, is a highly selective DA uptake blocker (Lewis et al., 1999) that has some cocaine-like behavioral effects (Spealman et al., 1989; Howell and Byrd, 1991). Like cocaine, GBR 12909 can serve as a positive reinforcer to maintain i.v. self-administration by animals (Bergman et al., 1989; Howell and Byrd, 1991; Skjoldager et al., 1993). However, GBR 12909 seems to be a weaker positive reinforcer than cocaine (Tella et al., 1991; Stafford et al., 2001; Woolverton et al., 2001). Similarly, several local anesthetics, as well as analogs of benztpine, are effective DA uptake blockers but weaker positive reinforcers than cocaine (Wilcox et al., 2000; Woolverton et al., 2001).

Research with compounds that block monoamine uptake but vary in reinforcing strength can help elucidate pharmacological mechanisms that contribute to their reinforcing strength and enhance our understanding of the pharmacology of drug abuse. As for any pharmacological effect, pharmacodynamics and pharmacokinetics are determinants of reinforcing strength of monoamine transporter ligands. It may be that a compound’s particular combination of monoamine actions influences reinforcing strength (Roberts et al., 1999). Cocaine is approximately equipotent at all three monoamine transporters (Carroll et al., 1995), whereas GBR 12909, in contrast, is more selective for the DAT (Lewis et al., 1999). Alternatively, pharmacological actions involving other nonmonoamine neurotransmitter systems may act to limit the self-administration of some DAT ligands (Wilcox et al., 2000; Ranaldi and Woolverton, 2002). Recently, it has been proposed that maximum DAT occupancy may be an important determinant of the subjective (Volkow et al., 1996, 1997) and reinforcing (Wilcox et al., 2002) effects of cocaine and

ABBREVIATIONS: DAT, dopamine transporter; DA, dopamine; (+)-CPCA, (+)-methyl 4β-(4-chlorophenyl)-1-methylpiperidine-3-α-carboxylate; LH, limited hold; TO, time-out; 5-HT, 5-hydroxytryptamine; CFT, 2β-carbamoxy-3β-(4-fluorophenyl)tropane; CI, confidence interval; S/C, striatal/cerebellar ratio; inj, injection; GBR 12909, 1-[2-[(bis[4-fluorophenyl]methoxy)ethyl]-4-(3-phenylpropyl)piperazine.
other DAT ligands. With regard to pharmacokinetics, GBR 12909 may be a weaker positive reinforcer than cocaine because of a slower onset of DAT binding (Pogun et al., 1991). The precise relationship between these pharmacological factors and reinforcing strength of monoamine transporter ligands remains largely speculative.

In addition to basic information about brain mechanisms and reinforcement, compounds that act primarily at monoamine transporters are being widely studied because of their potential as treatment medications for cocaine abuse. Compounds that bind the DAT but are weaker reinforcers than cocaine may prove useful in this regard (Mello and Negus, 1996; Howell and Wilcox, 2001). Numerous chemical approaches have been proposed for the development of novel medications (Newman, 1998; Carroll et al., 1999). Kozikowski and colleagues have synthesized piperidine-based cocaine analogs that vary in their potencies at the monoamine transporters (Kozikowski et al., 1998; Petukhov et al., 2002). One of these compounds, (+)-methyl 4β-(4-chlorophenyl)-1-methylpiperidine-3-α-carboxylate [(+)CPCA; Fig. 1], has shown promise as a potential treatment medication. In that study, (+)-CPCA was essentially equipotent to cocaine in blocking DA and norepinephrine uptake in vitro in rat brain tissue. However, it was substantially less potent than cocaine in blocking 5-HT uptake. The compound functioned as a positive reinforcer in monkeys responding under a fixed ratio schedule of reinforcement, but its relative strength as a positive reinforcer is unknown. In addition, there is no information currently available concerning the kinetics of transporter occupancy by (+)-CPCA.

The present study was designed to compare the strength of (+)-CPCA and cocaine as positive reinforcers in monkeys responding under a progressive ratio schedule of i.v. self-administration. Progressive ratio schedules allow the ranking of drugs according to their strength as positive reinforcers (Richardson and Roberts, 1996; Stafford et al., 1998). Because differences in monoamine transporter activity have been associated with differences in reinforcing strength (Roberts et al., 1999), we assessed affinity of (+)-CPCA for monoamine transporter sites in vitro in monkey brain tissue. Monkey brain tissue was used to allow more direct comparison to self-administration results. In addition, because the extent and rate of DAT occupancy may contribute to reinforcing strength, ex vivo binding methods were used in rats to compare rate and extent of DAT binding by cocaine and (+)-CPCA. Ex vivo binding has been used to characterize binding of a number of radioligands (Stockmeier et al., 1993) and has been applied to the study of various compounds to the DAT (Scheffel et al., 1991; Gatley et al., 1999).

**Materials and Methods**

**Self-Administration.** The subjects were five male (7.8–11.1 kg) rhesus monkeys (*Macaca mulatta*). Monkey AV88 was experimentally naive, whereas the other subjects had histories of drug self-administration. Most recently, monkey ROk2 had a history of self-administration of D2 agonists under a progressive ratio schedule of reinforcement (Woolverton and Ranaldi, 2002). Monkeys L638 and RJu2 had histories of self-administration of methamphetamine under a progressive ratio schedule of reinforcement and pretreatment with a 3-phenyltropane analog (Ranaldi et al., 2000). Monkey AP78 had a history of self-administration of cocaine/scopolamine mixtures under a progressive ratio schedule of reinforcement (Ranaldi and Woolverton, 2002). All monkeys were provided with sufficient food to maintain stable body weight (150–200 g/day; Teklad 25% Monkey Diet; Harlan/Teklad, Madison, WI). Water was continuously available. A vitamin supplement was provided three times per week.

The monkeys were housed in the experimental cubicles (1.0 m³; Plaslabs, Lansing, MI). Each monkey was fitted with a stainless steel restraint harness attached by a spring arm to the rear wall of the cubicle. The front door of the cubicle was transparent and the remaining walls were opaque plastic. Two response levers (PRL-001; BRS/LVE, Beltsville, MD) were mounted on the inside of the door, on either side of a food dish. Four jeweled stimulus lights, two red and two white, were mounted above each lever. Drug injections were delivered by a peristaltic infusion pump (Cole-Parmer Instrument, Chicago, IL). All events in an experimental session were controlled by a Macintosh computer with custom interface and software.

**Procedure.** Using strict aseptic techniques performed under ketamine and isoflurane anesthesia, a silicone catheter (0.26-cm o.d. × 0.076-cm i.d.; Cole-Parmer Instrument) was implanted into a jugular (internal or external) or femoral vein. Brachial veins were implanted with a tapered microrenethane catheter (0.08-cm o.d. × 0.04-cm i.d.; Braintree Scientific, Braintree, MA). The proximal end was inserted into the vein and threaded to terminate in the vena cava near the right atrium. The distal end of the catheter was passed subcutaneously to exit the monkey between the scapulae. After surgery the catheter was threaded through the spring arm, out the rear of the cubicle and connected to the peristaltic pump. In the event of catheter failure, surgery was repeated using another vein, after the veterinarian confirmed the health of the monkey.

Experimental sessions began at noon each day and were conducted 7 days/week. At the beginning of a session, the white lights were illuminated above both levers. Responding on the right lever under a progressive ratio schedule of reinforcement resulted in the delivery of an injection. Responding on the left lever was counted but had no other programmed consequence. The progressive ratio schedule has been described in detail previously (Wilcox et al., 2000). It consisted of five components, each made up of four trials, for a total of 20 available trials per day. The response requirement for the first component was 100 and doubled for each successive component. The same response requirement was in effect for each trial in a component, and a trial ended with a 10-s drug injection or the expiration of a 30-min limited hold (LH). During the injection the lights above both levers turned from white to red. There was a 30-min time-out (TO) after each drug injection or the expiration of an LH. If the

![Chemical structure of (+)-CPCA](image)
response requirement was not completed for two consecutive trials (i.e., the LH expired), or the animal took all 20 injections, the session ended.

In baseline sessions, cocaine (0.1 mg/kg/injection for RJu2, L637, and RlK2; 0.3 mg/kg/injection for AP13 and AV88) or saline was available for injection on alternate days until responding was stable (mean ± 2 injections) for at least three consecutive cocaine and saline sessions. At this point, the session sequence was changed to a double alternation, i.e., two consecutive cocaine sessions were followed by two consecutive saline sessions. When responding was again stable, test sessions were added to the daily sequence between two saline and two cocaine sessions. To prevent monkeys from learning this sequence and anticipating sessions, a randomly determined saline or cocaine baseline session was inserted after every other test session. During test sessions the monkeys had access to one of various doses of cocaine (0.01–1.0 mg/kg/injection) or (+)-CPCA (0.01–3.0 mg/kg/injection). After a test session, a monkey was returned to baseline sessions until cocaine- and saline-maintained responding was again stable. Doses of cocaine were tested twice, once the day after a cocaine baseline session and once the day after a saline baseline session. Because of limited drug supplies, doses of (+)-CPCA were generally tested once in each monkey. For cocaine, doses were tested in random order with the first dose for an individual monkey counterbalanced across monkeys. For (+)-CPCA, doses were tested in an ascending order in the first monkey tested then in a random order with the first dose for an individual monkey counterbalanced in the other monkeys.

After testing all doses of (+)-CPCA, the possibility that drug accumulation over a session decreased progressive ratio responding was examined in four monkeys by extending the TO after injections to 60 min. Cocaine was tested again, as described above, either at the dose that maintained the maximum number of injections (L637 and RlK2) as determined under standard conditions in stainless steel cages with water continuously available. They were fed a sufficient amount of Teklad Monkey Diet (Harlan, Indianapolis, IN) to maintain stable body weight, received fresh fruit 5 days/week, and a chewable multiple vitamin tablet 3 days/week.

Procedure. No monkeys were sacrificed specifically for this experiment but had been euthanized previously after all accessible veins had been used in self-administration studies. For euthanasia, monkeys were sedated with ketamine then given an overdose of i.v. pentobarbital. Brains were collected immediately (within 10 min) after sacrifice and the caudate nucleus, putamen, frontal cortex, and cerebellum were dissected (20–30 min) according to the atlas of Snider and Lee (1961). Immediately after dissection, tissue was frozen on aluminum foil over solid CO2 for 30 min, with no additional preparation, and then placed into a −80°C freezer until assay.

For binding studies, frozen tissue was thawed, homogenized in buffer (Woolverton et al., 2000) and centrifuged at 20,000g for 20 min at 4°C. The resulting pellet was resuspended in fresh buffer and centrifuged an additional one or two times, depending on the assay. After centrifugation, the pellet was resuspended at the appropriate tissue concentration for displacement or saturation assays.

Specific conditions for displacement and saturation assays were as previously published with minor changes (Table 1; Woolverton et al., 2000). For displacement studies the tissue was added to assays containing the radioligand and various concentrations of cocaine or (+)-CPCA dissolved in assay buffer. For saturation studies, the tissue was incubated with varying concentrations of [3H]CFT (0.1–25.6 nM), [3H]nisoxetine (0.1–12.8 nM), and [3H]paroxetine (0.006–25.6 nM). For displacement studies, concentrations of cocaine and (+)-CPCA ranged between 0.1 nM and 1 mM. For both saturation and displacement studies all assay tubes were brought to their final volume (1.0 ml for [3H]CFT, [3H]paroxetine; 500 µl for [3H]nisoxetine with the addition of buffer). All assays were initiated with the addition of tissue. Assays were incubated under conditions described in Woolverton et al. (2000). Reactions were terminated by rapid vacuum filtration using a 24-well cell harvester (Brandel Inc., Gaithersburg, MD) through presoaked GPC filters (Whatman, Maid-
stone, UK Table 1). The filters were rinsed with ice-cold buffer and deposited into Top Count deep-well plates (Packard Instrument Company, Inc., Downers Grove, IL). Five hundred microliters of Microscint-20 cocktail (Packard Instrument Company, Inc.) was added to each well. Bound radioactivity was determined using a Top Count scintillation counter (Packard Instrument Company, Inc.). Protein levels in tissue homogenate samples were determined using the bicinchoninic acid method (Smith et al., 1985; kits from Pierce Chemical, Rockford, IL). Absorbance (at 560 nm) was measured on a spectrophotometer (Beckman Coulter, Inc., Fullerton, CA). All saturation and displacement assays were performed in triplicate.

Data Analysis. Radioligand binding data were initially reduced and analyzed using iterative curve fitting (Prism 3.0; GraphPad Software). Kᵢ and Bₘₐₓ values, and their 95% confidence intervals (CIs), were derived from saturation studies. To compare the goodness-of-fit of one-site models to two-site models, one-site and two-site models with all Hill coefficients fixed to -1 were fit to displacement data. The one-site model was assumed unless the mean square error was significantly reduced by using a two-site model (p < 0.05 using a univariate F test). Kᵢ values and their 95% CIs were calculated for each compound from displacement studies.

Ex Vivo Binding

DAT binding was studied ex vivo using methods similar to those previously published using mice (Scheffel et al., 1991; Statthas et al., 1995; Gatley et al., 1999).

Subjects and Apparatus. The subjects were male Sprague-Dawley rats weighing between 250 and 300 g. They were initially housed in groups of three in plastic cages and with a 12-h light/dark cycle (lights on at 6:00 AM). Food and water were available ad libitum.

Procedure. Drugs were given i.v. via a surgically implanted catheter. For surgery, rats were anesthetized with pentobarbital (50 mg/kg i.p.) and a femoral catheter was implanted using standard techniques. The exteriorized tip of the catheter was sealed by heat-curing. After surgery, they were housed individually for 48 h then used experimentally.

To establish relative potency for DAT occupancy, injections of dose of cocaine or (+)-CPCA were given before [³H]CFT to permit maximum (or equilibrium) binding of cocaine and (+)-CPCA to the DAT before the injection of [³H]CFT. Initially, catheterized rats were placed in a plastic restrainer and injected i.v. (0.4 ml/rat/10 s) with either 10 μmol/kg cocaine or 30 μmol/kg (+)-CPCA. These doses were selected based upon preliminary studies with cocaine and the in vitro potency ratio between cocaine and (+)-CPCA. At various time points after drug injection (0.5–30 min), rats were injected with [³H]CFT, also i.v. over 10 s. Because preliminary studies showed that the striatal/cerebellar ratio (S/C) of [³H]CFT reached a maximum 45 min after injection of [³H]CFT, rats were decapitated at this time point. After decapitation, brains were removed and dissected into striatum (high DAT density) and cerebellum (no DAT, nonspecific binding). Striatum and cerebellum were weighed and placed into separate 5-ml glass vials. Solvable (10 μl/mg tissue) was added and the vial was allowed to sit for 24 h at room temperature. After 24 h, glacial acetic acid (1 μl/mg tissue) was added and 100 μl of the tissue solution was immediately pipetted into each well of 24-well scintillation plates (3–4 wells/sample). Microscint-20 cocktail (500 μl) was then added to each well and the plate was sealed. This preparation was allowed to sit for 4 h to further solubilize tissue and reduce chemiluminescence of the Microscint-20 cocktail. Radioactivity was then counted. Complete dose-response functions were then determined for each drug using the time points at which the decrease in [³H]CFT was maximal. ED₅₀ values were calculated for reduction in [³H]CFT binding.

To establish the time course of DAT binding, an injection of a selected dose of cocaine or (+)-CPCA was given at the time point at which [³H]CFT binding was asymptotic. The decrease in binding was measured at various time points after drug injection and compared with the same points after saline injection (Statthas et al., 1995).

Specifically, saline or ED₅₀ doses of cocaine or (+)-CPCA were given 45 min after injection of [³H]CFT. Animals were decapitated at various time points (0.5–120 min) after injection of test drugs.

Data Analysis. For each ligand, S/C was calculated. Data were normalized to S/C — 1 so that complete inhibition of binding approached zero. Transporter occupancy was calculated using the equation % occupancy = (A — x/A — B) × 100 (Gatley et al., 1999). In this equation, A and x are S/C measured after injection of radioligand alone and drug plus radioligand, respectively. B is the S/C measured after a high dose of cold GBR 12909, a selective DAT ligand, which is assumed to reflect 100% occupancy of the transporter. The difference between B and 1.0 presumably reflects differences in nonspecific binding. ED₅₀ values (the dose of competing drug displacing half the specific binding) and maximum occupancy were calculated using iterative curve fitting (Prism 3.0; GraphPad Software). Statistical significance of ED₅₀ differences was assessed using Student’s t test with significance set at the p < 0.05 level.

Time course data for inhibition of binding by cocaine and (+)-CPCA were converted to percentage of control with saline pretreatment data using the same time points as control. Data for cocaine and (+)-CPCA were compared using a two-way analysis of variance followed by adjusted Bonferroni t tests.

Drugs. Cocaine HCl was provided by the National Institute on Drug Abuse (Rockville, MD) and was dissolved in 0.9% saline for self-administration. (+)-CPCA was synthesized as described by Kozikowski et al. (1998) and was also dissolved in saline. Doses are expressed as the salt forms of the drugs. Molecular masses are 339 µg/µmol for cocaine and 304 µg/µmol for (+)-CPCA. Radioligands were purchased from PerkinElmer Life Sciences (Boston, MA). For binding studies, drugs were mixed fresh before each assay.

Results

Self-Administration. In baseline sessions, cocaine maintained an average of between 8.2 (AP13) and 17.8 (Rlk2) injections/session, whereas saline maintained between 1.0 (AP13) and 2.0 (L637) injections/session. Over the course of the experiment, responding in baseline sessions did not change systematically across monkeys (data not shown).

Cocaine functioned as a reinforcer in all monkeys and the mean dose-response function was asymptotic (Fig. 2). On an individual basis, responding increased with dose to an as-

![Graph 2](https://via.placeholder.com/150)

Fig. 2. Number of injections per session as a function of dose for cocaine and (+)-CPCA determined under a progressive ratio procedure in rhesus monkeys. Each point represents the mean number of injections for the five monkeys tested and vertical error bars represent the S.E.M. values. For cocaine, the effect of each dose was determined twice in each monkey. For (+)-CPCA, the effect of most doses was determined once in each monkey.
ymptote in four of the five monkeys and decreased again in the fifth (L637) at 1.0 mg/kg/injection (data not shown). (+)-CPCA also functioned as a positive reinforcer in all monkeys, and both mean and individual dose-response functions were biphasic. The mean ED₅₀ was 0.06 mg/kg/inj (0.01 S.E.M.) for cocaine and 0.21 mg/kg/inj (0.08 S.E.M.) for (+)-CPCA. On a molar basis, ED₅₀ values were 0.17 μmol/kg/inj (0.04 S.E.M.) for cocaine and 0.68 μmol/kg/inj (0.25 S.E.M.) for (+)-CPCA. The difference was not statistically significant (p = 0.08), primarily because ED₅₀ values for the two drugs were comparable in one monkey (L637). The mean maximum number of injections per session maintained was 16.5 (1.69 S.E.M.) for cocaine and 12.1 (1.48 S.E.M.) for (+)-CPCA. Differences between drugs in injection maximums were statistically signif-
ificant (p = 0.04). When the TO after injection was increased to 60 min the maximum was either unaffected or decreased (Table 2).

In Vitro Binding. Results of saturation studies using the different ³H-ligands in rhesus monkey brain are presented in Table 3. Both compounds inhibited binding of all three radioligands in a concentration-related manner. Displacement was consistent with a one-site model for all three radioligands. Cocaine had about a 2-fold higher affinity for the [³H]CFT site than did (+)-CPCA, whereas the compounds had similar affinities for the [³H]nisoxetine site (Table 4). For displacement of [³H]paroxetine, the affinity of cocaine was approximately 15-fold higher than that of (+)-CPCA.

Ex Vivo Binding. The maximum decrease in [³H]CFT binding by cocaine was seen when cocaine was given 3 min before [³H]CFT (data not shown). For (+)-CPCA, this value was 10 min. When various doses were administered at these pretreatment times, both cocaine and (+)-CPCA inhibited the binding of [³H]CFT in a dose-related manner. Occupancy of the DAT increased in a dose-related manner (Fig. 3). The ED₅₀ for cocaine was 8.82 μmol/kg and for CPCA was 34.3 μmol/kg. This difference was statistically significant (p = 0.03). Maximum occupancy was comparable for both drugs (p = 0.48). When the ED₅₀ of each drug was given at various times before sacrifice, DAT occupancy was greater for cocaine than for (+)-CPCA 2 min after injection (Fig. 4). The effects of the two drugs were not different at other time points.

Discussion

As in previous studies using this and other progressive ratio schedules (Stafford et al., 1998; Wilcox et al., 2000), cocaine functioned as a positive reinforcer and responding increased with dose to an asymptote. (+)-CPCA also functioned as a positive reinforcer under the present progressive ratio schedule, confirming and extending previous self-administration results with this compound in monkeys maintained under a fixed ratio schedule (A. Kozikowski, personal communication). Responding maintained by (+)-CPCA increased with dose then decreased at the highest dose tested. Although only tested in one monkey, it is likely that cocaine-maintained responding would have decreased again had doses of 1.0 mg/kg/injection or higher been tested. In all but one monkey, cocaine was approximately 4-fold more potent than (+)-CPCA as a reinforcer. Maximum levels of responding were higher for cocaine than for (+)-CPCA. Doubling the TO after an injection from 30 to 60 min failed to increase cocaine or (+)-CPCA-maintained responding, arguing that the maximum response maintained was not limited by the duration of action for either drug. These findings support the conclusion that cocaine was a stronger positive reinforcer than (+)-CPCA.

Clearly, reinforcing strength can vary with behavioral conditions (Katz, 1990). Because behavioral conditions were constant across the two drugs tested in the present experiment, it is possible to examine pharmacological mechanisms that may have contributed to differences in reinforcing strength. Pharmacodynamically, it has been suggested that the mix of effects on monoamine neurotransmission may influence reinforcing strength. More specifically, increased 5-HT relative to DA activity has been associated with diminished reinforcing strength (Vanover et al., 1992; Roberts et al., 1999). In monoamine uptake studies conducted in vitro in rat brain tissue, (+)-CPCA has been reported to have DA and norepinephrine activity comparable with that of cocaine, but to have diminished 5-HT activity (A. Kozikowski, personal communication). In vitro binding data in the present study extend that observation to transporter binding in monkey brain tissue. Other monoamine actions being equal, then, one would expect diminished 5-HT activity to enhance, or at least not diminish, the reinforcing strength of (+)-CPCA. That is, these findings argue against a conclusion that the mix of monoamine actions of (+)-CPCA was responsible for its reduced reinforcing strength relative to cocaine. It should also be considered that (+)-CPCA may have actions that cocaine lacks on other neurotransmitter systems that influence self-administration. For example, it has been suggested that sodium channel actions may limit the reinforcing effects of local anesthetics (Wilcox et al., 2000). Additionally, the reduced reinforcing strength of benztrone analogs that are DAT ligands may be related to anticholinergic actions.

Table 2

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Cocaine (30)</th>
<th>Cocaine (60)</th>
<th>(+)-CPCA (30)</th>
<th>(+)-CPCA (60)</th>
</tr>
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<tbody>
<tr>
<td>AP13</td>
<td>8.5 (0.3)</td>
<td>8 (0.3)</td>
<td>9.5 (1.0)</td>
<td>8 (1.0)</td>
</tr>
<tr>
<td>L637</td>
<td>18.5 (0.3)</td>
<td>11 (0.3)</td>
<td>12 (0.3)</td>
<td>9 (0.3)</td>
</tr>
<tr>
<td>AV88</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>RJ02</td>
<td>17 (0.1)</td>
<td>11 (0.1)</td>
<td>17 (0.3)</td>
<td>11 (0.3)</td>
</tr>
<tr>
<td>RfK2</td>
<td>19 (0.1)</td>
<td>2 (0.1)</td>
<td>10 (1.0)</td>
<td>10 (1.0)</td>
</tr>
</tbody>
</table>

N.D., not determined.

Table 3

<table>
<thead>
<tr>
<th>Site</th>
<th>Kᵦ (95% CI)</th>
<th>Bᵦ max (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>[³H]CFT</td>
<td>30.3 (26.5–34.1)</td>
<td>2119 (1942–2296)</td>
</tr>
<tr>
<td>[³H]Nisoxetine</td>
<td>1.63 (1.42–1.84)</td>
<td>44.5 (42.5–46.5)</td>
</tr>
<tr>
<td>[³H]Paroxetine</td>
<td>0.085 (0.045–0.12)</td>
<td>111 (66.5–155)</td>
</tr>
</tbody>
</table>

Results of saturation studies. Data represent means and 95% CI (n = 4) and were derived as described under Materials and Methods.
when an excess of GBR 12909, was given in combination with [3H]CFT. Note that abscissa is log scale. Each point is the mean of three to five rats and vertical lines represent the S.E.M. values. Data represent the mean and 95% confidence intervals (n = 4).

**Table 4**

<table>
<thead>
<tr>
<th>Drug</th>
<th>[3H]CFT $K_i$ (95% CI)</th>
<th>[3H]Nisoxetine $K_i$ (95% CI)</th>
<th>[3H]Paroxetine $K_i$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>167 nM (127–217)</td>
<td>1740 nM (1112–2722)</td>
<td>1386 nM (1070–1794)</td>
</tr>
<tr>
<td>(+)-CPCA</td>
<td>301 nM (243–373)</td>
<td>1475 nM (1012–2150)</td>
<td>18070 nM (11410–28620)</td>
</tr>
</tbody>
</table>

Fig. 3. Percentage of occupancy of the DAT as a function of drug dose for cocaine and (+)-CPCA in rats. Each point represents the S/C of radioactivity after drug + [3H]CFT, calculated as a percentage of the same ratio when an excess of GBR 12909, was given in combination with [3H]CFT. Each point is the mean of three to five rats and vertical lines represent the S.E.M. values.

Fig. 4. Time course of DAT occupancy by cocaine and (+)-CPCA in rats. Drugs were given 45 min after [3H]CFT and rats were sacrificed at the indicated time points. Each point represents the S/C of radioactivity after [3H]CFT + drug, calculated as a percentage of the same ratio when [3H]CFT followed by saline injections with the identical sacrifice times. Each point is the mean of three to five rats and vertical lines are the S.E.M. values. Note that abscissa is log scale. *, p < 0.05.

(2002) reported DAT occupancies of 64 to 75% and 94 to 99% for doses of cocaine or RTI-113, respectively, that maintained maximum rates of responding in monkeys. In the present study, the potency relationship between cocaine and (+)-CPCA was comparable in the self-administration and the occupancy assays. In addition, DAT occupancy by cocaine was about 60% at a dose of 10 μmol/kg (3.4 mg/kg), well within the range of doses shown to maintain responding in rats under a progressive ratio schedule of reinforcement (Roberts et al., 1999). Based on these data, there seems to be a good correspondence in the relationship potency in occupying the DAT and potency as a reinforcer that holds across species. However, in the present study cocaine and (+)-CPCA differed in their strength as reinforcers but exhibited comparable maximum occupancies of the DAT. Thus, the hypothesis that relative reinforcing strength was directly related to maximum occupancy of the DAT was not supported. Data from previous studies do not address this hypothesis. Additional research is required to further examine relationship between reinforcing strength and maximum DAT occupancy. Given that reinforcing strength is only one component of abuse liability, any relationship between DAT occupancy and abuse liability remains to be established.

Onset of action has also been proposed to be an important determinant of the reinforcing effect of drugs. Perhaps the strongest data in support of this notion have been collected in humans. More rapid onset of action has been associated with increased subjective effects of drugs in human subjects (deWit et al., 1993; Marsch et al., 2001). Although there is the suggestion of this effect in the animal literature (Balster and Schuster, 1973), a systematic analysis of the relationship between onset of action and reinforcing strength has not been undertaken. In a preliminary study, we found that monkeys given a choice between 0.1 mg/kg cocaine injected over 10 s and this same dose of cocaine injected over 30 or 100 s, preferred the more rapid injection. In the present study, cocaine occupied a higher proportion of DATs than did (+)-CPCA at 2 min after injection. At other time points, DAT occupancy was comparable for the two drugs. The precise mechanism accounting for this difference, e.g., differences in distribution or binding at the site of action, are unclear at this point. Assuming that the kinetic difference observed at the ED50 is comparable across the dose-response function, these data support the hypothesis that the difference in reinforcing strength between cocaine and (+)-CPCA is related to the slower onset of DAT occupancy by (+)-CPCA. More generally, these data suggest that reinforcing strength is directly related to rate of DAT occupancy over a time frame that is as short as the first 3 min after injection. Furthermore, our findings suggest that between-drug differences in rate of DAT occupancy as small as 1 min can influence relative reinforcing strength. Obviously, additional research is required to support or refute this conclusion. Nevertheless, the present data begin to provide an empirical time frame for the hypothesis that rate of onset contributes to reinforcing strength.

It has been amply demonstrated that among DAT ligands,
potency as a positive reinforcer is correlated with DAT affinity (Ritz et al., 1987; Bergman et al., 1989). However, the amount of behavior maintained by a drug is more nearly predicted by its relative strength as a reinforcer than by its potency. Clearly, the present experiment begins to quantify some of the factors, most particularly kinetics of onset, which may contribute to the reinforcing strength of monoamine transporter ligands.

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References


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