

Interaction of cocaine and dopamine transporter inhibitors on behavior and neurochemistry in monkeys

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Abstract

Drugs that target the dopamine transporter (DAT) have been proposed as pharmacotherapies to treat cocaine abuse. Accordingly, it is paramount to understand pharmacological interactions between cocaine and DAT inhibitors. The present study characterized acute interactions between cocaine and several DAT inhibitors (RTI-177, FECNT, RTI-112) that differed in selectivity for monoamine transporters on operant behavior and *in vivo* neurochemistry in squirrel monkeys. RTI-177 and FECNT, two DAT inhibitors with low affinity at norepinephrine transporters (NET), produced dose-dependent stimulant effects on behavior maintained by a fixed-interval schedule of stimulus termination. Compared to cocaine, RTI-177 and FECNT had a slower onset and longer duration of action. *In vivo* microdialysis in the caudate nucleus of awake monkeys confirmed dose-dependent increases in extracellular dopamine that corresponded to behavioral effects. Among the drugs characterized, RTI-112 is reportedly the least selective for binding to DAT, NET, and serotonin transporters (SERT). Interestingly, RTI-112 failed to produce significant behavioral-stimulant effects, and its effects on extracellular dopamine were highly variable across subjects. The results indicate that the pharmacological profile of DAT inhibitors may be influenced by actions at multiple monoamine transporters. Importantly, there was little evidence of additivity on behavioral or neurochemical measures when cocaine was administered in combination with behavioral-stimulant doses of the DAT inhibitors.

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1. Introduction

Cocaine is an equipotent inhibitor of neuronal dopamine (DAT), serotonin (SERT), and norepinephrine (NET) transporters (Heikkila and Manzino, 1984; Reith et al., 1986). However, inhibition of the DAT appears to be critical to the behavioral-stimulant and reinforcing effects of cocaine associated with its significant abuse

liability (Reith et al., 1985; Ritz et al., 1987; Kuhar et al., 1991). Preclinical studies have demonstrated a significant correlation between DAT occupancy and the behavioral-stimulant (Cline et al., 1992; Kuhar, 1993) and reinforcing (Ritz et al., 1987; Bergman et al., 1989; Wilcox et al., 1999) effects of a variety of DAT inhibitors from distinct structural classes. Moreover, neuroimaging studies in human cocaine users have found a significant correlation between DAT occupancy and the subjective high reported following administration of cocaine (Volkow et al., 1997) or methylphenidate (Volkow et al., 1999). Collectively, the results obtained in behavioral studies provide compelling evidence that dopamine plays a major role in the

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neuropharmacology and addictive properties of cocaine (Wise, 1984; Ritz et al., 1987; Woolverton and Kleven, 1988).

Given the obvious importance of the DAT in the addictive properties of cocaine, drugs that target the DAT and share some pharmacological properties with cocaine have been proposed as substitute agonist pharmacotherapies to treat cocaine abuse and dependence (Mello and Negus, 1996; Carroll et al., 1999; Howell and Wilcox, 2001). This emphasis highlights the importance of understanding pharmacological interactions between cocaine and DAT inhibitors on neurochemistry and behavior. However, published studies of drug interactions in animal models have been equivocal. Systemic administration of DAT inhibitors prior to a cocaine challenge caused additive or slightly infra-additive effects on measures of extracellular dopamine and locomotor activity in rats (Martin-Fardon et al., 1996; Tolliver et al., 1999). However, the phenylpiperazine, GBR 12909, has been reported to attenuate cocaine-induced increases in striatal dopamine in rats (Rothman et al., 1991; Baumann et al., 1994) at doses that suppressed cocaine self-administration in rhesus monkeys (Glowa et al., 1995). Interactions between cocaine and DAT inhibitors on *in vivo* neurochemistry have not been assessed in nonhuman primates.

The present study focused on the behavioral-stimulant effects of cocaine administered alone and in combination with several DAT inhibitors. A subset of experiments determined the effects of DAT inhibitor pretreatments on cocaine self-administration. Importantly, drug effects on behavior were complemented by *in vivo* neurochemistry in squirrel monkeys in order to identify potential mechanisms of action of acute pharmacological interactions between cocaine and DAT inhibitors. The DAT inhibitors under investigation included three novel cocaine analogs (RTI-177, FECNT and RTI-112) that exhibited high binding affinities at DAT but differed in their binding affinities at SERT and NET (Table 1). *In vitro* binding data indicated that RTI-177 was highly selective for DAT over SERT and NET (Kuhar et al., 1999). FECNT was less than 3-fold selective for DAT over SERT, but had very low affinity at NET (Goodman et al., 2000). RTI-112 was approximately 10-fold selective for DAT over SERT and approximately 30-fold selective for DAT over NET (Kuhar et al., 1999). All compounds had similar stimulant activity and time course of effects on

locomotor activity in rodents (Kimmel et al., 2001; Kimmel, unpublished data).

2. Methods

2.1. Subjects

Fifteen adult (3–15 years) male squirrel monkeys (*Saimiri sciureus*) weighing between 700 and 1500 g served as subjects. Monkeys were individually housed, had access to food twice daily (Small Monkey Chow, Harlan Teklad, Madison, WI; fresh fruit and vegetables), and had ad libitum access to water while in their home cage. All monkeys had prior exposure to cocaine and other drugs with either dopaminergic or serotonergic effects (Czoty et al., 2002). However, the microdialysis preparations were newly established in six subjects. Animal use procedures were in strict accordance with the NIH “Guideline for the Care and Use of Laboratory Animals” and were approved by the Institutional Animal Care and Use Committee of Emory University.

2.2. Apparatus

During all experimental sessions, monkeys were seated in a plastic chair placed inside a sound-attenuating and ventilated test chamber as previously described (Byrd, 1979). Each chair was equipped with stimulus lights, a white noise generator, and a tailstock. Catheterized and microdialysis animals were prevented from accessing the preparation with an adjustable Lexan waist plate and neck plate, respectively. During stimulus termination experiments, an electrical current generator was connected with snap leads to two brass plates that contacted a portion of the tail secured in the tailstock in order to deliver a 3–4 mA electric stimulus for 200 ms. During drug self-administration experiments, the distal end of the venous catheter was connected via polyvinyl chloride tubing to a motor-driven syringe (Harvard Apparatus Inc., Phd2000, Holliston, MA) to yield a precise injection volume of 0.2 ml over 7 s. During microdialysis experiments, a micro-infusion syringe pump (CMA/200, CMA/Microdialysis, North Chelmsford, MA) was connected to microdialysis probes via fluorinated ethylene propylene (FEP) tubing (CMA/Microdialysis, North Chelmsford, MA), which passed through a small hole in the top of the chamber. Equipment was interfaced with a computer through a MED Associates interface rack and controlled by commercially available software (MedPC, MED Associates Inc., St. Albans, VT).

2.3. Catheter implantation

Six monkeys were prepared with chronic indwelling intravenous catheters under aseptic surgical conditions. A

Table 1
Binding affinity at DAT, SERT and NET

	DAT	SERT	NET
RTI-177*	1	2420	504
FECNT ^a	8	21	>10,000
RTI-112*	1	11	36

^a Ki, nM (Goodman et al., 2000).

* IC₅₀ binding, nM (Kuhar et al., 1999).

catheter made of PVC tubing (0.38 mm ID; 0.76 mm OD) was inserted into either the femoral or jugular vein and the proximal end passed subcutaneously to an exit site in the intrascapular region. Preoperative antibiotic (Rocephin [ceftriaxone], 50 mg) and postoperative analgesic (Banamine [flunixin meglumine], 1.0 mg/kg) were administered according to veterinary staff direction. Animals were anesthetized with a cocktail of Telazol (tiletamine HCl and zolazepam HCl, 2 mg), ketamine HCl (15 mg) and atropine (0.1 mg). Anesthesia was maintained with additional 20–30 mg supplements of ketamine HCl as needed. After surgery, each monkey wore a small-primate nylon mesh jacket (Lomir Biomedical Inc., Malone, NY) at all times to protect the external portion of the catheter. Catheters were maintained with sterile heparinized saline (100 U/ml) and sealed with a stainless steel obturator when not in use.

2.4. Guide cannulae implantation

A stereotaxic device was used to implant guide cannulae (CMA/11, CMA/Microdialysis, North Chelmsford, MA) bilaterally in the caudate nuclei [from earbar: A/P±15.0, L/M±3.0, D/V, -15.0 (Emmers and Ackert, 1963)] under general anesthesia in six subjects as previously described (Czoty et al., 2000). Briefly, following induction of anesthesia with the cocktail of Telazol, ketamine, and atropine described above, inhaled isoflurane (1.0–2.0%) was administered to maintain anesthesia throughout the surgery. Guide cannulae were affixed to the skull with nylon screws (2.4 mm, Plastics One, Roanoke, VA) and cranio-plastic cement (Plastics One, Roanoke, VA). Preoperative antibiotic and postoperative analgesic were administered as described above. After surgery, a stainless steel stylet was placed in each cannula to protect the access site when not in use. Guide cannulae placement was later verified by visual inspection of well-defined anatomical landmarks observed with magnetic resonance imaging as previously described (Czoty et al., 2000).

2.5. Fixed-interval stimulus termination

Three monkeys were trained on a fixed-interval (FI) 300-s schedule of stimulus termination. Each experimental component consisted of a 300-s presentation of red stimulus lights, previously paired with an impending mild electric stimulus to the tail. After 300-s, the monkeys were required to complete one lever press during a 3-s limited hold. Successful completion resulted in a 15-s illumination of a white stimulus light, followed by a 60-s timeout during which stimulus lights were extinguished and responding had no scheduled consequences. Failure to respond during the limited hold resulted in delivery of a mild electric stimulus to the end of the tail followed by a 60-s timeout. Each experimental session consisted of 13 consecutive components.

2.5.1. Time course

Either drug or saline was administered i.m. immediately before monkeys were placed in the test chamber, and each dose was administered on at least two separate occasions. Response rates for each of the components comprising a daily test session were averaged individually across sessions and compared to the response rates following saline pretreatment.

2.5.2. Cumulative dosing

On each experimental day, intravenous catheters were connected to PVC tubing that exited the testing chamber. The tubing allowed experimenter access to the i.v. catheter for cocaine delivery. In order to determine the effects of cocaine over a wide range of doses, cumulative doses of cocaine were given on two different days. Thus, cumulative doses were as follows: [Experimental Day 1—0, 0.03, 0.1, 0.3 mg/kg or Experimental Day 2—0.03, 0.1, 0.3, 1.0 mg/kg cocaine]. Cocaine injections were given following components 1, 4, 7, and 10. Cocaine doses indicated represent the total amount of cocaine given. Response rate for the first component was not considered. Results were compared to those obtained when saline was administered at the same intervals described above.

2.5.3. Drug interactions

Either drug or saline was administered i.m. 30-min prior to initiation of an experimental session during which cocaine or saline was given cumulatively as described above. Drug pretreatment effects were compared to cumulative cocaine following saline pretreatment.

2.6. Second-order stimulus termination

Three monkeys were trained on a second-order FI(600-s)[(FR 20:S)] schedule of stimulus termination. Each test session began with a 600-s illumination of a red stimulus light. During this 600-s period, completion of 20 lever responses (FR 20) changed the stimulus light from red to white for 2-s. At the end of the 600-s interval, the subject had a 30-s limited hold in which to complete one FR 20. Completion of the FR before the limited hold elapsed changed the stimulus lights from red to white for 15-s and averted delivery of a mild electric stimulus. If an FR was not completed before the limited hold expired, a mild electric stimulus was delivered to the subject's tail. In either case, the limited hold was followed by a 60-s timeout period during which no stimulus lights were illuminated and lever responses had no scheduled consequences. Each daily session consisted of five consecutive components. Once animals demonstrated stable lever responding, a range of drug doses or saline was administered i.m. 30 min prior to the commencement of experimental sessions for three consecutive days. Due to concerns about adverse effects, each subject received the pretreatment dose of FECNT (0.17 mg/kg) on only 1 day.

2.7. Cocaine self-administration

Three monkeys were trained on a second-order FI(600-s) [(FR 20:S)] schedule of stimulus termination as described above. When their behavior had stabilized, intravenous catheters were implanted, and the method of reinforcement was changed so that successful completion of a FR during a 200-s limited hold resulted in i.v. delivery of 0.1 mg cocaine. All other schedule parameters were as described for second-order stimulus termination. Once animals demonstrated stable lever responding, a range of drug doses or saline was administered i.m. 30-min prior to the commencement of experimental sessions for three consecutive days. Due to concerns about adverse effects, one subject received FECNT (0.17 mg/kg) pretreatment on only 1 day.

2.8. Microdialysis

Six squirrel monkeys were surgically implanted with guide cannulae for commercially available microdialysis probes (CMA/11, CMA/Microdialysis, North Chelmsford, MA). Probes were inserted into the same access site on multiple occasions in unanesthetized monkeys over the course of several months. Previous work has demonstrated the feasibility of this approach in squirrel monkeys (Davis et al., 1997; Czoty et al., 2000, 2002). A microinfusion syringe pump continually perfused the probe with artificial cerebrospinal fluid (aCSF: NaCl (150 mM), KCl (3.0 mM), CaCl₂ (1.3 mM), MgCl₂ (1.0 mM), and NaHPO₄ (1.0 mM)) at a flow rate of 2.0 μ l/min. One hour after probe insertion, samples were collected every 10 min for 2.5–3.0 h. Dialysates were analyzed for dopamine using high-pressure liquid chromatography and electrochemical detection following a commercially available method (Application note 70-0318, ESA, Inc., Chelmsford, MA; Column: 3 mm i.d. \times 100 mm, 5 μ m C₁₈ stationary phase; Thermo Hypersil, Keystone Scientific Operations, Bellefonte, PA; Coulochem II, ESA, Inc., Chelmsford, MA; EZChrom, Scientific Software Inc., Pleasanton, CA). The mobile phase was commercially available (MD-TM, ESA, Inc., Chelmsford, MA), and the HPLC was optimized for catecholamine detection at a maximum sensitivity of 1 nM.

High-performance liquid chromatography (HPLC) and electrochemical detection were used to quantify levels of DA. The HPLC system consisted of a small-bore (3 mm i.d. \times 100 mm) column (5 μ m C₁₈ stationary phase; Thermo Hypersil, Keystone Scientific Operations, Bellefonte, PA) with a commercially available mobile phase (ESA, Inc., Chelmsford, MA) delivered by an ESA 582 solvent delivery pump at a flow rate of 0.3 ml/min. Samples (6 μ l) were mixed with 6 μ l of ascorbate oxidase, and 11 μ l of the mixture were injected into the HPLC system by a CMA/200 autosampler. Electrochemical analyses were performed using an ESA Coulochem II detector (ESA, Inc.). The neurochemical effects of the combinations were compared with neurochemical effects of cocaine following saline administration.

2.8.1. Time course

One hour after probe insertion, baseline dialysate concentrations were determined over three 10-min sampling periods. Either drug or saline was administered (i.m.) and dialysate was collected for an additional 120 min at 10-min intervals. Finally, to assess voltage dependency of dopamine release, aCSF with a high concentration of potassium (~75 mM) was perfused through the probe and dialysate was collected for a 10-min period. Only sites with a robust and reproducible dopamine response (>100% increase over the dopamine level of the previous sample) were included in the study.

2.8.2. Drug interactions

One hour after probe insertion, baseline dialysate concentrations were assessed over three 10-min sampling periods. Either a DAT inhibitor or saline was administered, and dialysate was collected for three additional 10-min intervals. Subsequently, cocaine was administered, and dialysate was collected for an additional 120 min at 10-min intervals. Finally, to assess voltage dependency of dopamine release, aCSF with a high concentration of potassium (~75 mM) was perfused through the probe and dialysate was collected for a 10-min period. Only sites with a robust and reproducible dopamine response (>100% increase over the dopamine level of the previous sample) were included in the study.

2.9. Statistical analyses

For each group of animals, repeated measures ANOVA was performed across doses of each drug. If a main effect of drug dose was present, post hoc analyses were performed with Tukey's corrections for multiple comparisons. In microdialysis experiments, saline control data were not acquired in subjects assigned to the FECNT treatment group before the surgical preparation failed. Accordingly, a between-subject design was employed for comparisons of each dose of FECNT to control data collected in a different group of subjects. All statistical tests were performed as indicated with commercially available statistical analysis software (SigmaStat; Systat Software, Inc., Point Richmond, CA).

2.10. Drugs

(2-Fluoroethyl)-2 β -carbomethoxy-3 β -(4-chlorophenyl)-nortropine, FECNT (Dr. Mark M. Goodman, Department of Radiology, Emory University, Atlanta, GA), cocaine HCl (National Institute on Drug Abuse, Rockville, MD), [(–)-3 β -(3'-methyl-4-chlorophenyl)tropane-2 β -carboxylic acid methyl ester]tartrate, RTI-112 and [3 β -(4-chlorophenyl)tropane-2 β -(3-phenylisoxazol-5-yl)]HCl, RTI-177 (Dr. F. Ivy Carroll, Research Triangle Institute, Research Park, NC) were dissolved in 0.9% sterile saline solution. Drug doses were calculated from the salt.

3. Results

3.1. Fixed-interval stimulus termination

Control data obtained from FI 300-s stimulus-termination experiments were consistent with previous reports of fixed-interval patterns of behavior in squirrel monkeys (Howell and Byrd, 1991). Doses found to produce the peak stimulant effect on stimulus termination behavior during cumulative administration of each drug were selected, along with a lower dose, in order to examine the ascending limb of the stimulant dose–effect relationship. All subjects successfully terminated most stimuli and electric stimulus presentation was infrequent.

3.1.1. Time course

The time course of behavioral-stimulant effects was determined for each DAT inhibitor (Fig. 1). For RTI-177, there was a significant main effect of dose [$F(2,48)=17.23$, $p=0.002$] and time [$F(12,48)=4.56$, $p<0.001$] but no significant interaction [$F(24,48)=0.89$, $p>0.64$]. For FECNT, there was a significant main effect of dose [$F(2,48)=26.95$, $p<0.01$] and time [$F(12,48)=2.44$, $p<0.05$] but no significant interaction [$F(24,48)=1.013$, $p>0.47$]. For RTI-112, there was no significant main effect of dose [$F(2,48)=6.213$, $p>0.05$] or time [$F(12,48)=1.174$, $p>0.35$] and no significant interaction [$F(24,48)=1.090$, $p>0.39$]. Although a dose of 0.03 mg/kg RTI-112 increased response rate by approximately 2-fold in each subject, large between-subject variability in raw baseline response-rates precluded statistical significance.

3.1.2. Interactions with cocaine

Pretreatment with each DAT inhibitor was compared to saline pretreatment over the entire cocaine dose–effect curve (Fig. 2). For interactions with RTI-177, there was a significant main effect of cocaine [$F(5,30)=3.03$, $p<0.05$] but no significant main effect of RTI-177 pretreatment across cocaine doses [$F(2,30)=2.21$, $p>0.19$] and no significant interaction [$F(10,30)=1.40$, $p>0.23$]. For interactions with FECNT, there was a significant main effect of cocaine [$F(5,30)=4.27$, $p<0.05$] and a significant interaction [$F(10,30)=4.14$, $p<0.005$] but no significant main effect of FECNT pretreatment across cocaine doses [$F(2,30)=4.767$, $p>0.06$]. Post hoc comparisons revealed that the combination of FECNT (0.1 mg/kg) and cocaine (0.01 mg/kg) significantly increased responding when compared to the response rate obtained following cocaine alone ($p<0.005$). For interactions with RTI-112, there was a significant main effect of cocaine [$F(5,30)=3.75$, $p<0.005$], a significant main effect of RTI-112 pretreatment across cocaine doses [$F(2,30)=7.40$, $p<0.05$] and a significant interaction [$F(10,30)=3.75$, $p<0.005$]. Post hoc comparisons revealed that the combination of RTI-112 (0.03 mg/kg) and cocaine (0.01 and 0.03 mg/kg) significantly increased responding when compared to the

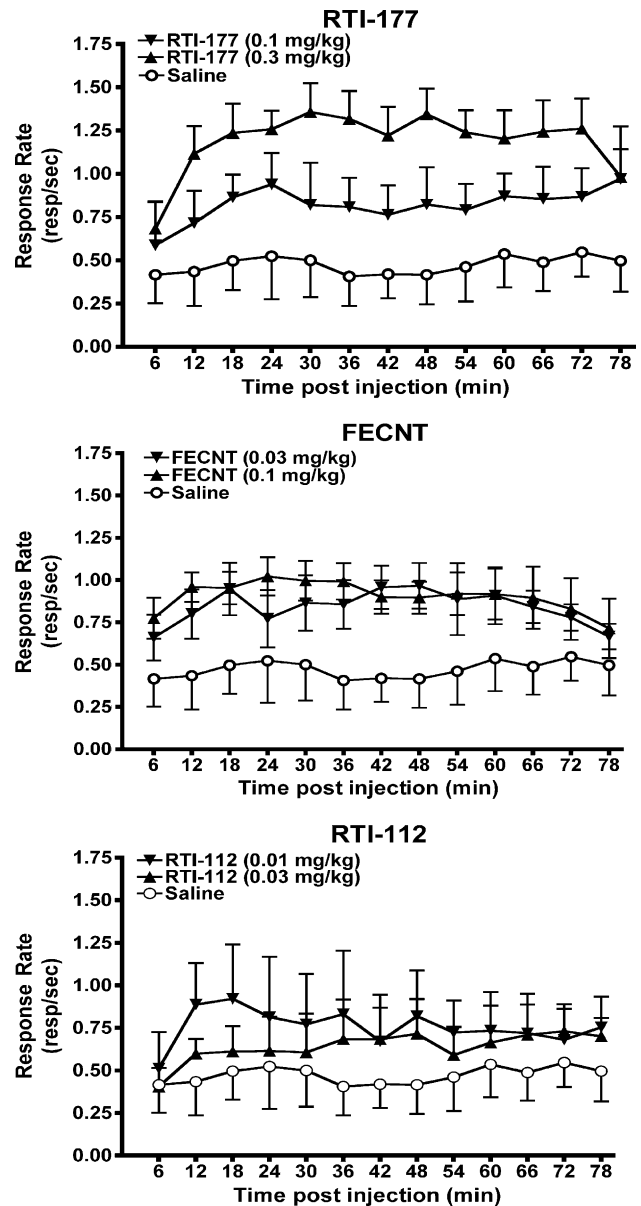


Fig. 1. Time course of drug effects on responding maintained by a fixed-interval schedule of stimulus termination in squirrel monkeys ($n=3$). Data points represent mean \pm S.E.M. rate of responding in each of 13 consecutive 5-min intervals. A 1-min timeout separated each fixed-interval component.

response rate obtained following cocaine alone ($p<0.005$ and 0.05, respectively). Also, the combination of RTI-112 (0.01 mg/kg) and cocaine (1.0 mg/kg) significantly increased responding when compared to the response rate obtained following cocaine alone ($p<0.005$).

3.2. Second-order stimulus termination and cocaine self-administration

Both cocaine self-administration and an identical second-order schedule of stimulus termination maintained responding in a manner consistent with previous studies utilizing second-order schedules in squirrel monkeys (Howell et al.,

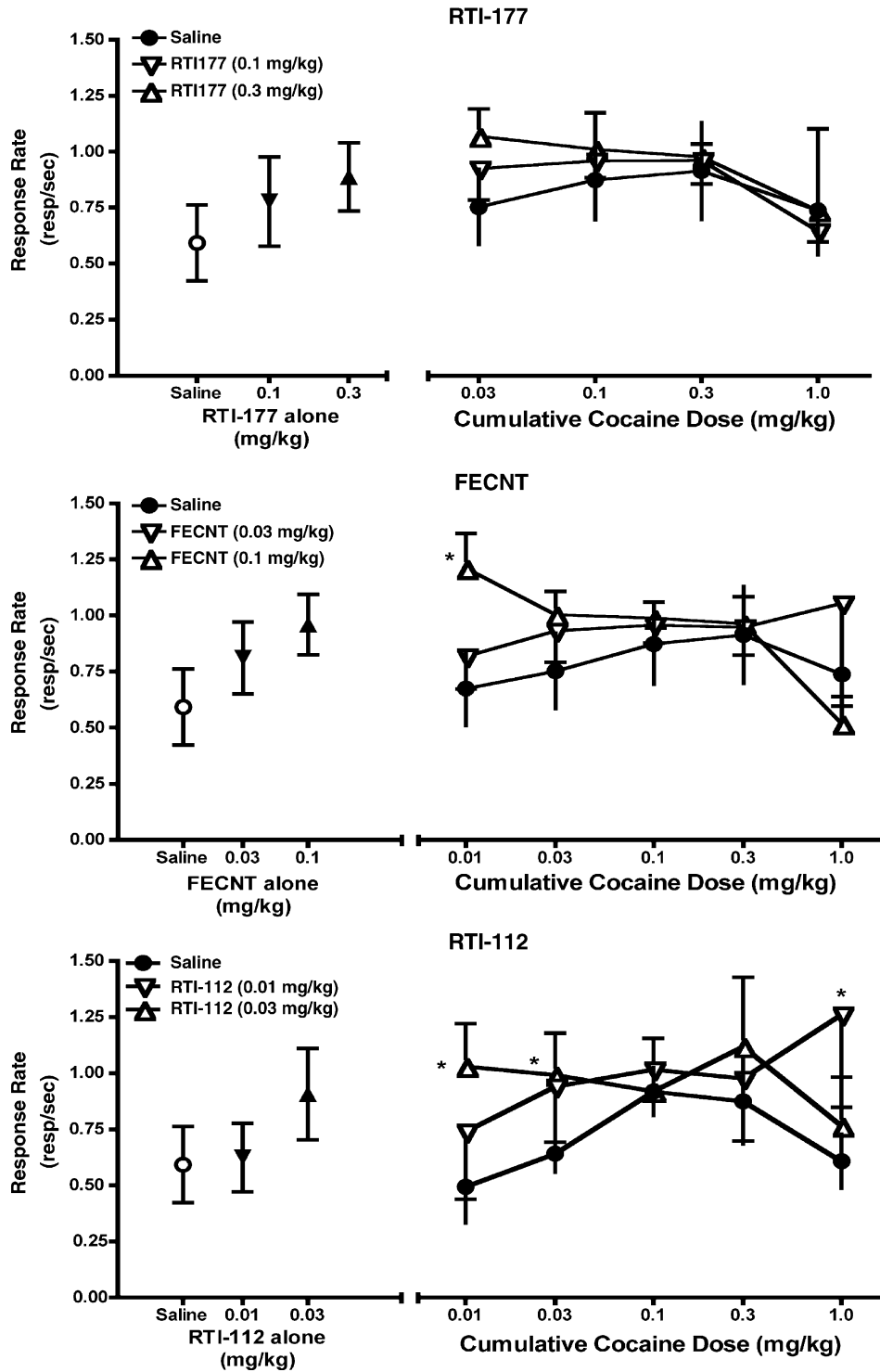


Fig. 2. Interactions between DAT inhibitors and cocaine on responding maintained by a fixed-interval schedule of stimulus termination in squirrel monkeys ($n=3$). Pretreatments were administered i.m. 30 min before the test session began. Cocaine was administered cumulatively during the test session via an indwelling i.v. catheter. Data points represent mean \pm S.E.M. rate of responding averaged for each dose of cocaine. Asterisks (*) represent individual points significantly different from saline control.

2000). Responding was maintained at a high rate with brief pauses occurring after each presentation of the paired stimulus. Pretreatment with each DAT inhibitor was compared to saline pretreatment under both schedules of reinforcement (Fig. 3).

RTI-177 had no significant effect on behavior maintained by stimulus termination [$F(5,10)=2.40, p>0.11$] or cocaine [$F(2,4)=0.11, p>0.90$]. FECNT had a significant effect on behavior maintained by stimulus termination [$F(4,8)=6.41, p<0.05$]. Post hoc analysis revealed that a

dose of 0.17 mg/kg significantly reduced response rate when compared to control rate ($p < 0.05$). FECNT had no effect on behavior maintained by cocaine [$F(3,6) = 1.96$, $p > 0.22$]. RTI-112 had a significant effect on behavior maintained by stimulus termination [$F(4,8) = 10.16$, $p < 0.005$]. Post hoc analysis revealed that a dose of 0.056 mg/kg significantly reduced response rate when compared to control rate ($p < 0.05$). RTI-112 had no effect on behavior maintained by cocaine [$F(3,6) = 0.46$, $p > 0.72$].

3.3. Microdialysis

To examine the neurochemical effects of behaviorally active doses of uptake inhibitors, the effects of RTI-177 (0.3 and 0.56 mg/kg), FECNT (0.03 and 0.1 mg/kg), RTI-112 (0.03 and 0.056 mg/kg) and cocaine (0.3 and 1.0 mg/kg) on extracellular dopamine were compared to saline treatment. To examine the neurochemical effects of uptake inhibitor interactions with cocaine, the effects of RTI-177 (0.3 mg/kg) and FECNT (0.03 and 0.1 mg/kg) administered prior to cocaine (0.3 or 1.0 mg/kg) were compared to effects following administration of cocaine alone (0.3 and 1.0 mg/kg).

The dopamine recovered in dialysate ranged from 1 to 3 nM at baseline conditions, and increased to a maximum of 12–25 nM following the most effective treatments. The drug effects were normalized to basal levels to account for individual differences in baseline dopamine.

3.3.1. Time course

The time course of drug-induced changes in extracellular dopamine was determined for each DAT inhibitor (Fig. 4). For RTI-177, there was a significant main effect of dose [$F(2,56) = 49.53$, $p < 0.005$] and time [$F(14,56) = 7.74$, $p < 0.001$], and a significant interaction [$F(28,56) = 2.28$, $p < 0.005$]. For FECNT, there was a significant main effect of dose [$F(2,56) = 6.43$, $p < 0.05$] and time [$F(4,56) = 4.51$, $p < 0.001$], and a significant interaction [$F(28,56) = 4.47$, $p < 0.001$]. For RTI-112, there was a significant main effect of time [$F(14,56) = 2.44$, $p < 0.05$] but no significant main effect of dose [$F(2,56) = 4.55$, $p > 0.09$] and no significant interaction [$F(28,56) = 1.50$, $p > 0.90$]. Although a dose of 0.056 mg/kg RTI-112 induced marked increases in extracellular dopamine, large between-subject variability precluded statistical significance. For cocaine, there was a significant main effect of dose [$F(2,56) = 11.13$, $p > 0.05$] and time [$F(14,56) = 20.05$, $p < 0.001$] and a significant interaction [$F(28,56) = 3.42$, $p < 0.001$].

3.3.2. Interactions with cocaine

Pretreatment with RTI-177 (0.3 mg/kg) was compared to saline pretreatment in combination with two doses of cocaine (0.3 and 1.0 mg/kg) (Fig. 5). For RTI-177 in combination with the lower dose of cocaine (0.3 mg/kg),

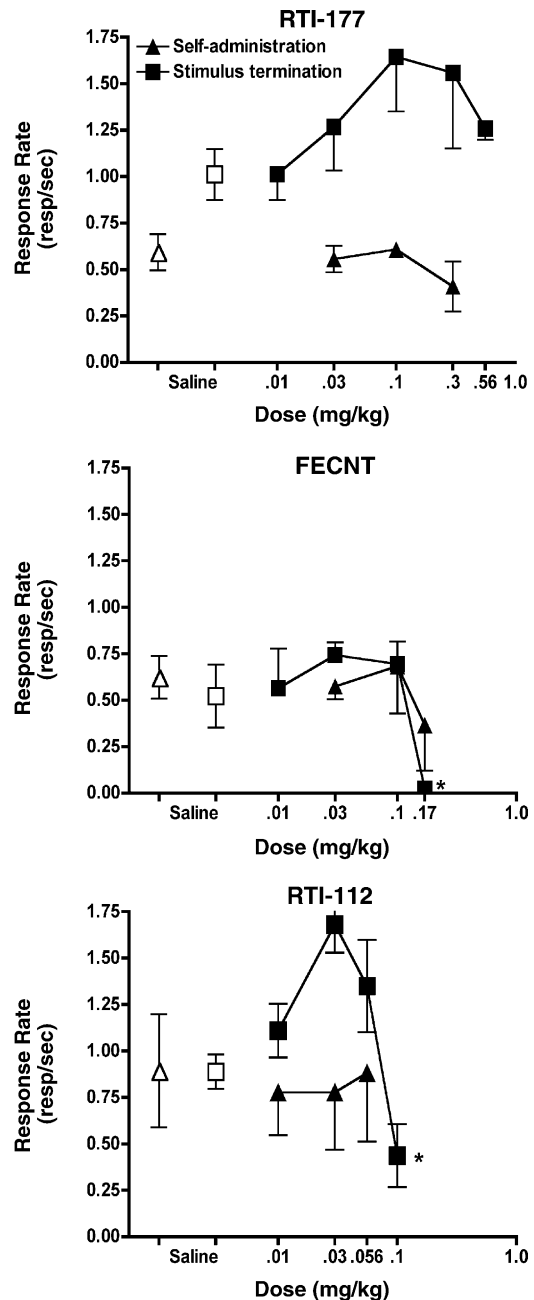


Fig. 3. Drug effects on responding maintained by a second-order schedule of stimulus termination ($n = 3$) or cocaine self-administration ($n = 3$) in squirrel monkeys. Doses of DAT inhibitors were administered i.m. 30 min before each session began. Data points represent mean \pm S.E.M. rate of responding under conditions specified. Asterisks (*) represent individual points significantly different from saline control.

there was a significant main effect of time [$F(11,44) = 7.69$, $p < 0.001$] but no significant main effect of drug treatment [$F(2,44) = 4.94$, $p > 0.08$] and no significant interaction [$F(22,44) = 0.69$, $p > 0.82$]. For RTI-177 in combination with the higher dose of cocaine (1.0 mg/kg), there was a significant main effect of time [$F(11,44) = 12.26$, $p < 0.001$] but no significant effect of drug treatment [$F(2,44) = 1.61$, $p > 0.30$] and no significant interaction [$F(22,44) = 0.96$, $p > 0.524$].

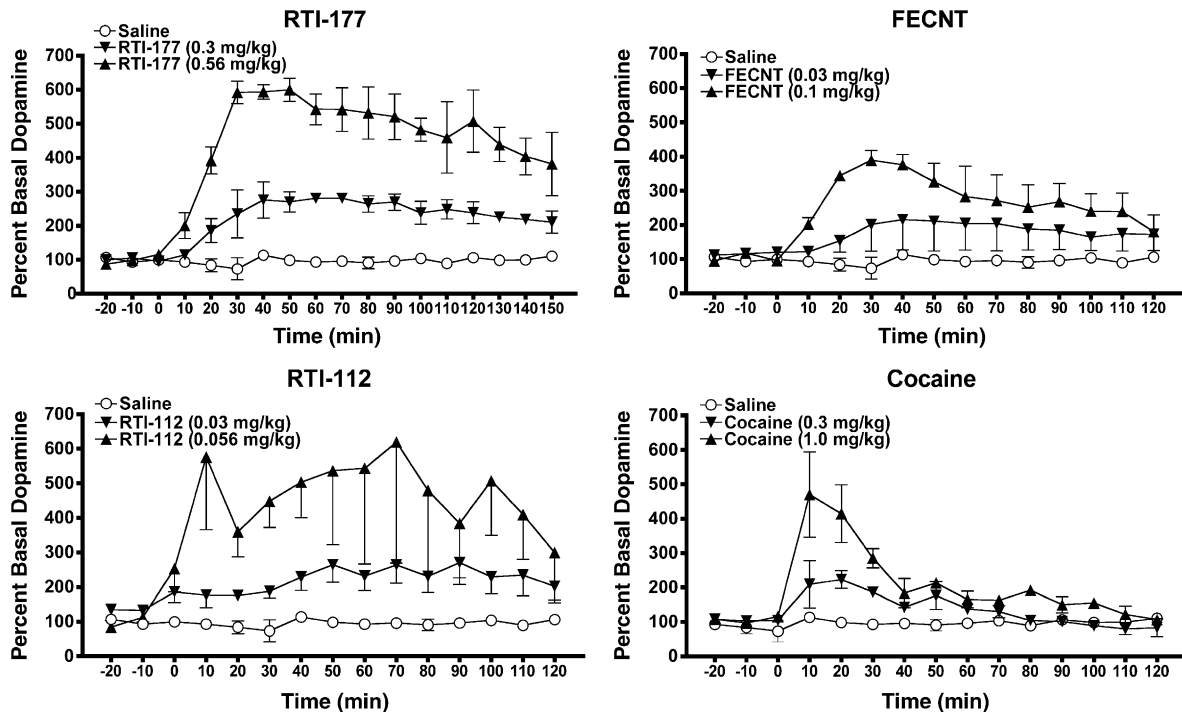


Fig. 4. Drug effects on extracellular dopamine in the caudate nucleus of unanesthetized squirrel monkeys as determined with *in vivo* microdialysis ($n=3$). Pretreatments were administered i.m. at time point zero. Data points represent mean \pm S.E.M. dopamine levels expressed as a percent of values obtained prior to drug administration.

Pretreatment of FECNT (0.03 and 0.1 mg/kg) was compared to saline pretreatment in combination with one dose of cocaine (0.3 mg/kg) (Fig. 6). For the lower dose of FECNT (0.03 mg/kg) in combination with cocaine, there was a significant main effect of time [$F(8,32)=2.68$, $p<0.05$] but no significant main effect of drug treatment [$F(2,32)=0.58$, $p>0.59$] and no significant interaction [$F(16,32)=0.48$, $p>0.94$]. For the higher dose of FECNT (0.1 mg/kg) in combination with cocaine, there was a significant main effect of time [$F(8,32)=24.17$, $p<0.001$] but no significant main effect of drug treatment [$F(2,32)=4.52$, $p>0.09$] and no significant interaction [$F(16,32)=0.72$, $p>0.75$].

4. Discussion

The present study characterized the effects of several DAT inhibitors on operant behavior and *in vivo* neurochemistry in nonhuman primates. The drugs investigated were tropane analogs of cocaine that had high affinity at DAT but differed in their relative potency in binding at DAT, SERT and NET. Both RTI-177 and FECNT produced significant dose-dependent stimulant effects on behavior maintained by a fixed-interval schedule of stimulus termination as reported previously for cocaine and other DAT inhibitors (Spealman et al., 1989; Howell and Byrd, 1991; Howell et al., 2000). RTI-112 induced less robust behavioral-stimulant effects that were not statistically significant. All drugs had slower onset and longer duration

of action compared to cocaine (Howell et al., 2000), consistent with reported drug effects on locomotor activity in rodents (Kimmel et al., 2001). *In vivo* microdialysis experiments in the caudate nucleus of unanesthetized monkeys confirmed that RTI-177 and FECNT induced significant, dose-dependent increases in extracellular dopamine. RTI-112 also induced marked increases in extracellular dopamine, but large between-subject variability precluded statistical significance. Importantly, the time course of drug effects on extracellular dopamine was consistent with the onset and duration of behavioral-stimulant effects for all drugs.

The similar pharmacological profile observed for the DAT inhibitors and cocaine suggests a common mechanism of action and predicts additivity of effects when DAT inhibitors are co-administered with cocaine. Interestingly, there was little evidence of additivity of drug effects on behavior observed in the present study. Following drug pretreatments that produced behavioral-stimulant effects when administered alone, a low dose of cocaine which had no behavioral effect when administered alone did produce significant increases in fixed-interval responding. However, response rate for the drug combinations did not differ from that observed for the pretreatment drugs alone. Thus, the apparent significant behavioral interactions between monoamine uptake inhibitors and cocaine in the present study are likely due to the behavioral-stimulant effect of the DAT inhibitor pretreatments. Clearly, the circumstances under which significant interactions were observed are limited.

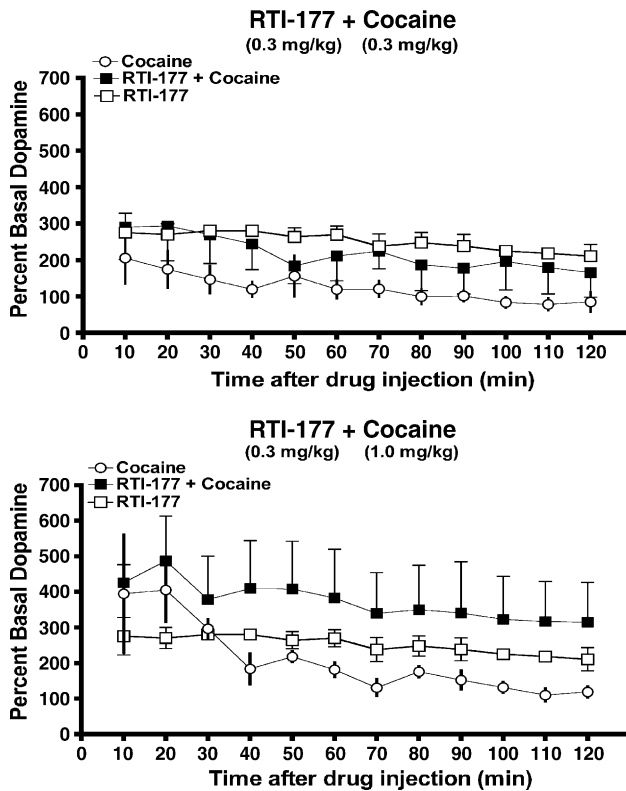


Fig. 5. Interactions between RTI-177 (0.3 mg/kg) and cocaine (0.3 and 1.0 mg/kg) on extracellular dopamine in the caudate of unanesthetized squirrel monkeys as determined with *in vivo* microdialysis ($n=3$). RTI-177 or saline was administered i.m. 30-min before time point zero. Cocaine or saline was administered at time point zero. Data points represent mean \pm S.E.M. dopamine levels expressed as a percent of values obtained prior to drug administration.

Similarly, there was no evidence for additivity of drug effects on *in vivo* neurochemistry. Pretreatment with either RTI-177 or FECNT failed to cause significant changes in cocaine-induced elevations in extracellular dopamine. Given that the effects of DAT inhibitors on extracellular dopamine are impulse-dependent and influenced by the prevailing tone of the system, it is not surprising that the combined effects of DAT inhibitors and cocaine may depend on the magnitude of effects induced by either drug alone. The latter consideration may account for equivocal results reported for interactions between DAT inhibitors and cocaine on extracellular dopamine in rodents (Rothman et al., 1991; Baumann et al., 1994; Martin-Fardon et al., 1996; Tolliver et al., 1999). Collectively, the interactions observed in the present study did not demonstrate robust additivity of effects on behavior or *in vivo* neurochemistry when behavioral-stimulant doses of DAT inhibitors were co-administered with cocaine in nonhuman primates.

The present study compared the effects of DAT inhibitor pretreatments on behavior maintained by identical second-order schedules of i.v. cocaine delivery or stimulus termination. Under these conditions, the DAT inhibitors did not selectively suppress cocaine-maintained responding.

In fact, FECNT and RTI-112 produced significant reductions in behavior maintained by stimulus termination at doses that had no significant effect on cocaine-maintained behavior. Higher pretreatment doses likely would have caused significant reductions in cocaine-maintained behavior, but concerns about the safety of high dose combinations with cocaine precluded testing higher doses of the DAT inhibitors. A recent study by Lindsey et al. (2004) demonstrated effective reduction of cocaine self-administration in rhesus monkeys following acute administration of RTI-177 (0.1 and 0.3 mg/kg) and RTI-112 (0.03 mg/kg) performing under a similar schedule of reinforcement. However, different sensitivity to these drugs in rhesus versus squirrel monkeys may explain disparities in the results of Lindsey et al. (2004) and those of the present study.

Published studies on selectivity of DAT-inhibitor pretreatment effects on cocaine-maintained behavior have reported equivocal results. The DAT inhibitor, GBR 12909, selectively attenuated cocaine-maintained behavior compared with food-maintained behavior at a low unit dose of cocaine but not at a higher unit dose (Glowa et al., 1995). However, additional studies with DAT inhibitors have demonstrated nonselective reductions in cocaine-maintained behavior and behavior maintained by alternative reinforcers,

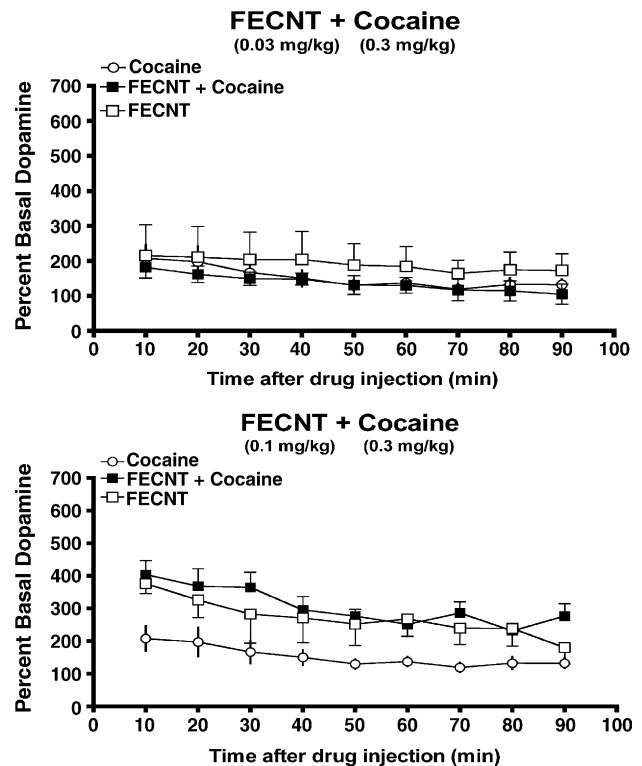


Fig. 6. Interactions between FECNT (0.03 and 0.1 mg/kg) and cocaine (0.3 mg/kg) on extracellular dopamine in the caudate of unanesthetized squirrel monkeys as determined with *in vivo* microdialysis ($n=3$). FECNT or saline pretreatment was administered i.m. 30-min before time point zero. Cocaine or saline was administered at time point zero. Data points represent mean \pm S.E.M. dopamine levels expressed as a percent of values obtained prior to drug administration.

including food and stimulus termination (Kleven and Woolverton, 1993; Glowa and Wojnicki, 1996; Howell et al., 2000). While selectivity of effects on drug-maintained behavior is desirable for potential pharmacotherapies, the nature of the alternative reinforcer and the behavior engendered are important considerations. Moreover, the types of side effects that are viewed as tolerable should be evaluated in the context of effectiveness in reducing drug use.

Three high-affinity DAT inhibitors were characterized that differed in their binding affinities at SERT and NET. Among the three cocaine analogs that were characterized, RTI-177 was the most selective for DAT, whereas RTI-112 was the least selective for the three monoamine transporters. FECNT exhibited high selectivity for DAT over NET but poor selectivity for DAT over SERT. Interestingly, the three compounds had a similar profile of behavioral and neurochemical effects. Although RTI-112 failed to induce significant behavioral-stimulant effects, it induced marked increases in extracellular dopamine. It was evident that large between-subject variability precluded statistical significance on neurochemical measures. A recent report compared the behavioral effects of RTI-177 and RTI-112 in rhesus monkeys (Lindsey et al., 2004). Both drugs induced dose-related reductions in cocaine self-administration behavior. However, the dose of RTI-177 that reduced responding by 50% (ED₅₀) resulted in DAT occupancy of approximately 70%. In contrast, the ED₅₀ dose of RTI-112 was below the limit of detection for DAT occupancy. Moreover, RTI-177 reliably maintained drug self-administration, whereas RTI-112 did not. Hence, it is apparent that the behavioral profile of DAT inhibitors may be influenced by actions at other monoamine transporters. The modest behavioral-stimulant effects and the large between-subject variability in neurochemical effects observed for RTI-112 in the present study may reflect complex actions at multiple monoamine transporters.

In summary, the DAT inhibitors under investigation exhibited a profile of effects on behavior and neurochemistry that was similar to cocaine in nonhuman primates. However, there was little evidence for additivity of effects on behavior or neurochemistry when DAT inhibitors were co-administered with cocaine. Among the three DAT inhibitors that were characterized, RTI-112 failed to induce significant behavioral-stimulant effects or reliable increases in extracellular dopamine. The latter results indicate that, in agreement with other lines of research, the pharmacological profile of DAT inhibitors may be influenced by actions at multiple monoamine transporters (Lile et al., 2003; Moron et al., 2002; Valentini et al., 2004; Czoty et al., 2002).

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