Relationship between rate of drug uptake in brain and behavioral pharmacology of monoamine transporter inhibitors in rhesus monkeys

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ABSTRACT

Although inhibition of dopamine transporters (DAT) and the subsequent increase in dopamine clearly play a role in the effects of psychomotor stimulants, the reinforcing effectiveness of DAT inhibitors varies. Previous studies suggest that pharmacokinetic and pharmacodynamic properties of these drugs account for this variability. The present studies compared the time course and behavioral effects of five phenyltropane analogs of cocaine with high affinity for DAT and varying time courses of action in rhesus monkeys. The rate of drug uptake in putamen was measured using positron emission tomography neuroimaging. The rank order of the time to peak drug uptake was cocaine<RTI-336<RTI-150<RTI-113<RTI-177. Cocaine and all five analogs fully substituted for the cocaine cue in animals trained to discriminate cocaine from saline. All of the drugs were self-administered under a progressive-ratio schedule of drug self-administration and reinstated previously extinguished self-administration maintained under a second-order schedule. The time to peak drug uptake corresponded closely with the time to peak discriminative stimulus effects, and there was a trend for the time of peak drug uptake to correspond negatively with the peak number of drug infusions. Collectively, these results indicate that the rate of drug entry in brain can play an important role in the behavioral pharmacology of psychomotor stimulants.

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1. Introduction

Although cocaine is a nonselective inhibitor of monoamine transporters, including dopamine, serotonin, and norepinephrine (Madras et al., 1989; Reith et al., 1986), the behavioral effects of cocaine associated with its abuse liability have been attributed primarily to its actions at the dopamine transporter (DAT) (Ritz et al., 1987). Preclinical studies have demonstrated a relationship between the potency of cocaine analogs at binding to the DAT in vitro and their potency in producing locomotor-stimulant effects in rodents (Cline et al., 1992; Kuhar, 1993). Similarly, a high correlation was found between the potency of cocaine analogs to displace cocaine in the caudate nucleus and the potency of these compounds to produce cocaine-like behavioral effects in squirrel monkeys (Bergman et al., 1989; Madras et al., 1989; Spealman et al., 1989). Cocaine and selective

DAT inhibitors typically exert similar effects on schedule-controlled behavior and are reliably self-administered in squirrel monkeys (Bergman et al., 1989; Howell and Byrd, 1991; Howell et al., 2000) and rhesus monkeys (Howell et al., 2007; Lindsey et al., 2004; Nader et al., 1997; Wilcox et al., 2005). The relevance of the DAT in the abuse liability of cocaine is supported further by neuroimaging studies. In human cocaine users, a significant correlation was observed between the level of DAT occupancy and the magnitude of the subjective high following administration of cocaine (Volkow et al., 1997) or the behavioral stimulant methylphenidate (Volkow et al., 1999).

Collectively, there is strong evidence to indicate a critical role for the DAT in the abuse liability of cocaine and related stimulants. However, DAT inhibitors differ in their effectiveness as behavioral stimulants and positive reinforcers. In order to produce equivalent increases in locomotor activity in rodents, selective DAT inhibitors must exhibit greater occupancy of DAT than does the nonselective monoamine transporter inhibitor cocaine (Rothman et al., 1992; Vaugeois et al., 1993). Some analogs of benztropine have high affinity for the DAT and inhibit dopamine uptake but generally have behavioral effects that differ from those of cocaine (Katz et al.,

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2004). In addition, several local anesthetics are effective DAT inhibitors, but are weaker reinforcers than cocaine in maintaining behavior under progressive-ratio or second-order schedules (Wilcox et al., 2005; Wilcox et al., 2000). Together, these studies clearly demonstrate that simple steady-state DAT occupancy cannot fully account for the reinforcing properties of psychomotor stimulants and that other pharmacological factors must be considered. In addition to complex pharmacological effects involving multiple sites of action, the time course of drug action may play an important role in determining the profile of behavioral effects induced by DAT inhibitors. Drugs that occupy DAT more rapidly produce more robust behavioral effects in rodents (Desai et al., 2005; Stathis et al., 1995). The more rapidly cocaine (Volkow et al., 2000) or methylphenidate (Volkow et al., 2002) enter the brain, the greater the reported "high" observed in humans. Accordingly, a slow onset of action may reduce the abuse liability of DAT inhibitors.

The purpose of the present study was to examine in rhesus monkeys the behavioral effects of cocaine and five phenyltropane analogs of cocaine with high affinity for the DAT and varying time courses of action. The time course of drug uptake in brain was determined with PET neuroimaging in awake subjects. The PET data were correlated with the rate of onset of peak discriminative stimulus effects in rhesus monkeys trained to discriminate cocaine from saline. In addition, the reinforcing effectiveness of the analogs was compared to that of cocaine using a progressive-ratio (PR) schedule of drug self-administration. Lastly, the effectiveness of the cocaine analogs in reinstating previously extinguished cocaine-maintained behavior was determined following drug administration as a priming bolus prior to the reinstatement sessions. The examination of the effects of non-contingent administration of cocaine analogs to animals with cocaine self-administration experience allowed us to assess whether drugs that engendered high rates of responding in the PR schedule of self-administration would also reinstate previously extinguished cocaine-maintained behavior. The time to peak uptake of drug in brain correlated significantly with the time to peak discriminative stimulus effects. There was a trend for the peak number of drug injections to decrease as the time to peak uptake in brain increased. In contrast, there was a trend for peak reinstatement effects to increase as time to peak uptake in brain increased. The results indicate that the time course of drug uptake in brain can predict drug effects on behavior that are associated with the abuse liability of stimulants. Determining the pharmacological properties that influence the abuse liability of behavioral stimulants may provide important insights regarding medications development for treating stimulant abuse.

2. Materials and methods

2.1. General methods

2.1.1. Subjects

PET neuroimaging, drug self-administration and reinstatement studies were conducted at the Yerkes National Primate Research Center, Emory University, in 7 female and 2 male adult rhesus monkeys (Macaca mulatta) weighing 7.5 to 13.0 kg. Each subject was housed individually and fed Purina monkey chow (Ralston Purina, St. Louis, MO), fruits, and vegetables. Water was continuously available. Each subject was prepared surgically with a chronic indwelling venous catheter under sterile conditions using a technique described previously (Lindsey et al., 2004). Catheters were flushed daily with 1.0 ml heparinized (100 units/ml) saline to maintain patency. Animal care procedures strictly followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Emory University.

Drug discrimination studies were conducted at McLean Hospital, Harvard University, in 6 adult male rhesus monkeys weighing 6.6 to

8.9 kg. Each monkey was maintained on a diet of 7–12 monkey biscuits (Purina Monkey Chow Jumbo #5037) and one piece of fresh fruit per day. During the week, all food was delivered after the experimental session, whereas on weekends, food was delivered between 9 a.m. and noon. Water was freely available at all times. Animal maintenance and research were conducted in accordance with the guidelines provided by the Committee on Laboratory Animal Resources. The facility was licensed by the United States Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee of Harvard University.

2.1.2. Drugs

Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD), RTI-112, RTI-113, RTI-150, RTI-177, and RTI-336 (Research Triangle Institute, Research Triangle Park, NC) were dissolved in 0.9% saline. All drug doses were determined as salts. The five phenyltropane analogs of cocaine were selected based on their high affinity for the DAT and varying time courses of action (Carroll et al., 2004; Kimmel et al., 2001; Kimmel et al., 2007; Kuhar et al., 1999) (Table 1).

2.2. PET neuroimaging

2.2.1. Apparatus

PET neuroimaging was performed at the Emory University PET Center on a Siemens 951 scanner. A set of Ge-68 ring sources was used for attenuation correction before injection of radiolabeled compounds. All images were reconstructed with measured attenuation correction, zoom factor 8, and Shepp–Logan reconstruction filter cut off at 1 cycle/cm. This produced images with an inplane pixel size of 1.17- and 8-mm resolution. The axial slice thickness was 3.375 mm. All images were decay-corrected to the time of injection. Regions of interest were manually drawn on the late images over the putamen and cerebellum. The regions of interest were then overlaid on all images to obtain time–activity curves.

2.2.2. Procedure

Uptake of the [^{11}C]-labeled compounds was characterized in a group of 3 rhesus monkeys in the awake state during image acquisition as described previously (Howell et al., 2002; Lindsey et al., 2004). Subjects were positioned in the PET scanner and a 15-min transmission scan was obtained for attenuation correction. Subsequently, a tracer dose (5 mCi) of the [^{11}C] labeled drug was administered as a rapid i.v. bolus in approximately 2.0 ml of saline. Image acquisition began coincident with the start of the injection and continued for 90 min.

2.2.3. Data analysis

Time–activity curves were determined separately for the putamen and cerebellum. Activity measures were standardized to body weight and dose of radioactivity injected to yield standard uptake values (SUV). Peak SUV values reported were derived from the earliest time point on the time–activity curves at which subsequent values were no more than 5% greater.

Table 1
Monoamine uptake inhibition (IC$_{50}$, nM)

Compound	[^{3}H]DA	[^{3}H]NE	[^{3}H]5HT
Cocaine[a]	310	221	260
RTI-112[a]	1.1	0.8	1.4
RTI-113[a]	3.0	31	229
RTI-150[a]	11	35	194
RTI-177[a]	1.9	3.8	235
RTI-336[b]	11.5	112	522

[a] Data from Kuhar et al. (1999). Uptake values were determined using rat tissue.
[b] Values supplied by NIDA under the Cocaine Treatment Discovery Program (CTDP). Uptake values were determined using cloned hDAT, hNET, and hSERT.

2.3. Drug discrimination

2.3.1. Apparatus

Each monkey was housed individually in a well-ventilated, stainless steel chamber. The home cages of all monkeys were modified to include an operant panel mounted on the front wall. Three square translucent response keys could be transilluminated by red or green stimulus lights (Superbright LED's). In addition, the operant panel supported an externally mounted pellet dispenser (Gerbrands, Model G5310, Arlington, MA) that delivered 1 gm fruit-flavored food pellets (Precision Primate Pellets Formula L/I Banana Flavor, P. J. Noyes Co., Lancaster, NH) to a food receptacle mounted on the cage beneath the operant response panel. Operation of the operant panels and data collection were accomplished with IBM-compatible computers and interface systems (Med Associates, St. Albans, VT) located in a separate room.

2.3.2. Discrimination training

Discrimination training was conducted 5 days/week during daily sessions composed of multiple cycles. Each cycle consisted of a 15-min time-out period followed by a 5-min response period. During the time-out, all stimulus lights were off, and responding had no scheduled consequences. During the response period, the right and left response keys were transilluminated red or green, and monkeys could earn up to 10 food pellets by responding under a FR 30 schedule of food presentation. For three monkeys in this study, the left key was illuminated green, and the right key was illuminated red. The colors of the response keys were reversed for the other three monkeys. On training days, monkeys were given an i.m. injection of either saline or 0.40 mg/kg cocaine 5-min after the beginning of each time-out period (i.e., 10 min before the response period). Following the administration of saline, only responding on the green key (the saline-appropriate key) produced food, whereas following administration of 0.40 mg/kg cocaine, only responding on the red key (the drug-appropriate key) produced food. Responses on the inappropriate key reset the FR requirement on the appropriate key. Sessions consisted of 1 to 5 cycles, and if the training dose of cocaine was administered, it was administered only during the last cycle. Thus, training days consisted of 0 to 5 saline cycles followed by 0 to 1 drug cycles.

Monkeys were considered to have acquired cocaine discrimination when the following three criteria were met for 7 of 8 consecutive training sessions: 1) the percent injection-appropriate responding prior to delivery of the first reinforcer was greater than or equal to 80% for all cycles; 2) the percent injection-appropriate responding for the entire cycle was greater than or equal to 90% for all cycles; 3) response rates during saline training cycles were greater than 0.5 responses per second.

2.3.3. Discrimination testing

Once monkeys met criterion levels of cocaine discrimination, testing began. Test sessions were identical to training sessions except that 1) 30 responses on either key produced food, and 2) cocaine, RTI-112, RTI-113, RTI-150, RTI-177 or RTI-336 was administered using either a cumulative dosing procedure or a time course procedure. In the cumulative dosing procedure, increasing doses of the test compound were administered at the beginning of each successive cycle, instead of saline or the cocaine training dose, and each successive dose increased the total cumulative dose by 0.25 or 0.5 log units. In the time course procedure, a single dose of the test compound was administered, and 5 min response periods were scheduled to begin after 10, 30, 100 and 300 min. For the cocaine analogs, additional response periods were scheduled to occur after 24 h (all five cocaine analogs) and 48 h (RTI-177) to characterize the full time course of these compounds. The time course of each cocaine analog was tested at two doses: (1) the lowest dose to produce complete substitution in all monkeys, and (2) a dose 1/4 log unit lower. The time course of the training dose of cocaine was also determined for comparison. A total of 6 monkeys was used for the study, and each cocaine analog was tested in a group of 4 monkeys. The potency and time course of cocaine were determined in all 6 monkeys.

Mean data from saline and drug cycles during the training day immediately preceding the initial test day served as the control data for the subsequent test day. If responding did not meet criterion levels of discrimination performance, then training was continued until criterion levels of performance were obtained for at least two consecutive days.

2.3.4. Data analysis

A test compound was considered to have substituted completely for cocaine if any dose produced ≥90% cocaine-appropriate responding. Discrimination ED_{50} values were defined as the dose of cocaine or cocaine analog that produced 50% cocaine-appropriate responding. ED_{50} values were determined by linear regression from the linear portion of the discrimination dose–effect curve in each monkey, and individual values were averaged to yield mean $ED_{50}s$ and 95% confidence limits. ED_{50} values for different drugs were considered to be significantly different if 95% confidence limits did not overlap. For the purposes of correlation with PET data, the rate of onset for discriminative stimulus effects of each cocaine analog was defined as the time of maximal effect produced by a dose 1/4 log unit below the dose that produced complete substitution in all monkeys. This dose was used because peak effects produced by this dose were less than the ceiling effect of the assay (100% cocaine-appropriate responding), and as a result, the time to peak effect was not obscured by the ceiling of the assay.

2.4. Progressive-ratio (PR) self-administration

2.4.1. Apparatus

Each monkey was seated in a commercially available primate chair (Primate Products, Redwood City, CA). A response panel with one lever was mounted on the front of the chair. Located above the lever in the center of the response panel were red and white stimulus lights. A Huber needle (Access Technologies) was inserted into the venous access port, and PVC tubing attached to the Huber needle was connected to a motor-driven syringe (Coulbourn Instruments, Allentown, PA) located outside of the chamber containing the drug solution. A volume of 2.0 ml/infusion was delivered over 7 s. Testing during daily 2-hour sessions occurred in a ventilated, sound-attenuating chamber. IBM-compatible computers controlled experimental events and recorded data.

2.4.2. Procedure

Experimental sessions were conducted 5 days/week. At the beginning of a session, the red light was illuminated and responding was reinforced under a PR schedule of i.v. drug delivery. Under baseline conditions, the PR procedure consisted of eight components, each made up of four trials, for a total of 32 trials each day. The response requirement for the first component was 15 and doubled for each successive component, so that the response requirement sequence was 15, 30, 60, 120, 240, 480, 960, and 1920. The same response requirement was in effect for each trial in a component, and a trial ended with a 10-s drug injection, with the expiration of a 15-min limited hold, or the end of the 2-h session. During the injection the light changed from red to white and responses had no scheduled consequence. There was a 30-s time-out after each drug injection or the expiration of a limited hold during which the light was off and responding had no scheduled consequence. If the response requirement was not completed for two consecutive trials (i.e., the limited hold expired), or the animal took all 32 injections, the session ended.
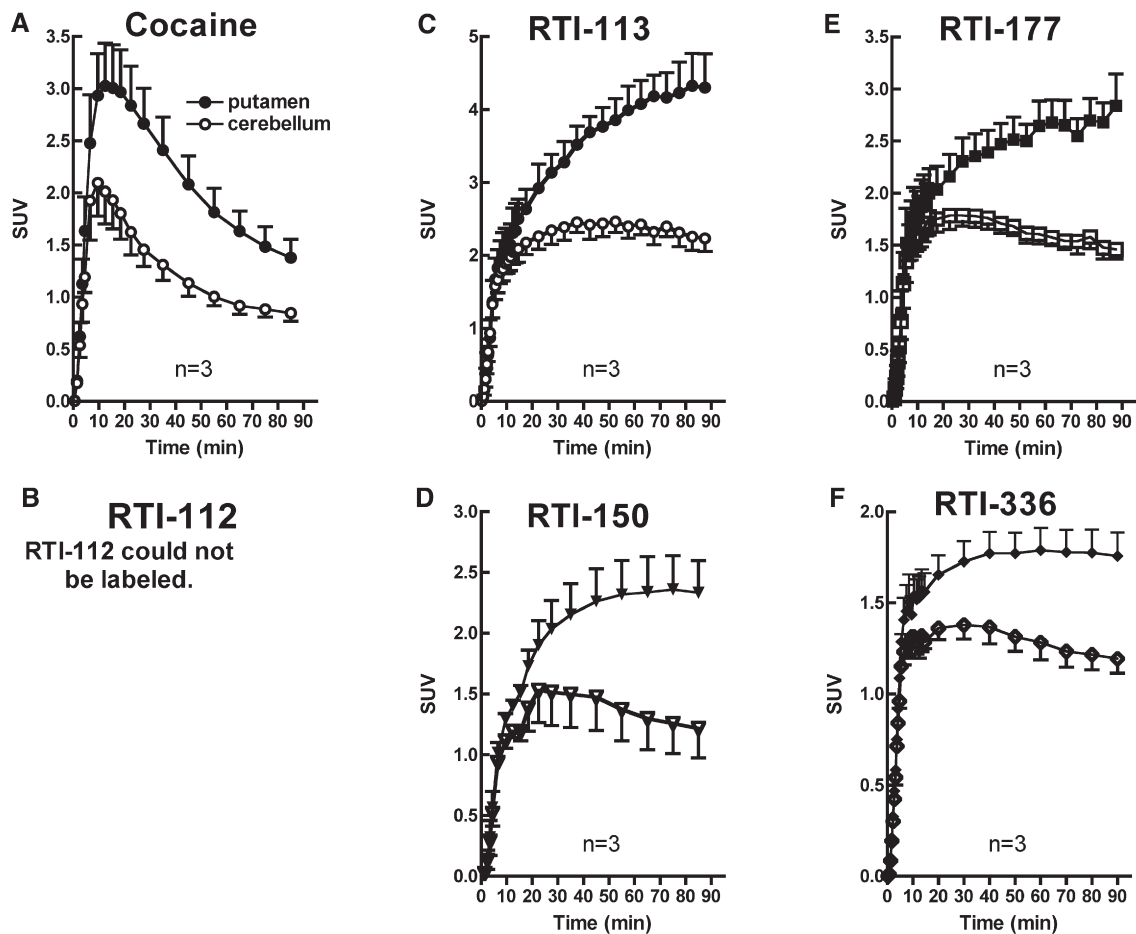
Fig. 1. Time course of uptake and clearance of [¹¹C] labeled cocaine and cocaine analogs. PET scans were acquired in awake subjects. RTI-112 could not be radiolabeled effectively and was therefore omitted from this assay. Abscissa: time in minutes. Ordinate: standard uptake value (SUV). Each point shows the mean ± SEM in three monkeys.

In training sessions, cocaine (0.10 mg/kg per injection) or saline was available for injection on alternate days until responding was stable for at least three consecutive cocaine and saline sessions. At this point, test sessions were added to the daily sequence and the session on the day after a test session was always a cocaine training session. That is, the session sequence became CSTCSCT where C indicates cocaine training sessions, S indicates saline baseline sessions and T indicates test sessions. Conditions of test sessions were identical to training sessions except a novel drug and/or dose was available under various PR sequences. The order in which the monkeys received the test drug doses was counterbalanced across animals. After a test condition, the monkeys were returned to baseline conditions, starting with a cocaine training session, until responding for cocaine and saline were stable again.

2.4.3. Data analysis

The number of infusions per session was plotted as a function of drug dose for individual subjects. The peak number of infusions obtained for each drug, regardless of dose, was analyzed using a repeated-measures one-way ANOVA followed by the Holm–Sidak method of all pairwise multiple comparison post-hoc procedure using SigmaStat v.3.0. "Infusions comparable to cocaine" was defined as equal to or greater than 75% of the maximal cocaine responding of 21 infusions, or equal to or greater than 15.8 infusions of the test compound.

2.5. Reinstatement

2.5.1. Apparatus

The apparatus and training equipment were identical to that described for the PR drug self-administration studies.

2.5.2. Training conditions

Subjects responded for i.v. infusions of cocaine under a second-order schedule of reinforcement with a fixed interval of 10 min. When the daily session began, the red light on the response panel was illuminated and indicated the availability of cocaine reinforcement. Each fixed ratio of 20 responses (FR20) completed during a 10-minute fixed interval changed the stimulus light from red to white for 2 s. The first FR20 completed after the 10-minute fixed interval had elapsed resulted in the delivery of 0.1 mg/kg of cocaine and changed the stimulus light from red to white for 15 s. There was a 30-second limited hold for completion of the first FR20 after the 10-minute fixed interval had elapsed, and drug was not delivered if the limited hold expired. After the drug delivery or the expiration of the limited hold, there was a 1-minute time-out during which all stimulus lights were extinguished and responding had no scheduled consequence. Subjects had the opportunity to take 5 injections in each daily session. Stable

Table 2
Average time to peak or plateau of [¹¹C]-labeled compounds in putamen or to peak of discriminative stimulus effects

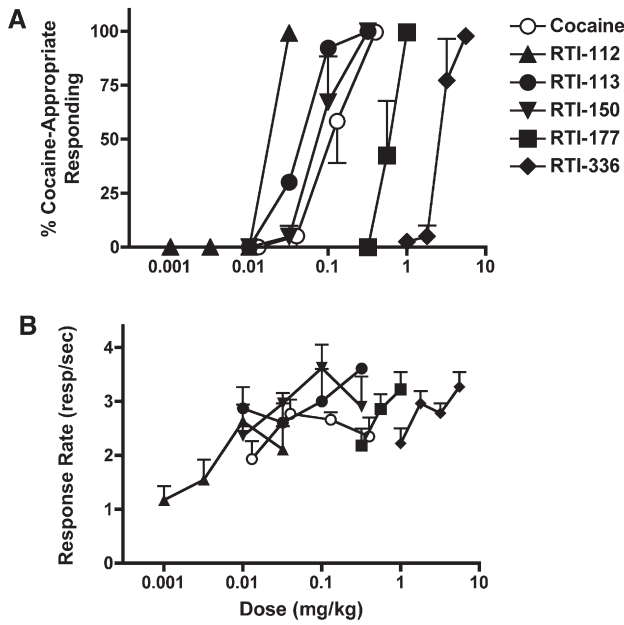| Compound	Time of peak of [¹¹C]-labeled compound (min)	Time to peak discrimination (min)
Cocaine	9.5	–
RTI-112	N.D.	30
RTI-113	62.5	100
RTI-150	45	30
RTI-177	87.5	300
RTI-336	40	30

Fig. 2. Effects of cocaine and cocaine analogs in rhesus monkeys trained to discriminate 0.4 mg/kg cocaine i.m. from saline. Abscissae: drug dose in mg/kg (log scale). Top ordinate: percent cocaine-appropriate responding. Bottom ordinate: response rate in responses/s. Each point shows the mean±SEM in six monkeys (cocaine) or four monkeys (cocaine analogs).

responding was defined as 20% or less variation in response rate for 0.1 mg/kg/inf cocaine under these conditions for five consecutive days.

2.5.3. Extinction

During the extinction phase, saline was substituted for cocaine in the syringe, but all other testing conditions remained the same. The criterion for successful extinction was a decrease in response rate to at least 25% of baseline for at least two consecutive test days.

2.5.4. Reinstatement

Drugs were administered i.v. non-contingently 5 min prior to the start of the session. As in the extinction phase, saline was available in the syringe for self-administration. Each dose was separated by at least two days of extinction (saline in syringe and no priming injection). The order in which the monkeys received the drug doses was counter-balanced across animals and all doses of a given drug were characterized one time before initiating testing with a different drug. Between drugs, baseline responding for cocaine was re-established with a unit dose of 0.1 mg/kg/infusion followed by extinction.

2.5.5. Data analysis

Response rate following non-contingent priming injections was derived as a percent of control rates maintained by cocaine self-administration, and plotted as a function of drug dose in individual subjects. "Fully reinstated responding" is defined as responding at 90% or greater of cocaine reinstated responding, while "partially reinstated responding" is defined as responding at levels between 50 and 90% of cocaine reinstated responding. The peak responding as a percent of control obtained for each drug, regardless of dose, was analyzed using a repeated-measures one-way ANOVA using SigmaStat v.3.0.

3. Results

3.1. PET neuroimaging

The time course for drug uptake in putamen and cerebellum for each of the [^{11}C]-labeled compounds is shown in Fig. 1. The average time to peak levels of cocaine was 9.5 min (Table 2), and cocaine levels dropped markedly after 40–50 min. In contrast, the time to peak levels of the cocaine analogs was considerably greater, and drug levels were sustained for the duration of the 90-min session. The rank order of the
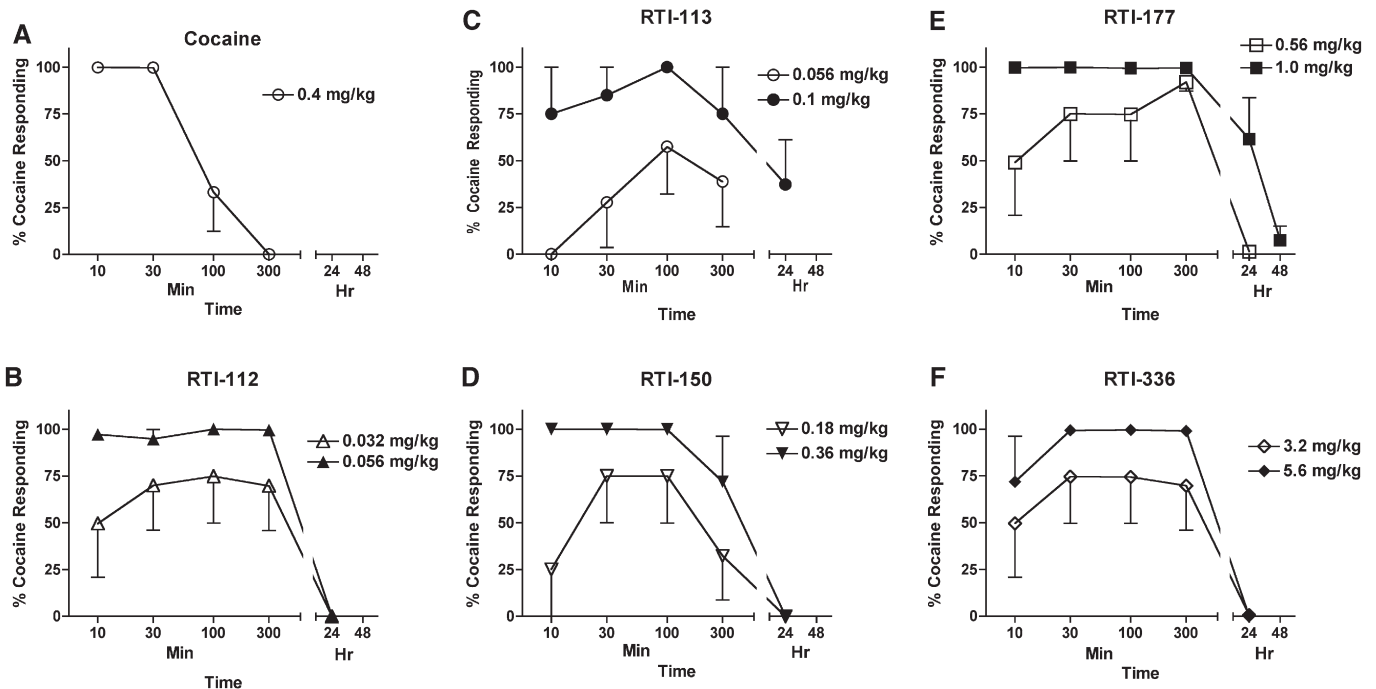

Fig. 3. Time course of cocaine and cocaine analogs in rhesus monkeys trained to discriminate 0.4 mg/kg cocaine i.m. from saline. Abscissae: time after drug injection in minutes (10–300) or hours (24–48). Ordinates: percent cocaine-appropriate responding. Each point shows the mean±SEM in six monkeys (cocaine) or four monkeys (cocaine analogs).

Table 3
ED_{50} values in mg/kg for cocaine and each RTI compound to substitute for cocaine in the cocaine-discrimination procedure

Drug	ED_{50} (95% CL)
Cocaine	0.11 (0.065–0.18)
RTI-112	0.018 (0.018–0.018)
RTI-113	0.043 (0.034–0.054)
RTI-150	0.084 (0.065–0.11)
RTI-177	0.59 (0.51–0.68)
RTI-336	2.7 (2.4–3.1)

95% confidence limits are shown in parentheses.

time to peak uptake was cocaine<RTI-336<RTI-150<RTI-113<RTI-177. The time course for drug uptake was very consistent across subjects for each of the compounds evaluated. Due to the short half-life of [^{11}C], the PET scan was limited to 90 minutes post-injection of the labeled compounds.

3.2. Drug discrimination

During the training days preceding test days, monkeys responded almost exclusively on the saline key during saline cycles (mean % saline-appropriate responding=99.99±0.01) and almost exclusively on the cocaine key during cocaine training (mean % cocaine-appropriate responding=99.69±0.20). Mean response rates were 2.19±0.17 and 2.50±0.41 responses/s during saline and drug training cycles, respectively.

Fig. 2 shows the effects of each compound on cocaine-appropriate responding and response rates. Cocaine and all five cocaine analogs produced dose-dependent increases in cocaine-appropriate responding and full substitution in all monkeys tested. The order of potency was RTI-112>RTI-113>RTI-150≥cocaine>RTI-177>RTI-336. At doses that substituted for cocaine, each compound tended to have no effect on or to increase response rates. None of the compounds produced noticeable overt behavioral effects across the dose range tested.

Fig. 3 shows the time course of a maximally effective dose of each compound (i.e. the lowest dose to substitute in all four monkeys tested). For the cocaine analogs, the time course of a dose 1/4 log unit lower than the maximally effective dose is also shown. Cocaine had a relatively short duration of action, and the discriminative stimulus effects of the training dose of cocaine were absent after 100 min in 4/6 monkeys and after 300 min in all monkeys. RTI-112, RTI-150 and RTI-336 had longer durations of action, with peak effects sustained for at least 300 min; however, the effects of the compounds were no longer apparent after 24 h. RTI-113 and RTI-177 had the longest durations of action. The highest doses of the compounds produced sustained effects in two out of
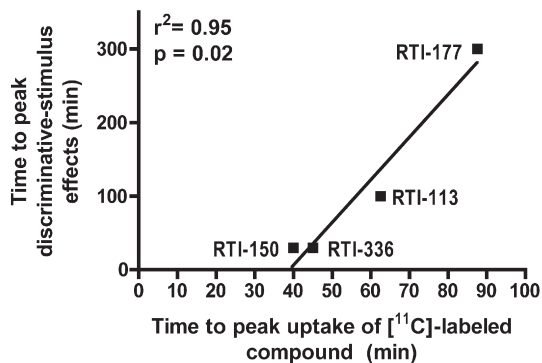
four monkeys (RTI-113) or three out of four monkeys (RTI-177) after 24 h. The cocaine-like discriminative stimulus effects of RTI-177 were no longer apparent after 48 h. The time to peak discriminative stimulus effects is shown in Table 2. The ED_{50} values for each drug were considered to be significantly different if 95% confidence limits did not overlap (Table 3). By this criterion, all compounds differed in potency except RTI-150 and cocaine.

Fig. 4 shows the correlation between the time to peak uptake of [^{11}C]-labeled compounds and the time to peak discriminative stimulus effects of the lower dose of each compound. A Pearson correlation analysis resulted in an r^2 value of 0.95 (p=0.02), indicating a significant correlation between these two measures. In addition, the slope was calculated to be 5.8, suggesting that decreases in the rate of peak uptake of the [^{11}C]-labeled compounds produces greater reductions in the rate of peak discriminative stimulus effects.

3.3. Progressive-ratio (PR) self-administration

In monkeys trained to self-administer cocaine under a PR schedule of reinforcement, cocaine and each of the cocaine analogs maintained a greater number of infusions per session than that maintained during saline extinction conditions (Fig. 5). While none
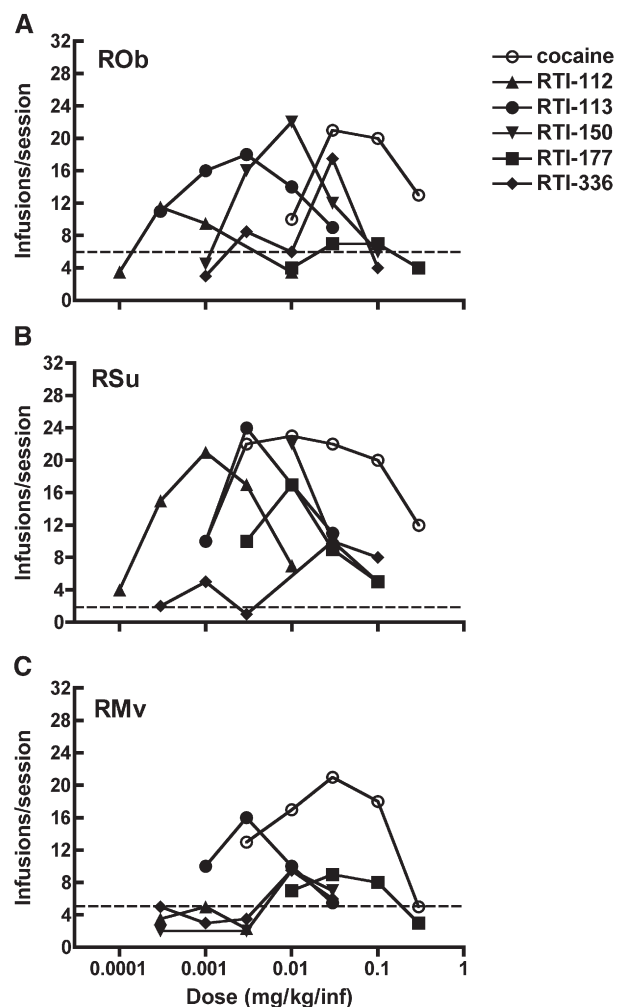
Fig. 4. Correlation between the time to peak uptake of [^{11}C]-labeled compounds and the time to peak discriminative stimulus effects, based on the data presented in Table 2. A Pearson correlation analysis resulted in an r^2 value of 0.77 (p=0.05), indicating a significant correlation between these two measures.

Fig. 5. Effects of cocaine and cocaine analogs on behavior maintained by a PR schedule of i.v. drug self-administration. Abscissae: drug dose in mg/kg (log scale). Ordinates: mean maximum number of infusions per session. Each data point shows the mean value in each of three individual monkeys. Dashed lines indicate number of infusions per session when saline was substituted for cocaine.

Table 4
Peak number of infusions in PR schedule of self-administration

	RSu	ROb	RMv	Mean±SEM
Saline	2	6	5	4.3±1.2[C]
Cocaine	23	21	21	21.7±0.7[S]
RTI-112	21	11.5	5	12.5±4.7[S,C]
RTI-113	24	18	16	19.3±2.4[S]
RTI-150	22	22	9.5	17.8±4.2[S]
RTI-177	17	7	9	11.0±3.1[C]
RTI-336	10	17.5	9.5	12.3±2.6[S,C]

S denotes significantly more infusions than saline and *C* denotes significantly fewer infusions than cocaine.

of the monkeys took the maximum number of infusions available for any drug, all three monkeys took at least 21 infusions of cocaine. RTI-112 maintained a maximum number of infusions comparable to cocaine in one out of the three monkeys, and RTI-113 maintained a maximum number of infusions comparable to cocaine in all three subjects. RTI-150 maintained a maximum number of infusions comparable to cocaine in two monkeys but very little responding in the third monkey. RTI-177 and RTI-336 each maintained very little responding in two monkeys but maintained a maximum number of infusions comparable to cocaine in the third monkey. The rank order for the average maximum number of infusions across all subjects was cocaine>RTI-113≥RTI-150>RTI-112=RTI-336≥RTI-177 (Table 4). A repeated-measures one-way ANOVA indicated that the mean number of infusions differed between the drugs ($F(6, 12)=5.363$, $p=0.007$). Post-hoc tests indicated that the animals self-administered significantly more infusions of cocaine and each of the analogs (except RTI-177) than they did saline. In addition, significantly fewer infusions of RTI-112, RTI-177, and RTI-336 were self-administered relative to cocaine. ED_{50} values were calculated for each drug by using linear interpolation of the ascending limb of the combined dose-response curve for all 3 animals, and the resulting order of potency was RTI-112>RTI-113>RTI-150>cocaine>RTI-336>RTI-177.

3.4. Reinstatement

All three monkeys reliably self-administered cocaine (0.1 mg/kg/infusion) under the second-order schedule of drug self-administration. Mean (±SD) rate of responding during the maintenance phase, expressed as the number of lever presses per second, was 0.67±0.05 for RLk, 1.7±0.23 for RSo, and 0.59±0.07 for RVt . Saline was substituted for cocaine until responding decreased to less than 20% of the response rate maintained by cocaine. Subsequently, when saline was administered as a non-contingent priming injection, responding was minimal for all subjects (Fig. 6). However, when multiple doses of cocaine were administered on separate occasions, at least one dose induced a robust reinstatement effect with rates of responding ranging from approximately 50–100% of pre-extinction baseline rates. RTI-112 administration partially reinstated responding in one monkey and completely reinstated responding in a second monkey, but not at all in a third animal. Similarly, RTI-113 fully reinstated responding in two monkeys, but not at all in the third animal. The effects of RTI-150 administration induced full reinstatement in only one of the three monkeys. Overall, RTI-177 administration induced robust reinstatement effects, with partial reinstatement in one monkey and full reinstatement in the other two animals. Lastly, RTI-336 administration induced partial reinstatement in one monkey, complete reinstatement in a second monkey, but none in the third animal. The rank order for the maximum response rate across all subjects was RTI-177>RTI-113>cocaine>RTI-336 ≥ RTI-112>RTI-150 (Table 5). A repeated-measures one-way ANOVA indicated that the peak responding did not differ between the drugs ($F(6, 12)=1.672$, NS). ED_{50} values were calculated by using linear interpolation of the ascending limb of the
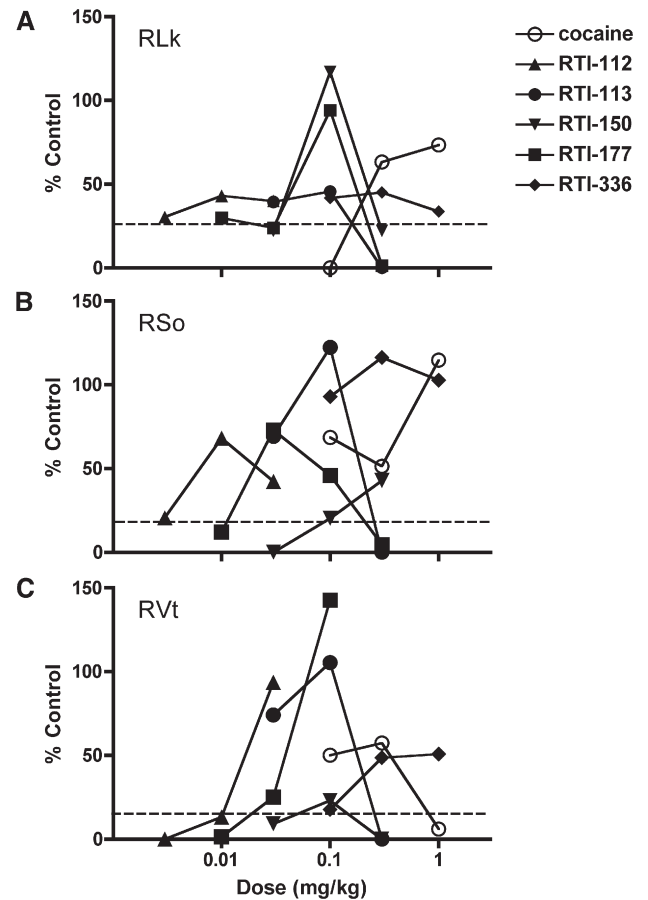

Fig. 6. Effects of cocaine and cocaine analogs on reinstatement of previously extinguished self-administration maintained by a second-order schedule of i.v. drug delivery. Abscissae: drug dose in mg/kg (log scale). Ordinates: response rate expressed as a percent of responding maintained by cocaine (0.1 mg/kg/injection) self-administration. Each data point shows responding following drug prime obtained in each of three individual monkeys. Dashed lines indicate effects of saline on reinstatement.

combined dose–response curve for all 3 animals, and the resulting order of potency was RTI-112>RTI-177>RTI-113>RTI-150=cocaine>RTI-336.

3.5. Correlational analyses

3.5.1. Time to peak uptake vs. behavioral effects

The time to peak drug uptake is correlated with the peak number of infusions in PR (Fig. 7A) and the peak reinstatement effect (Fig. 7B). In addition, the peak number of infusions in PR was correlated with the peak reinstatement effect (Fig. 7C). Although Pearson correlation analyses resulted in non-significant r^2 values in all three of these comparisons (Fig. 7A, B, and C), there was a trend towards a negative

Table 5
Peak responding (percent control) in reinstatement of previously extinguished self-administration behavior

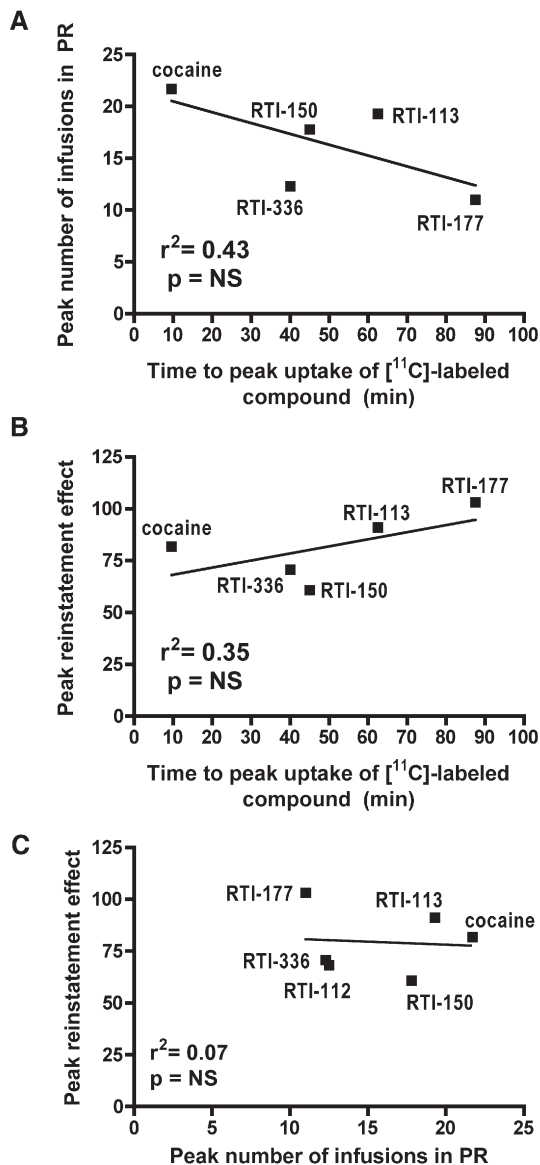|	RLk	RSo	RVt	Mean±SEM
Saline	20.4	19.0	16.2	18.5±1.2
Cocaine	73.5	114.7	57.3	81.8±17.1
RTI-112	43.1	68.1	93.7	68.3±14.6
RTI-113	45.7	122.3	105.5	91.2±23.3
RTI-150	116.9	42.8	23.0	60.9±28.6
RTI-177	94.0	73.0	142.7	103.2±20.7
RTI-336	45.1	116.3	50.8	70.7±22.9

Fig. 7. Correlation between the time to peak uptake of [^{11}C]-labeled compounds and the peak number of infusions during the PR schedule of drug self-administration (A), and the peak reinstatement effect (B). The Pearson correlation analyses resulted in non-significant r^2 values for both of the other comparisons.

correlation between drug uptake and self-administration in the PR paradigm and a trend towards a positive correlation between drug uptake and reinstatement. However, there was no correlation between the peak number of infusions in the PR paradigm and the peak reinstatement effect.

4. Discussion

The present study in rhesus monkeys compared the rate of drug uptake in brain with the discriminative stimulus, reinforcing, and reinstatement effects of several phenyltropane analogs of cocaine with high affinity for the DAT and varying time courses of action. The time to peak uptake of [^{11}C]-labeled drugs in putamen indicated that cocaine had the fastest rate of onset, followed by RTI-150 and RTI-336, with RTI-113 and RTI-177 having the slowest rates of onset. In the present studies, the time to peak drug uptake in putamen was predictive of the time to the peak discriminative stimulus effects in that cocaine had the most rapid rate of onset of discriminative

stimulus effects, while RTI-150 and RTI-336 had a slower rate of onset, and RTI-113 and RTI-177 had the slowest rate of onset. These data lend further support to the hypothesis that the rate of drug entry into brain may be one determinant of the abuse-related effects of psychomotor stimulants.

In the current studies, the rank order of the time to peak drug uptake in putamen was cocaine<RTI-150=RTI-336<RTI-113<RTI-177, which corresponds very closely with the rank order of the time to peak increases in extracellular dopamine in squirrel monkey caudate [cocaine<RTI-150=RTI-336<RTI-177] (Kimmel et al., 2007). In the latter study, the time to peak increases in caudate dopamine correlated positively and strongly (r^2=0.9419) with peak behavioral-stimulant effects. Taken together, these studies indicate that the rate of drug entry in the brain clearly influences the rate of change in extracellular dopamine and also the behavioral pharmacology of stimulants with prominent dopaminergic effects. In addition, these studies validate the use of PET imaging of radiolabeled drugs to provide a high-resolution measure of the kinetics of brain penetration of these drugs. That the rate of drug uptake into brain correlated strongly with the rate of onset in a behavioral assay of drug discrimination suggests that PET could also provide a high-resolution measure of the rate of onset of centrally-mediated abuse-related effects.

Cocaine and all of the cocaine analogs produced dose-dependent increases in cocaine-appropriate responding and full substitution in all monkeys tested under the drug discrimination protocol. Accordingly, the rate of drug uptake did not influence the peak discriminative stimulus effects, suggesting that rate of onset does not play a significant role in the efficacy of these phenyltropane analogs to substitute for cocaine. Similarly, the time to peak drug uptake measured by PET neuroimaging did not correlate significantly with peak reinforcing or reinstatement effects. However, there was a clear trend towards an inverse relationship between peak drug uptake and the peak number of infusions in the PR paradigm, such that the faster-onset drugs (cocaine, RTI-336, and RTI-150) produced greater levels of responding relative to the slower-onset drugs (RTI-113 and RTI-177). These data suggest that the rate of onset may play an important role in the reinforcing strength of psychomotor stimulants. Consistent with the latter interpretation, when cocaine was infused more slowly, it was found to be less effective as a reinforcer in nonhuman primates (Panlilio et al., 1998). Similarly, the cocaine analog HD-23, which takes 60 min to attain significant binding was not as reinforcing in rhesus monkeys as were cocaine or methylphenidate (Lile et al., 2003). The piperazine analog (+)-CPCA binds to DAT more slowly than cocaine and was found to be a less robust reinforcer than cocaine in nonhuman primates (Woolverton et al., 2002). In human studies, the rate of uptake in brain determined with PET neuroimaging closely paralleled the perception of the "high" following cocaine or methylphenidate administration (Volkow et al., 2002). Lastly, routes of administration (i.e. smoking, intravenous) that result in a more rapid entry of cocaine in brain produce greater "highs" than other routes of administration (i.e. oral) resulting in slower drug uptake (Volkow et al., 2000).

In addition to rate of onset, duration of action may also influence the behavioral effects of monoamine transporter inhibitors. In general, the longer-acting cocaine analogs were more effective in the reinstatement paradigm than were the shorter-acting drugs. In agreement with these results, the long-acting tropane analog PTT was more efficacious than cocaine in reinstating previously extinguished cocaine self-administration in rhesus monkeys (Lile et al., 2004). Several studies have also found an inverse relationship between the duration of action and the rate of self-administration. For example, PTT engenders lower rates of self-administration than does cocaine in rodents (Roberts et al., 1999) and nonhuman primates (Lile et al., 2002; Lile et al., 2000). Only a few studies have examined the role of duration of action on a PR schedule of self-administration,

but these studies suggest that duration of action does not affect the ability of a drug to maintain responding. In rodents, the long-acting phenylpiperazine GBR12909 was similarly efficacious to cocaine (Roberts, 1993) and the ultra-short-acting opioid remifentanil was similarly efficacious to heroin (Panlilio and Schindler, 2000). The long-acting tropane HD-60 was nearly as efficacious as cocaine in the PR paradigm in rhesus monkeys (Lile et al., 2003). Similarly, duration of action did not alter the discriminative stimulus effects of monoamine-selective tropanes in rodents (Carroll et al., 2004). In the present studies, all of the cocaine analogs fully substituted for the cocaine cue and reinstated previously extinguished cocaine-maintained behavior, despite differences in their duration of action. Collectively, the results suggest that rate of onset play a more prominent role in the behavioral effects of psychomotor stimulants than does duration of action.

In addition to the time course of effects, the pharmacological specificity of the cocaine analogs may influence the profile of behavioral effects. For example, a negative relationship was observed between the potencies of several cocaine- and amphetamine-like drugs in self-administration studies and their binding potencies to serotonin uptake sites (Ritz et al., 1987). Monoamine-releasing agents had decreased reinforcing effectiveness in rhesus monkeys when serotonin-releasing potency was increased relative to dopamine (Wee et al., 2005). In drug discrimination studies, substitution for cocaine was positively associated with selectivity for dopamine/norepinephrine versus serotonin release in rhesus monkeys (Negus et al., 2007). The behavioral and neurochemical profile of DAT inhibitors is also influenced by actions at multiple monoamine transporters in squirrel monkeys (Ginsburg et al., 2005). In the present study, all of the cocaine analogs had high affinity at the DAT but they differed in their relative affinity at the serotonin transporter (SERT) and norepinephrine transporter (NET). Among the five cocaine analogs that were characterized, RTI-336 was the most selective for DAT and RTI-112 was the least selective for the three monoamine transporters. The remaining three cocaine analogs exhibited approximately 2–10 fold selectivity for DAT over NET and 20–100 fold selectivity for DAT over SERT. Interestingly, cocaine and all five analogs produced dose-dependent increases in cocaine-appropriate responding and full substitution under the drug discrimination paradigm. Moreover, there was no obvious relationship between the pharmacological specificity of the cocaine analogs and their peak reinforcing or reinstatement effects. This outcome may reflect a complex interaction between pharmacokinetic and pharmacodynamic properties regarding their influence on the profile of behavioral effects. In general, the rank order of potencies was similar across the three behavioral paradigms. Hence, it is evident that the different outcome measures associated with abuse-related effects are closely interrelated.

In summary, the rate of drug entry in brain can play an important role in the behavioral pharmacology of psychomotor stimulants. One major finding of these studies is that the time to peak drug uptake in putamen was predictive of the time to peak discriminative stimulus effects. Another major finding is that there was a trend for the time of peak drug uptake to correspond negatively with the number of drug infusions earned in the progressive-ratio paradigm, suggesting that faster-onset drugs tend to be more reinforcing. Finally, the rank order of potencies was similar for drugs to produce discriminative stimulus, reinforcing, and reinstatement effects, indicating that the different outcome measures associated with abuse liability are closely interrelated. However, the peak reinforcing effects in the PR paradigm did not correlate with the peak reinstatement effects, suggesting that different mechanisms are involved in these drug effects. The present results further validate the use of PET neuroimaging of radiolabeled drugs as a tool to measure rates of drug uptake in brain and their relationship to abuse-related behavioral effects.

References

Bergman J, Madras BK, Johnson SE, Spealman RD. Effects of cocaine and related drugs in nonhuman primates. III. Self-administration by squirrel monkeys. J Pharmacol Exp Ther 1989;251:150–5.

Carroll FI, Pawlush N, Kuhar MJ, Pollard GT, Howard JL. Synthesis, monoamine transporter binding properties, and behavioral pharmacology of a series of 3beta-(substituted phenyl)-2beta-(3'-substituted isoxazol-5-yl)tropanes. J Med Chem 2004;47:296–302.

Cline EJ, Scheffel U, Boja JW, Carroll FI, Katz JL, Kuhar MJ. Behavioral effects of novel cocaine analogs: a comparison with in vivo receptor binding potency. J Pharmacol Exp Ther 1992;260:1174–9.

Desai RI, Kopajtic TA, French D, Newman AH, Katz JL. Relationship between in vivo occupancy at the dopamine transporter and behavioral effects of cocaine, GBR 12909 [1-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-4-(3-phenylpropyl)piperazine], and benztropine analogs. J Pharmacol Exp Ther 2005;315:397–404.

Ginsburg BC, Kimmel HL, Carroll FI, Goodman MM, Howell LL. Interaction of cocaine and dopamine transporter inhibitors on behavior and neurochemistry in monkeys. Pharmacol Biochem Behav 2005;80:481–91.

Howell LL, Byrd LD. Characterization of the effects of cocaine and GBR 12909, a dopamine uptake inhibitor, on behavior in the squirrel monkey. J Pharmacol Exp Ther 1991;258:178–85.

Howell LL, Czoty PW, Kuhar MJ, Carroll FI. Comparative behavioral pharmacology of cocaine and the selective dopamine uptake inhibitor RTI-113 in the squirrel monkey. J Pharmacol Exp Ther 2000;292:521–9.

Howell LL, Hoffman JM, Votaw JR, Landrum AM, Wilcox KM, Lindsey KP. Cocaine-induced brain activation determined by positron emission tomography neuroimaging in conscious rhesus monkeys. Psychopharmacology (Berl) 2002;159:154–60.

Howell LL, Carroll FI, Votaw JR, Goodman MM, Kimmel HL. Effects of combined dopamine and serotonin transporter inhibitors on cocaine self-administration in rhesus monkeys. J Pharmacol Exp Ther 2007;320:757–65.

Katz JL, Kopajtic TA, Agoston GE, Newman AH. Effects of N-substituted analogs of benztropine: diminished cocaine-like effects in dopamine transporter ligands. J Pharmacol Exp Ther 2004;309:650–60.

Kimmel HL, Carroll FI, Kuhar MJ. Locomotor stimulant effects of novel phenyltropanes in the mouse. Drug Alcohol Depend 2001;65:25–36.

Kimmel HL, O'Connor JA, Carroll FI, Howell LL. Faster onset and dopamine transporter selectivity predict stimulant and reinforcing effects of cocaine analogs in squirrel monkeys. Pharmacol Biochem Behav 2007;86:45–54.

Kuhar MJ. Neurotransmitter transporters as drug targets: recent research with a focus on the dopamine transporter. The Pharmacologist 1993;35:28–33.

Kuhar MJ, McGirr KM, Hunter RG, Lambert PD, Garrett BE, Carroll FI. Studies of selected phenyltropanes at monoamine transporters. Drug Alcohol Depend 1999;56:9–15.

Lile JA, Morgan D, Freedland CS, Sinnott RS, Davies HM, Nader MA. Self-administration of two long-acting monoamine transport blockers in rhesus monkeys. Psychopharmacology (Berl) 2000;152:414–21.

Lile JA, Morgan D, Birmingham AM, Wang Z, Woolverton WL, Davies HM, et al. The reinforcing efficacy of the dopamine reuptake inhibitor 2beta-propanoyl-3beta-(4-tolyl)-tropane (PTT) as measured by a progressive-ratio schedule and a choice procedure in rhesus monkeys. J Pharmacol Exp Ther 2002;303:640–8.

Lile JA, Wang Z, Woolverton WL, France JE, Gregg TC, Davies HM, et al. The reinforcing efficacy of psychostimulants in rhesus monkeys: the role of pharmacokinetics and pharmacodynamics. J Pharmacol Exp Ther 2003;307:356–66.

Lile JA, Morgan D, Birmingham AM, Davies HM, Nader MA. Effects of the dopamine reuptake inhibitor PTT on reinstatement and on food- and cocaine-maintained responding in rhesus monkeys. Psychopharmacology (Berl) 2004;174:246–53.

Lindsey KP, Wilcox KM, Votaw JR, Goodman MM, Plisson C, Carroll FI, et al. Effects of dopamine transporter inhibitors on cocaine self-administration in rhesus monkeys: relationship to transporter occupancy determined by positron emission tomography neuroimaging. J Pharmacol Exp Ther 2004;309:959–69.

Madras BK, Fahey MA, Bergman J, Canfield DR, Spealman RD. Effects of cocaine and related drugs in nonhuman primates. I. [3H]cocaine binding sites in caudate-putamen. J Pharmacol Exp Ther 1989;251:131–41.

Nader MA, Grant KA, Davies HM, Mach RH, Childers SR. The reinforcing and discriminative stimulus effects of the novel cocaine analog 2beta-propanoyl-3beta-(4-tolyl)-tropane in rhesus monkeys. J Pharmacol Exp Ther 1997;280:541–50.

Negus SS, Mello NK, Blough BE, Baumann MH, Rothman RB. Monoamine releasers with varying selectivity for dopamine/norepinephrine versus serotonin release as candidate "agonist" medications for cocaine dependence: studies in assays of cocaine discrimination and cocaine self-administration in rhesus monkeys. J Pharmacol Exp Ther 2007;320:627–36.

Panlilio LV, Goldberg SR, Gilman JP, Jufer R, Cone EJ, Schindler CW. Effects of delivery rate and non-contingent infusion of cocaine on cocaine self-administration in rhesus monkeys. Psychopharmacology (Berl) 1998;137:253–8.

Panlilio LV, Schindler CW. Self-administration of remifentanil, an ultra-short acting opioid, under continuous and progressive-ratio schedules of reinforcement in rats. Psychopharmacology (Berl) 2000;150:61–6.

Reith MEA, Meisler BE, Sershen H, Lajtha A. Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. Biochem Pharmacol 1986;35:1123–9.

Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 1987;237:1219–23.

Roberts DC. Self-administration of GBR 12909 on a fixed ratio and progressive ratio schedule in rats. Psychopharmacology (Berl) 1993;111:202–6.

Roberts DC, Phelan R, Hodges LM, Hodges MM, Bennett B, Childers S, et al. Self-administration of cocaine analogs by rats. Psychopharmacology (Berl) 1999;144:389–97.

Rothman RB, Grieg N, Kim A, DeCosta BR, Rice KC, Carroll FI, et al. Cocaine and GBR12909 produce equivalent motoric responses at different occupancy of the dopamine transporter. Pharmacol Biochem Behav 1992;43:1135–42.

Spealman RD, Madras BK, Bergman J. Effects of cocaine and related drugs in nonhuman primates. II. Stimulant effects on scheduled-controlled behavior. J Pharmacol Exp Ther 1989;251:142–9.

Stathis M, Scheffel U, Lever SZ, Boja JW, Carroll FI, Kuhar MJ. Rate of binding of various inhibitors at the dopamine transporter in vivo. Psychopharmacology (Berl) 1995;119:376–84.

Vaugeois JM, Bonnet JJ, Duterte-Boucher D, Costentin J. In vivo occupancy of the striatal dopamine uptake complex by various inhibitors does not predict their effects on locomotion. Eur J Pharmacol 1993;230:195–201.

Volkow ND, Wang GJ, Fischman MW, Foltin RW, Fowler JS, Abumrad NN, et al. Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 1997;386:827–30.

Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Logan J, Ding YS, et al. Blockade of striatal dopamine transporters by intravenous methylphenidate is not sufficient to induce self-reports of "high". J Pharmacol Exp Ther 1999;288:14–20.

Volkow ND, Wang GJ, Fischman MW, Foltin R, Fowler JS, Franceschi D, et al. Effects of route of administration on cocaine induced dopamine transporter blockade in the human brain. Life Sci 2000;67:1507–15.

Volkow ND, Fowler JS, Wang GJ, Ding YS, Gatley SJ. Role of dopamine in the therapeutic and reinforcing effects of methylphenidate in humans: results from imaging studies. Eur Neuropsychopharmacol 2002;12:557–66.

Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, Woolverton WL. Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. J Pharmacol Exp Ther 2005;313:848–54.

Wilcox KM, Rowlett JK, Paul IA, Ordway GA, Woolverton WL. On the relationship between the dopamine transporter and the reinforcing effects of local anesthetics in rhesus monkeys: practical and theoretical concerns. Psychopharmacology (Berl) 2000;153:139–47.

Wilcox KM, Kimmel HL, Lindsey KP, Votaw JR, Goodman MM, Howell LL. In vivo comparison of the reinforcing and dopamine transporter effects of local anesthetics in rhesus monkeys. Synapse 2005;58:220–8.

Woolverton WL, Ranaldi R, Wang Z, Ordway GA, Paul IA, Petukhov P, et al. Reinforcing strength of a novel dopamine transporter ligand: pharmacodynamic and pharmacokinetic mechanisms. J Pharmacol Exp Ther 2002;303:211–7.