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Relationship between injection duration, transporter occupancy and reinforcing strength of cocaine

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Abstract

Among drugs that can function as positive reinforcers, slower occupancy of central nervous system sites of action has been associated with diminished reinforcing strength. The present study examined the relative reinforcing strength of cocaine, and the rate of in vivo dopamine transporter binding, as a function of injection duration. Rhesus monkeys ($N=5$) were allowed to self-administer cocaine under a progressive-ratio schedule with doses injected over different times (10–600 s). An ex vivo dopamine transporter binding assay was used to examine kinetics of in vivo transporter occupancy by cocaine injected over the same times in rats. Cocaine was a weaker reinforcer, and dopamine transporter binding rate decreased, with slower injections. Maximum transporter binding was the same across injection durations. These results support the hypothesis that slower onset of action is associated with a slower transporter occupancy and diminished reinforcing strength. Relative strength as a reinforcer may not be determined by maximum occupancy, at least not exclusively.

Keywords: Cocaine; Pharmacokinetics; Occupancy; Reinforcement; Self-administration; Rhesus monkey

1. Introduction

Drugs that function as positive reinforcers can differ in their relative reinforcing strengths or efficacies. Pharmacodynamics, pharmacokinetics and behavioral conditions can all contribute to these between-drug differences. For example, there is evidence that agonist efficacy can be directly related to strength as a reinforcer (Winger et al., 1996; Weed et al., 1997). A more rapid onset of action has also been associated with greater reinforcing strength (Sellers et al., 1989; Gorelick, 1997). Data in support of this hypothesis have been collected for a variety of drugs in both human (de Wit et al., 1992, 1993; Marsch et al., 2001) and nonhuman subjects (Lile et al., 2002; Winger et al., 2002; Woolverton et al., 2002). Duration of action, on the other hand, has been reported not to influence reinforcing strength substantially (Panlilio and Schindler, 2000; Ko et al., 2002).

One of the difficulties in evaluating the relationship between onset of action and relative reinforcing strength using between-drug comparisons derives from the reality that there are virtually always pharmacodynamic differences between drugs that could also contribute to differences in reinforcing strength (e.g., Lile et al., 2003; Woolverton et al., 2002). One approach to this problem is to compare relative reinforcing strength of a single drug injected at different rates. Balster and Schuster (1973) studied cocaine self-administration by monkeys under a fixed-interval schedule with a time-out of 15 min after each injection. Responding maintained by cocaine decreased as injection duration increased from 5 to 200 s in a manner that was consistent with a decrease in dose at a constant injection rate. Similar results have been reported by Panlilio et al. (1998) using a fixed-ratio schedule of reinforcement. These data are consistent with the hypothesis that a slower onset of action is associated with reduced reinforcing strength. Experiments with human subjects have also used this approach and concluded that subjective effects of drugs decrease with slower rate of onset either orally (de Wit et al., 1992, 1993) or i.v. (Marsch et al., 2001).

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It is reasonable to assume that a slower onset of action is related to a reduced rate of occupancy of the central nervous system (CNS) site of action that is pharmacologically related to the reinforcing effect. For cocaine and cocaine-like drugs, there is strong evidence that brain dopamine transporters are a primary site of action for the reinforcing effect (e.g., Ritz et al., 1987; Bergman et al., 1989). Recent data are consistent with the hypothesis that a slower rate of dopamine transporter occupancy is associated with reduced reinforcing strength (Woolverton et al., 2002; Lile et al., 2002). As noted, although the drugs that have been compared share an action at dopamine transporters, pharmacodynamic differences between them may contribute to apparent differences in reinforcing strength (Lile et al., 2003). The purpose of the present study was to examine the relationship of onset of action and reinforcing strength using a single drug, cocaine, injected at different rates. To extend the results of Balster and Schuster (1973) and Panlilio et al. (1998), cocaine was made available under conditions explicitly designed and validated for the measurement of strength of a reinforcing effect. Rhesus monkeys were prepared with i.v. catheters and allowed to self-inject cocaine under a progressive-ratio schedule of reinforcement (Wilcox et al., 2000; Woolverton et al., 2002). Dose–response functions were determined for cocaine with doses injected over 10, 100, 300 or 600 s. To examine the relationship between rate of dopamine transporter occupancy and reinforcing strength, transporter binding was studied in rats using an ex vivo binding assay (Gatley et al., 1999; Woolverton et al., 2002) with cocaine doses infused over 10, 300 or 600 s.

2. Materials and methods

The animals used in this study were maintained in accordance with the United States Public Health Service Guide for Care and Use of Laboratory Animals, and all procedures were approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

2.1. Self-administration

2.1.1. Animals

The subjects were adult male rhesus monkeys (*Macaca mulatta*: $N=5$) that weighed between 7.8 and 10 kg at the start of the study. Each monkey was fed, at approximately 07:00 h, a sufficient amount of monkey chow (130–180 g/day, Teklad 25% Monkey Diet, Harlan/Teklad, Madison, WI) to maintain a stable body weight. In addition, each monkey was given fresh fruit daily and a chewable vitamin tablet every other day. Water was available continuously. All monkeys had a history of responding under the conditions of the present experiment . Most recently, all monkeys had been subjects in a study to compare self-administration of cocaine, phentermine and sibutramine under the present progressive-ratio schedule of reinforcement (Woolverton and Heal, unpublished). Previously, monkey L638, RJu2 and AV88 had a history of self-administration of dopamine transporter ligands (Woolverton et al., 2002), while monkeys L500 and H228 had a recent history of self-administration of cocaine/scopolamine mixtures (Ranaldi and Woolverton, 2002) under a progressive-ratio schedule of reinforcement.

2.1.2. Apparatus

Each monkey was fitted with a stainless-steel restraint harness and spring arm (E&H Engineering, Chicago, IL) which was attached to the rear of a ventilated experimental cubicle (1 m^3) in which the monkey lived during the experiment. Two response levers (BRS/LVE, PRL-001, Beltsville, MD), equipped with jeweled stimulus lights, were mounted on the inside of the transparent front of each experimental cubicle, 10 cm above the floor of the cubicle. Baseline drug injections were delivered at the rate of approximately 1.0 ml/10 s by a peristaltic pump (Model 7540X, Cole Parmer Instrument, Vernon Hills, IL) located outside the cubicle. Injections in test sessions were administered in a volume of 3.0 ml by either a variable speed peristaltic pump (Model 7553-70, Cole Parmer Instrument) or a syringe pump (Model 352, Sage Instruments, Freedom, CA). Programming and recording of experimental events were accomplished by a Macintosh computer and associated interfaces located in an adjacent room.

2.1.3. Procedure

An i.v. catheter had been surgically implanted into a major vein in each monkey. For internal and external jugular and femoral veins, a silicone catheter (0.076 cm i.d., 0.26 cm o.d.; Cole-Parmer, Chicago, IL) was used. For brachial veins, the catheter was Micro-Renethane (0.1 cm i.d., 0.2 cm o.d.; Braintree Scientific, Braintree, MA) drawn to a tapered tip after heating. The monkey was injected with a combination of ketamine hydrochloride (1.0 mg/kg, i.m.) and atropine sulfate (0.04 mg/kg, i.m.) followed in 20–30 min by inhaled isoflurane. After surgery, the monkey was returned to the experimental cubicle and the catheter was threaded through the spring arm, out the back of the cubicle and connected to the pump. An antibiotic was administered i.m. daily for 7 days after surgery to prevent infection. If a catheter became nonfunctional during the experiment, a new catheter was implanted as before following a 1–2 week period to allow any infection to clear. Catheters were filled between sessions with a solution of 20–40 units/ml heparin to prevent clotting at the catheter tip. Experimental sessions began at noon each day and were conducted 7 days per week.

In the experimental cubicle, there were two red and two white stimulus lights above each lever. At the

beginning of a session, the white lights were illuminated above both levers. Responding on the right lever under a progressive-ratio schedule of reinforcement resulted in the delivery of an injection. Responding on the left lever was counted but had no other programmed consequence. The progressive-ratio schedule has been described in detail previously (see Woolverton et al., 2002). It consisted of five components, each made up of four trials, for a total of 20 available trials/day. The response requirement for the first component was 100 and doubled for each successive component. The same response requirement was in effect for each trial in a component, and a trial ended with an injection or the expiration of a 30-min limited hold. During the injection, the lights above both levers turned from white to red. There was a 30-min time-out after each drug injection or the expiration of the limited hold. If the response requirement was not completed for two consecutive trials, or the animal took all 20 injections, the session ended.

In baseline sessions, cocaine (0.1 mg/kg/injection for L500; 0.3 mg/kg/injection for H228, RJu2, RIk2 and AV88) or saline was available under a double alternation sequence; that is, two consecutive cocaine sessions were followed by two consecutive saline sessions. When responding was stable (± 2 injections for at least eight consecutive sessions), test sessions were added to the daily sequence between two saline and two cocaine sessions. To help prevent monkeys from learning this sequence, a randomly determined saline or cocaine baseline session was inserted after every other test session. Thus, the final sequence of sessions was CSTSCTRCSTSCTR, where C, S, R and T denote, respectively, cocaine, saline, random and test sessions. During test sessions, the monkeys had available to them one of various doses of cocaine (0.01–1.0 mg/kg/injection) injected in a volume of 3.0 ml delivered over 10, 100, 300 or 600 s. Between test sessions, a monkey was returned to baseline conditions until cocaine- and saline-maintained responding were again stable. Doses of cocaine were tested twice, once the day after a cocaine baseline session, and once the day after a saline baseline session in random order. If the results of the two test sessions were widely disparate (≥ 5 injections), both test sessions were repeated. Injection durations were tested in an irregular order across monkeys, except that the 600-s injection was tested last in all monkeys. Monkey H228 was not tested at the 600-s injection because of a downward shift in baseline self-administration of cocaine.

2.1.4. Data analysis

Progressive-ratio data were analyzed as injections/session. This dependent measure has been shown to be comparable to the more traditional breakpoint measures and more amenable to statistical analysis (Depoortere et al., 1993; Rowlett et al., 1996). Means and S.E.M.s were calculated for each dose of each drug. ED_{50} values were individually calculated using the linear portion of the dose–response function (GraphPad Prism 3.0). The maximum number of injections maintained by any dose served as 100% for ED_{50} analysis. In addition, maximum number of injections, regardless of dose, was used as a measure of reinforcing strength. Statistical significance of differences between ED_{50}s and maximum injections was analyzed using one-way analysis of variance (ANOVA) for repeated measures. A significant ANOVA was followed by pairwise comparison using a paired *t*-test. Statistical significance was set at the $P = 0.05$ level. Monkey H228 was excluded from the statistical analysis because of his baseline shift.

2.2. Ex vivo binding

Dopamine transporter binding was studied ex vivo using methods similar to those previously published using mice (Gatley et al., 1999) and rats (Woolverton et al., 2002).

2.2.1. Subjects and apparatus

The subjects were male Sprague–Dawley rats weighing between 250 and 300 g. They were initially housed in groups of three in plastic cages and with a 12:12 light/dark cycle (lights on at 06:00). Food and water were available ad libitum.

2.2.2. Procedure

Drugs were given i.v. via a surgically implanted catheter. For surgery, rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and a femoral catheter was implanted using standard techniques (e.g., Panlilio and Schindler, 2000). The exteriorized tip of the catheter was sealed by heating. After surgery, they were housed individually for 48 h then used experimentally.

On experimental days, catheterized rats were placed in a plastic restrainer and injected i.v. with 10 μCi/rat of [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane (CFT; 0.4 ml/10 s) via the catheter in and returned to the cage. Preliminary studies showed that the striatal/cerebellar ratio of [^{3}H]CFT reached a maximum 45 min after injection of [^{3}H]CFT (Woolverton et al., 2002). Therefore, rats were injected with saline or 8.9 μmol/kg cocaine beginning 45 min after [^{3}H]CFT injection. This cocaine dose was selected based upon previous studies establishing this dose as the approximate ED_{50} for inhibiting [^{3}H]CFT binding in vivo (Woolverton et al., 2002). Doses were given in a maximum of 0.4 ml over either 10, 300 or 600s. The 10-s injections were given by hand and the 300- and 600-s injections were given via syringe pump (74900-00, Cole-Parmer). A 100-s injection was not studied because self-administration of cocaine was not decreased at this injection duration. Rats were decapitated at various time points after beginning the cocaine injection. After decapitation, brains were removed and dissect-

ed into striatum (high dopamine transporter density) and cerebellum (no dopamine transporter, nonspecific binding). Striatum and cerebellum were weighed and placed into separate 5-ml glass vials. Solvable (10 μl/mg tissue) was added and the vial was allowed to sit for 24 h at room temperature. After 24 h, glacial acetic acid (1 μl/mg tissue) was added and 200 μl of the tissue solution was immediately pipetted into each well of 24-well scintillation plates (3–6 wells/sample). Microscint-20 cocktail (1000 μl) was then added to each well and the plate was sealed. This preparation was allowed to sit for 24 h to reduce chemiluminescence of the microscint-20 cocktail. Radioactivity was then counted using a Top Count scintillation counter (Packard Instrument).

2.2.3. Data analysis

For all samples, striatal/cerebellar ratio was calculated and data were normalized to striatal/cerebellar ratio—1 so that complete inhibition of binding approached 0. Time course data for dopamine transporter binding by cocaine were converted to percent of control with saline pretreatment data at the same time points serving as control. Data for cocaine and saline were compared using a one-way ANOVA followed by adjusted Bonferroni *t*-tests. Slopes of time curves were calculated using the time points from the end of the injection to the point of maximum binding by least squares linear regression (Prism 3.0, Graphpad, San Diego, CA) and compared using one-way ANOVA. In slope calculations for 300- and 600-s injections, binding was assumed to be 1.0% and 2.0%, respectively, at the 30-s time point.

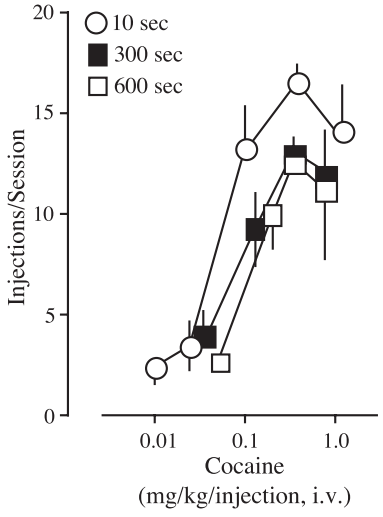

Fig. 1. The number of injections/session as a function of dose of cocaine injected over 10, 300 or 600 s, determined under a progressive-ratio schedule of reinforcement in rhesus monkeys. Each point represents the mean number of injections for the five monkeys, except for the 600-s condition where four monkeys were tested. Vertical error bars represent the S.E.M. The effect of each dose and duration was determined at least twice in each monkey.

Table 1
ED_{50} and maximum injections/sessions values for cocaine at different injection durations

Duration (s)	ED_{50} (mg/kg/inj) ± S.E.M.	Maximum ± S.E.M.
10 ($N=5$)	0.08 ± 0.02	18 ± 0.35
100 ($N=5$)	0.1 ± 0.03	16.9 ± 0.50
300 ($N=5$)	0.1 ± 0.04	15.4 ± 0.52[a,b]
600 ($N=4$)	0.09 ± 0.02	14.5 ± 1.21

[a] Significantly different from 10 s.
[b] Significantly different from 100 s.

2.3. Drugs

Cocaine HCl was provided by the National Institute on Drug Abuse (Rockville, MD) and was dissolved in 0.9% saline for self-administration. Radioligands were purchased from Perkin-Elmer (Boston, MA).

3. Results

Saline maintained between one and four injections/session under baseline conditions. The number of injections/session increased from saline levels with cocaine dose over low to intermediate doses (0.01–0.3 mg/kg/injection) at all injection durations (Fig. 1). Data for the 100-s injection were not different from the 10-s injection and, for clarity, are not shown in Fig. 1. The potency of cocaine did not vary as a function of injection duration [Table 1; $F(3,9)=1.64$, $P=0.25$]. However, there was a statistically significant effect of injection duration on injection maximum [Table 1; $F(3,9)=4.56$, $P=0.033$]. There was no difference between 10- and 100-s maximums ($t=1.62$, $df=3$, $P=0.203$). However, the maximum for the 300-s injection was significantly lower than the maximum for 10 s ($t=3.18$, $df=3$, $P=0.05$) and for 100 s ($t=4.08$, $df=3$, $P=0.03$). The maximum for the 600-s injection was lower than for any other injection duration but did not achieve statistical significance because of increased variability. This was due to one monkey, AV88, that took a maximum of 18 injections at this injection duration. When data from the other three monkeys were considered, the maximum at the 600-s injection was lower than at the 10-s injection ($t=7.79$, $df=2$, $P=0.016$) but not different from the other durations.

When 8.9 μmol/kg cocaine was injected over 10 s, significant dopamine transporter binding was noted at 2 min after injection (Fig. 2). Maximum binding was achieved 15 min post-injection, was unchanged 30 min after injection and waned after that (data not shown). When the same dose of cocaine was infused over 300 s, there was statistically significant binding 5 min after the start of the injection, and maximum binding was observed 25 min after the start of the injection. At 5, 10 and 15 min after the start of the injection, binding was lower for the 300-s injection than for the 10-s injection ($P<0.05$). For the 600-s injection, statistically

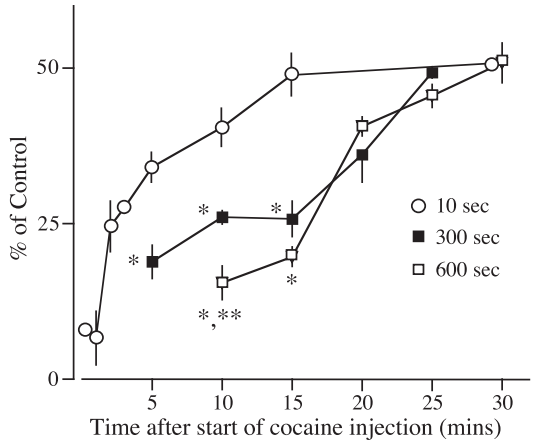

Fig. 2. Time course of dopamine transporter binding by cocaine in rat brain as a function of time from the end of a cocaine injection. Injections began 45 min after [^{3}H]CFT and rats were sacrificed at the indicated time points. Injection durations were 10, 300 or 600 s. Each point represents the striatal/cerebellar ratio of radioactivity after [^{3}H]CFT + drug, calculated as a percentage of the same ratio when [^{3}H]CFT was followed by saline injections with the identical sacrifice times. Each point is the mean of three to five rats and vertical lines are the S.E.M. *$P<0.05$ relative to a 10-s injection; **$P<0.05$ relative to a 300-s injection. Since the ED_{50} dose of cocaine was tested, the assay maximum is 50%.

significant binding was seen at the 15-, 20-, 25- and 30-min time points. Binding immediately after the end of the injection was significantly lower for the 600-s injection than for both the 10- and the 300-s injection ($P<0.05$) at the 10-min time point. At the 15-min time point, binding was lower than the 10-s injection ($P<0.05$), but from this point on 300- and 600-s data points were not different. Maximum binding was seen 15 min after beginning the 10-s injection, 25 min after beginning the 300-s injection and 30 min after beginning the 600-s injection. For all injection durations, comparable maximum occupancies were observed 30 min after the start of the injection. Slopes of the time functions were 2.65 for the 10-s injection (1.2–4.1, 95% c.l.), 1.66 for the 300-s injection (1.0–2.3, 95% c.l.) and 1.81 for the 600-s injection (1.3–2.3, 95% c.l.). Both the 300- and the 600-s slopes were different from the 10-s slope ($P<0.05$), but the 300- and 600-s slopes did not differ ($P>0.05$).

4. Discussion

As in previous studies using this and other progressive-ratio paradigms, cocaine functioned as a positive reinforcer in rhesus monkeys over a dose range of 0.03–1.0 mg/kg/injection (e.g., Stafford et al., 1998; Wilcox et al., 2000). Reinforcing effects were observed at injection durations that varied from 10 to 600 s. Variation in injection duration between 10 and 600 s did not affect the potency of cocaine as a positive reinforcer: cocaine ED_{50} values did not change with injection duration. However, the strength or efficacy of cocaine as a reinforcer, as measured by maximum levels of responding under a progressive-ratio schedule, was reduced with a slower injection. These results confirm previously published findings of decreased responding maintained by slower cocaine injections in monkeys (Balster and Schuster, 1973) and extend the conclusions of that study to conditions explicitly designed to examine relative reinforcing strength. Data with human subjects have demonstrated diminished subjective effects with slower injection rates of, e.g., morphine (Marsch et al., 2001), though the relationship between injection duration and reinforcing effect has not explicitly been studied in humans. Thus, the present experiment extends previous results by explicitly confirming the relationship between onset of binding at the CNS site and reinforcing strength. One potential concern with the injection duration approach used in this and previous studies is the possibility that metabolism during an extended injection could functionally decrease the total dose to which a subject is exposed. For the present study, the cocaine injections were short relative to the approximate 45-min elimination half-life of cocaine in monkeys, at least when given intramuscularly (Llamas et al., 1995), and the influence of metabolism, if any, should be small. The fact that potency of cocaine was unaffected by injection duration supports this argument.

Under the progressive-ratio schedule, it was necessary to increase injection duration to 300 s before a decrease in responding relative to the 10-s injection was evident. It should be noted that in a previous study using this method (Woolverton et al., 2002), we reported that the reinforcing strength of the phenylpiperidine (+)-methyl 4β-(4-chlorophenyl)-1-methylpiperidine-3-α-carboxylate was reduced relative to cocaine and associated with a diminished dopamine transporter binding over the first 2–3 min after injection. Thus, seemingly small decreases in onset of action can decrease reinforcing strength. In a preliminary study (Woolverton, unpublished), monkeys were given a choice between two injections of a dose of cocaine injected at different rates. In one monkey, a 10-s injection was preferred to a 30-s injection. In the second monkey, an increase in injection duration to 100 s was required to decrease the reinforcing effect of cocaine. Although preliminary findings, the duration values were lower than were effective in the present study. When considered with the present results, they raise the possibility that the influence of injection duration on reinforcing strength may depend on the nature of the choice available to the subject. Under the progressive-ratio schedule, the alternative to self-administering a 100-s injection was no injection, whereas under the choice schedule, the alternative to the 100-s injection was a 10-s injection. Even with substantial increases in response requirement under the progressive-ratio schedule, the 100-s injection maintained behavior comparable to the 10-s injection. That is, an injection that fails to maintain behavior when there is an alternative reinforcer available maintains substantial behavior when the strength of the alternative is decreased (see also, e.g., Campbell and Carroll, 2000; Nader

and Woolverton, 1991). In any case, the influence of the onset pharmacokinetics on reinforcing strength is likely dependent upon behavioral circumstances.

Although the strength of cocaine as a reinforcer was decreased with a slower injection, the present results suggest that there may be a "floor" on this effect, at least within the limits that were tested. That is, it may be that in the absence of an effective alternative reinforcer, relative reinforcing strength can be decreased with slower onset of action, to a point but not below. This is perhaps not surprising, considering that a number of drugs function as positive reinforcers in both humans and nonhumans when self-administered by the oral route, where onset is relatively slow (Meisch, 2001). Additionally, a number of dopamine transporter ligands have been shown to function as positive reinforcers despite very slow onset of dopamine transporter binding (Bergman et al., 1989; Lile et al., 2003; Spealman and Kelleher, 1981). We also have examined several dopamine transporter ligands that have been shown to have reinforcing effects in animals but have slow onsets of dopamine transporter binding (unpublished data).

The fact that our behavioral results were collected in monkeys while our kinetic results were collected in rats merits a caveat. With regard to behavior, it is well known that both species readily self-administer cocaine under progressive-ratio schedules of reinforcement, in a qualitatively similar manner (see, e.g., Roberts et al., 1999). However, dopamine transporter binding rate as a function of cocaine injection rate has not, to our knowledge, been studied in monkeys. Recent experiments comparing a kinetics of dopamine transporter ligands in rats to self-administration by monkeys have given no reason to suspect this comparison (e.g., Woolverton et al., 2002; Lile et al., 2003). Additionally, it should be noted that the important comparison in the present experiment is not across species, but across injection durations. In this regard, the data were remarkably consistent. It seems unlikely that the relative kinetics of different injection durations varies with species.

The extent of occupancy of the dopamine transporter has also been proposed to play a role in subjective effects and reinforcing effects of cocaine and other dopamine transporter ligands. Volkow et al. (1996, 2002) reported that the subjective effects of cocaine and methylphenidate increased with dopamine transporter occupancy. On the other hand, Wilcox et al. (2002) reported that cocaine maintained higher rates of self-administration in monkeys than did a comparison phenyltropane at doses that exhibited lower dopamine transporter occupancy than the comparator. The results of the present study, i.e., that self-administration and binding increase with dose, support the argument that reinforcing effect increases with dopamine transporter binding. However, the maximum dopamine transporter binding in the present study was the same at 30 min post-injection regardless of injection duration. Moreover, in previous studies with several different drugs, the compounds exhibited variable reinforcing strength with comparable maximum dopamine transporter occupancies (Lile et al., 2003; Woolverton et al., 2002). That is, relative reinforcing strength of dopamine transporter ligands can vary even when maximum occupancy is comparable. These findings imply that although reinforcing effect increases with dopamine transporter occupancy, relative strength as a reinforcer is not determined by maximum dopamine transporter occupancy, at least not exclusively.

In the present study, ex vivo binding data demonstrated differences in the slope of the functions relating dopamine transporter binding to time. That is, a slower rate of onset of dopamine transporter binding was associated with diminished reinforcing strength. A similar relationship has been demonstrated between plasma levels and subjective effects of morphine in humans (Marsch et al., 2001). The present experiment makes explicit the relationship between rate of brain dopamine transporter binding and reinforcing strength, in the absence of between-drug pharmacodynamic differences. Although rate of onset of a higher dose of cocaine would be expected to be greater, the relationship between the onsets of different injection durations would not be expected to change with dose. This and previous experiments suggest that decreased rate of onset over as little as the first 1–3 min after injection can substantially diminish reinforcing strength. Additionally, the present experiment, considered together with previous experiments with slow onset drugs, suggests that there may be a floor on this effect.

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