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

Rajeev I. Desai, Theresa A. Kopajtic, Dawn French, Amy H. Newman, and Jonathan L. Katz

Psychobiology (R.I.D., T.A.K., D.F., J.L.K.) and Medicinal Chemistry Sections (A.H.N.), Medications Discovery Research Branch, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Department of Health and Human Services, Baltimore, Maryland

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ABSTRACT

Analogs of benztropine (BZT) bind to the dopamine (DA) transporter and inhibit DA uptake but often have behavioral effects that differ from those of cocaine and other DA-uptake inhibitors. To better understand these differences, we examined the relationship between locomotor-stimulant effects of cocaine, 1-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-4-(3-phenylpropyl)piperazine (GBR 12909), and BZT analogs [(3α-[bis(4'-fluorophenyl)methoxy]-tropane) (AHN 1-055) and (N-allyl-3α-[bis(4'-fluorophenyl)methoxy]-tropane) (AHN 2-005)] and their in vivo displacement of the DA transporter ligand [125I]3-(4-iodophenyl)-tropan-2-carboxylic acid isopropyl ester hydrochloride (RTI-121) in striatum. Cocaine, GBR 12909, and BZT analogs each displaced [125I]RTI-121 and stimulated locomotor activity in a dose- and time-dependent manner. The time course revealed a slower onset of both effects for AHN 1-055 and AHN 2-005 compared with cocaine and GBR 12909. The BZT analogs were less effective than cocaine and GBR 12909 in stimulating locomotor activity. Locomotor stimulant effects of cocaine were generally greater than predicted by the regression of displacement of [125I]RTI-121 and effect at short times after injection and less than predicted at longer times after injection. This result suggests that the apparent rate of occupancy of the DA transporter, in addition to percentage of sites occupied, contributes to the behavioral effects of cocaine. The present results suggest that among drugs that act at the DA transporter, the slower apparent rates of occupancy with the DA transporter by the BZT analogs may contribute in an important way to differences in their effectiveness.

It has been well established that cocaine binds to dopamine (DA), serotonin, and norepinephrine transporters, resulting in an inhibition of reuptake of each of these monoamines (Heikkila et al., 1975; Kennedy and Hanbauer, 1983; Javitch et al., 1984; Madras et al., 1989). In addition, cocaine acts as a local anesthetic by binding to sodium channels (Reith, 1988). Despite this multiplicity of actions, most studies indicate that the behavioral effects of cocaine, including the reinforcing effects that are thought to underlie abuse liability, are mediated by the inhibition of DA transport (Ritz et al., 1987; Bergman et al., 1989; Volkow et al., 1997). For example, Ritz et al. (1987) showed a positive correlation between binding affinity at the DA transporter and potency for reinforcing effects among monoamine uptake inhibitors, whereas the relationships between affinities for norepinephrine or serotonin transporters and potencies for reinforcing effects were significantly weaker.

To better understand the role of the DA transporter in the effects of cocaine, a number of novel, high-affinity ligands that bind selectively to the DA transporter have been synthesized (Carroll et al., 1997; Newman and Kulkarni, 2002). Among these are analogs of benztropine (BZT), which have high binding affinity and selectivity for the DA transporter compared with the transporters of the other monoamines (Newman et al., 1995; Agoston et al., 1997). However, despite selective actions at the DA transporter, many of the BZT...
analogs have behavioral effects that are different from those of cocaine. For example, several analogs of BZT are less effective in stimulating locomotor activity compared with cocaine and fail to fully reproduce cocaine-like discriminative stimulus effects in rats trained to discriminate injections of cocaine from saline (Newman et al., 1994; Katz et al., 1999; Tolliver et al., 1999). Furthermore, BZT analogs are less effective than cocaine in maintaining high rates of responding in self-administration procedures (Woolverton et al., 2001). These findings suggest differences between these drugs and cocaine analogs in their interactions with the DA transporter (Vaughan et al., 1999; Reith et al., 2001; Chen et al., 2004), which may be responsible for the diminished cocaine-like pharmacological activity of the BZT analogs.

In vivo binding procedures have been used to assess relations between binding of ligands to relevant sites of action and various pharmacological effects. Importantly, Scheffel et al. (1991) have found a close relationship among potencies of cocaine and its analogs for displacement of radioligand from the DA transporter in vivo with their in vitro binding affinities. Additional studies have suggested that generally behavioral effects of various DA uptake inhibitors can be related to measures of DA transporter binding (Cline et al., 1992; Rothman et al., 1992; Vauegois et al., 1993; Gatley et al., 1999). Moreover, positron emission tomography studies in humans have reported that occupancy of more than 60 to 90% of DA transporters is required for abusers to report the subjective response compared with insufflated cocaine, which is dissolved in 0.9% NaCl. The BZT analogs are sonicated and heated for complete solubilization. Cocaine, GBR 12909, and the BZT analogs were administered by the i.p. route on a milligram/kilogram body weight basis in a volume of 1 ml/0.1 kg. [125I]RTI-121 was administered i.v. in 0.2 ml.

**Locomotor Activity.** For the assessment of horizontal locomotor activity (ambulation), mice were tested alone in clear acrylic experimental chambers (40 cm²). Around the outside of two perpendicular adjoining walls of the chambers were arrays of light-sensitive detectors, spaced 2.5 cm apart. Infrared light sources were mounted outside the opposing walls and directed at the detectors (Omnitech Electronics, Columbus, OH). Each interruption of a single light beam registered by the detectors resulted in the tabulation of one horizontal activity count. Mice were injected and immediately placed in the apparatus for 8 h. Total activity count data were collected every 10 min. Locomotor activity data were collected with behavior occurring continuously over time because the uptake and distribution of the drug to binding sites and the resulting association and dissociation are analogously continuous processes occurring in time, rendering the behavioral study similar to the binding study along this parameter. Mice were used only once, and each dose of the drug was studied in eight mice.

**In Vivo Binding of [125I]RTI-121.** Each animal received an i.v. injection of 2 μCi of [125I]RTI-121. Two hours after administration of [125I]RTI-121, the animals were sacrificed by cervical dislocation. In each mouse, displacement of [125I]RTI-121 was examined by giving an i.p. injection of one of the displacers at various doses and times relative to sacrifice. Cocaine (29, 59, and 118 μg/kg), GBR 12909 (6, 19, and 57 μmol/kg), AHN 1-055 (8, 25, and 71 μmol/kg), AHN 2-005 (7, 25, and 72 μmol/kg), or vehicle was examined. Displacement was examined at 5, 10, 30, and 125 min after cocaine, at 5, 30, 60, 125, 150, and 300 min after GBR 12909, at 10, 30, 150, and 210 min after AHN 1-055, and at 10, 30, 150, 210, and 240 min after AHN 2-005 injection. Each data point was determined in sets of 5 to 10 mice. Whole brains were rapidly removed, and striatum and cerebellum were dissected on ice. Following dissection, each brain region was placed into separate plastic vials (55 × 12 mm; Röhrten Tubes; Sarstedt, Aktiengesellschaft & Co., Nürnberg, Germany) and weighed, and tissue radioactivity was measured using an automated gamma counter (10/600 PLUS; MP Biomedicals, Irvine, CA).

**Analysis of Data.** For the analysis of in vivo binding data, regional radioactivity levels were divided by weight (gram) of the tissue (cpm/tissue weight). Specific binding was calculated as cpm/tissue weight in striatum divided by cerebellum minus 1 (S/C - 1), which is based on the observation that dopaminergic transporter sites are highly concentrated in the striatum and relatively absent in the cerebellum (Scheffel et al., 1991). These values were expressed as a percentage of specific binding after vehicle injection. Data were analyzed using two-way analysis of variance (ANOVA), and a post hoc Tukey’s test was used to determine significance of effects for individual doses at different time periods. The ED₅₀ dose was calculated as the dose producing 50% displacement of specific [125I]RTI-121 binding. Data for the effects of cocaine have been previously

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**Materials and Methods**

**Animals.** Male Swiss-Webster mice (Taconic Farms, Germantown, NY) weighing 25 to 40 g at the time of testing were used. Subjects were housed in groups of four in plastic cages with pine sawdust bedding and with food and water continuously available. All the subjects were kept in a colony room maintained at 21 ± 1°C under a 12-h light/dark cycle (lights on 7:00 AM). All the experiments were conducted during the light phase of the light/dark cycle, 8:00 AM to 3:00 PM, in a room that was separate from the housing room. Animal care procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, National Institute on Drug Abuse, Intramural Research Program.

**Drugs.** Drugs in the present studies were (-)-cocaine hydrochloride, GBR 12909 (Sigma-Aldrich, St. Louis, MO), and the N-methyl and N-allyl analogs of BZT (Agoston et al., 1997). The radioligand used in the in vivo binding studies was [125I]RTI-121 (specific activity, 2200 Ci/mmol; PerkinElmer Life and Analytical Sciences, Boston, MA) (Lever et al., 1996), which has a high selectivity and a high affinity for the DA transporter over other monoamine transporters and has been used to assess occupancy at the DA transporter by ligands that interact both rapidly and slowly with the DA transporter (Scheffel et al., 1992; Lever et al., 1996). In addition, Lever et al. (1996) found that nonmetabolized radioligand represented 85% of the signal observed. These characteristics make [125I]RTI-121 an ideal radiotracer to examine DA transporter occupancy by ligands. All the drug solutions that were administered to animals were prepared fresh daily in sterile water, except for cocaine, which was dissolved in 0.9% NaCl. The BZT analogs are sonicated and heated for complete solubilization. Cocaine, GBR 12909, and the BZT analogs were administered by the i.p. route on a milligram/kilogram body weight basis in a volume of 1 ml/0.1 kg. [125I]RTI-121 was administered i.v. in 0.2 ml.
reported in a preliminary report (Desai et al., 2005) and are included in this study for comparison with the other compounds.

The apparent rate of occupancy of each drug was calculated by determining the slope of the time course for displacement of \([^{125}\text{I}]\text{RTI-121}\). Initially, the data from all the time points were included in the analysis, and for each drug, it was determined whether there was a statistically significant deviation from linearity. Visual inspection of the curves indicated that deviations from linearity were likely caused by a decrease in displacement with drug elimination (cocaine) or by plateaus in the curves beyond the point of maximum displacement (GBR 12909, AHN 1-055, and AHN 2-005). Therefore, deviations from linearity were followed by a further analysis with the data from the time point furthest after injection eliminated. If a deviation from linearity remained, the process was repeated. Using this iterative process, we ensured that only the linear portions of the curve were analyzed. Because with AHN 1-055 and AHN 2-005 time points between 30 and 150 min were not assessed, the slopes of the time course for displacement of \([^{125}\text{I}]\text{RTI-121}\) for each drug using only the first two time points were determined to confirm observations about slope derived from time points that spanned 30 to 150 min.

Horizontal locomotor activity counts were analyzed using two-way ANOVA and post hoc Tukey’s test to determine significance of effects of individual doses at different time periods. Dose-effect curves for both locomotor activity and in vivo binding were analyzed using standard ANOVA and linear regression techniques. The ED50 values and their 95% confidence limits were determined from data using the linear portions of the dose-effect curves (Snedecor and Cochran, 1967). The ED50 dose was defined as the dose producing half of the drug’s own maximal effect.

The relationship between binding events at the DA transporter and behavioral outcome was examined by linear regression, as has been done previously (Ritz et al., 1987; Cline et al., 1992; Rothman et al., 1992; Vaugeois et al., 1993; Gatley et al., 1999; Volkow et al., 2002). Stimulation of locomotor activity, during the 10 min around each time point at which binding was assessed, was determined by subtracting the total number of ambulatory counts produced by the vehicle from the total ambulatory counts produced by the drug, similarly to the approach used by other groups (e.g., Vaugeois et al., 1993). DA transporter binding was calculated by subtracting the percentage displacement \([^{125}\text{I}]\text{RTI-121}\) produced by each drug from the percentage displacement \([^{125}\text{I}]\text{RTI-121}\) produced after the vehicle.

**Results**

**In Vivo Binding.** The cocaine-induced displacement of \([^{125}\text{I}]\text{RTI-121}\) in striatum (Fig. 1A) was significantly related to both dose (\(F_{3,148} = 86.3; p < 0.01\)) and time (\(F_{3,148} = 59.7; p < 0.01\)). ANOVA also showed a significant interaction of dose and time (\(F_{9,444} = 8.5; p < 0.01\)). Maximal displacement in striatum was obtained at the highest dose studied, 118 μmol/kg. Tukey’s post hoc analyses revealed that the displacement of \([^{125}\text{I}]\text{RTI-121}\) by cocaine was significant at 10, 30, and 125 min after injection at each of the doses studied. The effect at 30 min was significantly greater than at earlier and later time points at each of the doses, with the exception that there was no significant difference between displacement at 30 and 125 min after injection of the highest dose.

GBR 12909 also produced a dose- (\(F_{3,95} = 273; p < 0.001\)) and time-dependent (\(F_{5,95} = 39.3; p < 0.01\)) displacement of \([^{125}\text{I}]\text{RTI-121}\) in striatum (Fig. 1B), with a significant dose by time interaction (\(F_{15,95} = 10.4; p < 0.01\)). Significant dose-related displacement of \([^{125}\text{I}]\text{RTI-121}\) was obtained at all the time points after 5 min. The maximal displacement was obtained at 125 min after injection of 57 μmol/kg of GBR 12909. Tukey’s post hoc analyses indicated that with GBR 12909 there was no significant reduction in effect after the maximum, although there were significant differences between effects at 125 min and at 5 and 30 min after injection.

Displacement of \([^{125}\text{I}]\text{RTI-121}\) binding as a function of dose and time is shown for AHN 1-055 and AHN 2-005 in Fig. 1, C and D, respectively. For AHN 1-055, the effects of time (\(F_{3,135} = 34.1; p < 0.01\)) and dose (\(F_{3,135} = 96.4; p < 0.01\)) were significant, as they were for AHN 2-005 (time: \(F_{4,177} = 15.6; p < 0.01\); dose: \(F_{4,177} = 134; p < 0.01\)). The interaction of time and dose were also significant for the two drugs (AHN 1-055: \(F_{9,135} = 5.7; p < 0.01\); AHN 2-005: \(F_{12,177} = 6.9; p < 0.01\)). Significant and dose-related displacement of \([^{125}\text{I}]\text{RTI-121}\) binding in striatum was obtained with AHN 1-055 at all the time points after injection, with the maximum obtained at 76 μmol/kg at 150 min after injection. Post hoc analysis showed displacement at 150 min after injection was significantly greater than at earlier times at all but the lowest dose. For each dose, there was no significant difference between the levels of displacement at 150 and 210 min.

For AHN 2-005 (Fig. 1D), dose-related significant displacement of \([^{125}\text{I}]\text{RTI-121}\) binding in striatum was also obtained at all the time points after injection, with the maximum obtained 138 μmol/kg. At the 74-μmol/kg dose, maximal dis-
placement was obtained at 210 min after injection, and at this time point, post hoc analyses indicated that placement was significantly greater than at 10 and 30 min after injection. Post hoc analysis also showed that there were no significant differences in the levels of displacement obtained at 210 min and either 150 or 240 min after injection.

Figure 1 indicates that the change in displacement of $[^{125}I]$RTI-121 binding in striatum over time differed among the drugs. The dose producing maximal effects was further analyzed to determine the linear part of the time-response curve, and the slope of the linear portion of the curve was calculated to provide an apparent in vivo rate of occupancy. The apparent rate of occupancy was calculated using the highest doses of each of the drugs to minimize the different contributions of uptake and distribution processes for each drug. Table 1 shows that the highest apparent rate of occupancy was obtained for 118 $\mu$mol/kg of cocaine (2.04%/min) and that each of the other drugs (AHN 1-055 = 76 $\mu$mol/kg; AHN 2-005 = 74 $\mu$mol/kg; GBR 12909 = 57 $\mu$mol/kg) had a significantly slower apparent rate of occupancy, as indicated by 95% confidence limits that did not overlap with those for cocaine. Differences from cocaine in apparent rates of occupancy were as little as 1.65-fold for GBR 12909 to 6-fold for AHN 2-005. Analysis of the first two data points for each of the drugs confirmed the trends obtained across a wider range of time periods; however, there was more variability in these estimates using fewer data and a resulting overlap in 95% confidence limits for slopes (Table 1).

**Locomotor Activity.** Cocaine, as has been shown previously, increased ambulatory activity with a maximum of 662 cpm during the first 30 min of the session at 118 $\mu$mol/kg (Fig. 2A, downward triangles), the highest dose tested. Effects were significantly related to both time ($F_{47,1316} = 15.7; p < 0.05$), dose ($F_{3,28} = 11.0; p < 0.05$), and the interaction of the two ($F_{141,1316} = 2; p < 0.05$). Locomotor activity progressively decreased after the first 30 min, until reaching control levels at 170 min after injection ($t = 0.5; p = 0.615$). The lower doses, although stimulating activity to a lesser extent, also had effects that returned to control levels at about the same time and, similar to the highest dose, remained at control levels throughout the rest of the 8-h observation period (all $p$ values $\geq 0.054$; Fig. 2A, squares and upward triangles); occasional significant differences from vehicle were obtained toward the end of the observation period (all $p$ values $\leq 0.0459$), which were not systematically related to dose or time since injection.

GBR 12909 increased locomotor activity to a maximum of 567 cpm, which was obtained during the first 70 min of the session at 57 $\mu$mol/kg (Fig. 2B, downward triangles). After this time, the stimulation of locomotor activity progressively decreased but remained above control levels for the remainder of the observation period. ANOVA of the effects of GBR 12909 revealed significant effects of time ($F_{47,517} = 7.53; p < 0.05$), dose ($F_{4,18} = 59.5; p < 0.05$), and their interaction ($F_{188,846} = 1.82; p < 0.05$). The lowest dose of GBR 12909 (6 $\mu$mol/kg) generally failed to increase ambulatory activity throughout the 8-h observation period (Fig. 2B, squares). In contrast, at 19 $\mu$mol/kg GBR 12909 produced significant increases in locomotor activity from 20 through 160 min (all $p$ values $\leq 0.042$), which decreased to control levels at 170 min after injection ($t = 1.49; p = 0.136$). The highest dose of GBR

![Image](https://example.com/image.png)

**Fig. 2.** Time course of effects of cocaine, GBR 12909, AHN 1-055, and AHN 2-005 on locomotor activity in mice. Ordinates, horizontal locomotor activity counts per min; abscissae, time since injection and placement of subject in the experimental chamber. Each point represents the average effect determined in eight mice, for successive 10-min time periods up to 480 min (8 h) after injection. For clarity, the lowest doses tested of cocaine, AHN 1-055, and AHN 2-005 are not shown. Note that in contrast to cocaine, the duration of effects of the other drugs was greater, with maximal stimulation produced by AHN 1-055 and AHN 2-005 generally in the 2nd or 3rd hours and often diminished in the 7th or 8th hours after injection.

### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time Points over which Data Were Linear</th>
<th>Apparent Occupancy Rate</th>
<th>Initial Two Time Points</th>
<th>Apparent Occupancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% occupancy/min</td>
<td></td>
<td></td>
<td>% occupancy/min</td>
</tr>
<tr>
<td>Cocaine</td>
<td>5–30 min</td>
<td>2.04 (1.64–2.45)</td>
<td>5 and 10 min</td>
<td>3.03 (0.451–5.60)</td>
</tr>
<tr>
<td>GBR 12909</td>
<td>5–60 min</td>
<td>1.24 (0.952–1.53)</td>
<td>5 and 30 min</td>
<td>1.72 (0.882–2.55)</td>
</tr>
<tr>
<td>AHN 1-055</td>
<td>10–150 min</td>
<td>0.363 (0.272–0.455)</td>
<td>10 and 30 min</td>
<td>1.05 (0.216–1.88)</td>
</tr>
<tr>
<td>AHN 2-005</td>
<td>10–210 min</td>
<td>0.330 (0.239–0.420)</td>
<td>10 and 30 min</td>
<td>0.840 (0.0146–1.70)</td>
</tr>
</tbody>
</table>
Both AHN 1-055 and AHN 2-005 increased locomotor activity compared with vehicle-treated subjects (Fig. 2C and D, respectively). Maximal levels of locomotor activity that were less than those obtained with cocaine were found at about 60 min after injection and were sustained with some doses for the entire observation period. Two-way ANOVA of the effects of AHN 1-055 revealed significant effects of time ($F_{47,1,1316} = 1.8; p < 0.05$), dose ($F_{3,28} = 77.06; p < 0.05$), and their interaction ($F_{141,1316} = 1.94; p < 0.05$). For AHN 2-005, there were also significant effects of time ($F_{47,1,551} = 1.51; p < 0.05$), dose ($F_{1,33} = 41.68; p < 0.05$), and the interaction ($F_{188,1,551} = 1.20; p < 0.05$). The 25-μmol/kg dose of AHN 1-055 generally stimulated ambulatory activity from 30 to 450 min after injection (all $p$ values ≤ 0.0028; Fig. 3C, downward triangles). The 76-μmol/kg dose of AHN 1-055 (Fig. 2C, downward triangles) generally failed to stimulate locomotor activity for the first 220 min after injection (all $p$ values ≥ 0.1324) but significantly stimulated locomotor activity after that throughout the remainder of the 8-h observation period (all $p$ values ≤ 0.0264; Fig. 2C). For AHN 2-005, the 25-μmol/kg dose (Fig. 2D, upward triangles) generally stimulated locomotor activity throughout the 8-h observation period (all $p$ values ≤ 0.0289) with occasional exceptions (all $p$ values ≥ 0.1051) that were not systematically related to time or dose. The highest two doses of AHN 2-005 (Fig. 2D, downward triangle and diamond) also significantly stimulated ambulatory activity from 60 to 480 min (all $p$ values ≤ 0.045) after injection.

The dose-related effects of the drugs at times at which maximal $[^{125}\text{I}]$RTI-121 displacement occurred are shown in Fig. 3. For all the drugs, there was a significant effect of dose on locomotor activity (all $F$ values > 12.4; all $p$ values < 0.05), and there were significant differences in maximal behavioral effects of the compounds ($F_{13,68} = 28.1; p < 0.05$; Fig. 3, open symbols). The maximal stimulation of activity produced by 118 μmol/kg of cocaine was 627 cpm, which was greater than that produced by any dose of either BZT analog, although not different from that produced by the highest doses of GBR 12909 (post hoc Dunnett’s test, all $p$ values < 0.05, except 57 and 107 μmol/kg of GBR 12909). In contrast, the dose–response relationships for the stimulation of locomotor activity with $[^{125}\text{I}]$RTI-121 produced by cocaine at the highest dose studied was significantly lower than that produced by the highest doses of each of the other compounds examined (GBR 12909: $F_{1,12} = 10.0, p < 0.05$; AHN 1-055: $F_{1,18} = 42.1, p < 0.05$; AHN 2-005: $F_{1,18} = 18.2, p < 0.05$). No significant differences were obtained when comparing the maximum displacement of $[^{125}\text{I}]$RTI-121 produced by each of the drugs other than cocaine (all $F$ values$_{1,12} = 4.30$; all $p$ values > 0.05). At low to intermediate doses, there was a reasonably close correspondence between the binding and the behavioral effect of the drugs. For all the drugs, the potencies for the two effects were not significantly different (Fig. 3), and there was a close correspondence among the ED$_{50}$ values for these two effects (Table 2). However, at the highest doses there was less agreement between the binding and the behavioral effects of the drugs (Fig. 3). Particularly with the BZT analogs, there was a reduced stimulation of activity at the highest doses with a concomitant further increase in the displacement of $[^{125}\text{I}]$RTI-121.

Across all the time points at which in vivo binding was assessed, there was a significant positive correlation between displacement of $[^{125}\text{I}]$RTI-121 and locomotor-stimulant effects of cocaine, GBR 12909, AHN 1-055, and AHN 2-005 (Fig. 4). With GBR 12909 ($r = 0.841; p < 0.001$), AHN 1-055 ($r = 0.910; p < 0.001$), and AHN 2-005 ($r = 0.883; p < 0.001$), there was a close agreement between these two parameters of drug effect. However, with cocaine, a substantially lower $r$ value was obtained when correlating displacement of $[^{125}\text{I}]$RTI-121 with stimulation of locomotor activity ($r = 0.612; p = 0.0121$; Fig. 4). Interestingly, with cocaine, the

![Figure 3](https://i.imgur.com/3.png)  
**Fig. 3.** Dose-effect relationships for the stimulation of locomotor activity produced by cocaine, GBR 12909, AHN 1-055, and AHN 2-005 and the in vivo displacement of $[^{125}\text{I}]$RTI-121 binding. Ordinates, mean horizontal activity counts at times that produced maximum inhibition of $[^{125}\text{I}]$RTI-121 binding. Abscissae, i.p. dose in micromole/kilogram. Those times were for cocaine, 20 to 40 min; AHN 1-055, 140 to 160 min; and AHN 2-005, 200 to 220 min. Each point represents a mean of eight subjects. Note that across the doses studied, cocaine was more effective in stimulating locomotor activity than was GBR 12909, AHN 1-055, and AHN 2-005.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time</th>
<th>In Vivo Binding ED$_{50}$ Value</th>
<th>Behavioral ED$_{50}$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>30</td>
<td>37.9 (25.4–48.1) μmol/kg</td>
<td>47.8 (37.8–61.9)</td>
</tr>
<tr>
<td>GBR 12909</td>
<td>125</td>
<td>16.6 (14.6–18.8)</td>
<td>20.9 (15.2–28.0)</td>
</tr>
<tr>
<td>AHN 1-055</td>
<td>150</td>
<td>9.04 (6.26–11.8)</td>
<td>10.5 (8.21–14.0)</td>
</tr>
<tr>
<td>AHN 2-005</td>
<td>210</td>
<td>25.0 (22.5–27.9)</td>
<td>10.8 (5.59–19.6)</td>
</tr>
</tbody>
</table>

**Table 2.** Affinities and ED$_{50}$ values from in vivo binding studies and for locomotor stimulant effects

Numbers in parentheses represent 95% CL.
increase in locomotor activity observed within the first 10 min was significantly greater than what would be predicted from the linear regression (Fig. 4, top left panel, circles).

**Discussion**

The relationship between in vivo displacement of $^{[125]}$I-RTI-121 from the DA transporter and locomotor stimulant effects of cocaine, GBR 12909, and several BZT analogs was examined. All the compounds examined had in vitro effects of cocaine, GBR 12909, AHN 1-055, or AHN 2-005. Ordinates, difference between mean horizontal activity counts after drug and after saline. Abscissae, percentage of displacement of $^{[125]}$I-RTI-121. For each drug, the solid line represents the linear regression of percent occupancy of the DA transporter and horizontal locomotor activity. Dashed lines represent 95% confidence limits for the regression lines. Note that the locomotor stimulant effects of cocaine are less strongly related to DA transporter occupancy than are the effects of GBR 12909, AHN 1-055, and AHN 2-005.

Fig. 4. Relationship between percentage of displacement of radiolabeled RTI-121 from the DA transporter in striatum and locomotor stimulant effects of cocaine, GBR 12909, AHN 1-055, or AHN 2-005. Ordinates, difference between mean horizontal activity counts after drug and after saline. Abscissae, percentage of displacement of $^{[125]}$I-RTI-121. For each drug, the solid line represents the linear regression of percent occupancy of the DA transporter and horizontal locomotor activity. Dashed lines represent 95% confidence limits for the regression lines. Note that the locomotor stimulant effects of cocaine are less strongly related to DA transporter occupancy than are the effects of GBR 12909, AHN 1-055, and AHN 2-005.

and cocaine with regard to their distribution into brain (Raje et al., 2003). However, maximal brain/plasma ratios were observed after 1 h for AHN 2-005 and at 2 h for AHN 1-055, as opposed to 15 min for cocaine, suggesting some differences in their central nervous system distribution. Nonetheless, both AHN 1-055 and AHN 2-005 were detected in the brain shortly after injection and at concentrations approximating 4 to 15 μg/g (Raje et al., 2003) (Fig. 3), which corresponds to concentrations well above $K_i$ values for the drugs (Katz et al., 2004). Together, these data suggest that pharmacokinetics of uptake and distribution do not account for the differences from cocaine obtained in the present study.

The present results are consistent with those from previous studies (Agoston et al., 1997; Katz et al., 2004; Desai et al., 2005) in which these compounds, along with several other N-substituted BZT analogs, were significantly less effective than cocaine in stimulating locomotor activity. In those studies, the N-substituted BZT analogs were also generally less effective than cocaine in producing cocaine-like discriminative stimulus effects in rats. These findings together are consistent with several previous studies indicating decreased efficacy of BZT analogs compared with cocaine in producing prototypical psychomotor stimulant effects (Katz et al., 1999, 2004), including reinforcing effects (Woolverton et al., 2001).

Several previous studies have indicated that occupancy at the DA transporter by cocaine and other DA uptake inhibitors corresponds in general to their behavioral effects. For example, Cline et al. (1992) found that, among phenyltropane analogs, the potency for stimulation of locomotor behavior in mice was closely related to the potency for displacement of $^{3}$H]WIN 35,428 from DA transporter binding sites determined by in vivo methods similar to those of the present study. The results of the Cline et al. (1992) study are similar to several other studies (e.g., Bergman et al., 1989), including the original work of Ritz et al. (1987) and Kuhar et al. (1991) that reported the potency for reinforcing effects of various DA uptake inhibitors were related to their affinities for binding to the DA transporter.

However, several other studies have suggested complexities in the relationship between actions at the DA transporter and the behavioral effects of DA uptake inhibitors. For example, Vaugeois et al. (1993) found differences among DA uptake inhibitors in the degree of locomotor stimulation produced by the doses that produced 50% inhibition of specific $^{3}$H]GBR 12783 binding. Similarly, Rothman et al. (1992) showed in rats that several DA uptake inhibitors produced comparable levels of locomotor stimulation. However, the amount of displacement of $^{[125]}$I-RTI-121 that accompanied those effects was different for the different compounds. In particular, comparable locomotor stimulation was produced by cocaine at approximately 60% and by GBR 12909 at approximately 100% displacement of $^{[125]}$I-RTI-121. Along with the present results, these findings suggest that transduction of pharmacological effects from binding events at the DA transporter may involve intermediary steps that are modulated by the intrinsic nature of the ligand.

In the present study, the maximal effects of cocaine on behavior were obtained with about 70% displacement of $^{[125]}$I-RTI-121, whereas greater than 80% displacement was necessary for the maximal behavioral stimulant effects of GBR 12909. These results are consistent with the findings of Rothman et al. (1992), who showed differences between oc-
cupancy and behavioral effects for these two drugs. The maximal behavioral effects of AHN 1-055 and AHN 2-005 were obtained at values between those for cocaine and GBR 12909; however, for those drugs locomotor behavior decreased at the highest doses, which produced maximal occupancy. Similar effects have been reported with cocaine (Cline et al., 1992), suggesting that this aspect of the dose–effect relation does not distinguish cocaine from the BZT analogs.

Further examination of the present data provides more points of departure between levels of DA transporter occupancy and behavioral effects. For example, at 5 min after administration, cocaine significantly stimulated locomotor activity with 5 to 10% displacement of $^{[125]}$I-RTI-121. None of the other compounds were effective locomotor stimulants at that amount of displacement. We recently showed that the N-butyl analog of BZT, JHW 007, was minimally effective as a locomotor stimulant despite producing substantial displacement of $^{[125]}$I-RTI-121 (Desai et al., 2005). Collectively, these findings suggest that the relationship between DA transporter occupancy and behavioral effects of DA uptake inhibitors may depend on the compound being studied and, further, that factors in addition to levels of DA transporter occupancy are involved in the behavioral effects of DA uptake inhibitors.

The time course for displacement of $^{[125]}$I-RTI-121 in the present study revealed a difference in the apparent rates of DA transporter occupancy for cocaine, GBR 12909, AHN 1-055, and AHN 2-005. The apparent rate of occupancy was greatest for cocaine, followed by GBR 12909, AHN 1-055, and AHN 2-005. In addition, cocaine differed from the other compounds in that the amount of behavioral stimulation early after injection was greater than predicted from the regression analysis of stimulation of locomotor activity and occupancy at the DA transporter. These findings suggest that the apparent rate of occupancy is an important factor in the behavioral stimulation produced by cocaine and other DA uptake inhibitors and is consistent with a recent report by Volkow et al. (2000) that concluded that the rate at which the DA transporter is occupied plays a critical role in the subjective response to cocaine in humans. Conversely, Desai et al. (2005) recently reported that compared with cocaine, the slow apparent rate of occupancy at the DA transporter by the BZT analog JHW 007 may be responsible for JHW 007 being relatively devoid of cocaine-like behavioral effects.

Several studies have noted the importance of the kinetics of both the radiolabeled and displacing drug when assessing occupancy at the DA transporter (Gatley et al., 1996, 1997; Fowler et al., 1998). For example, drugs such as cocaine that have rapid association and dissociation kinetics have short periods at peak concentration at which the most effective competition with the radiotracer occurs. This kinetic profile can complicate assessments of DA transporter occupancy under nonequilibrium conditions, particularly when using radiotracers with slow association and dissociation kinetics (Gatley et al., 1996, 1997). The radiotracer used in the present study, $^{[125]}$I-RTI-121, has slow association and dissociation constants (Lever et al., 1996). However, in the present study, the time between administration of $^{[125]}$I-RTI-121 and sacrifice was always kept constant (i.e., near peak concentration at 120 min), and estimates of DA transporter occupancy were obtained by injecting the different doses of drugs at various times relative to sacrifice, minimizing problems introduced by different kinetic profiles.

Baumann and colleagues have noted some differences between analogs of GBR 12909 and cocaine and indeed have suggested that these compounds may block some of the effects of cocaine (e.g., Baumann et al., 1994). Previous studies of interactions of BZT analogs and cocaine in rats have shown either a potentiation of the effects of cocaine by BZT analogs (Tolliver et al., 1999) or no appreciable effects (Katz et al., 2004). The latter study examined interactions of N-substituted BZT analogs with cocaine in rats. However, in that study, BZT analogs were administered at the same time as cocaine. The present results suggest that the interactions of cocaine and BZT analogs may be more readily obtained hours after injection. Indeed, a preliminary study of the interaction of cocaine and the BZT analog JHW 007 at 270 min after the latter’s injection in mice showed an antagonism of the locomotor stimulant and discriminative stimulus effects of cocaine (Desai et al., 2005).

In summary, the present results with BZT analogs have important implications for the DA transporter hypothesis of the behavioral effects of cocaine. That hypothesis suggests that compounds that bind to the DA transporter and inhibit DA reuptake will have behavioral effects like those of cocaine (Kuhar et al., 1991). However, several BZT analogs have reduced behavioral effects compared with those of cocaine, and this occurs despite similar DA transporter occupancies. Thus, at least with analogs of BZT, a factor in addition to DA transporter occupancy appears to be involved in the production of behavioral effects. Together with previous studies (Rothman et al., 1992; Vaugeois et al., 1993; Volkow et al., 2000; Desai et al., 2005), the present results suggest that the apparent rate of occupancy may play an important role in the behavioral effects, and possibly the abuse liability, of DA uptake inhibitors, with slow rates of occupancy reducing abuse liability. Future studies will examine the present BZT analogs more fully in attempt to reveal further important differences between them and cocaine, and to assess their potential to serve as leads for the discovery of medications for cocaine abuse.

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References


effects of novel cocaine analogs: a comparison with in vivo receptor binding po-


