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EFFECTS OF N-SUBSTITUTED ANALOGUES OF BENZTROPINE: DIMINISHED COCAINE-LIKE EFFECTS IN DOPAMINE TRANSPORTER LIGANDS

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Diminished cocaine-like effects of benztropine analogues

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ABSTRACT

Previous studies demonstrated that analogues of benztropine (BZT) possess high affinity for the dopamine transporter, inhibit dopamine uptake, but generally have behavioral effects different from those of cocaine. One hypothesis is that muscarinic-M_1 receptor actions interfere with cocaine-like effects. Several tropane-nitrogen substitutions of 4',4"-diF-BZT have reduced M_1 affinity compared to the CH_3-analogue (AHN 1-055). All of the compounds displaced [^{3}H]WIN35,428 binding with affinities ranging from 11-108 nM. Affinities at norepinephrine ([^{3}H]nisoxetine) and serotonin ([^{3}H]citalopram) transporters ranged from 457-4810 and 376-3260 nM, respectively, and at muscarinic M_1 receptors ([^{3}H]pirenzepine) from 11.6 (AHN 1-055) to higher values reaching 1030 nM for the other BZT-analogues. Cocaine and AHN 1-055 produced dose-related increases in locomotor activity in mice, with AHN 1-055 less effective than cocaine. The other compounds were ineffective in stimulating activity. In rats discriminating cocaine (29 μmol/kg, IP) from saline, WIN35,428 fully substituted for cocaine, whereas AHN 1-055 produced a maximal substitution of 79%. None of the other analogues fully substituted for cocaine. WIN35,428 produced dose-related leftward shifts in the cocaine dose-effect curve, whereas selected BZT analogues produced minimal changes in the effects of cocaine. The results suggest that reducing M_1 affinity of 4',4"-diF-BZT with N-substitutions reduces effectiveness in potentiating the effects of cocaine. Further, although the BZT-analogues bind with high affinity at the dopamine transporter, their behavioral effects differ from those of cocaine. These compounds have reduced efficacy compared to cocaine, a long duration of action, and may serve as leads for the development of medications to treat cocaine abuse.

Novel analogues of benztropine (BZT; 3α-(diphenylmethoxy)-1H,5H-tropane) have been developed that have high-affinity for the dopamine transporter and are selective for the dopamine transporter over the other monoamine transporters (Kline et al., 1997; Newman et al., 1994, 1995; Newman and Kulkarni, 2002). Although these compounds bind to the dopamine transporter and inhibit the uptake of dopamine *in vitro*, their behavioral effects are generally different from those of the typical dopamine uptake inhibitors, for which cocaine is a prototype (Katz et al., 1999). Many of these compounds have relatively high affinity for muscarinic M1 receptors as well as the dopamine transporter, and it has been suggested that these other actions may interfere with the cocaine-like behavioral effects of BZT analogues (Katz et al., 1999). For example, 4'-Cl-BZT (4'-chloro-3α-(diphenylmethoxy)tropane) has a 30 nM affinity for the dopamine transporter which is comparable to the high-affinity binding of cocaine. However, 4'-Cl-BZT is only marginally efficacious as a stimulant of locomotor activity and does not produce cocaine-like discriminative-stimulus effects (Katz et al., 1999; Tolliver et al., 1999). Further, this compound does not maintain rates of responding as high as those maintained by cocaine in a "self-administration" paradigm (Woolverton et al., 2000) or break points as high as those for cocaine in a self administration progressive ratio procedure (Woolverton et al., 2001). As such, this and similar compounds suggest a challenge to the dopamine transporter hypothesis of the behavioral effects of cocaine. According to that hypothesis, compounds that bind to the dopamine transporter and inhibit dopamine uptake will have behavioral effects like those of cocaine (Kuhar et al., 1991). In addition, an understanding of the differences in pharmacological mechanisms of cocaine and the BZT analogues may provide insight into the neurobiological substrates that underlie the abuse liability of cocaine, and help understand the functioning of the dopamine transporter.

The present studies further examined the pharmacology of 4',4''-diF-BZT analogues (Figure 1) both alone and in combination with cocaine. The present report focuses on analogues substituted on the nitrogen, which are among the BZT analogues with the highest affinities for the dopamine

transporter (Newman and Kulkarni, 2002). These N-substituted analogues retained high affinity for the dopamine transporter and had a reduced affinity for muscarinic M_1 receptors (Agoston et al., 1997). As such, studies of these compounds will serve as additional assessments of the role of M_1 muscarinic activity in the behavioral effects of BZT analogs.

METHODS

Subjects. Studies in rats and mice were conducted, respectively, with male Sprague-Dawley and Swiss-Webster strains (Taconic Labs, Germantown, NY). All animals were housed in a temperature and humidity controlled vivarium with a 12 hr light/dark cycle (lights on 07.00 hr). All experiments were conducted during the light phase of the light/dark cycle, between 08:00 and 15:00. With the exception of the behavioral studies in rats, the subjects were group housed with unrestricted access to food and water.

[^{3}H]Nisoxetine Binding Assay. Membranes from frozen frontal cortex dissected from male Sprague-Dawley rats (Taconic Labs, Germantown, NY) were homogenized in 20 volumes (w/v) of 50 mM Tris containing 120 mM NaCl and 5 mM KCl (pH 7.4 at 25°C), using a Brinkman Polytron (at setting 6 for 20 sec). The tissue was centrifuged at 50,000 x *g* for 10 min at 4°C. The resulting pellet was resuspended in buffer and recentrifuged. The final pellet was resuspended in cold buffer to a concentration of 80 mg/ml (original wet weight).

Ligand binding experiments were conducted in assay tubes containing 0.5 ml buffer, 0.5 nM [^{3}H]nisoxetine (New England Nuclear, Boston MA), and 8 mg frontal cortex tissue. The reaction was started with the addition of the tissue and the tubes were incubated for 60 min at 0-4°C. The incubation was terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% polyethylenimine, using a Brandel Cell Harvester (Brandel Instruments Gaithersburg, Maryland). The filters were washed twice with 5 ml cold buffer, transferred to scintillation vials to which Beckman Ready Safe was added. Nonspecific binding was determined using 1 μM desipramine. Data were analyzed using GraphPad Prism software (San Diego, California).

[^{3}H]Citalopram Binding Assay. Membranes from frozen rat midbrain were homogenized in 20 volumes (w/v) of 50 mM Tris containing 120 mM NaCl and 5 mM KCl (pH 7.4 at 25º C), using a Brinkman Polytron (at setting 6 for 20 sec). The tissue was centrifuged at 20,000 x *g* for 10 min at 4º C. The resulting pellet was resuspended in buffer and recentrifuged. The final pellet was resuspended in cold buffer to a concentration of 15 mg/ml (original wet weight).

Ligand binding experiments were conducted in assay tubes containing 0.5 ml of buffer, 1.4 nM [^{3}H]citalopram (New England Nuclear, Boston MA), and 1.5 mg midbrain tissue. The reaction was started with the addition of the tissue and the tubes were incubated for 60 min at 25ºC (room temperature). The incubation was terminated by rapid filtration through Whatman GF/B filters (presoaked in 0.3% polyethylenimine in water) using a Brandel Cell Harvester (Brandel Instruments Gaithersburg, MD). The filters were washed twice with 5 ml cold buffer, transferred to scintillation vials to which Beckman Ready Safe was added. Nonspecific binding was determined using 10 µM fluoxetine (RBI, Natick, MA). Data were analyzed using GraphPad Prism software (San Diego, California).

[^{3}H]Pirenzepine Binding Assay. Membranes from frozen rat brains excluding cerebellum were thawed in ice-cold buffer (10 mM Tris-HCl, 320 mM sucrose, pH 7.4) and homogenized with a Brinkman polytron in a volume of 10 ml/gm of tissue. The homogenate was centrifuged at 1,000 x *g* for 10 min at 4ºC. The resulting supernatant was then centrifuged at 10,000 x *g* for 20 min at 4ºC. The resulting pellet was resuspended in a volume of 200 mg/ml in 10 mM Tris buffer (pH 7.4).

Ligand binding assays were conducted in tubes containing 0.5 ml of buffer (10 mM Tris-HCl, 5 mM $MgCl_2$), 3 nM [^{3}H]pirenzepine (New England Nuclear, Boston, MA), and 20 mg of brain tissue. The reaction was started with the addition of the tissue and the tubes were incubated for 60 min in a 37º C water bath. The incubation was terminated by the addition of 5 ml of ice-cold buffer (10 mM Tris-HCl, pH 7.4) and rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in 0.5% polyethylenimine) using a Brandel Cell Harvester (Brandel Instruments, Gaithersburg, MD). The filters were washed twice with 5 ml cold buffer, and transferred to scintillation vials to which absolute ethanol and Beckman Ready Safe was added. Quinuclidinyl benzilate (QNB), 100 µM final concentration, was used to determine non-specific binding. Data were analyzed by using GraphPad Prism software (San Diego, CA).

Receptor Screen. Three of the compounds, AHN 2-005, GA-I-103, and JHW 007, were screened for their activity at various receptor sites by examining their competition with the

appropriate radioligands (ProfilingScreen® procured from MDS Panlabs Pharmacology Services, Bothell, Washington). The screen consisted of assays designed to assess the activity of the compounds at 31 mammalian receptors. Each compound was tested in duplicate in each assay at a concentration of 10 μM. If at this concentration there was greater than 50% displacement of specific binding of ligand, the test was repeated in duplicate at the original concentration and at 10-, 100-, and 1000-fold lower concentrations to obtain an approximation of affinity for the site. Concurrent vehicle and reference standards were conducted with each assay, and the sites listed in Table 1 were targeted. Significant details of the procedures are also provided in the table. For other details, see the MDS Panlabs catalogue (MDS Panlabs Pharmacology Services, 2000).

For sites at which activity was identified, IC_{50} values and inhibition constants (K_i) were computed from the displacement data using a non-linear, least squares regression analysis (GraphPad Prism software, San Diego, CA). The K_i values were calculated using the equation of Cheng and Prusoff (1973) using the obtained IC_{50} value of the tested compound, the concentration of radioligand employed in the assay, and the MDS Panlabs historical value for the K_d of the ligand. Because IC_{50} were determined from four concentrations of cold compound, the derived binding constants should be interpreted as estimates.

---Insert Table 1 about here ---

Locomotor Activity. Mice were placed one at a time in clear acrylic chambers (40 cm^3) for the assessment locomotor activity on a horizontal plane. The acrylic chambers fit within monitors (Omnitech Electronics, Columbus, OH) which were equipped with light sensitive detectors, spaced 2.5 cm apart along two perpendicular walls. Mounted on the opposing walls and directed at the detectors were infrared light sources. One activity count was registered each time the subject interrupted a single light beam. Mice were injected (i.p. in volumes of 1 ml/100 g) and immediately placed in the apparatus for 1 hr, with activity counts totaled each 10 min. Each drug dose was studied in 8 mice, and mice were used only once. In experiments on the time course of effects mice were injected, immediately placed in the apparatus, and data were collected for 8 hr. All other

aspects of these experiments were identical to those in which activity was assessed for 60 min.

Cocaine Discrimination. Rats weighing 320-350 g served as subjects. They were fed daily about 15 g of standard lab chow at least 30 min after testing that maintained them at their individual weights throughout the study. Subjects were tested daily in two-lever operant-conditioning chambers (Med Associates, Model ENV 007, St. Albans, VT, USA) that were housed within light- and sound-attenuating enclosures. White noise was present throughout testing to mask extraneous sounds. Ambient illumination was by a lamp in the top center of the front panel (houselight). Levers were set 17 cm apart, with pairs of lamps (light-emitting diodes, LEDs) above each of the levers, also on the front panel. A downward force on either lever of 0.4 N through about 1 mm was defined as a response, and produced an audible click. Reinforced responses dispensed one 45-mg pellet (BioServe, Frenchtown NJ, USA) into a food tray centered between the levers on the front panel of the chamber. On-line experimental control and data collection were by PC MS-DOS computers with Med Associates interfacing equipment and operating software (Med Associates, St. Albans, VT).

Subjects were initially trained to press both levers under a 20-response fixed-ratio (FR 20) schedule of food reinforcement and to discriminate IP injections of 29 μmol/kg cocaine (10 mg/kg) from IP injections of saline. After cocaine injection, responses on only one lever were reinforced; after saline injection, responses on the other lever were reinforced. The assignment of cocaine- and saline-appropriate levers was counterbalanced across rats. Immediately after injection, rats were placed inside the experimental chambers. A 5-min time-out period, during which the houselight and LEDs were extinguished and responding had no scheduled consequences preceded the illumination of the houselight and the LEDs. Only responses on the appropriate lever were reinforced, and responses on the inappropriate lever reset the FR response requirement. Each food presentation was followed by a 20-sec time-out period during which all lights were off, and responding had no scheduled consequences. Sessions ended after 20 food presentations or 15 min, whichever occurred first. Training sessions with cocaine (C) and saline (S) injections were conducted daily 5 days per

week, and ordered in a double alternation sequence [e.g. ...SCCS...].

Testing was initiated when performances reached criteria of at least 85% appropriate responding overall and during the first FR 20 of the session over four consecutive sessions. Tests were conducted with different doses of cocaine, doses of the novel compounds, or combinations of doses administered prior to sessions. Selected doses of the test compounds were administered at different times up to 120 min after injection in order to examine the timecourse of the discriminative-stimulus effects. After a test session, a subject was required to meet the above performance criteria over two consecutive (cocaine and saline) training sessions in order to be tested again. Repeated test sessions were conducted, with at least two training sessions between tests, until entire dose-effects were determined in each subject. Test sessions were identical to training sessions, with the exception that 20 consecutive responses on either lever were reinforced.

For each of the rats studied in the cocaine-discrimination procedure, the overall response rate and the percentage of responses occurring on the cocaine-appropriate lever were calculated. The mean values were calculated for each measure at each drug dose tested. If less than half of the rats responded at a particular dose, no mean value was calculated for percentage of cocaine-appropriate responding at that dose. At least 20% cocaine-appropriate responding was adopted as a conservative criterion at which to assume a significant difference from saline; 80% or higher cocaine-appropriate responding was taken as similar to the training dose of cocaine, and intermediate levels of cocaine-appropriate responding were considered partial substitution.

Data Analyses. Each dose-effect curve was analyzed using standard ANOVA and linear regression techniques. Locomotor activity in mice was assessed with counts collected during each successive ten-min epoch; counts during the first and last 3 epochs of the 1-hr assessments were cumulated for separate analyses of the first and last 30 min. Effects of individual doses were determined significant by analysis of variance (ANOVA) and subsequent planned comparisons (Stevens, 1990). Results of cocaine discrimination studies were assessed with data collected during the entire session which lasted a maximum of 15 min. In general, the effects obtained in the first

and second 30-min periods were comparable though there were some quantitative differences. Therefore only the analyses of data from the 30-min period in which maximal stimulation was obtained are described. When locomotor stimulant effects were obtained, maximal effects were in the first 30 min after injection, and those data are shown in the figure.

Half-maximum stimulation of locomotor activity was calculated by adding the number of horizontal locomotor activity counts at the dose that produced the largest increase in activity to the number of counts after vehicle injection, and the sum was divided by two. The dose that produced this half maximal stimulation (ED_{50} value) was determined by linear regression. For cocaine discrimination, the dose producing a half maximal effect (50% cocaine-appropriate responding) was calculated. For these analyses, points on the linear part of the ascending portions of the dose-effect curves were used (Snedecor and Cochran, 1967). Pairs of ED_{50} values were considered to be significantly different if their 95% confidence limits did not overlap. In order to assess the degree of change in the cocaine dose-effect curve produced by co-administration of the BZT analogues, data were also analyzed by standard parallel-line bioassay techniques as described by Finney (1964). This analysis consists of a one-way ANOVA which determines whether the slopes of the two dose-response curves are significantly different from parallel, and fits a common slope to the two dose-response curves. It then compares the ratio of doses for a 50% effect to provide a value for relative potency as a measure of the degree of shift in the cocaine dose-effect curve. The relative potency value represents the dose of cocaine, in subjects co-administered one of the BZT analogues, equal to 1 μmol/kg of cocaine alone (i.e. a relative potency value of 0.5 indicates a 2-fold shift to the left of the cocaine dose-effect curve in the presence of the BZT analogue). A significant shift in the cocaine dose-effect curve is indicated when the 95% confidence limits for the relative potency ratio do not include the value 1.0. Differences in the effectiveness of selected pairs of drugs were assessed by comparing maximal effects with a Student's *t* test.

Drugs. The drugs studied were: (-)-cocaine HCl (Sigma, St. Louis, MO), and N-substituted analogues of 3α-diphenylmethoxytropane analogues. The synthesis of these analogues was

conducted in the Medicinal Chemistry Section of the NIDA Intramural Research Program, and has been described previously (Agoston et al., 1997). Substitutions examined in the present study were exclusively on the nitrogen and are shown in Figure 1. All drugs were dissolved in 0.9% NaCl or water, with heat and sonication, as necessary. The drugs were administered IP on the basis of body weight at 1 ml/kg (rats) or 10 ml/kg (mice).

--- Insert Figure 1 about here ---

RESULTS

Radioligand Binding Assays. The K_i values determined for the BZT analogues at norepinephrine and serotonin transporters ranged from 457 to 4810 and 376 to 3260 nM, respectively (Table 2). These values were considerably higher than those previously determined (Agoston et al., 1997) for displacement of [^{3}H]WIN 35,428 from the dopamine transporter, which ranged from 8.5 to 108 nM (Table 2). Consistent with the Agoston et al. results, N-substitutions for CH_3 decreased binding affinity at M_1 muscarinic receptors: the 12 nM affinity of the parent compound, AHN 1-055, was reduced to values ranging from 177 to 1030 nM (Table 1). In general, the N-substituted compounds retained selectivity for the dopamine transporter over the other monoamine transporters, and had increased selectivity for the dopamine transporter over muscarinic sites.

--- Insert Table 2 about here ---

The sites at which 10 μM of AHN 2-005, GA-I-103, or JHW 007 displaced ligand to less than 50% specific binding are shown in Table 3, along with approximate K_i values. Among the 31 sites evaluated, all three compounds had activity in the less than 10 nM range at central histamine H_1 receptors (K_i values ranged from 2.77 to 5.97 nM). Two of the compounds also had low nM affinity at sigma binding sites, with affinity at this site reduced for AHN 2-005 compared to the other compounds. Affinity in the range of 0.01 to 0.1 μM was obtained with two of the three compounds at α_1 adrenergic and dopamine D_{2L} receptors. In general, activity at the remaining sites was within the 0.1 to 10 μM range. Occasional lower values were obtained for one of the three compounds (e.g. 5-HT_2 and M_2 sites, Table 3).

--- Insert Table 3 about here ---

Locomotor Activity. Cocaine, as has been demonstrated previously, increased ambulatory activity with a maximum of 580 counts per min during the first 30 min of the session at 59 μmol/kg (Figure 2, filled circles; Table 4), which appeared to be the maximum stimulation as a higher dose had a reduced effect (Figure 2). In addition, AHN 1-055 at a dose of 25.4 μmol/kg also produced a

significant stimulation of activity (Figure 2, diamonds), though the maximal stimulation of 403 counts per min was significantly less ($t_{22} = 5.676$, $P < 0.0001$) than that produced by cocaine (Table 4). In contrast, none of the other 4',4"-diF- BZT analogues produced significant stimulation of activity that was greater than that observed after vehicle injection (Figure 2). Each of the drugs was examined at doses ranging from those having no effects on activity to those that decreased locomotor activity. Each of the compounds was active across a comparable range of doses, with the exceptions of GA-I-103 and JHW 005 which minimally decreased activity, and did so to a degree that was not statistically significant.

--- Insert Figure 2 and Table 4 about here ---

Cocaine Discrimination. As has been shown previously, there was a dose-related increase in the percentage of cocaine-appropriate responses in subjects trained to discriminate cocaine (29 μmol/kg) from saline (Figure 3, panels A-F, filled symbols). In addition, the phenyltropane analogue of cocaine, WIN 35,428 also fully substituted for cocaine (Figure 3, panel A, open circles). Several of the other N-substituted BZT analogues (AHN 1-055, AHN 2-003, AHN 2-005, JHW 005) also produced a level of substitution greater than that produced by saline, though none of these compounds fully substituted for cocaine (Figure 3, panel A, triangles, and panels B, C, and D, respectively). In contrast, JHW 007, GA-I-103, and JHW 025 did not produce substitution significantly greater than saline levels (Figure 3, panels E and F). The maximal substitution produced by any of the BZT analogues was 54% at 15.3 μmol/kg of AHN 2-003 (Table 5). Each of the compounds that did not fully substitute for cocaine was examined from doses having no effects to those producing pronounced decreases in response rates (Figure 3, panels G through L). An exception to this was JHW 025, a quaternary derivative, which was studied at doses as high as possible with restrictions imposed by its solubility.

--- Insert Figure 3 and Table 5 about here ---

The lack of full substitution for cocaine produced by the BZT analogues does not depend on the measure of substitution used. Other measures that have been used previously to assess the

substitution of the test drug for the training drug were also examined. These alternate measures were: 1) the percentage of responses on the cocaine-appropriate lever before the first reinforcer (Table 6, column B), 2) the percentage of subjects selecting the cocaine-appropriate lever (Table 6, column C), and 3) the percentage of subjects selecting the cocaine-appropriate lever on the first trial of the session (Table 6, column D). There was a very close correspondence among all of these alternative measures of maximal effect with the percentage of responses on the cocaine-appropriate lever tabulated over the entire session (Table 6, column A). The r^2 values for the correlations among all four measures of maximal effect were all above 0.980. All four of these measures indicated that, other than the N-methyl substituted analogue, none of the compounds had maximal effects comparable to those of cocaine or its analogue, WIN 35,428.

--- Insert Table 6 about here ---

Increasing the time between injection and testing did not significantly change the efficacy of AHN 2-003 and AHN 2-005, the two drugs for which the time courses of effects were assessed (Figure 4). With AHN 2-003 there was a trend towards an increase in the efficacy in producing cocaine-like discriminative stimulus effects, however that effect was not significant ($F_{3,43}$=2.642, p=0.061). Additionally, over the same time there was a trend towards a decrease in the efficacy of AHN 2-003 in reducing response rates ($F_{3,48}$=2.640, p=0.060; Figure 4). Similarly, with AHN 2-005 there were no significant changes in efficacy in producing cocaine-like discriminative stimulus effects ($F_{4,113}$=0.417, p=0.796) over the course of 120 min. As with AHN 2-003, there was a trend towards a reduction in the effects of the drug on response rates ($F_{4,116}$=2.431, p=0.051; Figure 4).

--- Insert Figure 4 about here ---

Drug Interactions. In general, treatment with the BZT analogues did not produce significant changes in the effects of cocaine, whereas WIN 35,428 produced a significant leftward shift in the cocaine dose-effect curve (Figure 5). The change in the effects of cocaine produced by WIN 35,428 was significant; the relative potency estimate (Table 7) of 574 had 95% CL values exclusive of 1.0 at a WIN 35,428 dose of 1.0 mg/kg. In contrast to the effects obtained with WIN

35,428, among the N-substituted analogues of BZT only AHN 1-055 (at 14.2 μmol/kg) and JHW 005 (at 18.7 μmol/kg) produced significant changes in the discriminative-stimulus dose effects of cocaine (Table 7 and Figure 5).

Table 7 shows the ED_{50} values for cocaine, either alone or in combination with the BZT analogues. Relative potency estimates with 95% CLs are also shown. The cocaine ED_{50} value was not significantly changed by the co-administration of any of the BZT analogs (note that 95% CLs overlapped). In addition, the relative potency estimates generally showed no significant change induced by administering a BZT analog with cocaine (95% CLs include the value 1.0). The exceptions were AHN 1-055 and JHW 005 which at the highest doses studied produced an approximate 2-fold leftward shift in the cocaine dose-effect curve for the discriminative-stimulus effects of cocaine. As indicated in Figure 5, AHN 2-005 produced a trend towards a shift to the left in the cocaine dose effect curve, however, this trend did not approach statistical significance as indicated by the relative potency value (Table 7). For JHW 007 and AHN 2-005 the highest dose studied in combination with cocaine produced an increased substitution when these drugs were examined in combination with the lowest dose (2.9 μmol/kg) of cocaine. This change resulted in a decrease in the slopes of the dose-effect curves, and a lack of significance of the linear regression (Table 7).

--- Insert Figure 5 and Table 7 about here ---

To ensure a comparability of doses of BZT analogues studied in combination with cocaine, the doses that were studied were selected based on their potency for decreasing response rates (Figure 3, panels G through L). Each compound was examined in combination with cocaine at doses that bracketed either their ED_{50} values, or the minimally effective dose, for decreasing response rates. The pharmacological equivalence of the doses examined in combination with cocaine can be seen in the lower panels of Figure 5. Each of the drugs produced a decrease in the slope of the dose effects of cocaine on response rates, as well as a generalized decrease in the rates of responding across the range of cocaine doses.

DISCUSSION

In the present study, the pharmacology of a selected series of 4',4"-diF, N-substituted analogues of BZT was evaluated. Each of these compounds had relatively high affinity for the dopamine transporter (Agoston et al., 1997). In addition to this action, the compounds were relatively selective among the monoamine transporters, having affinities at the norepinephrine and serotonin transporters that were relatively low compared to those for the dopamine transporter. The present results are consistent with previous studies that have demonstrated that N-methyl analogues of BZT can have high affinity and selectivity for the dopamine transporter compared to the norepinephrine and serotonin transporters (Newman et al., 1995). Among the previously studied N-methyl-BZT analogues was the parent compound of the present series, AHN 1-055, which was previously reported to have relatively high affinity for M_1 muscarinic receptors (Newman et al., 1995; Katz et al., 1999). In contrast, the present N-substituted BZT analogues have reduced affinity for M_1 receptors. For example, AHN 2-005 had approximately 100-fold lower affinity for M_1 muscarinic receptors and a four-fold higher affinity for the dopamine transporter than does the parent compound, BZT. Comparably increased selectivities were obtained with the other N-substituted analogues.

Activity at the dopamine transporter is generally thought to be sufficient for cocaine-like behavioral effects, and consequently actions at the dopamine transporter should result in compounds having cocaine-like behavioral effects. For example, phenyltropane analogues of cocaine generally have high affinity for the dopamine transporter, inhibit dopamine uptake *in vitro*, and produce cocaine-like behavioral effects, often with significantly greater potency than cocaine (Spealman et al., 1977, 1979; Fleckenstein et al., 1996, Carroll et al., 1995). Moreover, cocaine-like activity typically is produced by cocaine analogues with relatively high selectivity for the dopamine transporter (Kimmel et al., 2001). Thus, the selectivity of the BZT analogues for the dopamine transporter compared to the other monoamine transporters, would not by itself be expected to produce behavioral effects different from those of cocaine. The original observation that N-methyl

analogues of BZT were generally not as effective as other dopamine uptake inhibitors in producing cocaine-like behavioral effects was surprising (Newman et al., 1995), and has led to investigations into the mechanisms for the differences (Katz et al., 1999).

One explanation for the differences in behavioral effects between BZT analogues and cocaine-like stimulants is that other actions of the BZT analogues may interfere with the expression of cocaine-like effects. The high affinity for muscarinic M_1 receptors of the N-methyl analogues (Katz et al., 1999; Newman et al., 1995) was the focus of previous studies. In general, anticholinergics, such as atropine and scopolamine, have generally been reported to potentiate the behavioral effects of CNS stimulants (e.g. Carlton and Didamo, 1961; Scheckel and Boff, 1964). This type of interaction was also found for cocaine and either atropine or scopolamine using behavioral procedures identical to those used in the present study (Katz et al., 1999). Those results suggested it unlikely that antimuscarinic effects of BZT analogues could interfere with their effectiveness in producing the presently examined cocaine-like effects.

In the present study, N-substituted BZT analogues were examined for which modifications in structure resulted in a relatively low affinity for muscarinic M_1 receptors compared to BZT. These compounds retained their relatively high affinity and selectivity for the dopamine transporter, and were used to further examine the potential of M_1 activity to influence the behavioral effects of BZT analogues. Despite a relatively decreased muscarinic affinity, the N-substituted BZT analogues did not produce cocaine-like locomotor stimulant or discriminative-stimulus effects. These results are consistent with those reported previously (Katz et al., 1999; Katz et al., 2001) for the N-methyl analogues of BZT which had relatively high affinity for M_1 muscarinic receptors. AHN 1-055, which has 12 nM affinity for M_1 receptors, as in the previous study (Katz et al., 1999), produced some stimulation of locomotor activity and substituted partially (79%) for cocaine, effects that approached those of cocaine.

In a previous study AHN 1-055 fully substituted for cocaine when administered 90 min before testing its cocaine-like discriminative-stimulus effects. Along those lines, recent

pharmacokinetic studies (Raje et al., 2003) have indicated that the elimination of several BZT analogues (including AHN 1-055, AHN 2-003, AHN 2-005, JHW 007) from plasma and brain is substantially slower than that for cocaine. Thus, it is possible that providing greater amounts of time for uptake and distribution may have rendered the present compounds more effective in producing cocaine-like discriminative stimulus effects. In the present study, AHN 2-003 and AHN 2-005 were examined at time points up to 2 hours after injection without a significant increase in their cocaine-like discriminative-stimulus effects. In addition, we generally examined a range of doses of the compounds, from those having no effects to those virtually eliminating response rates. The decreases in response rates suggest that doses examined were more than adequate for a centrally mediated effect. The study by Raje et al. (2003) demonstrated that AHN 1-055, AHN 2-003, AHN 2-005, and JHW 007 readily and rapidly crossed the blood-brain barrier, having brain to plasma ratios either equal to, or greater than two-fold higher than that of cocaine. Taken together, the present studies on time course and the pharmacokinetic studies indicate that, while these compounds have a long duration of action, their uptake and distribution in brain is sufficient to rule out a limited uptake and distribution of the drugs as an explanation for their lack of substitution for cocaine.

It is currently not clear what differences in the pharmacology of AHN 1-055 and the N-substituted compounds account for their differences in behavioral effects. However, one possibility which is consistent with the literature (e.g. Carlton and Didamo, 1961; Scheckel and Boff, 1964) is that the antimuscarinic effects, rather than interfering with, contribute to the cocaine-like pharmacology of the BZT analogues with effects most closely resembling those of cocaine (see also Katz et al., 2001). Consistent with this hypothesis, the N-substituted BZT analogues that had reduced M_1 receptor affinity were uniformly less active as stimulant-like drugs than cocaine, its phenyltropane analogue, WIN 35,428, and AHN 1-055. However, BZT itself has high affinity for M_1 receptors, yet was relatively inactive as a cocaine-like agent (Katz et al., 1999). In contrast to the other compounds, BZT has relatively low affinity for the dopamine transporter. It seems reasonable to suggest that some minimal activity at the dopamine transporter is necessary for the potentiation of

cocaine-like effects by antimuscarinic activity.

A recent study of cocaine self administration in rhesus monkeys was designed to address the role of antimuscarinic actions in the reinforcing effects of BZT analogues. In that study, the addition of the anticholinergic, scopolamine, to the cocaine solution being self administered decreased the effectiveness of cocaine, and did not shift the cocaine dose-effect curve leftward (Ranaldi and Woolverton, 2002), differentiating reinforcing effects from other effects of cocaine. The lack of a potentiation of the reinforcing effects of cocaine by scopolamine suggests further that the anticholinergic component of action will not potentiate whatever reinforcing effects are exhibited by BZT analogues. As such, that finding is consistent with previous findings indicating a lesser effectiveness of BZT analogues in self administration compared to cocaine (Woolverton et al., 2000, 2001). Ranaldi and Woolverton further speculated that the decreased effectiveness of scopolamine-cocaine combinations compared to cocaine alone, could be due to an antagonism of the reinforcing effects of cocaine by scopolamine or an added punishment of cocaine-maintained behavior by the response-dependent administration of scopolamine. In a previous study (Wilson and Schuster, 1973) the effects of the anticholinergic, atropine, were examined in rhesus monkeys self administering cocaine. Atropine was administered before experimental sessions independently of responding, and therefore could not function as a punishing stimulus. In that study, the effects of atropine were similar to the effects of lowering the injected dose of cocaine. Thus, the most parsimonious interpretation of the results together suggests a physiological antagonism of the reinforcing effects of cocaine by the anticholinergic agents. However, as Ranaldi and Woolverton note, a more thorough study of alternative hypotheses is in order. Certainly, studies of self administration of the present N-substituted BZT analogues which have reduced affinity for muscarinic receptors, will add important information in assessing the role of antimuscarinic actions in the reinforcing effects of BZT analogues. Those studies are currently ongoing.

Activity at sites other than the monoamine transporter and muscarinic M_1 receptors was investigated for several of the compounds. Among the sites evaluated, activity in the less than 10

nM range was demonstrated for central histamine H_1 receptors. This activity was not surprising as the parent compound, BZT, has antihistaminic activity (Richelson, 1981). Nonetheless, it is possible that actions at histamine H_1 receptors contributed to the present effects, and the influence of activity at histamine H_1, H_2, and H_3 receptors on the effects of cocaine has been examined (Campbell et al., 2002). In general, antagonists at each of these receptors were inactive in significantly modifying the dose-effect curves for the discriminative-stimulus effects of cocaine. In addition, a large number of BZT analogues were assessed for their affinities at each of these histamine receptors and compared to their affinities for the dopamine transporter. Those compounds with higher affinity for the dopamine transporter than histamine receptors were no more likely to produce increases in locomotor activity than were compounds for which the affinity at a histamine receptor was greater than its affinity at the dopamine transporter. From these results together it appears unlikely that antihistaminic effects of BZT analogues could interfere with their effectiveness in producing the presently examined cocaine-like effects.

Two of the compounds (JHW 007 and GA-I-103) also had low nM affinity at sigma binding sites, which was greater than their affinity for the dopamine transporter. The other compound, AHN 2-005, had lower sigma receptor affinity than dopamine transporter affinity. Actions at sigma receptors have been noted previously to influence the behavioral effects of cocaine. For example, Menkel et al. (1991) found that relatively low doses of the sigma antagonists, rimcazole and BMY 14802, could block the locomotor stimulant effects of cocaine. In addition, other sigma antagonists have been demonstrated to alter locomotor stimulant and acute toxic effects of cocaine (e.g. Matsumoto et al., 2001). Moreover, subjects treated with sigma1 receptor antisense oligodeoxynucleotides show diminished effects of cocaine (Romieu et al., 2000). Using the present procedures, several analogs of the sigma antagonist, rimcazole, were found to diminish the behavioral effects of cocaine, though the effects were most pronounced in only one of the compounds (Katz et al, 2003). Taken together these results indicate that the possibility that the reduced cocaine-like effectiveness of the present N-substituted BZT analogs is due to their actions at

sigma receptors cannot be discounted and deserves further study.

Because of the reduced effectiveness among the present BZT analogues compared to cocaine, it was of interest to examine the interactions of these compounds with cocaine. Previous studies of interactions have found that BZT analogues shift the cocaine dose-effect curve for discriminative-stimulus effects to the left (Tolliver *et al.,* 1999; Katz et al., 2001). That leftward shift was not expected for compounds that bind to the same site as cocaine, but have reduced effectiveness. In the present study, WIN 35,428 produced a pronounced leftward shift in the cocaine dose-effect curve similar to those produced by other dopamine transport inhibitors (e.g. Holtzman, 2001; Katz et al., 2003). In contrast, the BZT analogues examined in this study failed to produce large or significant shifts to the left in the cocaine dose-effect curve. In order to ensure an assessment of doses that would be behaviorally active, the doses of BZT analogues that were studied in combination with cocaine were selected for the pharmacological equivalence of their effects on response rates. Thus, the present N-substituted analogues of BZT appeared to be qualitatively different from other dopamine uptake inhibitors as indicated by their lack of substitution for cocaine and a lack of potentiation of the behavioral effects of cocaine.

The lack of a significant shift leftward in the cocaine dose-effect curve in the present study with N-substituted BZT analogues distinguishes these compounds from other BZT analogues. As mentioned above, previous studies (Tolliver *et al.,* 1999; Katz *et al.*, 2001) have shown that BZT analogues shift the cocaine dose-effect curve leftward. It is possible that the antimuscarinic effects of the previously studied compounds contributed significantly to the leftward shift in the cocaine dose-effect curve, as was found with atropine and scopolamine (Katz *et al*., 1999). The general absence of a significant alteration in the effects of cocaine by the present N-substituted BZT analogues is being further investigated.

By analogy to treatments for heroin dependence, such as methadone and buprenorphine, the present results suggest that analogues of BZT may be useful as treatments for cocaine abuse, in situations in which an agonist treatment is indicated. Moreover, these compounds may possess a

preclinical profile indicative of therapeutic advantage over other dopamine uptake inhibitors in that they have significantly reduced effectiveness as stimulants compared to other ligands for the dopamine transporter. In addition, as mentioned above previous studies have indicated that BZT analogues are less effective in maintaining self administration than are either cocaine or another dopamine uptake inhibitor, GBR 12909 (Woolverton et al., 2000; 2001). These findings taken together suggest that BZT analogues may have potential as leads in the development of medications to treat cocaine abuse, and that these drugs may have reduced liability for their abuse. The reduced affinity for muscarinic M_1 receptors, as well as the sites examined in the broad screen of activity that was obtained with the present N-substituted analogues of BZT, suggests further that these particular compounds may be efficacious with a reduced liability for side effects.

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FOOTNOTES

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FIGURE CAPTIONS

Figure 1: Basic structures of cocaine and BZT analogues with substitutions on the nitrogen.

Figure 2: Dose-dependent effects of N-substituted BZT analogues on locomotor activity in mice. Ordinates: horizontal locomotor activity counts after drug administration in counts per sec. Abscissae: dose of drug in μmol/kg, log scale. Each point represents the average effect determined in 8 mice. The data are from the 30-min period immediately after drug administration. Vehicle values averaged 247 (±16.2) counts/min. Note that none of the BZT analogues produced a stimulation of activity that was equivalent to that of cocaine, and that among these only the AHN 1-055 significantly stimulated activity.

Figure 3: Effects of N-substituted BZT analogues in rats trained to discriminate injections of cocaine from saline. Ordinates for top panels: percentage of responses on the cocaine-appropriate key. Ordinates for the bottom panels: rates at which responses were emitted (as a percentage of response rate after saline administration). Abscissae: drug dose in μmol/kg (log scale). Each point represents the effect in 4 to 16 rats. Filled circles in each panel of the figure represent the effects of cocaine, replotted for reference. The percentage of responses emitted on the cocaine-appropriate key was considered unreliable, and not plotted, if fewer than half of the subjects responded at that dose. Note that only the cocaine analogue, WIN 35,428, fully substituted for cocaine, and among the N-substituted BZT analogues only AHN 1-055 approached the effects of cocaine.

Figure 4: Effects of N-substituted BZT analogues in rats trained to discriminate injections of cocaine from saline at various times after injection. Ordinates for top panels: percentage of responses on the cocaine-appropriate key. Ordinates for the bottom panels: rates at which responses were emitted (as a percentage of response rate after saline administration). Abscissae: drug dose in μmol/kg (log

scale). Each point represents the effect in 4 to 6 rats. Filled circles in each panel of the figure represent the effects of cocaine, redetermined during the course of assessing the effects of pretreatment time. Note that with pretreatments of up to 2 hr the effects of the drugs in substituting for cocaine were not significantly increased, and the effects on response rates tended to be diminished though this effect was not statistically significant.

Figure 5: Changes in the cocaine dose-effect curve for discriminative stimulus effects produced by pretreatments with WIN 35,428 or the N-substituted BZT analogues. Ordinates: percentage of responses on the cocaine-appropriate key. Abscissae: cocaine dose in μmol/kg (log scale). Each point represents the effect in 4 to 6 rats. The percentage of responses emitted on the cocaine-appropriate key was considered unreliable, and not plotted, if fewer than half of the subjects responded at that dose. Note that WIN 35,428 shifted the cocaine dose-effect curve to the left, whereas the BZT analogues were either inactive or only marginally shifted the cocaine dose-effect curve to the left.

Table 1: Assay Conditions for Activity at Various Receptor Sites by Examining Competition with the Appropriate Radioligands (ProfilingScreen®, MDS Panlabs Pharmacology Services, Bothell, Washington)

Assay Target	**Ligand**	**Non-Specific Binding**	**Tissue**	**Incubation**
Adenosine A_1	1 nM [^{3}H]DPCPX	10 μM PIA	Human recombinant CHO cells	90 min @ 25°C
Adenosine A_{2A}	0.05 μM [^{3}H]CGS-21680	10 μM NECA	Human recombinant HEK-293 cells	90 mm. @ 25°C
Adrenergic α_1, non-selective	0.25 nM [^{3}H]prazosin	0.1 μM prazosin	Rat brain	30 min @ 25°C
Adrenergic α_2, non-selective	0.7 nM [^{3}H]rauwolscine	1 μM yohimbine	Rat cortex	30 min @ 25°C
Adrenergic β_1	0.03 nM [^{125}I]-cyanopindolol	100 μM (s)-propranolol	Human recombinant CHO cells	120 min @ 22°C
Adrenergic β_2	0.2 nM [^{3}H]CGP-12177	10 μM ICI-118551	Human recombinant CHO cells	60 min @ 22°C
Ca^{++} Channel-L, dihydropyridine	0.1 nM [^{3}H]nitrendipine	1 μM nifedipine	Rat cortex	90 min @ 25°C
Dopamine D_1	1.4 nM [^{3}H]SCH 23390	10 μM (+)-butaclamol	Human recombinant CHO cells	120 min @ 37°C
Dopamine D_{2L}	2 nM [^{3}H]spiperone	10 μM haloperidol	Human recombinant CHO cells	120 min @ 25°C
Estrogen	0.5 nM [^{3}H]estradiol	5.8 μM diethylstilbestrol	Human recombinant sf9 cells	16 hr @ 4°C
$GABA_A$, Agonist Site	1 nM [^{3}H]muscimol	100 nM muscimol	Rat brain	10 min @ 0°C
$GABA_A$, Chloride Channel	3 nM [^{3}H]TBOB	200 μM picrotoxin	Rat cortex	15 min @ 22°C
Glucocorticoid	0.02 μM [^{3}H]dexamethasone	20 μM dexamethasone	Human Jurkat cells	120 min @ 25°C
Glutamate, NMDA, phencyclidine	4 nM [^{3}H]TCP	1 μM MK801	Rat cortex	45 min @ 25°C
Glutamate, non-selective	1.6 nM [^{3}H]L-glutamate	50 μM L-glutamate	Rat brain	10 min @ 37°C
Glycine, strychnine-sensitive	2 nM [^{3}H]strychnine	1 mM glycine	Rat spinal cord	10 min @ 4°C
Histamine H_1, central	3 nM [^{3}H]pyrilamine	1 μM pyrilamine	Guinea pig brain	60 min @ 25°C
Insulin	0.03 nM [^{125}I]insulin	1 μM insulin	Rat liver	l6 hr @ 4°C

Muscarinic M_2	0.29 nM [^{3}H]N-methylscopolamine	1 μM atropine	Human recombinant sf9 cells	60 min @ 27°C
Muscarinic M_3	0.29 nM [^{3}H]N-methylscopolamine	1 μM atropine	Human recombinant sf9 cells	60 min @ 27°C
Opiate-δ	0.9 nM [^{3}H]DPDPE	1 μM naltrindole	Human recombinant CHO cells	60 min @ 25°C
Opiate-κ	0.6 nM [^{3}H]U-69593	1 μM U-50488	Human recombinant CHO-K1 cells	120 min @ 25°C
Opiate-μ	0.6 nM [^{3}H]diprenorphine 2 nM [^{3}H]DAMGO*	0.5 μM DAMGO	Human recombinant CHO-K1 cells Guinea pig brain*	60 min @ 25°C 120 min @ 25°C*
Phorbol ester	3 nM [^{3}H]PDBu	1 μM PDBu	Mouse brain	60 min @ 37°C
K+ Channel [K_{ATP}]	5 nM [^{3}H]glyburide	1 μM glyburide	Hamster pancreatic β cells HIT-T15	120 min @ 22°C
Progesterone	2 nM [^{3}H]R-5020	410 nM R-5020	Calf uterus	16 hr @ 4°C
Serotonin 5-HT_1	2 nM [^{3}H]5-HT	10 μM 5-HT	Rat cortex	10 min @ 37°C
Serotonin 5-HT_2	0.5 nM [^{3}H]ketanserin	1 μM ketanserin	Rat brain	40 min @ 25°C
Sigma, non-selective	0.8 nM [^{3}H]DTG	10 μM (+)3-PPP	Guinea pig brain	30 min @ 25°C
Na+ Channel, site 2	1.5 nM [^{3}H]batrachotoxin	100 μM veratridine	Rat brain	30 min @ 37°C
Testosterone	2 nM [^{3}H]mibolerone	2 μM mibolerone	Rat ventral prostate	18 hr @ 4°C

*These parameters were used for JHW 007 only.

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TABLE 2: Potencies of N-Substituted BZT Analogues in Binding to the Dopamine, Norepinephrine, and Serotonin Transporters, and M_1 Muscarinic Receptors (K_i Values and SEM)

Compound	R-Substitution	DAT K_i Value (nM)[a]	M_1 K_i Value (nM)[a]	NET K_i Value (nM)	SERT K_i Value (nM)
Cocaine	--	187 (18.7)	61400 (10900)	3210 (149)	293 (30.0)
BZT	--	118 (10.6)	2.10 (0.294)	1390 (134)	31600 (5160)
AHN 1-055	CH_3	11.8 (1.30)	11.6 (0.930)	610 (80.5)	3260 (108)
AHN 2-003	H	11.2 (1.23)	203 (16.5)	457 (69.8)	922 (87.2)
AHN 2-005	allyl	29.9 (0.299)	177 (21.1)	1740 (242)	2850 (62.5)
JHW 007	butyl	24.6 (1.97)	399 (28.3)	1670 (232)	1350 (151)
JHW 005	benzyl	82.2 (12.3)	1030 (150)	4810 (657)	2090 (125)
GA-I-103	butylphenyl	8.51 (0.766)	575 (10.7)	2210 (239)	376 (51.8)
JHW 025	$(CH_3)_2{}^+I^-$	108 (13.0)	11.8 (0.587)	3350 (534)	1220 (166)

[a]Some of these values have been reported previously (Agoston et al., 1999; Newman et al., 1995; Katz et al., 1999).

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Table 3: Estimated K_i Values (in nM) of Selected N-Substituted BZT Analogues in Binding to Sites Examined in the Receptor Screen

Assay	**AHN 2-005**	**GA-I-103**	**JHW 007**
Adenosine A_1	>10,000	1660	>10,000
Adenosine A_{2A}	>10,000	>10,000	>10,000
Adrenergic α_1, non-selective	88.9	19.3	>10,000
Adrenergic α_2, non-selective	849	1910	1540
Adrenergic β_1	>10,000	1910	>10,000
Adrenergic β_2	>10,000	>10,000	>10,000
Ca^{++} Channel-L, dihydropyridine	1070	216	874
Dopamine D_1	230	334	327
Dopamine D_{2L}	234	20.5	47.1
Estrogen	>10,000	>10,000	>10,000
$GABA_A$, Agonist Site	>10,000	>10,000	>10,000
$GABA_A$, Chloride Channel	>10,000	>10,000	>10,000
Glucocorticoid	>10,000	>10,000	>10,000
Glutamate, NMDA, phencyclidine	>10,000	>10,000	>10,000
Glutamate, non-selective	>10,000	>10,000	>10,000
Glycine, strychnine-sensitive	>10,000	>10,000	2600
Histamine H_1, central	2.78	5.97	2.77
Insulin	>10,000	>10,000	>10,000
Muscarinic M_2	291	70.5	351
Muscarinic M_3	213	203	6940
Opiate-δ	>10,000	2680	>10,000
Opiate-κ	1940	1050	>10,000
Opiate-μ	2110	377	1140
Phorbol ester	>10,000	>10,000	>10,000
K+ Channel [K_{ATP}]	>10,000	>10,000	>10,000
Progesterone	>10,000	>10,000	>10,000
Serotonin 5-HT_1	>10,000	>10,000	>10,000
Serotonin 5-HT_2	30.9	108	104
Sigma, non-selective	76.5	5.94	2.15

Na+ Channel, site 2	504	41.4	123
Testosterone	>10,000	>10,000	>10,000

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Table 4: Comparisons of Potencies and Maximal Stimulant Effects on Locomotor Activity of N-Substituted BZT Analogues

	ED_{50} Value[a] (μmol/kg)	Maximal Stimulation (Counts/min)
Cocaine	*5.23* *(1.37 - 9.13)*	580 (±19.8) @ 58.8 μmol/kg
AHN 1-055	*7.78* *(4.86 - 12.4)*	402.7 (±19.2) @ 25.4 μmol/kg
AHN 2-003	45.4 (34.4 - 65.9)	No significant stimulant effects
AHN 2-005	73.4 (54.4 - 98.5)	No significant stimulant effects
JHW 007	Nonsignificant linear regression[b]	No significant stimulant effects
JHW 005	91.1 (31.6 - 17900)	No significant stimulant effects
GA-I-103	216 (117 - 660)	No significant stimulant effects

[a]Values in italics are for stimulant effects; all other values are for decreases in locomotor activity

[b]A nonsignificant linear regression precluded calculation of an ED_{50} value.

Table 5: Comparisons of Potencies and Maximal Effects of N-Substituted BZT Analogues in Substituting for Cocaine and Affecting Rates of Responding

Drug	% Cocaine		Response Rates
	ED_{50} Value (μmol/kg) & 95% CL	Maximum %	ED_{50} Value (μmol/kg) & 95% CL
Cocaine	9.68 (6.62 - 13.9)	98.0 ± 1.72 @ 10 mg/kg	Nonsignificant linear regression
WIN 35,428	1.13 (0.772 - 1.55)	100 ± 0 @ 3 mg/kg	Nonsignificant linear regression
AHN 1-055	12.3 (8.32 - 20.9)	78.8 ± 19.7 @ 17 mg/kg	15.6 (9.64 - 30.3)
AHN 2-003	19.9 (8.97 - 2481)	54.1 ± 20.4 @ 5.6 mg/kg	6.37 ($4.4x10^{-4}$ - 12.4)
JHW 007	No Substitution	8.22 ± 5.70 @ 3.0 mg/kg	Nonsignificant linear regression
AHN 2-005	7.59 (1.95 - 365)	42.3 ± 16.8 @ 10 mg/kg	19.4 (9.71 - 761)
JHW 005	12.2 (3.21 - $1.10x10^{5}$)	37.6 ± 23.9 @ 10 mg/kg	8.91 (4.95 - 22.6)
GA-I-103	No Substitution	10.1 ± 6.52 @ 5.6 mg/kg	6.58 (3.62 - 19.0)
JHW 025	No Substitution	7.43 ± 7.19 @ 5.6 mg/kg	Nonsignificant linear regression

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TABLE 6: Efficacy of N-Substituted BZT Analogues in Substituting for Cocaine in Rats: Comparisons of Different Methods of Assessing Efficacy

Compound	**A** % Cocaine Responses; Entire Session	**B** % Cocaine Responses; First Trial	**C** % Subjects Selecting Cocaine; Entire Session[a]	**D** % Subjects Selecting Cocaine; First Trial[a]
Cocaine	98.0 ± 1.72 @ 10 mg/kg	95.2 ± 4.85 @ 10 mg/kg	100 [8] @ 10 mg/kg	87.5 [8] @ 10 mg/kg
WIN 35,428	100 ± 0 @ 3 mg/kg	100 ± 0 @ 3 mg/kg	100 [4] @ 3 mg/kg	100 [4] @ 3 mg/kg
AHN 1-055	78.8 ± 19.7 @ 17 mg/kg	77.8 ± 19.5 @ 17 mg/kg	80 [5] @ 17 mg/kg	80 [5] @ 17 mg/kg
AHN 2-003	54.1 ± 20.4 @ 5.6 mg/kg	54.0 ± 20.4 @ 5.6 mg/kg	40 [5] @ 5.6 mg/kg	40 [5] @ 5.6 mg/kg
JHW 007	8.22 ± 5.70 @ 3.0 mg/kg	7.69 ± 5.44 @ 3.0 mg/kg	0 [9] @ 1.0 mg/kg	0 [9] @ 1.0 mg/kg
AHN 2-005	42.3 ± 16.8 @ 10 mg/kg	43.2 ± 16.4 @ 10 mg/kg	33 [9] @ 10 mg/kg	33 [9] @ 10 mg/kg
JHW 005	37.6 ± 23.9 @ 10 mg/kg	38.4 ± 23.5 @ 10 mg/kg	25 [4] @ 5.6 and 10 mg/kg	25 [4] @ 5.6 and 10 mg/kg

GA-I-103	10.1 ± 6.52 @ 5.6 mg/kg	10.24 ± 6.51 @ 5.6 mg/kg	0 [6] @ 5.6 mg/kg	0 [6] @ 5.6 mg/kg
JHW 025	7.43 ± 7.19 @ 5.6 mg/kg	10.9 ± 10.9 @ 5.6 mg/kg	0 [5] @ 5.6 mg/kg	0 [5] @ 5.6 mg/kg

[a]The number in brackets that follows the entry is the number of subjects responding at that dose.

Correlations Between Columns

B with A: R^2=0.999 ($F_{1,8}$=5267.2; P<0.001)

C with A: R^2=0.989 ($F_{1,8}$=659.6; P<0.001)

D with A: R^2=0.986 ($F_{1,8}$=479.2; P<0.001)

C with B: R^2=0.987 ($F_{1,8}$=547.1; P<0.001)

D with B: R^2=0.987 ($F_{1,8}$=519.3; P<0.001)

D with C: R^2=0.992 ($F_{1,8}$=862.0; P<0.001)

Table 7: Alterations in the Discriminative-Stimulus Potency of Cocaine Produced by Treatment with N-Substituted BZT Analogues. ED_{50} Values and Potencies Relative to Cocaine Alone (With 95% Confidence Limits) Are Shown.

Drug	**ED_{50} Value (μmol/kg)**	**Relative Potency Value[a]**
Cocaine	9.68 (6.62-13.9)	1.0
0.18 WIN 35,428 + Cocaine	5.83[b] (3.30 - 22.9)	1.24 (0.643 - 2.09)
1.77 WIN 35,428 + Cocaine	NS Regression	*574*[c] *(260 - 1810)*
0.76 AHN 1-055 & Cocaine	14.1 (8.15 - 37.6)	0.657 (0.353 - 1.14)
2.54 AHN 1-055 & Cocaine	9.85 (4.85 - 22.0)	0.903 (0.491 - 1.63)
3.6 AHN 1-055 & Cocaine	9.65 (6.39 - 13.7)	1.13 (0.750 - 1.75)
14.2 AHN 1-055 & Cocaine	Nonsignificant linear regression	1.94 (1.03 - 4.30)
2.73 AHN 2-003 + Cocaine	8.32 (4.88-14.2)	1.06 (0.601 - 1.87)
8.20 AHN 2-003 + Cocaine	6.97 (4.21-10.5)	1.26 (0.776 - 2.10)
15.3 AHN 2-003 + Cocaine	Nonsignificant linear regression	0.919 (0.508 - 1.68)
2.37 JHW 007 + Cocaine	13.4 (9.32-21.7)	0.682 (0.382 - 1.15)
7.11 JHW 007 + Cocaine	10.1	0.880

	(6.62-15.7)	(0.518 - 1.48)
23.7 JHW 007 + Cocaine	Nonsignificant linear regression	1.66[c] (0.766 - 4.20)
13.8 AHN 2-005 + Cocaine	6.94[b] (5.35-8.68)	1.29 (0.849 - 2.00)
24.6 AHN 2-005 + Cocaine	Nonsignificant linear regression	1.12[c] (0.490 - 2.69)
5.60 JHW 005 + Cocaine	6.53 (3.38-10.3)	1.33 (0.800 - 2.31)
10.5 JHW 005 + Cocaine	7.00 (3.00-12.5)	1.21 (0.696 - 2.19)
18.7 JHW 005 + Cocaine	Nonsignificant linear regression	*2.15[c,d] (1.04 - 5.77)*

[a]Significant relative potency values effects are indicated by italics.

[b]Value is an estimate due to a significant deviation from linearity.

[c]Value is an estimate due to a significant deviation from parallel.

[d]Value is an estimate due to a significant effect of preparations.

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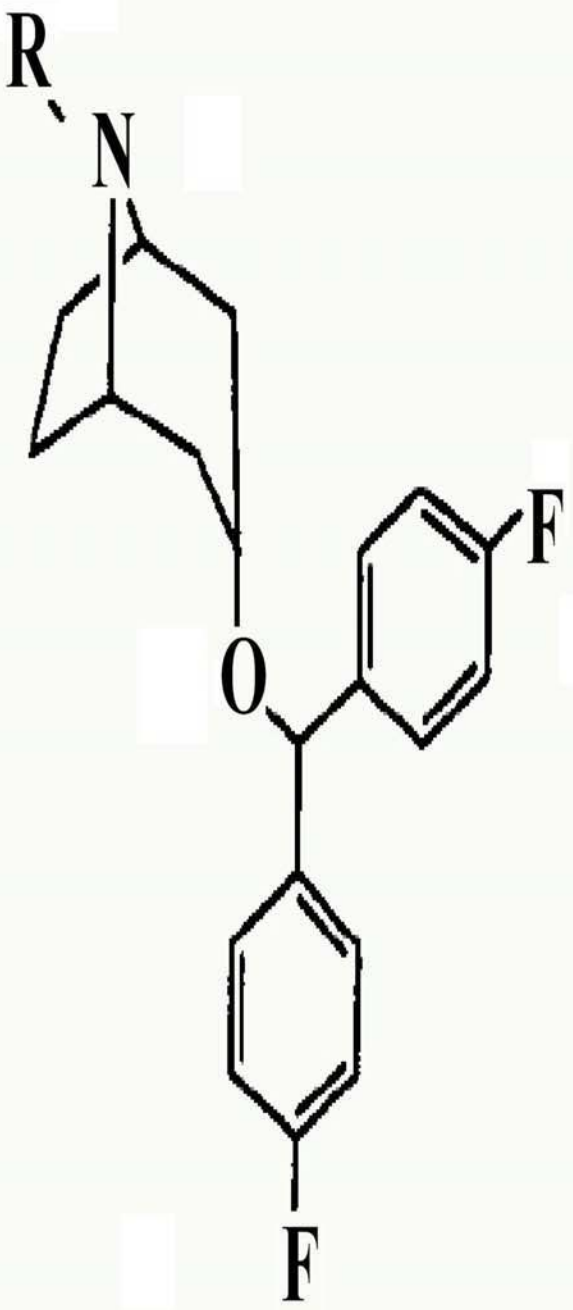

Compound	N-Substitution
AHN 1-055	CH_3
AHN 2-003	H
AHN 2-005	allyl
JHW 007	butyl
JHW 005	benzyl
GA-1-103	butylphenyl
JHW 025	$(CH_3)_2I^-$

Figure 1

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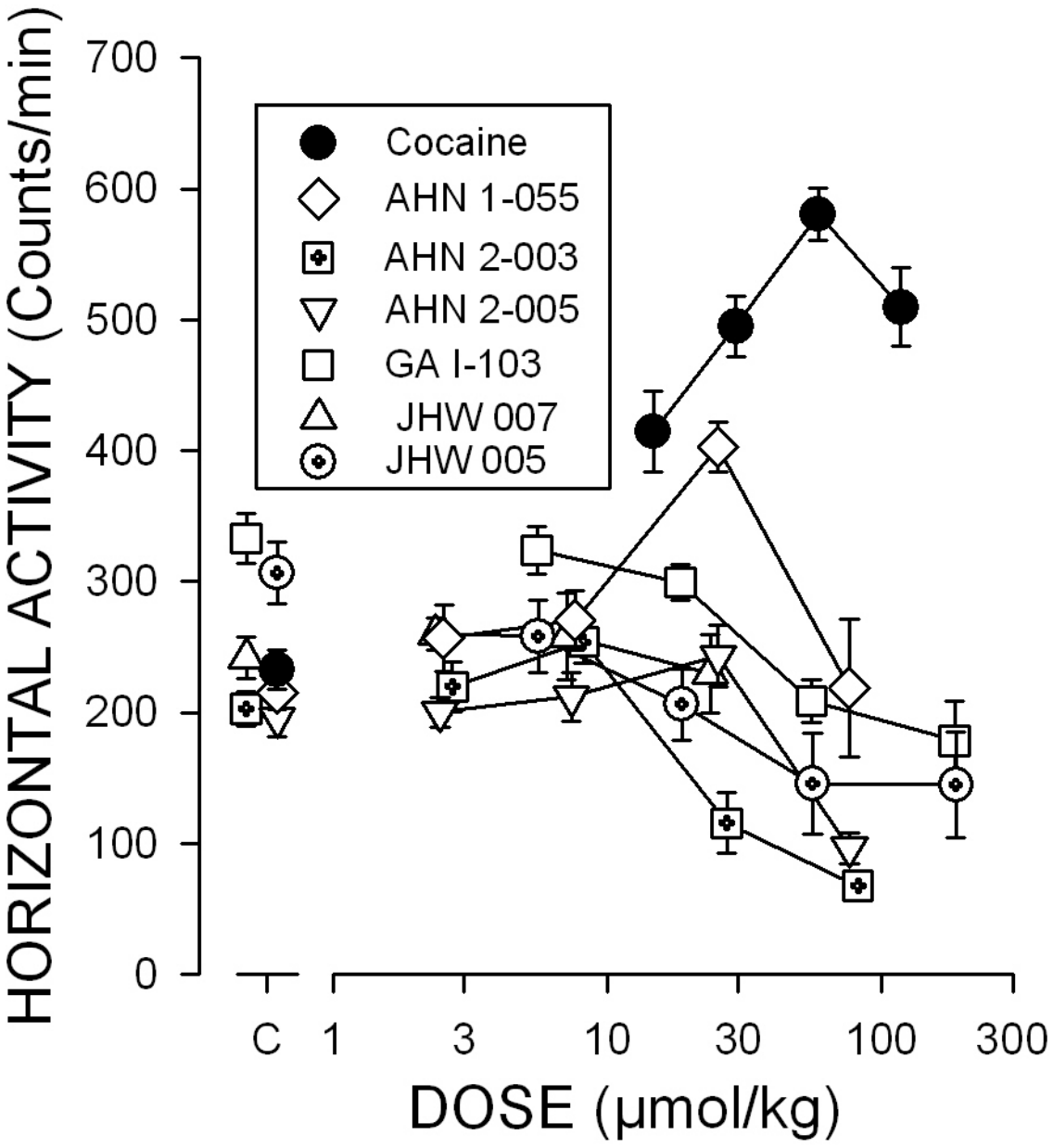

Figure 2

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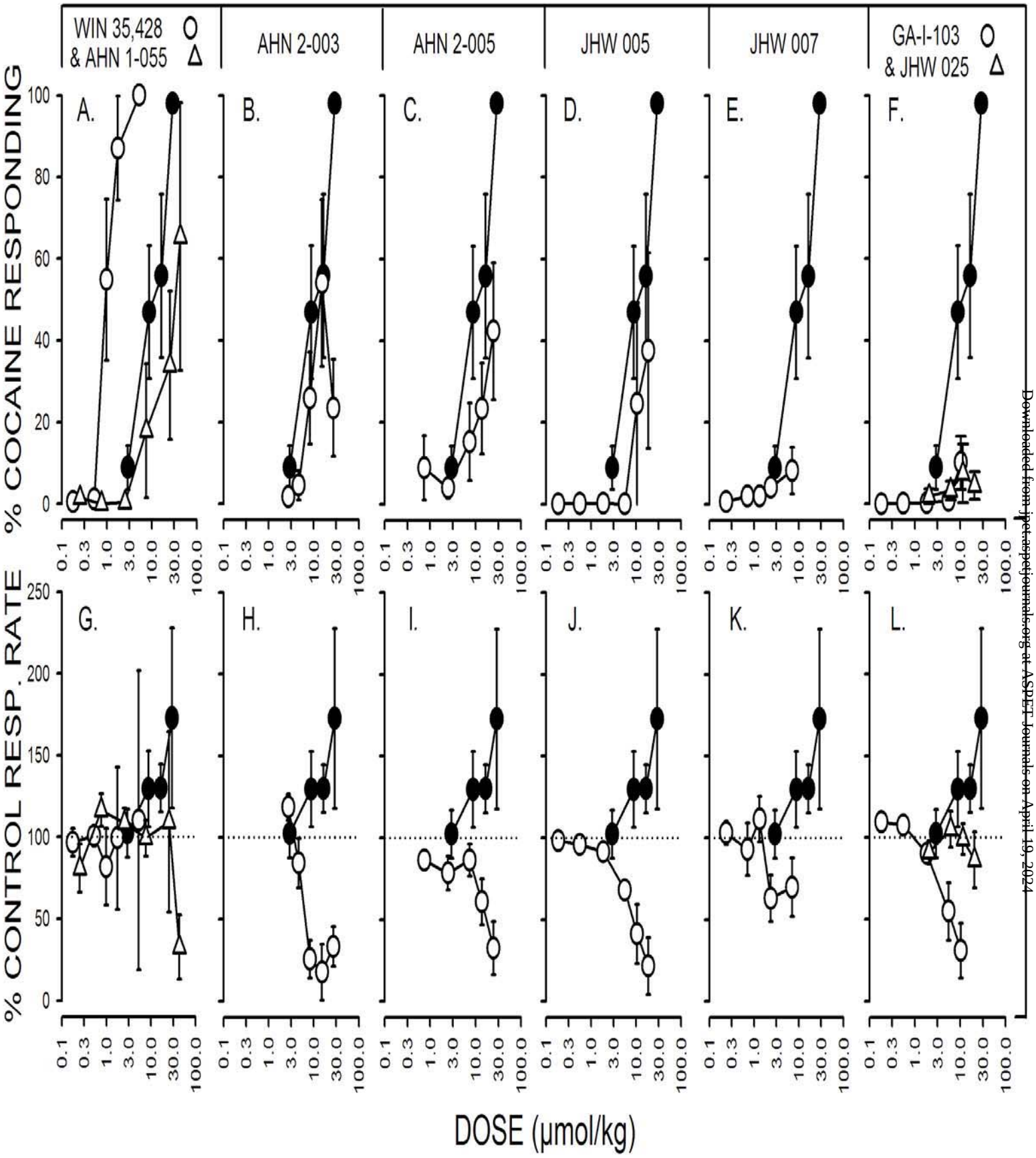

Figure 3

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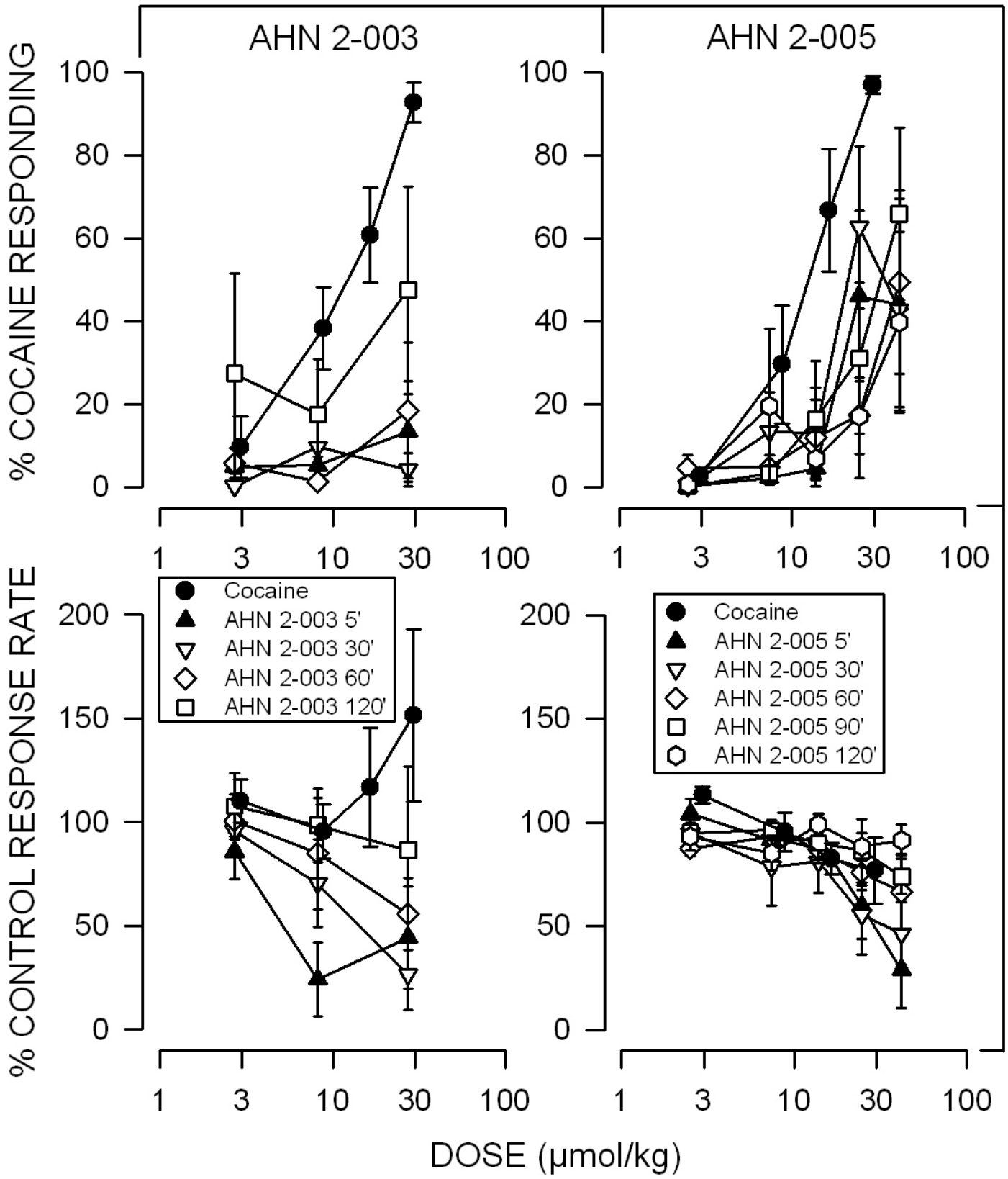

Figure 4

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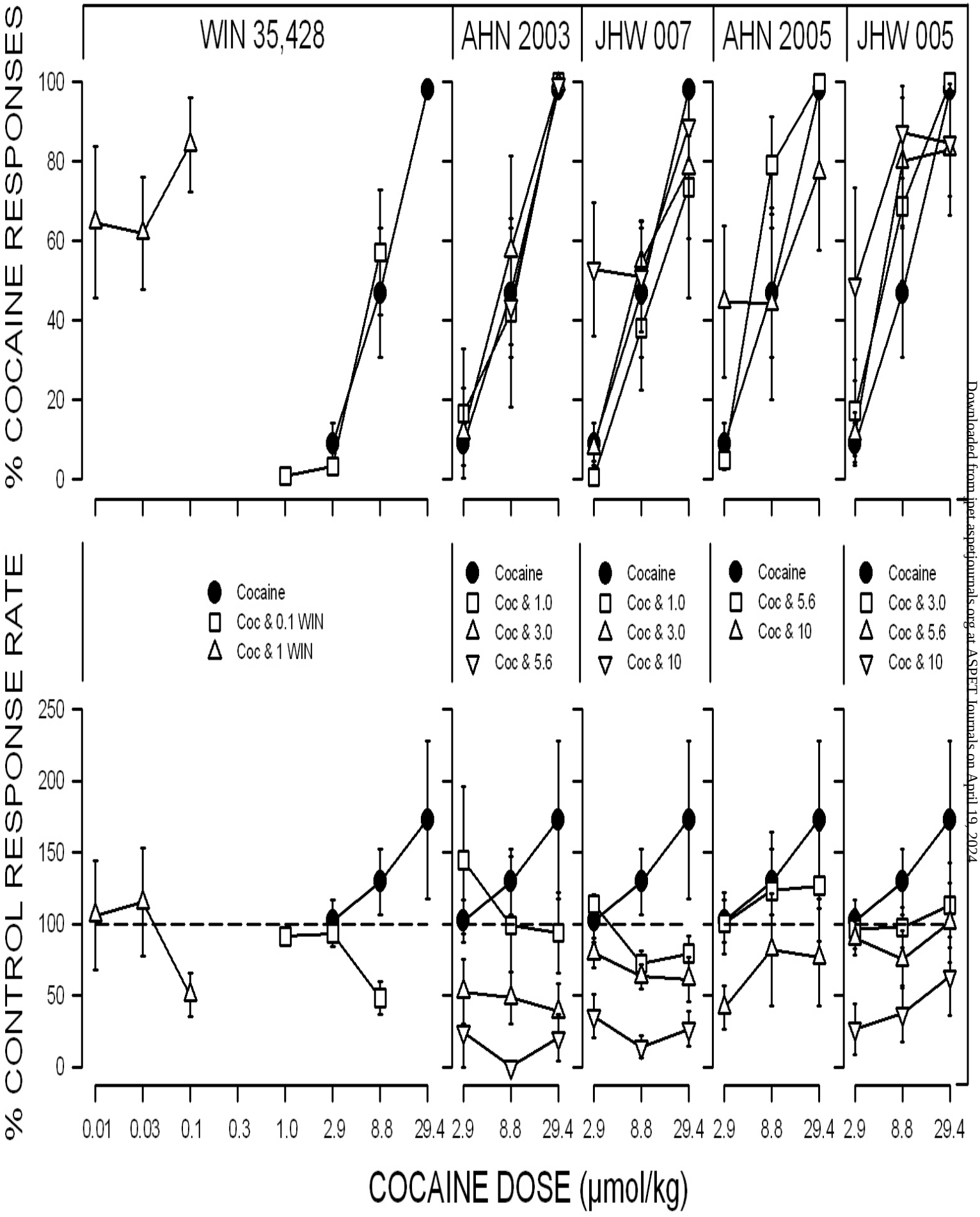

Figure 5