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FURTHER DELINEATION BETWEEN TYPICAL AND ATYPICAL DOPAMINE UPTAKE INHIBITORS: EFFECTS ON FOOD-MAINTAINED BEHAVIOR AND FOOD CONSUMPTION

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Abstract

The present studies compared the acute effects of benztropine (BZT) analogs (4-Cl-BZT, JHW 007, AHN 1-055), which are atypical DA uptake inhibitors, with those of the standard DA uptake inhibitors GBR 12909 and cocaine, on the reinforcing efficacy of food and food intake in male Sprague-Dawley rats. Repeated drug effects of JHW 007 on food intake were also determined. The number of ratios completed under a progressive-ratio (PR) schedule of food delivery was used as an index of reinforcing efficacy. Food intake was determined by measuring powdered laboratory-chow consumption during daily 40 min food-availability time periods. Under the PR schedule, cocaine and GBR 12909 dose-dependently increased the number of ratios completed. JHW 007 decreased ratios completed while neither 4-Cl-BZT nor AHN 1-055 increased ratios completed with a magnitude that approximated any of the increases produced by cocaine or GBR 12909. Acute administration of each drug dose-dependently decreased food intake; however, the BZT analogs were more potent than cocaine and GBR 12909. A reduction in food intake emerged after repeated administration of a low dose of JHW 007 indicated that. Future studies that compare JHW 007 to standard anorectic drugs (e.g., phentermine) and continue investigation of the repeated drug effects under similar experimental procedures are clearly warranted.

Keywords

Benztropine analogs; Atypical dopamine uptake inhibitors; Food intake; Progressive-ratio schedule; Dopamine transporter; AHN 1-055; JHW 007; 4-Cl-BZT; Cocaine; GBR 12909; rat

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INTRODUCTION

Previous studies have suggested that novel atypical DA uptake inhibitors, such as analogs of benztropine (BZT) that have a high affinity and selectivity for the DA transporter, paradoxically have low abuse potential of their own and have potential for the treatment of stimulant abuse (see review by Tanda et al., 2009a). For example, BZT analogs are less effective than cocaine in stimulating locomotor activity, producing cocaine-like discriminative-stimulus effects and in stimulating DA release in brain regions associated with reinforcement in rats (see review by Tanda et al., 2009a). In addition, BZT analogs are either less effective than cocaine in maintaining self-administration behavior (AHN 1-055) or are not self-administered (JHW 007, AHN 2-005), and pre-treatment with these compounds decreases cocaine self-administration (Hiranita et al., 2009; Ferragud et al., 2009) and can attenuate neurochemical effects of cocaine (Tanda et al., 2009b). A similar profile of behavioral and pharmacological effects of BZT analogs have been demonstrated with other psychostimulant drugs of abuse such as amphetamine and methamphetamine (Velazquez-Sanchez et al., 2011, 2013; Ferragud et al., 2014).

The current study was a continued investigation into the similarities and differences among effects of typical and atypical dopamine uptake inhibitors – particularly effects related to food reinforcement. Hiranita et al. (2009) examined effects of three BZT analogs (AHN 1-055, AHN 2-005, and JHW 007) under an FR 5 schedule of food reinforcement. Orally administered doses (10–100 mg/kg) of the latter two drugs did not appreciably alter response rates; however a number of unique experimental manipulations were conducted to facilitate matching of baseline response rates to the rate of cocaine self-administration (e.g., subjects were given their daily ration of food 60 min prior to treatment with the BZT analogs). Ferragud et al. (2014) found that the BZT analog JHW 007 (3 and 10 mg/kg) also did not appreciably alter responding maintained under an FR 3 schedule of sucrose delivery. The primary objective of the present study was to evaluate and compare the effects of several BZT analogs (AHN 1-055, JHW 007, 4-CI-BZT) to standard indirect monoaminergic agonists (cocaine and the more DA selective agent GBR 12909) on different assessments of food reinforcement – specifically procedures that are commonly used to assess appetite-suppressant effects of drugs that may provide indications for an anti-obesity medication (LeSage et al., 2004).

In Experiment 1, we determined the effects of the above mentioned compounds on behavior reinforced with food under a progressive-ratio schedule of food delivery. A typical progressive-ratio schedule is one in which the number of responses required to obtain food progressively increases after each food presentation. The highest ratio completed before lever pressing ceases within a defined time period (commonly referred to as a breakpoint) is considered an index of the efficacy of food as a reinforcer (Hodos, 1961; Hodos and Kalman, 1963). However, it is also important to note that drugs can have multiple behavioral effects and thus changes in performance under a PR schedule may be attributed to effects other than changes in reinforcer efficacy (e.g., changes in general activity or stereotypy, cf. Jones et al., 1995; Lesage et al., 2004). There is one previous study (Ferragud et al., 2014) that examined effects of one BZT analog (JHW 007, 3 and 10 mg/kg) under a PR schedule

of sucrose delivery and found that JHW 007 dose-dependently increased the number of ratios completed, suggesting an increase in the reinforcing efficacy of the sucrose solution.

Another common procedure used to assess appetite-suppressant effects of drugs is to examine food consumption during limited access periods. In Experiment 2, we assessed the acute effects of the above mentioned typical and atypical dopamine uptake inhibitors on food consumption during a 40-min daily-feeding period in food-deprived rats. Findings from the first two experiments indicated prominent effects consistent with appetite suppression for the BZT analog JHW 007. Therefore, a third experiment was conducted to determine the effects of chronic administration of JHW 007 (as anorectic drugs are typically taken repeatedly), at doses that produced minimal behavioral effects, to assess potential for appetite suppression without toxicity.

METHODS

Subjects

The subjects were male Sprague-Dawley rats, weighing 325–350 g (Taconic Farms, Hudson, NY) in experiment 1 (n=5), and 250–300 g (Charles River, Wilmington, MA, USA) in experiments 2 (n=8) and 3 (n=24). They were housed individually in a temperature and humidity-controlled vivarium under a 12-h light/dark cycle (lights on 07:00 h), and had unlimited access to water. In experiment 1, animals were fed approximately 10–15 g of standard Purina rat chow (Purina Mills Inc., St. Louis, MO, USA) 1 hour after each session; thus, each subject was food restricted for approximately 22 hr prior to the start of each experimental session. In experiments 2 and 3, animals were fed a daily ration of approximately 20 g of standard Purina rat chow until two weeks before testing. Care of the subjects was in accordance with the guidelines of the National Institutes of Health and the National Institute on Drug Abuse Intramural Research Program Animal Care and Use Program.

Apparatus

In experiment 1, experimental sessions were conducted in two-lever operant conditioning chambers (modified ENV 007, Med Associates, Inc., St. Albans, Vermont, USA) that were enclosed in ventilated, sound-attenuating cubicles and provided with white noise at all times to mask extraneous sounds (ENV-018, Med Associates, Inc.). The front panel of the operant chambers consisted of the two response levers, which were 10.2 cm apart, 2 pairs (one red and one green) of light-emitting diodes (LEDs) and house-lights, located directly above each response lever, and a food receptacle behind an approximate 5×5 cm opening centered between the two levers. Each lever press on either lever with a downward force of approximately 0.4 N through approximately 1 mm produced an audible click and was recorded as a response. A food pellet dispenser (ENV-203, Med Associates, Inc.) behind the front panel delivered 45-mg food pellets (Bio-Serv, Inc., Frenchtown, New Jersey, USA) into the receptacle. Experimental variables and data collection were controlled and recorded with desk-top computers, commercially available interfacing equipment, and operating software (Med Associates, Inc.).

In experiments 2 and 3, subjects were tested in plastic cages that were identical to the home cage (30 × 19 × 19 cm). Powdered food (100 g) was presented to each subject in feeding jars with a diameter of 8.5 cm and a height of 4.5 cm. A lid with a centered circular (diameter 3.3 cm) aperture limited spillage.

Experiment 1: Acute drug effects under a progressive-ratio schedule of food delivery

Subjects were initially trained to press a lever under a fixed-ratio 1 (FR 1) schedule of food reinforcement. Assignment of right or left levers was counterbalanced across subjects. After a 5-min time-out (TO) period, the house-light and each pair of red and green LEDs above the active lever were illuminated. A single active lever press turned off the LEDs, produced a 0.1 s flash of the houselight, and delivered a single food pellet. A 10-s TO period followed each pellet delivery, during which all lights inside the chamber were off and responses had no programmed consequences. Sessions lasted until 40 food pellets were presented.

The number of responses required for each pellet delivery was gradually increased across 10 sessions from FR 1 to FR 10. Approximately 15 training sessions were then conducted under an FR 10 schedule of food reinforcement, after which a progressive-ratio schedule was put into effect for the remainder of the study. Under this schedule, the number of required responses was two for the first ratio of the session and incremented exponentially after each pellet delivery such that the sequence was: 2, 4, 7, 11, 17, 25, 36, 50, 69, 95, 131, 178, 242, 328, 445, 603, 815, 1102, and 1489. Experimental sessions terminated after 2 h or if responding on the active lever ceased for 15 min. The last FR completed before session termination due to a 15-min pause in active lever pressing was defined as the “break point.”

Drug testing was initiated after approximately 25 sessions under the progressive-ratio schedule. Drug tests were generally conducted on Tuesdays and Fridays, with vehicle test sessions interposed between drug test days on Thursday. Drugs were tested in the following order, with the indicated pretreatment times and with cocaine tested once at the beginning and once at the end of the study: cocaine (5 min), GBR12909 (5 min), JHW 007 (5 min), AHN 1-055 (10 min), 4-Cl-BZT (10 min), cocaine (5 min). Individual doses of each drug were administered in a mixed sequence.

Experiment 2: Acute drug effects on feeding behavior

Two weeks before testing, all subjects were given daily access to 100 g of powdered Bio-Serv diet (Bio-Serv, Frenchtown, NJ, USA) for 40 minutes on weekdays and 20 g of standard laboratory chow on weekends. This feeding routine was in effect for the remainder of the experiment. Testing was conducted in a room separate from the colony room. Prior to test sessions, all rats were weighed and doses of each drug were administered only if the group mean body weight deviated less than 10% from the initial body weight. Test sessions were generally conducted on Tuesdays and Fridays, with vehicle test sessions interposed between drug test days on Mondays and Thursdays; no injections were given on Wednesdays. On test days, all rats received an injection of a dose of a test drug 10 min before the filled and pre-weighed feeding jars were placed inside the test cages. After 40 min, the jars were removed from the test cages, and weighed. Food intake was calculated by subtracting post- from pre-feeding values. Drugs were tested in the following order with

individual doses in a mixed sequence: cocaine, GBR12909, 4-Cl-BZT, JHW 007, and AHN 1-055.

Experiment 3: Repeated drug effects of JHW 007 on feeding behavior

Prior to repeated drug testing, subjects were given daily access to 100 g of powdered Bio-Serv diet (Bio-Serv, Frenchtown, NJ, USA) for 40 (2.37 $\mu\text{mol/kg}$ group) or 60 minutes (7.11 $\mu\text{mol/kg}$ group) on weekdays (see below for rationale in food access duration change) and 20 g of standard laboratory chow on weekends. Repeated drug testing was initiated after approximately 6 daily feeding sessions in which there were no trends in the average amount of food consumed for each subject. Food consumption was measured and calculated as in Experiment 2. Once repeated drug testing was initiated, daily access to 100 g of powdered Bio-Serv diet continued seven days per week for a maximum of 17 consecutive days. In a between-subjects design, 2.37 $\mu\text{mol/kg}$ JHW 007 (10-min pretreatment) was tested in 6 subjects while a separate group of subjects ($n=6$) received repeated saline injections. Following testing of 2.37 $\mu\text{mol/kg}$ JHW 007, a new group of subjects ($n=6$) were tested with 7.11 $\mu\text{mol/kg}$ JHW 007 (10-min pretreatment) while a separate group of subjects ($n=6$) received repeated saline injections. The duration of food access was increased for the 7.11 $\mu\text{mol/kg}$ group because the vehicle-control group that was compared to the 2.37 $\mu\text{mol/kg}$ JHW 007 group had lost weight during repeated testing (as shown in the results).

Drugs

Cocaine hydrochloride and GBR 12909 were obtained from Sigma-Aldrich (St. Louis, MO). The BZT analogs, 4'-chloro-3 α -(diphenylmethoxy)-tropane (4-Cl-BZT), 3 α -[bis-(4-fluorophenyl)methoxy]tropane (AHN 1-055), and N-(n-butyl)-3 α -[bis(4'-fluorophenyl)methoxy]-tropane (JHW 007) were synthesized in the Medicinal Chemistry Section (Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD) according to published procedures (Newman et al., 1994; Agoston et al., 1997). All drugs were dissolved in sterile water or 0.9% saline (NaCl), and were injected i.p.p at 1 ml/kg body weight. For complete solubilisation, GBR 12909 and BZT analogs were sonicated and heated. Doses of each drug are expressed in terms of the salt.

Data Analysis

Experiment 1—Response rates and number of ratios completed were the dependent measures. The number of completed ratios directly corresponds to the log transformation of the final ratio value obtained (Richardson and Roberts, 1996). The two determinations of cocaine were analyzed using a two-way repeated-measures ANOVA (within-subjects factors: time and drug dose). One-way repeated-measures ANOVA was used to analyze the effects of GBR 12909, AHN 1-055, JHW 007 and 4-Cl-BZT (within-subjects factor: drug dose) with these results detailed in the figure captions. Multiple-comparison tests were conducted using Bonferroni corrections. Data from the linear portions of the dose-effect curves for ratios completed were used to calculate an ED_{25} value (i.e., the dose that produced a 25% change from the number of ratios completed under vehicle-control conditions) and their 95% CLs. Significant differences for all analyses were based upon $p < 0.05$.

Experiment 2—The mean amount of food consumed (g) was the dependent measure. Each dose-effect curve for each drug was analyzed using standard ANOVA and linear regression techniques. Data from the linear portions of the dose-effect curves were used to calculate ED₅₀ values and their 95% confidence limits (CLs) (Snedecor and Cochran, 1967); where appropriate, one-way ANOVAs and Dunnett's *t*-tests were conducted. A significant difference in ED₅₀ values is indicated by 95% CLs that do not overlap. Significant differences for all analyses were based upon $p < 0.05$.

Experiment 3—The average body weight (g) and amount of food consumed (g) were the dependent measures. Each measure was analyzed with a two-way repeated measures ANOVA at each dose with a between-subjects factor of group (JHW 007 or saline) and within-subjects factor of session. Multiple-comparison tests were conducted using Holm-Sidak comparisons. Significant differences for all analyses were based upon $p < 0.05$.

RESULTS

Experiment 1: Acute drug effects under a progressive-ratio schedule of food delivery

Both cocaine and GBR 12909 generally increased response rates in a dose-dependent manner under the progressive-ratio schedule (Fig. 1, upper left panel), though the ANOVA showed only a significant main effect of dose for cocaine, indicating significant increases at the highest two doses. The BZT analogs produced a range of effects on response rates. AHN 1-055 (Fig. 1, lower left panel), like cocaine, significantly increased rates of responding, with the highest dose producing effects significantly different from vehicle. In contrast, 4-Cl-BZT (Fig. 1, upper right panel) and JHW 007 (Fig. 1, lower right panel) produced only dose-dependent and significant decreases in response rates. The multiple comparisons revealed that the highest dose of 4-Cl-BZT and the two highest doses of JHW 007 significantly decreased response rates.

Similar to the effects on response rate, both cocaine and GBR 12909 produced dose-dependent increases in the number of ratios completed under the progressive-ratio schedule (Fig. 2, upper left panel). The main effect of dose was significant for each drug, and multiple-comparison analyses revealed significant effects as indicated on the figures compared to vehicle-control values. These dose-dependent increases in ratios completed for cocaine and GBR 12909 were accompanied by dose-dependent decreases in the number of sessions that ended due to the "break point" criterion (see Table 1).

In contrast to the effects of cocaine and GBR 12909, the BZT analogs were substantially less effective in increasing the number of ratios completed in a session (Fig. 2). 4-Cl-BZT produced a significant increase in the number of ratios completed at a dose of 44.94 $\mu\text{mol/kg}$, however, that increase (~ 2 ratios on average) was less than that obtained with cocaine and GBR 12909, and was not obtained at higher doses (Fig. 2, upper right panel). There were no significant effects of AHN 1-055 on the number of ratios completed (Fig. 2, lower left panel), though there was a trend towards an increase at the highest dose studied. In contrast, JHW 007 (Fig. 2, lower right panel) significantly decreased the number of ratios completed at a dose of 23.70 $\mu\text{mol/kg}$. Further more, unlike cocaine and GBR 12909, test sessions generally terminated due to meeting the "break point" criterion, even as the dose of

each BZT analog increased (Table 1). The potency analysis (Table 2) indicates that the BZT analogs AHN 1-055 and 4-Cl-BZT were generally less potent in increasing the number of ratios completed than cocaine and GBR 12909. For all drugs, earned food pellets were consumed during the experimental sessions.

Experiment 2: Acute drug effects on feeding behavior

Acute administration of all of the drugs studied decreased food intake in a dose-dependent manner, with maximum decreases in food intake produced by the highest doses of each drug (Fig. 3). Over the domain of doses examined, all drugs appeared to be in the same range of effectiveness (Fig. 3). The ANOVAs confirmed significant effects of cocaine, GBR 12909, 4-Cl-BZT, AHN 1-055, and JHW 007 with significant reductions in food intake compared to control values, as indicated on the figure. The ED₅₀ values (Table 2) confirm that the BZT analogs were generally more potent in reducing food intake compared to both cocaine and GBR12909. Additionally, the potencies of cocaine and GBR 12909 for decreasing food intake were greater than their potencies for increasing the number of ratios completed under the progressive-ratio schedule. In contrast, the potencies of the BZT analogs for affecting performance under the progressive-ratio schedule were generally comparable to their potencies for decreasing food intake.

Experiment 3: Repeated drug effects of JHW 007 on feeding behavior

Across 17 daily sessions of repeated dosing with 2.37 $\mu\text{mol/kg}$ JHW 007 (Fig. 4; left column), both the average weight (before eating) of the subjects and amount of food consumed were no different from the vehicle group. The weights of subjects treated with 7.11 $\mu\text{mol/kg}$ JHW 007 (Fig. 4; right column top graph) showed a decreasing trend whereas those of subjects treated with vehicle showed an increasing trend. The ANOVA resulted in a significant interaction, with tests corrected for multiple comparisons showing significantly lower weights for the 7.11 $\mu\text{mol/kg}$ group across sessions 11 through 17. As seen in Fig. 4 (right column, lower graph), food intake also increased across sessions for the vehicle group, while food intake remained unchanged and relatively lower for the 7.11 $\mu\text{mol/kg}$ JHW 007 group. The ANOVA did not result in a significant interaction; however, tests corrected for multiple comparisons showed differences between groups for sessions 13 through 16. One subject in the 7.11 $\mu\text{mol/kg}$ JHW 007 group died before testing on day 17; that subject had the largest change in weight and its elimination from the group was the cause of the small increase in the group average for body weight and food consumed on session 17.

DISCUSSION

The performances under the progressive-ratio schedule are consistent with a number of previous studies that have reported that BZT analogs generally do not share all of the pharmacological and behavioral effects with cocaine, despite having a high affinity and selectivity for the DA transporter (Tanda et al., 2009a; Ferragud et al., 2009). In the present study, cocaine significantly increased response rates under the progressive-ratio schedule while GBR 12909 produced a dose-dependent trend for increased response rates. However, neither 4-Cl-BZT nor JHW 007 increased response rates under the progressive-ratio schedule, and only the highest dose of AHN 1-055 increased response rate to a degree

approaching that for cocaine. Among BZT analogs, AHN 1-055 often produces the most “cocaine-like behavioral effects” (e.g., in the amount of substitution for cocaine in a drug discrimination procedure, the extent of increased locomotion or in the degree of self-administration, see Tanda et al., 2009a).

Cocaine and GBR 12909 administration were found to significantly increase the number of ratios completed under a progressive-ratio schedule of food delivery. A similar finding has been described in other studies with intermediate doses of cocaine (Thompson, 1977; Poncelet et al., 1983; Jones et al., 1995; Sizemore et al., 2003) and other psychostimulants such as *d*-amphetamine and methylphenidate (Schulze et al., 1990; Mayorga et al., 2000). This effect appears at odds with the interpretation that performance under a progressive-ratio schedule is a pure indication of reinforcer efficacy since both drugs decreased food intake. It is not unheard of for multiple measures of behavioral constructs to be altered by independent variables in different manners (e.g. Brito, 1985). As drugs generally have multiple effects, it is likely that the behavioral stimulant effect of cocaine and GBR 12909 under the progressive-ratio schedule is distinct from any actions resulting in a decrease in the reinforcing effects of food. Similarly, LeSage et al. (2004) found that administration of the stimulant phentermine at intermediate doses decreased food intake in some subjects under free-feeding conditions, but increased ratios completed under a progressive-ratio schedule of food delivery.

In contrast to the effects of cocaine and GBR 12909 on the number of ratios completed, the BZT analogs produced a distinct profile of effects. The BZT analog JHW 007 decreased ratios completed, while 4-Cl-BZT and AHN 1-055 did not increase the number of ratios completed to the extent of cocaine and GBR 12909. Further, the comparisons of dose-dependent effects indicated that the BZT analogs 4-Cl-BZT and AHN 1-055 were generally less potent and less efficacious in increasing ratios completed under the progressive-ratio schedule than were cocaine and GBR 12909 - thus indicating further delineation between typical and atypical dopamine uptake inhibitors. The decrease in ratios completed following JHW 007 administration is in apparent conflict with a previous report (Ferragud et al., 2014) that JHW 007 dose-dependently increased reinforcers earned under a PR schedule of sucrose delivery. Reconciliation of the discrepant findings could be directed at rat strain comparison (hooded PVG vs. Sprague Dawley rats), or within-subject analyses of pretreatment times (60 vs. 5 min), food type (100 μ l of 10% sucrose administered in 5 drops vs. 45-mg food pellets) or break point criterion (1 h since last active lever response vs. 15 min; cf. Stafford and Branch, 1998).

Consistent with previous studies (van Rossum and Simons, 1969; Balopole et al., 1979; Cooper and van der Hoek, 1993, 1994), the indirect DA agonist cocaine and the selective DA uptake inhibitor GBR 12909 decreased food intake in a dose-related manner during acute drug testing. Administration of the BZT analogs 4-Cl-BZT, JHW 007, and AHN 1-055 also dose-dependently decreased food intake. The rank order of potency for decreasing food intake, based on ED₅₀, values was 4-Cl-BZT > AHN 1-055 $\approx$ JHW 007 > GBR 12909 $\approx$ Cocaine. Based on their high and selective *in vitro* binding affinities for the DA transporter (e.g., Katz et al., 1999, 2004), these findings suggest that, like cocaine and GBR 12909, BZT

analogues also produced reductions in food intake primarily through actions at the DA transporter, but with greater potency.

Since acute administration of JHW 007 showed the most consistent profile for appetite-suppressant effects under the PR schedule and during food intake testing, we further examined its effects during repeated treatment with focus on doses producing minimal acute behavioral effects to assess development of appetite-suppression without toxicity. Repeated administration of 7.11 $\mu\text{mol/kg}$ JHW 007 resulted in statistically significant reductions in body weight and food intake that emerged after approximately 11-13 consecutive days of drug exposure. Repeated administration at one third the dose (2.37 $\mu\text{mol/kg}$) showed no indication of changes relative to the control group in weight or food consumed with . However, both groups (vehicle and 2.37 $\mu\text{mol/kg}$) were losing weight throughout the study, likely because the duration of food access was shorter than in the subsequent study at the higher dose, in which control subjects gained weight. Future studies should begin to investigate the potential development of tolerance to the reduction in feeding behavior produced by JHW 007, which is a finding typical of CNS anorectic drugs (Choi et al 2002; Wellman et al 2003; Fernstrom and Choi, 2008), by comparing dose-effect curves before and after a repeated drug regimen.

Several researchers have suggested that the dopaminergic mechanisms involved in the abuse of psychomotor stimulant drugs are similar to those involved in feeding behavior and food reinforcement (Hernandez and Hoebel, 1988; Clifton and Kennett, 2006), although there are other pharmacological targets of interest (Palmiter, 2007; Bray and Greenway, 2007). Findings from the current study support further exploration of BZT analogues as appetite suppressants – in particular JHW 007. Future studies that compare JHW 007 to standard anorectic drugs (e.g., phentermine) and continue investigation of the repeated drug effects under similar experimental procedures are clearly warranted.

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Figure 1.

Effects of cocaine, GBR 12909, and BZT analogs on response rate during the PR schedule of food delivery. Vertical axes: response rate (responses/minute). Horizontal axes: drug dose in $\mu\text{mol/kg}$ (log scale). Each point represents an average ($\pm$ SEM) effect for five rats. The ANOVAs indicated significant effects of cocaine ($F_{5,20} = 15.61$; $p < 0.001$), but not GBR 12909 ($F_{4,16} = 2.36$; $p = 0.098$), AHN1-055 ($F_{5,20} = 4.27$; $p = 0.008$), 4-Cl-BZT ($F_{5,20} = 4.08$; $p = 0.010$), or JHW 007 ($F_{4,16} = 9.02$; $p < 0.001$). Statistically significant results of Bonferroni comparisons for individual doses relative to control injections are indicated by an asterisk.

Figure 2.

Effects of cocaine, GBR 12909, and BZT analogs on the number of ratios completed during the PR schedule of food delivery. Vertical axes: ratios completed. Horizontal axes: drug dose in μmol/kg (log scale). Each point represents an average (± SEM) effect for five rats. The effects of cocaine ($F_{5,20} = 51.78$; $p < 0.001$), GBR 12909 ($F_{4,16} = 13.07$; $p < 0.001$), 4-Cl-BZT ($F_{5,20} = 3.00$; $p = 0.035$), and JHW 007 ($F_{4,16} = 4.44$; $p = 0.013$) were significant, with Bonferroni comparisons indicating increases relative to control injections with cocaine, GBR 12909 and 4-Cl-BZT (with a smaller magnitude). The effect with JHW 007 was a significant decrease at 23.70 μmol/kg ($p < 0.05$), while 40.29 μmol/kg approached significance ($p = 0.05$). There were no significant effects of AHN 1-055 ($F_{5,20} = 2.25$; $p = 0.089$).

Figure 3.

Effects of cocaine (filled circle), GBR 12909 (open square), and BZT analogs 4-Cl-BZT (open upward triangle), JHW 007 (open downward triangle) and AHN 1-055 (open diamond) on food intake in rats given access to powdered laboratory chow over a period of 40 min after treatment. Vertical axes: mean food intake in g. Horizontal axes: drug dose in μmol/kg (log scale). Each data point represents an average ($\pm$ SEM) effect determined for at least five to eight subjects. The ANOVAs indicated significant effects of each compound as follows: cocaine ($F_{4,59} = 31.64$; $p < 0.05$), GBR 12909 ($F_{3,28} = 20.75$; $p < 0.05$), 4-Cl-BZT ($F_{4,35} = 23.63$; $p < 0.05$), AHN 1-055 ($F_{3,11} = 16.61$; $p < 0.05$), and JHW 007 ($F_{4,30} = 12.26$; $p < 0.05$). Dunnett's t -tests indicated statistically significant reductions in food intake compared to control injections, as indicated by an asterisk.

Figure 4.

Effects of 2.37 (filled triangle) and 7.11 (filled circle), µmol/kg JHW 007, and vehicle (open symbols) on body weight (upper row) and food intake (lower row) in rats given access to powdered laboratory chow over a daily period of 40 (2.37 µmol/kg) or 60 min (7.11 µmol/kg) during repeated drug treatments. Vertical axes: mean starting weight in g (top row) and mean food intake in g (bottom row). Horizontal axes: average body weight during baseline sessions (BL) prior to drug treatment and the number of repeated drug or vehicle sessions. Each data point represents an average (± SEM) effect determined for five to six subjects. The ANOVA indicated a significant interaction for starting weight at the 7.11 µmol/kg dose ($F_{16,159} = 2.56$; $p < 0.05$). Holm-Sidak multiple comparison tests indicated significant reductions in starting weight compared to the vehicle group across sessions 11 through 17. Although the ANOVA was not significant ($F_{16,159} = 1.33$; $p > 0.05$) for the amount of food consumed at 7.11 µmol/kg, Holm-Sidak multiple comparison tests indicated significant reductions in food consumed relative to the vehicle group across sessions 11 through 16. Statistically significant effects are denoted by an asterisk.

Table 1

The average percentage of sessions that ended due to the “break point” criterion as a function of dose (mg/kg) for cocaine, GBR 12909, and BZT analogs. Standard error of the mean (SEM) are indicated within parentheses.

Dose (mg/kg)	Cocaine	GBR12909	4-Cl-BZT	JHW 007	AHN 1-055
Vehicle	100.0	100.0	100.0	95.0 (5.0)	100.0
1.0	100.0	100.0	100.0	100.0	90.0 (10.0)
3.0	81.0 (4.8)	100.0	100.0	100.0	70.0 (12.2)
10.0	41.7 (5.3)	80.0 (20.0)	70.0 (20.0)	100.0	50.0 (22.4)
17.0	33.3 (9.1)	—	60.0 (18.7)	86.7 (8.2)	70.0 (20.0)
30.0	13.3 (8.2)	0.0	60.0 (24.5)	—	80.0 (20.0)

Table 2

The potencies of cocaine, GBR 12909, 4-Cl-BZT, JHW 007, and AHN 1-055 for ratios completed under the progressive-ratio schedule and for food intake. For performances under the progressive-ratio schedule ED₂₅ values (with 95% CLs) are shown. For suppression of food intake ED₅₀ values (with 95% CLs) are shown. A ratio indicating the relative potency between the two measures is also listed.

Drug	Progressive Ratio Schedule ED ₂₅ Value in $\mu\text{mol/kg}$ (95% CL)	Food Intake Suppression ED ₅₀ Value in $\mu\text{mol/kg}$ (95% CL)	Ratio
Cocaine	6.18 (4.27–8.21)	51.7 (44.0–60.9)	8.37
GBR 12909	7.56 (4.45–11.7)	48.0 (28.5–115)	6.35
4-Cl-BZT	14.2 (3.15–125)	11.5 (8.70–15.1)	0.81
JHW 007	18.8 (8.79–93.1) *	25.5 (16.3–53.4)	1.36
AHN 1-055	49.0 (23.3–1380)	19.3 (15.8–21.1)	0.39

* This value represents the minimum effective dose necessary to decrease the number of completed ratios.