Reduction of Cocaine Self-Administration and D3 Receptor-Mediated Behavior by Two Novel Dopamine D3 Receptor-Selective Partial Agonists, OS-3-106 and WW-III-55


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ABSTRACT

Dopamine D3 receptor (D3R)-selective compounds may be useful medications for cocaine dependence. In this study, we identified two novel arylamide phenylpiperazines, OS-3-106 and WW-III-55, as partial agonists at the D3R in the adenyl cyclase inhibition assay. OS-3-106 and WW-III-55 have 115- and 862-fold D3R:D2 receptor (D2R) binding selectivity, respectively. We investigated their effects (0, 3, 5.6, or 10 mg/kg) on operant responding by using a multiple variable-interval (VI) 60-second schedule that alternated components with sucrose reinforcement and components with intravenous cocaine reinforcement (0.375 mg/kg). Additionally, we evaluated the effect of OS-3-106 (10 mg/kg) on the dose-response function of cocaine self-administration and the effect of WW-III-55 (0–5.6 mg/kg) on a progressive ratio schedule with either cocaine or sucrose reinforcement. Both compounds were also examined for effects on locomotion and yawning induced by a D3R agonist. OS-3-106 decreased cocaine and sucrose reinforcement rates, increased latency to first response for cocaine but not sucrose, and downshifted the cocaine self-administration dose-response function. WW-III-55 did not affect cocaine self-administration on the multiple-variable interval schedule, but it reduced cocaine and sucrose intake on the progressive ratio schedule. Both compounds reduced locomotion at doses that reduced responding, and both compounds attenuated yawning induced by low doses of 7-OH-DPAT (a D3R-mediated behavior), but neither affected yawning on the descending limb of the 7-OH-DPAT dose-response function (a D2R-mediated behavior). Therefore, both compounds blocked a D3R-mediated behavior. However, OS-3-106 was more effective in reducing cocaine self-administration. These findings support D3Rs, and possibly D2Rs, as targets for medications aimed at reducing the motivation to seek cocaine.

Introduction

The dopamine D3 receptor (D3R) subtype is a target for developing novel therapeutic agents for the treatment of psychostimulant addiction (Levant, 1997; Luedtke and Mach, 2003; Joyce and Millan, 2005; Le Foll et al., 2005; Newman et al., 2005; Heidbreder and Newman, 2010; Blaylock and Nader, 2012; Cheung et al., 2012). One unique attribute of the D3R is that its level of expression is much higher in the mesolimbic pathway than in other dopaminergic pathways (Bouthenet et al., 1991; Murray et al., 1994; Levant, 1997), and the mesolimbic pathway has been implicated in drug addiction (Wise, 2004; Kalivas and Volkow, 2005). Other
evidence implicating the D3R in psychostimulant addiction includes the findings that D3R expression is upregulated in response to the administration of several drugs of abuse (Spangler et al., 2003; Vengeliene et al., 2006). For instance, Mash and colleagues (Staley and Mash, 1996; Segal et al., 1997) reported elevated D3R binding and mRNA expression in the ventral striatum of cocaine overdose fatalities. Furthermore, D3Rs are upregulated after cocaine self-administration in rodents, and the magnitude of that elevation appears to be related to measures of motivation to seek cocaine (Neiswander et al., 2004; Conrad et al., 2010).

One challenge facing the development of D3R-selective compounds is the high degree of amino acid sequence homology between the D3R and the dopamine D2 receptor (D2R) (Sokoloff et al., 1990). Nonetheless, several compounds with varying degrees of D3R selectivity have been identified. These D3R compounds decrease psychostimulant self-administration (Xi et al., 2005, 2006; Song et al., 2012) and drug-seeking behavior (Pilla et al., 1999; Vorel et al., 2002; Cervo et al., 2003; Di Ciano et al., 2003; Gilbert et al., 2005; Gál and Gyertyán, 2006; Xi et al., 2006; Gyertyán et al., 2007; Achat-Mendes et al., 2010; Blaylock et al., 2011; Higley et al., 2011).

We previously reported on the development of a series of arylamide phenylpiperazines that have high affinity for D3Rs, moderate D3R:D2R binding selectivity (i.e., 23–51-fold), and low log P values (2.9–3.5) (Chu et al., 2005; Kumar et al., 2009). We found that three of these compounds—WC10, WC26, and WC44—reduced cocaine self-administration (Cheung et al., 2012). Although the response rate under sucrose reinforcement was also reduced, the latency to respond (i.e., the time between lever insertion and the first response on the active lever) increased as a function of dose for WC26 and WC44 when the reinforcer was cocaine but not when the reinforcer was sucrose. It has been proposed that the selective increase in response latency for cocaine reflects a decrease in the motivation to seek cocaine (Olmstead et al., 2000; Cheung et al., 2012).

To examine further the structure-activity relationship of arylamide phenylpiperazines in modulating cocaine self-administration, two novel arylamide phenylpiperazines, OS-3-106 and WW-III-55, were evaluated. Competition curves were performed to determine the affinity of these compounds at dopamine D2 and D3 receptors. We then examined the ability of these two novel compounds to suppress response pounds at dopamine D2 and D3 receptors. We then examined administration, two novel arylamide phenylpiperazines, arylamide phenylpiperazines in modulating cocaine self-administration (WC26, and WC44 et al., 2009). We found that three of these compounds included the findings that D3R expression is upregulated in response to the administration of several drugs of abuse (Spangler et al., 2003; Vengeliene et al., 2006). For instance, Mash and colleagues (Staley and Mash, 1996; Segal et al., 1997) reported elevated D3R binding and mRNA expression in the ventral striatum of cocaine overdose fatalities. Furthermore, D3Rs are upregulated after cocaine self-administration in rodents, and the magnitude of that elevation appears to be related to measures of motivation to seek cocaine (Neiswander et al., 2004; Conrad et al., 2010).

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Finally, to elucidate whether effective doses of these compounds acted through D3Rs, D2Rs, or both, we examined their effects on the inverted U-shaped dose-response function for yawning induced by the mixed D2R/D3R agonist 7-OH-DPAT. Behavioral pharmacological studies suggest that the induction of yawning by low doses of D2R/D3R agonists is mediated via the D3R, whereas the induction of yawning by high doses of D2R/D3R agonists is mediated via the D2R. Thus, D2R/D3R agonists-induced yawning is an effective in vivo screen for D3R- versus D2R-mediated antagonistic effects of pharmacological treatments (Collins et al., 2005).

Materials and Methods

Pharmacological Evaluation of OS-3-106 and WW-III-55

Dopamine Receptor Binding Assays. A filtration binding assay characterized the binding of OS-3-106 and WW-III-55 to D2 and D3Rs. Competition curves were performed using [3H]IABN with human dopamine receptors stably expressed in human embryonic kidney (HEK) 293 cells. Stably transfected cells were harvested by centrifugation, and the cell pellet was resuspended in cold (4°C) homogenization buffer (50 mM Tris-HCl, pH 7.4, with 10 mM EDTA, 150 mM NaCl) by vortexing and then homogenizing with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged at 12,000g at 4°C, and the membrane pellet was resuspended in buffer and kept at −80°C. Tissue homogenates (50 μl) were suspended in 50 mM Tris-HCl/150 mM NaCl/10 mM EDTA buffer, pH 7.5 and incubated with 50 μl of [3H]IABN at 37°C for 60 minutes. Nonspecific binding was defined using 2 μM (+)-butaclamol. For competition experiments, the radioligand concentration was generally equal to the Kd value, and the concentration of the competitive inhibitor ranged over 5 orders of magnitude. Binding was terminated by the addition of cold-wash buffer (10 mM Tris-HCl/150 mM NaCl, pH 7.5) and filtration over a glass-fiber filter (Whatman no. 32; Whatman, Piscataway, NJ). Filters were washed, and the radioactivity was measured using a Packard gamma counter (GMI, Inc, Ramsey, MN) with an efficiency of 75%. The protein concentration of the membranes was determined using a bicinchoninic acid reagent (Pierce, Rockford, IL) and bovine serum albumin as the protein standard.

Data from competitive inhibition experiments were modeled using nonlinear regression analysis to determine the concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC50 value). Since transfected cells expressing receptor were used for this study, competition curves were modeled for a single site using eq. 1:

\[ B = \frac{B_0}{1 + (L/K_{IC50})} + B_{ns} \]

where B is the amount of ligand bound to tissue, B0 is the amount of ligand bound in the absence of competitive inhibitor, L is the concentration of the competitive inhibitor, Bns is the nonspecific binding of the radioligand (defined using a high concentration of a structurally dissimilar competitive inhibitor), and IC50 is the concentration of competitive inhibitor that inhibits 50% of the total specific binding. Data from competition dose response curves were analyzed using Tablecurve program (Jandel/Systat Software Inc., San Jose, CA). IC50 values were converted to equilibrium dissociation constants (Kd values) (Cheng and Prusoff, 1973) using 0.03 and 0.04 nM for the Kd value for [3H]IABN at human D2 and human D3 dopamine receptors, respectively.

Adenylyl Cyclase Assays and Data Analysis. Whole-cell cAMP accumulation was measured by an adaptation of the method of
Shimizu and co-workers (Shimizu et al., 1969). Transfected HEK 293 cells were treated with serum-free medium containing 2,3,5-triiodo-3'-H-adrenaline (ICN), and cells were incubated at 37°C for 75 minutes. The media were replaced with serum-free media containing 0.1 mM 3-isobutyl-1-methylxanthine (Sigma-Aldrich, St. Louis, MO) and drugs to a total volume of 500 μl and incubated at 37°C for 20 minutes. The reaction was stopped by the addition of 500 μl of 10% trichloroacetic acid and 1 mM cAMP. After centrifugation, the supernatants were fractionated using Dowex A-1-X8 and neutral alumina to separate the [3H]ATP and the [3H]cAMP. Individual samples were corrected for column recovery by monitoring the recovery of the cAMP using spectrophotometric analysis at optical density 259 nm.

**Evaluation of the Effects of OS-3-106 and WW-III-55 on Behavior**

**Animals.** Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were maintained on a 12-hour reverse light/dark cycle (lights on at 7:00 PM) and were given ad libitum food and water, unless otherwise specified. Subjects were acclimated to handling for approximately 2 minutes/day for at least 5 days before the start of the experiment or surgery. All procedures and housing conditions were in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996) and were approved by the Institutional Animal Care and Use Committee at Arizona State University.

**Drugs.** Cocaine hydrochloride (RTI International, Research Triangle Park, NC) was administered daily. Rats were given a minimum of 6 recovery days, during which they were handled briefly to administer the heparin. Rats were trained with sucrose pellets (45 mg; Bio-Serv, Frenchtown, NJ) as the reinforcer. The right lever was the active lever. There was a cue light above the active lever to signal reinforcer availability. The left lever was inactive. Pressing the active lever once after a variable interval produced the following consequences: 1) delivery of sucrose pellet, 2) retraction of both levers, 3) offset of the cue light, and 4) onset of a tone (500 Hz, 10 db above background) for 6 seconds. The house light turned on with the offset of the tone and remained on for the next 24 seconds to signal a time-out in the OS-3-106 experiment. In the WW-III-55 experiment, the time-out was increased to 54 seconds. After the time-out, the stimuli were reset: 1) the levers were reinserted, 2) the cue light above the active lever was illuminated, and 3) the house light was turned off. Therefore, in the OS-3-106 experiment, the levers were retracted after each reinforcer delivery for a 30-second time-out initially, which was subsequently increased to a 60-second time-out. A 60-second time-out was used throughout the WW-III-55 experiment. Initially, the schedule of reinforcement progressed within daily sessions from fixed-ratio 1 (FR1), to variable-interval 10-second (VI 10-second), to VI 30-second, and then finally to VI 60-second. The schedule was advanced during the session if the rat received five reinforcers within 40 minutes. Once the rat had ended the session on the VI 60-second schedule for three consecutive sessions, the VI 60-second schedule was in effect exclusively thereafter.

A rat remained food-restricted to 16 g/day until a criterion of ≥14 reinforcers was achieved during VI 60-second-only sessions. After the criterion was reached, the food available in the home cage was progressively increased across several sessions to 18 g/day, then to 22 g/day, then to ad libitum. Rats were trained given food ad libitum for the remainder of the experiment. All rats had reached ad libitum food by the 10th training session. Training in the sucrose-only phase continued until a stability criterion had been met. The stability criterion was met when the number of reinforcers earned in each of three consecutive sessions differed from the average of the three sessions by less than 15%. For the WW-III-55 experiment, sucrose training under a VI 60-second schedule resumed after recovery from surgery, until the 3-day stability criterion was met again.

Training then progressed to the cocaine-only phase, which occurred over sessions 13 to 14 in the OS-3-106 experiment and sessions 14–17 in the WW-III-55 experiment. In the cocaine-only phase, rats were trained with cocaine (0.75 mg/kg/0.1 ml i.v.) as the reinforcer in daily sessions that were identical to the sucrose-only sessions, except that the positions of the active and inactive levers (left/right) were reversed. Once the 3-day stability criterion was met, training progressed to the multiple-schedule phase, which occurred over sessions 19–29 in the OS-3-106 experiment and sessions 20–30 in the WW-III-55 experiment. The multiple schedule consisted of eight 15-minute components and a 1-minute time-out between components. The reinforcer available during each component alternated between sucrose and cocaine. The schedule contingency used in each component was the same as that used during the sucrose- and cocaine-only phases. Therefore, during a sucrose component, the right lever was active and the cue light above it was turned on. During a cocaine component, the
left lever was active and the cue light above it was turned on. Reinforcement was delivered using a VI 60-second schedule for all components. The cocaine dose was reduced to 0.375 mg/kg per 0.1 ml for the multiple-schedule phase. During the 1-minute time-out between components, both levers were retracted and all lights were turned off. The reinforcer available in the first component of each session was varied randomly between sessions.

Testing of OS-3-106 or WW-III-55 started after seven or more training sessions on the multiple schedule and once the 3-day stability criterion was met for both reinforcers. This occurred over sessions 30–36 in the OS-3-106 experiment and sessions 36–41 in the WW-III-55 experiment. For each subject, four doses of OS-3-106 or WW-III-55 (vehicle, 3, 5.6, 10 mg/kg) were tested across separate sessions using a within-subject design. Each dose was tested once for each subject. The order in which doses were given was counterbalanced between rats. Test sessions were identical to the training sessions, with the exception that test sessions were shortened to four 15-minute components with alternating reinforcers, resulting in a total test session length of 1 hour and 4 minutes. The reinforcer available during the first component (sucrose or cocaine) was counterbalanced within subject across test sessions. Between test sessions, rats were maintained under the multiple schedule used during training. The 3-day stability criterion was implemented between tests.

**Effect of OS-3-106 on the Cocaine Dose-Response Function on a VI 60-Second Schedule.** Because we found that 10 mg/kg OS-3-106 reduced cocaine self-administration on the multiple schedule and according to the progression of ratio requirement maintained on the VI 60-second reinforcement schedule, we further examined OS-3-106 on the dose-response function of cocaine self-administration. A new group of rats (n = 8) was used for this experiment. After recovery from catheter implantation surgery, rats were trained to self-administer cocaine (0.75 mg/kg per 0.1 ml i.v.) in two-hour daily sessions. The same training regimen and contingency as used in the cocaine-only phase were implemented. After a minimum of six training sessions on the VI 60-second schedule, a series of test days was initiated, provided the 3-day stability criterion had been met. The dose of cocaine available (intravenously) during the 1-hour test session was increased across test days in the following order: 0, 0.094, 0.188, 0.375, 0.75, and 1.5 mg/kg per 0.1 ml. Animals were tested twice at each cocaine dose, once after vehicle pretreatment and once after pretreatment with 10 mg/kg OS-3-106. Treatment order was counterbalanced between rats. Pretreatments were given intraperitoneally 5 minutes before the start of the test sessions. Rats were maintained under daily 2-hour sessions at the 0.75 mg/kg per 0.1-ml training cocaine dose between test days. The 3-day stability criterion was implemented between tests.

**Effect of WW-III-55 on Cocaine Reinforcement on a Progressive Ratio Schedule.** Since WW-III-55 had no effect on cocaine self-administration in the multiple schedule experiment (see Results), the same group of rats was tested for the effect of WW-III-55 observed in the PR experiment was specific to cocaine self-administration, a separate cohort of 10 rats was trained and tested under a PR schedule of sucrose reinforcement. Rats were restricted to 16 g of food/day beginning 2 days before the experiment. The rats were trained on a FR1 for four sessions, after which the ratio requirement increased by 1 every session until reaching FR5. The right lever was the active lever, the left lever was inactive, and each sucrose reinforcer was delivered with the same stimuli as in the previous experiments. Sessions were terminated once a rat attained 50 reinforcers. Once rats had reached the FR5, food in their home cages was progressively increased to ad libitum, which remained in effect throughout the rest of the experiment. All rats attained ad libitum food by session 14; they were then transitioned to the previously described PR schedule. Rats were trained on the PR schedule until the 3-day stability criterion was met (sessions 31–44). They were then tested twice: once with vehicle and once with 5.6 mg/kg WW-III-55. The order of treatment was counterbalanced between rats, and additional sessions were given between tests to reestablish stability. A new cohort of 40 rats was used to test the effect of OS-3-106 and WW-III-55. OS-3-106 and WW-III-55 were tested using a 5 × 2 mixed factorial design. Different groups of rats were assigned to receive one of five doses of 7-OH-DPAT (0, 0.01, 0.032, 0.1, 0.32 mg/kg s.c.; n = 8/group), and all were tested twice, receiving vehicle before one test and 10 mg/kg OS-3-106 (intraperitoneally) before the other test, with the order counterbalanced between rats. On test days, rats were first habituated to the locomotor activity chambers for 30 minutes. OS-3-106 or its vehicle was then administered, and the rats were returned to their home cages. Five minutes later, the assigned dose of 7-OH-DPAT was administered, the rats were immediately placed in the chambers, and yawning and locomotion were measured for 30 minutes. Rats were given at least 1 day off before test sessions.

A separate cohort of 22 rats was used to test the effect of WW-III-55. The same procedure was used as described already, except five doses of 7-OH-DPAT were tested within subjects (vehicle, 0.01, 0.032, 0.1, 0.32 mg/kg s.c.) in two groups of rats (n = 11). One group was pretreated with vehicle, and the other group was pretreated with 5.6 mg/kg WW-III-55 (intraperitoneally). The order of 7-OH-DPAT doses was counterbalanced across subjects.

**Effects of OS-3-106 and WW-III-55 on Locomotor Activity.** Locomotor activity tests were conducted in Plexiglas cages (25 × 46 × 20 cm) with a wire bar lid. A video tracking system (TopScan Realtime Option version 2.0; Clever Sys Inc, Reston, VA) produced a continuous record of the rats’ movement, reported as total centimeters traveled. The effects of OS-3-106 and systemic cocaine were tested across four sessions using a within-subject 2 × 2 factorial design. The same nine rats from the OS-3-106 multiple-schedule experiment were used. Testing started at least 7 days after the last session of that experiment. Rats received OS-3-106 (10 mg/kg) or its vehicle 5 minutes before testing, and under each of those conditions, they also received cocaine (15 mg/kg i.p.) or saline immediately before testing. The order of treatments was counterbalanced between rats. Each test session was 1 hour long, and rats were given 1 day off between test sessions.
The effect of WW-III-55 on spontaneous locomotor activity was tested in a new cohort of rats. Different groups of rats received one of four doses of WW-III-55: vehicle, 3, 5.6, and 10 mg/kg \( (n = 8, 7, 7, \) and 7, respectively). Test sessions lasted 1 hour.

**Data Analyses**

Response rate, reinforcement rate, and response latency were analyzed using repeated measures trend analyses (Keppel and Wickens, 2004; Howell, 2007). In the multiple schedule experiment, the dose of the D3R compound was a linear trend factor. In the cocaine dose-response experiment, the dose of cocaine was a polynomial trend factor. Response latency for each reinforcer type (sucrose or cocaine) was defined as the latency from the insertion of levers to the first response on the active lever. In the multiple VI 60-second schedule, the number of reinforcers and lever presses were totaled across the two components for each reinforcer type, whereas response latencies were averaged across the two components for each reinforcer type. If a rat failed to respond during a component in the multiple schedule experiment, the duration of the component (15 minutes) was used as the response latency. If a rat failed to respond during a session in the cocaine dose-response experiment, the duration of the session (1 hour) was used as the response latency. Because Mauchly’s test of sphericity confirmed the homogeneity of variance, response latencies were log10-transformed before statistical analysis and data presentation to improve the homogeneity of variance (Olmstead et al., 2000). Note that although trend analyses are immune to violations of variance homogeneity (Keppel and Wickens, 2004), improving variance homogeneity can increase the power to detect an effect. In the case of a significant interaction, \( t \)-tests were also used to examine simple effects at each dose of the D3R compound for the multiple schedule experiment or at each dose of cocaine for the cocaine dose-response experiment.

In addition, two analyses were carried out to examine whether the effects of OS-3-106 and WW-III-55 depended on the way the multiple VI 60-second schedule was run. The first analysis tested the hypothesis that the effects of the D3R compounds varied, depending on whether the session began with a cocaine component, with the four components being cocaine-sucrose-cocaine-sucrose or, if it began with a sucrose component, with the four components being sucrose-cocaine-sucrose-cocaine. The second analysis tested the hypothesis that the effects of the D3R compounds differed in the first half versus the last half of the session. The details and results of these two analyses are presented in the Supplemental Data.

For the PR schedule experiments, the final ratio achieved was analyzed using nonparametric equivalents of analysis of variance (ANOVA; Friedman’s test) and \( t \)-test (Wilcoxon’s signed-rank test) because it is a noncontinuous, nonnormally distributed random variable.

For the yawning experiments, polynomial trend analysis was used because 7-OH-DPAT has previously been shown to have an inverted U-shape dose-response function for yawning and a U-shape dose-response function for locomotor activity (Khroyan et al., 1995; Collins et al., 2005). Because yawning data are count data, the number of yawns was square-root transformed before statistical analysis to improve the homogeneity of variance (Moyano and Valencia, 2002). Spontaneous and cocaine-induced locomotor activity was analyzed using an \( n \times 2 \) ANOVA (OS-3-106) or linear-trend analysis (WW-III-55). In addition, planned comparisons between vehicle and each dose of the drug were conducted for all dose-response experiments using two-tailed \( t \)-tests.

**Results**

### In Vitro Pharmacological Evaluation of OS-3-106 and WW-III-55

The structures of the two arylamide phenylpiperazines used in this study are shown in Fig. 1, and their pharmacological properties are summarized in Table 1. OS-3-106 binds with high affinity \( (K_i \) value = 0.2 nM) at the D3R and exhibits 115-fold binding selectivity for the D3R compared with the D2R. Whereas WW-III-55 binds at the D3R with 100-fold lower affinity than OS-3-106 \( (K_i \) value = 20 nM), its D3R:D2R binding selectivity is estimated to be >800-fold.

Both compounds were found to be partial agonists at the D3R (58–68% intrinsic activity \( (IA) \) of the full agonist quinpirole) using a forskolin-dependent adenyl cyclase inhibition assay. OS-3-106 is also a partial agonist at the D2R (47% of quinpirole) (Table 1). It was not possible to determine the IA of WW-III-55 at the D2R because of its low affinity at that receptor subtype.

### In Vivo Behavioral Effects of OS-3-106 and WW-III-55

**Effects of OS-3-106 and WW-III-55 on Sucrose and Cocaine Reinforcement.** Analyses presented in the Supplemental data section (see Supplemental Figs. 1 and 2) suggest that the effects of OS-3-106 and WW-III-55 did not depend on whether the session began with a cocaine component or a sucrose component. Therefore, data from both types of sessions were combined for all subsequent analyses unless otherwise specified.

The effects of OS-3-106 on the total number of reinforcers delivered across the two 15-minute sucrose and cocaine components are shown in Fig. 2A. OS-3-106 dose dependently reduced reinforcement rates, and the slope of this reduction did not differ between reinforcer types. Trend analysis (4 OS-3-106 doses × 2 reinforcer types) found that OS-3-106 produced a significant linear trend \( (F_{1,8} = 33.68, P < 0.001) \), but there was no OS-3-106 × reinforcer-type interaction of linear trend. Planned \( t \)-tests comparing the effect of each dose of OS-3-106 against vehicle, for each reinforcer type (Fig. 2A), confirmed that reinforcement rates for both sucrose and cocaine were decreased by 10 mg/kg OS-3-106 \( (t_6 = 4.25, P = 0.003 \) and \( t_6 = 4.85, P = 0.001 \), respectively).

The effects of OS-3-106 on active and inactive lever presses totaled across the two components for each reinforcer type are shown in Fig. 2, B and C. An overall ANOVA with lever (active/inactive), reinforcer type (sucrose/cocaine), and OS-3-106 dose (four doses) as within-subject factors found a significant contrast effect of lever \( (F_{1,8} = 46.05, P < 0.001) \) but no lever × reinforcer type interaction. These results confirm that rats were able to discriminate between the active versus inactive lever in both sucrose and cocaine components. Trend analysis of active lever responses (4 OS-3-106 doses × 2 reinforcer types) found that OS-3-106 produced a significant linear trend...
(P<0.01), but there was no effect of reinforcer type or OS-3-106 × reinforcer type interaction (Fig. 2B). This finding suggests that OS-3-106 dose dependently reduced active lever response rates and that the slope of this reduction did not differ between reinforcer types. Planned t-tests found that 10 mg/kg OS-3-106 decreased response rate for sucrose relative to vehicle pretreatment (t8 = 2.73, P = 0.026). However, this effect failed to reach significance during the cocaine components (t8 = 2.05, P = 0.075).

Trend analysis of inactive lever responses, totaled across the two components for each reinforcer type, found no significant trends of OS-3-106 or reinforcer type and no significant interaction between the two factors (Fig. 2C).

The effects of OS-3-106 on response latency, averaged across the two components for each reinforcer type, are shown in Fig. 2D. The effect of OS-3-106 depended on whether the rat was responding for sucrose or cocaine, as trend analysis found a significant OS-3-106 × reinforcer type interaction (F1,8 = 28.92, P < 0.01). Separate trend analysis of each reinforcer type found that OS-3-106 increased response latency for cocaine (F1,8 = 35.43, P < 0.001) but not for sucrose (F1,8 = 3.09, P = 0.12). T-tests comparing response latency for sucrose versus cocaine at each dose of OS-3-106 found that response latency was higher for cocaine than for sucrose after pretreatment with 10 mg/kg OS-3-106 (t8 = 2.49, P = 0.037).

Planned t-tests for each reinforcer type found that response latency was higher for cocaine than for sucrose after pretreatment with 10 mg/kg OS-3-106 (t8 = 2.49, P = 0.037).

**TABLE 1**

Selectivity and intrinsic activity of OS-3-106 and WW-III-55

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<th>D3*</th>
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<td></td>
<td>Kd</td>
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<tr>
<td>OS-3-106</td>
<td>26.0 ± 2.4</td>
<td>47.8 ± 6.3</td>
<td>0.23 ± 0.02</td>
<td>58.8 ± 7.2</td>
</tr>
<tr>
<td>WW-III-55</td>
<td>17,098 ± 2677</td>
<td>***</td>
<td>19.8 ± 2.3</td>
<td>67.6 ± 12.5</td>
</tr>
</tbody>
</table>

*Transfected HEK 293 cells expressing either human D2long or D3 dopamine receptors were used.
*Kd values (mean ± S.E.M.) were determined with at least three competitive radioligand binding experiments using [125I]IABN as the radioligand.
*Adenylyl cyclase inhibition, expressed as percent intrinsic activity (% IA) relative to the full receptor agonist quinpirole, was determined using a forskolin-dependent adenylyl cyclase whole-cell assay at a concentration of test compound ≥10× Kd value. Mean values (± S.E.M.) were obtained with at least three experiments.
*K for D2long receptor, K for D3 receptor.
*Log P values are theoretically calculated using ChemDraw.
*** WW-III-55 %IA was not determined for D2R due to drug insolubility at 10× the Kd value concentration.

**Fig. 2.** Effect of OS-3-106 and WW-III-55 on operant responding (±S.E.M.) for sucrose and cocaine (0.375 mg/kg per infusion) on a multiple VI 60-second schedule. Doses of OS-3-106 (top row, n = 9) and WW-III-55 (bottom row, n = 10) were given in randomized order with additional stabilization sessions between tests. The number of reinforcers (A and E), active lever presses (B and F), and inactive lever presses (C and G) were totaled across the two 15-minute components for sucrose (gray squares) or cocaine (black circles) components. Response latency (D and H) was averaged between the two 15-minute components for each reinforcer type. Note that the ordinate for response latency is on a log scale. V, Vehicle. *Difference compared with vehicle pretreatment for sucrose (gray) or cocaine (black) components, P < 0.05. †, Main effect of reinforcer type, P < 0.05.
The effects of WW-III-55 on active and inactive lever presses totaled across the two components for each reinforcer type are shown in Fig. 2, F and G. An overall ANOVA with lever (active/inactive), reinforcer type (sucrose/cocaine) and WW-III-55 dose (four doses) as within-subject factors found a significant effect of lever ($F_{1,8} = 54.24, P < 0.001$) but no lever × reinforcer type interaction. These results confirm that rats were able to discriminate between the active versus inactive lever in both sucrose and cocaine components. Trend analysis of active lever responses found that an interaction between reinforcer type and the linear trend of the WW-III-55 dose ($F_{1,8} = 5.56, P = 0.046$) revealed that WW-III-55 produced a significant linear trend on active lever responding for sucrose ($F_{1,8} = 5.79, P = 0.043$) but not for cocaine. However, planned $t$-tests comparing the effect of each dose of WW-III-55 against vehicle, for each reinforcer type, found that only the decrease in sucrose response rate at the 10 mg/kg dose of WW-III-55 approached significance ($t_8 = 2.45, P = 0.047$). None of the doses of WW-III-55 affected cocaine reinforcement rates ($ps > 0.39$).

The effects of WW-III-55 on response latency, averaged across the two components for each reinforcer type, were shown in Fig. 2H. Trend analysis found a significant effect of reinforcer type only ($F_{1,8} = 9.42, P < 0.05$). This result suggests that the response latency was longer for sucrose than for cocaine, regardless of WW-III-55 dose, and that WW-III-55 did not alter the latency to respond. Planned $t$-tests comparing response latencies at each dose of WW-III-55 against vehicle also found no significant effects.

We conducted a further analysis to examine whether the effects of OS-3-106 and WW-III-55 were comparable during the first versus the last half of the session. The results are presented in the Supplemental data (Supplemental Figs. 3 and 4). The effect of OS-3-106 on reducing sucrose reinforcement rate and sucrose active lever response rates dissipated during the last half of the session compared with the first half. In contrast, the effect of OS-3-106 on reducing cocaine self-administration did not depend on session half. These results suggest that the effects of OS-3-106 were comparable between the two halves of the session for cocaine but not for sucrose. Although OS-3-106 had no effect on sucrose response latency averaged over the whole session, it increased sucrose response latency in the first half of the session but not in the last half (Supplemental Fig. 4, E and F).

The finding that OS-3-106 increased sucrose response latency in the first half of the session is surprising because a previous study found that WC26 and WC44, which are partial and full D3R agonists, respectively, did not increase sucrose response latency in either session halves (Figs. 4 and S1 in Cheung et al., 2012). However, in that study, sucrose was always available during components 1 and 3, whereas cocaine was always available during components 2 and 4. Therefore, we examined the effect of OS-3-106 on response latency at each individual component to facilitate comparisons. We treated the two factors—the dose of OS-3-106 and the reinforcer type factor—as between-subject factors because both of these factors were counterbalanced between subjects within a component. This resulted in $n = 4$ to 5. Trend analysis found that, similar to WC26 and WC44, OS-3-106 did not increase sucrose response latency in component 1 (i.e., the first active lever press of the session, $F_{1,14} = 1.05, P = 0.32$) (Fig. 3). The lack of effect of OS-3-106 is not due to its brain concentration being too low to alter behavior at the time of first response in component 1 because trend analysis found that OS-3-106 dose dependently increased cocaine response latency in component 1 ($F_{1,14} = 4.91, P < 0.05$; Fig. 3). The increase in cocaine response latency by 10 mg/kg OS-3-106 relative to vehicle also approached significance ($t_{2.04} = 2.01, P = 0.08$). Furthermore, response latency in component 1 was higher for cocaine than for sucrose at 10 mg/kg OS-3-106 ($t_{1,14} = 4.31, P < 0.05$, with variance inhomogeneity corrections). This result suggests that the lack of effect of OS-3-106 on sucrose response latency is not due to a ceiling effect. Additional trend analyses (Supplemental Fig. 5) found that OS-3-106 increased sucrose response latency only in component 2 and not in components 3 and 4. In contrast, OS-3-106 increased cocaine response latency in components 2 and 3, and its increase in component 4 also approached statistical significance ($P = 0.054$).

For WW-III-55, analysis shown in the Supplemental Data revealed that WW-III-55 had comparable effects in the first and the last half of the session, for both reinforcer types, for 1) reinforcement rate, 2) inactive lever response rate, and 3) latency.
response latency (Supplemental Figs. 3 and 4). WW-III-55's effect on active lever response rate for cocaine was comparable between the two halves of the session. However, WW-III-55 reduced active lever response rate for sucrose during the first, but not last, half of the session (Supplemental Fig. 3).

**Effect of OS-3-106 on the Cocaine Dose-Response Function on a VI 60-Second Schedule.** We further examined the effect of 10 mg/kg OS-3-106 on the cocaine dose response function on a VI 60-second schedule. The effect of OS-3-106 on the number of reinforcers (infusions) is shown in Fig. 4A. Trend analysis (six cocaine doses × 2 OS-3-106 treatments) found an interaction between OS-3-106 and the quadratic trend of cocaine dose ($F_{1,7} = 61.12$, $P < 0.001$). Subsequent analysis found that after vehicle pretreatment, the number of reinforcers showed a significant quadratic trend ($F_{1,7} = 500.67$, $P < 0.01$), in agreement with the inverted U-shaped dose-response functions typically observed with cocaine. In contrast, after OS-3-106 pretreatment, none of the polynomial trends was significant, although both linear and cubic trends approached significance ($ps < 0.063$). $T$-tests confirmed that after vehicle pretreatment, the number of reinforcers increased relative to the 0 mg/kg per infusion dose of cocaine at 0.094, 0.188, and 0.375 mg/kg per infusion doses of cocaine ($t_{8} = 9.85, 19.76$, and 9.04, respectively; $ps < 0.001$). The number of reinforcers was decreased at 1.5 mg/kg per infusion of cocaine ($t_{8} = 5.02$, $P < 0.01$). However, after OS-3-106 pretreatment, the number of reinforcers was increased only at 0.75 mg/kg per infusion of cocaine ($t_{8} = 2.35$, $P < 0.05$). Additional $t$-tests examining the effect of OS-3-106 at each dose of cocaine found that OS-3-106 reduced the number of cocaine infusions at 0, 0.094, 0.188, and 0.375 mg/kg per infusion of cocaine ($t_{8} = 5.51, 12.48, 11.83$, and 3.11 respectively, $ps < 0.05$). OS-3-106 did not increase the number of cocaine infusions relative to vehicle pretreatment at any of the cocaine doses.

OS-3-106 also reduced cocaine intake (Fig. 4B). Trend analysis found an interaction between OS-3-106 and the quadratic trend of cocaine dose ($F_{1,7} = 27.88$, $P < 0.01$). Subsequent analysis found that cocaine intake showed a significant linear trend after both vehicle and OS-3-106 pretreatments ($F_{1,7} = 149.23$ and 14.74 respectively, $ps < 0.01$). This result suggests that cocaine intake increased as a function of cocaine dose after both pretreatments. In addition, cocaine intake showed a quadratic trend after OS-3-106 pretreatment ($F_{1,7} = 14.39$, $P < 0.01$). $T$-tests examining the effect of OS-3-106 at each dose of cocaine found that OS-3-106 reduced cocaine intake at 0.094, 0.188, and 0.375 mg/kg per infusion of cocaine ($t_{8} = 12.64, 11.68$, and 3.64 respectively, $ps < 0.01$). OS-3-106 did not increase cocaine intake compared with vehicle pretreatment at any of the cocaine doses.

OS-3-106 also reduced active lever response rate (Fig. 4C). Trend analysis found an interaction between OS-3-106 and the quadratic trend of cocaine dose ($F_{1,7} = 52.61$, $P < 0.001$). Subsequent analysis found that cocaine intake showed a significant quadratic trend after vehicle pretreatments ($F_{1,7} = 49.83$, $P < 0.001$). However, none of the polynomial trends was significant after OS-3-106 pretreatment ($ps > 0.1$). $T$-tests confirmed that after vehicle pretreatment, the active lever response rate increased relative to 0 mg/kg per infusion of cocaine at 0.094, 0.188, and 0.375 mg/kg per infusion of cocaine ($t_{8} = 7.58, 4.94$, and 3.49, respectively; $ps < 0.05$). Active lever response rate was decreased at 1.5 mg/kg per infusion of cocaine ($t_{8} = 3.78$, $P < 0.01$). In contrast, after OS-3-106 pretreatment, active lever response rate was not altered by any of the cocaine doses ($ps > 0.30$). Additional $t$-tests examining the effect of OS-3-106 at each dose of cocaine found that OS-3-106 reduced the active lever response rate at 0, 0.094, 0.188, and 0.375 mg/kg per infusion of cocaine ($t_{8} = 2.87, 10.43, 6.93$, and 8.17 respectively; $ps < 0.05$).

OS-3-106 did not significantly affect the inactive lever response rate (Supplemental Fig. 6A). One subject was excluded because its data were >12 SD from the group mean. Trend analysis and $t$-tests found no significant effect of cocaine dose or OS-3-106.

OS-3-106 increased latency to the first response (see Supplemental Fig. 6B). Response latency data were averaged across cocaine doses because there were no cues signaling the upcoming cocaine dose at the beginning of the test sessions. $T$-test of the log10-transformed data found an increase by OS-3-106 ($t_{8} = 10.84$, $P < 0.001$), in agreement with results from the multiple schedule experiment.

![Fig. 4](image-url) Effect of 10 mg/kg OS-3-106 (± S.E.M.) on the number of reinforcers (A), cocaine intake (B), and active lever presses (C) for different doses of cocaine on a VI 60-second schedule ($n = 8$). Available cocaine doses were tested in an ascending dose order, whereas the order of vehicle and OS-3-106 pretreatment were counterbalanced between rats. Additional stabilization sessions were given between tests. *Difference compared with 0 mg/kg per infusion of cocaine dose after vehicle (gray) or OS-3-106 (black) pretreatment, $P < 0.05$. #, Difference between vehicle and OS-3-106 at a given cocaine dose, $P < 0.05$.
Effect of WW-III-55 on Cocaine Reinforcement on a PR Schedule. The dose-dependent effects of WW-III-55 on total active and inactive lever responses are shown in Fig. 5, A and B, respectively. Trend analysis found a significant linear trend of WW-III-55 on total active lever responses ($F_{1,7} = 6.42, P < 0.05$) but not on total inactive lever responses. Trend analysis also found that WW-III-55 dose dependently reduced total number of reinforcers (Fig. 5C; $F_{1,7} = 8.15, P < 0.05$). The effect of WW-III-55 on final ratio achieved is shown in Fig. 5D. Nonparametric ANOVA (Friedman’s test) found a significant effect of WW-III-55 ($P = 0.011$). Additional tests found that final ratio achieved was reduced by pretreatment with 5.6 mg/kg WW-III-55 (Wilcoxon signed rank test, $P = 0.012$). Finally, the effect of WW-III-55 on response latency on the active lever is shown in Fig. 5E. Trend analysis found no significant terms for the effect of WW-III-55.

Effect of WW-III-55 on Sucrose Reinforcement on a PR Schedule. The effects of 5.6 mg/kg WW-III-55 on total active and inactive lever responses for sucrose are shown in Fig. 6, A and B, respectively. T-tests failed to find significant effects of WW-III-55 on either measure. However, WW-III-55 reduced the total number of reinforcers obtained ($t_9 = 2.30, P = 0.047$; Fig. 6C). The effect of WW-III-55 on the final ratio achieved is shown in Fig. 6D. Wilcoxon signed rank test failed to detect a significant difference ($P = 0.108$). Finally, WW-III-55 had no significant effect on response latency on the active lever (Fig. 6E).

Yawning and Locomotor Activity in Response to OS-3-106 or WW-III-55 with and without 7-OH-DPAT. The effects of OS-3-106 (10 mg/kg) and WW-III-55 (5.6 mg/kg) on yawning induced by different doses of 7-OH-DPAT are shown in Fig. 7, A and C, respectively. For OS-3-106, trend analysis found a significant 7-OH-DPAT × OS-3-106 quadratic term interaction ($F_{1,35} = 11.18, P < 0.01$). The simple effects of 7-OH-DPAT were thus analyzed separately for pretreatment with vehicle versus pretreatment with OS-3-106. Trend analysis of the simple effect of 7-OH-DPAT after vehicle pretreatment (0 mg/kg OS-3-106) found a significant quadratic trend ($F_{1,35} = 16.81, P < 0.01$). This result suggests that low doses of 7-OH-DPAT increased yawning and that this increase was reduced at higher doses of 7-OH-DPAT. This observation is in agreement with the typical inverted U-shaped dose-response function of yawning induced by 7-OH-DPAT (Khroyan et al., 1995; Collins et al., 2005). T-tests showed that after vehicle pretreatment (0 mg/kg OS-3-106), yawning was increased by 0.01, 0.032, and 0.1 mg/kg 7-OH-DPAT ($t_{14} = 3.89, P = 0.002$; $t_{14} = 6.95, P < 0.001$; $t_{14} = 2.93, P = 0.011$, respectively). In contrast, trend analysis of the simple effect of 7-OH-DPAT after OS-3-106 pretreatment (10 mg/kg) revealed no significant terms, suggesting that OS-3-106 attenuated 7-OH-DPAT-induced yawning. T-tests found that none of the doses of 7-OH-DPAT significantly affected yawning after OS-3-106 pretreatment ($ps > 0.3$). Furthermore, tests of the simple effects of OS-3-106 at each dose of 7-OH-DPAT (t-tests) found that OS-3-106 reduced 7-OH-DPAT-induced yawning at the 0.01 and 0.032 mg/kg doses ($t_7 = 3.43, P = 0.011$ and $t_7 = 2.62, P = 0.034$, respectively). OS-3-106 did not significantly increase yawning at any of the 7-OH-DPAT doses.

Analysis of yawning after treatment with WW-III-55 showed a significant 7-OH-DPAT × WW-III-55 cubic term interaction ($F_{1,20} = 5.66, P < 0.05$). Figure 7C shows that the cubic term effect is due to an S-shaped dose-response function of 7-OH-DPAT after WW-III-55 pretreatment. Trend analysis of the simple effect of 7-OH-DPAT after vehicle pretreatment (0 mg/kg WW-III-55) showed a significant quadratic trend ($F_{1,10} = 19.23, P < 0.01$) but no significant cubic trend, in agreement with the OS-3-106 experiment described herein and the typical inverted U-shaped dose-response function of 7-OH-DPAT. Trend analysis of the simple effect of 7-OH-DPAT after WW-III-55 pretreatment (5.6 mg/kg) revealed significant quadratic ($F_{1,10} = 18.31, P < 0.01$) and cubic trends ($F_{1,10} = 7.87, P < 0.05$). These results suggest that WW-III-55 did not completely attenuate the ability of some doses of 7-OH-DPAT to induce yawning. T-tests found that after vehicle pretreatment (0 mg/kg WW-III-55), yawning was increased by 0.01, 0.032, and 0.1 mg/kg 7-OH-DPAT ($t_{10} = 4.10, P = 0.002$; $t_{10} = 8.13, P < 0.001$; $t_{10} = 3.39, P = 0.007$, respectively). In contrast, after WW-III-55 pretreatment, yawning was increased only by 0.032, 0.1, and 0.32 mg/kg 7-OH-DPAT ($t_{10} = 8.60, P < 0.001$; $t_{10} = 4.66, P = 0.001$; $t_{10} = 2.90, P = 0.016$, respectively). Furthermore, tests of the simple effects of WW-III-55 at each dose of 7-OH-DPAT (t-tests) found that WW-III-55 significantly reduced yawning induced by 0.01 mg/kg 7-OH-DPAT ($t_{10} = 2.92, P = 0.009$). WW-III-55 did not significantly increase yawning at any of the 7-OH-DPAT doses.

The effects of OS-3-106 (10 mg/kg) and WW-III-55 (5.6 mg/kg) on 7-OH-DPAT-induced changes in locomotor activity are shown in Fig. 7, B and D, respectively. For OS-3-106, trend analysis found a significant 7-OH-DPAT × OS-3-106 quadratic term interaction ($F_{1,35} = 7.44, P < 0.05$). Trend analysis

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Fig. 5. Effect of WW-III-55 on progressive ratio responding (± S.E.M.) for cocaine (0.375 mg/kg per infusion). Animals ($n = 8$) received doses of WW-III-55 in randomized order with additional stabilization sessions between tests. † Significant linear trend of WW-III-55, $P < 0.05$. * Difference compared with vehicle pretreatment, $P < 0.05$. [Image links to ASPET Journals on October 22, 2014]
of the simple effect of 7-OH-DPAT after vehicle pretreatment found a significant linear \( (F_{1,35} = 7.32, P < 0.05) \) and quadratic trend \( (F_{1,35} = 10.80, P < 0.01) \), which suggests that low doses of 7-OH-DPAT reduced locomotor activity but that this reduction was reversed with higher doses of 7-OH-DPAT, in agreement with the typical U-shaped dose-response of 7-OH-DPAT on locomotor activity (Khroyan et al., 1995; Collins et al., 2005). T-tests found that after vehicle pretreatment (0 mg/kg OS-3-106), locomotor activity was reduced by 0.032 and 0.1 mg/kg 7-OH-DPAT \( (t_{14} = 3.79, P = 0.002 \) and \( t_{14} = 4.45, P = 0.001 \), respectively). In contrast, after pretreatment with OS-3-106 (10 mg/kg), trend analysis of the simple effect of 7-OH-DPAT revealed no significant terms. This result suggests that OS-3-106 reduced locomotor activity regardless of 7-OH-DPAT dose, and t-tests revealed that after OS-3-106 pretreatment, none of the doses of 7-OH-DPAT significantly affected locomotor activity \( (ps > 0.12) \). Tests of the simple effects of OS-3-106 at each dose of 7-OH-DPAT \( (t\)-tests) found that OS-3-106 significantly reduced locomotor activity at the 0 and 0.32 mg/kg doses of 7-OH-DPAT \( (t_{7} = 2.75, P = 0.029 \) and \( t_{7} = 2.85, P = 0.025 \), respectively).

Analysis of locomotor activity after WW-III-55 (Fig. 7D) failed to find any 7-OH-DPAT × WW-III-55 interaction terms. However, there was a main effect of 7-OH-DPAT on the linear \( (F_{1,20} = 43.25, P < 0.001) \) and quadratic trend terms \( (F_{1,20} = 33.72, P < 0.001) \). These findings indicate that 7-OH-DPAT induced a U-shaped dose-response on locomotor activity and that pretreatment with WW-III-55 did not alter this dose-response relationship; \( t\)-tests comparing the effect of each dose of 7-OH-DPAT versus 0 mg/kg 7-OH-DPAT, with the WW-III-55 pretreatment factor collapsed, found that locomotor activity was decreased by all four doses of 7-OH-DPAT: 0.01, 0.032, 0.1, and 0.32 mg/kg \( (t_{21} = 4.77, 7.36, 9.18, \) and 0.32 mg/kg).
Effects of OS-3-106 and WW-III-55 on Locomotor Activity. The effects of OS-3-106 and cocaine on locomotor activity are shown in Fig. 8A. Repeated measures ANOVA (2 OS-3-106 doses × 2 cocaine doses) found a main effect of OS-3-106 (F_{1,8} = 88.19, P < 0.001) and a main effect of cocaine (F_{1,8} = 41.27, P < 0.001) but no significant interaction. These findings suggest that OS-3-106 decreased locomotion to the same degree regardless of pretreatment with cocaine versus saline, and cocaine increased locomotion to the same degree regardless of pretreatment with OS-3-106 versus vehicle.

The dose-effect function of WW-III-55 on spontaneous locomotor activity is shown in Fig. 8B. Trend analysis found that WW-III-55 produced a significant linear trend (F_{1,28} = 10.83, P < 0.01), suggesting that WW-III-55 reduced spontaneous locomotor activity. Planned t-tests comparing locomotor activity at each dose of WW-III-55 against vehicle found that 5.6 and 10 mg/kg WW-III-55 significantly decreased locomotor activity (t_{13} = 2.34, P = 0.036 and t_{13} = 2.96, P = 0.011, respectively).

Discussion

Like the arylamide phenylpiperazines WC10, WC26, and WC44 (Chu et al., 2005), OS-3-106 and WW-III-55 have log P values that predict the ability to cross the blood-brain barrier (Table 1). Both are partial agonists according to the adenylyl cyclase assay. OS-3-106 is 115-fold D3R:D2R selective, and WW-III-55 is 862-fold D3R:D2R selective. WW-III-55 is thus one of the most D3R-selective compounds reported in the literature, which makes it particularly useful for examining the effect of selective D3R partial activation.

OS-3-106 reduced cocaine self-administration in the multiple schedule. OS-3-106 also reduced responding for sucrose and locomotor activity; therefore, it could be argued that OS-3-106 reduced cocaine self-administration by impairing general motoric function. However, since OS-3-106 increased response latency for cocaine but not for sucrose in component 1, it is unlikely that motor impairment explains all the present findings. Instead, two lines of evidence suggest that OS-3-106 may be more effective in suppressing cocaine self-administration compared with responding for sucrose. First, OS-3-106 increased cocaine response latency throughout the session but increased sucrose response latency only during component 2. Second, OS-3-106 reduced the cocaine reinforcement rate throughout the session, but its reduction of sucrose reinforcement rate dissipated in the last half of the session. The differential effects of OS-3-106 on responding for cocaine versus sucrose suggest that motivation for cocaine is reduced. This result is consistent with previous reports that other D3R compounds attenuate cocaine seeking as assessed with the extinction/reinstatement model and with a second-order schedule (Pilla et al., 1999; Cervo et al., 2003; Gilbert et al., 2005; Gál and Gyertyán, 2006; Gyertyán et al., 2007; Martelle et al., 2007; however, see Achat-Mendes et al., 2009).

OS-3-106 suppressed the intake of several doses of cocaine without increasing the intake of low or high doses of cocaine. These findings suggest that OS-3-106 does not potentiate the reinforcing effects of cocaine. The D2/D3R partial agonist aripiprazole has also been reported to downshift the cocaine reinforcing effects of cocaine. The D2/D3R partial agonist aripiprazole has also been reported to downshift the cocaine reinforcing effects of cocaine. The D2/D3R partial agonist aripiprazole has also been reported to downshift the cocaine reinforcing effects of cocaine. These findings suggest that OS-3-106 does not potentiate the reinforcing effects of cocaine. The D2/D3R partial agonist aripiprazole has also been reported to downshift the cocaine reinforcing effects of cocaine.

Unlike OS-3-106, WW-III-55 did not reduce cocaine self-administration in the multiple schedule. This difference may be due to WW-III-55 having a lower D3R binding affinity. However, even the moderate dose of 5.6 mg/kg WW-III-55 reduced 7-OH-DPAT-induced yawn, locomotor activity, and cocaine self-administration under the PR schedule. WW-III-55 did not affect cocaine response latency in either multiple or PR schedules. It did, however, reduce the final ratio achieved on the PR schedule. Given WW-III-55’s high level of D3R:D2R selectivity (>800-fold), this result suggests that preferential blockade of D3Rs reduces the ability to sustain responding on a demanding schedule and may also be the mechanism through which the selective D3R partial agonist RGH-237 reduces cue- and cocaine-primed reinstatement of cocaine seeking (Gyertyán et al., 2007).
7-OH-DPAT-induced yawning was used as an in vivo screen for D3R versus D2R antagonism. D2R/D3R agonists such as 7-OH-DPAT produce an inverted-U-shaped dose-response function for yawning (Khroyan et al., 1995, 1997; Collins et al., 2005, 2007; Baladi et al., 2011). The ascending limb of the function is attenuated by D3R-selective antagonists, whereas yawning on the descending limb is increased by the D2R-selective antagonist L-741,626 (Collins et al., 2005, 2007; Blaylock et al., 2011). Both OS-3-106 (10 mg/kg) and WW-III-55 (5.6 mg/kg) reduced 7-OH-DPAT-induced yawning on the ascending limb, consistent with the D3R partial agonist CJ909 (Martelle et al., 2007), suggesting an attenuation of D3R activation. By contrast, OS-3-106 and WW-III-55 both failed to increase 7-OH-DPAT-induced yawning on the descending limb, suggesting no D2R antagonism. This result may be due to their high D3R:D2R selectivity. However, it is possible that D3R antagonism blocked disinhibition of yawning as a result of D2R antagonism (Collins et al., 2007). In vivo screens purported to be more selective for D2R effects, such as hypothermia (Collins et al., 2007), may allow potential D2R occupancy by OS-3-106 or WW-III-55 to be detected.

The yawn counts in the present experiment are low compared with those of some reports (Collins et al., 2005), although they are not atypical (Khroyan et al., 1995). This discrepancy may be due to differences in methods, such as the level of exposure to 7-OH-DPAT, because repeated exposure to 7-OH-DPAT can sensitize its ability to induce yawning (Khroyan et al., 1995).

Both OS-3-106 and WW-III-55 reduced sucrose reinforcement rates in the multiple schedule, and WW-III-55 also reduced the number of sucrose reinforcers in the PR schedule. There is evidence that food-related behavior is reduced by partial D3R activation (Martelle et al., 2007; Achat-Mendes et al., 2009), although it can also be reduced by full D3R agonists (Caine and Koob, 1995) and antagonists (Claytor et al., 2006; Higley et al., 2011; however, see Di Ciano et al., 2003). Further work is needed to clarify the role of D3Rs role in food-related behavior.

OS-3-106 and WW-III-55 reduced locomotor activity, although the effect of WW-III-55 appeared relatively weak. OS-3-106 attenuated, but failed to reverse completely, cocaine-induced hyperactivity, similar to the D3R partial agonist WC26 (Cheung et al., 2012). OS-3-106 and WW-III-55 may have reduced locomotor activity by partially activating D3Rs (Waters et al., 1993), although this is inconsistent with the report that RGH-237, a D3R-selective partial agonist, reduced cocaine seeking without reducing locomotor activity (Gyertyán et al., 2007). The side effects of OS-3-106 and WW-III-55 in reducing locomotor activity may, in part, contribute to their reduction of operant responding. However, it should be emphasized that OS-3-106 seemed more effective in reducing responding for cocaine than for sucrose, which suggests that OS-3-106 reduced the motivation to respond for cocaine, in addition to any nonspecific suppression of behavior related to the reduction of locomotor activity.

The greater D3R:D2R selectivity of WW-III-55 compared with OS-3-106 (862-versus 115-fold) may explain why WW-III-55 failed to reduce cocaine self-administration in the multiple schedule. Previous studies indicated that compounds with high D3R:D2R selectivity are less effective in reducing cocaine self-administration when response effort is low (Vorel et al., 2002; Di Ciano et al., 2003; Gál and Gyertyán, 2003, 2006; Gilbert et al., 2005; Xi et al., 2005, 2006; Gyertyán et al., 2007; Higley et al., 2011; however, see Song et al., 2012). In contrast, compounds with lower D3R:D2R selectivity seem more effective in attenuating cocaine self-administration. For example, the moderately (<51-fold) selective WC series compounds reduce cocaine self-administration in a multiple schedule similar to that used in the present study (Cheung et al., 2012). Furthermore, acute treatment with nonselective D2R/D3R partial agonists shifts preference from cocaine toward food (Thomsen et al., 2008) and reduces cocaine seeking (Khroyan et al., 2000; Feltenstein et al., 2009). One explanation for these findings is that D3Rs may play a pivotal role in the motivation to seek drug, whereas reinforcement may require concurrent activation of both D2Rs and D3Rs.

To the extent that co-occupancy of D2Rs and D3Rs is required to attenuate cocaine reinforcement, such interactions may occur in the same or different neurons or may involve D2R-D3R heterodimers (Maggio et al., 2003; Pou et al., 2012). In vivo positron emission tomography imaging using D2R- and D3R-selective imaging agents may aid the development of compounds with “threshold” D3R:D2R occupancy levels that can attenuate low response-demand cocaine self-administration while minimizing side effects (Mach et al., 2011; Tu et al., 2011). Such compounds may be useful in a two-stage therapeutic strategy. Early during recovery, when the relapse rate is high, moderately D3R:D2R-selective partial agonists might be administered to attenuate craving and cocaine reinforcement if relapse occurs. They may also help normalize the dopaminergic system (Fuchs et al., 2002; Neisewander et al., 2004). Later during recovery when prolonged abstinence has been achieved, a transition to more D3R-selective partial agonists or antagonists may be effective in attenuating seeking while minimizing extrapyramidal and risk-taking side effects associated with D2R/D3R activation (Fenu et al., 2009).

One issue that remains is whether the effects of D3R compounds observed in the present study change with repeated administration. Also, the rats used for two experiments (PR schedule with cocaine and locomotor testing with OS-3-106) had a more extensive cocaine history, which may affect D3R-mediated behavior (Blaylock et al., 2011). In conclusion, two novel ary lamide phenylpiperazines with >100-fold D3R:D2R selectivity and D3R partial agonistic activities attenuated cocaine self-administration. OS-3-106 was more effective in reducing cocaine self-administration compared with responding for sucrose in the multiple schedule. OS-3-106 also reduced cocaine self-administration over a range of cocaine doses. WW-III-55 failed to reduce cocaine intake in the multiple schedule despite doing so at a lower dose in the PR schedule. Both compounds reduced sucrose reinforcement rates and locomotor activity. Results from the yawning experiment provided evidence for D3R antagonism. WW-III-55 may be less effective than OS-3-106 in reducing cocaine self-administration as a result of its lower D3R binding affinity. Alternatively, concomitant D2R/D3R occupancy may have contributed to the ability of OS-3-106 to reduce cocaine self-administration. Taken together, the results of the present study add to the evidence that D3R is a viable target for reducing cocaine-related behaviors (Luendetka and Mach, 2003; Heidbreder and Newman, 2010; Blaylock and Nader, 2012).

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