Published in final edited form as:

Behav Pharmacol. 2012 June ; 23(3): 280–291. doi:10.1097/FBP.0b013e3283536d21.

Lack of Abuse Potential in a Highly Selective Dopamine D₃ Agonist, PF-592,379, in Drug Self-Administration and Drug Discrimination in Rats

Gregory T. Collins, PhD,

Alcohol & Drug Abuse Research Center, Division of Neuroscience, McLean Hospital - Harvard Medical School, Belmont, MA, USA

Paul Butler, PhD,

Pfizer Worldwide R&D, Ramsgate Road, Sandwich, Kent, CT13 9NJ, UK

Chris Wayman, PhD,

Pfizer Worldwide R&D, Ramsgate Road, Sandwich, Kent, CT13 9NJ, UK

Sian Ratcliffe, PhD,

Pfizer Worldwide R&D, Ramsgate Road, Sandwich, Kent, CT13 9NJ, UK

Paul Gupta, PhD,

Pfizer Worldwide R&D, Ramsgate Road, Sandwich, Kent, CT13 9NJ, UK

Geoffrey Oberhofer, and

Alcohol & Drug Abuse Research Center, Division of Neuroscience, McLean Hospital - Harvard Medical School, Belmont, MA, USA

S. Barak Caine, PhD

Alcohol & Drug Abuse Research Center, Division of Neuroscience, McLean Hospital - Harvard Medical School, Belmont, MA, USA

Abstract

Dopamine D₃-preferring agonists are commonly used to treat Parkinson's disease and restless leg syndrome; however, laboratory animal studies suggest that they may possess a moderate abuse potential. These studies aimed to compare the highly-selective, full D₃ agonist PF-592,379 to that of the less selective D₃ agonist 7-OH-DPAT, and the indirect dopamine agonist cocaine in drug self-administration and discrimination assays. Although rats readily acquired high rates of fixed ratio (FR)1 responding for cocaine, experimentally naïve rats failed to acquire responding when 7-OH-DPAT or PF-592,379 were made available during an 18-session acquisition period. Cocaine also maintained dose-dependent levels of responding when available under an FR5 or progressive ratio (PR) schedule of reinforcement. Although, 7-OH-DPAT maintained modest levels responding when substituted under an FR5, it failed to maintain significant levels of PR responding. PF-592,379 maintained saline-like rates of responding when substituted under FR5 or PR schedules of reinforcement. Similar behavioral profiles were observed in cocaine discrimination assays, with 7-OH-DPAT partially substituting for cocaine, and PF-592,379 producing saline-like effects over a wide range of doses. Together, the results of these studies

Corresponding Author: Gregory T. Collins, PhD., Alcohol & Drug Abuse Research Center, McLean Hospital, 115 Mill Street, Mail Stop 214, Belmont, MA 02478, 617-855-2611 (tel), 617-855-3865 (fax), gcollins@mclean.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

predict that highly selective D₃ agonists, such as PF-592,379, will have low abuse potential in humans.

Keywords

Drug Self-administration; Drug Discrimination; cocaine; PF-592,379; Dopamine D₃; Dopamine D₂; Rat

Introduction

The dopamine D₃ receptor has been hypothesized to play an important role in the reinforcing effects of drugs, and other abuse-related behaviors (for reviews see: Heidbreder, 2008; Heidbreder et al., 2005; Heidbreder and Newman, 2010; Newman et al., 2005). Although normally expressed at low levels relative to D₂ receptors, D₃ receptors display a much more restricted, limbic pattern of distribution, with high levels of expression observed in brain regions thought to be important for reward, such as the nucleus accumbens (Gurevich and Joyce, 1999; Levesque et al., 1992; Sokoloff et al., 1990; Stanwood et al., 2000). D₃-preferring agonists, such as quinpirole, quinlorane, and 7-OH-DPAT, have been shown to produce a variety of cocaine-like effects in both monkeys and rats, including dose-dependent leftward shifts in the dose-response curve for cocaine self-administration (Caine and Koob, 1995; Caine et al., 1999, 2000a), dose-dependent increases in cocaine-appropriate responding in drug discrimination assays (Acri et al., 1995; Baker et al., 1998; Barrett and Appel, 1989; Caine et al., 2000b; Katz and Witkin, 1992; Sinnott et al., 1999; Spealman, 1996), and the capacity to dose-dependently maintain self-administration when substituted for cocaine (Caine and Koob, 1993; Collins and Woods, 2007; Nader and Mach, 1996; Sinnott et al., 1999). Although these effects suggest that the D₃ receptor may be involved in mediating the reinforcing effects of cocaine, D₃-preferring and -selective antagonists have generally failed to inhibit the ongoing self-administration of cocaine when the response requirements are low (Achat-Mendes et al., 2010; Gal and Gyertyan, 2003; Xi and Gardner, 2007; Xi et al., 2005; Xi et al., 2006), raising the possibility that activation of the D₃ receptor is not necessary for the reinforcing effects of cocaine.

Interestingly, the capacity of D₃-preferring agonists to maintain self-administration appears to be dependent upon the animal's prior experience with cocaine self-administration (Collins and Woods, 2007; Nader and Mach, 1996), suggesting that their behavioral effects are altered following a history of cocaine administration. Consistent with these findings, experimenter- and self-administered cocaine has been shown to result in an increase in the density/availability/expression of striatal D₃ receptors in both rats (Collins et al., 2011; Conrad et al., 2010; Le Foll et al., 2005; Neisewander et al., 2004) and humans (Segal et al., 1997; Staley and Mash, 1996), as well as an enhancement of the D₃-mediated behavioral effects of D₃-preferring agonists rats and monkeys (Collins et al., 2011; Hamilton et al., 2010). Nevertheless, it is important to note that the D₃-preferring agonist quinlorane failed to maintain responding when substituted for cocaine in mice with a genetic deletion (knock out; KO) of the D₂ receptor (Caine et al., 2002).

Whilst these findings suggests that the reinforcing effects of D₃-preferring agonists are likely mediated by their actions at D₂, rather than D₃ receptors, the relative contributions of the D₂ and D₃ receptors in the discriminative stimulus effects of cocaine are less clear. For instance, not only are D₂- and D₃-preferring agonists equally effective at increasing cocaine-appropriate responding (Achat-Mendes et al., 2010), but D₃-preferring or selective antagonists and D₂-preferring antagonists are equally effective at antagonizing the interoceptive effects of cocaine (Achat-Mendes et al., 2010; Costanza et al., 2001; Martelle

et al., 2007), suggesting that both receptor subtypes may play a role in mediating the discriminative stimulus properties of cocaine.

Although the development of antagonists that are ~100-fold selective for the D₃ over D₂ receptor *in vitro* (Grundt et al., 2005; Reavill et al., 2000), has allowed for significant advances towards understanding the role of the D₃ receptor in abuse-related behaviors, antagonists with a similarly high degree of selectivity for the D₂ receptor do not exist. Moreover, although agonists have been described with *in vitro* radioligand binding selectivities of ~100-fold for the D₃ over D₂ (pramipexole; Millan et al., 2002), and ~200-fold for the D₂ over D₃ receptor (sumanirole; McCall et al., 2005), neither of these compounds are capable of selectively activating the D₃ or D₂ receptor over more than a 30-fold range of doses *in vivo* (Collins et al., 2007), making it difficult to parse the relative contributions of their D₃ and D₂ activities in self-administration and discrimination assays. Similar distinctions are commonly observed between the *in vitro* selectivities obtained using radioligand binding and functional assays (e.g., Sautel et al., 1995), suggesting that *in vitro* functional selectivities may provide a more accurate predictor of *in vivo* activity.

PF-592,379 is a novel agonist at the D₃ receptor that has been shown to possess a high degree of functional selectivity over both the D₂ (>470-fold) and D₄ (180-fold) dopamine receptor subtypes (Attkins et al., 2010). The availability of this highly selective D₃ agonist provides an opportunity to compare its reinforcing and discriminative effects with a mildly D₃-preferring agonist, 7-OH-DPAT, and with the indirect dopamine agonist, cocaine. First, we compared PF-592,379 to 7-OH-DPAT in order to determine its relative selectivity for the D₃ over D₂ receptor in binding and functional assays. Next, we compared the acquisition of self-administration for PF-592,379, 7-OH-DPAT, and cocaine to assess its abuse potential in drug naïve rats. A dose-response analysis of the capacity of PF-592,379, 7-OH-DPAT, and cocaine to maintain responding under fixed ratio (FR) 5 and progressive ratio (PR) schedules of reinforcement was also performed to allow for a comparison of each compound's relative reinforcing effectiveness. Finally, the capacities of PF-592,379 and 7-OH-DPAT to produce cocaine-like interoceptive effects were evaluated in rats that were trained to discriminate cocaine from saline, and compared the substitution profiles of the indirect dopamine agonist d-amphetamine, and the kappa (κ)-opioid agonist U50,488.

Methods

In Vitro Pharmacology

***In vitro* Binding Assays**—The binding affinities of PF-592,379 and 7-OH-DPAT were characterized at each the five dopamine receptor subtypes using the following radioligands: [³H]SCH23390 (D₁ and D₅, 70 Ci mmol, 1 nM), [³H]U-86170 (D₂, 62 Ci/mmol, 2 nM), and [³H]spiperone (D₃ and D₄, 96 Ci/mmol, 0.2 nM). CHO cells expressing recombinant human D₁, D₂, D₃, D₄, and D₅ receptors were rinsed with, and harvested in, ice-cold Ca²⁺/Mg²⁺-free phosphate-buffered saline prior to pelleting (500g, 5 min), resuspension in 25 mM Tris, 5 mM EDTA, and 5 mM EGTA, pH 7.5, and freezing the cells in liquid nitrogen. Upon thawing, the cells were homogenized and centrifuged at 1,000g to remove nuclei and unbroken cells, with the supernatant subsequently centrifuged at 47,000g. The membrane pellet was then washed with Tris, EGTA, EDTA, resuspended in 20 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM MgCl₂, and 1 mM EDTA, and frozen in liquid nitrogen prior to storage of membrane aliquots at -70°C. Membranes were then thawed and diluted into 20 mM HEPES, pH 7.4, 150 mM NaCl, 10 mM MgCl₂, or 1 mM EDTA, 10 mM MgSO₄, with binding reactions carried out in a total volume of 0.9 ml for 1 h at room temperature, and stopped by vacuum filtration. Nonspecific binding was assessed with 3 μM SCH23390 (D₁-like antagonist) or 3 μM haloperidol (D₂-like antagonist). Competition binding experiments employed 11 concentrations of PF-592,379 or 7-OH-DPAT run in duplicate. IC₅₀ values

were determined by fitting the data to a one-site model by nonlinear least-squares minimization, and K_i values were calculated with the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

In vitro Functional Assays—cAMP accumulation was measured using a 24-well plate with intact CHO cells at a density of 15,000 cells/well using methods identical to those of Atkins et al. (2010). Briefly, cells were plated 48 h prior to the experiment, and incubated in serum-free medium 1 h before the experiment. Fresh medium (0.5 ml) containing 100 μ M forskolin, 100 μ M isobutyl methylxanthine, and varying concentrations of 7-OH-DPAT or PF-592,379 were then added to each well. cAMP accumulation was allowed to proceed for 15 min at room temperature, with termination of the reactions achieved by removing the medium and adding 100 μ l of cold 7.5% trichloroacetic acid. Samples were diluted with 1.0 ml of 50 mM sodium acetate, pH 6.2, and aliquots were assayed by radioimmunoassay using the Biomedical Technologies Incorporated (Stoughton, MA) cAMP radioimmunoassay kit.

In Vivo Pharmacology

Subjects—Male Sprague-Dawley rats (280–330g on arrival) were obtained from Charles River (Wilmington, MA, USA), and allowed to acclimate to the laboratory prior to experimentation. All rats were individually housed in Plexiglas cages with kiln-dried pine shavings for bedding in a temperature ($\sim 22^\circ\text{C}$) and humidity ($\sim 55\%$) controlled environment under a 12-h light/dark cycle. Rats had free access to fresh tap water and standard fishmeal-based rat chow (Purina LabDiet 5001, PMI Nutrition International, Brentwood, MO), with weights increasing to 350–500g over the course of the studies. All procedures were performed in accordance with NIH Guide for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee.

Surgical Preparation—To allow for the intravenous self-administration of drugs, a chronic indwelling silastic catheter was implanted under isoflurane anesthesia. Catheters consisted of a 13-cm length of silastic tubing fitted to a 22-gauge guide cannula that was bent at a right angle, encased in dental cement, and anchored with a 0.5-in-diameter circular nylon mesh. The catheter was passed s.c. from the midscapular region and implanted in the right external jugular vein. Rats were allowed a recovery period of at least 7 days, with a prophylactic dose of ticarcillin (approximately 17 mg/kg; IV) dissolved in saline containing heparin (3 USP U/0.1 ml) delivered once daily for 5 days to prevent infection and aid in maintaining catheter patency. Thereafter, catheters were flushed daily with sterile physiological saline containing heparin (3 USP U/0.1 ml). Catheter patency was confirmed daily by checking if blood could be drawn back from the catheter. If blood could not be drawn, catheter patency was assessed by administering a mixture of midazolam and ketamine (0.075/1.5 mg) sufficient to produce anesthesia within ~ 3 sec in animals with patent catheters. Animals with non-functional catheters were either implanted with a second catheter in the left external jugular, or excluded from the study.

Apparatus—All self-administration studies were conducted in operant conditioning chambers (29.5 cm W $\times$ 23.5 cm D $\times$ 20 cm H), which were placed inside sound-attenuating cubicles equipped with a house light and an exhaust fan. Each chamber contained three response levers (ENV-110M; MED Associates Inc., Georgia, VT), with two situated on the front wall of the chamber (3.0 cm above the grid floor and 1.5 cm from the side walls), and a third located at the center of the rear wall (3.0 cm above the grid floor). A single white stimulus light (ENV-221M; MED Associates Inc., Georgia, VT) was located above each of the levers, and the illumination of the stimulus light above the active lever (center rear) served to signal that responding had scheduled consequences. A 3.3 rpm infusion pump (PHM-100; MED Associates Inc., Georgia, VT) was mounted inside each chamber to allow

for the i.v. delivery of drug or saline. Infusions were delivered through a single channel fluid swivel (Lomir Biomedical, Malone, NY) that mounted on a balance arm and attached to a customized spring lead with an inner tygon tubing (Brian Fromant, Cambridge, UK). The other end of the swivel was connected via tygon tubing to a 10 ml syringe situated in the infusion pump to allow for the automated delivery of intravenous injections. Completion of the specified number of responses (i.e., the ratio) on the active lever resulted in the delivery of one unit dose in a volume of 0.056 ml over 3.2 seconds. In conjunction with the start of the infusion, the session entered a 20-second timeout (TO) period, during which time the stimulus light was extinguished, and responding had no scheduled consequence. Following TO, the illumination of the stimulus light above the active lever signaled that responding once again had scheduled consequences.

Drug discrimination studies were performed in operant conditioning chambers that were identical to those described for self-administration studies, with the following exceptions: only two levers were present, and the drug infusion equipment were replaced with a pellet dispenser (ENV-203M; MED Associates Inc., Georgia, VT). Pellet dispensers delivered 45 mg sucrose pellets (A/I Rodent Pellets; P. J. Noyes Co., Lancaster, NH) into a receptacle that was positioned in between the two levers, and 2 cm above the floor. Stimulus lights above each of the levers served to signal that responding had scheduled consequences, however, they did not differentiate the active and inactive levers.

IV Drug Self-Administration Studies

Acquisition Studies—Three groups of 7 drug-naïve rats were used to compare the time course of the acquisition of responding for 0.32 mg/kg/inj of cocaine, 7-OH-DPAT or PF-592,379. These doses of cocaine and 7-OH-DPAT were chosen based on previous reports that they fall on the descending limbs of the dose-response curves in well trained rats (Caine et al., 1999). The dose of PF-592,379 was chosen based on the results of pharmacokinetic studies which suggest that a single i.v. dose of 0.32 mg/kg should produce estimated free drug plasma levels greater than the functional EC₅₀ of PF-592,379 at D3 receptors (Attkins et al., 2010). Each chamber was equipped with three levers, one active and two inactive. Responses on the active lever resulted in drug infusions, and were reinforced under a fixed ratio (FR) 1 TO20-sec schedule of reinforcement, whereas responses on either of the inactive levers were recorded, but had no scheduled consequence. Acquisition of responding was assessed over 18, 2-h sessions, conducted five days per week. The criteria for acquisition were defined as 15 injections per session, and 70% of responding on the active lever over at least three consecutive sessions. These criteria were based on rates of stable responding previously reported for rats maintained with 0.32 mg/kg/inj of either cocaine or 7-OHDPAT (Caine et al., 1999).

Upon completion of the *Acquisition Studies* any rat that retained health parameters within specified criteria as well as a functional catheter was progressed to the *Fixed Ratio and Progressive Ratio Dose-Response Studies*. Naïve animals were added as necessary and trained to respond for cocaine under similar parameters until sufficient group sizes were obtained to evaluate 7-OH-DPAT and PF-592,379 in cocaine-trained rats.

Fixed Ratio Dose-Response Studies—The capacities of cocaine, 7-OH-DPAT and PF-592,379 to maintain responding were examined using rats (n=6) that were trained to self-administer 1.0 mg/kg/inj cocaine under a FR5 TO20-sec schedule of reinforcement. Saline substitutions preceded the substitution of each dose of cocaine, 7-OH-DPAT, and PF-592,379 to allow the reinforcing effects of each dose to be evaluated from an extinction baseline (responding <50% of cocaine). Substitution sessions were initiated with a single non-contingent i.v. dose of the drug that would be available for self-administration, with

each dose available for a minimum of two consecutive 2-h sessions, and there were no stability criteria in place during substitutions (i.e., rats were returned to 1.0 mg/kg/inj cocaine after two consecutive sessions had been completed). The order of cocaine (0.032–1.0 mg/kg/inj; IV), 7-OH-DPAT (0.032–1.0 mg/kg/inj; i.v.), and PF-592,379 (0.01–1.0 mg/kg/inj; i.v.) doses was determined by a Latin square design, with responding for 1.0 mg/kg/inj cocaine re-established and then extinguished (i.e., saline) prior to the substitution of each dose (from saline). All doses of a particular drug were evaluated in succession prior to the evaluation of the next drug. The different unit doses were delivered by adjusting the duration of the infusion so that a 0.032 mg/kg injection was delivered in ~0.32 s, a 0.1 mg/kg injection was delivered in ~1.0 s, a 0.32 mg/kg injection was delivered in ~3.2 s, etc... with all infusion times adjusted for body weight. The initiation of the TO period always coincided with the start of the injection, and was held constant at 20 s regardless of dose.

Progressive Ratio Dose-Response Studies—Upon completion of the dose-response analysis under the FR5 schedule of reinforcement, cocaine, 7-OH-DAT, and PF-592,379 were also evaluated for their capacity to maintain responding under a progressive ratio (PR) schedule of reinforcement. Sessions were initiated with a single non-contingent IV dose of the drug that would be available for self-administration, and the response requirement was incremented after each injection according to a logit function (3, 9, 13, 16, 18...), with sessions terminating if a rat failed to complete a ratio within a 60-min period (i.e., 1-h limited hold), or after 6 h had elapsed, whichever occurred first. After baseline criteria for the PR schedule were met (i.e., at least 10 injections of 1.0 mg/kg cocaine earned over two consecutive sessions with less than 20% variation across sessions), saline was substituted for cocaine until responding occurred at <50% of that maintained by cocaine. Once extinction criteria were met, the reinforcing effects of cocaine (0.032–1.0 mg/kg/inj; n=4), 7-OH-DPAT (0.1–1.0 mg/kg/inj; n=5), and PF-592,379 (0.1–1.0 mg/kg/inj; n=4) were evaluated under the PR schedule of reinforcement. The order of drug and dose were determined by a Latin square design, with each dose available for at least two sessions, and separated by a redetermination of baseline responding for 1.0 mg/kg/inj cocaine and saline. The different unit doses were delivered by adjusting the infusion duration as described above, however, for PR studies the TO period only lasted as long as the infusion duration.

Drug Discrimination Studies

Training Procedures—Following initial shaping of lever responding, rats (n = 12) were trained to discriminate 5.6 mg/kg cocaine from saline using a two-lever discrimination task. On training days, rats received an i.p. injection of either saline or 5.6 mg/kg cocaine, with the sequence of injections randomized except for the following two restrictions: (1) the same injection could not be given for three consecutive sessions, and (2) during each block of 30 training sessions, the numbers of saline and training drug sessions were approximately equal. Five minutes after the injection of saline or cocaine, a 25-min response period began. During this period, the house light and stimulus lights were illuminated and 20 food pellets were available under a FR schedule of reinforcement. The FR was gradually increased from a FR 1 to a FR 10. The positions of the saline- and cocaine-appropriate levers were counterbalanced across rats, with ratio completion on the injection-appropriate lever resulting in the delivery of a single food pellet. Responses on the inappropriate lever reset the ratio requirement on the correct lever. If all 20 food pellets were earned prior to the end of the 25-min response period, the house light and stimulus lights were turned off and the session was terminated. Training sessions were conducted five days per week, and continued until the following three criteria were met for seven of eight consecutive sessions: (1) 80% of responding during the first ratio occurred on the injection-appropriate lever; (2) 90% of the total session responding occurred on the injection-appropriate lever; (3) all available food pellets were earned during saline training sessions.

Testing Procedures—Once training criteria were met, substitution tests began to evaluate the degree to which various drugs were able to produce responding on the cocaine-appropriate lever. The contingencies that were in place for the test sessions were identical to those that were in place during training, with the exception that food was delivered following the completion of an FR10 on either lever. Completion of a ratio on either lever reset the ratio requirements for both levers. The drugs and doses that were tested were as follows: the indirect dopamine agonist cocaine (1.0, 3.2, 5.6, and 10.0 mg/kg; i.p.), the D₃-selective agonist PF-592,379 (0.32, 1.0, 3.2, 10.0, 18.0, and 32.0 mg/kg; s.c.), the D₃-preferring agonist 7-OH-DPAT (0.1, 0.32, 0.56, 1.0, and 1.8 mg/kg; s.c.), the indirect dopamine agonist d-amphetamine (0.1, 0.32, 1.0, and 1.8 mg/kg; i.p.) as a positive control, and the κ -opioid agonist U50,488 (1.0, 3.2, and 10.0 mg/kg; i.p.) as a negative control. The s.c. route was used for the D₃ agonists to facilitate the direct comparison of active doses between the discrimination assay (current studies) and yawning assay (e.g., Collins et al., 2005, 2007, 2009). Test sessions were separated by at least two training sessions, and were typically conducted twice per week. Substitution tests were conducted only if performance during the intervening training sessions met the three criteria described above. If these criteria were not met, additional training sessions were run until responding once again met the criteria for stable discrimination.

Drugs

Cocaine HCl was supplied by NIDA/NIH, batch 11168-1022-43-3. 7-OH-DPAT (($\pm$)-7-Hydroxy-2-dipropylaminotetralin HBr; lot H8653-01K4028) and d-amphetamine sulfate (lot 074K1169) were purchased from Sigma. PF-592,379 (5-[(2*R*,5*S*)-5-methyl-4-propylmorpholin-2-yl]pyridin-2-amine) was provided by Pfizer Inc. (batch E010002137; 901/H/M/1). U50,488 (2-(3,4-dichlorophenyl)-N-methyl-N-[(1*R*,2*R*)-2-pyrrolidin-1-ylcyclohexyl]acetamide) was obtained from Tocris bioscience (batch 2A/79754). All doses reflect the salt form of the drugs. Sterile saline (0.9%) was used as a vehicle, with dose volumes of 1.0 ml/kg i.p. or s.c. for discrimination studies. Infusion volumes varied as a function of dose for i.v. self-administration studies, with the 0.32 mg/kg/inj dose used in the acquisition studies delivered in a volume of 0.056 ml over 3.2 s.

Data Analysis

For self-administration studies, data are presented as the mean number of responses or injections $\pm$ the standard error of the mean (SEM). For self-administration studies in naïve rats, the acquisition criteria were set at 15 injections per session, and 70% of responding on the active lever over at least three consecutive sessions. For substitution studies under the FR5, and PR schedules of reinforcement, the maintenance of significant levels of responding (e.g., lever presses, injections earned, or final ratios achieved) were determined by one-way ANOVA with repeated measures and Newman-Keuls post hoc tests (GraphPad Prism; GraphPad Software Inc., San Diego, CA). For discrimination studies, the data are presented as the mean $\pm$ SEM percent of responding that occurred on the cocaine-appropriate lever, and the mean $\pm$ SEM rate of responding (responses/s). The criterion for full substitution for the cocaine discriminative stimulus was 90% cocaine-appropriate responding.

Results

in vitro Experiments

The results of *in vitro* binding and functional studies comparing PF-592,379 to 7-OH-DPAT are shown in Table 1. *In vitro* binding assays showed that PF-592,379 selectively bound D₃ receptors with a high affinity. Although PF-592,379 also bound to D₄ receptors, it displayed a 19-fold binding selectivity for D₃ over D₄ receptors. PF-592,379 failed to bind D₂, D₁, or D₅ receptors at concentrations of up to 10 μ M, and thus was at least 46-fold selective for D₃

over D₂, D₁, and D₅ receptors. Although 7-OH-DPAT bound to D₃ receptors with a higher affinity than PF-592,379, it failed to do so selectively as it was found to bind to the high affinity site on D₂ receptors at similar concentrations. A similar distinction was observed with regard to the inhibition of forskolin-stimulated cAMP accumulation in CHO cells that stably expressed recombinant human D₃ or D₂ receptors. Not only did PF-592,379 function as a full agonist, but it also displayed a 180-fold functional selectivity for D₃ over D₄ receptors. As was observed in binding studies, PF-592,379 did not show any functional activity at D₂ receptors, suggesting that it displays an *in vitro* functional selectivity of at least 470-fold for the D₃ over D₂ receptors. 7-OH-DPAT displayed a much more limited *in vitro* functional selectivity for the D₃ receptor over both the D₂ (2-fold) and D₄ (35-fold) receptors.

Self-Administration Studies

Acquisition Studies in Naïve Animals—As shown in Figure 1 (left panels), experimentally naïve rats readily acquired responding when 0.32 mg/kg/inj cocaine was available under an FR1 schedule of reinforcement, with almost exclusive responding on the active lever observed by the end of the acquisition period. Although slight increases in active lever responding were observed when 7-OH-DPAT (0.32 mg/kg) was available for injection (Figure 1; center panels), responding was much more variable from day to day, and rats generally failed to display a preference for the active vs. inactive levers. Unlike with either cocaine, or 7-OH-DPAT, responding occurred at very low rates when 0.32 mg/kg/inj PF-592,379 was available for injection, with the average number of injections earned decreasing across the 18-day acquisition period. As shown in Table 2, although a high percentage (5 out of 7, or 71%) of rats acquired responding for cocaine, none of the experimentally naïve rats acquired responding when lever presses resulted in injections of 7-OH-DPAT or PF-592,379. Although 2 of the 7 rats that had access to 7-OH-DPAT met the injection criterion by the end of the acquisition period, it is important to note that these relatively high levels of 7-OH-DPAT intake (4.8 mg/kg or greater) were accompanied by large increases in inactive lever responding, and thus a low proportion of responding directed at the active lever.

Substitution Studies in Cocaine-Trained Animals—In addition to evaluating the acquisition of responding for 7-OH-DPAT and PF-592,379 in naïve rats, a dose-response analysis of the potential reinforcing effects of these compounds was also carried out in rats with a history of cocaine self-administration. As shown in Figure 2 (top panels), when available under an FR5 schedule of reinforcement, cocaine maintained dose-dependent levels of responding [$F(4,20)=16.4$; $p<0.001$], with doses of 0.1 and 0.32 mg/kg/inj maintaining responding at rates that were significantly greater than those maintained by saline ($p<0.001$ for both). Likewise, 7-OH-DPAT also maintained responding in a dose-dependent manner [$F(2,24)=5.0$; $p<0.01$], however, responding was lower than that maintained by cocaine, and only significantly greater than saline at the 0.1 mg/kg/inj dose ($p<0.05$). Unlike with cocaine and 7-OH-DPAT, FR5 responding maintained by PF-592,379 remained low, and was no different than saline when cocaine-trained rats were provided access to a wide range of PF-592,379 doses.

As shown in Figure 2 (bottom panels), a similar profile of responding was also observed when cocaine, 7-OH-DPAT, and PF-592,379 were available for responding under a PR schedule of reinforcement. Under this schedule, dose-dependent [$F(4,12)=41.3$; $p<0.001$], and monotonic increases in responding were observed when ratio completion resulted in cocaine injections maintained, with doses of 0.1 ($p<0.01$) and greater ($p<0.001$) maintaining significantly more responding than saline. Although slight increases in responding were observed, 7-OH-DPAT failed to maintain PR responding at levels that were significantly

different than saline. Low, saline-like levels of responding were observed when PF-592,379 was available for responding under a PR schedule of reinforcement. The average number of injections earned, responses emitted, and final ratio achieved for the maximally effective dose of each drug and saline are summarized in Table 3.

Drug Discrimination Studies

In addition to evaluating the capacity of PF-592,379 and 7-OH-DPAT to maintain responding in experimentally naïve and cocaine-trained rats, these studies also assessed the degree to which they were able to substitute for the interoceptive effects of cocaine in rats trained to discriminate 5.6 mg/kg; i.p. cocaine from saline (Figure 3). Dose-dependent increases in cocaine-appropriate responding were observed following the IP administration of either cocaine or d-amphetamine, with a full substitution (90% cocaine-appropriate responding) observed at doses of 3.2 and 1.0 mg/kg, respectively. Conversely, the κ -opioid agonist U50,488 resulted in nearly exclusive responding on the saline-appropriate lever when administered at doses up to those that suppressed responding to ~10% of baseline. Although dose-dependent increases in cocaine-appropriate responding were observed with 7-OH-DPAT (maximum of 59% cocaine-appropriate responding), cocaine-appropriate responding failed to exceed 90% at doses up to those that suppressed responding to ~10% of baseline. Unlike the intermediate levels of cocaine-appropriate responding that were observed with 7-OH-DPAT, nearly exclusive responding on the saline-appropriate lever (maximum of 3.5% cocaine-appropriate responding) was observed following administration of PF-592,379 at doses up to those suppressed responding to ~15% of baseline.

Discussion

Dopamine D₃-preferring agonists, such as 7-OH-DPAT and quinpirole, are known to maintain self-administration, and dose-dependently increase cocaine-appropriate responding in drug discrimination procedures in mice, rats, and non-human primates suggesting that they possess an increased potential for abuse in humans. However, owing to the limited selectivity of these compounds for the D₃ over D₂ receptors it is unclear which of these receptor subtypes is responsible for the abuse-related effects. The current studies compared the effects of the D₃-preferring agonist 7-OH-DPAT, and the novel D₃-selective agonist PF-592,379, to those of the indirect dopamine agonist cocaine in rat self-administration and drug discrimination assays with three main findings. First, unlike with cocaine, experimentally naïve rats failed to acquire responding for either 7-OH-DPAT, or PF-592,379 when injections were available under an FR1 schedule of reinforcement. Second, although 7-OH-DPAT maintained dose-dependent and significant levels of FR5 responding when it was substituted for cocaine, PF-592,379 failed to maintain responding that was any different from saline; neither maintained significant levels of responding under a PR schedule of reinforcement. Third, unlike 7-OH-DPAT, which produced a dose-dependent, but partial substitution for the interoceptive effects of cocaine, PF-592,379 failed to increase cocaine-appropriate responding when evaluated over a wide range of doses in the drug discrimination assay. When taken together, these findings not only support the notion that the abuse-related effects of D₂-like agonists are mediated by their actions at the D₂ receptor, but they also provide the first evidence to suggest that highly D₃-selective agonists would have a low abuse potential in humans.

In agreement with previous studies assessing the reinforcing effects of D₂-like agonists in drug naïve rhesus monkeys (Nader and Mach, 1996) or rats (Collins and Woods, 2007), 7-OH-DPAT and PF-592,379 (0.32 mg/kg/inj) both failed to support the acquisition of responding when available to naïve rats under an FR1 schedule of reinforcement, whereas responding was readily acquired when 0.32 mg/kg cocaine was available for injection. Although 7-OH-DPAT-maintained responding appeared to increase over time, a dose-

related increase in inactive lever responding was also observed suggesting that 7-OH-DPAT was unable to maintain responding in a schedule-appropriate and selective manner. In contrast, although PF-592,379-maintained responding occurred at moderate levels during the first two days of acquisition, responding decreased over time with rats generally earning fewer than 5 injections per session by the end of the acquisition period. While it is possible that rats may have acquired cocaine-like patterns of responding if larger unit doses of PF-592,379 (or lower unit doses of 7-OH-DPAT) had been evaluated, the doses available for acquisition were chosen based on their position on the descending limb of a self-administration dose-response curve (cocaine and 7-OH-DPAT - Caine and Koob, 1993; Collins and Woods, 2007), or their pharmacokinetic properties in rats (PF-592,379 - Attkins et al., 2010). With respect to PF-592,379, it is known to have good CNS penetration (CSF to unbound plasma concentration ratio of 0.8–1.0; unpublished observation), and to produce pharmacologically relevant blood levels following a single i.v. dose of 0.1 mg/kg, with estimated free drug plasma levels ~140-times greater than the EC₅₀ for PF-592,379 at D₃ receptors following a single i.v. dose of 2 mg/kg (Attkins et al., 2010). Thus, in addition to being well within the range of pharmacologically active doses, that rats earned approximately 3 mg/kg of PF-592,379 during the first few acquisition sessions suggests that the inability of PF-592,379 to support the acquisition of responding did not result from an inactive dose of PF-592,379 being earned.

Despite the limitations of single-dose acquisition studies, differential patterns of responding emerged when 7-OH-DPAT and PF-592,379 were evaluated in rats trained to self-administer cocaine under FR5 or PR schedules of reinforcement. Consistent with previous reports in rats (Caine and Koob, 1993), 7-OH-DPAT maintained dose-dependent and significant levels of responding when injections were available under the FR5 schedule of reinforcement, suggesting that it effectively reinforced responding. While consistent with previous reports in which cocaine self-administration has been shown to play an important role in establishing the response-maintaining effects of D₃-preferring agonists in mice, rats, and monkeys (Caine et al., 2002; Collins and Woods, 2007; Nader and Mach, 1996), 7-OH-DPAT failed to maintain significant levels of responding when substituted for cocaine under a PR schedule of reinforcement suggesting that 7-OH-DPAT functions as a weak reinforcer relative to other drug reinforcers, such as cocaine. Unlike 7-OH-DPAT, low, saline-like rates of responding were observed when the D₃-selective agonist PF-592,379 was substituted for cocaine under either an FR5 or PR schedule of reinforcement, with rats earning fewer than 5 injections per session despite the fact that doses as large as 1.0 mg/kg were available for injection. This inability of PF-592,379 to maintain responding when evaluated over a wide range of doses, and under a variety of self-administration paradigms, strongly suggests that the D₃-selective agonist is devoid of any significant reinforcing properties in rats.

In addition to self-administration tests, drug discrimination procedures are commonly used in assessing a drug's abuse potential as they allow for the identification of pharmacologic mechanism(s) of action that may result in an increased risk for abuse in humans. Unlike the indirect dopamine agonists, cocaine and d-amphetamine, which produced a nearly exclusive selection of the cocaine-appropriate lever, the D₃-preferring agonist 7-OH-DPAT was only capable of producing ~60% cocaine-appropriate responding when administered at a dose large enough to suppress responding. Although this partial effect is consistent with the partial to full substitution profiles commonly reported with other moderately D₃-preferring agonists (~3–30-fold selective for D₃ over D₂), such as 7-OH-DPAT, PD-128,907, quinpirole, and pramipexole (e.g., Acri et al., 1995; Baker et al., 1998; Barrett and Appel, 1989; Caine et al., 2000b; Filip and Przegalinski, 1997; Garner and Baker, 1999; Katz and Witkin, 1992; Terry et al., 1994; Witkin et al., 1991), it is important to note that substitution is typically observed at doses that are known to act at D₂ receptors *in vivo* (Collins et al.,

2005, 2007, 2009). Although 7-OH-DPAT and PF-592,379 both suppressed responding, large doses of 7-OH-DPAT also stimulated locomotor activity; an effect that is thought to be mediated by D₂ receptor activation (Millan et al., 2004). Conversely, when administered at doses as large as 32.0 mg/kg, PF-592,379 produced only a mild inhibition of locomotor activity suggesting that it lacked D₂ agonist activities. Thus, the inability of the D₃-selective agonist PF-592,379 to substitute for the discriminative stimulus effects of cocaine not only distinguishes PF-592,379 from other D₂-like agonists, but these findings also suggest that some degree of D₂ receptor activity is likely necessary for D₃-preferring agonists to produce cocaine-like interoceptive effects in rats.

In addition to this unique behavioral profile of activity, *in vitro* functional assays have also shown that PF-592,379 functions as a full agonist at the D₃ receptor with a functional selectivity of at least 470-fold for the D₃ over D₂ receptor (Attkins et al., 2010). This high degree of selectivity corresponds to PF-592,379 being at least 235-times more D₃-selective than 7-OH-DPAT (current data), and at least 16-times more D₃-selective than pramipexole (Blagg et al., 2007), both of which have been shown to substitute for cocaine in drug self-administration and discrimination assays in rats (Acri et al., 1995; Caine and Koob, 1993; Collins et al., 2012; Filip and Przegalinski, 1997). Given the high levels of predictive validity that are provided by these two assays (for recent reviews see; Carter and Griffiths, 2009; O'Connor et al., 2011), the inability of PF-592,379 to maintain self-administration or substituted for the interoceptive effects of cocaine strongly suggests that highly selective D₃ agonists, such as PF-592,379, will have a low potential for abuse. This lack of abuse potential with PF-592,379 has also been apparent in five clinical studies, conducted in healthy volunteers or patients with osteoarthritis, in which PF-592,379 failed to produce any adverse events suggestive of abuse potential. In contrast to the oxycodone comparator in two of the studies, PF-592,379 was not associated with any reports of euphoric mood, feeling drunk, or disorientation. Furthermore, in a study in which a cognitive test battery was utilized, PF-592,379 did not elicit any of the cognitive changes that are typically associated with drugs of abuse.

In summary, convergent lines of evidence were provided to suggest that the highly D₃-selective agonist PF-592,379 does not possess cocaine-like reinforcing or subjective effects that may lead to an increased potential for abuse in humans. Furthermore, even though naïve rats failed to acquire responding for either of the D₃ agonists, 7-OH-DPAT maintained FR5 responding in rats with a history of cocaine self-administration; PF-592,379 did not. Similarly, although the less selective D₃ agonist, 7-OH-DPAT, produced a dose-dependent substitution for discriminative stimulus effects of cocaine, PF-592,379 failed to do so suggesting that it is devoid of cocaine-like interoceptive effects. Despite the similarities between the behavioral profiles of cocaine and 7-OH-DPAT, it is important to note that 7-OH-DPAT only partially substituted for cocaine in the discrimination assay, and failed to maintain responding when it was available for injection under a PR schedule of reinforcement, suggesting that the abuse potential of even moderately D₃-preferring agonists is relatively low when compared to cocaine. Moreover, when taken together with the marked differences between the *in vitro* functional selectivities of these ligands, the distinct behavioral profiles observed for 7-OH-DPAT and PF-592,379 strongly suggest that the attributes of D₃-preferring agonists that may contribute to an increased abuse potential are mediated by their activities at the D₂, rather than D₃ receptors.

Acknowledgments

We thank Erica Campianelli, Matthew Waddell, John Rosanelli, Sarah Blumenthal, and Christian Moseley for expert technical assistance, and Inge Knudsen for assistance with manuscript preparation. Supported in part by NIDA/NIH T32-DA07252 (GTC) and the Harvard College Research Program (GO).

References

- Achat-Mendes C, Grundt P, Cao J, Platt DM, Newman AH, Spealman RD. Dopamine D3 and D2 receptor mechanisms in the abuse-related behavioral effects of cocaine: studies with preferential antagonists in squirrel monkeys. *J Pharmacol Exp Ther.* 2010; 334:556–565. [PubMed: 20494958]
- Acri JB, Carter SR, Alling K, Geter-Douglass B, Dijkstra D, Wikstrom H, Katz JL, Witkin JM. Assessment of cocaine-like discriminative stimulus effects of dopamine D3 receptor ligands. *Eur J Pharmacol.* 1995; 281:R7–R9. [PubMed: 7589197]
- Attkins N, Betts A, Hepworth D, Heatherington AC. Pharmacokinetics and elucidation of the rates and routes of N-glucuronidation of PF-592379, an oral dopamine 3 agonist in rat, dog, and human. *Xenobiotica.* 2010; 40:730–742. [PubMed: 20836725]
- Baker LE, Svensson KA, Garner KJ, Goodwin AK. The dopamine D3 receptor antagonist PNU-99194A fails to block (+)-7-OH-DPAT substitution for D-amphetamine or cocaine. *Eur J Pharmacol.* 1998; 358:101–109. [PubMed: 9808258]
- Barrett RL, Appel JB. Effects of stimulation and blockade of dopamine receptor subtypes on the discriminative stimulus properties of cocaine. *Psychopharmacology (Berl).* 1989; 99:13–16. [PubMed: 2528777]
- Blagg J, Allerton CM, Batchelor DV, Baxter AD, Burring DJ, Carr CL, Cook AS, Nichols CL, Phipps J, Sanderson VG, Verrier H, Wong S. Design and synthesis of a functionally selective D3 agonist and its in vivo delivery via the intranasal route. *Bioorg Med Chem Lett.* 2007; 17:6691–6696. [PubMed: 17976986]
- Caine SB, Koob GF. Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. *Science.* 1993; 260:1814–1816. [PubMed: 8099761]
- Caine SB, Koob GF. Pretreatment with the dopamine agonist 7-OH-DPAT shifts the cocaine self-administration dose-effect function to the left under different schedules in the rat. *Behav Pharmacol.* 1995; 6:333–347. [PubMed: 11224342]
- Caine SB, Negus SS, Mello NK, Bergman J. Effects of dopamine D(1-like) and D(2-like) agonists in rats that self-administer cocaine. *J Pharmacol Exp Ther.* 1999; 291:353–360. [PubMed: 10490924]
- Caine SB, Negus SS, Mello NK. Effects of dopamine D(1-like) and D(2-like) agonists on cocaine self-administration in rhesus monkeys: rapid assessment of cocaine dose-effect functions. *Psychopharmacology (Berl).* 2000a; 148:41–51. [PubMed: 10663416]
- Caine SB, Negus SS, Mello NK, Bergman J. Effects of dopamine D1-like and D2-like agonists in rats trained to discriminate cocaine from saline: influence of experimental history. *Exp Clin Psychopharmacol.* 2000b; 8:404–414. [PubMed: 10975632]
- Caine SB, Negus SS, Mello NK, Patel S, Bristow L, Kulagowski J, Vallone D, Saiardi A, Borrelli E. Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. *J Neurosci.* 2002; 22:2977–2988. [PubMed: 11923462]
- Carter LP, Griffiths RR. Principles of laboratory assessment of drug abuse liability and implications for clinical development. *Drug Alcohol Depend.* 2009; 105(Suppl 1):S14–S25. [PubMed: 19443137]
- Collins GT, Woods JH. Drug and Reinforcement History as Determinants of the Response-Maintaining Effects of Quinpirole in the Rat. *J Pharmacol Exp Ther.* 2007; 323:599–605. [PubMed: 17675585]
- Collins GT, Witkin JM, Newman AH, Svensson KA, Grundt P, Cao J, Woods JH. Dopamine Agonist-Induced Yawning in Rats: A Dopamine D3 Receptor-Mediated Behavior. *J Pharmacol Exp Ther.* 2005; 314:310–319. [PubMed: 15833897]
- Collins GT, Newman AH, Grundt P, Rice KC, Husbands SM, Chauvignac C, Chen J, Wang S, Woods JH. Yawning and hypothermia in rats: effects of dopamine D3 and D2 agonists and antagonists. *Psychopharmacology (Berl).* 2007; 193:159–170. [PubMed: 17393143]
- Collins GT, Truccone A, Haji-Abdi F, Newman AH, Grundt P, Rice KC, Husbands SM, Greedy BM, Enguehard-Gueiffier C, Gueiffier A, Chen J, Wang S, Katz JL, Grandy DK, Sunahara RK, Woods JH. Proerectile effects of dopamine D2-like agonists are mediated by the D3 receptor in rats and mice. *J Pharmacol Exp Ther.* 2009; 329:210–217. [PubMed: 19136638]

- Collins GT, Truong YN, Levant B, Chen J, Wang S, Woods JH. Behavioral sensitization to cocaine in rats: evidence for temporal differences in dopamine D(3) and D (2) receptor sensitivity. *Psychopharmacology (Berl)*. 2011; 215:609–620. [PubMed: 21207013]
- Collins GT, Cunningham AR, Chen J, Wang S, Newman AH, Woods JH. Effects of pramipexole on the reinforcing effectiveness of stimuli that were previously paired with cocaine reinforcement in rats. *Psychopharmacology (Berl)*. 2012; 219:123–135. [PubMed: 21701814]
- Conrad KL, Ford K, Marinelli M, Wolf ME. Dopamine receptor expression and distribution dynamically change in the rat nucleus accumbens after withdrawal from cocaine self-administration. *Neuroscience*. 2010; 169:182–194. [PubMed: 20435100]
- Costanza RM, Barber DJ, Terry P. Antagonism of the discriminative stimulus effects of cocaine at two training doses by dopamine D2-like receptor antagonists. *Psychopharmacology (Berl)*. 2001; 158:146–153. [PubMed: 11702088]
- Filip M, Przegalinski E. The role of dopamine receptor subtypes in the discriminative stimulus effects of amphetamine and cocaine in rats. *Pol J Pharmacol*. 1997; 49:21–30. [PubMed: 9431548]
- Gal K, Gyertyan I. Targeting the dopamine D3 receptor cannot influence continuous reinforcement cocaine self-administration in rats. *Brain Res Bull*. 2003; 61:595–601. [PubMed: 14519456]
- Garner KJ, Baker LE. Analysis of D2 and D3 receptor-selective ligands in rats trained to discriminate cocaine from saline. *Pharmacol Biochem Behav*. 1999; 64:373–378. [PubMed: 10515316]
- Grundt P, Carlson EE, Cao J, Bennett CJ, McElveen E, Taylor M, Luedtke RR, Newman AH. Novel heterocyclic trans olefin analogues of N-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl}arylcarboxamides as selective probes with high affinity for the dopamine D3 receptor. *J Med Chem*. 2005; 48:839–848. [PubMed: 15689168]
- Gurevich EV, Joyce JN. Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. *Neuropsychopharmacology*. 1999; 20:60–80. [PubMed: 9885786]
- Hamilton LR, Czoty PW, Gage HD, Nader MA. Characterization of the dopamine receptor system in adult rhesus monkeys exposed to cocaine throughout gestation. *Psychopharmacology (Berl)*. 2010; 210:481–488. [PubMed: 20401746]
- Heidbreder C. Selective antagonism at dopamine D3 receptors as a target for drug addiction pharmacotherapy: a review of preclinical evidence. *CNS Neurol Disord Drug Targets*. 2008; 7:410–421. [PubMed: 19128200]
- Heidbreder CA, Newman AH. Current perspectives on selective dopamine D(3) receptor antagonists as pharmacotherapeutics for addictions and related disorders. *Ann N Y Acad Sci*. 2010; 1187:4–34. [PubMed: 20201845]
- Heidbreder CA, Gardner EL, Xi ZX, Thanos PK, Mugnaini M, Hagan JJ, Ashby CR Jr. The role of central dopamine D3 receptors in drug addiction: a review of pharmacological evidence. *Brain Res Brain Res Rev*. 2005; 49:77–105. [PubMed: 15960988]
- Katz JL, Witkin JM. Effects of quinpirole and SKF 38393 alone and in combination in squirrel monkeys trained to discriminate cocaine. *Psychopharmacology (Berl)*. 1992; 107:217–220. [PubMed: 1352052]
- Le Foll B, Diaz J, Sokoloff P. A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning. *Neuroreport*. 2005; 16:175–178. [PubMed: 15671872]
- Levesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, Schott D, Morgat JL, Schwartz JC, Sokoloff P. Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-N,N-di-n-propyl-2-aminotetralin. *Proc Natl Acad Sci U S A*. 1992; 89:8155–8159. [PubMed: 1518841]
- Martelle JL, Claytor R, Ross JT, Reboussin BA, Newman AH, Nader MA. Effects of two novel D3-selective compounds, NGB 2904 [N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-9H-fluorene-2-carboxamide] and CJB 090 [N-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide], on the reinforcing and discriminative stimulus effects of cocaine in rhesus monkeys. *J Pharmacol Exp Ther*. 2007; 321:573–582. [PubMed: 17272677]
- McCall RB, Lookingland KJ, Bedard PJ, Huff RM. Sumanitrole, a highly dopamine D2-selective receptor agonist: in vitro and in vivo pharmacological characterization and efficacy in animal

models of Parkinson's disease. *J Pharmacol Exp Ther.* 2005; 314:1248–1256. [PubMed: 15980060]

Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. *J Pharmacol Exp Ther.* 2002; 303:791–804. [PubMed: 12388666]

Millan MJ, Seguin L, Gobert A, Cussac D, Brocco M. The role of dopamine D3 compared with D2 receptors in the control of locomotor activity: a combined behavioural and neurochemical analysis with novel, selective antagonists in rats. *Psychopharmacology (Berl).* 2004; 174:341–357. [PubMed: 14985929]

Nader MA, Mach RH. Self-administration of the dopamine D3 agonist 7-OH-DPAT in rhesus monkeys is modified by prior cocaine exposure. *Psychopharmacology (Berl).* 1996; 125:13–22. [PubMed: 8724444]

Neisewander JL, Fuchs RA, Tran-Nguyen LT, Weber SM, Coffey GP, Joyce JN. Increases in dopamine D3 receptor binding in rats receiving a cocaine challenge at various time points after cocaine self-administration: implications for cocaine-seeking behavior. *Neuropsychopharmacology.* 2004; 29:1479–1487. [PubMed: 15100700]

Newman AH, Grundt P, Nader MA. Dopamine D3 receptor partial agonists and antagonists as potential drug abuse therapeutic agents. *J Med Chem.* 2005; 48:3663–3679. [PubMed: 15916415]

O'Connor EC, Chapman K, Butler P, Mead AN. The predictive validity of the rat self-administration model for abuse liability. *Neurosci Biobehav Rev.* 2011; 35:912–938. [PubMed: 21036191]

Reavill C, Taylor SG, Wood MD, Ashmeade T, Austin NE, Avenell KY, Boyfield I, Branch CL, Cilia J, Coldwell MC, Hadley MS, Hunter AJ, Jeffrey P, Jewitt F, Johnson CN, Jones DN, Medhurst AD, Middlemiss DN, Nash DJ, Riley GJ, Routledge C, Stemp G, Thewlis KM, Trail B, Vong AK, Hagan JJ. Pharmacological actions of a novel, high-affinity, and selective human dopamine D(3) receptor antagonist, SB-277011-A. *J Pharmacol Exp Ther.* 2000; 294:1154–1165. [PubMed: 10945872]

Sautel F, Griffon N, Levesque D, Pilon C, Schwartz JC, Sokoloff P. A functional test identifies dopamine agonists selective for D3 versus D2 receptors. *Neuroreport.* 1995; 6:329–332. [PubMed: 7756621]

Segal DM, Moraes CT, Mash DC. Up-regulation of D3 dopamine receptor mRNA in the nucleus accumbens of human cocaine fatalities. *Brain Res Mol Brain Res.* 1997; 45:335–339. [PubMed: 9149110]

Sinnott RS, Mach RH, Nader MA. Dopamine D2/D3 receptors modulate cocaine's reinforcing and discriminative stimulus effects in rhesus monkeys. *Drug Alcohol Depend.* 1999; 54:97–110. [PubMed: 10217549]

Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature.* 1990; 347:146–151. [PubMed: 1975644]

Spealman RD. Dopamine D3 receptor agonists partially reproduce the discriminative stimulus effects of cocaine in squirrel monkeys. *J Pharmacol Exp Ther.* 1996; 278:1128–1137. [PubMed: 8819495]

Staley JK, Mash DC. Adaptive increase in D3 dopamine receptors in the brain reward circuits of human cocaine fatalities. *J Neurosci.* 1996; 16:6100–6106. [PubMed: 8815892]

Stanwood GD, Artymyshyn RP, Kung MP, Kung HF, Lucki I, McGonigle P. Quantitative autoradiographic mapping of rat brain dopamine D3 binding with [(125)I]7-OH-PIPAT: evidence for the presence of D3 receptors on dopaminergic and nondopaminergic cell bodies and terminals. *J Pharmacol Exp Ther.* 2000; 295:1223–1231. [PubMed: 11082459]

Terry P, Witkin JM, Katz JL. Pharmacological characterization of the novel discriminative stimulus effects of a low dose of cocaine. *J Pharmacol Exp Ther.* 1994; 270:1041–1048. [PubMed: 7932151]

Witkin JM, Nichols DE, Terry P, Katz JL. Behavioral effects of selective dopaminergic compounds in rats discriminating cocaine injections. *J Pharmacol Exp Ther.* 1991; 257:706–713. [PubMed: 1674533]

- Xi ZX, Gardner EL. Pharmacological actions of NGB 2904, a selective dopamine D3 receptor antagonist, in animal models of drug addiction. *CNS Drug Rev.* 2007; 13:240–259. [PubMed: 17627675]
- Xi ZX, Gilbert JG, Pak AC, Ashby CR Jr. Heidbreder CA, Gardner EL. Selective dopamine D3 receptor antagonism by SB-277011A attenuates cocaine reinforcement as assessed by progressive-ratio and variable-cost-variable-payoff fixed-ratio cocaine self-administration in rats. *Eur J Neurosci.* 2005; 21:3427–3438. [PubMed: 16026480]
- Xi ZX, Newman AH, Gilbert JG, Pak AC, Peng XQ, Ashby CR Jr. Gitajn L, Gardner EL. The novel dopamine D3 receptor antagonist NGB 2904 inhibits cocaine's rewarding effects and cocaine-induced reinstatement of drug-seeking behavior in rats. *Neuropsychopharmacology.* 2006; 31:1393–1405. [PubMed: 16205781]

Figure 1.

Acquisition of self-administration under an FR 1 schedule of reinforcement for 0.32 mg/kg/inj cocaine (Left Panels), 0.32 mg/kg/inj 7-OH-DPAT (Center Panels), and 0.32 mg/kg/inj PF-592-379 (Right Panels). Top row depicts the mean + SEM (n = 7 per drug) number of injections earned during 18 consecutive 2-hr sessions. The mean + SEM (n = 7 per drug) numbers of inactive lever presses are shown in the Bottom row.

Figure 2.

Dose-response curves for responding maintained by cocaine (Left Panels), 7-OH-DPAT (Center Panels), and PF-592-379 (Right Panels) when substituted for 1.0 mg/kg/inj cocaine under a FR 5 (Top Row), or PR (Bottom Row) schedule of reinforcement. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. One-way, repeated measures ANOVA with Newman-Keuls post hoc tests were used to determine doses that maintained significantly more responding than saline injections.

Figure 3.

Effects of the indirect dopamine agonists, cocaine and d-amphetamine, the κ -opioid agonist U50,488 (Left Panels), and the D3 agonists 7-OH-DPAT (Center Panels) and PF-592,379 (Right Panels) in rats trained to discriminate 5.6 mg/kg cocaine from saline. The dose-response curves for each of the drugs on the percent of responding that occurred on the cocaine-appropriate lever are shown in the top row, whereas the effects of each of the drugs on rates of responding are shown in the bottom row.

Table 1*In vitro* pharmacologic profile of PF-592,379 and 7-OH-DPAT at recombinant human dopamine receptors

Receptor	<i>in vitro Binding Affinity</i> <i>Geometric Mean (95%CI)</i>		<i>in vitro Functional Activity</i> <i>Geometric Mean (95%CI)</i>	
	PF-592,379	7-OH-DPAT	PF-592,379 ^I	7-OH-DPAT
hD ₂	IC ₅₀ 10 μM	K _{i(low)} = 410 nM K _{i(high)} = 1.6 nM	EC ₅₀ 10 μM	EC ₅₀ = 2.6 nM
hD ₃	K _i = 215 nM (157–293)	K _i = 1.9 nM	EC ₅₀ = 21 nM (18–30) E _{max} = 95% (89–103)	EC ₅₀ = 1.3 nM
hD ₄	K _i = 4165 nM (3670–4727)	K _i = 110 nM	EC ₅₀ = 3.9 μM (1.6–5.5)	EC ₅₀ 45.2 nM (15.1–135)
hD ₁	IC ₅₀ 10 μM	K _i = 2800 nM	<i>n.d.</i>	<i>n.d.</i>
hD ₅	IC ₅₀ 10 μM	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>

^I functional data for PF-592,379 from Attkins et al., 2010*n.d.* = not determined

Percentage of rats meeting the criteria for acquisition of self-administration of Cocaine, 7-OH-DPAT and PF-592,379 at 0.32 mg/kg per injection IV

Table 2

<i>Acquisition Criteria</i>	Cocaine		7-OH-DPAT		PF-592,379	
	% Met	Sessions to Criteria (SEM)	% Met	Sessions to Criteria (SEM)	% Met	Sessions to Criteria (SEM)
<i>15 Injections per Session</i>	86	11.8 (2.4)	28	15.5 (1.5)	14	3 (n.a.)
<i>70% Active Lever Selection</i>	71	10.4 (2.1)	0	n.a.	0	n.a.
<i>Both Criteria Met</i>	71	11.0 (2.3)	0	n.a.	0	n.a.

n.a. = not applicable

Table 3

Progressive ratio endpoints maintained by the maximally effective dose of cocaine, 7-OH-DPAT and PF-592,379 relative to saline

	Saline	Cocaine (1.0 mg/kg/inf)	7-OH-DPAT (0.32 mg/kg/inf)	PF-592,379 (0.1 mg/kg/inf)
Total Responses	30.4 (18.9)	1895.0 (687.3) **	690.5 (303.7)	18.5 (11.7)
Total Injections	2.5 (1.1)	31.0 (3.4) ***	17.2 (5.4)	1.3 (0.5)
Final Ratio Achieved	13.4 (2.8)	172.0 (40.4) ***	73.5 (29.7)	8.9 (2.1)

**
p<0.01;

p<0.001.

Significant difference from saline as determined by one-way ANOVA with repeated measures and Newman-Keuls post hoc tests.