Double Dissociation of the Effects of Haloperidol and the Dopamine D3 Receptor Antagonist ABT-127 on Acquisition vs. Expression of Cocaine-Conditioned Activity in Rats


Center for Neuroscience Studies (T.J.B., R.J.B.) and Departments of Psychology and Psychiatry (R.J.B.), Queen’s University, Kingston, Canada; Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, Ludwigshafen, Germany (A.B., K.D., B.B., L.U., A.H., H.S., G.G.); and Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, Illinois (J.P.S.)

Received June 11, 2010; accepted August 18, 2010

ABSTRACT

Dopamine receptors play a critical role in reward-related learning, but receptor subtypes may be differentially involved. D2-prefering receptor antagonists, e.g., haloperidol, attenuate acquisition of cocaine-conditioned motor activity at doses that fail to block expression. We compared haloperidol [4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one] with the D3 receptor-prefering antagonist 2,3-di-tert-butyl-6-[4-[3-(4,5-dimethyl-4-[1,2,4] triazol-3-ylisulfanyl)-propyl]-piperazin-1-yl]-pyrimidine hydrochloride (ABT-127), given at D3 receptor-selective doses [i.e., no displacement of \(^{3}H\)3,5-dichloro-N-[2S]-1-ethyl-2-pyrrolidinyl[methyl]-2-hydroxy-6-methoxybenzamide binding, no effects on \(\gamma\)-butyrolactone-induced striatal \(\Delta\)-3,4-dihydroxyphenylalanine; haloperidol accumulation; no attenuation of apomorphine-induced stereotypy]. We hypothesized that haloperidol and ABT-127 will produce a doubly dissociable effect on acquisition versus expression of cocaine-conditioned activity. Rats received three 1-h habitation sessions to activity monitors followed by three 1-h cocaine (10 mg/kg) conditioning sessions. The expression phase (no cocaine injections) took place 48 h later. Haloperidol (50 \(\mu\)g/kg) given during the conditioning phase blocked the acquisition of conditioned activity but failed to block the expression of conditioning when given on the test day. In contrast, ABT-127 (1.0 mg/kg), when given during conditioning, failed to block the acquisition of conditioned activity but blocked the expression of conditioning when administered on the test day. Results suggest that D2 receptors are more critically involved in acquisition than initial expression and D3 receptors are more critically involved in expression than acquisition of conditioned activity based on cocaine.

Introduction

A number of behavioral test procedures have been used to study the role of dopamine (DA) receptors in reward-related learning. One of the easiest and simplest ways to study this type of learning is conditioned activity (Pickens and Crowder, 1967). During this procedure the effects of a pro-DA drug such as cocaine are associated with environmental stimuli. In a subsequent test without drug, the stimulant locomotor response evoked by contextual stimuli alone is termed “conditioned activity.” From a reward-related learning point of view, the environment paired with increased DA neurotransmission produced by cocaine

RECEIVED DECEMBER 9, 2008; ACCEPTED MARCH 3, 2009

This work was supported by the Natural Sciences and Engineering Research Council of Canada (Grant 7881-10) (to R.J.B.).

This study, in part, has been presented previously: Banasikowski TJ, Bespalov A, Drescher K, and Beninger RJ (2007) Double dissociation of the effects of haloperidol and the dopamine D3 receptor-specific antagonist ABT-127 on acquisition vs. expression of cocaine conditioned activity, at 37th Annual Society for Neuroscience Meeting; 2007 Nov. 3–7; San Diego, CA. Society for Neuroscience, Washington, DC. Parts of this study were included in the MSc. thesis of T.J.B.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org doi:10.1124/jpet.110.171348.

The online version of this article (available at http://jpet.aspetjournals.org) contains supplemental material.

ABBREVIATIONS: DA, dopamine; ABT-127, 2,3-di-tert-butyl-6-[4-[3-(4,5-dimethyl-4-[1,2,4] triazol-3-ylisulfanyl)-propyl]-piperazin-1-yl]-pyrimidine hydrochloride; \(\Delta\)-DOPA, \(\Delta\)-3,4-dihydroxyphenylalanine; NSD-1015, 3-hydroxybenzylydrazine dihydrochloride; GBL, \(\gamma\)-butyrolactone 1,4-lactone, 4-butylactone; DHBA, 3,4-dihydroxybenzylamine; ANOVA, analysis of variance; SB-277011, trans-\(\mathrm{N}^{-}[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl][cyclohexyl]-4-quinoilinecarboxamide; BP 897, \(\Delta\)-[4-(2-naphthoylamo)butyl]-4-[2-methoxyphenyl]-1A-piperazine HCl; sal, saline; n.s., not significant; \(^{3}H\)raclopride, \(^{3}H\)3,5-dichloro-N-[2S]-1-ethyl-2-pyrrolidinyl[methyl]-2-hydroxy-6-methoxybenzamide; CPP, conditioned place preference; NAc, nucleus accumbens.
acquired an increased ability to elicit approach and other responses during conditioning (acquisition) that was manifested as conditioned activity during test (expression) (Beninger and Hahn, 1983).

DA receptors are of five subtypes grouped into two families, all of which are G protein-coupled (Jaber et al., 1996). The D1-like family includes D1 and D5 receptors and stimulates the second messenger enzyme adenyl cyclase and, consequently, CAMP. Conversely, the D2-like family includes D2, D3, and D4 receptors and inhibits adenyl cyclase (Anderson and Pierce, 2005).

Treatment with the D2-prefering receptor antagonists pimozide (Beninger and Hahn, 1983; Beninger and Herz, 1986), haloperidol [4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one], raclopride, or sulpiride (Fontana et al., 1993; Dias et al., 2006 but see Cervo and Samanin, 1996), but not metoclopramide (Mazurski and Beninger, 1991), given during the conditioning phase, prevented the acquisition of conditioned activity. However, during the test expression, pimozide (Beninger and Hahn, 1983; Beninger and Herz, 1986) and raclopride (Fontana et al., 1993 but see Cervo and Samanin, 1996) failed to block the expression of conditioned activity. Results suggest that D2 receptors play a more important role in acquiring conditioning than in its expression during the test phase (see Beninger and Basasiski, 2008).

D3 receptors may play a more important role in the expression than the acquisition of conditioned activity. The selective D3 antagonist trans-N-[4-2-(6-cyano-1,2,3,4-tetrahydrosoquinolin-2-yl)ethylcyclohexyl]-4-quinolinecarboxamide (SB-277011) blocked the expression of conditioned activity based on cocaine in mice (Le Foll et al., 2002) or nicotine in rats (Pak et al., 2006). Likewise, the dopamine D3 receptor-prefering partial agonist 1-(4-(2-naphthoylamo)butyl)-4-(2-methoxyphenyl)-1A-piperazine HCl (BP 897) blocked the expression of amphetamine- or cocaine-induced conditioned activity in rats (Aujla et al., 2002; Aujla and Beninger, 2004) and mice (Le Foll et al., 2002). Partial DA agonists are thought to moderately stimulate the receptor during times when endogenous DA tone is lacking or diminished but to antagonize the receptor from full activation during conditions of increased DA release produced by drug-associated cues (Gyertyan et al., 2007). Thus, BP 897 might have acted like a D3 receptor antagonist in these experiments. In the same studies, Aujla et al. (2002) and Aujla and Beninger (2004) found that BP 897 given during the pairing phase had no significant effect on the acquisition of conditioned activity.

The goal of this study was to compare 2,3-di-tert-butyl-6-[4-[3-(4,5-dimethyl-4H-1,2,4-triazol-3-ylsulfanyl)propyl]-piperazin-1-yl]-pyridimine hydrochloride (ABT-127), a novel DA D3-prefering receptor antagonist (Drescher et al., 2002; Unger et al., 2005), and haloperidol, a DA D2-prefering receptor antagonist (Vanhaue et al., 1999), in acquisition and expression of conditioned activity based on cocaine. In the present study, D3-selective doses of ABT-127 were established by using tests of apomorphine-induced stereotypy, basal and quinpirole-inhibited striatal L-DOPA accumulation, and striatal dopamine D2 receptor occupancy using [3H]3,5-dichloro-N-[(2S)-1-ethyl-2-pyrrolidinyl]methyl]-2-hydroxy-6-methoxybenzamine ([3H]raclopride). We hypothesized that systemic treatment with haloperidol will block acquisition but not expression of conditioned activity and systemic injections with ABT-127 will block the expression but not the acquisition of conditioned activity based on cocaine.

Materials and Methods

Tests of D2 receptor occupancy, L-DOPA accumulation, and amphetamine-induced stereotypy were carried out at Abbott Laboratories in Germany using male Sprague-Dawley rats. Rats were obtained from Janvier DeGeneste (St. Isle, France) (body weight of 195–245 g) and housed in a dedicated quiet room maintained on a 12:12 h light-dark schedule (lights on at 6:00 AM) with food and water available ad libitum. Maintenance, compound treatment, and sacrificing of animals were done in compliance with German Animal Protection law (Tierschutzgesetz, Neufassung vom 25.05.1998) and European Council directive 86/609. All experiments were conducted in accordance with Abbott Laboratories’ Animal Welfare Office and the National Institutes of Health Guide for Care and Use of Laboratory Animals in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All animals were treated according to approved protocols, and the experiments were conducted during the light portion of the light/dark cycle.

Conditioned activity studies were carried out at Queen’s University using male Wistar rats. Experimentally naive albino Wistar rats (n = 161) weighing 200 to 225 g upon arrival from Charles River Canada (Montreal, QC, Canada) were housed in pairs or threes in clear Plexiglas cages (45.0 × 25.0 × 22.0 cm). Average temperature in the colony room was 21°C, and humidity was 40 to 70% with reversed light/dark cycle (lights off from 7:00 AM to 7:00 PM). Rats were handled for approximately 1 min every day for 5 days before starting the experiment and maintained with food (LabDiet 5001; PMI Nutrition International, Brentwood, MO) and water continuously available. Treatment of rats was in accordance with guidelines of the Animals for Research Act, the Canadian Council on Animal Care and was approved by the Queen’s University Animal Care Committee.

Drugs. ABT-127 was synthesized at Abbott Laboratories (Ludwigshafen, Germany) and dissolved in distilled water. Haloperidol (Sigma-Aldrich, St. Louis, MO) was prepared in a 20% water solution of dimethyl sulfoxide (Sigma-Aldrich). Injections were administered intraperitoneally in a volume of 1 ml/kg. Apomorphine [6aR-6-methyl-5,6,6a,7-tetrahydroxy-4H-dibenzo(de,ge)quinoline-10,11-diol] (Sigma-Aldrich) was dissolved in 0.5% acetic acid. Quinpirole, 3-hydroxybenzylidene dihydrochloride (NSD-1015), 3,4-dihydroxybenzylamine (DHBA) (Sigma, Deisenhofen, Germany), and γ-butyrolactone, 1,4-lactone, 4-butyrolactone (GBL; Aldrich, Steinheim, Germany) were dissolved in distilled water. Cocaine hydrochloride [3β-hydroxy-1α,5α-hydroprop-2β-carboxylic acid methyl ester benzoate hydrochloride] (Sigma-Aldrich) was dissolved in 0.9% isotonic saline (sal).

Statistical Analysis. All statistical tests were carried out by using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). Hypothesis tests were completed by using α = 0.05, and pairwise comparisons were made with Tukey’s honestly significant difference test.

Experiment 1: ABT-127 and Haloperidol Binding Comparison. Cloned rat and human dopamine D3 and D2 receptors were stably expressed in Sf9 cells (rD3), and human embryonic kidney 293 cells (hD2L, rD2, hD3) were cultured in Dulbecco’s modified Eagle medium and Nut MIX F-12 medium supplemented with 10% fetal calf serum, 1 to 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (R15-802). Detached cells were pelleted at 750g for 5 min, and cell ghosts (permeabilized cells) were prepared by osmotic shock using lysis buffer containing 50 mM Tris-HCl, 10% glycerol, pH 7.4 for 20 min at 4°C, pelleted at 750g for 20 min at 4°C, and then stored at –80°C until use.

The affinities of the compounds for human and rat dopamine D3 or D2 receptors were measured by competition experiments against 0.1

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nM \textsuperscript{[125]}Iiodosulpride and 0.01 nM \textsuperscript{[125]}Iiodospiperone and 1 to 5 µg of cell protein/tube in a total volume of 0.25 ml of D3 or 1 ml of D2, respectively. Assay buffer contained 50 nM Tris-Cl, 120 mM NaCl, 5 mM KCl, 2 mM MgCl\textsubscript{2}, 2 mM CaCl\textsubscript{2}, and 0.1% bovine serum albumin, pH 7.4. Nonspecific binding was defined with 1 µM spiperone. Radioligand binding reactions were carried out at room temperature for 60 min and terminated by rapid filtering through Whatman (Clifton, NJ) GF/B glass fiber filters followed by 3-ml rinses with ice-cold buffer (50 nM Tris-Cl, pH 7.4). Data derived from liquid scintillation counting were analyzed by iterative nonlinear regression analysis (SAS Institute, Cary, NC). Fitting was performed according to formulas described by Feldman (1972). The test compounds were analyzed in triplicate at five to 11 concentrations ranging from 0.1 nM to 10 µM using half- or full-log increments in each receptor-binding assay.

**Experiment 2: Rat Striatal Dopamine D2 Receptor Occupancy (In Vivo Displacement of \textsuperscript{[3H]}raclopride).** Rats were randomly assigned to seven groups (n = 5 per group) and administered ABT-127 (3, 10, and 30 mg/kg i.p.), haloperidol (0.5 mg/kg i.p.), or saline, followed by high specific activity \textsuperscript{[3H]}raclopride (NEN, Boston, MA; 88.2 Ci/mmol, 8 animals) 60 min later. The animals were sacrificed (under slight carbon dioxide anesthesia) 90 min after administration of ABT-127, haloperidol, or saline. The striata (excluding nucleus accumbens) and the cerebellum of each hemisphere (excluding lobule 9 and 10) were immediately dissected, weighed, dissolved in 1 ml of Soluene-350 (PerkinElmer Life and Analytical Sciences), and transferred into a 60°C water bath for 4 h. Next, 15 ml of scintillation fluid (Ultima Gold XR; PerkinElmer Life and Analytical Sciences) was added, and the concentration of accumulated radioactivity (cpm/mg) was determined by liquid scintillation counting (TriCarb 2250 CA; Packard, Meriden, CT). The ratio of specific (striatal – cerebellar) to nonspecific binding (cerebellar) at the time of sacrifice was taken as a measure of available dopamine D2 receptors. Occupancy (%) in ABT-127- or haloperidol-treated animals was calculated as: occupancy (%) = 100 × (1 – D2 drug/D2 vehicle).

**Experiment 3: \textsuperscript{[3H]}DOPA Accumulation in the Rat Striatum.** Rats were assigned to the different treatment groups and received the following compounds: ABT-127 (3.16, 10, 31.6, and 100 mg/kg i.p.) 90 min before sacrifice, quinpirole (0.1 mg/kg i.p.) 45 min before sacrifice, and/or NSD-1015 (100 mg/kg i.p.) immediately followed by GBL (750 mg/kg i.p.) 30 min before sacrifice. All compounds were dissolved in distilled water. The rats were sacrificed by cervical dislocation. The brains were removed, and different brain regions were dissected on ice. Brain regions were rapidly frozen in liquid nitrogen and stored at −80°C until assayed. The brain tissue was homogenized (by 10-s sonification) in 1 ml of 0.1 M perchloric acid containing 50 ng/ml DHBA as internal standard. Homogenates were centrifuged at 10,000 g for 20 min, and the resulting supernatant was mixed with 500 µl of 2 M Tris acetate, pH 8.7, to adjust the pH to 8.4. Samples were added to 10 mg of aluminum oxide powder and mixed for 15 min. The aluminum oxide was spun down by centrifugation (30 s at 10,000 g) and then washed twice with 1 ml of water. The pellet was mixed with 200 µl of 0.2 M perchloric acid for 15 min. The samples were centrifuged at 10,000 g for 30 s, and the resulting supernatants were stored at −80°C until analyzed by high-performance liquid chromatography. The eluate (20 µl) was injected onto a C18 reverse-phase column (SUPELCOSIL LC-18, 3 µM, 15 cm × 4 mm; SUPELCO, Bellefonte, PA). The reference mixture used was 50 ng/ml DHBA, 50 ng/ml \textsuperscript{[3H]}DOPA, 50 ng/ml 3,4-dihydroxyphenylacetic acid, and 100 ng/ml DA. The mobile phase consisted of 90 mM NaH\textsubscript{2}PO\textsubscript{4}, 3 mM octyl sulfate, 20 mM Na\textsubscript{2}EDTA, and 50 mM triethylamine, and it contained 10% methanol. The high-performance liquid chromatography system consisted of an AS3000 autosampler (Thermo Fisher Scientific, Waltham, MA), a P1000 isocratic pump (Thermo Fisher Scientific), a PE Nelson 900 Series Interface (PerkinElmer, Ueberlingen, Germany), and an intro-electrochemical detector (Antec, Leyden, The Netherlands). As the analytical cell, a VT-03 cell (Antec) was used with a 3-mm glass carbon working electrode and a salt bridge Ag/AgCl reference electrode. Oxidation currents were measured at a working potential of 650 mV. Data were integrated and processed with TotalChrom chromatography software (PerkinElmer Life and Analytical Sciences). The \textsuperscript{[3H]}DOPA content was calculated as nanogram/milligram of tissue wet weight.

**Experiment 4: Apomorphine-Induced Stereotypy.** Rats were placed into individual experimental chambers (wire mesh cages of 20 × 26 cm turned upside down) for a 60-min habituation period. The chambers were then pretreated with ABT-127, and after 60 min they were administered apomorphine HCl (1.21 mg/kg s.c.). Control animals received the corresponding vehicle. The measurement of stereotypy was begun 10 min after apomorphine treatment, and the time intervals of 20, 30, 40, 50, and 60 min after apomorphine injection were assessed.

Stereotypy was scored from 0 to 4 according to the following criteria: 0, absence of stereotyped behavior, similar to control; 1, presence of continuous exploration, intermittent sniffing, and movements of head; 2, intense stereotyped movements of head and/or licking and sniffing, continuous exploration; 3, intense licking, with intense sniffing, or head weaving without exploration and locomotion; and 4, intense gnawing confined to a small area without exploration and locomotion.

The scoring of apomorphine-induced stereotypy was done by a technician who was unaware of the nature of the compound that was being tested. During the testing the experimental chambers were arranged in a circle around the experimenter who continually rated animals, going from one to the next; this technique further reduced any possibility of experimental bias. We have previously compared this procedure to one in which the rater is blind to all drug treatments and found no significant differences.

**Experiment 5: Conditioned Activity between Group Comparisons.** For conditioned activity six automated activity chambers were made of clear Plexiglas, measuring 41.0 × 50.0 × 37.0 cm high and housed in wooden, Styrofoam-insulated outer boxes. A set of seven infrared emitters and detectors was positioned at a height of 5.0 cm above the metal rod floor (three on each 41-cm side, four along the 50-cm front and back walls) spaced 10 cm apart. The photocells formed a matrix of 20 squares of equivalent dimensions (approximately 10 × 10 cm) over the chamber surface. The rat had to enter and leave a beam for an activity count to be recorded. Each chamber was illuminated with a 2.5-W incandescent bulb and ventilated by a small fan that also provided background noise. Beam breaks were recorded on an experimenter-controlled circuit board connected to a 1-GHz IBM (White Plains, NY)-compatible computer. For further details of the apparatus see Aujla et al. (2002).

The general experimental protocol consisted of three phases: habituation (three sessions), conditioning (three sessions in experiment 5; four sessions in experiment 6), and testing (one session). All sessions occurred during the dark phase (7:00 AM to 7:00 PM) and consisted of a 1-h placement into one of the activity chambers. An individual rat was always placed into the same chamber, and all groups included at least one rat tested in each of the six chambers.

Animals (n = 125) in all groups received three 1-h habituation trials, one each day, over 3 days during which no drug was administered. The conditioning phase began on the next day and consisted of three 1-h sessions, conditioning days 1 to 3, one every 48 h. The single 1-h test session took place 48 h after conditioning day 3.

**Experiment 5a: Establishing Conditioned Activity.** Two groups were used to establish the conditioned activity paradigm. The unpaired/sal group (n = 9) received saline before conditioning and test sessions, and cocaine (10 mg/kg) was injected in the home cage 1 h after each conditioning session. The paired/sal group (n = 9) received cocaine (10 mg/kg) before each conditioning session and saline before the test; this group was injected with saline in the home cage 1 h after conditioning sessions.

**Experiment 5b: Finding the Dose of Haloperidol in Conditioning Sessions.** Six groups (n = 9) were used to evaluate the
effects of haloperidol on the acquisition of conditioning activity. Groups were injected with haloperidol (0.1, 1.0, 25, 50, 75, and 150 μg/kg) 1 h before being placed into the activity chamber. Immediately before placement in the activity chambers rats received cocaine (10 mg/kg). These groups received a saline injection in their home cage 1 h after the end of each conditioning session. These groups are identified as hal x + paired/sal, where x indicates the dose of haloperidol in microgram/kilogram. On the test day all animals received saline immediately before placement in the activity chambers.

Experiment 5c: Finding the Dose of ABT-127 in the Test Session. Two groups (n = 5) were used to evaluate the effects of ABT-127 on the expression of conditioned activity. During conditioning, rats received cocaine (10 mg/kg) immediately before placement in the activity chambers. One hour after the end of each conditioning session rats received a saline injection in their home cage. On the test day animals were pretreated with ABT-127 (1.0 or 10 mg/kg) 30 min before being injected with saline and placed in the activity chambers. These groups are identified as paired/ABT x + sal, where x indicates the dose of ABT-127 in milligram/kilogram.

Experiment 5d: Haloperidol in the Test Session or ABT-127 in Conditioning. Two additional groups enabled a direct comparison between haloperidol and ABT-127 in acquisition versus expression of cocaine-conditioned activity based on the results from experiments 5b and 5c. One group (n = 8) evaluated the role of haloperidol on the test day (expression). The dose of haloperidol (50 μg/kg) was chosen on the basis of the results of experiment 5b (see below). During conditioning, rats received cocaine (10 mg/kg) immediately before placement in the activity chambers. One hour after the end of each conditioning session rats received a saline injection in their home cage. On the test day animals were pretreated with haloperidol (50 μg/kg) before being injected with saline and placed in the activity chambers. This group is identified as paired/hal 50 + sal.

One group (n = 9) examined the role of ABT-127 during conditioning (acquisition). The dose of ABT-127 (1.0 mg/kg) was chosen on the basis of the results of experiment 5c (see below). Rats were injected with ABT-127 (1.0 mg/kg) 30 min before being placed into the activity chamber. Immediately before placement in the activity chambers rats received cocaine (10 mg/kg). Rats received a saline injection in their home cage 1 h after the end of each conditioning session. The group is identified as ABT 1.0 + paired/sal. On the test day animals received saline immediately before placement in the activity chambers.

Experiment 5e: Haloperidol or ABT-127 in the Test Session After Conditioning with Saline. Two further groups (n = 9) evaluated the effects of haloperidol (50 μg/kg) or ABT-127 (1.0 mg/kg) during the test session after conditioning sessions with saline. During conditioning, rats received saline immediately before placement in the activity chambers. One hour after the end of each conditioning session rats received cocaine (10 mg/kg) in their home cage. On the test day animals were pretreated with haloperidol 1 h or ABT-127 30 min before being injected with saline and placed in the activity chambers. These groups are identified as unpaired/hal 50 + sal and unpaired/ABT 1.0 + sal. Activity data from the last habituation day were compared among the groups using one-way between-subjects ANOVA to test for pretreatment differences. Activity counts averaged across the three conditioning sessions and from the single test day were similarly analyzed by separate one-way-between-subjects ANOVA. Significant main effects were followed by pairwise comparisons.

Experiment 6: Conditioned Activity within Group Comparisons. The purpose of this experiment was to investigate the effects of haloperidol and ABT-127 on expression of conditioned activity in the same rats by using a counterbalanced repeated-measures design. Thus, rats received DA receptor antagonists only before the saline test session but not during the conditioning phase. Two groups of rats (n = 9) received three 1-h habituation sessions over 3 consecutive days. The conditioning phase began the next day and consisted of 1-h sessions, one each day. On conditioning days 1 to 4 (week 1), 5 to 8 (week 2), 9 to 12 (week 3), 13 to 16 (week 4), and 17 to 20 (week 5), the paired/sal group received cocaine (10 mg/kg) and the unpaired/sal group received saline immediately before being placed into the activity chambers. One hour after each conditioning session the unpaired/sal group received cocaine in their home cages and the paired/sal group received saline.

On test days 1, 2, and 4 (weeks 1, 2, and 4), all rats received a saline injection before placement into the activity chambers. On test day 3 (week 3) rats from the paired/sal (n = 5) and unpaired/sal (n = 5) groups were pretreated with 50 μg/kg of haloperidol (paired/hal 50 + sal; unpaired/hal 50 + sal) 1 h before saline injection and placement in the activity chamber. The remaining four animals in each group were pretreated with 1.0 mg/kg of ABT-127 (paired/ABT 1 + sal; unpaired/ABT 1 + sal) 30 min before the saline injection and placed in the activity chambers. Test day 5 (week 5) was like test day 3 except that animals that were previously pretreated with haloperidol on week 3 received ABT-127 and those pretreated with ABT-127 on week 3 received haloperidol. Activity data from the last habituation day were compared among the groups using one-way between-subjects ANOVA to test for pretreatment differences. Test-day activity data were analyzed by using mixed factorial ANOVA. Significant main effects were followed by pairwise comparisons.

Results

Experiment 1: ABT-127 and Haloperidol Binding Comparison. ABT-127 exhibited high affinity in the radioligand binding assays for cloned human dopamine D3 receptors (Kᵢ = 0.98 nM) (see Table 1). All inhibition curves were best-fit by a one-site model, which also was evident by the Hill slope coefficients approaching unity (not shown). ABT-127 had much lower affinity for the human dopamine D2L receptor (Kᵢ = 145 nM), resulting in a selectivity for dopamine D3 receptors of 148-fold. For the rat dopamine D2 and D3 receptors, respective Kᵢ values for ABT-127 were 52 and 2.3 nM, resulting in a selectivity ratio for the rat dopamine D3 over D2 receptors of approximately 23-fold. In contrast, haloperidol was binding with higher affinity to D2 receptors (both human and rat) with the D2 affinity being approximately 14-fold lower than that for D3 receptors (Table 1).

Experiment 2: Rat Striatal In Vivo Dopamine D2 Receptor Occupancy. Figure 1 displays the D2 receptor occupancy using [3H]raclopride. ANOVA revealed a significant effect of group (p < 0.0001). Post hoc pairwise comparisons found that significant displacement of [3H]raclopride was seen in rats treated with 10 and 30, but not 3, mg/kg of ABT-127.

Experiment 3: L-DOPA Accumulation in the Rat Striatum. Striatal L-DOPA accumulation was examined (Fig. 2). ANOVA revealed a significant effect of group (p < 0.0001). Post hoc pairwise comparisons found that significant displacement of [3H]raclopride was seen in rats treated with 10 and 30, but not 3, mg/kg of ABT-127.

<table>
<thead>
<tr>
<th>Receptor Subtype</th>
<th>ABT-127</th>
<th>Haloperidol</th>
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<tbody>
<tr>
<td>Human D3</td>
<td>0.98 (0.89–1.08)</td>
<td>7.6 (7.3–7.8)</td>
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<tr>
<td>Human D2L</td>
<td>145 (130–162)</td>
<td>0.56 (0.52–0.59)</td>
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<td>–0.07</td>
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<td>Rat D3</td>
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<tr>
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</tr>
<tr>
<td>D2/D3</td>
<td>~23</td>
<td>~0.07</td>
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p < 0.0001). Post hoc pairwise comparisons found that only high doses of ABT-127 (30 and 100, but not 3 or 10, mg/kg) were significantly different from the quinpirole group (p < 0.05). In addition, it was found that the quinpirole group was significantly different from both the NSD and NSD + GBL groups (p < 0.05).

Experiment 4: Apomorphine-Induced Stereotypy. ABT-127 at doses of 10 and 30 mg/kg, but not 3 mg/kg, significantly attenuated apomorphine-induced stereotypy in rats (F_{1,46} = 12.0; p < 0.0001; see Fig. 3).

Experiment 5: Conditioned Activity between Group Comparisons. On the last habituation day, when all groups were compared on activity levels ANOVA revealed no significant effect (F_{13,112} = 1.54; n.s.) (data not shown). In experiment 5a (establishing conditioned activity), animals conditioned with cocaine exhibited a marked increase in activity counts averaged across the three conditioning sessions compared with animals conditioned with saline (Fig. 4A). ANOVA revealed a significant group effect (F_{1,17} = 20.27; p < 0.001). On the test day, the paired/sal group continued to exhibited higher activity than the unpaired/sal group (Fig. 4B). ANOVA revealed a significant group effect (F_{1,17} = 39.27; p < 0.001). Thus, conditioned activity was observed.

In experiment 5b (finding the dose of haloperidol in conditioning sessions), activity averaged across the three conditioning sessions among the six groups conditioned with cocaine plus haloperidol revealed a dose-dependent effect on the unconditioned stimulant properties of cocaine (Fig. 4A). A one-way ANOVA including the unpaired and paired control groups from experiment 5a revealed a significant effect of group (F_{7,64} = 19.13; p < 0.001). Post hoc pairwise comparisons found that haloperidol at 0.1, 1.0, 25, and 50 μg/kg, but not 75 and 150 μg/kg, differed from the unpaired/sal group (p < 0.001) but did not differ significantly from the paired/sal group. During the test session (Fig. 4B), the magnitude of the conditioned activity effect seemed to decrease with increasing doses of haloperidol during conditioning. A one-way between-groups ANOVA, including the two groups (paired/sal and unpaired/sal) from experiment 5a, revealed a significant group effect (F_{7,64} = 7.73; p < 0.001). Post hoc pairwise comparison found that groups pretreated with cocaine plus 0.1, 1.0, 25 μg/kg, but not 50, 75, or 150 μg/kg, haloperidol during conditioning were different from unpaired/sal controls (p < 0.01). The paired/sal group differed from groups conditioned with cocaine plus 50, 75, and 150 μg/kg (p < 0.05), but not 0.1, 1.0, and 25 μg/kg, haloperidol. Results indicate that 50 μg/kg of haloperidol during conditioning did not block the unconditioned stimulant effect of cocaine but led to decreased conditioned activity during the test session. This dose was used in the subsequent studies.

In experiment 5c (finding the dose of ABT-127 in the test session), groups were conditioned with 10 mg/kg of cocaine...
for three sessions and pretreated with a dose of ABT-127 (1.0 or 10.0 mg/kg) on the saline test day. ANOVA revealed no significant difference in activity among the paired/ABT 10 + sal, paired/ABT 1 + sal, and paired/sal groups across the three conditioning sessions ($F_{2,24} = 0.261$; n.s.; Fig. 4A). However, on the test day, rats pretreated with either dose of ABT-127 exhibited an attenuation of activity compared with the paired/sal group (Fig. 4B). ANOVA including the two groups from experiment 5a (paired/sal and unpaired/sal) showed a significant group effect ($F_{3,32} = 12.11; p < 0.001$).

Post hoc pairwise comparisons found that the groups pretreated with 1.0 and 10.0 mg/kg ABT-127 were different from the paired/sal group ($p < 0.01$) but not significantly different from the unpaired/sal group, indicating that both doses of ABT-127 blocked conditioned activity during the test session. The lower dose (1.0 mg/kg) was used in subsequent studies.

In experiment 5d (haloperidol in the test session or ABT-127 in conditioning) we aimed to directly compare the effects of haloperidol and ABT-127 on acquisition and expression of conditioned activity. Based on the results obtained in experiments 5b and 5c, we evaluated the 50 mg/kg dose of haloperidol in the test and the 1.0 mg/kg dose of ABT-127 in conditioning. The results for these groups are shown in Fig. 4B along with the hal 50 paired/sal group from experiment 5b and the paired/ABT 1 + sal group from experiment 5c (see inset). Analysis of the four groups revealed that haloperidol blocked acquisition but not expression and ABT blocked expression but not acquisition (ANOVA) revealed a significant drug by phase interaction.

... significant ($p < 0.001$) and †, significant ($p < 0.01$) difference from unpaired/sal by ANOVA; †, significant ($p < 0.05$) difference from paired/sal by ANOVA.
that animals conditioned with saline and given haloperidol or ABT-127 during the test did not exhibit motor impairments.

Experiment 6: Conditioned Activity within Group Comparisons. The purpose of experiment 6 was to examine the effects of haloperidol and ABT-127 in the same animals during expression of conditioned activity using a counterbalanced repeated-measures design. The groups did not exhibit significant differences in activity during the last day of habituation ($F_{1,16} = 1.26$; n.s.) (data not shown). Rats conditioned with cocaine across weeks 1 to 5 showed greater activity in comparison to rats conditioned with saline [mean ($\pm$ S.E.M.) = 1320.57 ($\pm$ 57.36), 628.98 ($\pm$ 55.10), respectively; $F_{1,16} = 75.60$, $p < 0.001$]. On the saline test at the end of weeks 1, 2, and 4, the paired/sal group significantly higher activity than the unpaired/sal group showing the conditioning activity effect (Fig. 5). On test weeks 3 and 5, rats were administered haloperidol or ABT-127 in a counterbalanced activity effect depending on the week. A mixed factorial were necessary for significant inhibition of [$^3$H]raclopride binding and quinpirole-induced l-DOPA accumulation in the rat striatum, suggesting that higher doses of ABT-127 are needed to affect D2 receptor transmission in the rat brain. A displacement of [$^3$H]raclopride binding and attenuation of l-DOPA accumulation was observed with low doses of the dopamine D2-prefering receptor antagonist haloperidol (data not shown).

It is noteworthy that there are no good radioligand tools that would allow us to measure D2 versus D3 receptor occupancy. Both [$^3$H]raclopride that is commonly used in preclinical studies (Kapur et al., 2000; McCormick et al., 2009) and [$^1$C](+)-4-propyl-3,4,4a,5,6,10b-hexahydro-2H-naphth[1,2-b][1,4]oxazin-9-ol that was recently used in humans as a D3-prefering tool (Boileau et al., 2009; Graff-Guerrero et al., 2009) are not selective enough; therefore, additional methods/approaches are necessary. One such approach is to evaluate radioligand binding in a brain area that is enriched in one receptor subtype and not the other. This is why in vivo [$^3$H]raclopride binding was performed in the striatum where D2 expression is high and D3 expression is low but not absent (Bouthenet et al., 1991). Using this approach, ABT-127 (10 and 30 mg/kg) significantly reduced specific striatal [$^3$H]raclopride binding, suggesting that these doses occupied D2 receptors. Additional studies confirmed significant D2 receptor occupancy by high doses of ABT-127: antagonism of quinpirole-induced decrease of l-DOPA accumulation at 30 mg/kg and antagonism of apomorphine-induced stereotypy at 10 and 30 mg/kg. Brain concentrations achieved at the studied doses of ABT-127 would be quite informative. In an unpublished study, rats were dosed (intraperitoneally) with 30 mg/kg of ABT-127, resulting in approximately 54 nM free brain levels. Considering the in vitro binding data (rat D3, $K_i = 2.3$ nM; rat D2, $K_i = 52$ nM), it is quite likely that at 30 mg/kg ABT-127 reaches D2 receptor occupancy (~50%) that is significant enough to observe behavioral and neurochemical effects. These results suggest that the lower doses (1.0 mg/kg) of ABT-127 that were observed to be effective in conditioned activity studies were selective for D3 receptors.

In experiments 5 and 6, animals receiving cocaine during conditioning and saline in the test were more active in the test than control animals that had never received cocaine in the test environment; thus they acquired conditioned activity. Coadministration of cocaine plus haloperidol (50 µg/kg) during conditioning did not have a significant effect on the unconditioned stimulant properties of cocaine but blocked the acquisition of conditioned activity. Treatment with haloperidol (50 µg/kg) during the test session had no significant effect on the expression of conditioned activity. Coadministration of cocaine plus ABT-127 (1.0 mg/kg) during conditioning had no significant effect on the stimulant properties of

**Fig. 5.** Mean ($\pm$ S.E.M.) activity counts per session during the 1-h saline test from experiment 2 (within-group comparisons) for the group that received sal paired with the test environment but cocaine in their home cage (unpaired) and the group that received cocaine (10 mg/kg immediately before) paired with the test environment (paired) when tested with sal on test days 1, 2, and 4 averaged (unpaired/sal and paired/sal), when tested with ABT-127 (1.0 mg/kg 30 min before) plus sal (unpaired/ABT 1 + sal and paired/ABT 1 + sal) and when tested with haloperidol (hal; 50 µg/kg 1 h before) plus sal (unpaired/hal 50 + sal and paired/hal 50 + sal), $*,$ significant ($p < 0.01$) difference from unpaired/sal by ANOVA; $\dagger$, significant ($p < 0.05$) difference from unpaired/hal 50 + sal by ANOVA.
cocaine and did not disrupt the acquisition of conditioned activity. Treatment with ABT-127 (1.0 mg/kg) during the test session after conditioning with cocaine blocked the expression of conditioned activity. Thus, haloperidol blocked acquisition, but not expression, of conditioned activity based on cocaine, and ABT-127 blocked expression, but not acquisition, of conditioned activity based on cocaine, a double dissociation.

It is noteworthy that this double dissociation was observed using compounds with limited rat D2/D3 receptor selectivity. The current approach was based on identifying doses of ABT-127 that did not affect D2 receptor-mediated signaling. Cocaine conditioning experiments were conducted with a dose of ABT-127 that did not produce behavioral or neurochemical effects typical for D2 receptor blockade. Results were similar to what would be expected when using highly selective D3 receptor antagonists (see below).

The observation of enhanced activity in a drug-free state in a test environment previously paired with cocaine is consistent with reports of conditioned activity based on cocaine (Beninger and Herz, 1986) and other psychomotor stimulants (Pickens and Crowder 1967; Mazurski and Beninger, 1991). Control groups that received environment-saline pairings on conditioning days and then received cocaine in their home cage environment 1 h later did not exhibit increased activity during the test session, as reported previously for amphetamine (Mazurski and Beninger, 1991). Therefore, the effect of increased activity in rats having received environment-cocaine pairings can be attributed to the association of environmental stimuli with cocaine rather than a previous history of cocaine treatment.

The finding that haloperidol, given during cocaine conditioning, blocked the acquisition of conditioned activity agrees with other reports using D2-preferring receptor antagonists (Beninger and Hahn, 1983, Beninger and Herz, 1986; Fontana et al., 1993; Dias et al., 2006). Reimer and Martin-Iverson (1994) did not observe a block of acquisition of conditioned activity with haloperidol but they used one dose, and their purpose was explicitly to use a subthreshold dose of haloperidol that could be tested for synergistic effects with the calcium channel blocker nimodipine. Previous reports have found that the expression of conditioned activity based on cocaine or amphetamine is resistant to the effects of dopamine D2-preferring receptor antagonists (Beninger and Hahn, 1983, Beninger and Herz, 1986; Fontana et al., 1993). Inhibition of the unconditioned stimulant effect of cocaine was not necessary to block the acquisition of conditioned activity, as reported previously by Fontana et al. (1993). Thus, at appropriate dose levels, D2-preferring receptor antagonists block the acquisition but not the initial expression of conditioned activity based on amphetamine or cocaine.

The finding that ABT-127 blocked expression, but not acquisition, of conditioned activity is in agreement with the observation that the selective D3 receptor antagonist SB-277011 blocked the expression of conditioned activity based on cocaine in mice (Le Foll et al., 2002) or nicotine in rats (Pak et al., 2006). The dopamine D3 receptor-preferring partial agonist BP 897 is thought to moderately stimulate the receptor during times when endogenous DA tone is lacking or diminished but to antagonize the receptor from full activation during conditions of increased dopamine release produced by drug-associated cues (Gyertyán et al., 2007). Consistent with action as a receptor antagonist, BP 897 blocked expression of conditioned activity based on amphetamine or cocaine in rats or mice (Aujla et al., 2002; Le Foll et al., 2002). In the same study, Aujla et al. (2002) found that BP 897 given during the pairing phase had no significant effect on the acquisition of conditioned activity. Thus, D3 receptor antagonists block expression, but not acquisition of, conditioned activity at doses that have no significant effect on unconditioned activity.

It may be D3 receptors in the nucleus accumbens (NAC) and basolateral amygdala that play a role in the expression of conditioned activity. Thus, Aujla and Beninger (2004) found that bilateral injections of BP 897 into NAC or basolateral amygdala during expression of conditioned activity based on intra-NAC amphetamine blocked the effect. Similar injections of BP 897 into either structure during conditioning had no significant effect.

This is the first study to systematically compare the effects of a D2 and D3 receptor-preferring antagonist in acquisition and expression of conditioned activity, showing a double dissociation. Our results with conditioned activity are consistent with related studies using stimulant self-administration, reinstatement, or conditioned place preference (CPP) techniques. We recently reviewed these studies (Beninger and Banasikowski, 2008) and will provide an overview of the results here. In self-administration studies, when the maintenance of responding relied heavily on conditioned stimuli (e.g., on lean second-order schedules) D3 receptor antagonists (Di Ciano et al., 2003; Claytor et al., 2006; Di Ciano, 2008; but see Martelle et al., 2007) or partial agonists decreased responding (Pilla et al., 1999; Martelle et al., 2007). Likewise, in self-administration studies using progressive ratios, D3 agents decreased break points for cocaine (Xi et al., 2005) and nicotine (Ross et al., 2007). D3 receptor antagonists or partial agonists generally reduced the response-reinstating effects of cues associated with self-administered cocaine (Cervo et al., 2003; Gilbert et al., 2005) or ethanol (Vengeliene et al., 2006). Some studies have reported that D2 receptor antagonists also reduce the response-reinstating effects of cocaine-associated cues (Cervo et al., 2003; Gál and Gyertyán, 2006). Those authors suggest the possibility that D2 and D3 receptors may act at different sites in the brain but further studies are needed to delineate the possible role for D2 and D3 receptors in reinstatement of responding for cocaine produced by cocaine-associated cues.

In CPP studies, D3 receptor antagonists or partial agonists usually blocked expression based on cocaine (Vorel et al., 2002; Duarte et al., 2003; Cervo et al., 2005), nicotine (Pak et al., 2006), d-amphetamine (Aujla and Beninger, 2005), morphine (see Duarte et al., 2003; Frances et al., 2004; Vazquez et al., 2007), or heroin (Ashby et al., 2000), but not food (Duarte et al., 2003). The effects of D3 receptor antagonists or partial agonists on the acquisition of CPP based on cocaine (see Vorel et al., 2002; Duarte et al., 2003; Gyertyán and Gál, 2003; Cervo et al., 2005; Gyertyán et al., 2007), d-amphetamine (Aujla and Beninger, 2005), or opiates (Duarte et al., 2003; but see Ashby et al., 2000) often were nonsignificant.

Changes in D3 receptors have been seen in behaviorally conditioned animals. Rats with a history of cocaine self-administration, after undergoing 30 to 31 days of withdrawal, received a reinstatement test and were killed for assay of D3 receptor binding 24 h later. Increases were found
in the ventral caudate and NAc core (Neisewander et al., 2004). Le Foll et al. (2002) injected mice with cocaine or saline and exposed them to a test environment; saline-treated mice (the unpaired group) received cocaine later in the home cage. On the test day, mice were placed into the test environment after a saline injection for a 30-min session, during which conditioned activity was seen in the paired group, and then they were killed. Results revealed a significant increase in D3 (but not D1 or D2) receptor mRNA and receptors in NAc but not striatum of paired mice. Results showed that changes in D3 receptors were specific to mice that had received drug-environment pairings that produced conditioned activity and implicate D3 receptors in the expression of conditioned activity.

In summary, ABT-127 is a D3 receptor-prefering antagonist. The D2 receptor-prefering antagonist haloperidol blocks the acquisition of conditioned activity based on cocaine at a dose that does not significantly affect expression; the D3 receptor-prefering antagonist ABT-127 blocks the expression of conditioned activity at a dose that does not significantly block acquisition, a double dissociation. These results agree with a number of findings from conditioned activity, drug self-administration, and CPP studies that point to a more important role for D3 receptors in the expression of conditioned activity than in their acquisition. Some studies suggest that behavioral conditioning leads to changes in D3 receptors in the NAc and possibly the ventral striatum. The mechanism underlying the contribution of D3 receptors to the expression of conditioned behaviors remains to be elucidated.

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**Address correspondence to:** R. J. Beninger, Department of Psychology, Queen's University, 62 Arch St., Kingston ON, Canada K7L 3N6. E-mail: beninger@queensu.ca