Brexpiprazole II: Antipsychotic-Like and Procognitive Effects of a Novel Serotonin-Dopamine Activity Modulator

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ABSTRACT

Brexpiprazole (OPC-34712, 7-{4-[4-(1-benzothiophen-4-yl)piperazin-1-yl]butoxy}quinolin-2(1H)-one) is a novel serotonin-dopamine activity modulator with partial agonist activity at serotonin 1A (5-HT$_{1A}$) and D$_{2/3}$ receptors, combined with potent antagonist effects on 5-HT$_{2A}$-, α_{1B}-, and α_{2C}-adrenergic receptors. Brexpiprazole inhibited conditioned avoidance response (ED$_{50}$ = 6.0 mg/kg), apomorphine- or D-amphetamine-induced hyperactivity (ED$_{50}$ = 2.3 and 0.90, respectively), and apomorphine-induced stereotypy (ED$_{50}$ = 2.9) in rats at clinically relevant D$_2$ receptor occupancies. Brexpiprazole also potently inhibited apomorphine-induced eye blinking in monkeys. The results suggest that brexpiprazole has antipsychotic potential. Brexpiprazole induced catalepsy (ED$_{50}$ = 20) well above clinically relevant D$_2$ receptor occupancies, suggesting a low risk for extrapyramidal side effects. Subchronic treatment with phencyclidine (PCP) induced cognitive impairment in both novel object recognition (NOR) and attentional set-shifting (ID-ED) tests in rats. Brexpiprazole reversed the PCP-induced cognitive impairment in the NOR test at 1.0 and 3.0 mg/kg, and in the ID-ED test at 1.0 mg/kg. However, aripiprazole (10 mg/kg) was ineffective in both tests, despite achieving relevant D$_2$ occupancies. In the NOR test, the 5-HT$_{1A}$ agonist buspirone and the 5-HT$_{2A}$ antagonist M100907 [(R)-(2,3-dimethoxyphenyl)[1-(4-fluorophenethyl)piperidin-4-yl]methanol] partially but significantly reversed PCP-induced impairment. Furthermore, the effect of brexpiprazole was reversed by cotreatment with the 5-HT$_{1A}$ antagonist WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate). The results indicate that brexpiprazole has antipsychotic-like activity and robust efficacy in relevant models of cognitive impairment associated with schizophrenia. The effects of brexpiprazole in the cognitive tests are superior to those of aripiprazole. We propose that the pharmacologic profile of brexpiprazole be based on its balanced effects on 5-HT$_{1A}$, D$_2$, and 5-HT$_{2A}$ receptors, with possible modulating activity through additional monoamine receptors.

Introduction

The main strategy for treatment of schizophrenia is based on functional dopamine antagonism. In addition to D$_2$ receptor antagonism, almost all second-generation antipsychotics include antagonism of serotonin 2A (5-HT$_{2A}$) receptors and of α_1-adrenoceptors. Some compounds also affect a variety of other monoamine receptors, such as 5-HT$_{1A/6/7}$ receptors, α_2-adrenoceptors, and histamine and muscarinic receptors. The broad target effects are aimed at either improving efficacy (e.g., potential effects on affective symptoms or cognitive deficits) or mitigating adverse effects related to the central nervous system (CNS), such as extrapyramidal symptoms (EPS), or to the endocrine system, such as hyperprolactinemia (Arnt and Skarsfeldt, 1998; Roth et al., 2004; Arnt et al., 2008; Newman-Tancredi, 2010; Newman-Tancredi and Kleven, 2011).

ABBREVIATIONS: 5-HT, serotonin; CAR, continued avoidance response; CI, confidence interval; CNS, central nervous system; ED, extradimensional; EDR, extradimensional reversal; EPS, extrapyramidal symptoms; ID, intradimensional; ID2R, intradimensional reversal task; ID-ED, attentional set-shifting; LE, Long Evans; LH, Lister hooded; M100907, (R)-(2,3-dimethoxyphenyl)[1-(4-fluorophenethyl)piperidin-4-yl]methanol; MK-801, (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine; NOR, novel object recognition; PCP, phencyclidine; OPC-34712, 7-{4-[4-(1-benzothiophen-4-yl)piperazin-1-yl]butoxy}quinolin-2(1H)-one; ORM-10921, (1S,12bS)-1-(methoxymethyl)-1-methyl-2,3,4,6,7,12b-hexahydro-1H-benzofuro[2,3-a]quinolizine; SB-269970, (R)-3-{2-[2-(4-methylpiperidin-1-yl)ethyl]pyrrolidin-1-ylsulfonyl}phenol; SB-271046, 5-chloro-N-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methylbenzo[b]thiophene-2-sulfonamide hydrochloride; subPCP, subchronic phencyclidine; subVeh, subchronic vehicle; WAY100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate.

Because of tolerability issues, treatment with D_2 antagonists is not considered the optimal strategy to modulate dopaminergic activity, and the discovery and development of D_2 partial agonists has provided a well tolerated treatment with stabilizing effects on dopamine function (Stahl, 2001). So far, only one D_2 partial agonist, aripiprazole, with moderate D_2 intrinsic activity, has reached the market with the approved indications of schizophrenia and bipolar mania and as an add-on treatment of major depression (Fleischhacker, 2005). Another compound with D_2 intrinsic activity similar to aripiprazole is in development (e.g., cariprazine; Kiss et al., 2010; Citrome, 2013), but others with higher D_2 intrinsic activity have seen their development discontinued (e.g., bifeprunox; Newman-Tancredi et al., 2007) because of their insufficient clinical efficacy (Casey et al., 2008).

A key issue for D_2 partial agonism is to determine an optimal level of intrinsic activity (or relative efficacy at the D_2 receptors that would lead to a desirable stabilization of dopaminergic transmission). Too high D_2 intrinsic activity leads to a lack of robust clinical activity and to adverse effects related to increased D_2 receptor tonus, including nausea, vomiting, insomnia, and motor effects, such as hyperkinesias and restlessness (Fleischhacker, 2005; Casey et al., 2008), whereas excessive D_2 antagonist activity results in an increased risk for EPS and increased prolactin secretion (Casey, 1996).

In addition to optimizing D_2 intrinsic activity, modulating other neurotransmitter systems may contribute to improved efficacy and tolerability. The D_2 partial agonist aripiprazole shows 70–90% D_2 receptor occupancy at clinically relevant doses, a partial agonist effect at $5\text{-}HT_{1A}$ receptors, and some antagonism at $5\text{-}HT_{2A}$ receptors (Mamo et al., 2007; Dahan et al., 2009). However, the occupancies at $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptors are considerably lower than that at D_2 receptors in patients with schizophrenia (Mamo et al., 2007), suggesting the receptor profile might not be optimal for clinical efficacy. Accordingly, a broader target profile (e.g., on selected 5-HT receptor and α-adrenoceptor subtypes) may lead to improved clinical efficacy and tolerability in the treatment of schizophrenia, including efficacy on positive symptoms and cognitive deficits, the main factors determining functional outcome in schizophrenia (Green, 1996). A recent meta-analysis study suggested that $5\text{-}HT_{2A}$ antagonism may reduce D_2 antagonist–induced akathisia (Laoutidis and Luckhaus, 2014). Furthermore, a broad pharmacologic profile could offer a wider potential in the treatment of a variety of other CNS disorders and symptoms, such as major depressive disorder and anxiety disorders, including post-traumatic stress disorder (Roth et al., 2004; Arnt et al., 2008; Wong et al., 2008).

Brexpiprazole [OPC-34712, 7-{4-[4-(1-benzothiophen-4-yl)piperazin-1-yl]butoxy}quinolin-2(1H)-one] was discovered by Otsuka Pharmaceutical Co Ltd. (Tokyo, Japan) and is being developed in collaboration with H. Lundbeck A/S (Valby, Denmark). It is a novel serotonin-dopamine activity modulator, combining moderate-intrinsic activity $5\text{-}HT_{1A}$ receptor partial agonism and low-intrinsic activity D_2 receptor partial agonism with antagonist activity on a variety of 5-HT and α-adrenergic receptor subtypes. Its basic in vitro and in vivo pharmacologic profile has been presented in detail in an accompanying paper (Maeda et al., 2014).

In the present study, the in vivo behavioral pharmacologic characteristics of brexpiprazole are evaluated and compared with two other second-generation antipsychotics, aripiprazole and risperidone, using several preclinical animal models and tests relevant to schizophrenia, including apomorphine/D-amphetamine–induced behavioral disturbances in rats and monkeys, conditioned avoidance response (CAR), catalepsy tests, and subchronic phencyclidine (PCP)–induced cognitive impairments in rats.

Materials and Methods

Subjects

Male Wistar rats (CAR, apomorphine-induced hyperactivity and stereotyped behavior, and catalepsy; 126–200 g at time of testing [Japan SLC Inc., Shizuoka, Japan]; and amphetamine-induced hyperactivity and spontaneous locomotor activity; 150–175 g at time of testing [Charles River, Koln, Germany]), male Lister hooded (LH) rats (novel object recognition [NOR]; 220–240 g at time of testing [Charles River]), and male Long Evans (LE) rats (attentional set-shifting [ID-ED]; 180–280 g at the time of testing [Charles River, Wilmington, MA]) were used. Albino Wistar rats are commonly used for standard behavioral testing of CNS compounds, and the pigmented LH and LE strains are preferred for the cognitive tests. These latter strains have been shown to acquire cognitive tests faster and more reliably (Andrews et al., 1995). For this reason, the NOR test was validated using LH rats (Idris et al., 2010; Redrobe et al., 2010), and the ID-ED test was validated with either LH (Goetghebeur and Dias, 2009) or LE rats (Rodefer et al., 2008). However, only the LE rat strain was available in the People's Republic of China, so it was selected for use in the ID-ED test. Rats had food and water available ad libitum, except for specified periods in each test. They were housed 2–4 per cage in standard Makrolon type III cages and were maintained on a 12-hour light/dark cycle (lights on 6:00 or 7:00 AM) in environmentally controlled climate conditions.

Male Cynomolgus monkeys (5–7 years old; Japan Wild Animal Research Center Inc., Kagoshima, Japan; apomorphine-induced eye blinking) were also used. Monkeys were housed individually with 200 g of certified primate diet 5048 (PMI Nutrition International, Shoreview, MN) and 600 ml of tap water daily.

The care and handling of rats was in accordance with relevant guidelines: Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co, Ltd. (revised on 1 April 2004), the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act, Code of Federal Regulations Title 9, Chapter 1, Subchapter A for apomorphine-induced behavior, CAR, and catalepsy tests; the Danish Executive Order No. 1306 of November 23, 2007, for NOR, D-amphetamine–induced hyperactivity, and spontaneous locomotor activity tests; the Minister of Health's 2001 laboratory animal requirements for environment and housing facilities, and the People's Republic of China (GB 14925-2001) for the ID-ED test. The care and handling of monkeys was in accordance with Japan SLC Inc. Experimental Animal Welfare Policy.

Drugs

Brexpiprazole, aripiprazole, and risperidone were synthesized by Otsuka Pharmaceutical Co., Ltd. Apomorphine hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO). The D-amphetamine sulfate and buspirone hydrochloride were obtained from Sigma-Aldrich. Modafinil, PCP, and WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate) were synthesized by H. Lundbeck A/S. Apomorphine was dissolved in saline immediately before injection; brexpiprazole, aripiprazole, and risperidone were suspended in 5% gum arabic–distilled water solution for CAR, apomorphine, and catalepsy studies. In cognition tests, D-amphetamine, and spontaneous locomotor activity studies, brexpiprazole and aripiprazole were dissolved in minimum amounts of 1 mM methanesulphonic acid in 10% HP-β-cyclodextrin solution, adjusted to pH 5.0 using 0.1 mM NaOH. It was confirmed that there was no major difference in plasma

concentration of brexpiprazole at 3 mg/kg between the two vehicles (C. Bundgaard, unpublished data on file). Modafinil was suspended in 0.5% methylcellulose, and phencyclidine, D-amphetamine sulfate, buspirone, and WAY100635 were dissolved in saline. Doses of salts are expressed as free bases, except for tool compounds (e.g., apomorphine, D-amphetamine, PCP). Test compounds for in vivo studies were administered by mouth or subcutaneously in a volume of 5 ml/kg, except in monkey studies (1 ml/kg).

CAR in Rats

The shuttle box (dimensions: $46 \times 19.5 \times 20$ cm; Bio Medica, Osaka, Japan) was placed in a sound-attenuated chamber and subdivided into two compartments by a hurdle (elastic band, 1 mm in width, 3 cm in height). The shuttle box floor was made from stainless steel bars (diameter: 4 mm, and spaced at 1.2 cm). On the upper part of both side panels of chambers, small lamps were mounted, and a house buzzer was set in the center of the chamber ceiling. The position of the animal in the shuttle box was detected by a microswitch attached to the tilting floor. Rats were trained to avoid a scrambled electric shock delivered through the grid floor of the shuttle box. On the first day of training, the rats were habituated for 10 minutes to the shuttle box. From the second day, rats were exposed to a daily session of 20 trials for 7 consecutive days.

Each trial consisted of a 10-second warning tone (105-decibel tone) as a conditioned stimulus followed by a 10-second foot shock (1 mA) as an unconditioned stimulus and a 15- to 75-second (mean; 45 second) intertrial interval. The unconditioned stimulus was terminated when the animal jumped over the hurdle from one compartment to the other or after a cutoff time of 10 seconds. Each rat was placed in one of the compartments of the shuttle box and allowed free exploration for 1 minute before starting the trial.

During the training session, three kinds of responses were recorded: crossing in response to a conditioned stimulus alone was recorded as a CAR; crossing during the unconditioned stimulus presentation was recorded as an escape response; failure to react was recorded as an escape failure. When the animal completed 75% correct avoidances (15 CAR/20 trials) for three consecutive training sessions, it was used for evaluating the effects of compounds in test sessions. On the day after the last training day, well trained animals (4–6 rats per group) were administered the test compounds p.o., 1 hour before the test session, with the exception of aripiprazole, which was given p.o. 2 hours before testing. The test session consisted of 20 trials, and the CAR, escape responses, and escape failures of each animal were recorded. ED_{50} values with 95% confidence intervals (CI) were calculated by nonlinear regression analysis using SAS software (SAS Institute Japan, Tokyo, Japan).

Apomorphine-Induced Hyperactivity in Rats

Brexpiprazole or aripiprazole were administered p.o. at 1 and 2 hours, respectively, before apomorphine injection (0.25 mg/kg s.c.). Five rats were used in each dose group. Thirty minutes before the apomorphine injection, each rat was placed individually in a plastic circular chamber (diameter: 30 cm $\times$ height 30 cm) and acclimated to the new environment. Measurement of locomotor activity was counted over 1 hour starting immediately after the apomorphine injection. The locomotor detection system (Yamashita Giken, Tokushima, Japan) was composed of a fixed pivot at the center of each chamber with six microswitches fitted beneath the perimeter to detect turning movement as the animal passed over them. The apomorphine dose was selected to induce the maximum level of locomotor stimulation while avoiding the stationary stereotyped behaviors that emerge at higher doses (see the following section) (Costall and Naylor, 1973; Arnt et al., 1988; Kikuchi et al., 1995). The ED_{50} values with 95% confidence intervals were calculated by nonlinear regression analysis using SAS software.

Apomorphine-Induced Stereotyped Behavior in Rats

Rats were fasted for 16–20 hours before the administration of test compounds. Brexpiprazole and risperidone were administered p.o.

1 hour before the apomorphine injection (0.7 mg/kg s.c.). Aripiprazole was administered p.o. 2 hours before the apomorphine injection. Six rats were used in each dose group. To habituate them to the test environment, each rat was placed individually in an acrylic cylinder (diameter: 23 cm $\times$ height 30 cm) 30 minutes before the apomorphine-injection.

Stereotyped behavior was recorded by an observer blinded to the treatment groups (compound and dose), for a 1-minute interval every 10 minutes over the 20- to 40-minute period after the apomorphine injection. The total score for the three observations was calculated using the following scoring scale: 0, the appearance of the animals was the same as drug-naive rats; 1, discontinuous sniffing, constant exploratory activity; 2, continuous sniffing, periodic exploratory activity; 3, continuous sniffing, discontinuous biting, gnawing, or licking, and very brief periods of locomotor activity; 4, continuous biting, gnawing, or licking and no exploratory activity (Costall and Naylor, 1973; Arnt et al., 1988; Kikuchi et al., 1995). The dose of apomorphine was selected to induce an average behavioral score of 3 to 4 in the control groups. ED_{50} values with 95% CI were calculated by nonlinear regression analysis using SAS software.

Amphetamine-Induced Hyperactivity in Rats

Locomotor activity in rats was measured using activity boxes equipped with photocells sensitive to infrared light. The activity boxes (Makrolon type III cage, high model) were equipped with four infrared light sources and photocells placed 4 cm above the floor. The locomotor activity was quantified by counting the number of photobeam interruptions. Recording of an activity count required consecutive interruption of adjacent light beams, thus avoiding counts induced by stationary movements. Drug effects were studied as described by Arnt (1995). Briefly, brexpiprazole, aripiprazole, or vehicle was administered p.o. 2 hours before injection of D-amphetamine (0.5 mg/kg s.c.). Eight rats were used in each dose group. The activity was measured for a period of 2 hours. ED_{50} values with 95% CI were calculated by nonlinear regression using GraphPad Prism (version 4; GraphPad Software, San Diego, CA).

Spontaneous Locomotor Activity in Rats

The equipment described for assessing D-amphetamine–induced hyperactivity was used. Brexpiprazole, aripiprazole, or vehicle was administered p.o. 2 hours before placing them in the activity box for assessment of locomotor activity for a 1-hour recording period. Eight rats were used in each dose group. ED_{50} values with 95% confidence intervals were calculated by nonlinear regression using GraphPad Prism (version 4).

Catalepsy in Rats

Rats were fasted for 15 hours before administration of test compounds. Catalepsy was measured at 1, 2, 4, 6, and 8 hours after the administration of each test compound. Six rats were used in each dose group. Measurements were performed three times at each observation time point by an observer blinded to the treatment administered. Rats were forced to hang by their right forepaw on the upper edge of a steel can (diameter: 6 cm $\times$ height 10 cm). A catalepsy response was recorded when the animals remained in the unnatural vertical position for 30 seconds or longer. The potency was determined at the time point of maximum effect for each compound, and ED_{50} values were calculated by nonlinear regression analysis using SAS software.

Apomorphine-Induced Eye Blinking in Monkeys

Four monkeys were used in this study. Initially, they were trained to sit in a specially designed chair. The experiment consisted of four sessions, each involving 2-day trials, conducted at 5-day intervals. On the first day of each session, spontaneous eye blinking and apomorphine-induced eye blinking were measured. The next day, inhibition of apomorphine-induced eye blinking was measured after

pretreatment with brexpiprazole. The measurements were conducted according to the literature (Migler et al., 1993; Kleven and Koek, 1996).

Brexpiprazole or vehicle was administered 4 hours before apomorphine-injection through a catheter (8 French, length 40 cm), inserted through the nose into the stomach, using a volume of 1 ml/kg. Apomorphine (0.16 mg/kg) was injected intramuscularly into the femoral region. The number of eye blinks was counted over a 1-minute period, 5, 15, and 30 minutes after saline or apomorphine injection, by two observers blinded to the treatment administered. Counts at each time point were averaged, and the mean numbers of three time points were summed and expressed as the total score. Statistical significance between spontaneous and apomorphine-induced eye blinking was analyzed by two-tailed paired t test, and the ED_{50} value with 95% CI was calculated by nonlinear regression analysis using SAS software.

NOR in Rats Treated with Subchronic PCP

Test Protocol. NOR testing was performed as described by Redrobe et al. (2010). In brief, animals were habituated to the test arena (dimensions: $95 \times 45 \times 50$ cm) for 10 minutes on day 1. On day 2, individual animals were introduced to the arena for an acquisition session with two identical objects (two opaque Perspex pyramids, $10 \times 10 \times 6$ cm, or two domed glass paperweights, $8 \times 8 \times 8$ cm) for 3 minutes. The animals were then transferred to their home cages for a 1-hour intertrial interval. After this period, the animal was reintroduced into the arena for the test session. Here an object identical to the familiar objects used in the acquisition, as well as a novel object, were placed in the arena, and the animal was allowed to explore during a 3-minute test session. Behavior of rats was recorded by video, and object interaction was scored manually by an observer blinded to the treatment groups. Object exploration was defined as sniffing, licking, or touching the object while facing it. Animals were excluded from the data analysis if they did not reach both of the following criteria during the retention trial: 1) a total exploration time of 15 seconds or above, and 2) a minimum of 2-second exploration time on each object. Treatment groups/subjects and novel/familiar object identity and location were randomized across the experiment.

Drugs and Treatment Schedule. Rats received subchronic PCP (subPCP, 5 mg/kg i.p.) or saline (subchronic [subVeh], i.p.) twice daily at 7:00 AM and 7:00 PM for 7 days, followed by a washout period of 8–9 days before behavioral testing. On the test day, brexpiprazole, aripiprazole, or vehicle was administered p.o. 2 hours before the acquisition trial. M100907 [(R)-(2,3-dimethoxyphenyl)[1-(4-fluoro-phenethyl)piperidin-4-yl]methanol], buspirone, WAY100635, or saline were administered subcutaneously 30 minutes before the acquisition trial. The number of rats in each group is indicated in Figs. 1–3.

Data Analysis. Results are presented as 1) exploration time(s) of novel or familiar object and 2) discrimination index, calculated as the novel object exploration time (Tn) minus the familiar object exploration time (Tf) divided by the total exploration time (Tn + Tf).

Statistics. Paired t test was used to analyze exploration time. One-way analysis of variance followed by appropriate multiple comparison versus control post-hoc analysis (Bonferroni) was used to investigate statistical discrimination index and total exploration differences between test groups ($P < 0.05$).

Exposure. Blood samples were drawn from animals treated with test compounds after completion of the NOR assay ($n = 6$ per test compound group). Drug concentrations were determined in plasma using ultraperformance liquid chromatography followed by tandem mass spectrometry detection in positive-ion electrospray ionization mode. Brain homogenate was prepared by homogenizing the brain 1:4 (v/v) with water:2-propanol: dimethyl sulfoxide (50:30:20 v/v/v) followed by centrifugation and collection of the supernatant. Plasma and brain supernatant samples were frozen at −80°C until analysis.
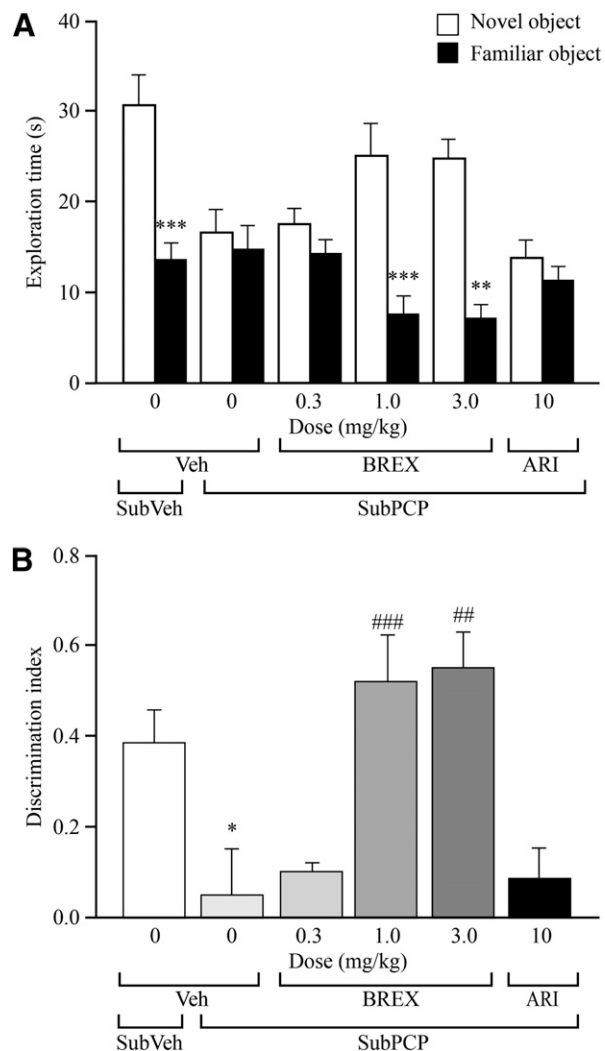

Fig. 1. Effects of brexpiprazole (BREX) and aripiprazole (ARI) on (A) exploration time(s) of familiar and novel objects and (B) discrimination index in a NOR task in rats treated with subchronic PCP. Brexpiprazole and aripiprazole were administered p.o. 2 hours before acquisition trial. Data represent mean ± S.E.M, $n = 11$ (subVeh/vehicle), $n = 11$ (subPCP/vehicle), $n = 10$ (brexpiprazole, 0.3 mg/kg), $n = 10$ (brexpiprazole, 1.0 mg/kg), $n = 6$ (brexpiprazole, 3.0 mg/kg), $n = 8$ (aripiprazole, 10 mg/kg). (A) $**P < 0.01$; $***P < 0.001$ versus familiar object exploration. (B) $*P < 0.05$ versus subVeh/vehicle group; $\#\#P < 0.01$; $\#\#\#P < 0.001$ versus subPCP/vehicle group.

Attentional Set-Shifting in Rats Treated with Subchronic PCP

This test was conducted according to Goetghebeur and Dias (2009) and Goetghebeur et al. (2010), which is a modified version of the protocol described by Birrell and Brown (2000).

Apparatus. The test apparatus (dimensions: $44 \times 64 \times 30$ cm) consisted of a three-compartment black box (a holding area and two choice compartments). A terra cotta pot (diameter: 11 cm), recessed into the floor of the test box, was placed in each choice area. Digging medium cues (e.g., HAMA plastic beads, paper confetti, paper clips, wall plugs) were added to the pots, odor cues (oils from The Body Shop International, London, UK) were applied around the rim of each pot, and all were novel to the rats.

Drugs and Treatment Schedule. Rats received subPCP (5 mg/kg i.p.) or subVeh (saline, i.p.), twice a day at 8:00 AM and 8:00 PM for 7 days, followed by a 7-day washout period before behavioral testing. Brexpiprazole and aripiprazole were administered subcutaneously 1 hour before test. Modafinil (64 mg/kg) was administered p.o. twice,
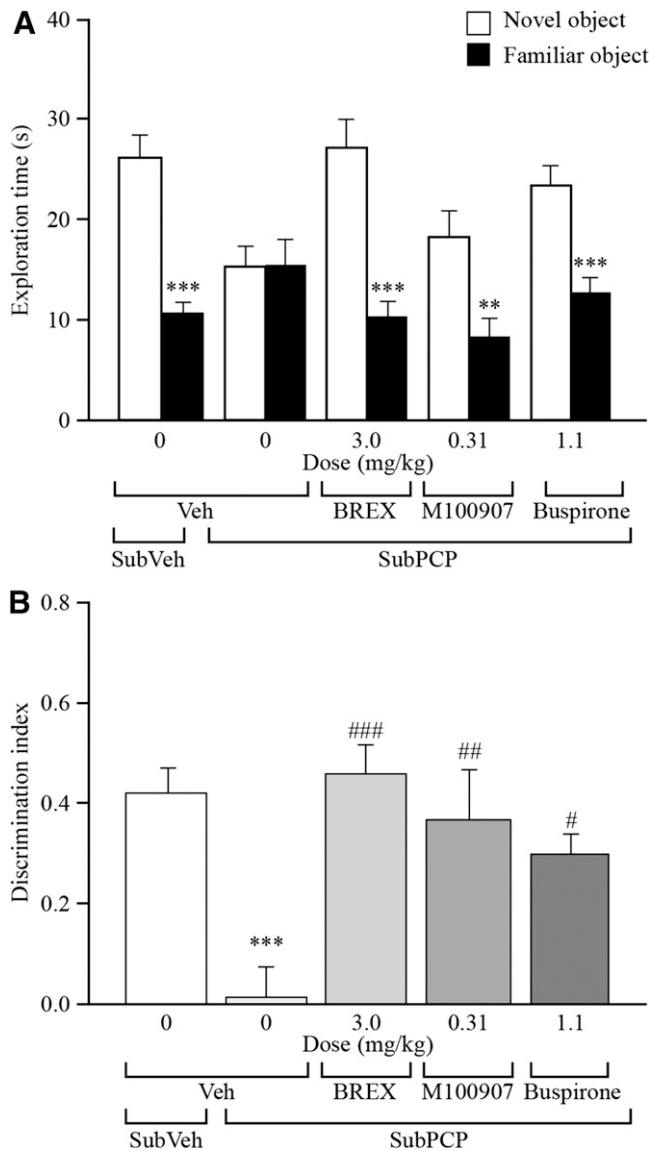
Fig. 2. Effects of M100907 and buspirone in comparison with brexpiprazole (BREX) on (A) exploration time(s) of familiar and novel objects and (B) discrimination index in a NOR task in rats treated with subchronic PCP. M100907 and buspirone were administered subcutaneously 30 minutes before acquisition trial. Brexpiprazole was administered p.o. 2 hours before acquisition trial. Data represent mean ± S.E.M., $n = 12$ (subVeh/vehicle), $n = 11$ (subPCP/vehicle), $n = 8$ (subPCP/brexpiprazole, 3.0 mg/kg), $n = 9$ (subPCP/M100907, 0.31 mg/kg), $n = 9$ (subPCP/buspirone, 1.1 mg/kg). (A) **$P < 0.01$; ***$P < 0.001$ versus familiar object exploration. (B) ***$P < 0.001$ versus discrimination index in the SubVeh/vehicle group; #$P < 0.05$; ##$P < 0.01$; ###$P < 0.001$ versus discrimination in the subPCP/vehicle group.

Fig. 3. Effects of brexpiprazole (BREX) alone and in combination with WAY100635 and of M100907 on (A) exploration time(s) of familiar and novel objects and (B) discrimination index in a NOR task in rats treated with subchronic PCP. M100907 and WAY100635 were administered subcutaneously 30 minutes before acquisition trial. Brexpiprazole was administered p.o., 2 hours before acquisition trial. Data represent mean ± S.E.M., $n = 12$ (subVeh/vehicle), $n = 10$ (subPCP/vehicle), $n = 9$ (subPCP/M100907, 0.013 mg/kg), $n = 6$ (subPCP/M100907, 0.31 mg/kg), $n = 10$ (subPCP/brexpiprazole, 3.0 mg/kg), $n = 11$ (subPCP/brexpiprazole, 3.0 mg/kg + WAY100635, 0.5 mg/kg). (A) *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus familiar object exploration. (B) *$P < 0.05$ versus SubVeh/vehicle group; #$P < 0.05$ versus SubPCP/vehicle group.

each at 32 mg/kg, once 30 minutes before the simple discrimination task and again 30 minutes before the fifth (intradimensional reversal task) discrimination stage because of its short plasma half-life in rats.

Behavioral Testing. On days 4–6 of the washout period, rats were habituated to the arena and learned to dig in test pots filled with cage bedding and food rewards (Honey Loops cereal; Kellogg's, Warrington, UK). On the final day of the washout period (day 7), all rats were presented with two different media and, thereafter, two different odors and were required to learn which of two media or two odors were associated with the food reward. On the day after the 7-day washout period, food-deprived rats were presented with a series of seven discrimination tasks: simple discrimination, compound discrimination, intradimensional shift 1 (ID1), intradimensional shift 2 (ID2), intradimensional reversal task (ID2R), extradimensional shift (ED), and
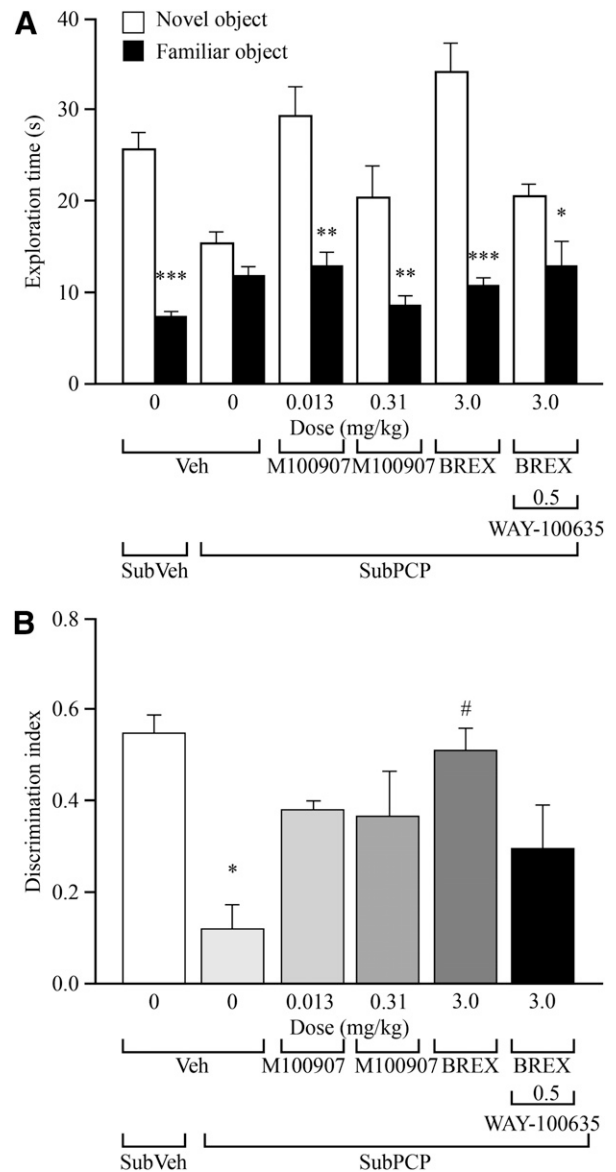
extradimensional reversal task (EDR). Rats only progressed from one discrimination task to the next (always presented in the same order) after reaching a criterion performance level of six consecutive correct responses. Rats were allowed one "discovery" trial at the start of each training or test discrimination task, in which they were allowed to self-correct a dig in the wrong pot.

The number of trials to reach criterion performance was recorded for each of the seven discrimination tasks presented (simple discrimination, compound discrimination, ID1, ID2, ID2R, ED, and EDR). Omissions were defined as an animal's refusal to participate in the task for more than 15 consecutive minutes. After an omission, animals were returned to their home cage to rest for approximately

30 minutes before testing was resumed by continuing the trial from the stage the animal had reached before its omission break. A cutoff time of 6 hours or 100 trials (whichever came first) was imposed, after which the animal was excluded from the study and replaced with another rat. Finally, the order and position in which the individual pots with various digging media/odor combinations appeared across the trials was irregular and random. Drug treatment was counterbalanced between rats, and the experimenter remained blinded to the treatment condition (both subchronic and acute treatment).

Drugs and Treatment Schedule. Rats received PCP (5 mg/kg i.p.) or saline (i.p.) twice a day at 8:00 AM and 8:00 PM for 7 days. Brexpiprazole and aripiprazole were administered subcutaneously 1 hour before the test. Modafinil was suspended in 0.5% methylcellulose to a concentration of 32 mg/ml. Because of its short plasma half-life in rats, modafinil (64 mg/kg) was administered p.o. twice, each at 32 mg/kg, once 30 minutes before the simple discrimination task and again 30 minutes before the fifth (ID2R) discrimination stage.

Statistical Analysis. The number of trials to reach the criterion (six consecutive correct trials) was analyzed with a two-way repeated measure analysis of variance (factor 1: discrimination stage; factor 2: drug treatment). Significant drug treatment $\times$ discrimination stage interactions were further analyzed using the Tukey post-hoc test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SigmaPlot (version 11; Systat Software, San Jose, CA). The experiment was performed by the Contract Research Organization Wuxi Apptech (Shanghai, People's Republic of China).

Results

Brexpiprazole Has Antipsychotic-Like Activity in Rats

CAR. Brexpiprazole induced a dose-dependent inhibition of CAR with an ED_{50} value of 6.0 mg/kg p.o., similar to risperidone ($ED_{50} = 3.3$ mg/kg p.o.; Table 1). Aripiprazole also inhibited CAR in a dose-dependent manner with an ED_{50} value of 23 mg/kg p.o., which is significantly less potent than that of brexpiprazole. Moreover, although brexpiprazole did not induce any nonspecific escape failures at the dose range tested (1.5–12 mg/kg p.o.), aripiprazole and risperidone induced escape failure at a rate of 5 and 30% at the highest doses used (60 and 10 mg/kg p.o.), respectively (data not shown).

Apomorphine-Induced Hyperactivity and Stereotyped Behavior. Brexpiprazole dose-dependently inhibited apomorphine-induced locomotor hyperactivity with an ED_{50} value of 2.3 mg/kg p.o. and apomorphine-induced stereotyped behavior with an ED_{50} value of 2.9 mg/kg p.o. (Table 1). In the same tests, aripiprazole was found to be slightly less potent than brexpiprazole, with ED_{50} values of 3.2 (locomotor hyperactivity) and 6.1 (stereotyped behavior) mg/kg. With similar potency to brexpiprazole, risperidone also inhibited apomorphine-induced stereotyped behavior with an ED_{50} value of 4.7 mg/kg p.o. (Table 1).

Amphetamine-Induced Hyperactivity. The difference in potency of the inhibitory effects of brexpiprazole and aripiprazole against hyperactivity induced by D-amphetamine ($ED_{50} = 0.92$ and 3.9 mg/kg, respectively) paralleled the results obtained with apomorphine. The inhibitory effect of brexpiprazole was more potent than that of aripiprazole (Table 1).

Inhibition of Spontaneous Locomotor Activity. Like other D_2 partial agonists and D_2 antagonists, brexpiprazole ($ED_{50} = 3.4$ mg/kg p.o.) and aripiprazole ($ED_{50} = 6.1$ mg/kg p.o.) inhibited spontaneous locomotor activity at slightly higher than or similar doses at which they inhibited the dopaminergic stimulant-induced behaviors (Table 1).

Catalepsy. Catalepsy was observed only at high doses of brexpiprazole and aripiprazole, with ED_{50} values of 20 and 42 mg/kg p.o., respectively (Table 1). The dose ratios of cataleptogenic activity against the inhibitory effect on dopaminergic stimulant-induced behavior and CAR tests were 3.3–21.7 for brexpiprazole and 1.8–13.1 for aripiprazole, respectively. In comparison, dose ratios of risperidone were 1.4–2.0 (Table 1).

Brexpiprazole Inhibits Apomorphine-Induced Eye Blinking in Monkeys

Apomorphine induces a characteristic eye blinking response in Cynomolgus monkeys (Table 2). Brexpiprazole potently antagonizes the apomorphine-induced eye blinking response with an ED_{50} value of 0.03 mg/kg (95% CI; 0.0004–0.08 mg/kg p.o.; for individual data, see Table 2).

Brexpiprazole Reverses Cognitive Deficits Induced by Subchronic PCP Treatment

Subchronic PCP treatment is known to impair cognitive performance in various tests (Rodefer et al., 2008; Neill et al., 2010). Two tests were selected for characterization of brexpiprazole and aripiprazole in the present studies: NOR and ID-ED.

TABLE 1

Overview of effects in rat test models of positive psychotic symptoms of schizophrenia and CNS side effects

Results are expressed as ED_{50} values (milligram per kilogram) with 95% CI. Drug pretreatment times are shown in parentheses with exceptions in footnotes.

Effect	No. in Group	ED_{50}		
		Brexpiprazole (1 h)[a]	Aripiprazole (2 h)[b]	Risperidone (1 h)[c]
		mg/kg p.o. (95% CI)		
Efficacy tests				
CAR	4–6	6.0 (4.3–9.7)	23 (20–27)	3.3 (2.0–7.6)
APO hyperactivity (0.25 mg/kg)	5	2.3 (1.2–3.1)	3.2 (1.9–5.0)	NT
APO stereotypy (0.7 mg/kg)	6	2.9 (2.2–3.8)	6.1 (5.2–7.2)	4.7 (2.9–6.9)
AMPH hyperactivity (0.5 mg/kg)	8	0.92 (0.6–1.5)	3.9 (2.0–7.7)	NT
CNS side effects				
Inhibition of spontaneous locomotor activity	8	3.4 (2.4–5.0)	6.1 (2.3–16)	NT
Catalepsy (at time of maximum effect)	6	20 (12–40)	42 (26–68)	6.6 (2.9–16)

AMPH, amphetamine; APO, apomorphine; NT, not tested.
[a]Except for spontaneous locomotor activity, amphetamine-induced hyperactivity (2 hours) and catalepsy (maximum effect at 6 hours).
[b]Except for catalepsy (maximum effect at 8 hours).
[c]Except for catalepsy (2 hours).

TABLE 2

Effect of brexpiprazole on apomorphine-induced eye blinking rate in Cynomolgus monkeys

Spontaneous and apomorphine- (0.16 mg/kg i.m.) induced scores are expressed for each individual monkey as the mean ± S.E.M. of observations in four test blocks. Individual scores in brexpiprazole-treated monkeys are presented for each monkey.

Effect	Total Counts of Eye Blinking			
	Animal 1	Animal 2	Animal 3	Animal 4
Spontaneous eye blinking	65 ± 4.5	19 ± 1.5	17 ± 2.3	38 ± 3.3
Apomorphine-induced eye blinking	112 ± 4.2[a]	61 ± 4.2[b]	60 ± 5.7[b]	85 ± 5.3[b]
Inhibitory effect of brexpiprazole (mg/kg p.o.) on apomorphine-induced eye blinking				
0.03	60	59	56	54
0.1	59	33	29	52
0.3	40	18	17	37
1.0	19	20	16	30

[a]$P < 0.05$, statistically significant compared with spontaneous eye blinking (paired t test, two-tailed).
[b]$P < 0.01$, statistically significant compared with spontaneous eye blinking (paired t test, two-tailed).

NOR. The test was performed in three separate experiments (Figs. 1–3). During the 3-minute test trial in each experiment, the subVeh rats, treated acutely with vehicle on the test day, spent significantly more time exploring a novel object than a familiar object ($P < 0.001$). In contrast, subPCP animals, treated acutely with vehicle on the test day, demonstrated significant impairment of NOR by spending approximately equal amounts of time exploring both a familiar and a novel object.

In the first experiment, brexpiprazole at doses of 0.3, 1.0, and 3.0 mg/kg p.o., and aripiprazole at a single high dose of (10 mg/kg p.o.) were studied. As shown in Fig. 1A (exploration time) and Fig. 1B (discrimination index, i.e., time spent exploring the novel versus familiar object, adjusted for total exploration time), acute administration of brexpiprazole (1.0 and 3.0 mg/kg p.o.) significantly attenuates subPCP-induced deficits in exploration ($P < 0.001$ and $P < 0.01$, respectively) and in discrimination index ($P < 0.001$ and $P < 0.01$, respectively). In contrast, aripiprazole (10 mg/kg p.o.) was ineffective. Neither brexpiprazole nor aripiprazole significantly affected total object exploration time at any dose tested (data not shown).

In the second experiment, the effect of brexpiprazole was compared with those of the 5-HT$_{2A}$ antagonist M100907 and the 5-HT$_{1A}$ partial agonist buspirone, as shown in Fig. 2, A and B. Acute administration of brexpiprazole (3.0 mg/kg p.o.), M100907 (0.31 mg/kg s.c.), and buspirone (1.1 mg/kg s.c.) attenuated subPCP-induced deficits in exploration, with statistical significance of $P < 0.001$, $P < 0.01$, and $P < 0.001$, respectively (Fig. 2A). Aripiprazole (20 mg/kg p.o.) was also included in this experiment, but less than half of the group (only four rats) complied with the test criteria; accordingly, the result was considered inconclusive and is not shown. However, plasma concentration of aripiprazole at that time was measured (Table 3). As shown in Fig. 2B, the subPCP-induced decrease in discrimination index is fully reversed by brexpiprazole at 3.0 mg/kg p.o. ($P < 0.001$), and partially but significantly attenuated by M100907 (0.31 mg/kg s.c., $P < 0.01$) and buspirone (1.1 mg/kg s.c., $P < 0.05$). At the doses tested, none of the drugs had any significant effects on total object exploration time (data not shown).

In the third experiment, the goal was to extend the dose range studied for M100907 and to explore whether the 5-HT$_{1A}$ antagonist WAY100635 would attenuate the effect of brexpiprazole. Figure 3A shows that subPCP-induced deficits in novel object exploration were significantly attenuated by M100907

(0.013 and 0.31 mg/kg s.c., $P < 0.01$ and $P < 0.01$, respectively), brexpiprazole (3.0 mg/kg p.o., $P < 0.001$), and brexpiprazole (3.0 mg/kg p.o.) coadministered with WAY100635 (0.5 mg/kg s.c., $P < 0.05$). Also, as shown in Fig. 3B, the decrease in discrimination index induced in the subPCP group was significantly attenuated after acute administration of brexpiprazole (3.0 mg/kg p.o., $P < 0.05$). In contrast, acute administration of M100907 (0.013 and 0.31 mg/kg s.c.) failed to significantly reverse discrimination index, although a numeric reversal was shown. Finally, when brexpiprazole (3.0 mg/kg p.o.) was coadministered with WAY100635 (0.5 mg/kg s.c.), it no longer reversed the subPCP-induced decrease in discrimination index. Total exploration time was significantly increased in the third experiment for brexpiprazole (3.0 mg/kg p.o.) and M100907 (0.013 mg/kg s.c.), compared with the subPCP-Veh group ($P < 0.05$), whereas M100907 (0.31 mg/kg s.c.) and brexpiprazole (3.0 mg/kg p.o.) cotreatment with WAY100635 (0.5 mg/kg s.c.) had no significant effect on object exploration time (data not shown).

Plasma Exposure. After completion of the NOR experiments—about 3 hours after administration of compounds—plasma concentrations of brexpiprazole and aripiprazole were measured. Mean plasma levels of brexpiprazole were 11, 31, and 74–113 (range based on three experiments) ng/ml at 0.3, 1.0, and 3.0 mg/kg p.o., respectively (Table 3). The mean plasma levels of aripiprazole were 40 and 158 ng/ml at 10 and 20 mg/kg p.o., respectively.

ID-ED. Figure 4 shows that subPCP treatment leads to a specific impairment in ED set-shifting 1 week after PCP withdrawal. The impairment is measured as an increased

TABLE 3

Plasma exposures of brexpiprazole and aripiprazole in rat NOR studies

See Figs. 1–3. The results show mean ± S.E.M. plasma concentrations from groups of six rats, measured after completion of NOR test. Blood samples were taken approximately 3 hours after drug administration.

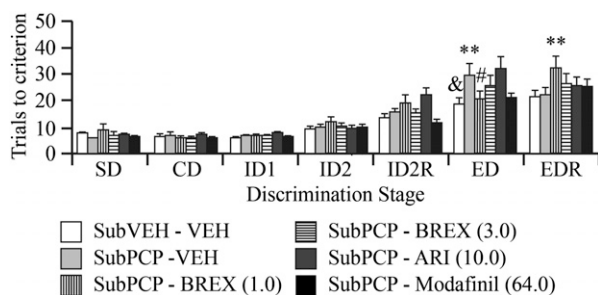| Treatment (Dose)	Plasma Concentration		
	Experiment 1	Experiment 2	Experiment 3
mg/kg p.o.	*ng/ml ± S.E.M.*		
Brexpiprazole			
0.3	11 ± 1.3	—	—
1.0	31 ± 2.7	—	—
3.0	113 ± 36	74 ± 7.9	89 ± 10
Aripiprazole			
10	40 ± 1.5	—	—
20	—	158 ± 29	—

Fig. 4. Effects of brexpiprazole (BREX) and aripiprazole (ARI) on ID-ED task in rats treated with subchronic PCP. Brexpiprazole and aripiprazole were administered subcutaneously 60 minutes before the test. The positive reference compound modafinil was administered p.o. 30 minutes before test and again 30 minutes before reaching the ED discrimination stage. Data represent mean ± S.E.M.; $n = 10$ for each treatment group. For explanation of task series, see *Materials and Methods*. $^{\&}P < 0.05$ versus subVeh/vehicle in ID2 stage; $^{**}P < 0.01$ versus subVeh/vehicle in the ED stage; $^{\#}P < 0.05$ versus SubPCP/vehicle in the ED stage.

number of trials to learn the ED task compared with rats treated with subchronic saline ($P < 0.01$). Furthermore, this confirms the formation of an attentional set shifting as a validation criteria for the test because the control rats treated with subVeh needed significantly more trials to learn the ED task than the preceding task, the ID2 task ($P < 0.05$).

Brexpiprazole significantly reversed the subPCP-induced impairment in the ED task at a dose of 1.0 mg/kg s.c., whereas the decrease in the number of trials needed to reach the criterion measured at a higher dose (3.0 mg/kg s.c.) was not statistically significant (Fig. 4). Aripiprazole (10 mg/kg s.c.) did not alter the PCP-induced impairment. Interestingly, the reversal of the PCP-induced impairment by the reference drug modafinil (64 mg/kg p.o.) did not reach statistical significance ($P = 0.053$). In addition to its effect on the ED stage performance, brexpiprazole (1.0 mg/kg s.c.) also significantly increased the number of trials needed to reach criterion in the EDR.

Discussion

Brexpiprazole, a novel serotonin-dopamine activity modulator, was developed and optimized to achieve clinical efficacy with minimal EPS potential. Its target optimization included D_2 partial agonism with low intrinsic activity, 5-HT$_{1A}$ partial agonism, and antagonism at 5-HT$_{2A}$, α_{1B}- and α_{2C}-adrenergic receptors (Maeda et al., 2014), supporting a favorable antipsychotic profile with low EPS risk and potential to treat core symptoms in schizophrenia, including cognitive deficits (Arnt and Skarsfeldt, 1998; Drouin et al., 2002; Marcus et al., 2010; Newman-Tancredi and Kleven, 2011; Sallinen et al., 2013).

Brexpiprazole showed antipsychotic-like activity in tests established for efficacy of D_2 antagonists on positive symptoms (Table 1), and efficacious doses show D_2 receptor occupancies within the clinically-relevant dose range for treatment of schizophrenia (Maeda et al., 2014). The inhibitory activity of brexpiprazole on drug-induced hyperactivity is slightly more potent than that on spontaneous locomotor activity, suggesting specific antipsychotic-like efficacy.

Classic animal tests of positive psychotic symptoms are usually unable to reveal differences between antipsychotic drugs (Arnt and Skarsfeldt, 1998). In contrast, the catalepsy test differentiates among antipsychotics to predict EPS

potential. Unlike haloperidol, both aripiprazole (Kikuchi et al., 1995) and risperidone (Arnt and Skarsfeldt, 1998) have shown separation between antipsychotic-like and cataleptogenic activity. Although the potency of brexpiprazole to induce antipsychotic-like activity (ED$_{50}$ = 0.92–6.0 mg/kg) is in the same range as that predicting in vivo D_2 receptor occupancy (ED$_{50}$ = 2.5 mg/kg p.o.; Maeda et al., 2014), cataleptogenic activity is only induced at high dosages (ED$_{50}$ = 20 mg/kg), likely due to D_2 partial agonism and its 5-HTergic activities (Kikuchi et al., 1995; Meltzer 1999). This suggests that, similar to aripiprazole, brexpiprazole has low potential to induce EPS at relevant clinical exposures leading to 60–90% D_2 receptor occupancy (Yokoi et al., 2002). Moreover, the potent inhibition by brexpiprazole of the apomorphine-induced eye blinking in monkeys is consistent with a low intrinsic activity at D_2 receptors because D_2 partial agonists with moderate intrinsic activity increase the eye-blinking rate (Kleven and Koek, 1996) whereas D_2 antagonists have inhibitory effects (Karson, 1983; Elsworth et al., 1991).

A major unmet medical need in the treatment of schizophrenia is improvement of cognitive dysfunction. Cognitive function has been identified as the primary factor determining functional outcome for schizophrenia patients, and efficacies of present treatments are limited (Green, 1996; Keefe and Harvey, 2012; Keefe et al., 2013). In recent years, focus on cognitive domains has increased (e.g., the MATRICS initiative [Measurement and Treatment Research to Improve Cognition in Schizophrenia]; Green et al., 2004; Young et al., 2009), and several animal models have been developed to improve the translational predictability to clinical effects.

The most widely used models presently apply either subPCP or subchronic ketamine, inducing enduring cognitive impairment across several cognitive domains (Neill et al., 2010; Nikiforuk and Popik, 2012). SubPCP-induced cognitive deficits are associated with changes in brain function, resembling many of the changes in patients with schizophrenia (Neill et al., 2010). Furthermore, the subPCP model has the advantage that evaluation of the effects of test compounds are conducted after withdrawal of PCP, thus avoiding risk for confounding effects through drug-drug interactions with the tool compound (PCP). In the present study, brexpiprazole and aripiprazole have been studied in two different cognitive tests, the NOR and the ID-ED tests, which are regarded as relevant for evaluating cognitive deficits in schizophrenia and other CNS disorders and require episodic memory and executive function (problem solving; cognitive flexibility), respectively (Green et al., 2004; Young et al., 2009).

Brexpiprazole fully reversed subPCP-induced impairments in the NOR test at 1.0–3.0 mg/kg with good reproducibility. These doses are within the dose range for antipsychotic-like efficacy (Table 1), suggesting that positive symptoms and cognitive impairment can be treated within a similar dose range. By comparing exposure analyses with the in vivo binding data in the adjoined paper (Maeda et al., 2014), receptor occupancies are estimated at 35% (1.0 mg/kg) and 64–76% (3.0 mg/kg) for D_2 receptors and 25% (1.0 mg/kg) and 45–55% (3.0 mg/kg) for 5-HT$_{2A}$ receptors, respectively. In addition, a moderate to high occupancy for 5-HT$_{1A}$ receptors is also predicted, based on the high in vitro affinity for this receptor (5-HT$_{1A}$ ≥ D_2 = 5-HT$_{2A}$) and the potency obtained by the ex vivo binding study (Maeda et al., 2014).

In contrast to brexpiprazole, and in spite of sufficient plasma exposure at clinically relevant doses of aripiprazole (10 and 20 mg/kg) predicting high D_2 receptor occupancies

(75 and 96%, respectively; Maeda et al., 2014), no significant effect of aripiprazole was detected in the NOR test (Fig. 1). In addition, receptor occupancies of aripiprazole at 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors were estimated to be much lower (4–18% for 5-HT$_{2A}$ receptors at 10–20 mg/kg; Maeda et al., 2014).

Pharmacologic analyses of targets involved in brexpiprazole efficacy in the NOR test suggest that both 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors are contributors. The 5-HT$_{1A}$ partial agonist buspirone had a significant effect (though did not fully reverse the subPCP deficit), and the 5-HT$_{1A}$ antagonist WAY100635 partially, but significantly, reversed the effect of brexpiprazole. Similar to buspirone, the selective 5-HT$_{2A}$ antagonist M100907 had partial effects at a dose range displaying 5-HT$_{2A}$ receptor occupancies of 50% and higher (Idris et al., 2010). It is unlikely that D$_2$ partial agonism is an essential effect, as aripiprazole was ineffective, despite a similar D$_2$ occupancy to brexpiprazole. Other supportive data have suggested 5-HT$_{1A}$ agonism as the preferential target reversing subPCP-induced cognitive impairment, in particular the results of experiments in which buspirone and another 5-HT$_{1A}$ partial agonist, tandospirone, were also effective (Horiguchi et al., 2012; Horiguchi and Meltzer, 2012). It has also been suggested that 5-HT$_{2A}$ blockade contributes to the ability of atypical antipsychotics to improve impairment of NOR caused by subPCP treatment (Meltzer et al., 2011). However, the contribution of 5-HT$_{2A}$ antagonism is ambiguous; some studies show efficacy levels comparable with those of the present study, but others do not (Grayson et al., 2007; Redrobe et al., 2010; Meltzer et al., 2011; Horiguchi et al., 2012).

Brexpiprazole also showed significant procognitive activity in the ID-ED test model of executive function in subPCP-treated rats whereas aripiprazole was again ineffective at the dose tested. The effect is a selective improvement of ID-ED–shift task performance. Notably, brexpiprazole (1.0 mg/kg) also increased trials to criterion in the EDR stage. The cause of this finding is currently unknown, but it could be a result of decreased motivation to perform this stage of the task. Importantly, the decreased EDR performance found here is not considered to be a general effect on reversal learning because no effect of brexpiprazole was found on the ID2R stage. This test model has shown ability to differentiate among antipsychotics with different pharmacologic profiles. It has been reported that haloperidol is ineffective whereas risperidone, olanzapine, and clozapine show nonsignificant trends toward improved ED-shift performance, and other antipsychotics, sertindole, and quetiapine show full reversal (Rodefer et al., 2008; Goetghebeur and Dias, 2009; Nikiforuk and Popik, 2012). In addition, studies of selective 5-HT receptor subtype antagonists indicate that the 5-HT$_{2A}$ antagonist M100907 has borderline effects similar to risperidone whereas a 5-HT$_6$ antagonist [SB-271046, 5-chloro-N-[4-methoxy-3-(1-piperazinyl) phenyl]-3-methylbenzo[b]thiophene-2-sulfonamide hydrochloride] and 5-HT$_7$ antagonist [SB-269970, (R)-3-{2-[2-(4-methyl-piperidin-1-yl)ethyl]pyrrolidin-1-ylsulfonyl}phenol] replicate the profile of sertindole (Rodefer et al., 2008; Nikiforuk et al., 2013). Therefore, the effect of brexpiprazole in this model would be associated with its potent 5-HT$_{2A}$ and modest 5-HT$_6$ receptor antagonist activity (Maeda et al., 2014). Although contribution of 5-HT$_7$ receptors is unlikely in rats based on low ex vivo binding potency, its nanomolar affinity (Maeda et al., 2014) may be associated with the procognitive effect in humans. Selective 5-HT$_{1A}$ agonists have not been studied in the ID-ED model, but

based on our present results and those of others (Horiguchi et al., 2012) on the effects of 5-HT$_{1A}$ agonists in the NOR model, the marked efficacy of brexpiprazole suggests that a combined 5-HT$_{1A}$ agonism and 5-HT$_{2A}$ antagonism is a likely mechanism of action in the ID-ED model. More precise dissection of receptor mechanisms involved will require further studies of selective agents, but it should also be mentioned that the potent α_{2C}-antagonist activity of brexpiprazole (Maeda et al., 2014) may play a role. First, it has been shown that the nonselective α_2-adrenoceptor antagonist atipamezole (Blaxall et al., 1991) improves ID-ED–shift performance in naive rats (Lapiz and Morilak, 2006). Furthermore, the selective α_{2C}-antagonist ORM-10921 [(1S,12bS)-1-(methoxymethyl)-1-methyl-2,3,4,6,7,12b-hexa-hydro-1H-benzofuro[2,3-a]quinolizine] reverses cognitive deficits induced by acute treatment with MK-801 [dizocilpine, (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine] (Sallinen et al., 2013).

Contrary to our results with aripiprazole, more positive results have been reported elsewhere. In subPCP-treated mice in the NOR test, aripiprazole reversed deficits, which were prevented by cotreatment with WAY100635, suggesting mediation by 5-HT$_{1A}$ receptors (Nagai et al., 2009). The reason for the lack of efficacy in this study is unknown, but may be due to differences of species, experimental design, and pharmacologic profile.

In summary, the present study suggests that brexpiprazole is a promising novel antipsychotic drug with robust efficacy on positive symptoms and cognitive impairment, with a favorable CNS safety profile in animal models. This is probably due to its broad serotonin-dopamine-system modulating activity as well as its antagonism of relevant noradrenergic receptors.

Authorship Contributions

Participated in research design: Maeda, McQuade, Kikuchi, Stensbøl, Lerdrup, Bundgaard.
Conducted experiments: Maeda, Sugino, Akazawa, Amada.
Performed data analysis: Maeda, Sugino, Akazawa, Amada, Lerdrup, Bundgaard.
Wrote or contributed to the writing of the manuscript: Maeda, Arnt, Lerdrup, Bundgaard, Stensbøl.

References

Andrews JS, Jansen JH, Linders S, Princen A, and Broekkamp CLE (1995) Performance of four different rat strains in the autoshaping, two-object discrimination, and swim maze tests of learning and memory. *Physiol Behav* **57**:785–790.
Arnt J (1995) Differential effects of classical and newer antipsychotics on the hypermotility induced by two dose levels of D-amphetamine. *Eur J Pharmacol* **283**:55–62.
Arnt J, Bang-Andersen B, Dias R, and Bøgesø KP (2008) Strategies for pharmacotherapy of schizophrenia. *Drugs Future* **33**:777–791 DOI: 10.1358/dof.2008.033.09.1236966.
Arnt J, Bøgesø KP, Hyttel J, and Meier E (1988) Relative dopamine D1 and D2 receptor affinity and efficacy determine whether dopamine agonists induce hyperactivity or oral stereotypy in rats. *Pharmacol Toxicol* **62**:121–130.
Arnt J and Skarsfeldt T (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology* **18**:63–101.

Blaxall HS, Murphy TJ, Baker JC, Ray C, and Bylund DB (1991) Characterization of the alpha-2C adrenergic receptor subtype in the opossum kidney and in the OK cell line. *J Pharmacol Exp Ther* **259**:323–329.

Birrell JM and Brown VJ (2000) Medial frontal cortex mediates perceptual attentional set shifting in the rat. *J Neurosci* **20**:4320–4324.

Casey DE (1996) Side effect profiles of new antipsychotic agents. *J Clin Psychiatry* **57** (Suppl 11):40–45, discussion 46–52.

Casey DE, Sands EE, Heisterberg J, and Yang HM (2008) Efficacy and safety of bifeprunox in patients with an acute exacerbation of schizophrenia: results from a randomized, double-blind, placebo-controlled, multicenter, dose-finding study. *Psychopharmacology (Berl)* **200**:317–331.

Citrome L (2013) Cariprazine: chemistry, pharmacodynamics, pharmacokinetics, and metabolism, clinical efficacy, safety, and tolerability. *Expert Opin Drug Metab Toxicol* **9**:193–206.

Costall B and Naylor RJ (1973) On the mode of action of apomorphine. *Eur J Pharmacol* **21**:350–361.

Dahan L, Husum H, Mnie-Filali O, Arnt J, Hertel P, and Haddjeri N (2009) Effects of bifeprunox and aripiprazole on rat serotonin and dopamine neuronal activity and anxiolytic behaviour. *J Psychopharmacol* **23**:177–189.

Drouin C, Darracq L, Trovero F, Blanc G, Glowinski J, Cotecchia S, and Tassin JP (2002) α_{1b}-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. *J Neurosci* **22**:2873–2884.

Elsworth JD, Lawrence MS, Roth RH, Taylor JR, Mailman RB, Nichols DE, Lewis MH, and Redmond DE, Jr (1991) D1 and D2 dopamine receptors independently regulate spontaneous blink rate in the vervet monkey. *J Pharmacol Exp Ther* **259**: 595–600.

Fleischhacker WW (2005) Aripiprazole. *Expert Opin Pharmacother* **6**:2091–2101.

Goetghebeur P and Dias R (2009) Comparison of haloperidol, risperidone, sertindole, and modafinil to reverse an attentional set-shifting impairment following subchronic PCP administration in the rat—a back translational study. *Psychopharmacology (Berl)* **202**:287–293.

Goetghebeur PJD, Lerdrup L, Sylvest A, and Dias R (2010) Erythropoietin reverses the attentional set-shifting impairment in a rodent schizophrenia disease-like model. *Psychopharmacology (Berl)* **212**:635–642.

Grayson B, Idris NF, and Neill JC (2007) Atypical antipsychotics attenuate a subchronic PCP-induced cognitive deficit in the novel object recognition task in the rat. *Behav Brain Res* **184**:31–38.

Green MF (1996) What are the functional consequences of neurocognitive deficits in schizophrenia? *Am J Psychiatry* **153**:321–330.

Green MF, Nuechterlein KH, Gold JM, Barch DM, Cohen J, Essock S, Fenton WS, Frese F, Goldberg TE, and Heaton RK, et al. (2004) Approaching a consensus cognitive battery for clinical trials in schizophrenia: the NIMH-MATRICS conference to select cognitive domains and test criteria. *Biol Psychiatry* **56**:301–307.

Horiguchi M, Hannaway KE, Adelekun AE, Jayathilake K, and Meltzer HY (2012) Prevention of the phencyclidine-induced impairment in novel object recognition in female rats by co-administration of lurasidone or tandospirone, a 5-HT(1A) partial agonist. *Neuropsychopharmacology* **37**:2175–2183.

Horiguchi M and Meltzer HY (2012) The role of 5-HT1A receptors in phencyclidine (PCP)-induced novel object recognition (NOR) deficit in rats. *Psychopharmacology (Berl)* **221**:205–215.

Idris N, Neill J, Grayson B, Bang-Andersen B, Witten LM, Brennum LT, and Arnt J (2010) Sertindole improves sub-chronic PCP-induced reversal learning and episodic memory deficits in rodents: involvement of 5-HT(6) and 5-HT (2A) receptor mechanisms. *Psychopharmacology (Berl)* **208**:23–36.

Karson CN (1983) Spontaneous eye-blink rates and dopaminergic systems. *Brain* **106**:643–653.

Keefe RS, Buchanan RW, Marder SR, Schooler NR, Dugar A, Zivkov M, and Stewart M (2013) Clinical trials of potential cognitive-enhancing drugs in schizophrenia: what have we learned so far? *Schizophr Bull* **39**:417–435.

Keefe RS and Harvey PD (2012) Cognitive impairment in schizophrenia. *Handbook Exp Pharmacol* **213**:11–37.

Kikuchi T, Tottori K, Uwahodo Y, Hirose T, Miwa T, Oshiro Y, and Morita S (1995) 7-(4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]butyloxy)-3,4-dihydro-2(1*H*)-quinolinone (OPC-14597), a new putative antipsychotic drug with both presynaptic dopamine autoreceptor agonistic activity and postsynaptic D2 receptor antagonistic activity. *J Pharmacol Exp Ther* **274**:329–336.

Kiss B, Horváth A, Némethy Z, Schmidt E, Laszlovszky I, Bugovics G, Fazekas K, Hornok K, Orosz S, and Gyertyán I, et al. (2010) Cariprazine (RGH-188), a dopamine D(3) receptor-preferring, D(3)/D(2) dopamine receptor antagonist-partial agonist antipsychotic candidate: in vitro and neurochemical profile. *J Pharmacol Exp Ther* **333**:328–340.

Kleven MS and Koek W (1996) Differential effects of direct and indirect dopamine agonists on eye blink rate in cynomolgus monkeys. *J Pharmacol Exp Ther* **279**:1211–1219.

Lapiz MDS and Morilak DA (2006) Noradrenergic modulation of cognitive function in rat medial prefrontal cortex as measured by attentional set shifting capability. *Neuroscience* **137**:1039–1049.

Laoutidis ZG and Luckhaus C (2014) 5-HT2A receptor antagonists for the treatment of neuroleptic-induced akathisia: a systematic review and meta-analysis. *Int J Neuropsychopharmacol* **17**:823–832

Maeda K, Sugino H, Akazawa H, Amada N, Shimada J, Futamura T, Yamashita H, Ito N, McQuade RD, and Mørk A, et al. (2014) Brexpiprazole I: in vitro and in vivo characterization of a novel serotonin-dopamine activity modulator. *J Pharmacol Exp Ther* **350**:589–604.

Mamo D, Graff A, Mizrahi R, Shammi CM, Romeyer F, and Kapur S (2007) Differential effects of aripiprazole on D(2), 5-HT(2), and 5-HT(1A) receptor occupancy in patients with schizophrenia: a triple tracer PET study. *Am J Psychiatry* **164**: 1411–1417.

Marcus MM, Wiker C, Frånberg O, Konradsson-Geuken A, Langlois X, Jardemark K, and Svensson TH (2010) Adjunctive α2-adrenoceptor blockade enhances the antipsychotic-like effect of risperidone and facilitates cortical dopaminergic and glutamatergic, NMDA receptor-mediated transmission. *Int J Neuropsychopharmacol* **13**:891–903.

Meltzer HY (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology* **21**(2, Suppl)106S–115S.

Meltzer HY, Horiguchi M, and Massey BW (2011) The role of serotonin in the NMDA receptor antagonist models of psychosis and cognitive impairment. *Psychopharmacology (Berl)* **213**:289–305.

Migler BM, Warawa EJ, and Malick JB (1993) Seroquel: behavioral effects in conventional and novel tests for atypical antipsychotic drug. *Psychopharmacology (Berl)* **112**:299–307.

Nagai T, Murai R, Matsui K, Kamei H, Noda Y, Furukawa H, and Nabeshima T (2009) Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D1 and serotonin 5-HT1A receptors. *Psychopharmacology (Berl)* **202**:315–328.

Neill JC, Barnes S, Cook S, Grayson B, Idris NF, McLean SL, Snigdha S, Rajagopal L, and Harte MK (2010) Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. *Pharmacol Ther* **128**:419–432.

Newman-Tancredi A (2010) The importance of 5-HT$_{1A}$ receptor agonism in antipsychotic drug action: rationale and perspectives. *Curr Opin Investig Drugs* **11**: 802–812.

Newman-Tancredi A, Cussac D, and Depoortere R (2007) Neuropharmacological profile of bifeprunox: merits and limitations in comparison with other third-generation antipsychotics. *Curr Opin Investig Drugs* **8**:539–554.

Newman-Tancredi A and Kleven MS (2011) Comparative pharmacology of antipsychotics possessing combined dopamine D$_2$ and serotonin 5-HT$_{1A}$ receptor properties. *Psychopharmacology (Berl)* **216**:451–473.

Nikiforuk A, Kos T, Fijał K, Hołuj M, Rafa D, and Popik P (2013) Effects of the selective 5-HT7 receptor antagonist SB-269970 and amisulpride on ketamine-induced schizophrenia-like deficits in rats. *PLoS ONE* **8**:e66695.

Nikiforuk A and Popik P (2012) Effects of quetiapine and sertindole on subchronic ketamine-induced deficits in attentional set-shifting in rats. *Psychopharmacology (Berl)* **220**:65–74.

Redrobe JP, Bull S, and Plath N (2010) Translational aspects of the novel object recognition task in rats abstinent following sub-chronic treatment with phencyclidine (PCP): effects of modafinil and relevance to cognitive deficits in schizophrenia. *Front Psychiatry* **1**:146.

Rodefer JR, Nguyen TN, Karlsson JJ, and Arnt J (2008) Reversal of subchronic PCP-induced deficits in attentional set shifting in rats by sertindole and a 5-HT6 receptor antagonist: comparison among antipsychotics. *Neuropsychopharmacology* **33**:2657–2666.

Roth BL, Sheffler DJ, and Kroeze WK (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev Drug Discov* **3**:353–359.

Sallinen J, Holappa J, Koivisto A, Kuokkanen K, Chapman H, Lehtimäki J, Pieponen P, Mijatovic J, Tanila H, and Virtanen R, et al. (2013) Pharmacological characterisation of a structurally novel α2C-adrenoceptor antagonist ORM-10921 and its effects in neuropsychiatric models. *Basic Clin Pharmacol Toxicol* **113**:239–249.

Stahl SM (2001) Dopamine system stabilizers, aripiprazole, and the next generation of antipsychotics, part 2: illustrating their mechanism of action. *J Clin Psychiatry* **62**:923–924.

Wong EH, Nikam SS, and Shahid M (2008) Multi- and single-target agents for major psychiatric diseases: therapeutic opportunities and challenges. *Curr Opin Investig Drugs* **9**:28–36.

Yokoi F, Gründer G, Biziere K, Stephane M, Dogan AS, Dannals RF, Ravert H, Suri A, Bramer S, and Wong DF (2002) Dopamine D$_2$ and D$_3$ receptor occupancy in normal humans treated with the antipsychotic drug aripiprazole (OPC 14597): a study using positron emission tomography and [11C]raclopride. *Neuropsychopharmacology* **27**:248–259.

Young JW, Powell SB, Risbrough V, Marston HM, and Geyer MA (2009) Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. *Pharmacol Ther* **122**:150–202.

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