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1. Title Page

Brexpiprazole II: Antipsychotic-like and pro-cognitive effects of a novel serotonin-dopamine activity modulator

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2. Running title page

a) Running title: Brexpiprazole, a novel serotonin-dopamine activity modulator

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c) The number of text pages: 24

The number of tables: 3

The number of figures: 4

The number of references: 51

The number of words in abstract: 248

The number of words in introduction: 651

The number of words in discussion: 1496

d) A list of nonstandard abbreviations

5-HT, serotonin

ANOVA, analysis of variance

b.i.d., twice daily

CAR, continued avoidance response

CD, compound discrimination

CNS, central nervous system

CS, conditioned stimulus

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DA, dopamine

D_{2L}, long form of human D_2 receptor

ED, extradimensional

EDR, extradimensional reversal

EPS, extrapyramidal symproms

ID, intradimensional

ID-ED, attentional set-shifting

LE, Long Evans

LH, Lister hooded

NMDA, N-methyl-D-aspartate

NOR, novel object recognition

PCP, phencyclidine

PET, positron emission tomography

SD, simple discrimination

subPCP, subchronic phencyclidine

subVeh, subchronic vehicle

Tf, familiar object exploration time

Tn, novel object exploration time

US, unconditioned stimulus

e) A recommended section assignment: Neuropharmacology

3. 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 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 low risk for extrapyramidal side effects. Sub-chronic 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, partially, but significantly, reversed PCP-induced impairment. Furthermore, the effect of brexpiprazole was reversed by co-treatment with the 5-HT_{1A} antagonist WAY-100635.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. It is proposed that the pharmacological profile of brexpiprazole is based on its balanced effects on 5-$HT_{1A,}$ D_2, and 5-HT_{2A} receptors, with possible modulating activity through additional monoamine receptors.

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4. 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 5-HT_{2A} receptors and of α_1-adrenoceptors, and some compounds also affect a variety of other monoamine receptors, such as 5-$HT_{1A/6/7}$ receptors, α_2-adrenoceptors, 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 central nervous system (CNS)-related adverse effects, such as extrapyramidal symptoms (EPS) or endocrine side effects such as hyperprolactinemia (Arnt and Skarsfeldt, 1998; Roth et al., 2004; Arnt et al., 2008; Newman-Tancredi, 2010; Newman-Tancredi and Kleven, 2011).

Due to 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, bipolar mania and as add-on treatment for major depression (Fleischhacker, 2005). Another compound with similar D2 intrinsic activity 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), due to lack of sufficient 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

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adverse effects related to increased D_2 receptor tonus, e.g. 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 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-HT_{1A} receptors and some antagonism at 5-HT_{2A} receptors (Mamo et al., 2007; Dahan et al., 2009). However, the occupancies at 5-HT_{1A} and 5-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-HT_{2A} antagonism may reduce D_2 antagonist-induced akathisia (Laoutidis and Luckhaus, 2013). Furthermore, a broad pharmacological 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; Wong et al., 2008; Arnt 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., and is being developed in collaboration with H. Lundbeck A/S. It is a novel serotonin-dopamine activity modulator, combining moderate-intrinsic activity 5-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* pharmacological profile has been presented in detail in an accompanying paper (Maeda et al., submitted).

In the present study, the *in vivo* behavioral pharmacological 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.

5. Methods

Subjects

Male Wistar rats (Japan SLC Inc., Shizuoka, Japan [CAR, apomorphine-induced hyperactivity and stereotyped behavior, and catalepsy; 126–200g at time of testing]; Charles River, Germany [Amphetamine-induced hyperactivity and spontaneous locomotor activity; 150–175g at time of testing]), male Lister Hooded (LH) rats (Charles River, Germany; NOR; 220–240g at time of testing) and male Long Evans (LE) rats (Charles River, Wilmington MA, USA; ID-ED;180–280g at the time of testing) were used. Albino Wistar rats are commonly used for standard behavioral testing of CNS compounds, while 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 (Redrobe et al., 2010; Idris et al., 2010), while the ID-ED test was validated with either LH (Goetghebeur et al., 2009) or LE rats (Rodefer et al., 2008). However, only the LE rat strain was available in China, and accordingly 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 Macrolon type III cages and were maintained on a 12 h light/dark cycle (lights on 6 or 7 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 Inc.) 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 01 Apr 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 23 November 2007 for novel object recognition (NOR), d-amphetamine-induced hyperactivity, and spontaneous locomotor activity tests; the Minister of Health (2001) Laboratory Animal - Requirements of environment and housing facilities, and Peoples republic of China (GB 14925-2001) for the attentional set-shifting (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, Tokushima, Japan). Apomorphine hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO, USA). D-amphetamine sulphate and buspirone hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO). Modafinil, PCP, and WAY-100635 oxalate were synthesized by H. Lundbeck A/S. Apomorphine was dissolved in saline immediately before injection, while 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-beta-cyclodextrin solution, adjusted to pH 5 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 (unpublished data on file). Modafinil was suspended in 0.5% methylcellulose, and phencyclidine, d-amphetamine sulphate, buspirone, and WAY 100635 were dissolved in saline. Doses of salts are expressed as

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free bases, except for tool compounds. Test compounds for *in vivo* studies were administered p.o. or s.c. in a volume of 5 ml/kg, except in monkey studies (1 ml/kg).

CAR in Rats

The shuttle box (dimensions: 46 × 19.5 × 20 cm, BIO MEDICA, Ltd, 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 micro-switch 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 min 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 s warning tone (105 dB tone) as a conditioned stimulus (CS) followed by a 10 s foot shock (1 mA) as an unconditioned stimulus (US) and a 15–75 s (mean; 45 s) inter-trial interval. The US was terminated when the animal jumped over the hurdle from one compartment to the other or after a cut-off time of 10 s. Each rat was placed in one of the compartments of the shuttle box and allowed a free exploration for 1 min before starting the trial.

During the training session, three kinds of responses were recorded: crossing in response to a CS alone was recorded as a CAR; crossing during the US 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 / group) were administered the test compounds p.o., 1 h before the test session, excepting aripiprazole, which was given p.o., 2 h 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 were calculated by non-linear regression analysis using SAS software (SAS institute Japan, Tokyo, Japan).

Apomorphine-induced Hyperactivity in Rats

Brexpiprazole or aripiprazole were administered p.o., 1 and 2 h, respectively, before apomorphine injection (0.25 mg/kg, s.c.). Five rats were used in each dose group. Thirty min before apomorphine-injection, each rat was placed individually in a plastic circular chamber (diameter 30 cm × height 30 cm) and acclimated to the new environment. Measurement of locomotor activity was counted over 1 h starting immediately after apomorphine injection. The locomotor detection system (Yamashita Giken, Tokushima, Japan) was composed of a fixed pivot at the center of each chamber with six micro-switches fitted beneath the perimeter to detect turning movement as the animal passes 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 next section; Costall and Naylor, 1973; Kikuchi et al., 1995; Arnt et al., 1988). ED_{50} values with 95% confidence intervals were calculated by non-linear regression analysis using SAS software.

Apomorphine-induced Stereotyped Behavior in Rats

Rats were fasted for 16–20 h before administration of test compounds. Brexpiprazole and risperidone were administered p.o., 1 h before apomorphine injection (0.7 mg/kg, s.c.).

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Aripiprazole was administered p.o., 2 h before apomorphine injection. Six rats were used in each dose group. In order to habituate to the test environment, each rat was placed individually in an acrylic cylinder (diameter 23 cm × height 30 cm) 30 min before apomorphine-injection.

Stereotyped behavior was recorded by an observer blinded to the treatment groups (compound and dose), for a 1 min interval every 10 min over the 20–40 min period following 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, no exploratory activity (Costall and Naylor, 1973; Kikuchi et al., 1995; Arnt et al., 1988). The dose of apomorphine was selected to induce an average behavioral score of 3–4 in the control groups. ED_{50} values with 95% confidence intervals were calculated by non-linear 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 (Macrolon 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 photo-beam 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 h before injection of d-amphetamine (0.5 mg/kg, s.c.). Eight rats were used in each dose group. The activity was

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measured for a period of 2 h. ED_{50} values with 95% confidence intervals were calculated by non-linear regression using GraphPad Prism software (v 4).

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 h before placing them in the activity box for assessment of locomotor activity for a 1 h recording period. Eight rats were used in each dose group. ED_{50} values with 95% confidence intervals were calculated by non-linear regression using GraphPad Prism software (v 4).

Catalepsy in Rats

Rats were fasted for 15 h before administration of test compounds. Catalepsy was measured at 1, 2, 4, 6 and 8 h 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 × height: 10 cm). A catalepsy response was recorded when the animals remained in the unnatural vertical position for 30 s or longer. The potency was determined at the time point of maximum effect for each compound, and ED_{50} values were calculated by non-linear 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 following day, inhibition of apomorphine-induced eye blinking was measured after pre-treatment with brexpiprazole. The measurements were conducted according to the literature (Migler et al., 1993; Kleven and Koek, 1996).

Brexpiprazole or vehicle was administered 4 h before apomorphine-injection through a catheter (8Fr, length 40 cm), inserted through the nose into the stomach, using a volume of 1 ml/kg. Apomorphine (0.16 mg/kg) was injected i.m. into the femoral region. The number of eye blinks was counted over a 1 min period, 5, 15, and 30 min 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% confidence intervals was calculated by non-linear regression analysis using SAS software.

NOR in Rats Treated with Subchronic PCP

Test protocol: NOR testing was performed as described in Redrobe *et al.* (2010). In brief, animals were habituated to the test arena (dimensions: 95 × 45 × 50 cm) for 10 min 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 × 10 × 6 cm, or two domed glass paperweights, 8 × 8 × 8 cm) for 3 min. The animals were then transferred to their home cages for a 1 h inter-trial interval. Following this period, the animal was re-introduced 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 min

test session. Behaviour 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: (A) a total exploration time of 15 s or above, (B) a minimum of 2 s 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 (subVeh, i.p.) twice daily at 7 AM and 7 PM for 7 days, followed by a washout period of 8–9 days prior to behavioural testing. On the test day, brexpiprazole, aripiprazole, or vehicle was administered p.o., 2 h prior to the acquisition trial. M100907, buspirone, WAY-100635, or saline were administered s.c., 30 min prior to the acquisition trial. The number of rats in each group is indicated in the legends to Figs 1–3.

Data analysis: Results are presented as: (i) exploration time(s) of novel or familiar object and (ii) 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 analyse exploration time. One-way ANOVA 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 LC chromatography followed by tandem mass

spectrometry detection in positive-ion electrospray ionisation 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.

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 2 choice compartments). A terracotta pot (diameter: 11 cm), recessed into the floor of the test box, was placed in each choice area. Digging medium cues (for example HAMA plastic beads, paper confetti, paper clips, wall plugs) were added to the pots, odor cues (oils from The Body Shop®, 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.), b.i.d. at 8 AM and 8 PM for 7 days, followed by a 7-day washout period prior to behavioural testing. Brexpiprazole and aripiprazole were administered s.c., 1 h before test. Modafinil (64 mg/kg) was administered p.o. twice, each at 32 mg/kg - once 30 min prior to the simple discrimination task and again 30 min before the fifth (ID2R) discrimination stage, due to its short plasma half-life in rats.

Behavioral testing: On days 4–6 of the washout period, rats were habituated to the arena and learnt to dig in test pots filled with cage bedding and food rewards (Honey Loop cereal). 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 (SD), compound discrimination (CD), 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 (SD, CD, 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 min before testing was resumed by continuing the trial from the stage the animal had reached prior to its omission break. A cut-off time of 6 h 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 counter-balanced between rats and the experimenter remained blinded to the treatment condition (both subchronic and acute treatment).

Drugs and treatment schedule: Rats received PCP i.p. (5 mg/kg) or saline i.p. twice daily (b.i.d.) at 8 AM and 8 PM for 7 days. Brexpiprazole and aripiprazole were administered s.c., 1 h before test. Modafinil was suspended in 0.5% methylcellulose to a concentration of 32 mg/ml.

Due to its short plasma half-life in rats, modafinil (64 mg/kg) was administered p.o. twice, each at 32 mg/kg - once 30 min prior to the simple discrimination task and again 30 min 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 ANOVA (factor 1: discrimination stage; factor 2: drug treatment). Significant drug treatment × discrimination stage interactions were further analyzed using the Tukey post-hoc test. The level of significance was $P < 0.05$. All statistical analyses were performed using SigmaPlot (version 11). The experiment was performed by the Contract Research Organization Wuxi Apptech (Shanghai, China).

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6. 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, while brexpiprazole did not induce any non-specific 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 3.1 (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 mg/kg 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 to which they inhibit 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 3.3–8.7 for brexpiprazole and 1.8–13 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 % confidence intervals 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.

NOR: The test was performed in three separate experiments (Figs. 1–3). During the 3 min 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. 2A and 2B. 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 and, 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).

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In the third experiment, the goal was to extend the dose range studied for M100907 and to explore whether the 5-HT_{1A} antagonist, WAY-100635, would attenuate the effect of brexpiprazole. Fig. 3A shows that subPCP-induced deficits in novel object exploration were significantly attenuated by M100907 (0.013 and 0.31 mg/kg, s.c.; $P < 0.01$, $P < 0.01$, respectively), brexpiprazole (3.0 mg/kg, p.o., $P < 0.001$), and brexpiprazole (3.0 mg/kg, p.o.) co-administered with WAY-100635 (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 co-administered with WAY-100635 (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.) co-treatment with WAY-100635 (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, i.e. about 3 h 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): Fig. 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 number of trials to

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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 since 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., while 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.

7. 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., submitted), supporting a favorable antipsychotic profile with low EPS risk and potential to treat core symptoms in schizophrenia, including cognitive deficits (Drouin et al., 2002; Arnt and Skarsfeldt, 1998; 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., submitted). The inhibitory activity of brexpiprazole on drug-induced hyperactivity is slightly more potent than that on spontaneous locomotor activity, suggesting specific antipsychotic-like efficacy.

Classical 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. While potency of brexpiprazole to induce antipsychotic-like activity (ED_{50} 0.9-6.5 mg/kg) is in the same range as that predicting *in vivo* D_2 receptor occupancy (ED_{50} 2.5 mg/kg; Maeda et al., submitted), cataleptogenic activity is only induced at high dosages (ED_{50} 20 mg/kg), likely due to partial D_2 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, since D_2 partial agonists with moderate intrinsic activity increase eye blinking rate (Kleven and Koek, 1996), while 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. 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., submitted), receptor occupancies are estimated at 35% (1 mg/kg) and 64–76% (3 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-$HT_{1A} \geq D_2 =$ 5-HT_{2A}) and the potency obtained by the *ex vivo* binding study (Maeda et al., submitted).

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., submitted), no significant effect of aripiprazole was detected in NOR test (Figure 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., submitted).

Pharmacological 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 WAY-100635 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 showing efficacy levels comparable with those of the present study, whilst others do not (Grayson et al., 2007; Meltzer et al., 2011; Horiguchi et al., 2012, Redrobe et al., 2010).

Brexpiprazole also showed significant procognitive activity in the ID-ED test model of executive function in subPCP-treated rats, while 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, since no effect of brexpiprazole was found on the IDR stage. This test model has shown ability to differentiate among antipsychotics with different pharmacological profiles. It has been reported that haloperidol is ineffective, while risperidone, olanzapine, and clozapine show non-significant trends towards 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, while a 5-HT_6 antagonist (SB-271046) and 5-HT_7 antagonist (SB-269970) replicate the profile of sertindole (Rodefer et al., 2008; Nikiforuk et al., 2013). Therefore, the

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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., submitted). Although contribution of 5-HT_7 receptors is unlikely in rats based on low *ex vivo* binding potency, its nanomolar affinity (Maeda et al., submitted) 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., submitted) may play a role: First, it has been shown that the non-selective 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 reverses cognitive deficits induced by acute treatment with MK-801 (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 co-treatment with WAY-100635, 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 pharmacological 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.

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9. Authorship Contributions.

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Performed exposure analysis: Bundgaard

Wrote or contribute to the writing of the manuscript: Maeda, Arnt, Lerdrup, Bundgaard, Stensbøl

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12. Legends to figures.

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 h prior to 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.

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 s.c., 30 min prior to acquisition trial. Brexpiprazole was administered p.o., 2 h prior to 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 WAY-100635 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 WAY-100635 were administered s.c., 30 min prior to acquisition trial. Brexpiprazole was administered p.o., 2 h prior to 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) + WAY-100635 (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.

Fig. 4. Effects of brexpiprazole (BREX) and aripiprazole (ARI) on attentional set shifting (ID-ED) task in rats treated with subchronic PCP. Brexpiprazole and aripiprazole were administered s.c. 60 min prior to the test. The positive reference compound modafinil was administered p.o. 30 min prior to test and again 30 min prior to reaching the ED discrimination stage. Data represent mean ± S.E.M.; n = 10 for each treatment group. For explanation of task series, see Methods section.

&*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.

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13. Tables.

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 (mg/kg) with 95% confidence intervals. Drug pretreatment times are shown in parentheses with exceptions in footnotes.

Test	n/group	ED_{50} (mg/kg, p.o.) (95% Confidence Intervals)		
		Brexpiprazole (1h)[a]	Aripiprazole (2h)[b]	Risperidone (1h)[c]
Efficacy tests				
CAR	n = 4–6	6.0 (4.3–9.7)	23 (20–27)	3.3 (2.0–7.6)
APO hyperactivity (0.25 mg/kg)	n = 5	2.3 (1.2–3.1)	3.2 (1.9–5.0)	NT
APO stereotypy (0.7 mg/kg)	n = 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)	n = 8	0.92 (0.6–1.5)	3.9 (2.0–7.7)	NT
CNS side effects				
Inhibition of Spontaneous Locomotor Activity	n = 8	3.4 (2.4–5.0)	6.1 (2.3–16)	NT
Catalepsy (at max effect time)	n = 6	20 (12–40)	42 (26–68)	6.6 (2.9–16)

a) except for spontaneous locomotor activity, amphetamine-induced hyperactivity (2 h) and catalepsy (maximum effect at 6 h).

b) except for catalepsy (maximum effect at 8 h).

c) except for catalepsy (2 h).

AMPH, amphetamine; APO, apomorphine; NT, not tested.

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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. * $P<0.05$ and ** $P<0.01$ indicates statistical significance compared with spontaneous eye blinking (paired t-test, two-tailed).

		Total counts of eye blinking (mean ± S.E.M.)			
Animal ID		1	2	3	4
Spontaneous eye blinking		65 ± 4.5	19 ± 1.5	17 ± 2.3	38 ± 3.3
Apomorphine-induced eye blinking		112 ± 4.2*	61 ± 4.2**	60 ± 5.7**	85 ± 5.3**
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

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Table 3. Plasma exposures of brexpiprazole and aripiprazole in rat NOR studies (see figs 1, 2, and 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 h after drug administration.

Treatment (dose, mg/kg, p.o.)		Plasma concentration (ng/ml ± S.E.M.)		
		Experiment 1	Experiment 2	Experiment 3
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	-

Figure 1

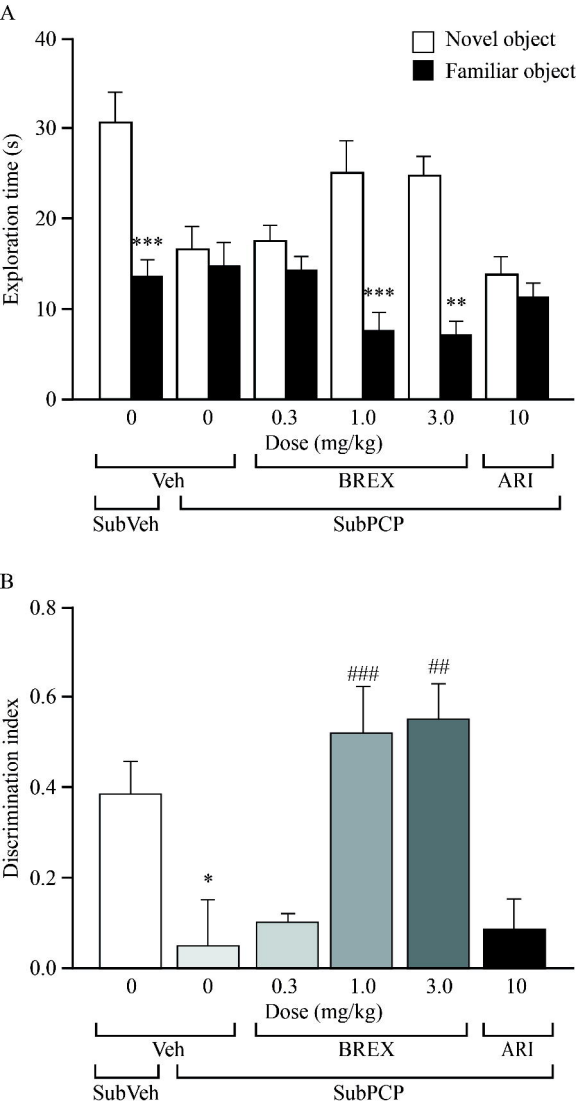
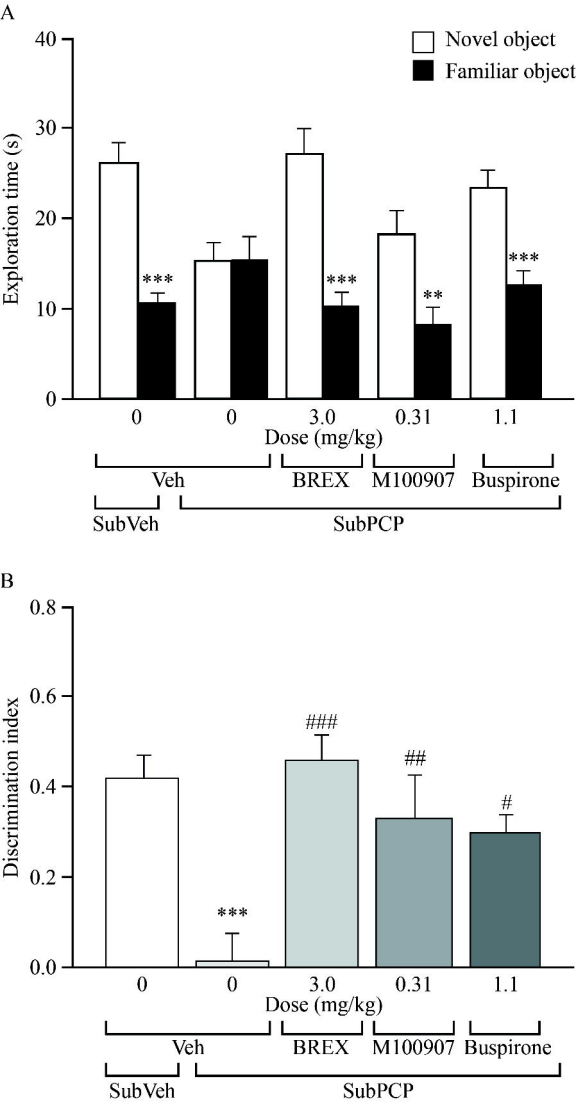

A
Novel object
Familiar object
Exploration time (s)
40
30
20
10
0
***
***
**
0
0
0.3
1.0
3.0
10
Dose (mg/kg)
Veh
BREX
ARI
SubVeh
SubPCP
B
Discrimination index
0.8
0.6
0.4
0.2
0.0
*
###
##
0
0
0.3
1.0
3.0
10
Dose (mg/kg)
Veh
BREX
ARI
SubVeh
SubPCP

Figure 2


A
Novel object
Familiar object
Exploration time (s)
40
30
20
10
0
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0
0
3.0
0.31
1.1
Dose (mg/kg)
Veh
BREX
M100907
Buspirone
SubVeh
SubPCP
B
Discrimination index
0.8
0.6
0.4
0.2
0.0
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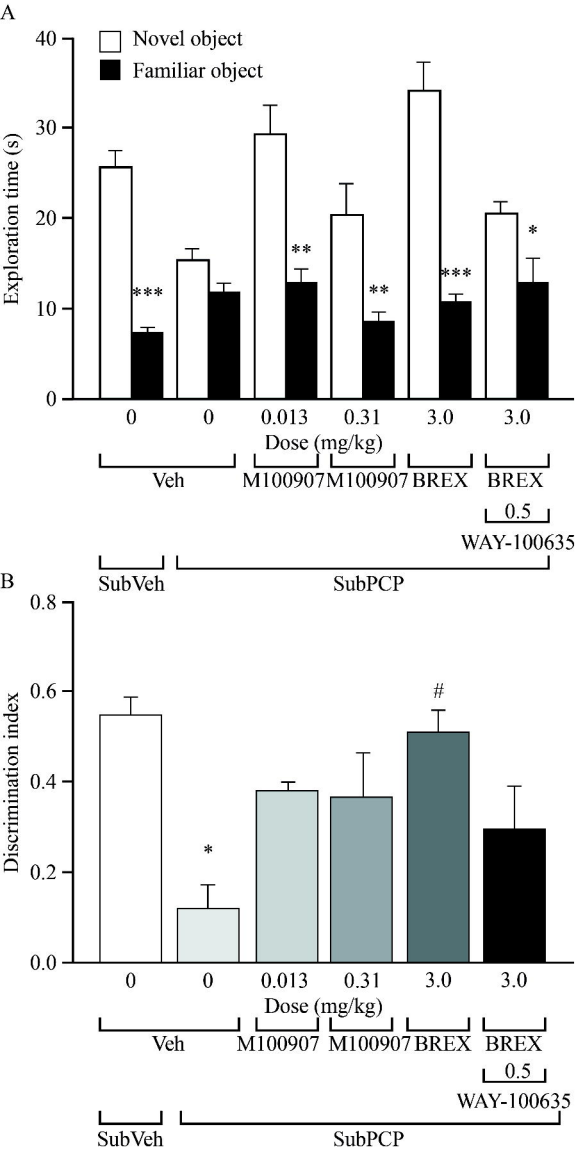
###
##
#
0
0
3.0
0.31
1.1
Dose (mg/kg)
Veh
BREX
M100907
Buspirone
SubVeh
SubPCP

Figure 3


A
Novel object
Familiar object
Exploration time (s)
40
30
20
10
0
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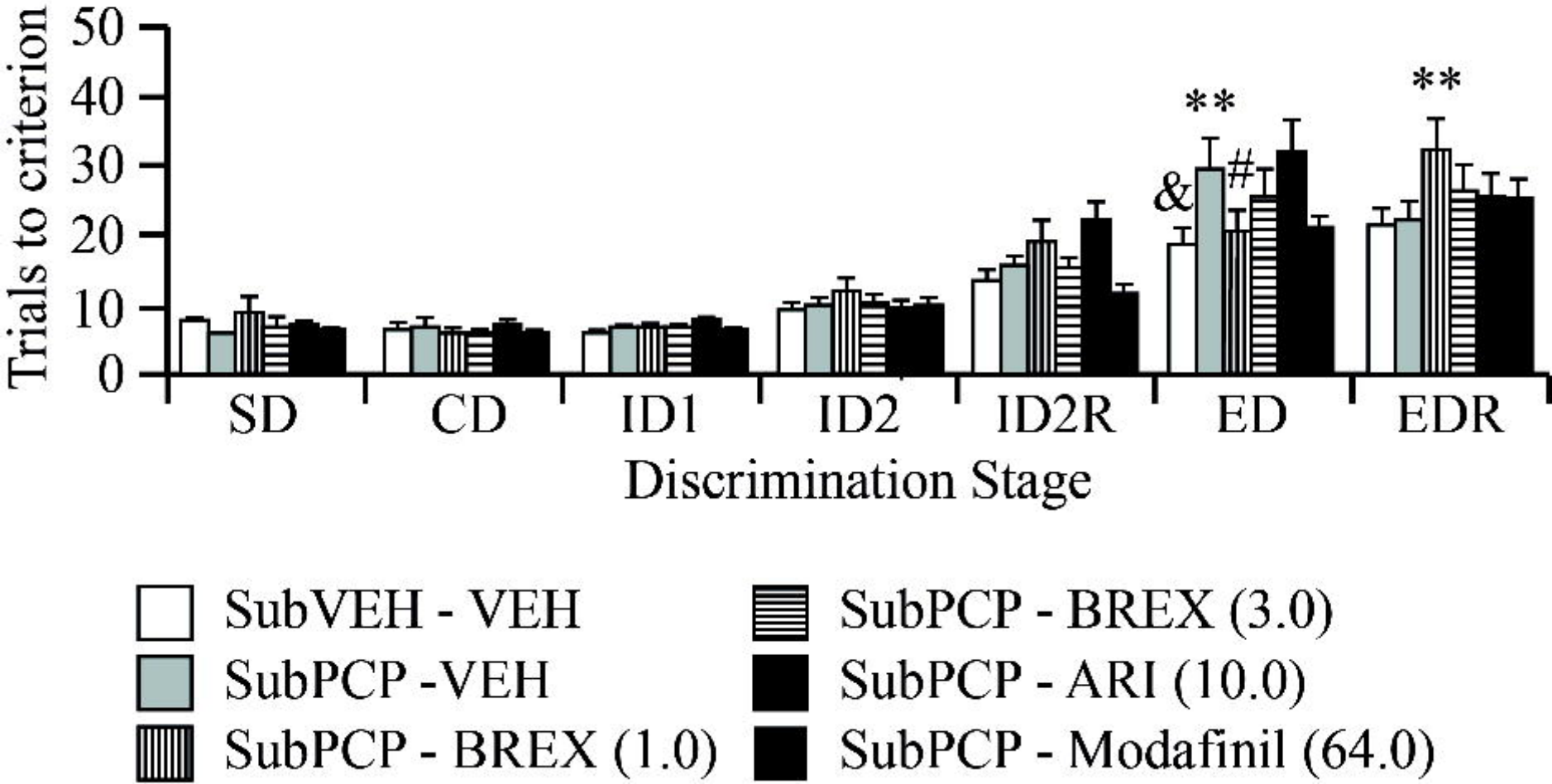
*
0
0
0.013
0.31
3.0
3.0
Dose (mg/kg)
Veh
M100907
M100907
BREX
BREX
0.5
WAY-100635
SubVeh
SubPCP
B
Discrimination index
0.8
0.6
0.4
0.2
0.0
*
#
0
0
0.013
0.31
3.0
3.0
Dose (mg/kg)
Veh
M100907
M100907
BREX
BREX
0.5
WAY-100635
SubVeh
SubPCP

Figure 4


Trials to criterion
50
40
30
20
10
0
SD
CD
ID1
ID2
ID2R
ED
EDR
Discrimination Stage
**
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&
#
SubVEH - VEH
SubPCP -VEH
SubPCP - BREX (1.0)
SubPCP - BREX (3.0)
SubPCP - ARI (10.0)
SubPCP - Modafinil (64.0)