The phosphodiesterase 10A selective inhibitor TAK-063 improves cognitive functions associated with schizophrenia in rodent models

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Abbreviations:

ASST, attentional set-shifting task
CD, compound discrimination
5-CSRTT, five-choice serial reaction time task
EDS, extra-dimensional shift; ICR, imprinting control region
IDS, intra-dimensional shift
ITI, inter-trial interval
MED, minimum effective dose
MK-801,

\[(5R,10S)-(+)\)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine\]

MSN, medium spiny neuron
NDI, novelty discrimination index
NMDA, N-methyl-D-aspartate
NORT, novel object recognition task
PCP, phencyclidine hydrochloride
PDE, phosphodiesterase
PET, positron emission tomography
SD, simple discrimination
TAK-063

[1-[2-fluoro-4-(1H-pyrazol-1-yl)phenyl]-5-methoxy-3-(1-phenyl-1H-pyrazol-5-yl)pyridazin-4(1H)-one]

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Neuropharmacology
Abstract

Cognitive deficits in various domains, including recognition memory, attention, impulsivity, working memory, and executive function, substantially affect functional outcomes in patients with schizophrenia. TAK-063 [1-[2-fluoro-4-(1H-pyrazol-1-yl)phenyl]-5-methoxy-3-(1-phenyl-1H-pyrazol-5-yl)pyridazin-4(1H)-one] is a potent and selective phosphodiesterase 10A inhibitor that produces antipsychotic-like effects in rodent models of schizophrenia. We evaluated the effects of TAK-063 on multiple cognitive functions associated with schizophrenia using naïve and drug-perturbed rodents. TAK-063 at 0.1 and 0.3 mg/kg p.o. improved time-dependent memory decay in object recognition in naïve rats. TAK-063 at 0.1 and 0.3 mg/kg p.o. increased accuracy rate, and TAK-063 at 0.3 mg/kg p.o. reduced impulsivity in a five-choice serial reaction-time task in naïve rats. N-methyl-D-aspartate receptor antagonists such as phencyclidine and MK-801 [(5R,10S)-(+)5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine] were used to induce working memory deficits relevant to schizophrenia in animals. TAK-063 at 0.3 mg/kg p.o. attenuated both phencyclidine-induced working memory deficits in a Y-maze test in mice and MK-801-induced working memory deficits in an eight-arm radial maze task in rats. An attentional set-shifting task using subchronic
Phencyclidine-treated rats was used to assess the executive function. TAK-063 at 0.3 mg/kg p.o. reversed cognitive deficits in extra-dimensional shifts. These findings suggest that TAK-063 has a potential to ameliorate deficits in multiple cognitive domains impaired in schizophrenia.

**Key words**

Phosphodiesterase 10A, TAK-063, schizophrenia, cognitive functions
Introduction

Schizophrenia is a chronic debilitating disorder comprising the following three symptomatic domains: positive symptoms, negative symptoms, and cognitive deficits (van Os and Kapur, 2009). Typical and atypical antipsychotics with dopamine D₂ receptor antagonistic activity are primary medications for the treatment of schizophrenia. They have been known to show some efficacy for positive symptoms, but their efficacy for other outcomes is considerably limited. In particular, these medications do not address the negative symptoms and cognitive deficits present in patients with schizophrenia (Green et al., 2004; Harvey et al., 2004; Kern et al., 2004; Keefe et al., 2007). The cognitive domains affected in schizophrenia, such as attention, working memory, reasoning and problem solving, processing speed, visual learning and memory, verbal learning and memory, and social cognition, were identified by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative (Nuechterlein et al., 2004; Young et al., 2009). Accumulating clinical evidence has indicated that cognitive deficits in schizophrenia are closely related to functional outcomes (Sharma and Antonova, 2003; Green et al., 2004). Therefore, better-tolerated therapeutic agents with the potential to address cognitive deficits are needed for the treatment of schizophrenia.
Phosphodiesterase 10A (PDE10A) is a dual-substrate PDE that hydrolyzes both cAMP and cGMP, and is selectively expressed in medium spiny neurons (MSNs) in the striatum of mammalian brains (Seeger et al., 2003; Xie et al., 2006). Striatal outputs mediated by MSNs are mainly divided into two pathways, namely dopamine D₂ receptor–expressing indirect pathway and D₁ receptor–expressing direct pathway (Graybiel, 1990; Graybiel, 2000; Bertran-Gonzalez et al., 2010). These pathways are considered critical for modulating learning behavior via corticostriatal circuits (Graybiel, 2000). In fact, gene targeting and pharmacological studies have shown that each pathway plays a distinct role in learning behavior (Hikida et al., 2010). PDE10A is expressed in both pathways and, thus, PDE10A inhibition and the resulting elevation of striatal cyclic nucleotide levels activate these pathways (Suzuki et al., 2015). Integrated striatal outputs following inhibition of PDE10A may improve cognitive functions by modifying cortical activity. Accordingly, several PDE10A inhibitors have been shown to improve cognitive functions in animal models (Rodefer et al., 2005; Grauer et al., 2009; Smith et al., 2013; Jones et al., 2015).

TAK-063

[1-[2-fluoro-4-(1H-pyrazol-1-yl)phenyl]-5-methoxy-3-(1-phenyl-1H-pyrazol-5-yl)pyridazin-4(1H)-one] is a potent, selective, and orally active PDE10A inhibitor (Kunitomo et
TAK-063 selectively bound to native PDE10A in the rodent brain and the PDE10A occupancy of TAK-063 in the rat striatum at 0.3 and 3 mg/kg orally (p.o.) was 26% and 77%, respectively (Harada et al., 2015). TAK-063 at 0.3 and 1 mg/kg p.o. increased cAMP and cGMP levels in the rodent striatum and upregulated phosphorylation levels of cAMP response element-binding protein and of (±)-α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid receptor subunit GluR1. Evaluation of pathway-specific markers (substance P mRNA for the direct pathway and enkephalin mRNA for the indirect pathway) revealed that TAK-063 at 1 mg/kg p.o. activated both the direct and indirect pathway MSNs in rats. TAK-063 at 0.3 mg/kg p.o. and higher suppressed MK-801–induced hyperlocomotion without affecting plasma prolactin or glucose levels at doses up to 3 mg/kg p.o. TAK-063 at 3 mg/kg p.o. elicited a weak cataleptic response in rats compared with current antipsychotics (Suzuki et al., 2015). The potential of TAK-063 to improve cognitive deficits in schizophrenia remains to be evaluated. TAK-063 is currently in a phase 2 clinical trial with reduction in the Positive and Negative Symptom Scale scores as the primary outcome measures (ClinicalTrials.gov Identifier: NCT02477020).

To investigate the effects of TAK-063 on various cognitive domains, including recognition memory, attention, impulsivity, working memory, and executive function,
we performed a novel object recognition task (NORT), a five-choice serial reaction time task (5-CSRTT), a Y-maze spontaneous alternation test, a radial arm maze task, and an attentional set-shifting task (ASST) in naïve rats or an N-methyl-
\textsubscript{D}-aspartate (NMDA) receptor antagonist-induced rodent models of schizophrenia (Enomoto et al., 2007; Coyle, 2012). Here we report preclinical evidence that TAK-063 can improve cognitive deficits in schizophrenia.
Materials and Methods

Animals. Male imprinting control region (ICR) mice and Long–Evans rats, and Sprague–Dawley (SD) rats were supplied by CLEA Japan, Inc. (Tokyo, Japan), Japan SLC, Inc. (Hamamatsu, Japan), and Charles River Laboratories Japan Inc. (Yokohama, Japan), respectively. Animals were housed in a light-controlled room (12-h light/12-h dark cycle with lights on at 07:00 AM). Animals were used in experiments after at least 1 week of acclimation. Food and water were provided ad libitum, except where noted. The care and use of animals for NORT, 5-CSRTT, Y-maze, and radial arm maze tasks were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited (Fujisawa, Japan). Female hooded Lister rats were supplied by Harlan Laboratories UK, Ltd. (Bicester, UK) for experiments using ASST. The study was conducted at the University of Bradford in accordance with the Animals Scientific Procedures Act and was approved by the Ethical Review Panel of the University of Bradford.

Chemicals. TAK-063 was synthesized by Takeda Pharmaceutical Company Limited (Kunitomo et al., 2014). TAK-063 was suspended in 0.5% (w/v) methylcellulose in distilled water and was orally administered (p.o.). (+)-MK-801 hydrogen maleate
(MK-801

\[(5R,10S)-(+)\)-5-methyl-10,11-dihydro-5\(H\)-dibenzo[a,d]cyclohepten-5,10-imine\];

Sigma-Aldrich, St Louis, MO) was dissolved in saline for subcutaneous administration (s.c.). Phencyclidine hydrochloride (PCP, Sigma-Aldrich) was dissolved in saline and was administered s.c. for the radial arm maze task and intraperitoneally (i.p.) administered for ASST. Risperidone (Sigma-Aldrich) was dissolved in acetic acid and was made up to volume with saline. Subsequently, p\(H\) was adjusted to 6 using 0.1 M NaOH, and solutions were administered i.p. to rats. Mice and rats were treated with compound solutions at volumes of 20 and 2 mL/kg body weight, respectively, except where noted.

**NORT.** Experiments were performed using 7-week-old male Long–Evans rats as described previously (Bevins and Besheer, 2006) with minor modifications. Briefly, rats were allowed to habituate to the behavioral test room environment for more than 1 h on day 1, and then were allowed to habituate to the empty test box (a gray-colored polyvinyl chloride box of 40 \(\times\) 40 \(\times\) 50 cm) for 10 min individually. Testing comprised two 3-min trials for acquisition and retention that were separated by a 48-h inter-trial interval (ITI). On day 2, rats were allowed to explore two identical objects (A1 and A2)
for 3 min in the acquisition trial. On day 4, rats were again allowed to explore a familiar
object (A3) and a novel object (B) for 3 min in the retention trial. Object exploration
was defined as licking, sniffing, or touching the object with forelimbs while sniffing.
Leaning against the object to look upward and standing or sitting on the object were
excluded. Exploration times were scored manually for each object (A1, A2, A3, and B)
in each trial. The investigator who conducted the measurement was blinded to the
treatment. TAK-063 was administered p.o. at 0.03, 0.1, or 0.3 mg/kg 120 min prior to
acquisition trials. The novelty discrimination index (NDI) was calculated as exploration
time for novel object/total exploration time.

5-CSRTT. Thirty-two male Long–Evans rats at 7 weeks of age were trained for this
study. During training, food was restricted to 85% of their free-feeding body weight.
Rats were then required to discriminate between short visual stimuli that occur
randomly in one of five apertures. Training and testing were conducted in four operant
chambers enclosed in sound-attenuating boxes (Med Associates Inc., St Albans, VT).
Each chamber contained a curved wall with five contiguous apertures. Food pellets
were delivered automatically into a magazine located in the opposite wall, and a
photocell beam was used to detect head entries into the magazine. An initial pellet was
delivered into the magazine at the start of each session to initiate the first trial. After ITI, a light stimulus was presented in one of the five apertures. Correct responses (e.g., responses in the illuminated aperture) resulted in the delivery of a food pellet into the magazine. Incorrect responses (e.g., responses in non-illuminated apertures), omissions (failure to respond during the limited hold), and premature responses (responses occurring prior to the presentation of the stimulus) were punished with 5-s timeout periods, extinction of the house light, and no delivery of food. Percent accuracy rates were calculated as the number of correct responses / (the number of correct responses + the number of incorrect responses) × 100. Each session lasted for 35 min or until 100 trials were completed. Durations of light stimuli were initially set at 30 s, then gradually decreased to 2 s during the training period. Duration of limited holds was kept for 5 s throughout the study. Rats received five training sessions per week until they achieved criteria for performance (>75% accuracy and <20 omissions) over 2 consecutive days. The training was continued to maintain the performance of rats until use.

Sixteen rats (within group of 32 trained rats) were used for the fixed ITI test at 13 months of age. These 16 rats were also used to assess the effects of other drugs than TAK-063 on 5-CSRTT performance at between ages 7 and 11 months. These rats received a 2-month washout period prior to the start of the study with TAK-063.
Another 16 rats were used only for characterization of TAK-063 in variable ITI tests at 6 months of age. On the testing day, a fixed ITI schedule was used with 0.5-s stimuli and 5-s ITI to assess attention, and impulsivity was assessed using a variable ITI schedule with 0.5-s stimuli at 4, 5, 7, or 10 s (Navarra et al., 2008; Robinson, 2012). Equal numbers of each of the four ITIs were randomly presented over 100 trials.

TAK-063 (0.1 and 0.3 mg/kg p.o.) or vehicle was administered 120 min before the test in crossover fashion, separated by a washout period of 1 week. The investigator who conducted the measurement was blinded to the treatment.

**Y-maze spontaneous alternation test.** Experiment was performed using 7-week-old male ICR mice as described previously (Hughes, 2004), with minor modifications. To measure spontaneous alternation behavior, the Y-maze was performed using 40-× 4-cm (length × width, respectively) arms with 12-cm high walls. TAK-063 (0.1 and 0.3 mg/kg p.o.) and PCP (2 mg/kg s.c. as a salt) were administered at 60 and 30 min, respectively, before testing. Animals were placed in one of three arms and were allowed to explore the maze for 5 min. Alternations and total numbers of arm choices were recorded. The investigator who conducted the measurement was blinded to the treatment. Spontaneous alternation rates were calculated as the ratio of numbers of arm choices that differed...
from the previous two choices to the total number of arm entries. Percent alternation was defined as the ratio of actual (total alternations) to possible alternations (total arm entries − 2) × 100.

**Radial arm maze task.** The experiment was performed using 9-week-old male Long–Evans rats as described previously (Zajaczkowski et al., 1996) with some modifications. To evaluate working memory, an eight-arm radial maze with arms of 50 cm in length, 10 cm in width, and 40 cm in height was elevated 50 cm above the floor. After fasting for 24 h, food was restricted to 85% of their free-feeding body weight on the first day of exposure to the maze. Three food pellets (45 mg each) served as reinforcement and were provided in food cups at the ends of each arm during testing. On the first day of habituation to the maze, reinforcements were placed near the entrance and at the mid-points of each arm. Three rats were placed in the maze and were allowed to explore and consume pellets for 8 min. On the second day of habituation, rats were placed to allow consumption of pellets at mid-points and from food cups at the ends of each arm for 5 min. Training sessions commenced on the third day, and pellets were placed in food cups at the ends of each arm. Rats were placed in the maze facing away from the experimenter and toward the same arm for the start of each trial. A timer
was started, and each arm entry was recorded in sequence. Rats were allowed to choose until all eight arms were entered into and all pellets were consumed or until 5 min had elapsed. Entries into previously chosen arms were counted as errors. If an animal failed to enter all eight arms in 5 min, omitted arms were also counted as errors. Rats received one training session per day, and training was continued until rats achieved the learning criterion, which was defined as ≤2 errors on 2 consecutive days. On the day prior to drug testing, baseline error levels were qualified as ≤2. TAK-063 (0.1 and 0.3 mg/kg p.o.) or vehicle was administered 150 min before the test, and MK-801 (0.08 mg/kg s.c. as a salt) or saline was administered 30 min before the test. The investigator who conducted the measurement was blinded to the treatment.

**ASST.** Overall order of discriminations presented to rats in ASST was previously described (McLean et al., 2008). ASST was conducted by b-neuro™ (The University of Manchester, UK). Female hooded-Lister rats (200–220 g) were treated twice daily for 7 days with vehicle (saline) or PCP (2 mg/kg i.p. as a salt) in volumes of 1 mL/kg body weight followed by a 7-day drug-free period. The set-shifting apparatus was previously described (McLean et al., 2008). Rats were tested in the ASST procedure 24 h after training. Rats were administered risperidone or TAK-063 at 60 or 90 min before testing,
respectively. The investigator who conducted the measurement was blinded to the treatment. Trials were initiated by raising both dividers to give access to both digging bowls (only one was baited). The first stage was the simple discrimination (SD), which was identical to the SD in the training session on the previous day, except new exemplars were used. Testing continued until rats reached the criterion of six consecutive correct responses. Subsequently, rats performed a series of discriminations in each test. Overall order of discriminations presented to rats in the attentional set-shifting task was previously described (McLean et al., 2008). For the compound discrimination (CD), a second dimension was introduced (odor), and correct and incorrect exemplars remained the same (digging medium). For reversals, exemplars and relevant dimensions remained the same (medium), but the rats had to learn that the baited odor had been switched. New exemplars were used for intra-dimensional shift (IDS) and extra-dimensional shift (EDS). For EDS, the previously irrelevant parameter (odor) was made relevant. It has been shown that rats find the difficulty of discriminating between medium-to-odor and odor-to-medium changes (Birrell and Brown, 2000; McLean et al., 2008). The number of trials taken to achieve the criteria (six consecutive correct choices) was counted in each discrimination test.
Spontaneous locomotor activity. Locomotion was measured using a SUPERMEX spontaneous motor analyzer (Muromachi Kikai Co. Ltd., Tokyo, Japan) for rats and an MDC system (BrainScienceIdea Co. Ltd., Osaka, Japan) for mice. Animals (male ICR mice at 7 weeks of age or SD rats at 9 weeks of age) were treated with either vehicle or TAK-063. The investigator was blinded to the treatment. After a pretreatment time, animals were transferred to the test chamber (length × width × height: 24 × 37 × 30 cm for rats and 36 × 22 × 13.5 cm for mice). Sixty-minute and 120-min pretreatment time was used in the mouse and rat studies. Activity counts were recorded in successive 1-min bins and then cumulative counts during 120 min were calculated.

Statistics. Pairwise differences between groups were identified using Student’s t test (for homogeneous data) or Aspin–Welch test (for non-homogeneous data). In experiments with multiple doses of test compounds, differences were identified using two-tailed Williams’ test (for homogeneous data) or two-tailed Shirley–Williams test (for non-homogeneous data). Values of $P \leq 0.05$ were considered significant. In 5-CSRTT experiments, the data were tested by crossover analysis of variance (ANOVA). The contrast test was performed to compare the mean in the vehicle with that in each TAK-063–treated animal and was considered significant when $P \leq 0.05$
(versus the vehicle-treated group). In ASST experiments, we compared the performance in between the IDS and EDS phases in the control group by paired t test only to validate our experimental conditions for assessing the attentional set shift. Differences were considered significant when $P \leq 0.05$. For comparing several treatments with the vehicle-treated group, Dunnett’s test was used and differences were considered significant when $P \leq 0.05$. 
Results

Effects of TAK-063 on Recognition Memory Assessed by a NORT in Rats.

TAK-063 at 0.3 mg/kg p.o. increased striatal cAMP and cGMP levels and produced potent antipsychotic-like effects in rodents (Suzuki et al., 2015); thus, doses of ≤0.3 mg/kg p.o. were used in the cognitive studies. TAK-063 at 0.1 and 0.3 mg/kg p.o. did not affect spontaneous locomotor activity in rats (Supplemental Fig. 1A). NORT experiments were performed in naïve rats to assess the effects of TAK-063 on recognition memory performance. Acquisition trials were initiated 120 min after the administration of TAK-063 at 0.03, 0.1, and 0.3 mg/kg p.o. No significant differences in total interaction times with objects were observed between the four groups in the acquisition trial (Fig. 1A). After an interval time of 48 h, vehicle-treated rats showed no significant preferential exploration of either object during the retention trial (Fig. 1, B and C), suggesting natural memory decay of the familiar object. In contrast, TAK-063–treated rats spent significantly more time exploring the novel object than the familiar one ($P \leq 0.01$, at 0.1 mg/kg p.o., $P \leq 0.05$, at 0.3 mg/kg p.o.). TAK-063 also significantly increased the NDI in retention trials at doses of 0.1 and 0.3 mg/kg p.o. ($P \leq 0.05$; Fig. 1C).
Effects of TAK-063 on Attention and Impulsivity Assessed by 5-CSRTT in Rats.

Originally used as a continuous performance test in humans, 5-CSRTT is known to be an assessment of sustained attention in rodents (Robbins, 2002). Two conditions (fixed ITI and variable ITI) were used to evaluate the effects of TAK-063 on attention and impulsivity. Rats were trained to achieve criteria for performance (>75% accuracy and <20 omissions under the conditions with 2-s duration of light stimuli and fixed 5-s ITI) over 2 consecutive days.

In a fixed ITI test, duration of light stimuli was reduced from 2 to 0.5 s and ITI was kept at 5 s. Under these conditions, rats needed to be highly attentive to a brief light stimulus (0.5 s) to choose the correct position. Thus, this test may be sensitive to assess the effect of a drug on attention. Treatment with TAK-063 at 0.1 and 0.3 mg/kg p.o. significantly increased accuracy rates compared with vehicle treatment ($P \leq 0.05$, at 0.1 mg/kg p.o., $P \leq 0.01$, at 0.3 mg/kg p.o.; Fig. 2A). TAK-063 also significantly increased latencies of food pellet retrieval following correct responses (magazine latencies, $P \leq 0.05$, at 0.1 mg/kg p.o., $P \leq 0.05$, at 0.3 mg/kg p.o.; Fig. 2B). Omission rates were not significantly affected by treatment with TAK-063 (Fig. 2C). TAK-063 at 0.3 mg/kg p.o. significantly decreased the numbers of premature responses ($P \leq 0.01$; Fig. 2D).

In a variable ITI test, duration of light stimuli was reduced to 0.5 s and the brief light
stimulus (0.5 s) was presented in an unpredictable manner (4-, 5-, 7-, or 10-s ITI).

Under these conditions, rats must maintain attention for variable duration and that led to an increase in the impulsive responses (Robbins, 2002). As expected, premature responses were dramatically increased under these conditions compared with those in fixed ITI tests from about 7 to 40 times (Fig. 2D and 3D). TAK-063 at 0.3 mg/kg p.o. significantly decreased the increased premature responses, from about 40 to 25 times ($P \leq 0.05$; Fig. 3D). Accuracy, magazine latency, and omission rates were not significantly affected by treatment with TAK-063 (Fig. 3, A–C). These results suggest that TAK-063 improves attention and reduces impulsivity in naïve rats.

Effects of TAK-063 on PCP-Induced Spatial Working Memory Deficits Assessed by Y-Maze Tests in Mice. NMDA receptor antagonists such as PCP and MK-801 were known to cause schizophrenia-like symptoms, including cognitive deficits in clinical and preclinical studies (Coyle, 2012). To evaluate the effects of TAK-063 on working memory deficits such as those occurring in schizophrenia, animals pretreated with an NMDA receptor antagonist were used. Initially, mouse Y-maze tests were conducted to evaluate spatial working memory in rodents. TAK-063 at 0.1 and 0.3 mg/kg p.o. did not affect spontaneous locomotor activity in mice (Supplemental Fig. 1B). After the
administration of PCP to mice, the numbers of total entries into arms was significantly increased ($P \leq 0.01$; Fig. 4A). Pretreatment with TAK-063 at 0.3 mg/kg p.o. significantly inhibited PCP-induced increases in total entries ($P \leq 0.05$; Fig. 4A). In Y-maze tests, untreated mice tend to visit the arms one after the other, and typically exhibit an alternation rate of about 60-70% (Belzung, 1999). In fact, the alternation rate in control mice was 66.5 ± 2.4% under experimental conditions (Fig. 4B). Treatment with PCP significantly decreased spontaneous alternation rates ($P \leq 0.01$; Fig. 4B). This PCP-induced decrease was dose-dependently ameliorated by pretreatment with TAK-063 with a minimum effective dose (MED) of 0.3 mg/kg p.o. ($P \leq 0.05$; Fig. 4B). These data suggest that TAK-063 attenuates PCP-induced spatial working memory deficits in the Y-maze test.

Effects of TAK-063 on MK-801–Induced Spatial Working Memory Deficits

Assessed by the Eight-Arm Radial Maze Task in Rats. The effects of TAK-063 on MK-801–induced impairment of spatial working memory were examined in a radial arm maze task. During test sessions, all rats in the control group found and consumed all pellets in the maze without retracing errors (Fig. 5). Treatment with MK-801 produced significant increases in the numbers of errors ($P \leq 0.05$; Fig. 5) and
pretreatment with TAK-063 at 0.3 mg/kg p.o. produced significant decreases in the numbers of errors produced by MK-801 ($P \leq 0.05$; Fig. 5).

**Effects of TAK-063 on Disturbed Executive Function in Subchronic PCP-Treated Rats using ASST.** To assess the effects of TAK-063 on executive function, an ASST was conducted in rats treated sub-chronically with PCP. Rats treated with PCP show sustained cognitive deficits similar to those shown in patients with schizophrenia (Neill et al., 2010). Subjects with schizophrenia exhibit poorer IDS/EDS performance compared to healthy controls (Young et al., 2009); thus, ASST paradigm is validated by the significant difference in trials to reach the criterion between the EDS and IDS phases. More trials to reach the criterion in EDS phase were needed compared with those in IDS phase, suggesting that rats had formed an attentional set to the initial stimulus dimension (Birrell and Brown, 2000). In the test session, we confirmed that saline-treated rats needed more trials to reach the criterion in the EDS phase than in the IDS phase ($P \leq 0.01$; Fig. 6). Subchronic PCP-treated rats showed markedly greater impairments in the EDS phase than did saline-treated rats ($P \leq 0.01$; Fig. 6). Moreover, PCP-induced increases in the numbers of trials required to meet the criterion in the EDS phase were dose-dependently reduced by acute treatment with TAK-063 and risperidone.
(\(P \leq 0.01\); Fig. 6). The MED of TAK-063 was 0.3 mg/kg p.o. No significant differences were observed in any other parameters in this task. These results suggest that TAK-063 ameliorates impairments of executive function in subchronic PCP-treated rats.

**Discussion**

The potential effects of TAK-063 on cognitive improvement in schizophrenia was investigated by studying its effects on various cognitive domains that are known to be disrupted in schizophrenia, including recognition memory, attention, impulsivity, working memory, and executive function (Goldman-Rakic, 1994; Cirillo and Seidman, 2003; Kaladjian et al., 2011). PDE10A inhibition can affect motor function that may impact the results of cognitive tasks in rodents. In fact, some signs of motor decrease were observed in the magazine latency in 5-CSRTT with fixed ITI (Fig. 2B) and total entry in mice Y-maze test (Fig 4A), but not in other tests, such as exploration time in NORT (Fig. 1A) and magazine latency in 5-CSRTT with variable ITI (Fig. 3B). Increase in magazine latency observed in the fixed ITI 5-CSRTT was significant but very small (only about 0.2 s), and the decrease in the total entry in mice treated with PCP in the Y-maze test can be secondary to TAK-063’s antipsychotic effects. In fact, TAK-063 suppressed MK-801–induced hyperlocomotion in rodents (Suzuki et al., 2007).
Moreover, TAK-063 at doses we used in this study did not significantly affect spontaneous locomotor activity in both mice and rats (Supplemental Fig. 1). Therefore, possible cognitive improvement by TAK-063 may not be affected by modulation of motor function.

The NORT with naïve rats was used to measure recognition memory (Mumby and Pinel, 1994; Albasser et al., 2009; Rios Valentim et al., 2009). In agreement with previous studies with various PDE10A inhibitors (Grauer et al., 2009; Smith et al., 2013; Jones et al., 2015), TAK-063 at 0.1 and 0.3 mg/kg p.o. enhanced retention of recognition memory in rats. 5-CSRTT has been used to measure attention-related functions in rodents (Robbins, 2002). To evaluate the effects of TAK-063 on attention and impulsivity, two conditions (fixed ITI and variable ITI) were used. In a fixed ITI test, duration of light stimuli was reduced from 2 to 0.5 s, and rats needed to be highly attentive to choose the correct position. Thus, this test may be sensitive to assess the effect of a drug on attention. TAK-063 at 0.1 and 0.3 mg/kg p.o. significantly increased accuracy rates and magazine latencies, and TAK-063 at 0.3 mg/kg p.o. significantly decreased the numbers of premature responses under fixed ITI conditions. To further evaluate the effects of TAK-063 on impulsivity, variable ITI tests were performed. In these tests, rats must maintain attention for variable durations and that led to an increase
in the baseline levels of premature responses (Navarra et al., 2008; Robinson, 2012).

Under these conditions, TAK-063 at 0.3 mg/kg p.o. significantly decreased the numbers
of premature responses compared to vehicle. These results suggest that TAK-063
improved attention and impulsivity, although further studies will be needed to fully
characterize the effects of TAK-063 on attention because 1) unlike the fixed ITI test,
TAK-063 did not increase the accuracy rate in the variable ITI test, 2) TAK-063 showed
a tendency to increase omissions, and 3) the 5-CSRTT is a complex operant
conditioning task. Differences of effect of a drug on accuracy rate were also reported in
other studies (Bizarro et al., 2004).

Acute treatment with the D2 receptor antagonist haloperidol also significantly
decreased the numbers of premature responses and increased magazine latencies in a
5-CSRTT using naïve rats; however, unlike TAK-063, haloperidol also significantly
decreased accuracy rates (Paine and Carlezon, 2009). PDE10A inhibition by TAK-063
may have a different pharmacological profile from those of current antipsychotics on
attention and impulsivity.

Non-competitive NMDA receptor antagonists are known to change both human and
animal behavior and induce schizophrenia-like symptoms, including positive symptoms,
negative symptoms, and cognitive deficits (Javitt and Zukin, 1991; Coyle, 2012).
Therefore, NMDA receptor antagonist–treated animals have been widely used as models of schizophrenia (Noda et al., 1995; Sams-Dodd, 1996; Nilsson et al., 1997; Enomoto et al., 2005; Enomoto et al., 2007). To evaluate the effects of TAK-063 on the spatial working memory deficits produced by an NMDA receptor antagonist, Y-maze test and radial arm maze task were used (Lalonde, 2002; Hughes, 2004; Abdul-Monim et al., 2006). TAK-063 at 0.3 mg/kg p.o. significantly attenuated NMDA antagonist–induced working memory deficits in both tasks.

The effects of TAK-063 on executive functions were investigated using rats treated subchronically with PCP. The ASST measures the ability of an animal to learn a rule and form an attentional set within the same sorting category and the ability to shift attentional sets between different sorting categories (Birrell and Brown, 2000). The ASST is comparable to the human CANTAB ID/ED task (Downes et al., 1989), in which patients with schizophrenia exhibit impaired executive function (Berg, 1948; Pantelis et al., 1999; Tyson et al., 2004). In agreement with results reported with the PDE10A inhibitor, papaverine, in this model (Rodefer et al., 2005), TAK-063 at 0.3 mg/kg p.o. significantly ameliorated deficits in the EDS phase in subchronic PCP-treated rats. Note that the PDE10A selectivity of papaverine is poor (Zhang et al., 2011) and this is the first data showing that the PDE10A selective inhibitor can improve
executive function in PCP-treated rats. In this test, risperidone at 0.1 mg/kg i.p. also showed significant efficacy in the EDS phase. This dosage of risperidone may not be high enough to produce its antipsychotic-like effects based on the reported <50% dopamine D₂ receptor occupancy in the rat striatum (Zhang and Bymaster, 1999).

Moreover, haloperidol was ineffective in ASST (McLean et al., 2008). Therefore, efficacy of risperidone in the ASST could be mediated through mechanisms other than dopamine D₂ receptor antagonism or combination of lower levels of dopamine D₂ receptor blockage plus something unknown. Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study showed no meaningful procognitive efficacy of risperidone at 1.5-6 mg where risperidone produces a potent antipsychotic effect in patients with schizophrenia (Stroup et al., 2003; Keefe et al., 2007; Lieberman and Stroup, 2011); however, its efficacy on cognitive functions at sub-therapeutic dosages has not been established. Further pre-clinical and clinical studies will be needed to understand the potential of sub-therapeutic dosages of risperidone and of translational predictability of the ASST with subchronic PCP-treated rats.

Expression of PDE10A is highly restricted to the striatum and, in fact, radiolabeled TAK-063 selectively accumulated in the striatum in both in vitro and in vivo autoradiography experiments (Harada et al., 2015). The striatum and frontal cortex are
highly interconnected via neuronal circuitry (Haber, 2003; Simpson et al., 2010), and
the striatum plays a general role in learning and cognition (Graybiel, 2005). Recent
studies have shown that convergent inputs of direct and indirect pathways contribute to
learning (Hikida et al., 2010; Yawata et al., 2012). PDE10A is reported to be expressed
in both direct and indirect pathway MSNs (Nikiforuk et al., 2015; Duinen et al., 2015;
Wilson et al., 2015). The cell type-specific translational profile using translating
ribosome affinity purification techniques also suggests that PDE10A is expressed in
both pathways (Doyle et al., 2008). In line with these reports, TAK-063 upregulated
mRNA expressions of pathway-specific markers for both direct and indirect pathways
(substance P and enkephalin) (Suzuki et al., 2015). Thus, augmented striatal outputs
following inhibition of PDE10A by TAK-063 may modulate cortical activity and
contribute to cognitive functions via corticostriatal circuits. Studies with high temporal-
and spatial-resolution methods such as electroencephalography and pharmacological
magnetic resonance imaging indicated the effects of TAK-063 on cortical activity
(Tomimatsu Y, Cash D, Suzuki M, Bernanos M, Simmons C, Williams S, and Kimura H,
manuscript in preparation).

In the present study, TAK-063 improved the performance of mice and rats in various
cognitive domains that are impaired in schizophrenia, including recognition memory,
attention, working memory, and executive function. These preclinical data suggest that TAK-063 has the potential to treat cognitive deficits in schizophrenia. Procognitive effects of TAK-063 in these domains were observed at around 0.3 mg/kg p.o. TAK-063 at this dose also showed a potent antipsychotic-like effect in rodents (Suzuki et al., 2015). Thus, TAK-063 may improve multiple symptomatic domains, i.e. positive symptoms and cognitive impairments, in schizophrenia at similar dose levels. The PDE10A occupancy of TAK-063 at 0.3 mg/kg p.o. was 26% in rats (Harada et al., 2015).

A nonhuman positron emission tomography (PET) study using [11C]T-773, an original PET tracer for PDE10A, demonstrated that TAK-063 occupies striatal PDE10A in a dose-dependent manner (Takano A, Stepanov V, Nakao R, Amini N, Gulyás B, Kimura H, and Halldin C, manuscript in preparation). These results will be helpful for dose setting in future clinical trials. TAK-063 is currently in clinical development (ClinicalTrials.gov Identifier: NCT 02477020).

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**Authorship Contributions**

*Participated in research design:* K. Suzuki, Shiraishi, Kimura.

*Conducted experiments:* K. Suzuki, Harada, N. Suzuki, Shiraishi.


*Wrote or contributed to the writing of the manuscript:* K. Suzuki, Shiraishi, Kimura.
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Footnotes

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Figure Legends

Fig. 1. Effects of TAK-063 on a novel object recognition task in naïve rats. TAK-063 was orally administered at 0.03, 0.1, and 0.3 mg/kg 120 min prior to the acquisition trial. (A) Total exploration times for two identical objects in the acquisition trial. (B) Exploration times for familiar and novel objects in the retention trial. (C) Novel discrimination index (NDI). Data are presented as mean ± S.E.M. (n = 10 in each group); **P \leq 0.01; *P \leq 0.05 (vs familiar object; paired t test); #P \leq 0.05 (vs vehicle group; two-tailed Williams’ test).

Fig. 2. Effects of TAK-063 on performance in a five-choice serial reaction time task under a 5 sec fixed inter-trial interval schedule in naïve rats. (A) TAK-063 at 0.1 and 0.3 mg/kg p.o. significantly increased accuracy. (B) TAK-063 at 0.1 and 0.3 mg/kg p.o. significantly increased magazine latencies (latencies of food pellet retrieval following correct responses). (C) Neither dose of TAK-063 significantly increased omission rates. (D) TAK-063 at 0.3 mg/kg p.o. significantly decreased the numbers of premature responses. Data are represented as mean ± S.E.M. (n = 16 in each group); ##P \leq 0.01; #P \leq 0.05 (versus vehicle group; crossover ANOVA followed by a contrast test).
**Fig. 3.** Effects of TAK-063 on the five-choice serial reaction time task under the variable inter-trial interval (ITI) schedule in naïve rats. ITI was taken randomly for 4, 5, 7, and 10 s. (A) TAK-063 at 0.1 and 0.3 mg/kg p.o. did not significantly affect accuracy rates. (B) Magazine latencies were not significantly different between groups. (C) TAK-063 at 0.3 mg/kg p.o. did not significantly increase omission rates. (D) TAK-063 at 0.3 mg/kg p.o. significantly decreased the numbers of premature responses. Data are represented as mean ± S.E.M. (n = 16 in each group); #P ≤ 0.05 (versus vehicle group; crossover ANOVA followed by a contrast test).

**Fig. 4.** Effects of TAK-063 on phencyclidine (PCP)-induced deficits in mouse Y-maze spontaneous alternation tests. (A) Total entries in the Y-maze test; control animals were administered vehicle and saline (white column). Vehicle-treated animals were administered vehicle and PCP (black column). TAK-063 at 0.3 mg/kg reduced the PCP-induced increase in total arm entries. (B) TAK-063 inhibited PCP-induced decreases in spontaneous alternation rates in a Y-maze test at 0.3 mg/kg p.o. Data are represented as mean ± S.E.M. (n = 10 in each group); **P ≤ 0.01 (versus control group; Student’s t test), #P ≤ 0.05 (versus vehicle + PCP group; two-tailed Williams’ test).
Fig. 5. Effects of TAK-063 on working memory errors in rat radial arm maze task.

Control animals were administered vehicle and saline, and vehicle-treated animals were injected with vehicle and MK-801 (black column). All rats in the control group took all pellets without errors. TAK-063 at 0.3 mg/kg p.o. significantly attenuated the numbers of errors produced by MK-801. Numbers of errors are represented as mean + S.E.M. (n = 4 in control group, n = 7 in vehicle + MK-801 group, n = 6 in TAK-063 + MK-801 groups); *P ≤ 0.05 (versus control group; Aspin–Welch test), #P ≤ 0.05 (versus vehicle + MK-801 group; two-tailed Shirley–Williams test).

Fig. 6. Effects of the acute administration of TAK-063 on sub-chronic phencyclidine (PCP)-induced deficits in the rat attentional set-shifting task. Control (white column) and treatment groups were administered saline and PCP twice daily for 7 days. TAK-063 at 0.03, 0.1, and 0.3 mg/kg p.o. or risperidone at 0.1 mg/kg i.p. was administered 90 or 60 min before the test, respectively. Acute treatments with TAK-063 at 0.3 mg/kg p.o. or risperidone at 0.1 mg/kg significantly decreased the numbers of trials required to reach EDS phase criteria in subchronic PCP-treated rats. All data are expressed as mean + S.E.M. (n = 9 in TAK-063 0.03-mg/kg group, n = 10 in other groups); ††P ≤ 0.01 (versus IDS in control group; paired t test), †††P ≤ 0.01 (versus
control group; Student’s $t$ test), **$P \leq 0.01$ (versus vehicle group; Dunnett’s test). SD: simple discrimination; CD: compound discrimination; Rev: reversals; IDS: intra-dimensional shift; EDS: extra-dimensional shift.
**Fig. 4**

![Bar graphs showing total entries and alternation ratio for different treatments](image)

**A**
- Total entries
  - Control
  - Vehicle
  - 0.1 TAK-063 (mg/kg p.o.)
  - 0.3 TAK-063 (mg/kg p.o.)
  - PCP (2 mg/kg s.c.)

**B**
- Alternation ratio
  - Control
  - Vehicle
  - 0.1 TAK-063 (mg/kg p.o.)
  - 0.3 TAK-063 (mg/kg p.o.)
  - PCP (2 mg/kg s.c.)

Annotations:
- *: Significant difference
- #: Trend towards significant difference

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Fig. 5

Number of errors

Control  Vehicle  0.1  0.3

TAK-063 (mg/kg p.o.)

MK-801 (0.08 mg/kg s.c.)
Fig. 6

Trials to criterion

SD  CD  Rev1  IDS  Rev2  EDS  Rev3

Control  Vehicle  TAK-063 0.03 mg/kg p.o.  TAK-063 0.1 mg/kg p.o.  TAK-063 0.3 mg/kg p.o.  Risperidone

††  **  ##  **

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The phosphodiesterase 10A selective inhibitor TAK-063 improves cognitive functions associated with schizophrenia in rodent models

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Supplemental Fig. 1

A Rat

B Mouse

Supplemental Fig. 1. The effect of TAK-063 on locomotor activity in a novel environment in male SD rats (A) or ICR mice (B). After a pretreatment time, animals were transferred to the test chamber. Accumulated activity counts during the 120 min were calculated and are indicated as mean ± SEM (n = 6 in rats, n = 10 in mice). There was no significant difference between the groups by two-tailed Williams's test (P > 0.05).