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The effects of PRX-07034, a novel 5-HT₆ antagonist, on cognitive flexibility and working memory in rats

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Abstract

Rationale—Accumulating evidence indicates that schizophrenia and autism spectrum disorder patients are marked by cognitive deficits in working memory and strategy switching. There is accumulating evidence that 5-hydroxytryptamine (5-HT)₆ receptors may serve as a useful target to improve cognitive functioning.

Objectives—In the present experiments, the novel 5-HT₆ antagonist, PRX-07034, was examined for its selectivity of the 5-HT₆ receptor, as well as its effect on delayed spontaneous alternation and strategy switching.

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Methods—The binding affinity of PRX-07034 to the 5-HT₆ receptor, other 5-HT receptors, as well as other G-protein coupled receptors, ion channels, and transporters was evaluated. Cyclic AMP production was measured from transfected HEK-293 cells. In separate behavioral experiments, rats received different doses of PRX-07034 (0.1, 1, or 3 mg/kg, i.p.) 30 min prior to delayed spontaneous alternation testing or prior to the acquisition and switch phases in a place–response switch test.

Results—The results indicated that PRX-07034 is both a potent ($K_i = 4\text{--}8\text{ nM}$) and highly selective 5-HT₆ receptor antagonist (100-fold selectivity for the 5-HT₆ receptor compared to 68 other GPCRs, ion channels, and transporters, except D₃ ($K_i = 71\text{ nM}$) and 5-HT_{1B} ($K_i = 260\text{ nM}$) receptors. For cyclic AMP quantification, PRX-07034 demonstrated antagonist activity ($IC_{50} = 19\text{ nM}$) without an effect on basal levels and did not show any agonist activity up to $10\text{ }\mu\text{M}$. PRX-07034 at 1 and 3 mg/kg (but not 0.1 mg/kg) significantly enhanced delayed spontaneous alternation. The drug at 1 and 3 mg/kg also enhanced switching between a place and response strategy, but did not affect initial learning of either a place or response discrimination.

Conclusions—These findings demonstrate that PRX-07034 is a selective 5-HT₆ receptor antagonist that may represent a novel treatment for enhancing working memory and cognitive flexibility.

Keywords

Serotonin; Learning; Memory; Set-shifting; Schizophrenia; Autism spectrum disorder

Introduction

The 5-HT₆ receptor has been identified as a potential target for treatment of cognitive deficits in schizophrenia (Mitchell and Neumaier 2005). Further interest has emerged from results showing that 5-HT₆ receptors are localized in the frontal cortex, striatum, and hippocampus (Woolley et al. 2004). These brain areas show abnormalities in neuropsychiatric disorders including schizophrenia and autism spectrum disorder, as well as playing an important role in working memory and cognitive flexibility (Harrison and Weinberger 2005; Langen et al. 2009; Schumann et al. 2010). Thus, targeting 5-HT₆ receptors could prove beneficial in reducing working memory and cognitive flexibility deficits in various disorders.

Experiments investigating the effects of 5-HT₆ receptor antagonists in learning and memory have led to mixed results. In several studies, treatment with a 5-HT₆ receptor antagonist has enhanced learning and memory (King et al. 2004; Mitchell et al. 2006; Rogers and Hagan 2001; Woolley et al. 2001; Riemer et al. 2003; Foley et al. 2004; Hirst et al. 2006) or attenuated a scopolamine-induced deficit in memory (Woolley et al. 2003; Lieben et al. 2005; Hirst et al. 2006). Other experiments found that 5-HT₆ receptor antagonists failed to improve memory, either alone (Woolley et al. 2003; Mitchell et al. 2006) or against scopolamine-induced deficits (Lindner et al. 2003). The reason for the conflicting results is unclear because the majority of these studies have used the same 5-HT₆ receptor antagonists (Ro 04–6790 and/or SB-271046); thus, the specific 5-HT₆ receptor antagonist used cannot explain the difference in findings. However, 5-HT₆ receptor antagonists improve cognition more readily when reversing scopolamine-induced deficits rather than enhancing performance in normal animals (e.g., Woolley et al. 2003). Therefore, one possibility for inconsistent findings is that 5-HT₆ receptor antagonists may be most effective when attenuating an already existing deficit.

Less is known about the effects of a 5-HT₆ receptor antagonist on shifting strategies or choice patterns. One study found that the 5-HT₆ receptor antagonist, SB-399885, enhanced

reversal learning in rats but only produced a modest improvement in an extra-dimensional set shift (Hatcher et al. 2005). More recently, treatment with the 5-HT₆ receptor antagonist, SB-271046, was reported to attenuate a PCP-induced impairment in set-shifting (Rodefer et al. 2008). Further understanding the effects of a 5-HT₆ receptor antagonist on a strategy-switching test may be particularly relevant because previous studies showed that schizophrenia or autism spectrum disorder subjects have set-shifting deficits (Elliot et al. 1995; Pantelis et al. 1999; Jazbec et al. 2007; Corbett et al. 2009). Further, it is not yet clear whether a specific 5-HT₆ receptor antagonist can improve both working memory and cognitive flexibility to serve as a single treatment for alleviating multiple cognitive deficits.

To better understand the role of 5-HT₆ receptors in learning and memory, the present experiments investigated the selectivity of a novel drug, PRX-07034, for 5-HT₆ receptors in a radioligand binding study, ability to penetrate the brain, as well as examining its effects on working memory and strategy switching.

Materials and methods

All studies were carried out in accordance with the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals adopted by the US National Institutes of Health.

Drug

PRX-07034 was mixed in sterile water and injected intraperitoneal for all behavioral experiments. Doses were based on previous experiments that examined the effects of 5-HT₆ receptor antagonists on learning and memory (King et al. 2004; Woolley et al. 2003). The chemical structure of PRX-07034 was previously published (Johnson et al. 2008).

Radioligand binding studies

All radioligand binding studies were carried out by CEREP (Paris, France) using a similar procedure as described previously (McCreary et al. 2007). The binding assay protocol includes a minimum of 6-control wells (non-specific and total binding) plus an 8-point dose-response of the relevant reference compound. Binding affinity was initially estimated at 1 μ M. A specific K_i was calculated using Cheng-Prusoff equation in all cases where greater than 50% displacement was observed. The binding affinity of other 5-HT receptors as well as 59 other proteins, including other G-protein coupled receptors, ion channels, and transporters was evaluated. When available, the experiments were carried out using recombinant human receptors cloned into various cell types (see Table 1 and Supplementary Table 1).

cAMP production in transfected cells

Procedures were similar to those described previously (Mohler et al. 2007). Briefly, to test 5-HT₆ receptor cAMP production, HEK-293 cells were transiently transfected with 5-HT₆ receptors. HEK-293 cells were then plated into 24-well clusters (70,000 cells/well). After 24 h, cAMP production levels were determined by stimulation for 5 min with the appropriate concentrations of 5-HT, 0.1 mM L-ascorbic acid, and RO-20-1724, a phosphodiesterase inhibitor, in 250 μ l of HEPES-buffered saline (20 mM HEPES, 150 mM NaCl, 4.2 mM KCl, 0.9 mM CaCl₂, 0.5 mM MgCl₂, 0.1% glucose, 0.1% BSA) at 37°C. Quantification of cAMP production was performed by Homogenous Time Resolved Fluorescence™ (HTRF) using the cAMP Dynamic kit (CIS Bio International, Bagnols sur Ceze, France) according to instructions of the manufacturer. Functional activity for Dopamine 3 (D3), histamine 2 (H2), and opioid μ -receptors was carried out by CEREP. Each experiment included a minimum of 6-control wells (background, and maximal signal with and without vehicle) plus an 8-point

dose–response of the relevant reference compound. Methods are as previously described for D3 (Missale et al. 1998), H2 (Leurs et al. 1994), and μ -receptors (Wang et al. 1994).

Brain penetration assay

Rats received an intraperitoneal injection of either vehicle ($n = 3$) or PRX-07034 3 mg/kg ($n = 3$). Thirty minutes following treatment, rats were decapitated, their brains removed, and approximately 1 ml of blood was collected from each rat. For brain sample preparation, whole rat brains were weighed and homogenized (Polytron) on ice. Brain homogenate (100 μ l) was portioned out, and 5 μ L of 1 μ M erythromycin was added to each as the internal standard. Standard curve and recovery samples were spiked into control rat brain homogenate with varying amounts of PRX-07034 (0 to 198 ng/10 mg of brain). Acetonitrile was added to each vial to precipitate the protein. The samples were centrifuged (Eppendorf 5804R) for 10 min at $8,000 \times g$, and the solvent was removed from the pellet and dried under a stream of nitrogen. A similar method was used for serum sample preparation as brain sample preparation except that 100 μ L of serum was portioned out for sampling.

One-microliter samples were analyzed with a Shimadzu UHPLC (Nexera LC-30 AD) system by injecting each sample onto a Shimadzu Shimpack XR-ODS III reverse phase column (2×50 mm, 1.6 μ m packing). The column was kept at 40°C using a gradient elution consisting of 0.2% formic acid in deionized water (A) and 0.2% formic acid in acetonitrile (B). Linear gradient was from 28% B to 50% B over 2 min at 0.3 mL/min, and the column was re-equilibrated at 28% B for 1.5 min. A Shimadzu (LCMS-8030) triple quadrupole mass spectrometer equipped with an electrospray source analyzed the MRM transitions m/z 454 to 199 and m/z 734 to 158 for the protonated molecules of PRX-07034 and the internal standard, respectively. Area under the curve was calculated to determine PRX-07034 concentrations in the samples. The lower detection limit for serum and brain tissue was 0.092 ng/100 μ L and 0.091 ng/10 mg, respectively.

Behavioral pharmacology experiments

Subjects

Male Long–Evans rats (Charles River Laboratories, Indianapolis, IN, USA) weighing approximately 350 g were individually housed in plastic cages in a temperature-controlled room with a 12-h light:dark cycle (lights on at 0700 hours). For the spontaneous alternation test, rats had free access to food and water except for the 24-h preceding testing, at which time, they were restricted to 12 g of food. In the strategy-switching experiments, rats were restricted to 85% of their ad libitum weight prior to testing as described in detail below. In all behavioral experiments, rats were tested during the light phase consistent with previous experiments (Baker et al. 2011; Mohler et al. 2007).

Apparatus

A black plastic four-arm cross maze was used for all behavioral testing. The height of the maze walls was 15.0 cm, and each of the arms measured 55-cm long $\times$ 10-cm wide. There was a food well (3.2 cm diameter $\times$ 1.6 cm high) in each arm located 3 cm away from the end wall. The hole in the food well measures 2.3 cm in diameter and was 1.6 cm deep. The maze was elevated 72 cm above the floor in a room with extra-maze cues.

Behavioral testing

To test the effect of PRX-07034 treatment on delayed spontaneous alternation, each rat was placed in the testing room 5 min prior to testing. For the test, a rat was allowed to freely choose an arm. After each choice, a block was placed into that arm for 30 s to prevent exiting the arm. This represented the delay period. The block was a 21.5×12 -cm piece of

plastic. After the 30-s delay, the block was removed, and a rat was allowed to enter another arm. The number and sequence of arm entries were recorded. An arm entry was recorded when all four paws entered an arm.

An alternation was defined as entry into four different arms on overlapping quadruple sets of arm entries (e.g., a quadruple set consisting of arm choices A, D, C, B was an alternation, but a quadruple set consisting of arm choices A, D, A, C was not). The percent alternation score is equal to the ratio of (actual alternations/possible alternations) multiplied by 100. The test session lasted 15 min. Rats that made less than 11 arm entries were excluded from the analysis. This criterion is similar to that used in previous experiments (Mohler et al. 2007). Thirty minutes before testing, rats received an intraperitoneal injection of one of the following treatments: (1) vehicle ($n = 10$), (2) PRX-07034 0.1 mg/kg ($n = 9$), (3) PRX-07034 1 mg/kg ($n = 9$), or (4) PRX-07034 3 mg/kg ($n = 7$).

The effect of PRX-07034 was tested on place and response acquisition, as well as a switch between a place and response strategy. Separate groups of rats were used in each study. One week prior to training, each rat was handled daily for 5 min and food restricted to maintain their weight at 85% of their free-feed weight. Each rat had free access to water throughout the study. Food restriction continued until the completion of behavioral testing. Rats were trained to recover a half piece of Froot Loops cereal (Kelloggs, Battle Creek, MI, USA) from each food well in 15-min daily sessions as described previously (Baker et al. 2011). On the final day of training, a rat's turn bias (left or right) was determined as described before (Baker et al. 2011).

One study tested PRX-07034 on place acquisition and switch to a response strategy. Each rat was tested across two consecutive days. In the first test phase, each rat had to learn a place discrimination. Rats were started pseudorandomly from one of two different arms such that any start arm was not used in more than three consecutive trials. The two start arms ("east" and "west") were always opposite each other. A black plastic block was placed in the entrance of the maze arm opposite to that of the start arm, giving the maze a T-shape and always making the choice arms the "north" and "south" arms. In the place acquisition phase, a rat was required to use extramaze cues to enter the choice arm containing half a piece of cereal, e.g., "north" arm. If a rat chose the correct arm, the trial was terminated after a rat consumed the cereal piece. If a rat chose the incorrect arm, the trial was terminated after a rat reached the unbaited food well. Between trials, a rat was placed on top of its home cage which sat by the maze. The maze was then wiped down with 2% Quatricide between trials to reduce the use of odor cues and re-baited if necessary. The inter-trial interval was approximately 15 s. To minimize the use of intramaze cues, the maze was rotated 90° every fourth trial. The criterion for acquisition of the place discrimination was ten consecutive correct trials. On the second day of testing (switch from place discrimination to a response strategy), a rat was required to turn in the same direction (left or right) from each start arm in order to receive a cereal reinforcement. Learning criterion was achieved when a rat made ten consecutive correct trials. Past studies showed through probe trials removal of extramaze cues or transferring of the maze to different test rooms that the place test requires the use of extramaze cues to learn and the response test requires the use of egocentric/body information to learn (Ragozzino et al. 1999).

Thirty minutes prior to each phase, rats received an intraperitoneal injection of the designated treatment for that phase. The rats were divided into the following groups: (1) vehicle–vehicle ($n = 11$), (2) PRX-07034 1.0 mg/kg–vehicle ($n = 9$), (3) PRX-07034 3.0 mg/kg–vehicle ($n = 9$), (4) vehicle–PRX-07034 0.1 mg/kg ($n = 6$), (5) vehicle–PRX-07034 1.0 mg/kg ($n = 10$), or (6) vehicle–PRX-07034 3.0 mg/kg ($n = 8$). PRX-07034 was mixed in sterile water.

In the switch phase, errors were recorded as described previously (Hill et al. 2006; Ragozzino et al. 2003). Briefly, for approximately half the trials, a rat had to turn in the opposite direction in the switch phase than in the place acquisition phase. For example, a rat might have to learn to always enter the “north” arm in the place acquisition phase. In the switch to response, a rat now had to learn to always turn left. Thus, when a rat was started from the “east” arm, it now had to switch from turning right into the “north” arm and now turn left. Trials when place and response strategies were placed in opposition were used to analyze “perseverative” and “regressive” errors. Trials were separated into consecutive blocks of four trials each. A perseverative error was defined as initially entering the incorrect arm in three or more trials per block of four trials as in previous experiments (Ragozzino et al. 2009). Thus, if a rat initially chose the previously correct choice on the majority of trials in a given block, it was considered perseveration. Once a rat made less than three errors in a block, all subsequent errors were no longer counted as perseverative errors. Errors on all future trials were considered regressive errors when a rat reverted back to the previously correct choice on those trials that required the opposite turn as on the place version. During the shift, a third type of error could be made if a rat turned in the opposite direction on the trials in which the turn in the switch to the response strategy was the same required in the place acquisition. These errors are referred to as never-reinforced errors because a rat was never reinforced for this choice either on the acquisition or switch phase (Baker et al. 2011).

Another study tested the effect of PRX-07034 on acquisition of a response strategy and switch to a place discrimination. In this experiment, rats were started on the response discrimination followed by a switch to the place discrimination. All other aspects of the testing procedure were as described above. In the switch to the place version, perseveration and regressive errors were analyzed from the trials in which a rat was required to enter the arm opposite to the turn direction required during response acquisition. The treatment groups in this experiment were as follows: (1) vehicle–vehicle ($n = 8$), (2) PRX-07034 1.0 mg/kg–vehicle ($n = 8$), (3) PRX-07034 3.0 mg/kg–vehicle ($n = 6$), (4) vehicle–PRX-07034 0.1 mg/kg ($n = 8$), (5) vehicle–PRX-07034 1.0 mg/kg ($n = 6$), and (6) vehicle–PRX-07034 3.0 mg/kg ($n = 8$).

Statistical analysis

In the spontaneous alternation test, a one-way analysis of variance was used to identify differences in percent alternation scores and number of arm entries across groups. Newman–Keuls post-hoc tests were used to compare differences between specific treatment groups. In the strategy switching tests, one-way analyses of variance were used to identify differences in trials to criterion for acquisition and switch phases. Separate analyses of variance were carried out to determine group differences in perseverative, regressive, and never-reinforced errors. Newman–Keuls post-hoc tests were used to determine significant differences between treatment groups.

Results

Binding affinity, functionality, and specificity for the 5-HT₆ receptor

PRX-07034 demonstrated high affinity for the 5-HT₆ receptor ($K_i = 4\text{--}8$ nM) compared to that of other 5-HT receptors (see Table 1). A large panel of in vitro receptor binding assays was performed to evaluate binding affinity to non-serotonin receptors. PRX-07034 displayed good selectivity for the 5-HT₆ receptor, showing binding affinity of $K_i > 1$ μ M for 57 different GPCRs, ion channels, and transporters (see Supplementary Table 1). Moderate binding affinity was detected for the dopamine D3 receptor ($K_i = 71$ nM). PRX-07034 also

demonstrated moderate binding affinity for the histamine H₂ and opioid μ -receptors (K_i = 640 and 450 nM, respectively).

The functional activity (agonist or antagonist) of PRX-07034 was evaluated by measuring the cAMP stimulation or inhibition in HEK-293 cells following exposure to the compound. PRX-07034 demonstrated antagonist activity (IC_{50} = 19 nM) and did not have an effect on basal levels, indicating that PRX-07034 does not possess any inverse agonist activity. PRX-07034 did not show any agonist activity up to 10 μ M. In functional assays, PRX-07034 demonstrated low antagonist activity for the dopamine D₃ receptor (IC_{50} =4.8 μ M) and very low agonistic activity for the opioid μ -receptor (EC_{50} = 19 μ M). In addition, PRX-07034 showed no agonist or antagonist activity for the histamine H₂ receptor in a concentration up to 10 μ M. One possible explanation for the moderate binding affinity with no functional activity may be species differences of the receptor used in each test (guinea pig striatum and recombinant human in CHO cells, respectively; see Preuss et al. 2007). However, overall, these results demonstrate that PRX-07034 is a selective antagonist for the 5-HT₆ receptor.

Brain/serum ratio

As described below, PRX-07034 3 mg/kg was the highest effective dose to enhance working memory and strategy switching. To determine the brain penetration of the drug, rats were treated with 3 mg/kg with serum collected, and the brain was removed 30 min post-injection. Mean ($\pm$ EM) serum levels were 549.0 $\pm$ 68.3 ng/mL serum. Mean brain levels were 55 $\pm$ 15.7 ng/g tissue. The brain:serum ratio for PRX-07034 at 3 mg/kg was 0.1:1.

The effect of PRX-07034 on delayed spontaneous alternation

PRX-07034 treatment significantly enhanced delayed alternation in a dose-dependent manner (see Fig. 1). PRX-07034 treatment at 0.1 mg/kg led to alternation scores comparable to that of controls. In contrast, PRX-07034 at 1 and 3 mg/kg enhanced alternation scores. The difference in alternation scores among the groups was significant, $F(3, 20)=11.72$, $P<0.01$. Post-hoc tests indicated that PRX-07034 1 and 3 mg/kg significantly improved alternation scores vs. vehicle controls and the 0.1-mg/kg dose ($P_s<0.01$). PRX-07034 at 0.1 mg/kg was comparable to that of controls. However, PRX-07034 treatment across all doses led to a similar number of arm entries as that of the control group. Thus, PRX-07034 improved delayed spontaneous alternation in a dose-dependent fashion without an effect on arm entries.

The effect of PRX-07034 on place acquisition and a switch to a response strategy

All groups acquired the place discrimination in a comparable number of trials (see Fig. 2a). However, PRX-07034 at 1 and 3 mg/kg administered prior to the switch phase reduced the number of trials to criterion (see Fig. 2b). An ANOVA revealed that the difference in trials to criterion among the groups was significant, $F(5, 47)=10.80$, $P<0.01$. Post-hoc tests revealed that the vehicle-PRX-07034 1 mg/kg and vehicle-PRX-07034 3 mg/kg groups significantly decreased trials to criterion in the switch phase compared to that of all other groups ($P_s<0.05$). In contrast, the vehicle-PRX-07034 0.1 mg/kg group reached criterion in a similar number of trials as controls.

Analysis of the errors, revealed that there was a significant effect of treatment on perseverative errors, $F(5, 47)=6.72$, $P<0.01$ (see Fig. 2c). A post-hoc test indicated that the vehicle-PRX-07034 3 mg/kg group committed significantly fewer perseverative errors compared to that of all other groups, except the vehicle-PRX-07034 1 mg/kg groups ($P_s<0.01$). There was also a significant difference in the number of regressive errors among the groups, $F(5, 47)=5.60$, $P<0.01$. Post-hoc tests indicated that PRX-07034 1 and 3 mg/kg significantly reduced regressive errors compared to that of the vehicle-vehicle group.

($P_s < 0.01$), as well as the PRX-07034 1 mg/kg–vehicle, PRX-07034 3 mg/kg–vehicle, and vehicle–PRX-07034 0.1 mg/kg groups ($P_s < 0.05$). There was not a significant effect of treatment for never-reinforced errors.

The effect of PRX-07034 on response acquisition and switch to a place discrimination

All the groups acquired the response strategy in a comparable number of trials (see Fig. 3a). In the switch phase, PRX-07034 at 1 and 3 mg/kg administered prior to this phase reduced the number of trials to criterion. The difference in trials to criterion among the groups was significant, $F(5, 38) = 25.68$, $P < 0.01$. Post-hoc tests revealed that the vehicle–PRX-07034 1 mg/kg and vehicle–PRX-07034 3 mg/kg groups significantly improved performance in the switch phase compared to that of all other treatment groups ($P_s < 0.05$).

In the switch phase, there was a significant effect of treatment on perseverative errors, $F(5, 38) = 4.51$, $P < 0.01$ (see Fig. 3b). Post-hoc tests revealed that the vehicle–PRX-07034 1 mg/kg and the vehicle–PRX 3 mg/kg groups significantly reduced perseverative errors compared to that of all other treatment groups ($P_s < 0.05$). There was also a significant difference in the number of regressive errors among the groups, $F(5, 38) = 12.64$, $P < 0.01$. Post-hoc tests indicated that PRX-07034 at 1 and 3 mg/kg significantly reduced regressive errors compared to that of all other treatment groups ($P_s < 0.01$). There was not an overall effect of treatment on never-reinforced errors.

Discussion

The present findings indicate that PRX-07034 is a highly selective and potent 5-HT₆ receptor antagonist that enhances working memory and strategy switching. The selectivity of PRX-07034 as a 5-HT₆ receptor antagonist was indicated by a more than 100-fold difference in affinities for the 5-HT₆ receptor compared with that of all other 5-HT receptor subtypes, except for the 5-HT_{1A} and 5-HT_{1B} receptors. Furthermore, PRX-07034 had an equal to or greater 100-fold selectivity for the 5-HT₆ receptor compared to that of 68 other receptors, ion channels, and transporters in a selectivity panel. Despite the high selectivity and potency of PRX-07034, it had a limited brain penetration. Although, the actual brain penetration was similar to that or slightly greater than other 5-HT₆ receptor antagonists reported to enhance cognition (Ahmed et al. 2005).

In addition to receptor binding specificity, PRX-07034 significantly enhanced delayed spontaneous alternation task in a dose-dependent manner. PRX-07034 did not affect the number of arm entries, indicating that enhanced alternation performance was not related to a general change in locomotion. Consistent with this task having a mnemonic component, inserting a delay between arm choices significantly reduces alternation performance (Ragozzino et al. 1996; Mohler et al. 2007). This enhancement in spontaneous alternation observed with acute PRX-07034 treatment is comparable to previous studies demonstrating that other 5-HT₆ receptor antagonists improve short-term memory (Woolley et al. 2001, 2003; Foley et al. 2004; King et al. 2004; Mitchell et al. 2006). However, several earlier investigations indicated that treatment with a 5-HT₆ receptor antagonist reverses a drug-induced recognition memory impairment or object recognition deficit produced by a 4-h inter-trial interval. Therefore, this is one of the few experiments demonstrating that treatment with a 5-HT₆ receptor antagonist alone under shorter delays enhances working memory. Taken together with previous experiments, the findings suggest that treatment with a 5-HT₆ receptor antagonist may be effective for individuals who suffer from working memory deficits such as in schizophrenia and autism (Minor and Park 1999; Pukrop et al. 2003; Reilly et al. 2006; Steele et al. 2007; Corbett et al. 2009).

PRX-07034 also enhanced strategy switching. The enhancement is comparable to that observed with SB-399885 on a reversal learning test (Hatcher et al. 2005) and SB-271046 which attenuates a PCP-induced impairment in set-shifting (Rodefer et al. 2008). This effect of PRX-07034 was selective to the switch phase and did not affect initial learning. During the switch phase, PRX-07034 significantly reduced perseverative and regressive errors. This pattern of results suggests that PRX-07034 improves the ability to initially inhibit a previously relevant strategy and/or generate a new strategy, as well as enhance the ability to maintain a new strategy once selected. Thus, PRX-07034 affects multiple processes to enhance cognitive flexibility and therefore may prove beneficial in alleviating cognitive flexibility impairments in a variety of conditions marked by strategy switching deficits.

The finding that PRX-07034 reduces both perseverative and regressive errors may also provide some insight to where this drug is acting centrally to enhance cognitive flexibility. More specifically, several experiments found that lesions or drug infusions into prefrontal cortex subregions selectively affect perseveration, but do not generally affect the learning of a new strategy once selected (Ragozzino et al. 1999, 2003; Ragozzino 2002; Chudasama and Robbins 2003; Kim and Ragozzino 2005; Floresco et al. 2006, 2009). In contrast, manipulations of the dorsomedial striatum also impair set-shifting, but selectively affect the ability to reliably execute the new strategy once selected (Ragozzino et al. 2002). Because PRX-07034 affected both the ability to initially shift away from a strategy, as well as maintain a new strategy once selected, PRX-07034 may block 5-HT₆ receptors in both the prefrontal cortex and striatum to produce a more rapid shift in learning a new strategy while also inhibiting a previously relevant strategy. This is consistent with 5-HT₆ receptors showing a high density in the striatum and a moderate density in the frontal cortex (Ward et al. 1995).

Serotonin 6 receptor blockade may influence cognitive flexibility by modulating acetylcholine release in the frontal cortex and striatum. Previous experiments have demonstrated that acetylcholine output in these brain areas is important for a switch in choice patterns (Ragozzino and Choi 2004; Boix-Trelis et al. 2007; Ragozzino et al. 2009). Moreover, enhanced frontal cortex acetylcholine release occurs after 5-HT₆ receptor antagonist treatment in vivo (Riemer et al. 2003), as well as in vitro in cortical and striatal slices (Marcos et al. 2006). Alternatively, 5-HT₆ receptor blockade may directly influence glutamate transmission. 5-HT₆ receptor antagonist treatment increases brain glutamate levels (Dawson et al. 2001). Furthermore, the NMDA receptor antagonist MK-801 prevents a 5-HT₆ receptor antagonist-induced memory improvement (King et al. 2004). Future experiments that directly examine the effects of a 5-HT₆ receptor antagonist on specific brain neurotransmitter systems during cognitive tests can shed light on the mechanism(s) by which a 5-HT₆ receptor antagonist improves working memory and strategy switching.

Two recent studies report that 5-HT₆ receptor agonists improve learning and memory (Burnham et al. 2010; Kendall et al. 2011). It is not clear how similar behavioral effects might be observed with both 5-HT₆ agonists and antagonists, although regional differences in serotonergic tone and therefore sensitivity to 5-HT₆ agonists and antagonists may be one possibility (Fone 2008). In support of this, combining a non-effective dose of a 5-HT₆ agonist and an antagonist improves cognition, possibly through differential activity on GABA and glutamate transmission, respectively (Kendall et al. 2011).

Overall, the findings indicate that PRX-07034 improves performance in working memory and strategy switching. As patients with neuropsychiatric disorders often show impairments in both cognitive domains, PRX-07034 may potentially provide relief for these deficits. Future work will be critical to characterize the range of cognitive domains that PRX-07034

affects, as well as the neurotransmitter systems modulated by 5-HT₆ receptor antagonists such as PRX-07034.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. PRX-07034 treatment enhanced delayed spontaneous alternation performance in a cross-maze. **a** Percent alternation scores in the delayed spontaneous alternation test. ** $P < 0.01$ vs. vehicle controls and PRX-07034 0.1 mg/kg group. The *dashed line* denotes chance performance (approximately 22.1%). **b** Mean number of arm entries in the delayed spontaneous alternation test. *Veh* vehicle

Fig. 2. PRX-07034 treatment selectively enhanced a switch to a response strategy. Treatment given during a particular test phase is in *bold type*. **a** Trials to criterion during the acquisition of a place discrimination. **b** Trials to criterion during the switch to a response discrimination; ** $P < 0.01$. **c** Analysis of errors during the switch to a response strategy; † $P < 0.01$ vs. Veh–Veh, PRX-07034 1.0 mg/kg–Veh, PRX-07034 3.0 mg/kg–Veh, and Veh–PRX-07034 0.1 mg/kg. Veh vehicle

Fig. 3.

PRX-07034 treatment selectively enhanced a switch to a place discrimination. Treatment administered during a specific test phase is in *bold type*. **a** Trials to criterion during acquisition of a response discrimination. **b** Trials to criterion during the switch to a place discrimination; ** $P < 0.01$. **c** Analysis of errors during the switch to a place strategy; * $P < 0.05$ vs. Veh–Veh, PRX-07034 1.0 mg/kg–Veh, PRX-07034 3.0 mg/kg–Veh, and Veh–PRX-07034 0.1 mg/kg; ** $P < 0.01$ vs. Veh–Veh, PRX-07034 1.0 mg/kg–Veh, PRX-07034 3.0 mg/kg–Veh, and Veh–PRX-07034 0.1 mg/kg. Veh vehicle

Table 1

Receptor binding profile for PRX-07034

Receptor	Ligand	Tissue/receptor source	PRX-07034 Ki/IC ₅₀ ^a
5-HT ₆	Serotonin/LSD	Human cDNA	4–8 nM
5-HT _{1A}	8-OH-DPAT	Human cDNA	420 nM
5-HT _{1B}	Serotonin/cyanopindolol	Rat cerebral cortex	260 nM
5-HT _{1D}	Serotonin/antagonist not tested	Bovine caudate	2.8 μM
5-HT _{2A}	Ketanserin	Human cDNA	2.5 μM ^a
5-HT _{2B}	Agonist not tested/LSD	Human cDNA	2.5 μM ^a
5-HT _{2C}	Mesulergine	Human cDNA	3.7 μM ^a
5-HT ₃	MDL 72222/BRL 43694	Human cDNA	>5 μM ^a
5-HT ₄	Agonist not tested/GR113808	Human cDNA	>5 μM ^a
5-HT _{5A}	Serotonin/LSD	Human cDNA	>5 μM ^a
5-HT ₇	Serotonin/LSD	Human cDNA	>5 μM ^a
Other GPCRs			
Dopamine D ₃	(+)Butaclamol	Human cDNA	71 nM
Histamine H ₂	Cimetidine	Guinea pig cerebellum	0.64 μM
Opioid μ	DAMGO	Human cDNA	0.45 μM

All binding studies were carried out by CEREP

LSD lysergic acid diethylamide, *DAMGO* [D-Ala2, *N*-MePhe4, Gly-ol]-enkephalin^aBinding affinity estimated based on percent displacement at PRX-07034