Title Page

Attenuation of phencyclidine-induced object recognition deficits by the combination of atypical antipsychotic drugs and pimavanserin (ACP 103), a 5-hydroxytryptamine2A receptor inverse agonist


Division of Psychopharmacology, Vanderbilt University School of Medicine, Nashville, TN 37212 USA. (SS, MH, MH, ZL, HYM)

Organon Schering Plough Laboratories Ltd, Newhouse, Lanarkshire, Glasgow, ML1 5SH, UK. (MS)

School of Pharmacy, University of Bradford, Bradford, BD7 1DP, UK. (JCN)
Running Title Page

Running Title: 5HT2AR antagonism and reversal of PCP deficits in NOR

Corresponding author
Herbert Y Meltzer, M.D.,
Vanderbilt Psychiatric Hospital,
1601 23rd Ave South, Suite 306,
Nashville TN 37212,
Phone number: +1-615-327-7049,
Fax 615-327-7093,
Email address: Herbert.meltzer@vanderbilt.edu

Figures: 8
Tables: 1
Text pages 23

Word Count
Abstract 250
Introduction 683
Discussion 1479
References 38

Abbreviations
APDs: atypical antipsychotic drugs; DA: dopamine; serotonin (5-HT); NDMC: N-desmethyclozapine; NOR: novel object recognition; PCP: phencyclidine
Abstract

Sub-chronic administration of the NMDA receptor antagonist, phencyclidine (PCP), in rodents has been shown to produce impairment in novel object recognition (NOR), a model of visual learning and memory. We tested the hypothesis that the selective 5-HT_{2A} inverse agonists, pimavanserin and M10097, would potentiate sub-effective doses of atypical antipsychotic drugs (APDs) to reverse the NOR deficits. Methods: Female rats received vehicle or PCP (2 mg/kg bid) for 7 days, followed by 7 day washout. Pimavanserin (3 mg/kg) or M100907 (1 mg/kg) alone, or four atypical APDs, risperidone (0.05-0.1 mg/kg), melperone (1-3 mg/kg), olanzapine (1-2 mg/kg), or N-desmethylclozapine (1-2 mg/kg), and the typical APD, haloperidol (0.05-0.1 mg/kg), were administered alone, or in combination with pimavanserin or M100907, prior to NOR testing. The exploration times of objects during 3 min acquisition and retention trials, separated by a 1 min interval, were compared by ANOVA. Results: Vehicle-, but not PCP-treated, animals, explored the novel object significantly more than the familiar, in the retention trial (p<0.05-0.01). Pretreatment with the higher doses of the atypical APDs, but not pimavanserin, M100907 or haloperidol alone, reversed the effects of PCP. The effect of risperidone was blocked by haloperidol pretreatment. Co-administration of pimavanserin or M100907, with ineffective doses of the atypical APDs, but not haloperidol, also reversed the PCP-induced deficit in NOR. These results support the importance of 5-HT_{2A} receptor blockade relative to D_{2} receptor blockade in the ability of atypicals to ameliorate the effect of subchronic PCP, a putative measure of cognitive dysfunction in schizophrenia.
INTRODUCTION

Deficits in multiple domains of cognition, including visual learning and memory, is a characteristic feature of schizophrenia (Meltzer and McGurk, 1999). Atypical antipsychotic drugs (APDs), but less so typical APDs, have usually, but not always, been found to produce significant improvement in some domains of cognition in schizophrenia patients (Meltzer and McGurk, 1999; Keefe et al., 2007). These classes of APDs differ in the relatively higher affinity for 5-hydroxytryptamine (5-HT)\textsubscript{2A} compared to dopamine (DA) D\textsubscript{2} receptors of the atypical APDs (Meltzer et al., 1989; Schotte et al., 1996; Meltzer and Huang, 2008). Microdialysis studies in rats have shown that the higher affinity for 5-HT\textsubscript{2A} compared to D\textsubscript{2} receptors of the atypical APDs contributes to their ability to enhance cortical and hippocampal DA efflux. This effect has been suggested to be important to their ability to enhance cognitive function in man and rodent models of cognitive impairment (Moghaddam and Bunney, 1990; Kuroki et al., 1999; Liegeois et al., 2002) and hypodopaminergic activity in the cortex has been postulated to be a major factor in the cognitive impairment of schizophrenia (Goldman-Rakic and Selemon, 1997).

Hypoglutamatnergic function, particularly in the frontal cortex, has also been suggested to be a major factor in the cognitive impairment of schizophrenia (Coyle, 2006). Acute and sub-chronic administration of non-competitive NMDA antagonists, e.g. phencyclidine (PCP) and MK-801, has been reported to produce impairments in visual and learning memory, attention, reasoning and problem solving, working memory, and social cognition (Stefani and Moghaddam, 2002; Rodefer et al., 2008; Abdul-Monim et al., 2007; Grayson et al., 2007; McLean et al., 2009), thus, providing an animal model for related deficits in schizophrenia and the efficacy of atypical APDs to partially improve those deficits. Clozapine, but not the typical antipsychotic, haloperidol, has been reported to reverse cognitive deficits induced by sub-chronic PCP treatment in novel object recognition.
(NOR) and attentional set shifting tasks (Grayson et al., 2007; Hashimoto et al., 2005; Nagai et al., 2009). Sub-chronic treatment with PCP has been reported to decrease basal and stress-evoked DA utilization in rat and primate prefrontal cortex (Jentsch et al., 1997; 1999) while acute administration of PCP enhances cortical DA (Adams and Moghaddam 1998). The latter does not appear to be related to its ability to disrupt cognitive performance (Adams and Moghaddam 1998). The ability of clozapine, but not haloperidol, to reverse the cognitive impairment due to sub-chronic PCP treatment in rodents has also been suggested to be due to restoration of cortical dopaminergic neurotransmission (Elsworth et al., 2008). Sub-threshold doses of the typical APD, haloperidol, and various atypical APDs, in combination with any of three 5-HT2A receptor inverse agonists, e.g. M100907, eplivanserin (SR-43469-B), or pimavanserin (N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N'-(4-(2-methylpropyloxy)phenylmethyl) carbamide (2R,3R)-dihydroxybutanedioate (2:1); ACP103) enhance cortical DA efflux (Liegeois et al., 2002; Bonacorsso et al., 2002; Li et al., 2005). Pimavanserin, which has high affinity for rodent and human 5-HT2A 5-HT2C receptors with minimal affinity and functional activity for other rat and human monoaminergic receptors (Vanover et al., 2006) was recently shown to potentiate the ability of risperidone, but not haloperidol, to treat psychosis in acutely psychotic patients with schizophrenia (Meltzer et al., in preparation). If enhanced DA efflux in the cortex or hippocampus were sufficient to reverse the effects of sub-chronic PCP on NOR, then the combination of a 5-HT2A inverse agonist such as pimavanserin and either haloperidol or an atypical APD would be predicted to reverse the effect of sub-chronic PCP to impair NOR.

This study was designed to test whether pimavanserin alone, three other atypical APDs (olanzapine, melperone, and N -desmethylclozapine (NDMC), the major metabolite of clozapine) as well as risperidone or haloperidol, or the combination of pimavanserin and these atypical APDs or haloperidol, are able to reverse the PCP-induced deficit in NOR. We also tested whether M100907, another 5-HT2A inverse agonist (Schmidt et al., 1992) in combination with sub-effective
dose of risperidone, could substitute for pimavanserin in improving the PCP-induced NOR deficit. Finally, we tested the prediction that haloperidol and risperidone in combination would be ineffective to overcome the effect of PCP on NOR because of excessive D$_2$ receptor blockade produced by pretreatment with haloperidol.
METHODS

Subjects and housing conditions

88 female Long-Evans rats from two separate batches (Zivic-Miller Laboratories, Porterville, PA) weighing 220±15g were used as subjects. Rats were housed in groups of five (cages measured 38 x 59 x 24 cm) and kept under standard laboratory conditions on a 12hr light:dark cycle, lights on at 0700 hr. Temperature and humidity conditions were 21 ± 2 °C and 40-50% respectively. All testing was carried out in the light phase. Food and water were available ad libitum. All experiments were conducted in accordance with the Vanderbilt animal committee regulations.

Drugs and treatment

48 rats were randomly assigned to two treatment groups. 16 were treated with vehicle [saline, intraperitoneally (i.p.)] and 32 with PCP (2 mg/kg, i.p.) twice daily for seven days.

PCP was dissolved in distilled water and administered in a volume of 1ml/kg i.p. Haloperidol and pimavanserin were dissolved in distilled water; olanzapine and risperidone were dissolved in a small amount of 0.1 M phosphoric acid and the pH was adjusted to 6–7 with 0.1 N NaOH. NDMC and M100907 were dissolved in a small amount of 0.1 M phosphoric acid and the pH was adjusted to 6–7 with 0.1 N NaOH. All doses are calculated as base equivalent weight and were administered i.p. All injections were given 30 min apart. Dose and pre-treatment times for haloperidol, risperidone and olanzapine were based on previous studies of their ability to enhance cortical DA efflux (Kuroki et al., 1999). The doses of pimavanserin and NDMC, M100907 and melperone were based on previous microdialysis experiments showing potentiation of DA and...
ACh efflux in the rat brain when administered in combination with sub-threshold doses of APDs (Kuroki et al., 1999; Ichikawa et al., 2002; Li et al., 2005).

Each rat was tested four times in the NOR paradigm. To reduce carryover effects, a seven day washout period was given between each of the test sessions. The criterion for continuing to test the rats was based on mean total exploration time in the acquisition phase $\geq 8 \pm 2$ seconds. If a rat did not explore at least that amount, they were excluded from the analysis. This happened rarely and not enough to affect the ability to utilize the remaining animals for analysis. All experiments consisted of 6-8 rats.

Animal Group 1:

Experiment I Effect of pimavanserin to reverse PCP-induced deficit in NOR

Following a 7 day washout period, groups of vehicle- and PCP- treated rats were treated with pimavanserin (3mg/kg) or acute vehicle (saline) 30 min prior to behavioural testing in NOR.

Experiment II Effect of risperidone at sub effective and effective dose, and pimavanserin augmentation to reverse PCP-induced deficit in NOR

After a 7 day wash out period, PCP-treated rats were administered risperidone (0.05 mg/kg and or 0.1mg/kg), alone or pimavanserin (3 mg/kg) plus 0.05mg/kg risperidone

Experiment III: Effect of haloperidol with and without pimavanserin augmentation to reverse PCP-induced deficit in NOR

After another 7 day washout period, PCP-treated. rats were administered haloperidol 0.05 or 0.1 mg/kg), alone or in combination with pimavanserin (3 mg/kg) or acute vehicle (saline, i.p.).

Experiment IV: Effect of olanzapine with and without pimavanserin augmentation to reverse PCP-induced deficit in NOR
Following another 1 week washout period after each test, the same rats were tested in the NOR paradigm following treatment with olanzapine (1 mg/kg) and pimavanserin (3 mg/kg) either alone or in combination.

*Animal Group II*

A second group of 40 rats were then subject to the same PCP or vehicle treatment regime as described above.

Overall, in this within subjects design, each rat was tested four times in the NOR test. To reduce carryover effects a seven day washout period was given in between each of the test sessions. Our criterion for continuing to test the rats was based on mean total exploration time in the acquisition phase ≥ 8 ± 2 seconds. Animals were no longer tested if total exploration time was less. Haloperidol and pimavanserin were dissolved in distilled water and olanzapine and risperidone were dissolved in a small amount of 0.1 M phosphoric acid and the pH was adjusted to 6–7 with 0.1 N NaOH.

*Experiment V: Effect of N-desmethylclozapine (NDMC) with and without pimavanserin augmentation to reverse PCP-induced deficit in NOR*

Following the 7 day washout period, rats were tested in the NOR paradigm following treatment with NDMC (1 mg/kg), pimavanserin (3mg/kg) or both and acute vehicle (saline, i.p.). All drugs or vehicle were given 30 min prior to behavioural testing.

*Experiment VI: Effect of melperone with and without pimavanserin augmentation to reverse PCP-induced deficit in NOR*

Following a 7 day washout period, the same rats were tested in the NOR paradigm following treatment with either melperone (0.1mg/kg), or both pimavanserin (3mg/kg) and melperone (0.1mg/kg) and acute vehicle (saline, i.p.)
Experiment VII: Effect of M100907 alone and risperidone plus M100907 to reverse PCP-induced deficit in NOR

Following another 7 day washout period, the rats were treated M100907 (0.1mg/kg) alone and with risperidone (0.05mg/kg).

Experiment VIII: Effect of haloperidol pretreatment on effect of risperidone to reverse PCP-induced deficit in NOR

After another one week washout, PCP-treated rats were given vehicle or haloperidol (0.1mg/kg), 30 minutes before risperidone (0.1 mg/kg).

Drugs

Pimavanserin and N-desmethylclozapine was provided by ACADIA Pharmaceuticals, Torrence, CA. Clozapine was obtained from Novartis, risperidone from Janssen Pharmaceuticals, melperone from Pharmacia-Upjohn, and M100907 from Aventis.

Novel object recognition

Apparatus

The object recognition test was performed in an open-field comprising a square box made of Plexiglas (52 × 52 × 31 cm) placed 37cm above the floor on an immovable stand. The floor of the box was white with black gridlines forming nine identical squares on it. All other walls were black. A video camera connected to a video recorder and monitor was positioned above the box. The objects used for the test consisted of four heavy pyramidal structures made of metal or Perspex that could not be displaced by the animals. Care was taken to ensure that these objects were not of any natural significance to the rats.
Object Recognition Testing

Testing was carried out according to a previously validated method (Grayson et al., 2007). The rats were familiarised to the test environment and NOR arena prior to the test day. Habituation consisted of placing the subjects in the empty NOR arena for one hour, on the day before the test day (day 1). Prior to behavioural testing on day 2, rats were given a further 3min habituation.

For each experimental trial following the 3min habituation period, the rats were given two 3min trials (T1 and T2), separated by a 1min inter-trial interval in the home cage during which the objects were changed and the arena was cleaned. In Trial 1 (T1) or the acquisition trial, the animals were allowed to explore two identical objects (A1 and A2) for 3min. In the second trial (T2) or the retention trial, the animals explored a familiar object (A) from T1 and a novel object (B) for 3 min. The familiar object presented during T2 was a duplicate of the object presented in T1 in order to avoid any olfactory trails.

Data Collection

Behaviour in all trials was recorded on video for subsequent blind scoring for the following parameters, total exploration time of both objects in the acquisition trial (s), total exploration time of objects in the retention trial (s),

Object exploration is defined by animals licking, sniffing or touching the object with the forepaws whilst sniffing, but not leaning against, turning around, standing or sitting on the object. The exploration time (s) of each object in each trial was recorded using two stopwatches.
**Statistical Analysis**

All data are expressed as mean ± SEM (n=6-8 per group). Exploration data were analysed by a repeated measures two-way ANOVA. This detected the main effect of drug treatment, main effect of the task (exploration of both objects) and the interaction between drug treatment and the two trials (acquisition and retention). Further analysis by a post-hoc Bonferroni t-test was carried out, if a significant effect was detected by the ANOVA, which compared the time spent exploring the novel and familiar object.
RESULTS

Experiment 1

Effect of Pimavanserin on PCP-induced deficit in acquisition trial-T1

An overall two-way ANOVA revealed that neither sub-chronic PCP nor acute pimavanserin treatment in the vehicle or PCP-treated rats produced any significant effect on object exploration in the acquisition trial of the test ($F_{2,15} = 3.13; p=0.078$; Fig. 1a). Rats from all treatment groups spent equal times exploring both objects in this phase of the trial. Furthermore, a one-way ANOVA on the total time spent exploring both objects, in both trials, showed an overall significant effect of pimavanserin alone in vehicle or PCP-treated rats to increase exploration time. Identical results were obtained with perphenazine, (obtained from sigma aldrich) another typical APD, at a dose of 0.2 mg/kg (study done in a 3rd group of animals; data not presented).

Effect of Pimavanserin alone on PCP-induced deficit in the retention trial-T2

A two-way ANOVA revealed a significant interaction effect of drug treatment and object exploration time in the retention phase of the NOR task ($F_{2,16} = 18.4; p<0.001$; Fig. 1b). Further analysis revealed that the control group (vehicle-treated animals) and vehicle + pimavanserin group had a clear preference for the novel compared to the familiar object, i.e. spent significantly ($p<0.001$) more time exploring the novel versus familiar object (Fig 1b). This effect was abolished in rats treated with PCP, i.e. these rats spent a similar amount of time exploring both objects. Furthermore, treatment with pimavanserin alone at 3 mg/kg did not attenuate the behavioural deficit induced by PCP. As can be seen from Fig 1b, pimavanserin, 3 mg/kg, alone was ineffective
in reversing the PCP deficit, in that the rats again spent nearly equal time exploring the novel compared with the familiar object. Identical results were obtained with perphenazine, another typical APD, at a dose of 0.2 mg/kg (data not presented).

Experiment 2

Effect of risperidone and pimavanserin in acquisition trial-T1

An overall two-way ANOVA demonstrated that neither risperidone alone nor its coadministration with pimavanserin produced a significant effect on left and right object exploration in the acquisition trial in vehicle- or PCP-treated rats. All treatment groups spent equivalent time exploring both identical objects in this phase of the test (F_{4, 27}=1.54, NS; Fig 2a)

Effect of risperidone and pimavanserin in retention trial-T2

A two-way ANOVA revealed a significant interaction between drug treatment and object exploration time in the retention phase of the NOR task (F_{4, 27}= 5.44, p<0.001). Risperidone alone (0.1 mg/kg) significantly improved the PCP-induced NOR deficit (p<0.05) but risperidone 0.05 mg/kg did not. However, risperidone (0.05 mg/kg) in combination with pimavanserin significantly restored greater exploration time of the novel object (p<0.001; Fig 2b).

Experiment 3

Effect of olanzapine and pimavanserin in acquisition trial-T1
An overall two-way ANOVA showed a significant interaction between object exploration and drug treatment \( (F_{4, 32} = 9.89; p<0.001; \text{Fig 3a}) \). Although all treatment groups spent equal times exploring both objects in this phase of the trial, there was an overall effect of drug treatment in that co-administration of pimavanserin 3 mg/kg, with olanzapine (1mg/kg)significantly increased the total object exploration time when compared to the other groups \( (p<0.001) \).

Effect of olanzapine and pimavanserin in retention trial-T2

The overall two-way ANOVA revealed a statistically significant interaction effect of drug treatment \( (F_{4, 32}= 5.18; \text{Fig 3b}) \). Olanzapine 1 mg/kg when administered alone did not attenuate NOR whereas a higher dose (2mg/kg) significantly improved the deficit \( (p<0.05) \). However, olanzapine (1 mg/kg), in combination with pimavanserin (3 mg/kg) reversed the PCP-induced deficit in exploration time of the novel relative to the familiar object \( (p<0.01) \).

**Experiment 4**

Effect of NDMC and pimavanserin in acquisition trial-T1

Overall ANOVA revealed no effect of drug treatment on exploration time of either object in the acquisition trial by pimavanserin or NDMC \( (F_{4, 30} = 2.27, \text{NS}) \).

Effect of NDMC and pimavanserin in retention trial-T2

An overall ANOVA of the exploration times in the retention phase showed a significant interaction between drug treatment and object exploration \( (F_{4, 30}= 3.37, p=0.01; \text{Fig 4b}) \). There was no significant difference between time spent exploring the novel vs familiar object in PCP-treated rats
that had received NDMC alone at 1 mg/kg. However, further post hoc analysis revealed that rats that had received the combination of pimavanserin (3mg/kg) and NDMC (1mg/kg) successfully discriminated between the novel and familiar objects (p<0.01). There was a trend for NDMC alone, (2mg/kg), to restore NOR in PCP-treated animals (p=0.07, Fig 4b).

Experiment 5

Effect of melperone in acquisition trial

An overall two-way ANOVA revealed that neither melperone 1-3 mg/kg, alone or in combination with pimavanserin at 3 mg/kg, (Fig 5a) produced any significant effect on object exploration in the acquisition trial of the NOR test (F₄, ₂₇=1.63, NS). Rats from all treatment groups explored the two identical objects for equal times in this phase of the trial.

Effect of melperone in retention trial

An overall ANOVA on the exploration times in the retention trial showed a significant interaction between drug treatment and object exploration (F₄, ₂₇= 4.17, p=0.01; Fig 5b). Melperone, 3 mg/kg, alone but not melperone 1 mg/kg restored NOR in PCP-treated animals (p<0.001). Further post hoc analysis revealed that, while there was no significant difference between time spent exploring the novel vs familiar object in PCP-treated rats that had received melperone alone at 1 mg/kg, rats that had received the combination of pimavanserin and melperone 1 mg/kg successfully discriminated between the novel and familiar objects (p<0.01).
Experiment 6

Effect of M100907 and risperidone in acquisition trial

An overall two-way ANOVA once again revealed no significant effect of drug treatment on object exploration in the acquisition trial ($F_{3, 31}=0.22$; NS; Fig 6a). Rats from all treatment groups spent almost equal times exploring the two identical objects in this phase of the test.

Effect of M100907 and risperidone in retention trial

A two-way ANOVA showed a significant interaction between object and drug treatment ($F_{3, 31}=6.66$; $p<0.05$). This was followed by post-hoc t-test which showed that the combination of M100907 (0.1mg/kg) and risperidone (0.05mg/kg) successfully reversed the PCP-induced deficit in NOR (Fig 6b, $p<0.05$). This effect was similar to that of pimavanserin on sub threshold dose of risperidone (Fig 2a, b) in improving NOR deficits.

Experiment 7

Effect of haloperidol and pimavanserin in the acquisition trial-T1

An overall two-way ANOVA revealed that neither haloperidol 0.1 mg/kg alone, nor haloperidol 0.05 or 0.1 mg/kg, in combination with pimavanserin 3 mg/kg, produced any significant interaction effect on object exploration in the acquisition trial of the NOR test ($F_{4, 31}=0.61$, NS; Fig 7a). Rats from all treatment groups spent almost equal times exploring the two identical objects in this phase of the test.
Effect of haloperidol and pimavanserin in the retention trial-T2

An overall two-way ANOVA revealed a significant interaction effect of drug treatment in this phase of the task ($F_{4, 31} = 7.28$, $p < 0.001$; Fig 7b). Further analysis using post-hoc t-tests revealed a significant difference between time spent exploring the novel object compared to the familiar object only in the vehicle-treated group ($p < 0.01$). Haloperidol 0.1 mg/kg did not affect the exploration times of the PCP-treated rats in the retention phase. Further, haloperidol 0.05 or 0.1 mg/kg in combination with pimavanserin (3mg/kg) did not affect the exploration times of the PCP-treated rats, in the retention phase.

Experiment 8

Effect of haloperidol + risperidone in acquisition trial-T1

An overall two-way ANOVA demonstrated that the combination of haloperidol 0.05 mg/kg and risperidone 0.1 mg/kg had no significant effect on left and right object exploration in the acquisition trial in vehicle or PCP-treated rats. Rats from all treatment groups spent equivalent time exploring both the objects in this phase of the trial ($F_{3, 21} = 0.24$, NS, Fig 8a).

Effect of haloperidol + risperidone in retention trial-T2

A two-way ANOVA revealed no significant interaction effect of drug treatment on object exploration time in the retention phase of the NOR task ($F_{3, 24} = 1.69$, NS; Fig 8b). However, although the interaction was not significant, there was a significant effect on exploration of novel vs familiar objects ($F_{1, 24} = 9.05$, $p < 0.01$). Post hoc analysis revealed that vehicle-treated animals
spent more time exploring the novel compared to the familiar object (p<0.05). This effect was abolished in the PCP-treated animals, as was the case in all previous trials, and was reinstated by risperidone (p<0.05). Importantly, pre-treatment with 0.05mg/kg haloperidol blocked the ability of 0.1 mg/kg risperidone to restore the ability of PCP-treated rats to distinguish novel from familiar objects (Fig 8b).
DISCUSSION

The major findings of this study are that the atypical APDs, olanzapine, melperone, NDMC, and risperidone, but not the typical APD, haloperidol, significantly reversed the impairment in the retention phase of NOR subsequent to sub-chronic treatment with PCP in female rats. The selective 5-HT$_{2A}$ inverse agonists, pimavanserin and M100907, alone, at doses which fully occupy 5-HT$_{2A}$ receptors (Kehne et al., 1996; Vanover et al., 2006) did not attenuate the NOR deficit in PCP-treated rats. However, the combination of pimavanserin with doses of the four atypical APDs which alone had no effect, but not the typical APDs haloperidol or perphenazine (data not shown), restored NOR performance in the PCP-treated rats. M100907, another 5-HT$_{2A}$ inverse agonist, and a sub-effective dose of risperidone were also able to reverse the effect of sub-chronic PCP on the retention phase of the NOR. Furthermore, it was found that haloperidol pre-treatment blocked the ability of risperidone to reverse PCP-induced deficits in NOR.

The same animals were used for up to four studies, separated by a week’s time, in this, as well as previous studies (Grayson et al., 2007). There was no evidence that the subjects in the study, with or without PCP treatment, were able to remember prior exposure to the objects based upon performance during the acquisition phase which did not vary during the month. We validated this methodology by conducting experiment 8 as the last of four experiments with one group of animals and the first experiment in another group of animals, and obtained the same results (data not presented).

The doses of the atypical APDs studied here which were effective to reverse the impairment in NOR are those we have previously shown enhance cortical DA efflux in normal rats (Kuroki et al., 1999; Ichikawa et al., 2002; Li et al., 2005). The doses of haloperidol or perphenazine are also
effective to block the effects of amphetamine on locomotor activity and produce small increases in cortical DA efflux (Kuroki et al., 1999). The dose of pimavanserin employed here has been reported to achieve essentially 100% 5-HT<sub>2A</sub> receptor occupancy (Vanover et al., 2006). Clozapine also reverses the effect of subchronic PCP using the same pre-treatment schedule (Grayson et al., 2007) and produces a marked increase in cortical DA efflux (Moghaddam and Bunney, 1990; Ichikawa et al., 2001). Another atypical antipsychotic drug, lurasidone, also produces a dose-dependent reversal of the effect of subchronic PCP on NOR (Horiguchi and Meltzer, unpublished data). Quetiapine and aripiprazole, two other atypical antipsychotic drugs, also reverse the effect of a sub-chronic PCP regimen on NOR in rodents (Tanibuchi et al., 2009; Nagai et al., 2009). The ability of eight atypical antipsychotic drugs, which are 5-HT<sub>2A</sub> antagonists, to reverse the effects of sub-chronic PCP suggests that this may be a general property of atypical antipsychotic drugs which achieve 5-HT<sub>2A</sub> and D<sub>2</sub> receptor blockade. The results reported here are consistent with the ability of clozapine, the prototypical atypical APD, to reverse the effect of sub-chronic treatment with PCP in rodents or monkeys in a variety of tests that require learning and memory (Elsworth et al., 2008; Grayson et al., 2007; Hashimoto et al., 2005; McLean et al., 2009). However, Rodefer et al., (2008) reported that sertindole, another serotonin-dopamine multireceptor antagonist, but not clozapine, olanzapine or risperidone, was able to reverse the effect of sub-chronic PCP on an attentional set shifting task in rats.

To further clarify the importance of the ratio of 5-HT<sub>2A</sub> to D<sub>2</sub> receptor blockade as a component of the action of atypical APDs to reverse the PCP-induced NOR deficit, doses of the atypical APDs were decreased 50-67%, to reduce occupancy of both D<sub>2</sub> and 5-HT<sub>2A</sub> receptors, at which point they were ineffective to reverse the NOR deficit. At these doses, the occupancy of rat cortical 5-HT<sub>2A</sub> receptors by risperidone and olanzapine are ~50% and ~60%, respectively, while those of striatal D<sub>2</sub> receptors are ~15 and ~50%, respectively (Schotte et al., 1996). These occupancies are significantly lower than the levels thought to be necessary to treat patients with schizophrenia.
The ability of pimavanserin and M100907, to restore the activity of the atypical APDs suggests that more complete blockade of 5-HT$_{2A}$ receptors is required for them to be effective to reverse the effects of PCP. It is noteworthy that M100907 and pimavanserin effectively block the effects of acute PCP stimulation of locomotor activity, considered a model of psychosis (Maurel-Remy et al., 1995; Vanover et al., 2006; Gardell et al., 2007) as well as their ability to block NMDA antagonist effects on firing rate of pyramidal neurons (Wang and Liang 1998). The 5-HT$_{2A}$ receptor has been shown to play a key role in the transport and dynamic regulation of NMDA receptors in cortical pyramidal neurons (Yuen et al., 2008). 5-HT$_{2A}$ agonists which are hallucinogens modulate NMDA receptor-mediated neurotransmission in the prefrontal cortex, effects which are blocked by M100907 (Arvanov et al., 1999). The effect of 5-HT$_{2A}$ receptor blockade to modulate glutamatergic currents may contribute to the procognitive effects of atypical APDs and selective 5-HT$_{2A}$ inverse agonists. The importance of 5-HT$_{2A}$ receptor blockade to the action of atypical APDs to counteract NMDA-receptor action in NOR is consistent with the extensive evidence linking 5-HT$_{2A}$ receptor mechanisms to glutamatergic function.

The ability of pimavanserin to restore the efficacy of atypical APDs after sub-chronic PCP treatment indicates that 5-HT$_{2A}$ receptor stimulation persists even after sub-chronic PCP treatment. Choi et al., (2009) recently reported that the PCP regimen used here did not alter levels of 5-HT$_{2A}$, D$_2$ or D$_4$ receptors, while down-regulating D$_1$ receptors in the caudate-putamen and increasing 5-HT$_{1A}$ receptor binding in the mPFC and dorso-lateral cortex. We have shown that 5-HT$_{1A}$ agonism and 5-HT$_{2A}$ blockade are synergistic actions of atypical APDs (Ichikawa et al, 2001). Pimavanserin and M100907 alone were ineffective in restoring performance in the NOR test in the PCP-treated rodents, indicating that reversing cognitive impairment due to subchronic PCP treatment, unlike blocking effect of acute PCP on neuronal firing and locomotor activity, requires more than 5-HT$_{2A}$ receptor blockade. The inability of low dose haloperidol plus pimavanserin to reverse the effect of subchronic PCP treatment indicates that reversal of the
impairment of NOR requires more than $D_2$ and $5-HT_{2A}$ receptor blockade, which rules out enhanced cortical DA efflux as being sufficient as well. Preliminary studies from this laboratory have found that even after subchronic PCP, risperidone is able to enhance cortical DA efflux to an extent similar to that of control rats but the effect of haloperidol and pimavanserin in the PCP-treated rats has not yet been studied (Snigdha and Meltzer, unpublished data). However, since pretreatment with haloperidol blocked the effect of risperidone to reverse the effect of PCP treatment on NOR, without compromising motor function, it is apparent that the extent of $D_2$ receptor blockade to that of $5-HT_{2A}$ receptor blockade, is a relevant factor.

Taken together, these results indicate that more potent blockade of $5-HT_{2A}$ than $D_2$ receptor blockade is necessary, but not sufficient, for reversal of PCP-induced impairment by an atypical APD of the $5-HT_{2A}/D_2$ type. It is likely that additional pharmacologic features of the atypical APDs not shared by haloperidol contribute to their effectiveness as monotherapy, and their potentiation following pre-treatment with pimavanserin. Some of the atypical APDs which reverse the effects of PCP have $D_3$ and $D_4$ receptor affinities that might possibly contribute to their efficacy even at the subthreshold dose (for Ki values see Table 1). Wang et al., (2006) reported that 3 days treatment with PCP, as well as acute PCP, impaired D4 receptor modulation of NMDA receptors as well as D4 regulation of CaMKII activity. The effect of PCP on D4-mediated stimulation of NMDA receptors was reversed by clozapine (Wang et al., 2006). It is possible that the atypical APDs do not utilize the same mechanisms to potentiate $5-HT_{2A}$ and $D_2$ receptor blockade to reverse the NOR impairment due to PCP. It is noteworthy that a variety of mechanisms, including but not limited to D1 agonism, alpha-2 adrenoceptor agonism, PDE10A inhibition, $\alpha_7$ nicotinic receptor agonism (Hashimoto et al., 2008), glycine transporter-1 (GlyT-1) inhibition, have been shown to reverse the effects of sub-chronic PCP treatment. Further study is needed to integrate these mechanisms into a unified model. This might lead to more effective treatments for cognitive impairment in schizophrenia.
In conclusion atypical, but not typical, APDs, are able to restore the deficit in NOR memory produced by sub-chronic PCP treatment in rodents. This restoration requires extensive blockade of 5-HT$_{2A}$ receptors, which may be achieved by co-administration of 5-HT$_{2A}$ receptor inverse agonists, and sub-therapeutic doses of atypical APDs. These findings may be relevant to the disputed ability of atypical APDs to improve declarative memory in schizophrenia. Extensive blockade of D$_2$ receptors prevents the efficacy of atypical APDs in this model, which may have implications for combining typical and atypical drugs in patients with schizophrenia.

Acknowledgements

We thank Graham Pearson at Bradford for making the NOR chamber and Dr Hitoshi Hashimoto for his inputs to this manuscript.
References


Elsworth JD, Jentsch JD, Morrow BA, Redmond DE, Jr., Roth RH (2008) Clozapine normalizes prefrontal cortex dopamine transmission in monkeys sub-chronically exposed to phencyclidine. *Neuropsychopharmacology* 33:491-496.

improves the antipsychotic efficacy and side-effect profile of haloperidol and risperidone in experimental models. *J Pharmacol Exp Ther* 322: 862-870.


Schmidt CJ, Fadayel GM, Sullivan CK, Taylor VL (1992) 5-HT2 receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the


Legends for Figures

Fig. 1a The effect of acute administration of pimavanserin (3 mg/kg, i.p.) following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of two identical objects in the 3 min acquisition trial-T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± SEM of exploration time (s) n=6-8 rats per group.

Fig. 1b The effect of acute administration pimavanserin (3 mg/kg, i.p.) following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of a novel and a familiar object in the 3 min retention trial-T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± SEM of exploration time (s) n=6-8 rats per group. ***p<0.001 significant increase in time exploring the novel compared with familiar object, Bonferroni t-test.

Fig. 2a The effect of acute administration of risperidone (0.05-0.1mg/kg, i.p.), pimavanserin (3mg/kg, i.p) and risperidone (0.05mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of two identical objects in the 3 min acquisition trial-T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± SEM of exploration time (s) n=6-8 rats per group.

Fig. 2b The effect of acute administration of risperidone (0.05-0.1mg/kg, i.p.), pimavanserin (3mg/kg, i.p) and risperidone (0.05mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of a novel and a familiar object in the 3 min retention trial-T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± SEM of exploration time (s) n=6-8 rats per group. *p<0.05;
**p<0.001**: significant increase in time exploring the novel compared with familiar object, Bonferroni t-test.

Fig. 3a The effect of acute administration of olanzapine (1mg/kg, i.p.), and olanzapine (1mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of two identical objects in the 3 min acquisition trial-T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± SEM of exploration time (s) n=6-8 rats per group.

Fig. 3b The effect of acute administration of olanzapine (1-2 mg/kg, i.p.), and olanzapine (1mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of a novel and a familiar object in the 3 min retention trial-T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± SEM of exploration time (s) n=6-8 rats per group. *p<0.05, **p<0.01 significant increase in time exploring the novel compared with familiar object, Bonferroni t-test.

Fig. 4a The effect of acute administration of NDMC (1-2mg/kg, i.p.), and NDMC (1mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of two identical objects in the 3 min acquisition trial-T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± SEM of exploration time (s) n=6-8 rats per group.

Fig. 4b The effect of acute administration of NDMC (1-2mg/kg, i.p.), and NDMC (1mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of a novel and a familiar object in the 3 min retention trial-T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± SEM of
exploration time (s) n=6-8 rats per group. **p<0.01 significant increase in time exploring the novel compared with familiar object, Bonferroni t-test.

Fig. 5a The effect of acute administration of melperone (1-3 mg/kg, i.p.), and melperone (1mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of two identical objects in the 3 min acquisition trial-T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± SEM of exploration time (s) n=6-8 rats per group.

Fig. 5b The effect of acute administration of melperone (1-3 mg/kg, i.p.), and melperone (1mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of a novel and a familiar object in the 3 min retention trial-T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± SEM of exploration time (s) n=6-8 rats per group. **p<0.01 or ***p<0.001: significant increase in time exploring the novel compared with familiar object, Bonferroni t-test.

Fig. 6a The effect of acute administration of M100907 (0.1mg/kg, i.p.), and M100907 (0.1mg/kg, i.p.) + risperidone(0.05 mg/kg,i.p) following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of two identical objects in the 3 min acquisition trial-T1 in a novel object recognition test in female Long-Evans rats. Data are shown as mean ± SEM of exploration time (s) n=7-8 rats per group.

Fig. 6b The effect of acute administration M100907(0.1mg/kg, i.p.), and M100907(0.1mg/kg, i.p.) risperidone (0.05 mg/kg,i.p)following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 day followed by a 7 day drug-free period) on exploration of a novel and a familiar object in the 3 min retention trial-T2 in a novel object recognition test in female Long-Evans rats. Data are expressed as mean ± SE
of exploration time (s) n=7-8 rats per group *p<0.05; **p<0.01 significant increase in time exploring the novel compared with familiar object, Bonferroni t-test

Fig. 7a The effect of acute administration of haloperidol (0.1mg/kg, i.p.) and haloperidol (0.05-0.1mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of two identical objects in the 3 min acquisition trial-T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± SEM of exploration time (s) n=6-8 rats per group.

Fig. 7b The effect of acute administration haloperidol (0.1mg/kg, i.p.) and haloperidol (0.05-0.1mg/kg) + pimavanserin (3mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of a novel and a familiar object in the 3 min retention trial-T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± SEM of exploration time (s) n=6-8 rats per group. **p<0.01: significant increase in time exploring the novel compared with familiar object, Bonferroni t-test.

Fig. 8a The effect of acute administration of risperidone (0.1mg/kg) and haloperidol (0.05mg/kg) + risperidone (0.1mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of two identical objects in the 3 min acquisition trial-T1 in a NOR test in female Long-Evans rats. Data are shown as mean ± SEM of exploration time (s) n=6-8 rats per group.

Fig. 8b The effect of acute administration risperidone (0.1mg/kg) and haloperidol (0.05mg/kg) + risperidone (0.1mg/kg), following sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7 days followed by a 7 day drug-free period) on exploration of a novel and a familiar object in the 3 min retention trial-T2 in a NOR test in female Long-Evans rats. Data are expressed as mean ± SEM of
exploration time (s) n=6-8 rats per group. *p<0.05 significant increase in time exploring the novel compared with familiar object, Bonferroni t-test.
<table>
<thead>
<tr>
<th>Drug</th>
<th>5-HT2A</th>
<th>D2</th>
<th>5-HT1A</th>
<th>5-HT2C</th>
<th>5-HT6</th>
<th>5-HT7</th>
<th>α1</th>
<th>α2</th>
<th>D1</th>
<th>D3</th>
<th>D4</th>
<th>H1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pimavanserin</td>
<td>0.13</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>#</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>73</td>
<td>0.15</td>
<td>2600</td>
<td>10,000</td>
<td>5,000</td>
<td>263</td>
<td>26</td>
<td>1600</td>
<td>21</td>
<td>21</td>
<td>15</td>
<td>3002</td>
</tr>
<tr>
<td>Melperone</td>
<td>32</td>
<td>199</td>
<td>2200^</td>
<td>1342</td>
<td>1254</td>
<td>578</td>
<td>180</td>
<td>150</td>
<td>&gt;1000</td>
<td>4.9</td>
<td>230</td>
<td>580</td>
</tr>
<tr>
<td>NDMC</td>
<td>10.9^</td>
<td>115^</td>
<td>13.9</td>
<td>11.9</td>
<td>9.4</td>
<td>14.9</td>
<td>105</td>
<td>138</td>
<td>14.3^</td>
<td>34</td>
<td>20</td>
<td>3.4</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>3.0</td>
<td>7.5</td>
<td>&gt;1000^</td>
<td>24</td>
<td>6</td>
<td>104</td>
<td>60</td>
<td>140</td>
<td>39</td>
<td>49</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.20</td>
<td>1.60</td>
<td>250</td>
<td>32</td>
<td>25</td>
<td>1.4</td>
<td>0.7</td>
<td>75</td>
<td>21</td>
<td>2.8</td>
<td>18.6</td>
<td>88</td>
</tr>
</tbody>
</table>

ND = not determined; * = >1000; # = 88% inhibition at 10μM (Vanover et al., 2006)

All other data are obtained from the NIMH Psychoactive Drug Screening Program web site (http://pdsp.med.unc.edu/) and are almost all for rat cloned receptors except ^ which are from human cloned receptors
Fig 1a (left) and 1b (right)
Fig 2a (left) and 2b (right)
Fig 3a (left) and 3b (right)
Fig 4a (left) and 4b (right)
Fig 5a (left) and 5b (right)
Fig 6a (left) and 6b (right)
Fig 7a (left) and 7b (right)
Fig 8a (left) and 8b (right)