Title: D_1-like receptor activation improves PCP-induced cognitive deficits in animal models: implications for mechanisms of improved cognitive function in schizophrenia.

Samantha L. M^{c}Lean[1], Nagi F. Idris[1], Marie L. Woolley[2], Joanna C. Neill[1]

[1]Bradford School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP, UK

[2]GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK

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Samantha McLean
Bradford School of Pharmacy
The University of Bradford
Bradford
West Yorkshire
BD7 1DP
UK
Tel: 01274 234668
E-mail: s.l.mclean@bradford.ac.uk

Abstract

Phencyclidine (PCP) produces cognitive deficits of relevance to schizophrenia in animal models. The aim was to investigate the efficacy of the D_1-like receptor agonist, SKF-38393, to improve PCP-induced deficits in the novel object recognition (NOR) and operant reversal learning (RL) tasks. Rats received either sub-chronic PCP (2 mg/kg) or vehicle for 7 days, followed by a 7-day washout. Rats were either tested in NOR or the RL tasks. In NOR vehicle rats successfully discriminated between novel and familiar objects, an effect abolished in PCP-treated rats. SKF-38393 (6 mg/kg) significantly ameliorated the PCP-induced deficit ($P<0.01$) an effect significantly antagonised by SCH-23390 (0.05 mg/kg), a D_1-like receptor antagonist ($P<0.01$). In the RL task sub-chronic PCP significantly reduced performance in the reversal phase ($P<0.001$); SKF-38393 (6.0 mg/kg) improved this PCP-induced deficit, an effect antagonised by SCH-23390 ($P<0.05$). These results suggest a role for D_1-like receptors in improvement of cognitive function in paradigms of relevance to schizophrenia.

Keywords D_1 receptors; Schizophrenia; Recognition memory; Reversal learning; Female rat; Phencyclidine

1. Introduction

Cognitive dysfunction in schizophrenia is becoming an increasingly important therapeutic target as one reason for the residual disability of schizophrenia appears to be the long-standing cognitive deficits of the disorder (Green and Nuechterlein, 2004). The MATRICS initiative (Measurement and Treatment Research to Improve Cognition in Schizophrenia) aims to facilitate the development of better treatments targeted at cognition (Marder and Fenton, 2004). It has been often reported that atypical antipsychotics have some beneficial effect on cognitive deficits (Hagger *et al.*, 1993; Buchanan *et al.*, 1994; Rossi *et al.*, 1997; Meltzer and McGurk, 1999; Harvey *et al.*, 2004). However, the effect is small (Lieberman, 2006; Keefe *et al.*, 2007) and hence there remains a great unmet need for novel antipsychotics to improve cognitive function.

There is mounting evidence for the role of dopamine dysregulation in the prefrontal cortex (PFC) in schizophrenia (for review see Goldman-Rakic *et al.*, 2004). It has been suggested that the negative symptoms and cognitive deficits seen in schizophrenia may arise from a dopaminergic deficit in the prefrontal cortex i.e. hypofrontality (Davis *et al.*, 1991), whereas the positive symptoms are related to hyperactivity of sub-cortical dopaminergic neurons (Grace, 1991). In keeping with this, inhibitors of catechol-O-methyltransferase (COMT), the primary enzyme responsible for metabolic degradation of dopamine specifically in the medial prefrontal cortex (mPFC), have been shown to improve cortical processing in both humans (Apud *et al.*, 2007) and rats (Tunbridge *et al.*, 2006).

Spano *et al.* (1978) proposed the existence of two populations of dopamine receptors after it was shown that dopamine both stimulated and inhibited adenylate cyclase (AC) activity (Brown and Makman, 1972; Kebabian *et al.*, 1972). D_1 and D_5

receptors belong to the D_1-like family in that they stimulate adenylate cyclase (AC), whereas D_2, D_3 and D_4 receptors inhibit AC. D_1-like receptors are predominantly found in the PFC, while D_2-like receptors are expressed in sub-cortical regions (see Guillin *et al.,* 2007), although D_4 receptors are present in the PFC and hippocampus (Lahti *et al.,* 1998). In keeping with the dopaminergic hypothesis of schizophrenia current antipsychotics attenuate positive symptoms by blocking sub-cortical D_2 receptors (Seeman *et al.,* 1975; Creese *et al.,* 1976) but these drugs have, at best, only limited efficacy at treating cognitive deficits.

Mounting evidence suggests that the D_1 receptor in the mPFC may be important in regulating cognitive function in schizophrenic patients. Okubo and colleagues (1997) reported a down-regulation of D_1 binding in the PFC of treatment-free/-naïve schizophrenic patients. Another study has demonstrated an association between genetic risk for schizophrenia and alterations in cortical D_1 receptor binding (Hirvonen *et al.,* 2006). It has also been shown that D_1 receptors are more abundant then D_2 receptors in the PFC of non-human primates (Lidow *et al.,* 1991), and this D_1 receptor subfamily has been implicated in working memory functions of the PFC (Arnsten *et al.,* 1994, Sawaguchi and Goldman-Rakic, 1991) one aspect of cognition impaired in schizophrenia. Thus it is possible that stimulation of the D_1 receptor may represent a potential strategy for treating cognitive deficits associated with schizophrenia. Indeed, D_1 agonists have been highlighted as a molecular target for cognitive enhancement in schizophrenia (see Gray and Roth, 2007).

Phencyclidine (PCP) is a non-competitive NMDA receptor antagonist, which has been shown to produce enduring cognitive deficits similar to those observed in schizophrenia (Javitt and Zukin, 1991) particularly when administered sub-chronically rather than acutely (Jentsch and Roth, 1999). Repeated and intermittent

administrations of PCP have been shown to reduce dopamine turnover in the PFC of rats and monkeys (Jentsch *et al.,* 1997a,b); moreover, the use of a sub-chronic PCP regimen has been suggested to provide a superior pharmacological model of the hypodopaminergic state seen in schizophrenia (see Jentsch and Roth, 1999). Sub-chronic PCP also causes reduced density of parvalbumin-immunoreactive neurons (Abdul-Monim *et al.,* 2007) and brain-derived neurotrophic factor (BDNF) levels in cortical regions (Snigdha *et al.,* 2007a) in rats. Indeed the sub-chronic PCP dosage regime has been well-validated in our laboratory producing enduring cognitive deficits which can be reversed by atypical but not classical antipsychotics in NOR (Grayson *et al.,* 2007), reversal learning (Abdul-Monim *et al,.* 2006, 2007) and attentional set-shifting (McLean *et al.,* 2008) tasks. Sub-chronic PCP also produces social behaviour deficits in our laboratory which are improved by atypical but not by classical antipsychotics (Snigdha and Neill, 2008a; 2008b). Using this model we have observed cognitive deficits in NOR lasting up to 5 months following the last dose of PCP (Grayson *et al.* unpublished observations). As many atypical antipsychotics have affinity for a multitude of receptors, much research is now focusing on identifying specific receptor subtypes as potential novel targets and on the development of selective compounds (Gray and Roth, 2007) for the treatment of cognitive dysfunction in schizophrenia.

The core aim of this study was to utilise the selective D_1-like receptor agents SKF-38393 and SCH-23390 to elucidate the role of D_1-like receptors in cognition using two rodent tests validated in our laboratory, the NOR test and the operant reversal learning task, which are both tests highlighted by the MATRICS initiative as being relevant translational models for studying visual learning and memory and reasoning and problem solving respectively (see Hagan and Jones, 2005). It is

expected that SKF-38393 will ameliorate the sub-chronic PCP-induced deficit, and that the antagonist SCH-23390 will block these effects. Both ligands shall be referred to as D_1-like throughout as SKF-38393 and SCH-23390 have been reported to have similar Ki values at D_1 and D_5 receptors; SKF-38393 having reported Ki values of 26nM and 80nM at D_1 and D_5 receptors respectively (Neumeyer *et al.*, 2003; Qandil *et al.*, 2003), whilst SCH-23390 has reported Ki values of 0.37nM and 0.47nM for D_1 and D_5 receptors respectively (Lawler *et al.*, 1999).

We also sought to determine if the stage of oestrous had any effect on reversal learning ability since an interaction between gonadal steroids, in particular oestrogen and cognitive function has previously been reported (see Cahill, 2006 for review). However, we have previously shown no effect of oestrous cycle on novel object recognition (Sutcliffe *et al.*, 2007). It is important for each task to determine whether the oestrous cycle has an effect, therefore this was assessed here in reversal learning.

2. Experimental Procedures

Subjects and housing conditions

Two cohorts of fifty female hooded-Lister rats, 100 in total (Harlan, UK) housed in groups of four or five were used as subjects, rats weighed between 200-250 g. Animals were maintained under standard laboratory conditions at a temperature of 21°C (±2°C) and humidity of 40–50%. They were maintained on a 12-h/12-h light/dark cycle (lights on at 0700 hours) and experimental procedures were performed during the light phase. Cohort 1 were allowed free access to food, while cohort 2 prior to operant training and testing, were gradually food deprived to approximately 90% of free-feeding body weight; reduced body weight was maintained by restricting the amount of food (standard laboratory chow, Special Diet

Services, Essex, UK) given to each rat per day (12 g/day). The availability of water was not restricted. Experiments were conducted in accordance with the Animals Scientific Procedures Act, UK, 1986, and approved by the University of Bradford ethics review process.

Drugs

The study design involved ten groups (n = 10 per group initially); rats were given either 2 mg/kg PCP (*n* = 80) or vehicle (0.9 % saline; *n* = 20) by the intraperitoneal (i.p.) route twice daily for seven days. Dosing with sub-chronic PCP or vehicle was followed by a washout period of a further seven days. PCP hydrochloride (Sigma, UK) was dissolved in 0.9 % saline. SKF-38393 hydrochloride (Research Biochemicals International, MA, USA) and SCH-23390 hydrochloride (Tocris, UK) were dissolved in saline (0.9 %) and were administered via the i.p. route in a volume of 1 ml/kg. SCH-23390 or vehicle was given 20 min prior to SKF-38393 or vehicle and rats were tested 30 min following this treatment. All drug doses were calculated as base equivalent weight.

Hersi and colleagues (1995) showed that a dose of 3.0 mg/kg (i.p.) of SKF-38393 in male Long-Evans rats increased acetylcholine release by two-fold over baseline, thus we carried out a dose response with 3.0 mg/kg as the middle dose (0.75, 1.5, 3.0, 6.0 mg/kg) in the reversal learning task. The most efficacious dose (6.0 mg/kg) was then used in the novel object test. This dose (6.0 mg/kg) was also shown to improve a scopolamine-induced impairment in a T-maze working memory test (Amico *et al.*, 2007). Two doses of SCH-23390 (0.025, 0.05 mg/kg) were tested in the NOR experiment to determine which dose was required to antagonise the effect of SKF-38393. Once it was determined that 0.05 mg/kg of SCH-23390 reversed the

effect of SKF-38393 only this dose of the antagonist was tested in the second reversal learning experiment. 0.05 mg/kg SCH-23390 has also been shown to transiently inhibit acetylcholine release (Hersi *et al.*, 1995).

Novel Object Recognition Task

Cohort 1 was tested in the novel object recognition (NOR) task as described by Grayson *et al.* (2007). Rats were habituated to the test box for 20 minutes on 3 consecutive days. Following a 3-min habituation session on the day of testing each rat was placed in the NOR chamber (52 cm wide x 40 cm high x 52 cm long) and exposed to two identical objects (A1 and A2) for a period of 3 minutes. The objects used were opaque plastic pyramids, small glass jars, cola cans and striped plastic bottles and rats showed equal exploration of these objects in validation experiments in our laboratory (Grayson unpublished findings). The heights of the objects were comparable (10 ± 2 cm) and they were heavy enough not to be displaced by the animals, to achieve this, some objects were filled with NaCl. The rats were then returned to their home cage for an inter-trial interval of 1 min, the entire box was cleaned, both objects removed and one replaced with an identical familiar copy and one with a novel object. Following the ITI, rats were returned to explore the familiar (A) and a novel object (B) in the test box for a 3-min retention trial. The location of the novel object in the retention trial was randomly assigned for each rat using a Gellerman schedule. All experiments were filmed and video recorded for subsequent behavioural analysis by an experimenter blind to the treatments. Locomotor activity was also recorded; this was evaluated by scoring the total number of sectors or line crossings by the animal in both acquisition and retention trials. The exploration time

(E in sec) of each object in each trial was recorded manually using two stopwatches and the following factors were calculated.

The total exploration time of both objects in the acquisition trial ($E_{A1}+E_{A2}$)

The total exploration time of both objects in the retention trial (E_A+E_B).

Discrimination Index DI = $(E_B-E_A)/(E_A+E_B)$ and represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects in the retention trial.

Experimental design

Cohort 1 was used for the novel object recognition test. Eight rats were used per group. Sub-chronic PCP-treated rats were administered SKF-38393 (6.0 mg/kg, i.p.) or vehicle in the presence or absence of SCH-23390 (0.025 and 0.05 mg/kg, i.p.). SCH-23390 (0.025 mg/kg, i.p.) was also administered alone. The drug treatment given to each rat (within each home cage) was randomised.

Reversal learning

Cohort 2 was tested in the reversal learning task as described in detail by Abdul-Monim *et al.*, 2003 and Idris *et al.*, 2005. All rats were tested in one of eight operant chambers (constructed in-house). Each chamber (29 × 30 × 30 cm) consisted of Plexiglas walls and ceiling, and a metal grid floor over sawdust. A hinged Plexiglas panel (6 × 6 cm) provided access to a food hopper containing food pellets (45 mg Noyes pellets, Sandown Scientific, UK). Two retractable levers (4 × 2 cm) were positioned on either side of the food hopper. A red light emitting diode (LED) was positioned centrally above each lever and a house light was located in the ceiling of each chamber. The chambers were placed individually within ventilated sound-

attenuating hardboard boxes (69 × 38 × 42 cm) containing a Perspex window to allow viewing. A small fan was built into each chamber to mask external noise. Each animal was tested in the same operant chamber throughout the study. All boxes were controlled by Med-PC software (Version 2.0 for DOS or Med-PC for Windows, Med Associates, Inc. Lafayette, Indiana). Programmes were written using Medstate notation.

Following habituation to the operant chambers, rats were trained to respond for food on a fixed ratio 1 (FR1) schedule of reinforcement with both levers active, as previously described (Abdul-Monim *et al.*, 2003). Rats were trained to press either the left or right lever for food delivery according to a visual cue (LED on or off). The experimental session was terminated following a total of 128 lever presses, which took approximately 30 min. Rats were trained once daily for 5 days and this was repeated until rats had reached criterion, i.e. 90% correct responding for three consecutive days.

The day before each reversal task session, a full 30-min operant training session (as described above) was conducted in order to ensure stable responding, i.e. 90% correct responding. The reversal-learning session involved animals being first exposed to a 5-min period during which the active lever was the same as on the previous training day. During this period, responses on both correct and incorrect levers were recorded. This part of the session was termed the initial phase. This was followed by a 2-min time-out period, which was signalled by the house light being turned off. The 2-min time-out period acts as a cue that the rule is about to change. In the subsequent 5-min period, the active lever was reversed. Responses made on the correct and incorrect levers were again recorded. This second period was termed the reversal phase. Animals undertook several of these reversal-learning sessions before

beginning the drug studies in order to ensure that they attained a stable level of performance, i.e. 90% correct responding and at least 25 lever presses in total, in both the initial and reversal phases of the task.

Experimental design—drug studies

Cohort 2 was used for the reversal learning experiments; eight to ten trained rats were used per group in the drug studies. Rats were tested on a cycle of 4 days (previously described by Idris *et al.,* 2005). On day 1 each animal had a 30-min operant training session. The following day, animals received the appropriate drug(s) and undertook a reversal-learning session. On day 3 and day 4, each animal underwent a further operant training session and reversal learning session, respectively, in order to ensure that responding was back to normal after the drug treatment.

The first experiment was carried out before sub-chronic PCP treatment to assess the effect of the oestrous cycle in untreated rats. Vaginal smears were taken between 09:00 h and 11:00 h. This involved the insertion of 0.2 ml of 0.9 % saline solution into the vagina by means of a thin plastic pipette. Histological examination of the vaginal lavage was carried out by light microscopy (Finger, 1969). Each sample was classified as being in one of the following phases: pro-oestrus, oestrus, di-oestrus or met-oestrus.

Following sub-chronic PCP treatment a dose-response was carried out to SKF-38393, and this was followed by a further experiment where SCH-23390 was also administered in the presence of SKF-38393. The dose-response to SKF-38393 was carried out over 2 experiments, in the first experiment 0.75, 1.5, and 3.0 mg/kg (i.p.) were tested followed by a later experiment with 6.0 mg/kg (i.p.) subsequently the data were combined which meant there were 20 rats in the vehicle and PCP groups. The

drug treatment given to each rat (and within each home cage) over the course of these experiments was randomised.

Data and statistical analysis

Data for the novel object task i.e. time at novel versus familiar was analysed using paired t-tests, and the discrimination indices were compared using a one-way ANOVA followed by post-hoc Bonferroni's multiple comparison test. Data for percentage correct responding in the reversal learning task was calculated using the number of presses on the correct lever divided by the total number of presses multiplied by 100. The percent correct responding data was arcsine transformed and analysed by one-way ANOVA followed by post-hoc Dunnett's t-test. Reversal phase performance was compared to initial phase responding using a paired t-test. The total number of lever presses was calculated by adding the correct and incorrect presses together within the 5-min test session, this was used to assess whether drugs had caused any sedation or behavioural impairment.

3. Results

Novel object recognition – effects of SKF-38393 and SCH-23390

There was no significant difference in time spent exploring the two identical objects during the acquisition trial in any of the treatment groups (Fig 1a). In the retention trial, vehicle-treated rats explored the novel object significantly more than the familiar object ($P < 0.05$); this effect was abolished in sub-chronic PCP-treated rats (Fig 1b). The ability to distinguish between novel and familiar objects was restored following administration with SKF-38393 (6.0 mg/kg, i.p.; $P < 0.05$); this effect was antagonised by SCH-23390 at 0.025 and 0.05 mg/kg (i.p.). SCH-23390 alone had no

effect on PCP-treated rats in the retention trial. A one-way ANOVA revealed a significant effect of treatment [$F_{(5,47)} = 7.37$, $P < 0.001$] on the discrimination index (DI). The DI for the PCP-treated and SCH-23390 0.025 mg/kg alone groups were significantly reduced compared to the vehicle group to -0.05 and -0.07 respectively from 0.5 ($P < 0.01$); SKF-38393 significantly improved the PCP-induced deficit ($P < 0.01$) with a DI of 0.5, and this effect was significantly antagonised to -0.08 in the presence of SCH-23390 at 0.05 mg/kg ($P < 0.01$; Fig 1c) but not SCH-23390 at 0.025 mg/kg ($P = 0.216$). There was no effect on locomotor activity assessed by the number of line crossings in the initial and retention trials (Fig 1d).

Reversal learning - Effect of oestrous cycle

There was no significant effect of oestrous cycle on performance of either the initial or reversal phases of the reversal learning task (Fig 2) and there was also no effect on the total number of lever presses (table 1).

Reversal learning - Effects of SKF-38393 and SCH-23390

For percentage correct responding, a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P < 0.001$) and at SKF-38393 (0.75, 1.5 mg/kg, i.p.; $P < 0.05$). A one-way ANOVA in the reversal phase showed a significant effect of treatment ($F_{5,78} = 7.38$, $P < 0.001$). Post-hoc analysis revealed that SKF-38393 at 6.0 mg/kg (i.p.) significantly improved the PCP-induced deficit ($P < 0.05$; Fig 3). There was no significant effect on total lever pressing in the initial or reversal phases, suggesting that neither locomotor capacity nor motivation were affected (table 2).

In a second experiment a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P < 0.001$; Fig 4). A one-way ANOVA in the reversal phase showed a significant effect of responding ($F_{4,47} = 14.63$, $P < 0.001$). Post-hoc analysis showed that SKF-38393 (6.0 mg/kg, i.p.) significantly improved the PCP-induced deficit ($P < 0.001$), and that SCH-23390 (0.05 mg/kg, i.p.) significantly antagonised this effect ($P < 0.001$). A one-way ANOVA on the total lever pressing in the reversal phase showed a significant interaction ($F_{4,47} = 6.14$, $P < 0.01$) and post-hoc analysis showed a significant ($P < 0.01$) reduction in lever pressing following SCH-23390 treatment compared to the vehicle group (table 3).

4. Discussion

In the current set of experiments we examined the efficacy of the D_1-like receptor agonist, SKF-38398, in improving cognition in two rodent tests of cognitive dysfunction of relevance to schizophrenia. Our data show that SKF-38393 significantly improved a sub-chronic PCP-induced deficit in the novel object recognition and reversal learning tests, an improvement which was subsequently antagonised by the D_1-like receptor antagonist, SCH-23390.

Furthermore, we demonstrate that reversal learning is unaffected by the stage of the oestrous cycle in our female rats. This is supported by another study from our laboratory showing that stage of oestrous cycle had no effect on episodic memory as measured in the NOR task (Sutcliffe *et al.,* 2007). Sex differences in cognition and the role of gonadal steroids, in particular oestrogen and progesterone, is an important area of research (Cahill, 2006). Indeed, we have demonstrated enhanced performance of female rats in the NOR task compared with their male counterparts (Sutcliffe *et al.,*

2007), an impairment in episodic memory induced by ovariectomy which is restored by subsequent administration of oestradiol (Sutcliffe *et al.,* submitted) and an interaction between oestradiol and PCP in the NOR task (Sutcliffe *et al.,* 2008). In depth analysis of this subject is beyond the scope of this manuscript, however as natural fluctuations in gonadal steroids over the course of the oestrous cycle do not affect performance in either the NOR or reversal learning tasks. There is certainly no disadvantage in using females, indeed they confer many advantages to the use of males. For example we have found females to be better at NOR compared with males (Sutcliffe *et al.,* 2007), and we have demonstrated that female rats are more sensitive to PCP in other cognitive tasks (McLean *et al.,* 2007).

In both tests of cognition there was a significant impairment produced by sub-chronic PCP, as we have consistently demonstrated in NOR (Grayson *et al.,* 2007; Neill *et al.,* 2007; Snigdha *et al.,* 2007b) and reversal learning (Abdul-Monim *et al.,* 2006; 2007; Neill *et al.,* 2008). In the NOR test vehicle-treated rats explored the novel object significantly more than the familiar object in the retention trial, whereas PCP-treated rats could not distinguish between the novel and familiar objects, suggesting they did not recognise the familiar object. The mechanism for this effect of PCP is not established yet, however recent work from our laboratory showed that the PCP-induced deficit is maintained following a 1 hour inter-trial interval and may be due to increased susceptibility to distraction during the inter-trial interval (Grayson *et al.,* 2008). In the reversal learning task, the PCP-treated rats were unaffected in the initial phase, but demonstrated reduced correct responding in the reversal phase. This suggests that when the rule changes PCP-treated rats do not switch to respond on the new correct lever. We have also demonstrated reduced density of parvalbumin-immunoreactive neurons in the hippocampus and M1 (motor area 1) region of the

frontal cortex following sub-chronic PCP treatment in the rat (Abdul-Monim *et al.*, 2007). Sub-chronic PCP also caused significant reductions in brain-derived neurotrophic factor (BDNF) levels in several cortical regions (Snigdha *et al.*, 2007a). These behavioural and neurochemical data suggest that our sub-chronic PCP dosage regimen provides a good clinical correlate as similar findings were demonstrated in post-mortem brain tissue analysis from schizophrenic patients (Benes *et al.*, 1991; Benes and Berretta, 2001) and in behavioural impairments assessed using the cued reinforcement reaction time task (Murray *et al.*, 2008) and the Wisconsin Card Sorting Test (Haut *et al.*, 1996; Pantelis *et al.*, 1999).

The dopamine D_1-like agonist, SKF-38393, significantly improved novel object recognition performance and reversal learning ability in the PCP-treated rats. In the case of reversal learning, attenuation of the PCP-induced deficit was dose-dependent. Administration of the D_1-like receptor antagonist SCH-23390 fully reversed these improvements in both tests of cognition. This data is in agreement with a study by Granon and colleagues (2000), whereby infusions of SKF-38393 and SCH-23390 into the PFC improved and then respectively attenuated accuracy in the five-choice attentional task. Hersi and co-workers (1995) found that SKF-38393 improved cognitive performance in the Morris water maze in rats with age-related impairments. In the current study we have used a sub-chronic PCP regimen to induce schizophrenia-like deficits in cognition, however, amphetamine can also induce cognitive impairments in tests of working memory (Castner *et al*., 2005), attentional set-shifting (Fletcher *et al.*, 2005), and reversal learning (Idris *et al.*, 2005). A study by Fletcher and colleagues found that amphetamine-induced impairments were reversed by infusing SKF-38393 into the mPFC (Fletcher *et al.*, 2007). This effect of

SKF-38393 to improve cognitive function in amphetamine-treated rats is in agreement with the effect observed in our sub-chronic PCP-treated rats.

It is important to note that we have examined the effect of SCH-23390 alone in PCP-treated rats in order to eliminate the possibility that SCH-23390 could have affected cognitive performance alone. In the reversal learning task, it appears that there was a small but non-significant effect of SCH-23390 (0.05 mg/kg) to impair performance in the initial phase, an effect not observed following sub-chronic PCP treatment alone. This trend was supported by a study showing that intracortical infusions of SCH-23390 impaired performance accuracy in the five-choice attentional task (Granon *et al.,* 2000). Therefore, blockade of D_1-like receptors with SCH-23390 alone may impair cognition, but this was not observed in the current experiments.

SKF-38393 also has an affinity for 5-HT_{2C} receptors (then classified as 5-HT_{1C}; Briggs *et al.,* 1991), albeit reduced affinity compared with D_1-like receptors. It is unlikely that 5-HT_{2C} receptors play a critical role in the effects of SKF-38393 as its effects in both tasks were fully reversed by SCH-23390 which has been shown to be selective for D_1-like receptors, however further experiments are required to investigate this possibility since 5-HT_{2C} receptor agonists do show efficacy in pre-clinical tasks of cognition (Siuciak *et al.,* 2007). It was previously shown that 3.0 mg/kg of SKF-38393 increased acetylcholine (ACh) release by two-fold over baseline (Hersi *et al.,* 1995), although the improvement in reversal learning induced by this dose did not reach statistical significance in the current study. However, the most efficacious dose in our reversal learning experiment was 6.0 mg/kg; this dose was also shown to improve a scopolamine-induced impairment in a T-maze working memory test (Amico *et al.,* 2007). In our NOR experiment it was demonstrated that the most

efficacious dose of SCH-23390 was 0.05 mg/kg, and this dose has also been shown to transiently inhibit acetylcholine release (Hersi *et al.,* 1995).

Cognitive impairments observed in the Wisconsin Card Sorting Task (Kashima, 1991) were positively correlated with a down-regulation of D_1 binding in the PFC of treatment-free/-naïve schizophrenic patients (Okubo *et al.,* 1997); thus identifying the D_1 receptor as a particularly relevant target for schizophrenia (Gray and Roth, 2007). The prefrontal cortex is critically involved in reversal learning; it has been shown more specifically that lesions of the orbital PFC impair reversal learning ability (McAlonan and Brown, 2003; Tait and Brown, 2007). Damage to the PFC has also been shown to impair recognition memory tasks (Kolb *et al.,* 1994; Meunier *et al.,* 1997). The hippocampus is also a critical brain region for learning and memory. The density of D_1 receptors is considerably higher than D_2 receptors throughout the cortex with differential distribution in the rat mPFC (Vincent *et al.,* 1995), and low levels of D_2 receptors have been detected in the hippocampus (Meador-Woodruff *et al.,* 1989; Levey *et al.,* 1993). The majority of dopamine receptors in the hippocampus are from the D_1-like subfamily of receptors (Dawson *et al.,* 1986), with a high density of the D_5 receptor (Ciliax *et al.,* 2000). Furthermore, it has been reported that administration of SKF-38393 increased hippocampal acetylcholine (ACh) release in young and aged rats (Hersi *et al.,* 1995), which improved performance in the Morris water maze, thus suggesting a mechanism for the cognitive enhancing effects of SKF-38393. Dihydrexidine, a full D_1-like receptor agonist has been shown to increase extracellular concentrations of cortical ACh to around 300% of baseline values; an effect which was completely blocked by SCH-23390, and coincided with an improvement in cognitive performance assessed using a passive-avoidance paradigm (Steele *et al.,* 1997). Indeed, it has also been

demonstrated that atypical antipsychotics such as clozapine, olanzapine, risperidone and ziprasidone can increase levels of ACh in the mPFC (Ichikawa *et al.,* 2002). This suggests a beneficial mechanism of action in that D_1-like agonists could improve cognition in a similar manner to some atypical antipsychotics.

Positive symptoms are induced by elevation of dopamine in the limbic system, whereas negative and cognitive symptoms are due, at least in part, to decreased dopamine prefrontal function (Davis *et al.,* 1991). As agents which increase prefrontal dopamine turnover may have beneficial effects on cognition it would seem profitable to explore direct stimulation of the D_1 receptor in the PFC. Indeed, D_1 agonists have been highlighted as a molecular target for cognitive enhancement in schizophrenia (see Gray and Roth, 2007). Subsequently, a single administration of the full D_1-like receptor agonist, dihydrexidine, produced increased perfusion in D_1 rich PFC regions in patients with schizophrenia (Mu *et al.,* 2007); suggesting that agents increasing transmission at dopamine D_1 receptors could have potential in the pharmacotherapy of schizophrenia through a variety of mechanisms. However, the use of D_1 receptor agonists as therapeutic agents poses difficulties for pharmacologists as dopamine function in the PFC seems to follow an "inverted-U" dose-response relationship whereby increases or decreases from an optimal level result in cognitive impairment (Goldman-Rakic *et al.,* 2000). In addition, chronic treatment with D_1 agonists may lead to the down-regulation of D_1 receptors which could in turn worsen cognition. Though these limitations have been identified, a sensitising regimen of D_1 agonist treatment has substantial potential for providing an adjuvant treatment. Currently, the full D_1 agonist dihydrexidine (DAR-0100) is in clinical trials for this indication (George *et al.,* 2007).

In conclusion, in the present study we have demonstrated that the D_1-like agonist, SFK-38393, improves a PCP-induced deficit in both the novel object recognition and the reversal learning tests. The cognitive enhancing effect of SFK-38393 in both tests was significantly antagonised by the D_1-like receptor antagonist SCH-23390. These data provide evidence supporting the role of D_1-like receptors in improvement of cognitive deficits in schizophrenia as recently proposed by Goldman-Rakic *et al.*, 2004.

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Reference List

Abdul-Monim, Z., Reynolds, G.P., Neill, J.C. (2003) The atypical antipsychotic ziprasidone, but not haloperidol, improves PCP-induced cognitive deficits in a reversal learning task in the rat. Journal of Psychopharmacology 17, 57-66.

Abdul-Monim, Z., Reynolds, G.P., Neill, J.C. (2006) The effect of atypical and classical antipsychotics on sub-chronic PCP-induced cognitive deficits in a reversal-learning paradigm. Behavioural Brain Research 169, 263-273.

Abdul-Monim, Z., Reynolds, G.P., Neill, J.C. (2007) Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alteration in parvalbumin-immunoreactive expression in the rat. Journal of Psychopharmacology 21, 198-205.

Amico, F., Spowart-Manning, L., Anwyl, R., Rowan, M.J. (2007) Performance- and task-dependent effects of the dopamine D_1/D_5 receptor agonist SKF 38393 on learning and memory in the rat. European Journal of Pharmacology 577, 71-77.

Apud, J.A., Mattay, V., Chen, J., Kolachana, B.S., Callicott, J.H., Rasetti, R. *et al*, (2007) Tolcapone improves cognition and cortical information processing in normal human subjects. Neuropsychopharmacology 32, 1011-1020.

Arnsten, A.F.T., Cai, J.X., Murphy, J.L., Goldman-Rakic, P.S. (1994) Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. Psychopharmacology 116, 143-151.

Benes, F.M., McSparren, J., Bird, E.D., San Giovanni, J.P., Vincent, S.L. (1991) Deficits in small interneurons in prefrontal and cingulated cortices of schizophrenic and schizoaffective patients. Archives of General Psychiatry 48, 996-1001.

Benes, F.M., Berretta, S. (2001) GABAergic interneurones: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology 25, 1-27.

Briggs, C.A., Pollock, N.J., Frail, D.J., Paxson, C.L., Ralowski, R.F., Kang, C.H., Kebabian, J.W. (1991) Activation of the 5-HT1c receptor expressed in *Xenopus* oocytes by the benzazepines SCH 23390 and SKF 38393. British Journal of Pharmacology 104, 1038-1044.

Brown, J.H., Makman, M.H. (1972) Stimulation by dopamine of adenylate cyclase in retinal homogenates and of adenosine-3':5'-cyclic monophosphate formation in intact retina. Proceedings of the National Academy of Sciences of the U.S.A. 69, 539-543.

Buchanan, R.W., Holstein, C., Breier, A. (1994) The comparative efficacy and long-term effect of clozapine treatment on neuropsychological test performance. Biological Psychiatry 36, 717-725.

Cahill, L. (2006) Why sex matters for neuroscience. Nature Reviews Neuroscience 7, 477-484.

Castner, S.A., Vosler, P.S., Goldman-Rakic, P.S. (2005) Amphetamine sensitization impairs cognition and reduces dopamine turnover in primate prefrontal cortex. Biological Psychiatry 57, 743-51.

Ciliax, B.J., Nash, N., Heilman, C., Sunahara, R., Hartney, A., Tiberi, M. *et al.* (2000) Dopamine D(5) receptor immunolocalization in rat and monkey brain. Synapse 37, 125-145.

Creese, I., Burt, D.R., Snyder, S.H. (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 19, 481-483.

Davis, K.L., Kahn, R.S., Ko, G., Davidson, M. (1991) Dopamine in schizophrenia: A review and reconceptualization. American Journal of Psychiatry 148, 1474-1486.

Dawson, T.M., Gehlert, D.R., McCabe, R.T., Barnett, A., Wamsley, J.K. (1986) D1 dopamine receptors in the rat brain: a quantitative autoradiographic analysis. Journal of Neuroscience 10, 3247-3253.

Finger, F. (1969) Estrus and general activity in the rat. Journal of Comparative and Physiological Psychology 68, 461-6.

Fletcher, P.J., Tenn, C.C., Rizos, Z., Lovic, V., Kapur, S. (2005) Sensitization to amphetamine, but not PCP, impairs attentional set shifting: reversal by a D1 receptor agonist injected into the medial prefrontal cortex. Psychopharmacology 183, 190-200.

Fletcher, P.J., Tenn, C.C., Sinyard, J., Rizos, Z., Kapur, S. (2007) A sensitizing regimen of amphetamine impairs visual attention in the 5-choice serial reaction time test: reversal by a D1 receptor agonist injected into the medial prefrontal cortex. Neuropsychopharmacology 32, 1122-1132.

George, M.S., Molnar, C.E., Grenesko, E.L., Anderson, B., Mu, Q., Johnson, K., *et al.* (2007) A single 20 mg dose of dihydrexidine (DAR-0100), a full dopamine D(1) agonist, is safe and tolerated in patients with schizophrenia. Schizophrenia Research 93, 42-50.

Goldman-Rakic, P.S., Muly, E.C. III, Williams, G.V. (2000) D(1) receptors in prefrontal cells and circuits. Brain Research. Brain Research Reviews 31, 295-301.

Goldman-Rakic, P.S., Castner, S.A., Svensson, T.H., Siever, L.J., Williams, G.V. (2004) Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. Psychopharmacology 174, 3-16.

Grace, A.A. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. Neuroscience 41, 1-24.

Granon, S., Passetti, F., Thomas, K.L., Dalley, J.W., Everitt, B.J., Robbins, T.W. (2000) Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. Journal of Neuroscience 20, 1208-1215.

Gray, J.A., Roth, B.L. (2007) Molecular targets for treating cognitive dysfunction in schizophrenia. Schizophrenia Bulletin 33, 1100-1119.

Grayson, B., Kirun, A., Neill, J.C. (2008) PCP impairs recall in an episodic memory task: Influence of distraction. Journal of Psychopharmacology 22(Suppl):MA10.

Grayson, B., Idris, N.F., Neill, J.C. (2007) Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the NOR task in the rat. Behavioural Brain Research 184, 31-8.

Green, M.F., Nuechterlein, K.H. (2004) The MATRICS initiative: developing a consensus cognitive battery for clinical trials. Schizophrenia Research 72, 1-3.

Guillin, O., Abi-Dargham, A., Laruelle, M. (2007) Neurobiology of dopamine in schizophrenia. International Review of Neurobiology 78, 1-39.

Hagan, J.J., Jones, D.N. (2005) Predicting drug efficacy for cognitive deficits in schizophrenia. Schizophrenia Bulletin 31, 830-853.

Hagger, C., Buckley, P., Kenny, J.T., Friedman, L., Ubogy, D., Meltzer, H.Y. (1993) Improvement in cognitive functions and psychiatric symptoms in treatment-refractory schizophrenic patient receiving clozapine. Biological Psychiatry 34, 702-712.

Harvey, P.D., Meltzer, H., Simpson, G.M., Potkin, S.G., Loebel, A., Siu, C., Romano, S.J. (2004) Improvement in cognitive function following a switch to ziprasidone from conventional antipsychotics, olanzapine, or risperidone in outpatients with schizophrenia. Schizophrenia Research 66, 101-113.

Haut, M.W., Cahill, J., Cutlip, W.D., Stevenson, J.M., Makela, E.H., Bloomfield, S.M. (1996) On the nature of Wisconsin Card Sorting Test performance in schizophrenia. Psychiatry Research 65, 15-22.

Hersi, A.I., Rowe, W., Gaudreau, P., Quirion, R. (1995) Dopamine D1 receptor ligands modulate cognitive performance and hippocampal acetylcholine release in memory-impaired aged rats. Neuroscience 69, 1067-74.

Hirvonen, J., van Erp, T.G., Huttunen, J., Aalto, S., Någren, K., Huttunen, M., Lönnqvist, J., Kaprio, J., Cannon, T.D., Hietala, J. (2006) Brain dopamine d1 receptors in twins discordant for schizophrenia. American Journal of Psychiatry 163, 1747-53.

Ichikawa, J., Dai, J., O'Laughlin, I.A., Fowler, W.L., Meltzer, H.Y. (2002) Atypical, but not typical, antipsychotic drugs increase cortical acetylcholine release without an effect in the nucleus accumbens or striatum. Neuropsychopharmacology 26, 325-339.

Idris, N.F., Repeto, P., Neill, J.C., Large, C.H. (2005) Investigation of the effects of lamotrigine and clozapine in improving reversal-learning impairments induced by acute phencyclidine and D-amphetamine in the rat. Psychopharmacology 179, 336-348.

Javitt, D.C., Zukin, S.R., (1991) Recent advances in the phencyclidine model of schizophrenia. American Journal of Psychiatry 148, 1301-8.

Jentsch, J.D., Tran, A., Le, D., Youngren, K.D., Roth, R.H. (1997a) Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilisation and impairs prefrontalcortical-dependent cognition in the rat. Neuropsychopharmacology 17, 92-99.

Jentsch, J.D., Redmond, D.E. Jr, Elsworth, J.D., Taylor, J.R., Youngren, K.D., Roth, R.H. (1997b) Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. Science 277, 953-955.

Jentsch, J.D., Roth, R.H. (1999) The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20, 201-225.

Kashima, H. (1991) Frontal dysfunction of chronic schizophrenia-the pros and cons in neuropsychological assessment. Japanese Journal of Psychopharmacology 11, 83-88.

Kebabian, J.W., Petzold, G.L., Greengard, P. (1972) Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain and its similarity to the "dopamine receptor". Proceedings of the National Academy of Sciences of the U.S.A 69, 2145-2149.

Keefe, R.S., Bilder, R.M., Davis, S.M., *et al.*, (2007) Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE Trial. Archives of General Psychiatry 64, 633-47.

Kolb, B., Buhrmann, K., McDonald, R., Sutherland, R.J. (1994) Dissociation of the medial prefrontal, posterior parietal, and posterior temporal cortex for spatial navigation and recognition memory in the rat. Cerebral Cortex 4, 664-680.

Lahti, R.A., Roberts, R.C., Cochrane, E.V., Primus, R.J., Gallager, D.W., Conley, R.R., Tamminga, C.A. (1998) Direct determination of dopamine D4 receptors in normal and schizophrenic postmortem brain tissue: a [^{3}H]NGD-94-1 study. Molecular Psychiatry 3, 528-533.

Lawler, C.P., Prioleau, C., Lewis, M.M., Mak, C., Jiang, D., Schetz, J.A. *et al.* (1999) Interactions of the novel antipsychotic aripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. Neuropsychopharmacology 20, 612-627.

Levey, A.I., Hersch, S.M., Rye, D.B., Sunahara, R.K., Niznik, H.B., Kitt, C.A. *et al.* (1993) Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. Proceedings of the National. Academy of Sciences of the U.S.A 90, 8861-8865.

Lidow. M.S., Goldman-Rakic, P.S., Gallager, D.W., Rakic, P. (1991) Distribution of dopaminergic receptors in the primate cerebral cortex: quantitative autoradiographic analysis using [3H]raclopride, [3H]spiperone and [3H]SCH23390. Neuroscience 40, 657-671.

Lieberman, J.A. (2006) What the CATIE study means for clinical practice. Psychiatric Services 57, 1075.

Marder, S.R., Fenton, W. (2004) Measurement and treatment research to improve cognition in schizophrenia: NIMH MATRIICS initiative to support the development of agents for improving cognition in schizophrenia. Schizophrenia Research 72, 5-9.

McAlonan, K., Brown, V.J. (2003) Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. Behavioural Brain Research 146, 97-103.

McLean, S.L., Shemar, G.K., Idris, N.F., Marshall, K.M., Neill, J.C. (2007) Deficits in attentional set-shifting in rats following sub-chronic phencyclidine (PCP) administration, effect of gender. European Neuropsychopharmacology, 17 (Supp 4): S275.

McLean, S.L., Beck, J.P., Woolley, M.L., Neill, J.C. (2008) A preliminary investigation into the effects of antipsychotics on sub-chronic phencyclidine-induced deficits in attentional set-shifting in female rats. Behavioural Brain Research 189, 152-158.

Meador-Woodruff, J.H., Mansour, A., Bunzow, J.R., Van Tol, H.H., Watson, Jr. S.J., Civelli, O. (1989) Distribution of D2 dopamine receptor mRNA in rat brain. Proceedings of the National. Academy of Sciences of the U.S.A 86, 7625-7628.

Meltzer, H.Y., McGurk, S.R. (1999) The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. Schizophrenia Bulletin 25, 233-255.

Meunier, M., Bachevalier, J., Mishkin, M. (1997) Effects of orbital frontal and anterior cingulate lesions on object and spatial memory in rhesus monkeys. Neuropsychologia 35, 999 -1015.

Murray, G.K., Clark, L., Corlett, P.R., Blackwell, A.D., Cools, R., Jones, P.B., Robbins, T.W., Poustka, L. (2008) Incentive motivation in first-episode psychosis: a behavioural study. BMC Psychiatry 8, 34.

Mu, Q., Johnson, K., Morgan, P.S., Grenesko, E.L., Molnar, C.E., Anderson, B., Nahas, Z., Kozel, F.A., Kose, S., Knable, M., Fernandes, P., Nichols, D.E., Mailman, R.B., George, M.S. (2007) A single dose of the full D1 dopamine agonist dihydrexidine (DAR-0100) increases prefrontal perfusion in schizophrenia. Schizophrenia Research 94, 332-341.

Neill, J.C., Shahid, M., Marston, H., *et al.* (2007) Farampator (ORG 24448), an allosteric AMPA receptor potentiator, improves a sub-chronic PCP-induced deficit in working memory in the rat. Schizophrenia Bulletin 33, 533.

Neill, J.C., Idris, N.F., Arnt, J. (2008) Sertindole, 5-HT2A and 5-HT6 antagonists improve a subchronic phencyclidine-induced reversal learning deficit in rats. Schizophrenia Research, 102(Suppl 2):60.

Neumeyer, J.L., Kula, N.S., Bergman, J., Baldessarini, R.J. (2003) Receptor affinities of dopamine D1 receptor-selective novel phenylbenzazepines. European Journal of Pharmacology 474, 137-140.

Okubo, Y., Suhara, T., Suzuki, K., Kobayashi, K., Inoue, O., Terasaki, O. *et al.* (1997) Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. Nature 385, 634-636.

Pantelis, C., Barber, F.Z., Barnes, T.R.E., Nelson, H.E., Owen, A.M., Robbins, T.W. (1999) Comparison of set shifting ability in patients with chronic schizophrenia and frontal lobe damage. Schizophrenia Research 37, 251-70.

Qandil, A.M., Lewis, M.M., Jassen, A., Leonard, S.K., Mailman, R.B., Nichols, D.E. (2003) Synthesis and pharmacological evaluation of substituted napth[1,2,3-*de*]isoquinolines (dinapsoline analogues) as D1 and D2 dopamine receptor ligands. Bioorganic and Medicinal Chemistry 11, 1451-1464.

Rossi, A., Mancini, F., Stratta, P., Mattei, P., Gismondi, R., Pozzi, F., Casacchia, M. (1997) Risperidone, negative symptoms and cognitive deficit in schizophrenia: an open study. Acta Psychiatrica Scandinavica 95, 40-43.

Sawaguchi, T., Goldman-Rakic, P.S. (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251, 947-950.

Seeman, P., Chan-Wang, M., Tedesco, J., Wang, K. (1975) Brain receptors for antipsychotic drugs and dopamine: Direct binding assay. Proceedings of the National. Academy of Sciences of the U.S.A 72, 4376-4380.

Siuciak, J.A., Chapin, D.S., McCarthy, S.A., Guanowsky, V., Brown, J., Chiang, P., Marala, R., Patterson, T., Seymor, P.A., Swink, A., Iredale, P.A. (2007) CP-809, 101, a selective 5-HT_{2C} agonist, shows activity in animal models of antipsychotic activity. Neuropharmacology 52, 279-290.

Snigdha, S., Neill, J., Shahid, M., Cruise, L., Henry, B. (2007a) Brain-derived neurotrophic factor downregulation in rat brain following sub-chronic phencyclidine administration. European Neuropsychopharmacology 17(Suppl. 4):S258.

Snigdha, S., Shahid, M., Grayson, B., Marston, H.M., Neill, J.C. (2007b) A potential role for Glycine Transport Inhibitors in ameliorating cognitive dysfunction in Schizophrenia: effects of Org 25935. Schizophrenia Bulletin 33, 546.

Snigdha, S., Neill, J.C. (2008a) Efficacy of antipsychotics to reverse phencyclidine-induced social interaction deficits in female rats – a preliminary investigation. Behavioural Brain Research 187, 489-494.

Snigdha, S., Neill, J.C. (2008b) Improvement of phencyclidine-induced social behaviour deficits in rats: Involvement of 5-HT(1A) receptors. Behavioural Brain Research 191, 26-31.

Spano, P.F., Govoni, S., Trabucchi, M. (1978) Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system. Advances in Biochemical Psychopharmacology 198, 155-165.

Steele, T.D., Hodges, D.B. JR, Levesque, T.R., Locke, K.W. (1997) D1 agonist dihydrexidine releases acetylcholine and improves cognition performance in rats. Pharmacology Biochemistry and Behavior 58, 477-483.

Sutcliffe, J.S., Marshall, K.M., Neill, J.C. (2007) Influence of gender on working and spatial memory in the novel object recognition task in the rat. Behavioural Brain Research 177, 117-25.

Sutcliffe, J.S., Rhaman, F., Marshall, K.M., Neill, J.C. (2008) Oestradiol attenuates the cognitive deficit induced by acute phencyclidine treatment in mature female hooded-Lister rats. Journal of Psychopharmacology, doi:10.1177/0269881107083839

Tait, D.S., Brown, V.J. (2007) Difficulty overcoming learned non-reward during reversal learning in rats with ibotenic acid lesions of orbital prefrontal cortex. Annals of the New York Academy of Sciences 1121, 407-20.

Tunbridge, E.M., Harrison, P.J., Weinberger, D.R. (2006) Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biological Psychiatry 60, 141-151.

Vincent, S.L., Khan, Y., Benes, F.M. (1995) Cellular colocalization of dopamine D1 and D2 receptors in rat medial prefrontal cortex. Synapse 19, 112-120.

Tables

Table 1: The influence of stage of the oestrous cycle on total lever pressing.

Stage of cycle	Initial phase	Reversal phase
Di-oestrous	29.1± 0.3	28.0± 0.4
Met-oestrous	28.4± 0.6	29.0± 0.5
Pro-oestrous	28.4± 0.2	28.3± 0.3
Oestrous	28.7± 0.3	28.5± 0.4

Table 2: The effect of SKF-38393 on total lever pressing.

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	27.0± 0.3	27.0± 0.2
vehicle + sub-chronic PCP	26.9± 0.2	26.6± 0.2
0.75 mg/kg + sub-chronic PCP	25.9± 0.3	26.3± 0.2
1.5 mg/kg + sub-chronic PCP	26.1± 0.2	26.1± 0.2
3.0 mg/kg + sub-chronic PCP	25.8± 0.3	26.0± 0.1
6.0 mg/kg + sub-chronic PCP	26.8± 0.5	27.3± 0.4

Table 3: The effect of SKF-38393 and SCH-23390 on total lever pressing.

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	27.9± 0.5	26.9± 0.3
vehicle + sub-chronic PCP	27.0± 0.4	26.9± 0.4
6.0 mg/kg SKF38393 + sub-chronic PCP	26.1± 0.6	27.8± 0.4
0.05 mg/kg SCH23390 + sub-chronic PCP	24.2± 1.5*	20.2± 2.1***
0.05 mg/kg SCH23390 + 6.0 mg/kg SKF38393 + sub-chronic PCP	25.0± 0.9	24.6± 1.6

Figures

Fig 1a

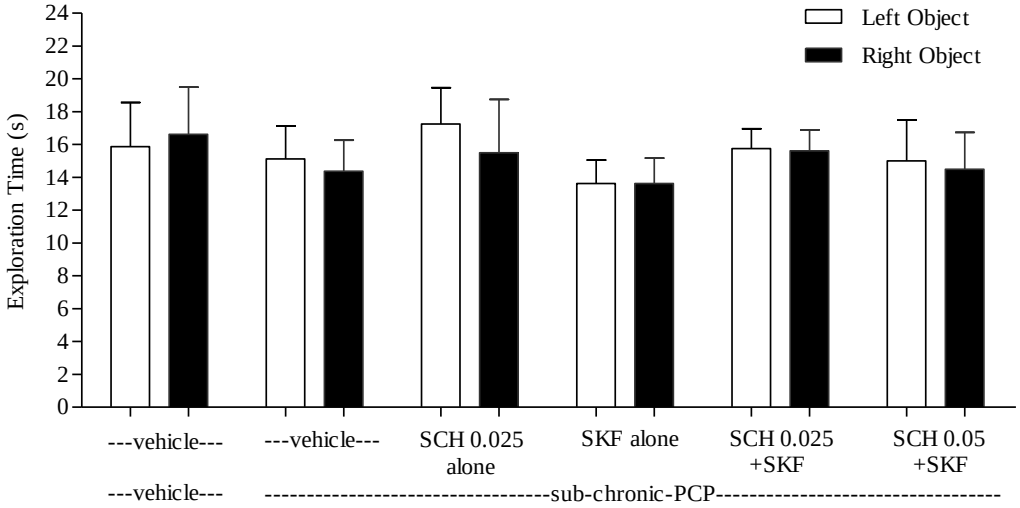
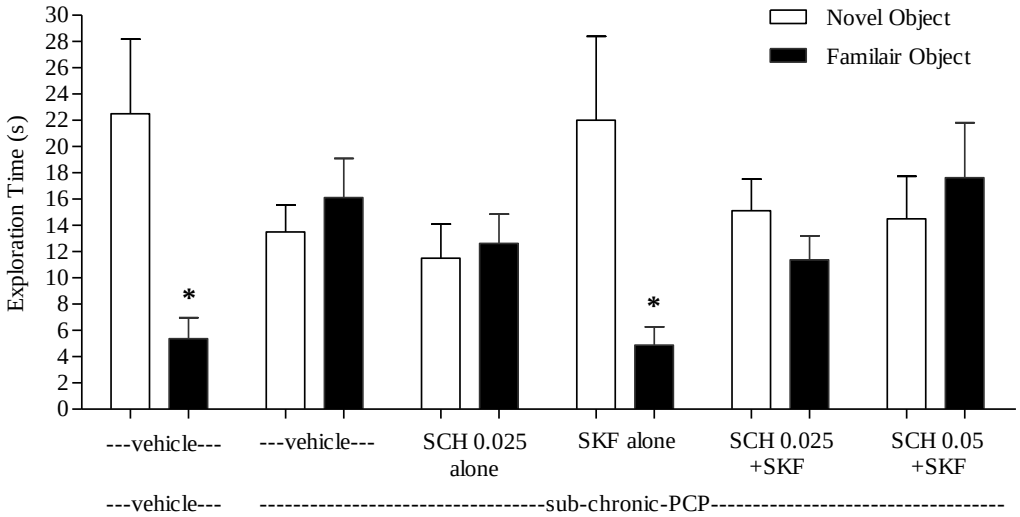


Fig 1b



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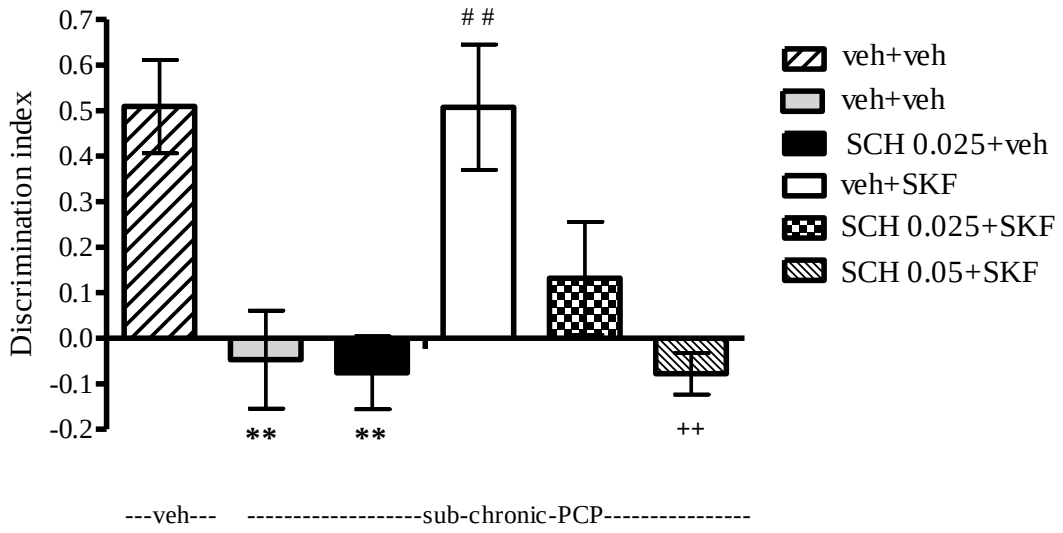
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Fig 1d

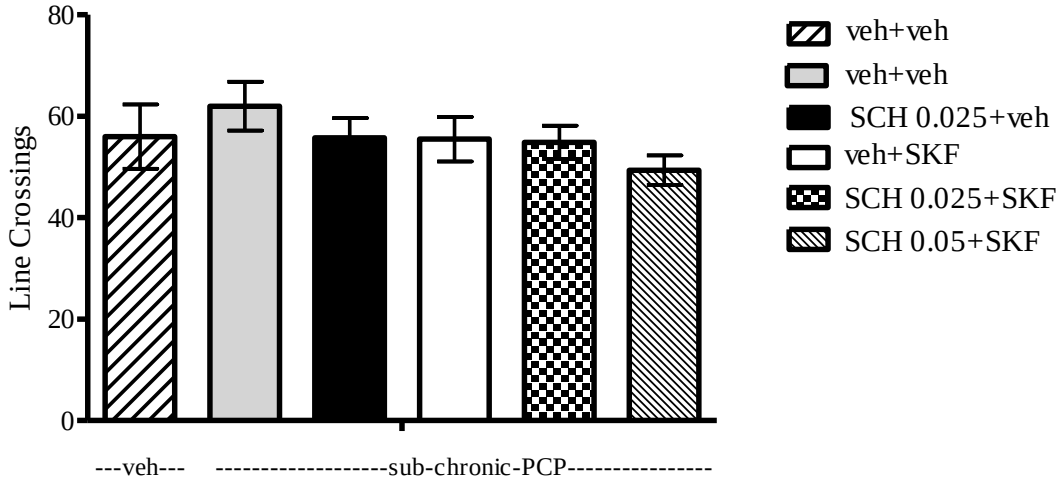
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Fig 2

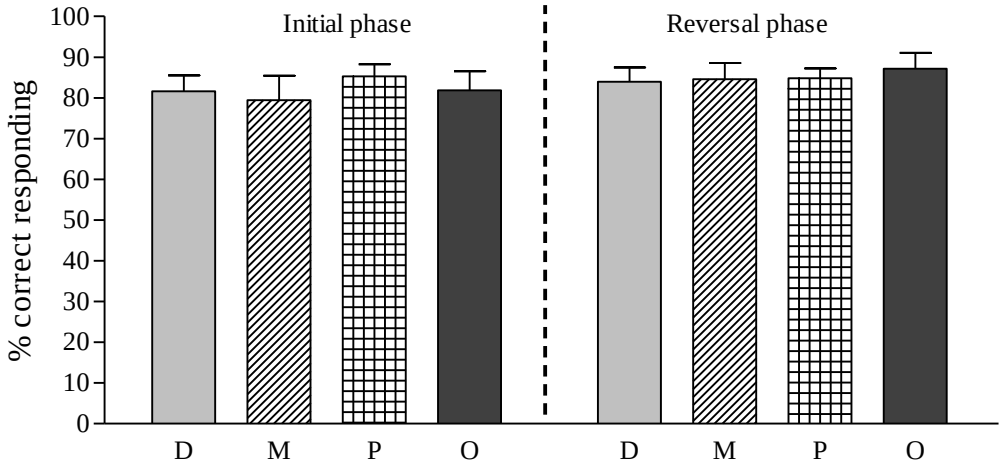
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Fig 3

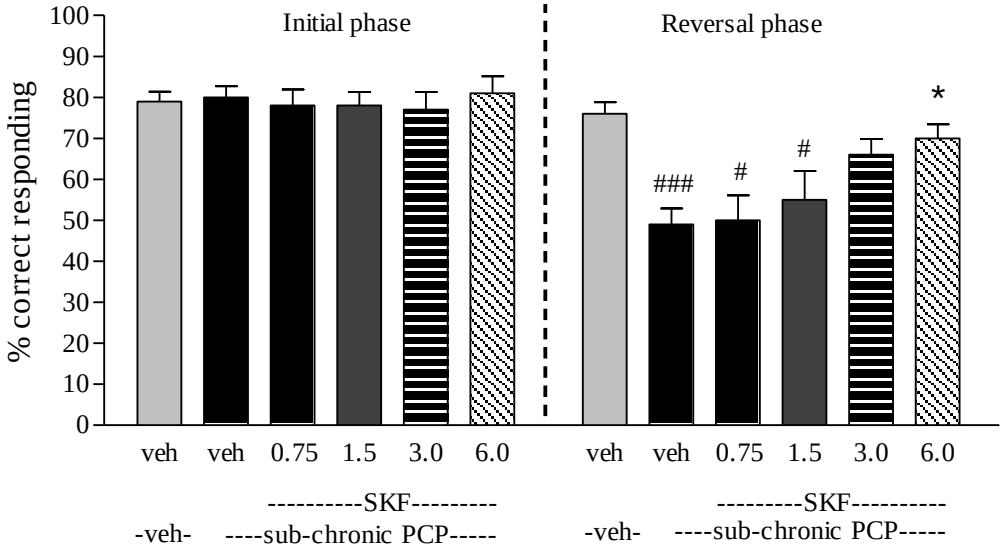
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Fig 4

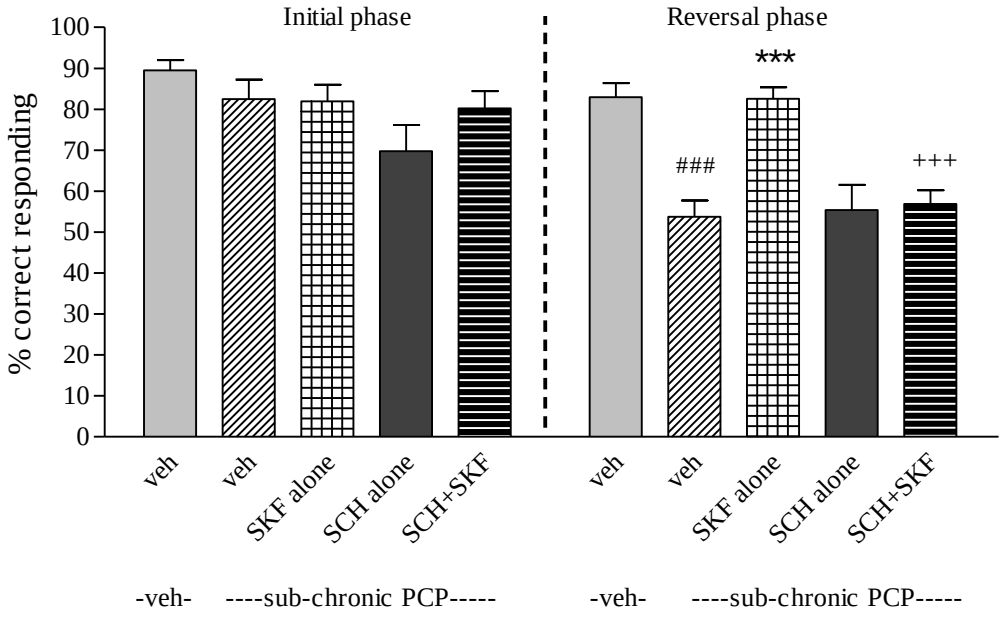

Table and Figure Legends

Table 1: The effect of stage of the oestrous cycle on the total number of lever presses in the reversal learning paradigm. Data are expressed as the mean ± sem total number of lever presses (n=6-9 per group) in the initial and reversal phase of the task.

Table 2: The effect of SKF-38393 (0.75, 1.5, 3.0, 6.0 mg/kg) and sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on the total number of lever presses in the reversal learning paradigm. Data are expressed as the mean ± sem total number of lever presses (n=9-20 per group) in the initial and reversal phase of the task.

Table 3: The effect of SKF-38393 (6.0 mg/kg), SCH-23390 (0.05 mg/kg) and sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on the total number of lever presses in the reversal learning paradigm. Data are expressed as the mean ± sem total number of lever presses (n=9-10 per group) in the initial and reversal phase of the task. Dunnett's t-test showed a significant reduction in lever pressing compared to vehicle (*P<0.05, ***P=0.001).

Figure 1a: The effect of treatment with vehicle or SCH-23390 (0.025, 0.05 mg/kg, i.p., 30 min prior to testing) followed by treatment with vehicle or SKF-38393 (6 mg/kg, i.p., 20 min prior to testing) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) in the acquisition phase of the novel object recognition task. Data are shown as mean ± s.e.m of time spent exploring the objects (n=8 per group).

Figure 1b: The effect of treatment with vehicle or SCH-23390 (0.025, 0.05 mg/kg, i.p., 30 min prior to testing) followed by treatment with vehicle or SKF-38393 (6.0 mg/kg, i.p., 20 min prior to testing) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) in the retention phase of the novel object recognition task. Data are shown as mean ± s.e.m of time spent exploring the objects (n=8 per group). Paired t-tests showed that the exploration time of the novel object was significantly greater then the familiar object (*P<0.05).

Figure 1c: The effect of treatment with vehicle or SCH-23390 (0.025, 0.05 mg/kg, i.p., 30 min prior to testing) followed by treatment with vehicle or SKF-38393 (6.0 mg/kg, i.p, 20 min prior to testing) on the deficit produced by sub-chronic PCP (2.0

mg/kg, twice daily for 7 days, i.p.) in the novel object recognition task. Data are shown as mean DI ± s.e.m (n=8 per group). The discrimination indices were analysed by post-hoc Bonferroni's multiple comparison test. The DI for the PCP-treated group and SCH-23390 alone were significantly impaired compared to the vehicle group (**P<0.01), SKF-38393 significantly improved the PCP-induced deficit ($^{\#\#}$P<0.01), and this effect was significantly blocked in the presence of SCH-23390 at 0.05 mg/kg ($^{++}$P<0.01).

Figure 1d: The effect of treatment with vehicle or SCH-23390 (0.025, 0.05 mg/kg, i.p., 30 min prior to testing) followed by treatment with vehicle or SKF-38393 (6.0 mg/kg, i.p, 20 min prior to testing) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on locomotor activity (line crossings) in the novel object recognition task. Data are shown as mean line crossings ± s.e.m (n=8 per group).

Figure 2: The influence of stage of the oestrous cycle on performance of the reversal phase of the reversal learning task. Data are shown as mean ± s.e.m. of percent correct responding (n = 6-9). Stages of the cycle were defined as di-oestrous (D), met-oestrous (M), pro-oestrous (P) and oestrous (O). No significant differences between the groups in the initial phase or reversal phases.

Figure 3: The effect of SKF-38393 (0.75, 1.5, 3.0, 6.0 mg/kg) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± s.e.m. of percent correct responding (n=9-20). A paired t-test showed a significant reduction in

performance of the reversal phase compared with the initial phase; $^{###}P<0.001$, $^{#}P<0.05$. Dunnett's t-test showed significant improvement in responding compared to PCP alone in the reversal phase at 6.0 mg/kg of SKF-38393; *P<0.05.

Figure 4: The effect of SKF-38393 (6.0 mg/kg) and SCH-23390 (0.05 mg/kg) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± s.e.m. of percent correct responding (n=9-10). Significant reduction in performance of the reversal phase compared with the initial phase ($^{###}P<0.001$). Post-hoc Bonferroni's multiple comparison test showed that SKF-38393 (6.0 mg/kg) significantly improved the PCP-induced deficit (***P<0.001), and that this deficit was significantly antagonised by SCH-23390 (0.05 mg/kg); $^{+++}P<0.001$.