Title: Activation of α7 nicotinic receptors improves phencyclidine-induced deficits in cognitive tasks in rats: implications for therapy of cognitive dysfunction in schizophrenia

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Abstract

Rationale: Nicotinic α7 acetylcholine receptors (nAChRs) have been highlighted as a target for cognitive enhancement in schizophrenia. Aim: To investigate whether the deficits induced by sub-chronic phencyclidine (PCP) in reversal learning and novel object recognition could be attenuated by the selective α7 nAChR full agonist, PNU-282987. Methods: Adult female hooded-Lister rats received sub-chronic PCP (2 mg/kg) or vehicle i.p. twice daily for seven days, followed by 7-days washout. In cohort 1, PCP-treated rats then received PNU-282987 (5, 10, 20 mg/kg; s.c.) or vehicle and were tested in the reversal learning task. In cohort 2, PCP-treated rats received PNU-282987 (10 mg/kg; s.c.) or saline for 15 days and were tested in the novel object recognition test on day 1 and on day 15, to test for tolerance. Results: Sub-chronic PCP produced significant deficits in both cognitive tasks ($P<0.01$-0.001). PNU-282987 attenuated the PCP-induced deficits in reversal learning at 10 mg/kg ($P<0.01$) and 20 mg/kg ($P<0.001$), and in novel object recognition at 10 mg/kg on day 1 ($P<0.01$) and on day 15 ($P<0.001$). Conclusions: These data show that PNU-282987 has efficacy to reverse PCP-induced deficits in two paradigms of relevance to schizophrenia. Results further suggest that 15 day daily dosing of PNU-282987 (10 mg/kg s.c.) does not cause tolerance in rat. This study suggests that activation of α7 nAChRs, may represent a suitable strategy for improving cognitive deficits of relevance to schizophrenia.

Keywords: Reversal learning; Novel object recognition; α7 nACh receptor; Phencyclidine; Female rat; Cognition; Schizophrenia

Introduction

Acetylcholine (ACh) is known to play an important role in various domains of cognition, particularly attention, learning, and memory (Friedman, 2004). Indeed, cholinergic dysfunction has been shown to be central to the pathophysiology of Alzheimer's disease and has also been postulated to contribute to the cognitive deficits observed in various neuropsychiatric disorders, including schizophrenia (Burghaus et al., 2000; Friedman, 2004). It is widely known that smoking rates in individuals with schizophrenia are higher than in the general population, perhaps suggesting that individuals may be self-medicating with nicotine (see Kumari and Postma, 2005). Nicotinic acetylcholine receptors (nAChRs) are ionotrophic receptors with a pentameric structure composed of alpha and beta subunits, and are highly expressed in the hippocampus, cortex, striatum, and thalamus (Breese et al., 2000; Freedman et al., 1995). The most prevalent nAChRs in the brain are the α4β2 and α7 subtypes, both of which have been shown to have reduced numbers in post-mortem studies of schizophrenia patients (Breese et al., 2000; Freedman et al., 1995). It has been suggested that these receptor subtypes play a role in cognition (Chan et al., 2007; Gray and Roth, 2007; Schreiber et al., 2002). The role of α7 nACh receptors in cognition, is further supported by evidence showing that α7 nACh receptor agonists and positive allosteric modulators improve performance in various tests of working and recognition memory (Pichat et al., 2007; Chan et al., 2007; Timmerman et al., 2007; Ng et al., 2007; Bitner et al., 2007; Redrobe et al., 2009; Hashimoto et al., 2008; Hauser et al., 2009). For a recent review of the involvement of α7 nACh receptors in cognitive processing of relevance to schizophrenia, see Leiser et al. 2009.

Our laboratory aims to model, in rats, the seven domains of cognition highlighted by the MATRICS initiative as being impaired in schizophrenia patients

(Green et al., 2004; Marder and Fenton, 2004). In our hands, this has predominantly involved the implementation of an operant reversal learning task (Abdul-Monim et al., 2003; 2006; Idris et al., 2005; 2009; McLean et al., 2009a; 2009b), the attentional set-shifting task (McLean et al., 2008; 2010) and the ethologically relevant, novel object recognition task (Grayson et al., 2007; Idris et al., 2009; McLean et al., 2009a). We have repeatedly demonstrated that a sub-chronic PCP dosage regimen produces robust deficits in these behavioural tests (for review see Neill et al., 2010); and that these deficits are accompanied by reductions in parvalbumin-immunoreactive neurons in the hippocampus and M1 (motor area 1) region of the frontal cortex (Abdul-Monim et al., 2007) and brain-derived neurotrophic factor (BDNF) levels in several cortical regions (Snigdha et al., 2007).

PNU-282987 is a selective agonist of the human and rat α7 nAChR (Bodnar et al., 2005; Hajos et al., 2005). It has been shown to activate the α7-5-HT_3 chimera with an EC_{50} value of 128 nM, and to evoke rapidly desensitizing currents in cultured rat hippocampal neurons. The compound does not interact with α4β2 channels, does not antagonise α3β4 or α1β1γδ nAChRs and only antagonises 5-HT_3 receptors at the higher concentrations, with an EC_{50} value of 4541 nM (Bodnar et al., 2005). The compound was shown to reverse an amphetamine-induced gating deficit in PPI in rats at 1 and 3 mg/kg i.v., and to improve a scopolamine-induced deficit in a continuous Y-maze task in mice at 10 mg/kg i.p. (Redrobe et al., 2009).

The aim of this study was to investigate the role of α7 nACh receptors to improve cognitive function of relevance to schizophrenia by using the selective α7 nAChR agonist, PNU-282987. This compound was tested in the reversal learning and novel object recognition tasks in the sub-chronic PCP model of schizophrenia. Active plasma and brain levels were determined. It has been suggested that nicotinic

agonists may produce tolerance (become less efficacious following chronic dosing) due to sustained activation and/or desensitisation of the nAChR, and subsequent neuroadaptations (Harris et al., 2004; Quick and Lester, 2002; Smith et al., 2002; White and Levin, 2004). To address this issue of tolerance, PNU-282987 was tested following 15 days once daily treatment in the novel object recognition task.

Materials and Methods

Subjects and housing conditions

Two cohorts of female hooded-Lister rats (Harlan, UK) housed in groups of four or five were used as subjects. Animals initially weighing 200-220 g 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 (50 rats) used in the reversal learning task was 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. Cohort 2 (30 rats) used in novel object recognition test had free access to food and water. Experiments were conducted in accordance with the Animals (Scientific Procedures) Act UK (1986), and approved by the University of Bradford ethical review process.

Female rats were used in this study as we have previously shown that females can outperform their male counterparts in novel object recognition (Sutcliffe et al., 2007) and are more sensitive to our PCP dosing regimen in the attentional set-shifting task (McLean et al., 2007). Importantly, we have previously demonstrated that the

stage of the oestrous cycle does not affect cognitive performance in either the novel object recognition or reversal learning tasks (Sutcliffe et al., 2007; McLean et al., 2009a).

Reversal learning task

Cohort 1 was tested in the reversal learning task as described in detail by Abdul-Monim et al. (2003) and Idris et al. (2005). Following habituation to the operant chambers (29×30×30 cm), 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. 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 3 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

starting 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. The entire shaping period requires 10-12 weeks of training; this was followed by pre-treatment with either 2.0 mg/kg PCP or vehicle (0.9% saline) by the intraperitoneal (i.p.) route twice daily for seven days, followed by at least a 7-day washout period. The pharmacological studies are detailed under experimental design.

Novel object recognition

Cohort 2 were pre-treated with either 2.0 mg/kg PCP or vehicle (0.9% saline) by the i.p. route twice daily for seven days, followed by a 7-day washout period and were then tested in the novel object recognition (NOR) task as described by Grayson et al. (2007). Briefly, rats were habituated to the test box for 20 min 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 × 40 cm high × 52 cm long) and exposed to two identical objects (A1 and A2) for a period of 3 min. 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 rats were then returned to their home cage for an inter-trial interval (ITI) 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 (sec) of each object in each trial was recorded manually using two stopwatches and the discrimination index (DI) was calculated [DI = (time at the novel object - time at the familiar object)/total retention trial exploration time]. The DI 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-drug studies

In reversal learning, 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 α7 nAChR agonist PNU-282987 (5, 10, 20 mg/kg) or vehicle and undertook a reversal-learning session. On day 3 and day 4, each animal underwent a further operant training session and reversal task session, respectively, in order to check that the baseline level of responding was regained following the drug treatment. In the novel object recognition test, the dose of 10 mg/kg PNU-282987 was selected based on the reversal learning data. Rats were tested acutely (on day 1) and following 15-day treatment with vehicle or PNU-282987. Different objects were used in the test on day 15. In each experiment the drug treatment given to each rat (and within each home cage) was randomised.

Drugs

Rats were pre-treated with either 2.0 mg/kg PCP or vehicle (0.9% saline) 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 at least a further seven days. PCP hydrochloride (Sigma, UK) was dissolved in 0.9% saline. PNU-282987 ([N-[(3R)-1-Azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide Hydrochloride]) was supplied by Johnson and Johnson (Belgium) and was dissolved in isotonic water and given in a volume of 1 ml/kg via the s.c. route, and was administered 1 hour before testing, see PK data below to explain the choice of this pre-treatment time and route of administration. The study design for each experiment is shown in table 1.

Pharmacokinetics analysis

The plasma and brain pharmacokinetics of PNU-282987 were studied in satellite groups of female Lister Hooded rats (190-240 g, Harlan, UK). The compound was administered at 10 mg/kg using the same vehicle (5% Glucose in water, as a suspension), dose volume (1 ml/kg) and dose route (s.c.) as the main pharmacological studies. From each individual animal (n=3 per time point), blood samples were collected at 15 and 30 min, 1, 2, 4, and 7 h after dose administration. Animals were sacrificed under anaesthesia and blood was collected by exsanguination into 10 ml BD vacutainers™ K3E (Becton Dickinson). In a second study, blood and brain tissue were harvested to determine the brain/plasma ratio. Briefly, blood samples were placed immediately on melting ice and plasma was obtained following centrifugation at 4 ºC for 10 minutes at approximately 1900 *x g*. All samples were shielded from daylight and stored at ~ -18 º C prior to analysis. After thawing, tissue samples were homogenized in demineralised water (1/9w/v or + 3 ml if tissue weight < 0.33 g). Plasma and brain tissue homogenate samples were analysed using an LC-MS/MS method. The lower limit of quantification (LLOQ) was 2.00 ng/ml for plasma and 20 ng/g for brain tissue. A limited pharmacokinetic analysis (non-compartmental) was

performed using WinNonlin™ Professional (Version 5.2.1). Data are shown in figure 4 and table 3.

Data and statistical analysis

Reversal learning percent correct data was arcsine transformed and analysed by a one-way ANOVA followed by post-hoc Dunnett's 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 the drugs induced any sedation or motor impairments. Data for the novel object task i.e. time at the novel versus familiar objects were analysed using paired t-tests, and the discrimination indices and line crossing data were compared using a one-way ANOVA followed by post-hoc Dunnett's t-test.

Results

Effect of acute PNU-282987 on reversal learning

For percent 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 ($P<0.001$). A one-way ANOVA in the reversal phase showed a significant interaction ($F_{4,47} = 10.69$, $P<0.001$). Post-hoc analysis revealed that PNU-282987 significantly improved the PCP-induced deficit at 10 mg/kg ($P<0.01$) and 20 mg/kg ($P<0.001$, fig 1). 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). On days 3 and 4, cognitive beneficial effects of PNU-282987 were no longer apparent (data not shown).

Effect of acute PNU-282987 treatment on novel object recognition (NOR)

There was no significant difference in time spent exploring the two identical objects during the acquisition trial in any of the treatment groups (fig 2a). In the retention trial, vehicle treated rats explored the novel object significantly more than the familiar object ($P<0.001$); this effect was abolished in sub-chronic PCP-treated rats (fig 2b). The ability to discriminate between the novel and familiar objects was restored following administration of PNU-282987 (10 mg/kg, s.c.; $P<0.001$). A one-way ANOVA revealed a significant effect of treatment ($F_{2,27} = 7.27$, $P<0.01$) on the discrimination index (DI). The DI for the PCP-treated group was significantly reduced compared to the vehicle group to 0.01 from 0.36 ($P<0.01$); PNU-282987 significantly improved the PCP-induced deficit ($P<0.01$) with a DI of 0.40 (fig 2c). There was no effect on locomotor activity assessed by the number of line crossings in the acquisition plus retention trial (fig 2d).

Effect of 15-day treatment with PNU-282987 on novel object recognition

There was no significant difference in time spent exploring the two identical objects during the acquisition trial in any of the treatment groups (fig 3a). In the retention trial, vehicle treated rats explored the novel object significantly more than the familiar object ($P<0.001$); this effect was abolished in sub-chronic PCP-treated rats (fig 3b). The ability to discriminate between the novel and familiar object was restored following administration of PNU-282987 (10 mg/kg, s.c.; $P<0.01$). A one-way ANOVA revealed a significant effect of treatment ($F_{2,24} = 17.37$, $P<0.001$) on the discrimination index (DI). The DI for the PCP-treated group was significantly reduced compared to the vehicle group to -0.07 from 0.41 ($P<0.001$); PNU-282987 significantly improved the PCP-induced deficit ($P<0.001$) with a DI of 0.20 (fig 3c).

There was no effect on locomotor activity assessed by the number of line crossings in the acquisition plus retention trial (fig 3d).

Active plasma and brain levels

Plasma and brain samples were taken from separate cohorts of hooded-Lister rats, following the s.c. dosing of 10 mg/kg PNU-282987. The plasma time-concentration plot is shown in figure 4. The concentrations and calculated pharmacokinetics parameters are listed in table 3. These analyses illustrate that PNU-282987 dosed from a suspension at 1 mg/ml dose volume is rather slowly absorbed from the s.c. site into plasma, with a T_{max} of 1.7 h after dosing. Plasma levels then decline monoexponentionally with a half life of 1.9 h. In a second study, brain T_{max} is reached later than in plasma indicating a lag time between the plasma and brain compartments. Having reached C_{max}/T_{max}, levels in the brain then follow those in plasma and decline with a similar half life of 1.9 h. The tissue to plasma ratio based on area under the curve (AUC) is 2.7. Absolute plasma concentrations, at 1 h following 10 mg/kg s.c. dosing of PNU-282987 are 744 ng/ml which correlates with 2.86 μM total concentrations in plasma. At 24 h after dosing, PNU-282987 levels were below the quantification limit.

Discussion

The current experiments showed that PNU-282987 attenuated the sub-chronic PCP-induced deficit in reversal learning when administered acutely and in novel object recognition following acute and 15-day administration.

Sub-chronic PCP-treated rats demonstrated reduced accuracy in the reversal phase only; performance in the initial phase was unaffected, suggesting that when the

rule changes PCP-treated rats do not switch to respond on the new correct lever. This is a robust and long-lasting (up to 6 months post-PCP treatment, Idris, personal communication) effect continually produced by our laboratory (Abdul-Monim et al., 2006; Idris et al. 2009; McLean et al., 2009a; 2009b) and is also in agreement with results from other laboratories (Jentsch and Taylor, 2001). Sub-chronic PCP treatment did not affect total lever pressing, suggesting that neither locomotor activity nor motivation were affected, thus the apparent cognitive deficits in the reversal phase were not due to any other effects of PCP. PNU-282987 dose-dependently improved the impairment produced by PCP with significant improvements at 10 and 20 mg/kg without causing any impairment in lever pressing. To our knowledge, our data is the first to show that activation of the α7 channel by an α7 agonist, reverses the PCP-induced deficit in a reversal learning task. Effective performance in the reversal learning task requires intact cognitive ability; thus animals are required to demonstrate flexibility, attention, motivation, and ability to suppress a previously learned response and implement a new one (Jones et al., 1991). The reversal learning task has been highlighted by the MATRICS initiative as a test of reasoning and problem solving i.e. executive function. Similar tests in schizophrenia patients, such as the Wisconsin Card Sorting Test, require intact functioning of the PFC (Deicken et al., 1995; Dias et al., 1997). It has been shown more specifically that lesions of the orbital prefrontal cortex impair reversal learning ability (McAlonan and Brown, 2003; Tait and Brown, 2007). Furthermore, in the PFC both α4β2 and α7 nAChRs have been implicated in attentional performance and cognition (Hahn et al., 2003; Chan et al., 2007). This current data suggests that α7 nAChR activation may enhance cognitive flexibility. Preliminary data also supporting a role for α7 nACh receptors in executive function have been presented in an attentional set-shifting model (Rodefer

et al., 2007, SfN abstract). The efficacy of PNU-282987 in our reversal learning model is therefore of particular importance for demonstrating a role for activation of α7 nAChRs in alleviating cognitive deficits in schizophrenia.

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 discriminate between the novel and familiar objects, suggesting they did not recognise the familiar object. It has been suggested that the brain areas involved in recognition memory include the prefrontal cortex (PFC) and perirhinal cortex (Miller *et al.*, 1996; Xiang and Brown, 2004; Winters and Bussey, 2005). Furthermore, dopamine hypofunction in the PFC is thought to have a major role in the aetiology of negative symptoms and cognitive dysfunction of schizophrenia (Abi-Dargham and Moore, 2003; Stone *et al.*, 2007). Although the mechanism for the effect of PCP is not yet established; recent work from our laboratory showed that the PCP-induced deficit is accompanied by impaired dopamine neurotransmission in the PFC during the retention trial of the task (Snigdha et al., 2008). This result was also recently confirmed in a second study in our laboratory (McLean, unpublished observations), suggesting a critical role for prefrontal dopamine in object recognition memory; dysfunction of this system in the PCP model provides further support for its validity for mimicking cognition in schizophrenia. PNU-282987 (10 mg/kg) significantly improved object recognition following an acute dose and following 15-day treatment, without affecting the number of line crossings, suggesting that the drug did not cause sedation. The fact that PNU-282987 still improved the ability to distinguish between familiar and novel objects following 15-day treatment suggests that tolerance to the beneficial cognitive effects of PNU-282987 did not develop, and that the α7 nAChRs did not become chronically desensitised, and that alterations in receptor expression,

receptor internalisation or other neuroadaptations were minimal. As we investigated only a single dose in these studies, our data do not exclude that the dose response or effective active window was not shifted to the right, i.e. that the compound had become less efficacious at lower dose and plasma exposures. Testing of a wider dose range would be required to exclude this possibility. The deficit induced by PCP in this task was maintained at 15 days, again demonstrating the robust nature of its effects to impair cognition of relevance to schizophrenia. It would have been interesting to assess if PNU-282987 is pro-cognitive by testing it alone; however, in the reversal learning task this is unlikely to reveal any improvement compared to the vehicle group as the rats are highly trained to reach criterion, thus a ceiling effect is likely to be observed. In the NOR task the compound could be tested using a longer inter-trial interval, for example 6 hours, by which time the vehicle rats are unable to discriminate between objects, to assess whether the compound improves natural cognitive decline over time. The hypothesis tested in that study is different from the one being tested here, i.e. here we assess efficacy in a model of cognitive deficits in schizophrenia whereas in "normal" animals this is a test of improvement of normal cognitive function.

Activity of α7 agonists has been documented in different versions of the novel object recognition test (Pichat et al., 2007; Wishka et al., 2006; Boess et al., 2007; Hashimoto et al., 2008; Hauser et al., 2009; Roncarati et al., 2009). Most of these models use acute pharmacological challenges to induce a cognitive impairment in the animal, or the model is based on spontaneous forgetting. It has been shown that SSR180711 (3 mg/kg) significantly improved a PCP-induced deficit (10 mg/kg/day for 10 days) in the performance of mice in a continuous Y-maze task (Thomsen et al., 2009). Interestingly, it was also shown that co-administration of SSR180711 (3

mg/kg) with PCP (10 mg/kg) for 10 days prevented a reduction in parvalbumin mRNA expression in the PFC, in addition to preventing the behavioural deficit in the Y-maze task (Thomsen et al., 2009).

Pharmacokinetic analysis of PNU-282987 in the hooded-Lister rat, following 10 mg/kg s.c. dosing, allowed us to translate active doses to active plasma concentrations. We have shown PNU-282987 to normalise the PCP-induced deficit in reversal learning and novel object recognition at 10 mg/kg s.c., which corresponds to 2.86 μM total concentrations in plasma. The dose of 5 mg/kg s.c., assuming dose linearity and corresponding to 1.35 μM in plasma, was inactive in the reversal learning task. The lowest efficacious plasma concentration of PNU-282987 in both tests (2.86 μM) is relatively close to the reported efficacious plasma level of 0.7 - 2.1 μM (associated with dosing of 1 and 3 mg/kg i.v.) in the amphetamine-induced auditory evoked potential deficit model in rat (Walker et al., 2006; Bodnar et al., 2005). Furthermore, PNU-282987 failed to show cognitive benefits in the reversal learning task at 24 and 48 h after dosing, indicating that cognitive benefits are lost when the compound is eliminated from the plasma and brain. This suggests that PNU-282987 does not elicit protracted cognitive effects such as described for several other nicotinic agonists (Bucafusco et al., 2005).

PNU-282987 has also shown beneficial effects in other cognitive paradigms in rodents. Previously, central injections of PNU-282987 into the frontal cortex have been reported to improve reference and working memory performance in the radial arm maze in rats (Chan et al., 2007). Furthermore, PNU-282987 (10 mg/kg; i.p.) was reported to improve a scopolamine-induced deficit in a continuous Y-maze task in mice (Redrobe et al., 2009). Other studies using PNU-282987 at 1 mg/kg i.v., have reported an enhancement in amphetamine-induced theta and gamma oscillations in the

CA3 region of the hippocampus and entorhinal cortex; these frequencies of oscillations and these brain regions are believed to be important for cognitive processing (Hajós et al., 2005; Hoffmann et al., 2005). Furthermore, we have shown gamma oscillations to be reduced following sub-chronic PCP treatment in the CA3 region of the hippocampus (McLean et al., 2009c). PNU-282987 has also been shown to increase c-Fos (at 20 mg/kg s.c.) in the PFC and the NAc shell in rat, while the NAc core and the dorsolateral striatum were unaffected (Hansen et al., 2007; Sumner et al., 2004). Elevation of c-Fos levels in these regions is often used as an indicator of atypical antipsychotic activity (Robertson et al., 1994).

In summary, PNU-282987 improved the PCP-induced deficit in both reversal learning and novel object recognition, two tests that assess cognitive ability following the induction of prefrontal hypofunction, and hence may be of particular relevance to cognitive dysfunction in schizophrenia. The activity of PNU-282987 in the reversal learning task suggests that activation of α7 nAChRs may be beneficial in executive function. The pro-cognitive effect of PNU-282987 following 15-day treatment, as observed in the novel object recognition task, suggests that repeated activation of this target does not evoke tolerance. In conclusion, agonists of α7 nAChRs may offer a novel therapy for cognitive dysfunction in schizophrenia and other disorders.

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Conflict of Interest

Drs Pemberton, Lesage and Mackie are employees of Johnson & Johnson Pharmaceutical Research and Development.

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Legends

Table 1: The behavioural experimental design showing the number of rats in each treatment group with each behavioural task.

Table 2: The effect of PNU-282987 (5, 10, 20 mg/kg; s.c.) and sub-chronic PCP (2mg/kg, twice a day for 7 days, i.p.) on the total number of lever presses in a reversal learning paradigm. Data are expressed as the mean ± S.E.M. total number of lever presses (n=9-10) in the initial and reversal phase of the task.

Table 3: Plasma concentrations and some basic pharmacokinetic parameters after single s.c. administration of PNU-282987 at 10 mg/kg in the female hooded-Lister rat (n=3).

Figure 1: The influence of PNU-282987 (5, 10, 20 mg/kg; s.c.) on the effect of sub-chronic PCP (2 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. **(a)** Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=9-10). **(b)** Data are shown as mean ± s.e.m. percentage correct responding (n=9-10). Paired t-test showed a significant deficit in the reversal phase compared to the initial phase; $^{###}P<0.001$. Post-hoc Dunnett's t-test showed PNU-282987 significantly improved responding compared to PCP alone in the reversal phase at 10 mg/kg (**P<0.01) and 20mg/kg (***P<0.001).

Figure 2: The effect of acute treatment with PNU-282987 (10 mg/kg, s.c.) in sub-chronic PCP (2 mg/kg, twice daily for 7 days, i.p.) treated rats in the novel object recognition task. Data are expressed as the mean ± S.E.M. (n=9-10 per group). **(a)** Mean exploration time of identical objects in the acquisition phase. **(b)** Mean exploration time of a familiar object and a novel object in the retention trial. Data were analysed by Student's t-test. ***P<0.001; Significant difference between time spent exploring the familiar and novel object. **(c)** The discrimination index (DI). Data were analysed by one way ANOVA and post-hoc Dunnett's t-test. **P<0.01; significant decrease in DI compared to the vehicle group. $^{\#\#}$P<0.01; significant increase in DI compared to the PCP group. **(d)** Total number of line crossings in the novel object recognition task.

Figure 3: The effect of 15-day treatment with PNU-282987 (10 mg/kg, s.c.) in sub-chronic PCP (2 mg/kg, twice daily for 7 days, i.p.) treated rats in a novel object recognition task. Data are expressed as the mean ± S.E.M. (n=9-10 per group). **(a)** Mean exploration time of identical objects in the acquisition phase. **(b)** Mean exploration time of a familiar object and a novel object in the retention trial. Data were analysed by Student's t-test. **P<0.01-***P<0.001; Significant difference between time spent exploring the familiar and novel object. **(c)** The discrimination index (DI). Data were analysed by one-way ANOVA and post-hoc Dunnett's t-test. ***P<0.001; significant decrease in DI compared to the vehicle group. $^{\#\#\#}$P<0.001; significant increase in DI compared to the PCP group. **(d)** Total number of line crossings in the novel object recognition task.

Figure 4: Mean plasma (n=3) concentration time profile after a single s.c. administration of PNU-282987 at 10 mg/kg dosed in the female hooded-Lister rat.

Tables

Table 1: Behavioural experimental design.

Experiment	Pre-treatment	Drug treatment
Reversal learning	10 Vehicle	10 Vehicle
	40 Sub-chronic PCP	10 Vehicle 10 PNU-282987 (5 mg/kg) 10 PNU-282987 (10 mg/kg) 10 PNU-282987 (20 mg/kg)
Novel object recognition	10 Vehicle	10 Vehicle for 15 days
	20 Sub-chronic PCP	10 Vehicle for 15 days 10 PNU-282987 (10 mg/kg) for 15 days

Table 2: The effect of PNU-282987 on total lever pressing in the reversal learning task.

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	27.6± 0.4	27.5± 0.2
vehicle + sub-chronic PCP	27.8± 0.1	28.0± 0.3
5.0 mg/kg + sub-chronic PCP	27.1± 0.3	27.6± 0.4
10.0 mg/kg + sub-chronic PCP	27.1± 0.3	27.4± 0.3
20.0 mg/kg + sub-chronic PCP	27.3± 0.3	27.4± 0.4

Table 3: Concentrations and pharmacokinetic parameters of PNU-282987 (10 mg/kg; s.c.)

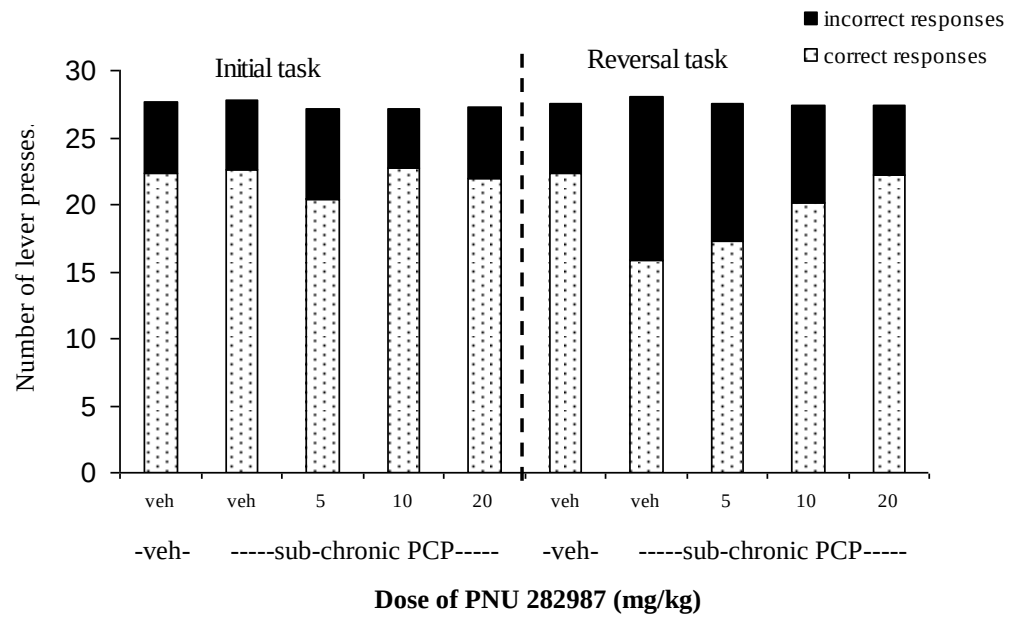
Exposure in rat after subcutaneous administration of PNU-282987

Time (h)	**A1**	**A2**	**A3**	**mean SC**		**s.d.**
Body Weights (g)	**188**	**199**	**206**	**198**	±	**9**
0.25	404	209	231	**281**	±	**107**
0.5	757	398	302	**486**	±	**240**
1	1110	666	456	**744**	±	**334**
2	1040	744	505	**763**	±	**268**
4	407	279	416	**367**	±	**77**
7	86.5	62.3	160	**103**	±	**51**
C_{max} (ng/ml)	1110	744	505	**786**	±	**305**
T_{max} (h)	1.0	2.0	2.0	**1.7**	±	**0.6**
$t_{1/2}$ (h)	1.4	1.4	2.9	**1.9**	±	**0.9**
time points $t_{1/2}$	2-7	2-7	2-7			
$AUC_{0\text{-last}}$ (ng.h/ml)	3707	2455	2487	**2883**	±	**714**
last time point AUC	7	7	7			
$AUC_{0\text{-inf}}$ (ng.h/ml)	3881	2580	3163	**3208**	±	**651**
MRT (h)	2.7	2.9	4.9	**3**	±	**1.2**

Figures

Figure 1

(a)



(b)

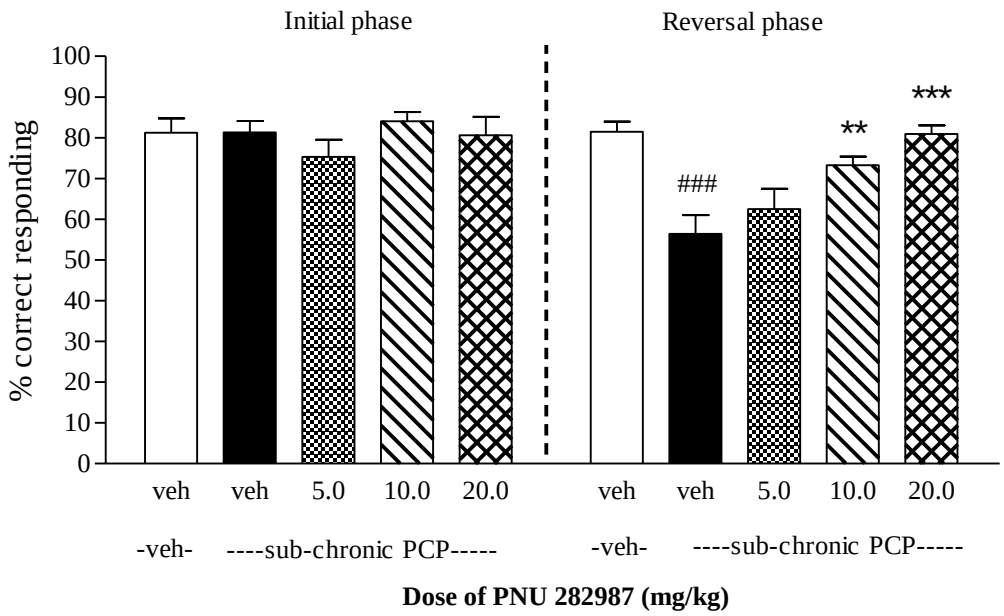
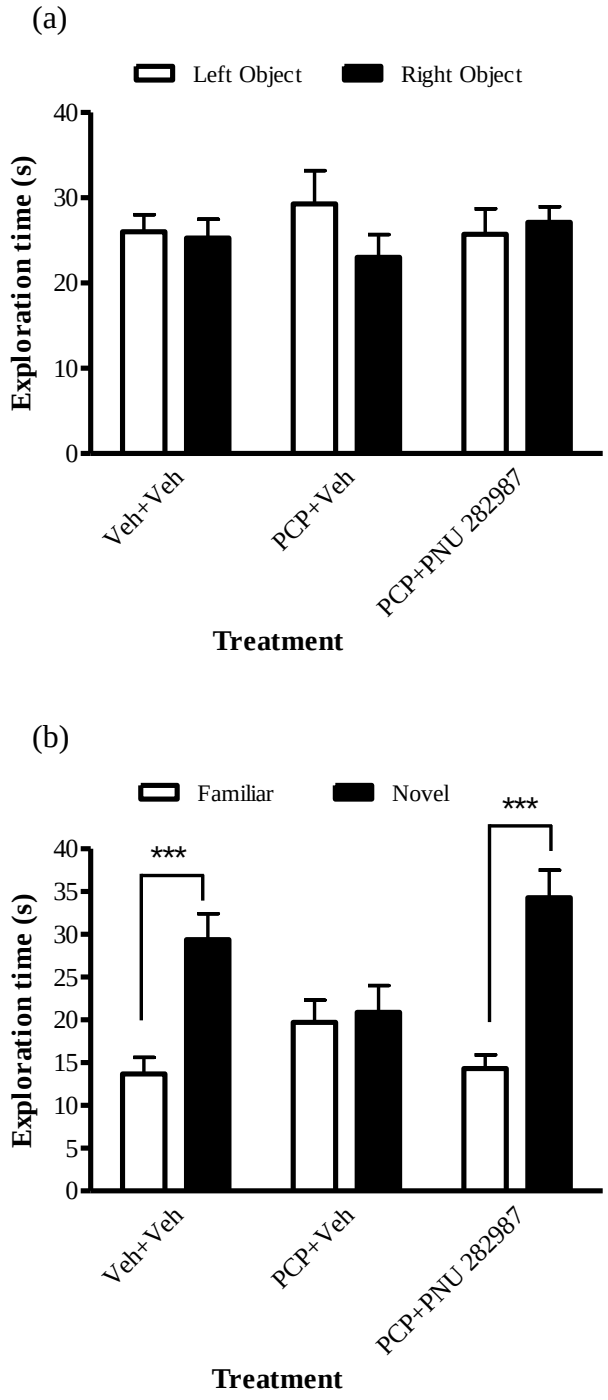
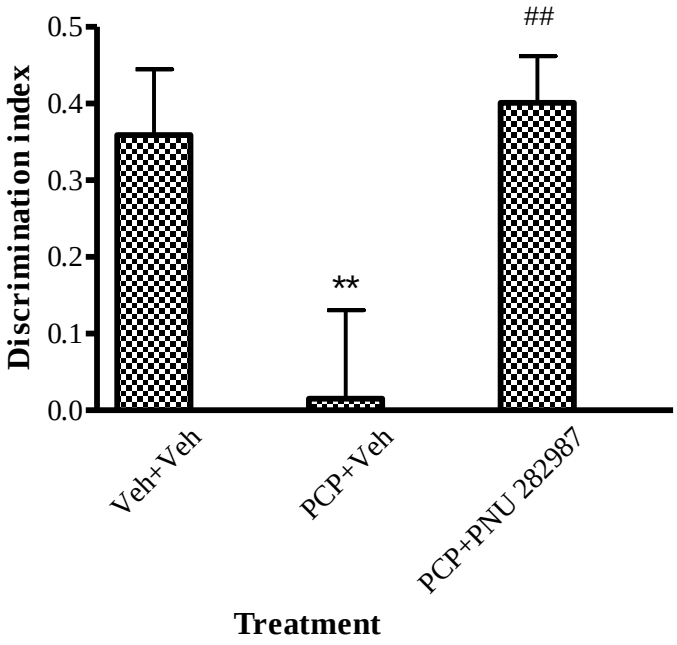


Figure 2


(a)
Left Object
Right Object
Exploration time (s)
40
30
20
10
0
Veh+Veh
PCP+Veh
PCP+PNU 282987
Treatment
(b)
Familiar
Novel
***
***
Exploration time (s)
40
35
30
25
20
15
10
5
0
Veh+Veh
PCP+Veh
PCP+PNU 282987
Treatment

(c)


0.5
0.4
0.3
0.2
0.1
0.0
Discrimination index
**
##
Veh+Veh
PCP+Veh
PCP+PNU 282987
Treatment

(d)

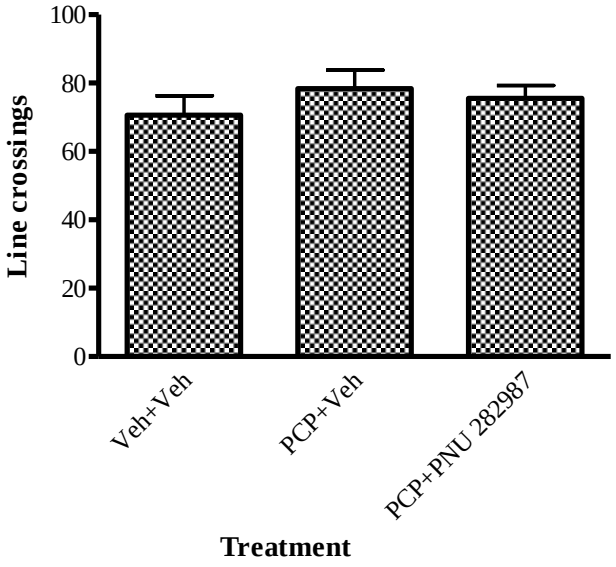
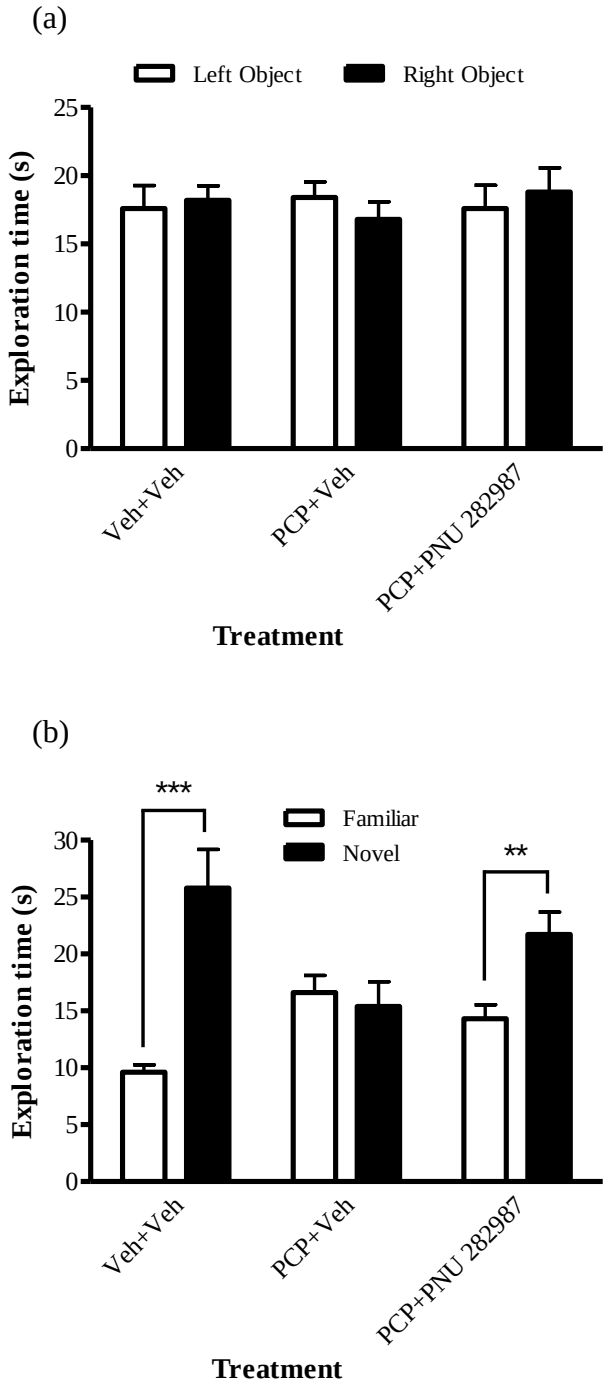

100
80
60
40
20
0
Line crossings
Veh+Veh
PCP+Veh
PCP+PNU 282987
Treatment

Figure 3


(a)
Left Object
Right Object
Exploration time (s)
25
20
15
10
5
0
Veh+Veh
PCP+Veh
PCP+PNU 282987
Treatment
(b)
***
**
Familiar
Novel
Exploration time (s)
30
25
20
15
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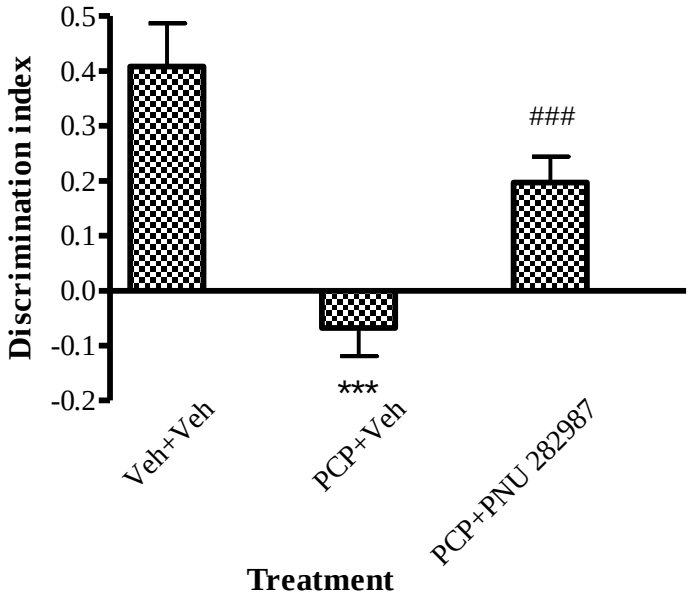
5
0
Veh+Veh
PCP+Veh
PCP+PNU 282987
Treatment

(c)


Discrimination index
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
###
***
Veh+Veh
PCP+Veh
PCP+PNU 282987
Treatment

(d)

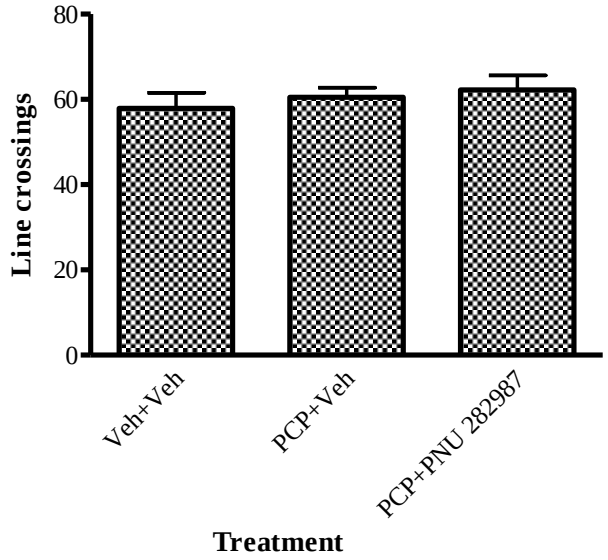

Line crossings
80
60
40
20
0
Veh+Veh
PCP+Veh
PCP+PNU 282987
Treatment

Figure 4