Title: Nicotinic α7 and α4β2 agonists enhance the formation and retrieval of recognition memory: potential mechanisms for cognitive performance enhancement in neurological and psychiatric disorders

Running title: Reversal of delay-induced object recognition deficits

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Abstract

Cholinergic dysfunction has been shown to be central to the pathophysiology of Alzheimer's disease and has also been postulated to contribute to cognitive dysfunction observed in various psychiatric disorders, including schizophrenia. Deficits are found across a number of cognitive domains and in spite of several attempts to develop new therapies, remain an unmet clinical need.

In the current study we investigated the efficacy of donepezil, risperidone and selective nicotinic α7 and α4β2 receptor agonists to reverse a delay-induced deficit in recognition memory. Adult female hooded-Lister rats received drug treatments and were tested in the novel object recognition (NOR) task following a 6 hour inter-trial interval (ITI). In all treatment groups there was no preference for the left or right identical objects in the acquisition trial. In the retention trial vehicle, risperidone (0.16mg/kg), PNU-282987, an α7 agonist (5mg/kg) and RJR-2403, an α4β2 agonist (1mg/kg) treated rats were unable to discriminate between the novel and familiar objects following a 6 hour ITI. In contrast, donepezil (1.0mg/kg), PNU-282987 (10mg/kg) and RJR-2403 (0.1mg/kg) treated rats spent significantly more time exploring the novel compared to the familiar object following the 6 hour ITI, indicative of enhanced cognitive performance ($P<0.05$-$P<0.01$). Interestingly, these compounds were efficacious when administered either before the acquisition *or* the retention trial of the task, suggesting an important role for nicotinic receptor subtypes in the formation and retrieval of recognition memory.

Keywords: Object recognition memory; female rat; delay-dependent deficits; α7 nicotinic receptors; α4β2 nicotinic receptors

Highlights

- Object recognition memory decays to 0 after a 6 hour ITI in female hL rats

- Activation of nicotinic α7 and α4b2 receptors restores this memory
- Donepezil restores this memory while risperidone is ineffective

1. Introduction

Cholinergic dysfunction has been shown to be central to the pathophysiology of Alzheimer's disease and is postulated to contribute to the cognitive deficits observed in various neuropsychiatric disorders, including schizophrenia (Burghaus et al., 2000; Friedman, 2004). Indeed, nicotine has been widely reported to improve cognitive function in humans and experimental animals (for review, see Levin et al., 2006), and adds support to the self-medication hypothesis to explain the high rates of smoking in schizophrenia patients (Zabala et al., 2009) although there may be alternative explanations for this (see recent discussion in the Lancet, Gage and Munafo, 2015; Fergusson et al. 2015). Of the nicotinic acetylcholine receptors (nAChRs), the most prevalent subtypes in the brain are comprised of α4β2 and α7 subunits (Paterson and Nordberg, 2000; Dani and Bertrand, 2007) and it has been suggested that they play an important role in cognition (Chan et al., 2007; Gray and Roth, 2007; Schreiber et al., 2002). These receptors are highly expressed in the hippocampus, cortex, striatum, thalamus and ventral tegmentum (Breese et al., 2000; Freedman et al., 1995; Gotti et al., 2006). Both subtypes have been shown to be reduced in post-mortem studies of schizophrenia patients (Breese et al., 2000; Freedman et al., 1995). Post-mortem studies of Alzheimer's disease patients have revealed a reduction in the α4β2 subtype (Warpman and Nordberg, 1995) while studies showing changes in α7 receptor levels in this illness are conflicting (see Toyohara and Hashimoto, 2010 for review).

Visual recognition memory is impaired in both schizophrenia (Calkins et al., 2005) and Alzheimer's disease patients (Parra et al., 2010). Recognition memory in rats can be tested using the novel object recognition (NOR) task, a spontaneous and ethologically

relevant paradigm based on the natural preference of rats to explore a novel object more than a familiar one after a specified time delay (Ennaceur and Delacour, 1988). Indeed, NOR has been listed by the TURNS initiative as relevant for studying visual learning and memory deficits in schizophrenia (TURNS.ucla.edu) and may be used to study cognitive deficits occurring in a range of disorders (Grayson et al., 2014). In our laboratory, we have extensively shown that sub-chronic phencyclidine (PCP) treatment impairs object recognition memory following a 1 min inter-trial interval-ITI (Damgaard et al., 2010, 2011; Grayson et al., 2007; Idris et al., 2010; McLean et al., 2009, 2011b; Snigdha et al., 2010, 2011). We have also demonstrated the efficacy of a wide range of selective receptor targets to reverse these sub-chronic PCP-induced deficits (see Neill et al., 2010 for review) as have other labs using the same paradigm (see Meltzer et al., 2013 for review). However, in order to investigate the efficacy of pro-cognitive drugs in a non-disease model we require rats to forget previously experienced situations or objects. In this scenario, the deficit is induced by increasing the ITI following acquisition and prior to the retention trial of the task. Previous studies in our laboratory have shown that, using this approach, female rats can recall objects up to a 4 hour ITI (Sutcliffe et al., 2007), an effect that was abolished following a 6 hour ITI (McLean et al., 2010a).

The aim of the current study was to investigate the ability of novel pharmacological agents and established drugs to enhance cognitive performance, with particular focus on targets of relevance to Alzheimer's disease and schizophrenia in this paradigm. The effects of the AChE inhibitor, donepezil, the atypical antipsychotic, risperidone, the α7 nACh receptor agonist, PNU-282987, and the α4β2 nACh receptor agonist, RJR-2403, on deficits in object recognition memory following a 6 hour ITI were investigated in female hooded-Lister rats. In order to ascertain whether these receptor mechanisms positively affect the formation or

retention of recognition memory, we administered these compounds both before the acquisition trial and in a separate experiment, before the retention trial of the test.

2. Materials and Methods

2.1 Subjects and housing conditions

Three cohorts of 20, 30 and 50 female hooded-Lister (hL) rats (Harlan, UK) housed in groups of five were used as subjects. Animals initially weighing 220-250 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 light/dark cycle (lights on at 0700 hours) and experimental procedures were performed during the light phase. Rats had free access to food (standard laboratory chow, Special Diet Services, Essex, UK) 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 recall information about a particular object for longer than male rats and that stage of the oestrous cycle does not affect cognitive performance in several tasks such as novel object recognition (Sutcliffe et al., 2007) and reversal learning (McLean et al., 2009).

2.2 Novel object recognition

Rats were tested in the novel object recognition (NOR) task as described in detail by McLean et al. (2011a, b). Briefly, rats in home cage groups 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 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 6 hours, 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 and a novel object 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 number of 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 D1 score was calculated [D1 = (time at the novel object - time at the familiar object)]. The D1 represents the difference in time spent exploring the novel and familiar objects.

2.3 Experimental design and dose selection

In each experiment the drug treatment given to each rat (and within each home cage) was randomised. For experiment 1, 20 rats were used and studies testing the effects of donepezil and risperidone separately were combined, therefore the vehicle group was n=20. The doses of donepezil (1 mg/kg) and risperidone (0.16 mg/kg) were selected based on their efficacy to reverse a sub-chronic PCP-induced deficit in female hooded-Lister rats in reversal learning in our laboratory (McLean et al., 2011a; Idris et al., 2011). For experiment 2, 30 rats were used and studies testing the effects of PNU-282987 and RJR-2403 separately were combined, therefore the vehicle group was n=17 (3 rats failed to explore the objects and were excluded from the final analysis). The doses of PNU-282987 were selected based on efficacy to reverse a sub-chronic PCP-induced deficit in reversal learning in our laboratory (McLean et

al., 2011b). The doses of RJR-2403 were selected based on efficacy to improve working memory in the odour span task (Rushforth et al., 2010). In experiments 1 and 2 where animals were re-used, at least one week separated each part of the study to allow drug wash-out, different objects were used for the second part of the study and dosing was fully randomised as described above. Experiment 3 was carried out in a separate cohort of 50 rats, with donepezil (1mg/kg), risperidone (0.16 mg/kg), PNU-282987 (10 mg/kg) and RJR-2403 (0.1 mg/kg) administered prior to the retention trial. In this experiment only the active doses from the prior experiments were used in an attempt to detemine which specific memory processes are affected by these compounds.

2.4 Drugs

Donepezil hydrochloride monohydrate (Sigma, UK) was dissolved in 0.5% carboxymethylcellulose and given in a volume of 1 ml/kg via the intraperitoneal (i.p.) route. Risperidone (Sigma, UK) was dissolved in a minimum volume of acetic acid, made up to volume with distilled water and pH adjusted to 6 with 0.1M NaOH. Risperidone was administered in a volume of 1 ml/kg via the i.p. route. PNU-282987 (Tocris, UK) was dissolved in isotonic water and given in a volume of 1 ml/kg via the sub-cutaneous (s.c.) route. RJR-2403 oxalate (Tocris, UK) was dissolved in 0.9% saline and given in a volume of 1 ml/kg via the s.c. route. All drug doses were calculated as base equivalent weight and were administered 30 min before the acquisition trial, with the exception of PNU-282987 which was administered 60 min before the acquisition trial. In experiment 3 all drugs were given 30 min before the retention trial, except PNU-282987 which was administered 60 min before the retention trial.

2.5 Data and statistical analysis

Data for the novel object task i.e. time at the novel versus familiar objects was analysed using paired t-tests. The D1 scores, line crossing data and total exploration time data were analysed using a one-way ANOVA followed by post-hoc Dunnett's t-test.

3. Results

3.1 Effects of donepezil and risperidone when administered before acquisition (experiment 1)

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 and risperidone treated rats showed no preference for the novel object, in contrast donepezil treated rats explored the novel object significantly more than the familiar object ($P<0.01$; fig 1b). The D1 score in the donepezil group was significantly increased compared to the vehicle group ($P<0.01$; table 1). Donepezil had no effect on locomotor activity assessed by the number of line crossings in the acquisition and retention trials; however, risperidone significantly reduced line crossings in the acquisition trial compared to the vehicle group ($P<0.01$; table 2). This was accompanied by a significant reduction in total object exploration time in the acquisition ($P<0.05$; table 3).

3.2 Effects of PNU-282987 and RJR-2403 when administered before acquisition (experiment 2)

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, PNU-282987 (5 mg/kg) and RJR-2403 (1.0 mg/kg) treated rats showed no preference for the novel object, in contrast PNU-282987 (10 mg/kg) and RJR-2403 (0.1 mg/kg) treated rats explored the novel object significantly more than the familiar object ($P<0.05$; fig 2b). The D1 scores were increased, but this effect failed to achieve statistical significance ($F_{(4,51)}=1.4$;

P=0.25; table 1). PNU-282987 had no effect on locomotor activity as assessed by the number of line crossings in the acquisition and retention trials; however, RJR-2403 at 1.0 mg/kg significantly reduced line crossings in the acquisition ($P<0.01$) and the retention trial ($P<0.05$) compared to the vehicle group (table 2). Total object exploration time in the acquisition and retention trials was unaffected by all of these compounds (table 3).

3.3 Effects of donepezil, risperidone, PNU-282987 and RJR-2403 when administered before retention (experiment 3)

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 and risperidone treated rats again showed no preference for the novel object, while donepezil, PNU-282987 and RJR-2403 treated rats explored the novel object significantly more than the familiar object ($P<0.05$-$P<0.01$; fig 3b). The D1 scores in the donepezil, PNU-282987 and RJR-2403 treated groups were significantly increased compared to the vehicle group ($P<0.05$; table 1). Risperidone significantly reduced line crossings ($P<0.001$; table 2) and the total object exploration time ($P<0.01$; table 3) in the retention trial compared to the vehicle group.

4. Discussion

In the present study we demonstrate that deficits in object recognition memory can be induced in "normal" rats following a 6 hour ITI, and that these deficits are reversed by the AChE inhibitor, donepezil, currently licensed for the treatment of mild to moderate Alzheimer's disease (Birks, 2006). We have also demonstrated the efficacy of PNU-282987 and RJR-2403 in this task; and to our knowledge, this is the first study to report the pro-cognitive effects of activating α7 or α4β2 receptors to reverse delay-induced deficits in object recognition memory in female rats.

We have repeatedly shown, in 3 different cohorts of rats, that a 6 hour ITI induces deficits in object recognition memory. These deficits were reversed by the selective nicotinic agonists PNU-282987 (10 mg/kg) and RJR-2403 (0.1 mg/kg), without affecting locomotor activity or total object exploration. We have not conducted dose response curves and have selected single effective doses for the retrieval experiment, 3. This is not ideal for a pharmacological study, however we have carefully selected doses from activity in other paradigms as described in the methods section and in the next sections, very often from our own work using female hL rats.

In our laboratory we have previously found that PNU-282987 at 10 mg/kg, the effective dose here, is able to reverse sub-chronic PCP-induced deficits in reversal learning performance and NOR following a 1 min ITI (McLean et al., 2011b). The positive allosteric modulator of a7 nicotinic receptors, PNU-120596, reversed the PCP-induced deficit in attentional set shifting in female hL rats (McLean et al. 2012), most recently we demonstrated efficacy of the α7 nACh receptor agonist, EVP-6124 to reverse PCP-induced NOR deficits, again in female hL rats (unpublished findings).

Our data are supported by a study in the odour-span working memory task, showing that both agonists at the same doses (10 mg/kg and 0.1 mg/kg) improved working memory in unimpaired rats (Rushforth et al., 2010). Furthermore, the selective α4β2 nAChR ligand, sazetidine-A, was found to reverse scopolamine and dizocilpine-induced attentional deficits in the signal detection task in female rats (Rezvani et al., 2011). Supporting this, unpublished studies from our laboratory support attentional enhancement following α7 receptor agonism in a rat model. Several recent studies add support to these findings, eg the α7 positive allosteric modulator, Lu AF58801, attenuated a sub-chronic PCP-induced deficit in NOR (Eskildsen et al., 2014) and the α7 nicotinic receptor agonist PHA-568487 ameliorated a sub-chronic MK-801-induced deficit in spatial memory (Karamihalev et al., 2014). Selective α7

nicotinic agonists, including PNU-282987 were found to improve scopolamine-induced deficits in a continuous Y-maze task (Redrobe et al., 2009). Recently, selective agonists at the α7 nACh receptor have also improved cognition in other models of impairment; for example GTS-21 improved spatial learning and memory in aged rats cognitively impaired by isoflurane (Kong et al., 2015), and PHA-543613 improved an $A\beta_{25-35}$-mediated deficit in Morris water maze performance in mice (Sadigh-Eteghad et al., 2015).

In summary, there is a wealth of preclinical evidence supporting the hypothesis that activation of these nicotinic receptor subtypes, particularly the α7 subtype can enhance cognitive performance in patient populations. A comprehensive review by Wallace et al. (2011) summarises the current α7 and α4β2 nAChR targets in various stages of development for cognitive impairments in schizophrenia, Alzheimer's disease and ADHD. Indeed, the α4β2 nAChR agonist, AZD3480, is in Phase II clinical trials for Alzheimer's disease (http://clinicaltrials.gov/show/NCT01466088). The partial α4β2 nAChR agonist and full α7 nAChR agonist varenicline was shown to have a beneficial effects on cognition in patients with schizophrenia when used in addition to antipsychotic drug treatment (Shim et al., 2012). An exploratory trial supported the potential benefits of the α7 nAChR partial agonist, TC-5619, for cognitive dysfunction and negative symptoms in schizophrenia (Lieberman et al., 2013). Phase II and III studies are also on-going to evaluate the α7 nAChR agonist, EVP-6124, for cognitive dysfunction in Alzheimer's disease (http://clinicaltrials.gov/show/NCT01073228) and as an adjunctive therapy in schizophrenia (http://clinicaltrials.gov/show/NCT01716975). A recent proof of concept trial has indicated the pro-cognitive effects of EVP-6124 in patients with schizophrenia when used alongside their usual antipsychotic treatment (Preskorn et al., 2014). Furthermore, a phase II trial has also supported the efficacy of EVP-6124 to improve cognition in patients with schizophrenia when used as an adjunctive therapy (Keefe et al., 2015). It remains to be determined how

these compounds improve cognitive performance, however the animal studies below provide some answers.

It is thought that the brain regions recruited for successful performance in the object recognition task depend upon the duration of the ITI. Rats with hippocampal lesions exhibit impairments in object recognition following ITIs greater than 15 min, but not short intervals of less than 15 min (Clark et al., 2000). In support of this, intra-hippocampal administration of the NMDA antagonist, APV, was reported to impair object recognition memory with a long (3 hour), but not short (5 min) ITI (Baker and Kim, 2002). Results from these studies suggest that reversal of our 6 hour delay-induced deficit, by PNU-282987 and RJR-2403, is mediated via activation of the hippocampus. PNU-282987 is a selective agonist of the human and rat α7 nAChR (Bodnar et al., 2005; Hajós et al., 2005); and RJR-2403 is an agonist with high selectivity and potency for α4β2 nAChR (Lippiello et al., 1996). Both α7 and α4β2 nicotinic receptors are found in the rat hippocampus (Séguéla et al., 1993; Wada et al., 1989) and are believed to contribute to the release of acetylcholine and glutamate (Changeux et al., 1998; Mansvelder et al., 2002; Wonnacott, 1997). Activation of these receptors may improve cognitive function by several mechanisms; α4β2 agonists have been shown to stimulate the release of dopamine, noradrenaline, and acetylcholine in the hippocampus and frontal cortex in rats (Bontempi et al., 2001). In addition, activation of α7 nAChRs has been shown to modulate the release of glutamate (Dickinson et al., 2008), GABA (Arnaiz-Cot et al., 2008), and dopamine (Quarta et al., 2009) in the PFC, hippocampus and striatum. Our data, together with previous preclinical and clinical results, strongly supports the role of α7 and α4β2 nAChRs in improving cognitive performance.

In our current study, risperidone did not improve the delay-induced deficit in object recognition. We have consistently demonstrated that a low dose of risperidone improves object recognition memory (Grayson et al., 2007), reversal learning (McLean et al., 2010b)

and attentional set shifting (McLean et al., 2008) deficits induced by sub-chronic PCP in female hL rats. It is important to note that the dose we use is not sufficient to block dopamine D2 receptors and is more likely to overcome the PCP deficit through other mechanisms (see Neill et al. 2014 for a discussion of this topic). Even this low dose reduces object exploration and clearly has non-specific behavioural effects, as evidenced by the data shown here, however we usually observe efficacy even under these conditions in the PCP model, so are confident of its lack of efficacy in the present study. Others have shown efficacy against MK801 (Bubenikova-Valesova et al., 2008) and tryptophan-depletion (Jenkins et al., 2010) induced deficits in animal studies. This suggests that, although risperidone may be efficacious in reversing cognitive deficits in a disease model, it is not pro-cognitive in "normal" animals when the deficit is induced by task manipulation. Therefore, the 6 hour ITI NOR test may have particular value in its ability to differentiate between antipsychotics and other pharmacological agents. Furthermore, the test may be useful for detecting targets for Alzheimer's disease as we have demonstrated the efficacy of donepezil (1 mg/kg) to reverse the delay-induced cognitive impairment. In a previous study we have also found an improvement in this task in rats treated with tacrine and memantine (McLean et al., 2011c).

In previous studies, donepezil has been found to reverse sub-chronic PCP-induced (McLean et al., 2011a; Kunitachi et al., 2009; Le Cozannet et al., 2010) and scopolamine-induced deficits (Lenz et al., 2012) in rodents. In support of our data in a model of delay-induced deficits, donepezil (0.1-3 mg/kg) alleviates object recognition deficits following a 4 hour inter-trial interval (Kendall et al., 2011). Furthermore, donepezil (0.3 mg/kg) improves object-place-context recognition performance in control rats (Le Cozannet et al., 2010) a task of episodic memory and of particular relevance to impairments observed in Alzheimer's disease. The current study investigated the effects of drug administration at different stages of the NOR task. The delay-dependent impairment in recognition memory is thought to

result from decay in memory of the familiar object (Ennaceur, 2010). Donepezil and the α7 and α4bβ2 nAChR agonists were found to improve object recognition memory when given both before the acquisition *and* the retention trials of the NOR task, suggesting that these targets have a role in both formation and retrieval memory processes.

In summary, these data demonstrate, for the first time, the efficacy of α7 and α4bβ2 nAChR agonists to reverse delay-induced deficits in object recognition memory in female hL rats. Importantly, risperidone was found not to attenuate the impairment, whereas the Alzheimer's disease treatment, donepezil, was effective to improve the delay-induced deficit, suggesting that the delay-dependent NOR task may be useful to detect targets for Alzheimer's disease and novel compounds with cognitive performance enhancing properties.

Conflict of Interest Statement

JCN has received expenses to attend conferences and fees for lecturing and consultancy work (including attending advisory boards) from the manufacturers of various neuropsychiatric drugs.

The other authors have no conflict of interest.

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Table 1: D1 scores for experiments 1-3.

Treatment Group	D1 Score
Vehicle	1.25 ± 1.6
Donepezil 1 mg/kg	11.3 ± 3.0**
Risperidone 0.16 mg/kg	5.9 ± 3.3
Vehicle	2.1 ± 2.9
PNU-282987 5 mg/kg	-2.8 ± 5.7
PNU-282987 10 mg/kg	7.1 ± 3.1
RJR-2403 0.1 mg/kg	8.1 ± 2.9
RJR-2403 1 mg/kg	1.1 ± 2.7
Vehicle	-1.4 ± 1.9
Donepezil 1 mg/kg	5.4 ± 1.9*
Risperidone 0.16 mg/kg	-2.0 ± 1.3
PNU-282987 10 mg/kg	6.4 ± 1.8*
RJR-2403 0.1 mg/kg	6.6 ± 2.2*

Table 1: D1 scores expressed as the mean ± S.E.M. for experiments 1-3. Experiment 1 shows the D1 score for donepezil (1.0 mg/kg, i.p., n=10), risperidone (0.16 mg/kg, i.p., n=10) and vehicle-treated (n=20) rats. Experiment 2 shows the D1 scores for PNU-282987 (5 and 10 mg/kg, s.c., both n=10), RJR-2403 (0.1 and 1 mg/kg, s.c., n=10 and n=9 respectively) and vehicle-treated (n=17) rats. Experiment 3 shows the D1 scores following acute treatment before the retention trial, of donepezil (1.0 mg/kg, i.p., n=10), risperidone (0.16 mg/kg, i.p., n=8), PNU-282987 (10 mg/kg, s.c., n=10) and RJR-2403 (0.1 mg/kg, s.c., n=10) in vehicle-treated (n=9) rats. *P<0.05-**P<0.01; significant increase in D1 score compared to the respective vehicle group.

Table 2: Locomotor activity measured by line crossings for experiments 1-3.

	Line crossings	
	Acquisition	Retention
Vehicle	38.2 ± 1.7	36.8 ± 1.8
Donepezil 1 mg/kg	41.5 ± 3.4	38.8 ± 2.4
Risperidone 0.16 mg/kg	26.6 ± 2.7**	44.6 ± 3.9
Vehicle	47.9 ± 3.8	50.6 ± 1.9
PNU-282987 5 mg/kg	54.6 ± 3.6	49.9 ± 4.9
PNU-282987 10 mg/kg	54.8 ± 4.3	48.9 ± 2.7
RJR-2403 0.1 mg/kg	41.6 ± 2.9	55.0 ± 7.5
RJR-2403 1 mg/kg	30.9 ± 1.7**	34.8 ± 4.1*
Vehicle	36.4 ± 4.6	36.7 ± 3.0
Donepezil 1 mg/kg	34.6 ± 3.6	43.9 ± 2.2
Risperidone 0.16 mg/kg	36.5 ± 2.7	19.5 ± 2.4***
PNU-282987 10 mg/kg	42.1 ± 1.6	29.0 ± 2.0
RJR-2403 0.1 mg/kg	43.0 ± 2.6	37.3 ± 2.4

Table 2: Locomotor activity (LMA) in the acquisition and retention trials of the NOR task expressed as mean number of line crossings ± S.E.M. for experiments 1-3. Experiment 1 shows the LMA for donepezil (1.0 mg/kg, i.p., n=10), risperidone (0.16 mg/kg, i.p., n=10) and vehicle-treated (n=20) rats. Experiment 2 shows the LMA for PNU-282987 (5 and 10 mg/kg, s.c., both n=10), RJR-2403 (0.1 and 1 mg/kg, s.c., n=10 and n=9 respectively) and vehicle-treated (n=17) rats. Experiment 3 shows the LMA following acute treatment before the retention trial of donepezil (1.0 mg/kg, i.p., n=10), risperidone (0.16 mg/kg, i.p., n=8), PNU-282987 (10 mg/kg, s.c., n=10) and RJR-2403 (0.1 mg/kg, s.c., n=10) in vehicle-treated (n=9) rats. *P<0.05-***P<0.001; significant reduction in line crossings compared to the respective vehicle group.

Table 3: Total exploration times for experiments 1-3.

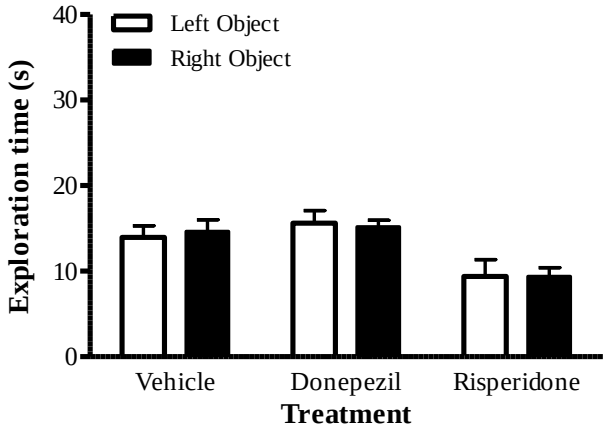
	Total exploration time (s)	
	Acquisition	Retention
Vehicle	28.6 ± 2.1	33.0 ± 2.2
Donepezil 1 mg/kg	30.8 ± 2.0	40.7 ± 4.4
Risperidone 0.16 mg/kg	18.8 ± 2.8*	32.7 ± 3.5
Vehicle	28.9 ± 3.0	35.0 ± 2.9
PNU-282987 5 mg/kg	37.0 ± 4.4	35.8 ± 4.0
PNU-282987 10 mg/kg	29.6 ± 2.8	39.5 ± 3.6
RJR-2403 0.1 mg/kg	28.2 ± 4.1	33.1 ± 4.1
RJR-2403 1 mg/kg	28.9 ± 2.8	24.2 ± 3.6
Vehicle	28.4 ± 3.2	25.2 ± 2.6
Donepezil 1 mg/kg	27.5 ± 2.8	26.2 ± 1.7
Risperidone 0.16 mg/kg	20.3 ± 2.3	11.0 ± 1.9**
PNU-282987 10 mg/kg	28.6 ± 3.8	21.0 ± 2.7
RJR-2403 0.1 mg/kg	30.9 ± 3.1	25.2 ± 2.9

Table 3: Mean total exploration times ± S.E.M. for experiments 1-3. Experiment 1 shows the total exploration times for donepezil (1.0 mg/kg, i.p., n=10), risperidone (0.16 mg/kg, i.p., n=10) and vehicle-treated (n=20) rats. Experiment 2 shows the total exploration times for PNU-282987 (5 and 10 mg/kg, s.c., both n=10), RJR-2403 (0.1 and 1 mg/kg, s.c., n=10 and n=9 respectively) and vehicle-treated (n=17) rats. Experiment 3 shows the total exploration times following acute treatment before retention trial of donepezil (1.0 mg/kg, i.p., n=10), risperidone (0.16 mg/kg, i.p., n=8), PNU-282987 (10 mg/kg, s.c., n=10) and RJR-2403 (0.1 mg/kg, s.c., n=10) in vehicle-treated (n=9) rats. *P<0.05-**P<0.01; significant reduction in total exploration time compared with vehicle.

Figures

Figure 1 – Effects of donepezil and risperidone administered before acquisition

(a)



(b)

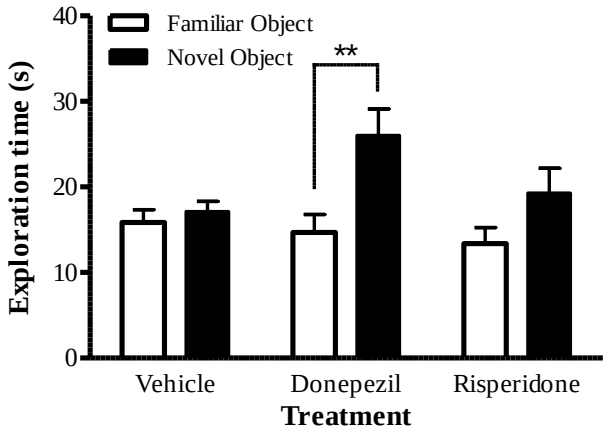
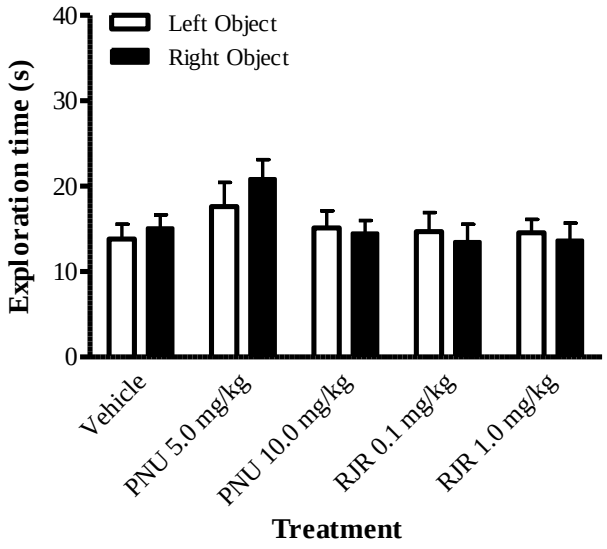


Figure 2 – Effects of PNU-282987 and RJR-2403 administered before acquisition

(a)



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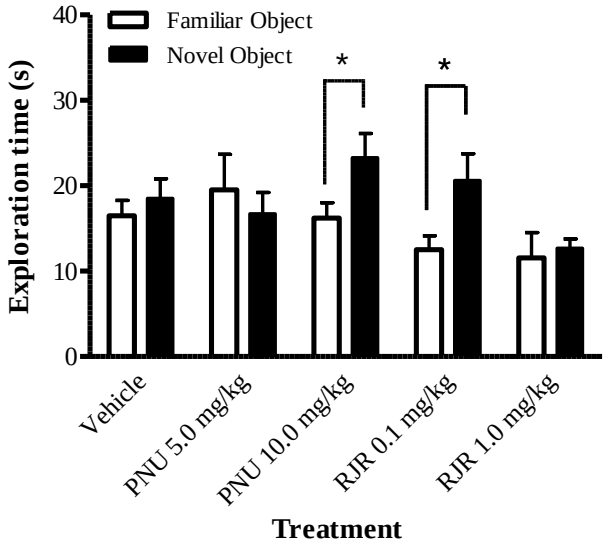
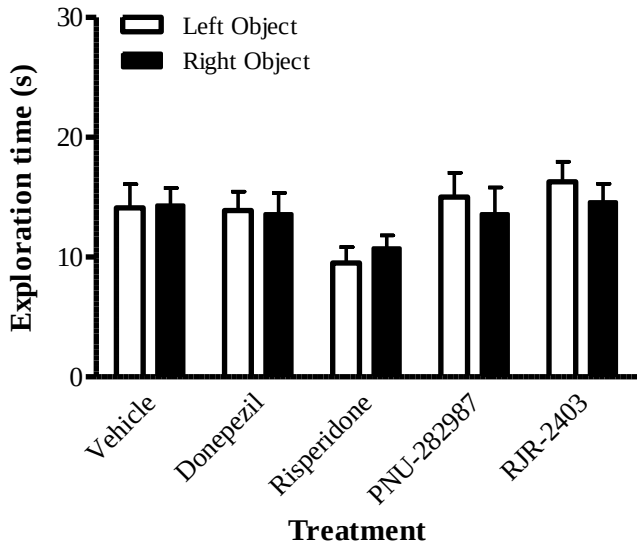


Figure 3 – Effect of the active dose of all compounds when administered before retention.

(a)



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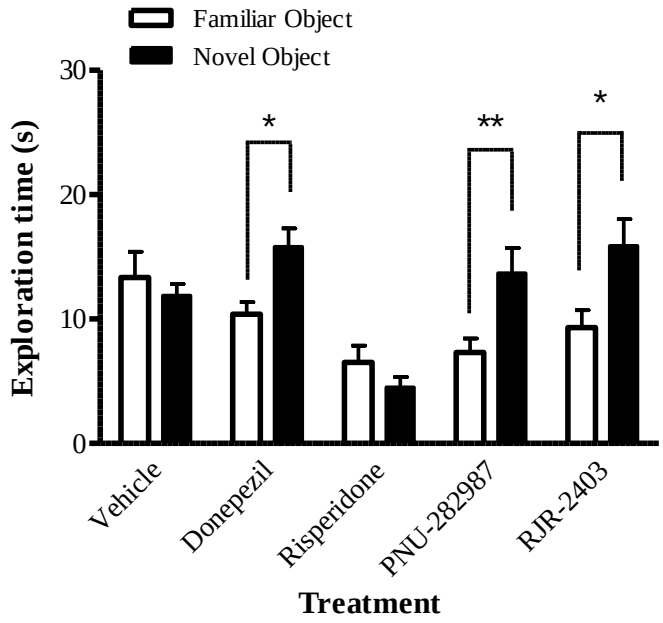

Figure legends

Figure 1: The effect of acute treatment before the acquisition trial with donepezil (1.0 mg/kg, i.p., n=10), risperidone (0.16 mg/kg, i.p., n=10) and vehicle-treated (n=20) rats in the NOR task. Data are expressed as the mean ± S.E.M. **(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 following a 6 hour inter-trial interval. Data were analysed by paired Student's t-test. **P<0.01; Significant difference between time spent exploring the familiar and novel object.

Figure 2: The effect of acute treatment before the acquisition trial with PNU-282987 (5 and 10 mg/kg, s.c., both n=10), RJR-2403 (0.1 and 1 mg/kg, s.c., n=10 and n=9 respectively) and vehicle-treated (n=17) rats in the NOR task. Data are expressed as the mean ± S.E.M. **(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 following a 6 hour inter-trial interval. Data were analysed by paired Student's t-test. *P<0.05; Significant difference between time spent exploring the familiar and novel object.

Figure 3: The effect of acute treatment before the retention trial with donepezil (1.0 mg/kg, i.p., n=10), risperidone (0.16 mg/kg, i.p., n=8), PNU-282987 (10 mg/kg, s.c., n=10) and RJR-2403 (0.1 mg/kg, s.c., n=10) in vehicle-treated (n=9) rats in the NOR task. All drugs were administered 30 min before the retention phase except PNU-282987 which was administered 60 min before retention. Data are expressed as the mean ± S.E.M. **(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 following a 6 hour inter-trial interval. Data were analysed by paired Student's t-test. *P<0.05, **P<0.01; Significant difference between time spent exploring the familiar and novel object.