



NICOTINE IMPROVES WORKING MEMORY SPAN CAPACITY IN RATS FOLLOWING SUB-CHRONIC KETAMINE EXPOSURE

Samantha L Rushforth, Thomas Steckler, Mohammed Shoaib

► To cite this version:

Samantha L Rushforth, Thomas Steckler, Mohammed Shoaib. NICOTINE IMPROVES WORKING MEMORY SPAN CAPACITY IN RATS FOLLOWING SUB-CHRONIC KETAMINE EXPOSURE. *Neuropsychopharmacology*, 2011, 10.1038/npp.2011.224 . hal-00683160

HAL Id: hal-00683160

<https://hal.science/hal-00683160>

Submitted on 28 Mar 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

NICOTINE IMPROVES WORKING MEMORY SPAN CAPACITY IN RATS FOLLOWING SUB-CHRONIC KETAMINE EXPOSURE

Samantha L Rushforth, Thomas Steckler¹, Mohammed Shoaib

Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, NE2 4HH

¹ Janssen Research & Development, Beerse, Belgium.

***Correspondence: Mohammed Shoaib, Psychobiology Research Laboratories, Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, NE2 4HH**

Tel: +44 191 222 7839, E-mail: mohammed.shoaib@newcastle.ac.uk

Keywords: Odour Span Task · Ketamine · Nicotine · LY404039 · Clozapine · Working memory

Title : 95 characters

Abstract : 239/250

Text : 4261

Figures : 4

Tables : 0

Supplementary Materials : 0

Abstract

Ketamine, an NMDA receptor antagonist produces cognitive deficits in humans in a battery of tasks involving attention and memory. Nicotine can enhance various indices of cognitive performance including working memory span capacity measured using the odour span task (OST). This study examined the effects of a sub-chronic ketamine treatment to model cognitive deficits associated with schizophrenia and to evaluate nicotine, antipsychotic clozapine and the novel mGlu2/3 agonist LY404039 in restoring OST performance. Male hooded Lister rats were trained in the OST, a working memory task involving detection of a novel odour from an increasing number of presented odours until they exhibited asymptotic levels of stable performance. Sub-chronic ketamine exposure (10 & 30mg/kg i.p. for 5 consecutive days) produced a dose-dependent impairment that was stable beyond 14 days following exposure. In one cohort, graded doses of nicotine (0.025-0.1mg/kg) administered acutely restored performance in ketamine-treated animals while significant improvements in odour span were observed in control subjects. In a second cohort of rats, acute tests with clozapine (1-10mg/kg) and LY404039 (0.3-10mg/kg) failed to reverse ketamine-induced deficits in doses that were observed to impair performance in the control groups. These data suggest that sub-chronic ketamine exposure in the OST presents a valuable method to examine novel treatments to restore cognitive impairments associated with neuropsychiatric disorders such as schizophrenia. Moreover, it highlights a central role for neuronal nicotinic receptors as viable targets for intervention that may be useful adjuncts to the currently prescribed anti-psychotics.

Introduction

Schizophrenia is a debilitating disorder affecting approximately 1% of the population: A multi-faceted disease with wide-ranging symptoms that comprise positive (auditory hallucinations, disorganised and deluded thoughts), negative (flattening of affect, apathy and anhedonia) and cognitive deficits (attention and memory) (Perala *et al*, 2007). These cognitive deficits are considered to be the core disabling feature of the disease and yet remain inadequately treated by pharmacological or behavioural therapeutic approaches (Javitt, 1999; Weinberger and Gallhofer, 1997). Of the various cognitive domains impaired (Buchanan *et al* 2005), working memory span capacity deficits have been reported in schizophrenic patients (Gold *et al*, 2010).

Dysregulation of glutamatergic transmission is proposed to play a crucial role in cognition. In both animals and humans sub-anaesthetic doses (0.1mg/kg), of NMDA receptor antagonist phencyclidine (PCP) can induce symptoms of schizophrenia, including negative and cognitive symptoms (Luby *et al*, 1959; Murray, 2002; Nabeshima *et al*, 2006). Ketamine is a less potent analogue of PCP with a shorter half-life (Anis *et al*, 1983). Thus, the psychotomimetic effects observed following acute sub-anaesthetic doses are transient and reversible. Ketamine is thought to induce cognitive deficits by initially binding to NMDA receptors on pyramidal cells which in turn causes disinhibition of GABA fast-spiking (FS) interneurons; producing a net increase of glutamatergic transmission to, which acts to increase transmission at non-NMDA receptors such as AMPA and kainate, which in turn increases dopamine release in the PFC; thought to be responsible for causing cognitive deficits (Jedema and Moghddam, 1996; Moghaddam *et al*, 1997).

In healthy subjects, a sub-anaesthetic dose of ketamine induces a wide-range of symptoms indistinguishable from those present in schizophrenia (Krystal *et al*, 1994b; Parwani *et al*, 2005). Krystal *et al* (1994) demonstrated that ketamine (0.1 and 0.05 mg/kg), administered to healthy subjects could dose-dependently induce positive, negative and cognitive symptoms (Krystal *et al*, 1994b). Schizophrenic patients also experienced exacerbated symptoms upon ketamine administration in a double-blind, placebo-controlled study (Malhotra *et al* 1997). Likewise, thirteen neuroleptic-free patients treated with sub-anaesthetic ketamine experienced exacerbated psychotic symptoms and further cognitive impairment (Malhotra *et al*, 1997b).

Despite the successful use of NMDA receptor antagonists to model symptoms of schizophrenia, it is only recently that a glutamatergic compound has been tested for treatment of schizophrenia with any success. LY404039 is a highly selective agonist for metabotropic glutamate 2/3 (mGlu2/3)

receptors, developed by Eli Lilly and currently in Phase II clinical trials for treatment of schizophrenia. Patil et al (2007) carried out a double-blind, placebo-controlled study with LY2140023 (an oral pro-drug of LY404039) in patients with schizophrenia, using olanzapine as an active control. Patients treated with LY2140023 or olanzapine showed statistically significant improvement in both positive and negative symptoms when compared to placebo. However, a follow up study revealed negative results and so further research is necessary to elucidate whether there is a role for mGlu2/3 receptors in terms of treatment for cognitive deficits (Patil *et al*, 2007).

The odour span task (OST) is a non-spatial task that assesses span capacity of working memory which is not dependent on the hippocampus (Dudchenko *et al*, 2000a). In the OST, rats are trained to dig in bowls of scented woodchip for food rewards. Upon retrieval of the reward, another bowl containing a differently scented woodchip is added. The novel bowl is the only bowl baited and must be selected over the previously sampled bowl. With each correct response, another bowl is added to assess the number of odours which can be remembered by an individual rat in a given trial. The number of odours remembered (span) provides a measure of working memory span capacity.

We have previously used the OST to demonstrate that nicotine, and specific nicotinic receptor agonists can improve working memory span capacity in rats. Drug naïve rats trained in the odour span task when administered nicotine (0.05 and 0.1 mg/kg), the $\alpha 4\beta 2$ agonist metanicotine or the $\alpha 7$ agonist (compound A), all significantly improved working memory span capacity (Rushforth *et al*, 2010).

Nicotine, known primarily for its addictive properties has proven beneficial effects on cognition in smokers (Atzori. *et al*, 2008; Newhouse *et al*, 2004) and non-smokers (Foulds *et al*, 1996; Froeliger *et al*, 2009). Since the vast majority (~80%) of schizophrenic patients smoke compared to the general population (~30%), it has been proposed that schizophrenic smokers may self-medicate with nicotine to ameliorate deficits in cognition (Bidzan, 2007; Zabala *et al*, 2009).

The present experiments aimed to assess whether sub-chronic exposure to ketamine presents a viable model to examine cognitive deficits on working memory span capacity associated with schizophrenia in rats. Using the OST, any impairment by ketamine on working memory span capacity was examined for restoration by acutely treating with nicotine. Comparisons with clozapine and the novel mGlu 2/3 receptor agonist LY404039 provided a translational perspective on ketamine-induced impairment from their relative efficacy to restore OST performance.

Methods

Animals

Two cohorts of twenty four male hooded Lister rats (Harlan, UK) each weighing approximately 175 g at the beginning of training were used. Animals were housed in groups of four under standard conditions (a temperature regulated room with a 12 hour light/dark cycle, lights on at 0700 h). Rats were food restricted at approximately 8 weeks for the duration of the study with weight monitored daily and amount of food adjusted to allow for natural growth. Under this schedule no animals showed a weight of less than 85% *ad libitum* body weight. Animals were permitted free access to water in the home cage and all testing was conducted in the light phase of the 12 hour light/dark cycle. The experiment was carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986.

Equipment

All training and testing took place on a wooden platform covered in black vinyl 93 cm square with 5 cm raised border. This was elevated 83 cm from the floor by placing on a table. The bowl locations were evenly spaced around the platform. Once training began, the position of the platform and table was kept the same throughout. Opaque ceramic dishes were used to house the scented woodchip used for the task. The 24 following odours were used in the task: rosemary, mint, onion powder, oregano, cinnamon, thyme, mixed spice, chinese-5-spice, paprika, fenugreek, nutmeg, garlic powder, caraway seed, celery salt, tea leaves, ginger (ASDA own brand), cocoa powder, cumin, coffee powder, coriander, parsley, sage, dill, lemon tea, (Lift®). All were Tesco own brand or Schwarz® except as indicated. Three grams of each odour was mixed with 100 g of woodchip and 9 crushed Nestlé® Cheerio's (added to minimize the likelihood of the rat digging in the correct bowl through scent of the food reward).

Shaping and acquisition of the Non-Matching-to-Sample (NMTS) rule

Rats were handled for approximately 5 minutes daily during the week prior to the start of training. The initial training sessions took place in a cage on top of the platform. Rats were trained to dig in unscented woodchip for a food reward (half a Nestlé® Cheerio). Once reliably digging in the woodchip for the food reward, scented bowls were introduced. The animal dug in the first bowl, retrieved the food reward and was then removed from the platform. The first bowl was relocated and a second differently-scented bowl was added. The rat had to smell both odours and then dig in the bowl containing the novel odour as this was the only bowl baited. Each rat took part in up to 10

trials per session for 3 sessions (time limited to 15 minutes), until they had learned the NMTS rule. The odours used were chosen randomly each day from the 24 mentioned above using a random letter list. All animals were exposed to all 24 scents within the 3 sessions.

Odour Span Task (OST) Training

In this phase, rats were introduced to the experimental platform where they underwent one further day of NMTS training with only two bowls at a time. From then on, the Span Task proceeded as the NMTS task but after a correct choice was made on the second bowl, an additional bowl, containing a novel scent was added. This meant the animal had to assess all three bowls and choose the third bowl. This was on the basis of scent alone as all bowls were relocated to prevent spatial cues aiding the choice. This procedure was repeated until 10 bowls were present or the rat had spent 15 minutes on the platform. This was the case despite any errors in sample choice. If a mistake was made, the animal was removed from the platform, the bowls relocated and the animal reintroduced. Once a correct choice was made, the rat was permitted to carry on to the next level. At this point, bowls were relocated but kept in reasonably close proximity (a half-OST: locations limited to numbers 1-12 on the OST board). This was done so the animals learned more quickly, (approximately 4 sessions) that they must sniff all odours before making a choice, rather than attempting to dig in every bowl. Following four half-OST sessions, animals took part in the full OST, in which bowls could be located in any of the 1 to 24 spaces and the task was ended once a mistake was made. This continued for a further 9-10 sessions, until demonstrating asymptotic performance. The main measured parameter in this task is the 'span': determined as the number of correct, consecutively chosen bowls minus 1 (as the first bowl generates no memory load). Other parameters measured included time to first sample and total time spent on the platform. Asymptotic performance was determined as achieving a span of 5 over in at least two consecutive sessions and fluctuating within a minimum of 3 spans over four consecutive sessions, based on criteria outlined by Dudchenko et al (2000) (Dudchenko *et al*, 2000b). For example, 3554, 5756, and 8868 were all acceptable span patterns for four sessions.

Probe Sessions

In this study, at random points during the training sessions, the reward for a correct choice was dropped into the bowl *after* a correct choice was made. Animals still chose correctly in each case, indicating that they were responding to the olfactory cues provided and not to the scent of the

reward. Occasionally bowls and scented woodchip were replaced during the trial, to ensure animals were not scent-marking and using this to identify the novel bowl.

Sub-chronic exposure to ketamine

In both experiments, once performance was stable, the rats were pseudo-randomised into performance-matched groups of 8 (first cohort) or 6 (second cohort) and treated with vehicle or ketamine (10 or 30 mg/kg IP) daily for 5 consecutive days. At least 2 days were allowed following the last ketamine injection to ensure 'washout' before tests with nicotine or the antipsychotic compounds.

Tests on OST

Testing was conducted every third day, with a rest day for washout followed by a training session prior to retesting. On test days, the maximum number of bowls used was increased from 10 to 15, to allow for any enhancing effect of compounds tested to be revealed. In a randomised order, acute doses of nicotine (0.025, 0.05, and 0.1 mg/kg SC) were evaluated on the OST given 10 minutes before placement on the platform. The following day the rats were rested to allow for 'washout' followed by retraining on the OST prior to the next test. Similarly, in the second experiment, tests were conducted with the atypical antipsychotic clozapine (1, 3 and 10 mg/kg IP) and the novel mGlu2/3 receptor agonist LY404039 (0.3, 1, 3, 10 mg/kg SC) administered 45 and 30 minutes prior to testing respectively. As before, doses of the antipsychotic compounds were tested acutely in a randomised sequence that occurred every third day which allowed 'washout' of the test drug followed by retraining on the OST prior to the next test. In all the above tests, the experimenter was blinded to which dose of test compound was administered and the previous history of sub-chronic exposure.

Statistical Analysis

The odour span from each group of rats was analysed using a two-way, repeated measures ANOVA with dose as the within-subject factor, and ketamine treatment as the between-subjects factor. Differences between groups and comparisons from vehicle were conducted using Bonferroni *post-hoc* tests where appropriate. Statistical significance was defined at $p < 0.05$. All analyses were performed using SPSS for Windows (SPSS Inc., V15.0).

Drugs

Ketamine (Sigma Aldrich, U.K.) was dissolved in water for injection and pH adjusted to ~pH6 with 0.1M NaOH. In the preliminary experiment, 10 or 30mg/kg doses were administered once daily for 5 consecutive days. In the second experiment, 10mg/kg was used for all animals as the first experiment revealed this induced sufficient deficits in task performance. Nicotine hydrogen tartrate (Sigma Aldrich, U.K.) was dissolved in water for injection and the pH adjusted to 7 with NaOH solution. Nicotine in doses of 0.025, 0.05 or 0.1mg/kg was administered SC 10 minutes before testing on the OST. Clozapine (Eli Lilly, synthesised by Janssen Pharmaceuticals, Belgium) was dissolved in 0.1M HCl, then adjusted to physiological pH of 7. Clozapine in doses of 1, 3 and 10mg/kg were given IP 45 minutes before testing on the OST. LY404039 (Eli Lilly, synthesised by Janssen Pharmaceuticals, Belgium) was dissolved in NaOH and pH balanced to around 6. LY404039 in doses of 0.3, 1.0, 3.0 and 10.0mg/kg were given 30 minutes prior to testing. Doses of both clozapine and LY404039 were based according to pharmacokinetic parameters derived by Janssen Research & Development, Beerse, Belgium and previously published literature (Abdul-Monim *et al* 2006; Rorick-Kehn *et al* 2007). All values are expressed as those of the base.

Results

Acquisition of the Odour Span Task

Animals were trained in a non-matching-to sample rule for approximately four training sessions. They were then moved on to a half OST until they achieved a span of 5 (figure 1). From here, they were trained in the full OST until demonstrating asymptotic performance: a span of 5 on at least two consecutive sessions, which does not fluctuate more than 3 spans over 4 consecutive sessions (figure 1).

Ketamine induces deficits in OST performance that persist over 21 days

Prior to sub-chronic treatment, mean odour span for the whole group of rats was 6.9 ± 0.3 (mean \pm SEM of the five runs prior to treatment). Mean odour span for control, ketamine 10 mg/kg and ketamine 30 mg/kg were 6.9 ± 0.5 , 7.0 ± 0.4 and 6.9 ± 0.6 respectively. Animals were performance matched to ensure no significant difference in the mean span between the experimental groups before treatment ($F(2,21)=0.003$, n.s.). Ketamine treatment for 5 days with either 10 or 30 mg/kg dose resulted in substantial impairment on odour span detection ($F(1,15)=372.6$, $p<0.001$) as measured over 8 days following the last dose of ketamine (figure 2).

Nicotine 0.025, 0.05 and 0.1 mg/kg dose-dependently enhance OST performance in both control and ketamine-treated animals

Acute administration of nicotine at 0.025, 0.05 and 0.1 mg/kg produced a dose-dependent increase in task performance in both control and ketamine treated animals ($F(3,45)=28.2$, $p<0.001$). These improvements are illustrated in figure 3. There was no interaction between the two factors ($F(6,45)=1.73$, n.s.), suggesting that nicotine improved OST performance irrespective of pretreatment. The 0.1 mg/kg nicotine dose was the most effective in enhancing performance by improving span by 4 in both vehicle- and ketamine-treated groups.

Neither clozapine nor LY404039 reverses ketamine-induced deficits in the OST.

Five test sessions following to ketamine exposure confirmed impairments in odour span detection, reduced to 3.3 ± 0.8 compared to the control group that exhibited a baseline mean span of 8.0 ± 1.2 . Treatment with acute doses of clozapine (1, 3 and 10 mg/kg) or vehicle impaired performance of

control animals, but ketamine-treated animals were unaffected (figure 4). A two-way repeated measures ANOVA revealed clozapine to impair OST performance, ($F(3,30)=6.2$, $p<0.01$), which interacted with ketamine exposure ($F(3,30)=4.6$, $p<0.05$). When analysed within group using a one way ANOVA for repeated measures, clozapine had no significant effect on OST performance in animals previously exposed to ketamine, ($F(3,23)=0.095$, n.s.), but caused a dose-dependent impairment in performance of control animals ($F(3,23)=10.9$, $p<0.001$).

In another set of animals, ketamine exposure also impaired performance, reducing span to 3.0 ± 0.7 compared to the control group that exhibited baseline span levels of 7.7 ± 0.6 . Treatment with acute doses of LY404039 (0.3, 1, 3 or 10mg/kg) or vehicle did not improve performance, in fact the highest dose impaired performance (figure 4). A two-way repeated measures ANOVA revealed LY404039 a significant effect on OST performance ($F(4,40)=12.3$, $p<0.001$), along with a significant interaction with ketamine ($F(4,40)=3.5$, $p<0.01$). Further analysis using a one way ANOVA to compare the effects of LY404039 on each subgroup revealed LY404039 to cause a dose-dependent impairment in performance of control animals ($F(4,29)=5.60$, $p<0.01$), and a small but significant effect of LY404039 was seen in ketamine-treated animals ($F(4,29)=38$, $p<0.05$). However, post-hoc analysis did not reveal significant effects of LY404039 when compared to vehicle treatment (figure 4).

Discussion

Sub-chronic ketamine exposure produced a long-lasting impairment of OST performance in rats; the animals remained stable for up to 21 days from treatment. The most significant finding to emerge from this study was the relative efficacy of nicotine to improve the odour span achieved in both control and ketamine-treated rats, improvements which were dose-related and a maximal effect achieved at the 0.05mg/kg dose. Under these conditions, clozapine with the mGlu2/3 receptor agonist LY404039 were not effective in restoring performance in the ketamine-treated rats and remarkably impaired performance in control animals.

The persistence of cognitive deficits produced by sub-chronic exposure to sub-anaesthetic doses of ketamine is not well documented. However, Becker *et al* (2003) injected rats with 30mg/kg ketamine i.p. daily for 5 days; the same regimen as employed in this study. They report that four weeks after the last injection of ketamine, glutamate binding in the prefrontal cortex was decreased by 25% but remained unchanged in both the hippocampus and striatum (Becker *et al*, 2003). This may explain the long-lasting effects of treatment with ketamine in this study.

Ketamine impairs cognition in both humans and animals (Becker *et al*, 2003; Buccafusco and Terry Jr, 2009; Ghoneim *et al*, 1985; Krystal *et al*, 1994a; Krystal *et al*, 2005; Malhotra *et al*, 1997a; Malhotra *et al*, 1996; Newcomer *et al*, 1999; Verma and Moghaddam, 1996). Verma and Moghaddam (1996) treated rats with ketamine 10, 20 and 30mg/kg i.p. prior to a spatial/non spatial short-term memory task which resulted in significant deficits in performance (Verma and Moghaddam, 1996). More recently, Buccafusco *et al* (2009) gave sub-anaesthetic doses of ketamine to monkeys trained in a computer-assisted delayed response task. Ketamine reduced accuracy in performance but did not affect processing speed. This effect was also fully reversed by the $\alpha 7$ nAChR partial agonist DMXB-A ([3-[(3E)-3-[(2,4-dimethoxyphenyl) methylidene]-5,6-dihydro-4H-pyridin-2-yl]pyridine]) (Buccafusco and Terry, 2009). These basic findings translate well; Malhotra *et al* (1996) administered sub-anaesthetic doses of ketamine to 15 healthy volunteers and observed impaired performance on various measures of attention and memory (Malhotra *et al*, 1996). In a later study, this group also reported on ketamine further impairing cognitive performance in schizophrenic patients as well as exacerbating symptoms of psychosis (Malhotra *et al*, 1997b). These clinical findings agree with the present observed results suggesting that ketamine exposure in the OST appears to be a valuable, clinically relevant model for modelling cognitive deficits in rodents. However, in a double blind, cross over clinical trial, though DMXB-A improved attention and working memory in the first treatment

arm, the MATRICS cognitive measures showed no significant difference over the three treatment arms when compared to placebo (Freedman *et al*, 2008).

Nicotine can enhance cognition in smokers, non-smokers, laboratory animals previously exposed to nicotine and animals which are nicotine naïve (for review see (Levin *et al*, 2006b)). Nicotine has also been shown to enhance cognitive function in schizophrenic patients (Barr *et al*, 2008; Jacobsen *et al*, 2004; Smith *et al*, 2002; Smith *et al*, 2006). Similarly, George *et al* (2001) demonstrated smoking abstinence significantly reduced visuospatial working memory in schizophrenic smokers but not control smokers. The schizophrenic smokers also experienced a beneficial effect on spatial working memory after smoking a cigarette; this effect was not observed in control subjects (George *et al*, 2002). These results translate well with the present observations in ketamine-treated rats and supports previous reports on improvements with nicotine in non-compromised subjects (Rushforth *et al*, 2010).

Nicotine has previously been reported to improve cognitive performance specifically in animal models of working memory. Levin *et al* (1997, 1998) examined the effect of both acute and chronic nicotine administration on working and reference memory in a 16-arm radial maze. In both cases, nicotine caused significant improvements in working, but not reference memory. In acute tests, nicotine administration was also shown to significantly attenuate impairments produced by mecamylamine (Levin *et al*, 1997; Levin *et al*, 1996). Further support comes from a study by Young *et al* (2007a) in which nicotine could restore OST performance in transgenic mice (Young *et al*, 2007a). Further experiments with repeated nicotine tests will inform on how persistent these improvements are on working memory of span capacity.

From a neurobiological perspective, Dudchenko *et al* (2000) discovered that non-spatial span capacity of working memory in rats is not reliant on an intact hippocampus; hippocampal lesions did not impair OST performance. Several studies have confirmed a significant contribution of the medial prefrontal cortex (mPFC) in an olfactory serial reversal and delayed alternation tasks (Kinoshita *et al*, 2008; Yoon *et al*, 2008). The mPFC of rodents is considered to be functionally homologous to the primate dorsolateral PFC, which also has a role in attention and working memory (Dalley *et al*, 2004; Groenewegen and Uylings, 2000). Interestingly, Dade *et al* (2000) have shown that the dorsolateral pre-frontal cortex is involved in olfactory working memory in humans. Using positron emission tomography to measure cerebral blood flow changes in 12 volunteers during an olfactory working memory task, they observed significant activation of the prefrontal cortex, specifically the dorsolateral and ventrolateral frontal cortex (Dade *et al*, 2001; Dalley *et al*, 2004). This and other animal studies suggest that despite the involvement of multiple brain regions in cognition, the

modulatory effect of nicotine on non-spatial span capacity of working memory in the work presented here is likely to be mediated through the nAChRs in the PFC (Granon *et al*, 1995; Levin, 1992). In further support of this, Turchi and Sarter (2000) using the rodent OST found significant contribution of cholinergic fibres in the PFC to span capacity of working memory function and attention. Infusion of ¹⁹²IgG-saporin into the basal forebrain resulted in an 80% reduction in density of fronto-dorsal acetylcholinesterase-positive fibres (Turchi and Sarter, 2000) which correlated significantly with a decline in olfactory span performance. These studies highlight the importance of both prefrontal cortical involvement and the necessity of cholinergic processes in olfactory working memory of span capacity. Enhanced activation as a result of nicotine administration may therefore enhance olfactory working memory and attention in both compromised and normal rodents by increasing cholinergic input to the mPFC and thus increasing excitatory synaptic strength (Laroche *et al*, 2000; Laroche *et al*, 1990). This finding is supported by Young *et al* (2007b) who found that $\alpha 7$ nAChR knock-out mice were impaired on the OST, though they suggested that this difference was largely a result of impaired attention rather than working memory required to maintain an adequate span capacity.

In this set of experiments, one objective was to compare the effects of nicotine against clozapine, known for its clinical efficacy against positive symptoms for its ability to restore ketamine-induced deficits in the OST. Clozapine has been reported to reverse deficits on various cognitive domains; clozapine restored FG1742-induced delayed memory deficits in monkeys (Murphy *et al*, 1997), attentional deficits produced by blockade of NMDA in the prefrontal cortex of rats (Baviera *et al* (2008) and PCP-induced deficits on reversal learning in rats (Abdul-Monim *et al* 2006). While these latter reports highlight the effects of clozapine on attentional and executive function, other studies focussing on spatial working memory generally have been less conclusive highlighting the lack of translation to the clinical situation. Levin and Christopher (2006) found clozapine impaired rather than improved spatial working memory in the rat neonatal hippocampal lesion model of schizophrenia (Levin and Christopher 2006). The present finding that clozapine was not effective against ketamine-induced deficits and impaired performance in control subjects in the OST supports previous findings (Addy and Levin, 2002; Gray *et al*, 2009; Levin *et al*, 2006a; Pocivavsek *et al*, 2006). This inconsistency of clozapine on working memory is also apparent clinically (Meltzer and McGurk, 1999; Rosenheck *et al*, 1999).

Clinically, clozapine is administered repeatedly which takes several weeks before minimal effects on cognitive performance are observed. Clozapine's inability to enhance performance in the OST may

be due to experimental design; all antipsychotics given clinically do not display immediate effects. In future, repeated administration would perhaps allow clozapine to be more effective.

LY404039, like clozapine, was also ineffective in restoring ketamine-induced non-spatial span capacity of working memory deficits. However, if the dose of ketamine used to induce deficits was reduced, perhaps a significant effect of LY404039 may have become apparent. Additionally, as with most antipsychotics, Patil et al (2007) report a significant clinical effect of LY404039 at week 4 of treatment (Patil *et al*, 2007). This suggests acute doses may be insufficient to overcome deficits.

In summary, ketamine has been shown to induce significant deficits in non-spatial working memory performance in the OST. This was fully reversed with acute doses of nicotine, which also improved performance in non-compromised subjects. Neither clozapine nor LY404039 restored performance in ketamine treated rats tested on the OST. Taken together with the nicotine data, the present findings support the use of ketamine in the OST to model cognitive deficits associated with schizophrenia and also suggest that nicotinic agonists may be useful as an adjunct therapy to the currently prescribed anti-psychotics.

Disclosure

All authors report no biomedical financial interests or potential conflicts of interest.

Acknowledgments

This research was supported by a research studentship grant awarded by Janssen Research & Development, Beerse, Belgium.

References

Abdul-Monim Z, Reynolds GP, Neill JC (2006) The effect of atypical and classical antipsychotics on sub-chronic PCP-induced cognitive deficits in a reversal-learning paradigm. *Behav Brain Res.* **169**:263-73

Addy N, Levin E (2002). Nicotine interactions with haloperidol, clozapine and risperidone and working memory function in rats. *Neuropsychopharmacology* **27**: 534-541.

Anis N, Berry S, Burton N, Lodge D (1983). The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *Br J Pharmacol* **79**: 565-575.

Atzori. G, Lemmonds. CA, Kotler. ML, Durcan. MJ, Boyle. J (2008). Efficacy of a nicotine (4 mg)-containing lozenge on the cognitive impairment of nicotine withdrawal. *Journal of Clinical Psychopharmacology* **28**: 667-674.

- Barr R, Culhane M, Jubelt L (2008). The effects of transdermal nicotine on cognition in nonsmokers with schizophrenia and nonpsychiatric controls. *Neuropsychopharmacology* **33**: 480-490.
- Baviera M, Invernizzi RW, Carli M (2008) Haloperidol and clozapine have dissociable effects in a model of attentional performance deficits induced by blockade of NMDA receptors in the mPFC. *Psychopharmacology (Berl)* **196**(2):269-80.
- Becker A, Peters B, Schroeder H (2003). Ketamine-induced changes in rat behaviour: A possible animal model of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* **27**: 687-700.
- Bidzan L (2007). [A review of the effects of nicotine on schizophrenia]. *Psychiatr Pol* **41**(5): 737-744.
- Breier A, Buchanan R, Kirkpatrick B (1994). Effects of clozapine on positive and negative symptoms in outpatients with schizophrenia. *American Journal of Psychiatry* **151**: 20-26.
- Buccafusco J, Terry Jr A (2009). A reversible model of the cognitive impairment associated with schizophrenia in monkeys: Potential therapeutic effects of two nicotinic acetylcholine receptor agonists. *Biochem Pharmacol* **Epub ahead of print**.
- Buccafusco JJ, Terry AV, Jr. (2009). A reversible model of the cognitive impairment associated with schizophrenia in monkeys: potential therapeutic effects of two nicotinic acetylcholine receptor agonists. *Biochem Pharmacol* **78**(7): 852-862.
- Dade L, Zatorre R, Evans A, Jones-Gotman M (2001). Working Memory in Another Dimension: Functional Imaging of Human Olfactory Working Memory *NeuroImage* **14**: 650-660.
- Dalley J, Cardinal R, Robbins T (2004). Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev* **28**: 771-784.
- Dudchenko PA, Wood ER, Eichenbaum H (2000a). Neurotoxic Hippocampal Lesions Have No Effect on Odor Span and Little Effect on Odor Recognition Memory But Produce Significant Impairments on Spatial Span, Recognition and Alternation. *Journal of Neuroscience* **20**: 2964 - 2977.
- Dudchenko PA, Wood ER, Eichenbaum H (2000b). Neurotoxic hippocampal lesions have no effect on odor span and little effect on odor recognition memory but produce significant impairments on spatial span, recognition, and alternation. *J Neurosci* **20**(8): 2964-2977.
- Foulds J, Stapleton J, Swettenham J, Bell N, McSorley K, Russell MA (1996). Cognitive performance effects of subcutaneous nicotine in smokers and never-smokers. *Psychopharmacology (Berl)* **127**(1): 31-38.
- Freedman R, Olincy A, Buchanan RW, Harris JG, Gold JM, Johnson L, *et al* (2008). Initial phase 2 trial of a nicotinic agonist in schizophrenia. *Am J Psychiatry* **165**(8): 1040-1047.
- Froeliger B, Gilbert DG, McClernon FJ (2009). Effects of nicotine on novelty detection and memory recognition performance: double-blind, placebo-controlled studies of smokers and nonsmokers. *Psychopharmacology (Berl)* **205**(4): 625-633.

George T, Vessicchio J, Termine A, Sahady D (2002). Effects of smoking abstinence on visuospatial working memory function in schizophrenia. *Neuropsychopharmacology* **26**: 75-85.

Ghoneim M, Hinrichs J, Mewaldt S, Petersen R (1985). Ketamine: Behavioral effects of subanesthetic doses. *J Clin Psychopharmacol* **5**: 70-77.

Granon S, Poucet B, Thinus-Blanc C, Changeux JP, Vidal C (1995). Nicotinic and muscarinic receptors in the rat prefrontal cortex: differential roles in working memory, response selection and effortful processing. *Psychopharmacology (Berl)* **119**: 139-144.

Gray G, van den Buuse M, Scarr E (2009). Clozapine reverses schizophrenia-related behaviours in the metabotropic glutamate receptor 5 knockout mouse: association with N-methyl-D-aspartic acid receptor up-regulation. *International Journal of Neuropsychopharmacology* **12**: 45-60.

Groenewegen H, Uylings H (2000). The prefrontal cortex and the integration of sensory, limbic and autonomic information. *Prog Brain Res* **126**: 3-28.

Jacobsen L, D'Souza D, Mencl W, Pugh K, Skudlarski P, Krystal J (2004). Nicotine effects on brain function and functional connectivity in schizophrenia. *Biol Psychiatry* **55**: 850-858.

Javitt D (1999). Treatment of negative and cognitive symptoms. *Curr Psychiatry Rep* **1**: 25-30.

Jedema HP, Moghddam B (1996). Characterization of excitatory amino acid modulation of dopamine release in the prefrontal cortex of conscious rats. *J Neurochem* **66**(4): 1448-1453.

Kinoshita S, Yokoyama C, Masaki D (2008). Effects of rat medial prefrontal cortex lesions on olfactory serial reversal and delayed alternation tasks. *Neurosci Res* **60**: 213-218.

Krystal J, Karper L, Seibyl J (1994a). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. *Arch Gen Psychiatry* **51**: 199-214.

Krystal J, Karper L, Seibyl J, Freeman G, Delaney R, Bremner J, *et al* (1994b). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* **51**: 199-214.

Krystal J, Perry EJ, Gueorguieva R (2005). Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. *Arch Gen Psychiatry* **62**: 985-994.

Laroche S, Davis S, Jay T (2000). Plasticity at Hippocampal to prefrontal cortex synapses: dual roles in working memory and consolidation. *Hippocampus* **10**: 438-446.

Laroche S, Jay T, Thierry A (1990). Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. *Neurosci Lett* **114**: 184-190.

Levin E (1992). Nicotinic systems and cognitive function. *Psychopharmacology (Berl)* **108**: 417-431.

Levin E, Christopher N (2006). Effects of clozapine on memory function in the rat neonatal hippocampal lesion model of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* **30**: 223-229.

Levin E, Kaplan S, Boardman A (1997). Acute nicotine interactions with nicotinic and muscarinic antagonists: working and reference memory effects in the 16-arm radial maze. *Behav Pharmacol* **8**: 236-242.

Levin E, Kim P, Meray R (1996). Chronic nicotine effects on working and reference memory in the 16-arm radial maze: interactions with D1 agonist and antagonist drugs. *Psychopharmacology (Berl)* **127**: 25-30.

Levin E, McClernon F, Rezvani A (2006b). Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. *Psychopharmacology (Berl)* **184**: 523-539.

Luby E, Cohen B, Rosenbaum F, Gottlieb J, R K (1959). Study of a new schizophrenomimetic drug, Sernyl. *Arch Neurol Psychiatry* **81**: 363-369.

Malhotra A, Pinals D, Adler C (1997a). Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology* **17**: 141-150.

Malhotra A, Pinals D, Adler C, Elman I, Clifton A, Pickar D, *et al* (1997b). Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology* **17**: 141-150.

Malhotra A, Pinals D, Weingartner H (1996). NMDA receptor function and human cognition: The effects of ketamine in healthy volunteers. *Neuropsychopharmacology* **14**: 301-307.

Meltzer H, McGurk S (1999). The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. *Schizophr Bull* **25**: 233-255.

Moghaddam B, Adams B, Verma A, Daly D (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* **17**: 2921-2927.

Murphy B, Roth R, Arnsten A (1997). Clozapine reverses the spatial working memory deficits induced by FG7142 in monkeys. *Neuropsychopharmacology* **16**: 433-437.

Murray JB (2002). Phencyclidine (PCP): a dangerous drug, but useful in schizophrenia research. *J Psychol* **136**(3): 319-327.

Nabeshima T, Mouri A, Murai R, Noda Y (2006). Animal model of schizophrenia: dysfunction of NMDA receptor-signaling in mice following withdrawal from repeated administration of phencyclidine. *Ann N Y Acad Sci* **1086**: 160-168.

Newcomer J, Farber N, Jevtovic-Todorovic V (1999). Ketamine-Induced NMDA Receptor Hypofunction as a Model of Memory Impairment and Psychosis. *Neuropsychopharmacology* **20**: 106-118.

Newhouse PA, Potter A, Singh A (2004). Effects of nicotinic stimulation on cognitive performance. *Curr Opin Pharmacol* **4**(1): 36-46.

- Parwani A, Weiler M, Blaxton T, Warfel D, Hardin M, Frey K, *et al* (2005). The effects of a subanesthetic dose of ketamine on verbal memory in normal volunteers. *Psychopharmacology (Berl)* **183**: 265-274.
- Patil S, Zhang L, Martenyi F, Lowe S, Jackson K (2007). Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. *Nature Medicine* **13**: 1102-1107.
- Perala J, Suvisaari J, Saarni S, Kuoppasalmi K, Isometsä E, Pirkola S (2007). Lifetime prevalence of psychotic and bipolar I disorders in a general population. *Arch Gen Psychiatry* **64**: 19-28.
- Pocivavsek A, Laura Icenogle L, Levin E (2006). Ventral hippocampal $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptor blockade and clozapine effects on memory in female rats. *Psychopharmacology* **188**: 597-604.
- Rezvani AH, Tizabi Y, Getachew B, Hauser SR, Caldwell DP, Hunter C, *et al* (2008). Chronic nicotine and dizocilpine effects on nicotinic and NMDA glutamatergic receptor regulation: interactions with clozapine actions and attentional performance in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **32**(4): 1030-1040.
- Rorick-Kehn LM, Johnson BG, Knitowski KM, Salhoff CR, Witkin JM, Perry KW, Griffey KI, Tizzano JP, Monn JA, McKinzie DL, Schoepp DD (2007). In vivo pharmacological characterization of the structurally novel, potent, selective mGlu2/3 receptor agonist LY404039 in animal models of psychiatric disorders. *Psychopharmacology (Berl)* **193**(1):121-36.
- Rosenheck R, Dunn L, Peszke M (1999). Impact of clozapine on negative symptoms and on the deficit syndrome in refractory schizophrenia. *American Journal of Psychiatry* **156**: 88-93.
- Rushforth SL, Allison C, Wonnacott S, Shoaib M (2010). Subtype-selective nicotinic agonists enhance olfactory working memory in normal rats: a novel use of the odour span task. *Neurosci Lett* **471**(2): 114-118.
- Smith R, Singh A, Infante M, Khandat A (2002). Effects of cigarette smoking and nicotine nasal spray on psychiatric symptoms and cognition in schizophrenia. *Neuropsychopharmacology* **27**: 479-497.
- Smith R, Warner-Cohen J, Matute M, Butler E, Kelly E (2006). Effects of nicotine nasal spray on cognitive function in schizophrenia. *Neuropsychopharmacology* **31**: 637-643.
- Turchi J, Sarter M (2000). Cortical cholinergic inputs mediate processing capacity: effects of 192 IgG-saporin-induced lesions on olfactory span performance *European Journal of Neuroscience* **12**: 4505 - 4514.
- Verma A, Moghaddam B (1996). The role of excitatory amino acids in prefrontal cortex function as assessed by spatial delayed alternation performance in rats: modulation by dopamine. *J Neurosci* **16**: 373-379.
- Weinberger D, Gallhofer B (1997). Cognitive function in schizophrenia. *Int Clin Psychopharmacol* **12**: S29-36.
- Yoon T, Okada J, Jung M (2008). Prefrontal cortex and hippocampus subserve different components of working memory in rats. *Learn Mem* **19**: 97-105.

Young JW, Kerr LE, Kelly JS, Marston HM, Spratt C, Finlayson K, *et al* (2007a). The odour span task: a novel paradigm for assessing working memory in mice. *Neuropharmacology* **52**(2): 634-645.

Young JW, Crawford N, Kelly JS, Kerr LE, Marston HM, Spratt C, Finlayson K, Sharkey J. (2007b) Impaired attention is central to the cognitive deficits observed in alpha 7 deficient mice. *Eur Neuropsychopharmacol.* **17**(2):145-55

Zabala A, Eguiluz JI, Segarra R, Enjuto S, Ezcurra J, Pinto AG, *et al* (2009). Cognitive performance and cigarette smoking in first-episode psychosis. *Eur Arch Psychiatry Clin Neurosci* **259**(2): 65-71.

Legends for figures

FIGURE 1 Acquisition of the odour span task (n=24), over 18 training sessions including 4 specific sessions each on the NMTS and ½ OST stages and 10 sessions on the full task. The dashed line depicts the mean span attained by the group towards the final stages of training. Each data point depicts the mean \pm SEM.

FIGURE 2 Treatment with sub-chronic ketamine at both 10 and 30mg/kg induced significant deficits in OST task performance stable over 8 days, (n=8). ***denotes statistical significance from vehicle-treated group ($p<0.001$). Each data point depicts the mean \pm SEM.

FIGURE 3 The effect of two doses of ketamine (10 and 30mg/kg) on span length in the OST. Both groups of animals (n=8) treated with ketamine exhibited significantly decreased span length when compared to control animals. This deficit in span length was reverse by ketamine administration in a dose dependent manner. Nicotine also improves span length in control animals. Each bar depicts the mean \pm SEM. ***denotes statistical significance from vehicle treatment ($p<0.001$).

FIGURE 4 Neither clozapine (A) nor LY404039 (B) significantly reverse deficits in OST task performance. Both produce significant deficits in performance of control animals in a dose-dependent fashion (n=6/group). Each data point depicts the mean \pm SEM. ***denotes statistical significance between group ($p<0.001$). ***denotes statistical significance ($p<0.01$).







