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# Nicotine Improves Working Memory Span Capacity in Rats Following Sub-Chronic Ketamine Exposure

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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 odor span task (OST). This study examined the effects of a sub-chronic ketamine treatment to model cognitive deficits associated with schizophrenia, and to evaluate the effectiveness of 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 odor from an increasing number of presented odors until they exhibited asymptotic levels of stable performance. Sub-chronic ketamine exposure (10 and 30 mg/kg i.p. for 5 consecutive days) produced a dose-dependent impairment that was stable beyond 14 days following exposure. In one cohort, administration of graded doses of nicotine (0.025–0.1 mg/kg) acutely restored the performance in ketamine-treated animals, while significant improvements in odor span were observed in control subjects. In a second cohort of rats, acute tests with clozapine (1–10 mg/kg) and LY404039 (0.3–10 mg/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.

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## 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, disorganized 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 behavioral 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).

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Dysregulation of glutamatergic transmission is proposed to have a crucial role in cognition. In both animals and humans, sub-anesthetic doses (0.1 mg/kg) of NMDA-receptor antagonist phencyclidine 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 analog of phencyclidine with a shorter half-life (Anis *et al*, 1983). Thus, the psychotomimetic effects observed following acute sub-anesthetic 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 interneurons, producing a net increase of glutamatergic transmission, which acts to increase transmission at non-NMDA receptors such as AMPA and kainate, which in turn increases dopamine release in the prefrontal complex (PFC), which is thought to be responsible for causing cognitive deficits (Jedema and Moghddam, 1996; Moghaddam *et al*, 1997).

In healthy subjects, a sub-anesthetic dose of ketamine induces a wide range of symptoms that are indistinguishable from those present in schizophrenia (Krystal *et al*, 1994; Parwani *et al*, 2005). Krystal *et al* (1994)

demonstrated that administration of ketamine (0.1 and 0.05 mg/kg) to healthy subjects could dose-dependently induce positive, negative, and cognitive symptoms (Krystal *et al*, 1994). Schizophrenic patients also experienced exacerbated symptoms upon ketamine administration in a double-blind, placebo-controlled study (Malhotra *et al*, 1997). Likewise, 13 neuroleptic-free patients treated with sub-anesthetic ketamine experienced exacerbated psychotic symptoms and further cognitive impairment (Malhotra *et al*, 1997).

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 is 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 with 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 odor span task (OST) is a non-spatial task that assesses the 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 odors that can be remembered by an individual rat in a given trial. The number of odors 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. When drug-naïve rats trained in the OST were administered nicotine (0.05 and 0.1 mg/kg), the $\alpha4\beta2$ agonist metanicotine, or the $\alpha7$ agonist (compound A), all showed significant improvement in their 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). As the vast majority ($\sim$80%) of schizophrenic patients smoke compared with the general population ($\sim$30%), it has been proposed that schizophrenic smokers may self-medicate with nicotine to ameliorate the 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 of working memory span capacity by ketamine 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.

## MATERIALS AND METHODS

### Animals

Two cohorts of 24 male hooded Lister rats (Harlan, UK), each weighing approximately 175 g at the beginning of the training, were used. Animals were housed in groups of four under standard conditions (a temperature-regulated room with a 12-h light/dark cycle, lights on at 0700 hours). Rats were food-restricted at approximately 8 weeks for the duration of the study, with weights monitored daily and amount of food adjusted to allow for natural growth. Under this schedule, no animal 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-h light/dark cycle. The experiment was carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986.

### Equipment

All training and testing were done on a wooden platform covered in black vinyl, 93-cm square with a 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 the 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 following 24 odors 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, and lemon tea (Lift). All were Tesco-owned brands or Schwarz, except as indicated. Three grams of each odor was mixed with 100 g of woodchip and nine crushed Nestlé Cheerios (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 min daily during the week before the start of training. The initial training sessions took place in a cage on top of the platform. The rats were trained to dig in an unscented woodchip for a food reward (half a Nestlé Cheerio). Once they reliably started 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 odors and then dig in the bowl containing the novel odor, as this was the only bowl baited. Each rat took part in up to 10 trials per session for three sessions (time limited to 15 min),

until they had learned the NMTS rule. The odors 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 three sessions.

### 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 that 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 min 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 were relocated, and the animal was 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 that the animals learned more quickly (approximately four sessions) to sniff all odors 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 1–24 spaces, and the task was ended once a mistake was made. This continued for a further 9–10 sessions, until asymptotic performance was demonstrated. The main measured parameter in this task was the 'span': determined as the number of correct and 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 in at least two consecutive sessions and fluctuating within a minimum of 3 spans over four consecutive sessions based on the criteria outlined by 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 that animals were not scent-marking and using this to identify the novel bowl.

### Sub-Chronic Exposure to Ketamine

In both experiments, once the performance was stable, the rats were pseudo-randomized into performance-matched groups of eight (first cohort) or six (second cohort), and treated with vehicle or ketamine (10 or 30 mg/kg i.p.) 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 before 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 randomized order, acute doses of nicotine (0.025, 0.05, and 0.1 mg/kg s.c.) were evaluated on the OST given 10 min before placement on the platform. The following day, the rats were rested to allow for 'washout' followed by retraining on the OST before the next test. Similarly, in the second experiment, tests were conducted with the atypical antipsychotic clozapine (1, 3, and 10 mg/kg i.p.) and the novel mGlu2/3 receptor agonist LY404039 (0.3, 1, 3, and 10 mg/kg s.c.) administered 45 and 30 min before testing, respectively. As before, doses of the antipsychotic compounds were tested acutely in a randomized sequence that occurred every third day, which allowed 'washout' of the test drug followed by retraining on the OST before the next test. In all the above tests, the experimenter was blinded to the dose of test compound being administered and the previous history of sub-chronic exposure.

### Statistical Analysis

The odor span from each group of rats was analyzed by 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 calculated 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, UK) was dissolved in water for injection and pH was adjusted to $\sim$6 with 0.1 M NaOH. In the preliminary experiment, 10 or 30 mg/kg doses were administered once daily for 5 consecutive days. In the second experiment, 10 mg/kg was used for all animals, as the first experiment revealed that this induced sufficient deficits in task performance. Nicotine hydrogen tartrate (Sigma Aldrich) was dissolved in water for injection and the pH was adjusted to 7 with NaOH solution. Nicotine in doses of 0.025, 0.05, or 0.1 mg/kg was administered s.c. 10 min before testing on the OST. Clozapine (Eli Lilly, synthesized by Janssen Pharmaceuticals, Belgium) was dissolved in 0.1 M HCl, and then adjusted to a physiological pH of 7. Clozapine in doses of 1, 3, and 10 mg/kg were given i.p. 45 min before testing on the OST. LY404039 (Eli Lilly, synthesized by Janssen Pharmaceuticals, Belgium) was dissolved in NaOH and pH was balanced to around 6. LY404039 in doses of 0.3, 1.0, 3.0, and 10.0 mg/kg were given 30 min before testing. Doses of both clozapine and LY404039 were based according to the 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 were expressed as those of the base.

## RESULTS

### Acquisition of the OST

Animals were trained according to a NMTS rule for approximately four training sessions. They were then moved onto a half OST until they achieved a span of 5 (Figure 1). From here, they were trained in the full OST until asymptotic performance was demonstrated: a span of 5 on at least two consecutive sessions, which does not fluctuate more than 3 spans over four consecutive sessions (Figure 1).

### Ketamine Induces Deficits in OST Performance that Persist Over 21 Days

Before sub-chronic treatment, the mean odor span for the whole group of rats was $6.9 \pm 0.3$ (mean ± SEM of the five runs before treatment). The mean odor spans for control, ketamine 10 mg/kg, and ketamine 30 mg/kg were $6.9 \pm 0.5$, $7.0 \pm 0.4$, and $6.9 \pm 0.6$, 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 a substantial impairment of odorspan 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 the span by 4 in both vehicle- and ketamine-treated groups.

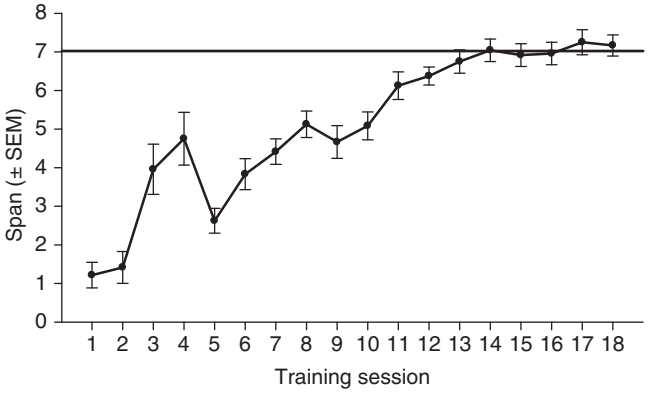


**Figure 1** Acquisition of the OST ($n = 24$) over 18 training sessions, including 4 specific sessions each on the NMTS and $\frac{1}{2}$ OST stages and 10 sessions on the full task. The dashed line depicts the mean span attained by the group towards the final stages of training. Each data point depicts the mean ± SEM.

### Neither Clozapine Nor LY404039 Reverses Ketamine-Induced Deficits in the OST

Five test sessions following ketamine exposure confirmed impairments in odor span detection, being reduced to $3.3 \pm 0.8$, compared with the control group that exhibited a baseline mean span of $8.0 \pm 1.2$. Treatment with acute

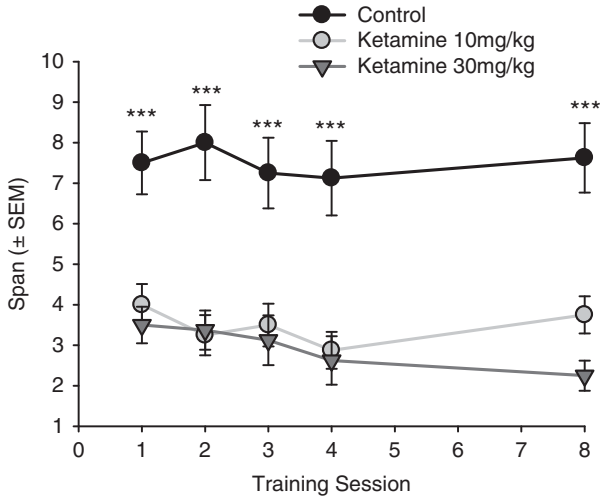


**Figure 2** Treatment with sub-chronic ketamine at both 10 and 30 mg/kg induced significant deficits in OST task performance stable over 8 days ($n = 8$). *** denotes statistical significance from the vehicle-treated group ($p < 0.001$). Each data point depicts the mean ± SEM.

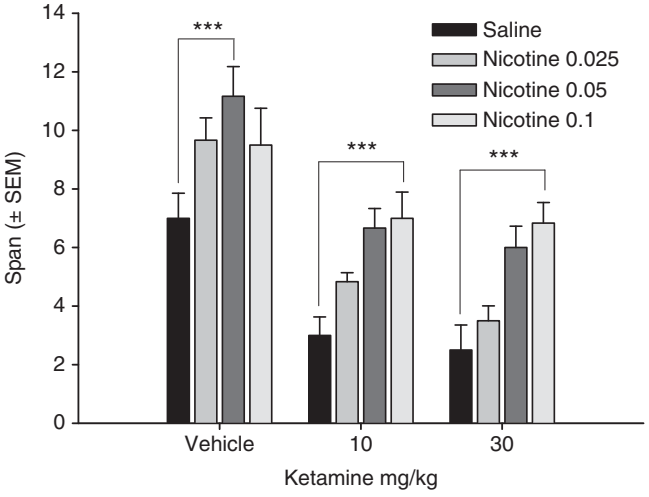


**Figure 3** The effect of two doses of ketamine (10 and 30 mg/kg) on span length in the OST. Both groups of animals ($n = 8$) treated with ketamine exhibited significantly decreased span length when compared with control animals. This deficit in span length was reversed by ketamine administration in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

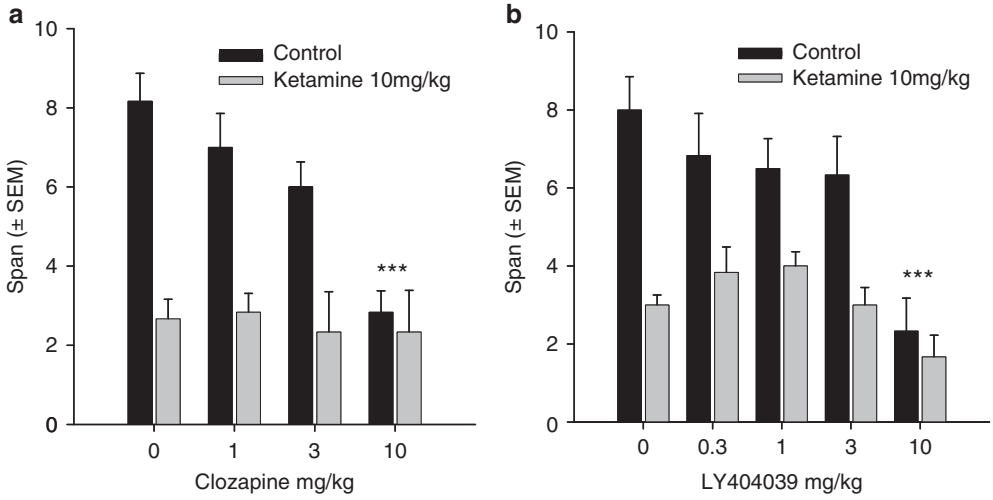


**Figure 4** Neither clozapine (a) nor LY404039 (b) significantly reverses deficits in OST task performance. Both produce significant deficits in performance of control animals in a dose-dependent manner ($n = 6$/group). Each data point depicts the mean ± SEM. *** denotes statistical significance between groups ($p < 0.001$). *** denotes statistical significance ($p < 0.01$).

doses of clozapine (1, 3, and 10 mg/kg) or vehicle impaired the performance of control animals, but ketamine-treated animals were unaffected (Figure 4). A two-way repeated-measures ANOVA revealed that clozapine impaired OST performance ($F(3,30) = 6.2$, $p < 0.01$), which interacted with ketamine exposure ($F(3,30) = 4.6$, $p < 0.05$). When analyzed within the group using a one-way ANOVA for repeated measures, clozapine had no significant effect on OST performance in animals that were 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 the span to $3.0 \pm 0.7$, compared with the control group that exhibited baseline span levels of $7.7 \pm 0.6$. Treatment with acute doses of LY404039 (0.3, 1, 3, or 10 mg/kg) or vehicle did not improve the performance; in fact the highest dose impaired the performance (Figure 4). A two-way repeated-measures ANOVA revealed LY404039 to have 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 the performance of control animals ($F(4,29) = 5.60$, $p < 0.01$), and a small but significant effect of LY404039 was seen in the ketamine-treated animals ($F(4,29) = 38$, $p < 0.05$). However, *post-hoc* analysis did not reveal significant effects of LY404039 when compared with 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 that emerged from this study was the relative efficacy of nicotine in improving the odor span achieved in both control and ketamine-treated rats, improvements that were dose-related, and a maximal effect achieved at the 0.05-mg/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 the performance in control animals.

The persistence of cognitive deficits produced by sub-chronic exposure to sub-anesthetic doses of ketamine is not well documented. However, Becker *et al* (2003) injected rats with 30 mg/kg ketamine i.p. daily for 5 days; the same regimen as employed in this study. They reported that 4 weeks after the last injection of ketamine, glutamate binding in the PFC was decreased by 25% but remained unchanged in both the hippocampus and striatum (Becker *et al*, 2003). This might 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, 2009; Ghoneim *et al*, 1985; Krystal *et al*, 1994, 2005; Malhotra *et al*, 1996, 1997; Newcomer *et al*, 1999; Verma and Moghaddam, 1996). Verma and Moghaddam (1996) treated rats with ketamine 10, 20, and 30 mg/kg i.p. before a spatial/non spatial short-term memory task, which resulted in significant deficits in performance (Verma and Moghaddam, 1996). More recently, Buccafasco and Terry (2009) gave sub-anesthetic doses of ketamine to monkeys trained in a computer-assisted delayed response task. Ketamine reduced accuracy in performance but did not affect the processing speed. This effect was also fully reversed by the $\alpha 7$ nAChR partial agonist DMXB-A ([3-[(3E)-3-[(2,4-dimethoxy-phenyl) methylidene]-5,6-dihydro-4H-pyridin-2-yl]pyridine]) (Buccafusco and Terry, 2009). These basic findings translate well; Malhotra *et al* (1996) administered sub-anesthetic doses of ketamine to 15 healthy volunteers, and observed an 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 the cognitive performance in schizophrenic patients, as well as exacerbating symptoms of psychosis (Malhotra *et al*, 1997). These clinical findings agree with the present observed results

suggesting that ketamine exposure in the OST appears to be a valuable and clinically relevant model for modelling cognitive deficits in rodents. However, in a double-blind, crossover 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 with placebo (Freedman *et al*, 2008).

Nicotine can enhance cognition in smokers, non-smokers, laboratory animals previously exposed to nicotine, and in animals that are nicotine-naive (for a review, see Levin *et al*, 2006). Nicotine has also been shown to enhance cognitive function in schizophrenic patients (Barr *et al*, 2008; Jacobsen *et al*, 2004; Smith *et al*, 2002, 2006). Similarly, George *et al* (2002) demonstrated that smoking abstinence significantly reduced visuospatial working memory in schizophrenic smokers but not in 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 support 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 in reference memory. In acute tests, nicotine administration was also shown to significantly attenuate impairments produced by mecamylamine (Levin *et al*, 1996, 1997). 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 the working memory span capacity.

From a neurobiological perspective, Dudchenko *et al* (2000a, b) discovered that the 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 PFC (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* (2001) have shown that the dorsolateral PFC 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 a significant activation of the PFC, 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 the 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 a significant contribution of cholinergic fibers in the PFC to the span capacity of working memory function and attention. Infusion of $^{192}$IgG-saporin into the basal forebrain resulted in an 80% reduction in density of fronto-dorsal acetlycholinesterase-positive fibers (Turchi and Sarter, 2000), which correlated significantly with a decline in olfactory span performance. These studies highlight the importance of both PFC involvement and the necessity of cholinergic processes in olfactory working memory span capacity. An 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, thereby increasing the excitatory synaptic strength (Laroche *et al*, 1990, 2000). This finding is supported by Young *et al* (2007b), who found that $\alpha$7 nAChR knockout 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 with those of clozapine, which is 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 the FG1742-induced delayed memory deficits in monkeys (Murphy *et al*, 1997), attentional deficits produced by blockade of NMDA in the PFC of rats (Baviera *et al*, 2008), and phencyclidine-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 focusing on spatial working memory generally have been less conclusive, highlighting the lack of translation to the clinical situation. Levin and Christopher (2006) found that 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 and Rezvani, 2006; 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 might 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 might have become apparent. Additionally, as with most antipsychotics, Patil *et al* (2007) report a significant clinical effect of LY404039 at week 4 of the

treatment (Patil *et al*, 2007). This suggests that acute doses might 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 the performance in non-compromised subjects. Neither clozapine nor LY404039 restored the 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 might be useful as an adjunct therapy to the currently prescribed anti-psychotics.

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## DISCLOSURE

The authors declare no conflict of interest.

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