Targeting the Alpha7 Nicotinic Receptor Subtype to Restore Working Memory Deficits in Rats Following Sub-chronic Ketamine Exposure

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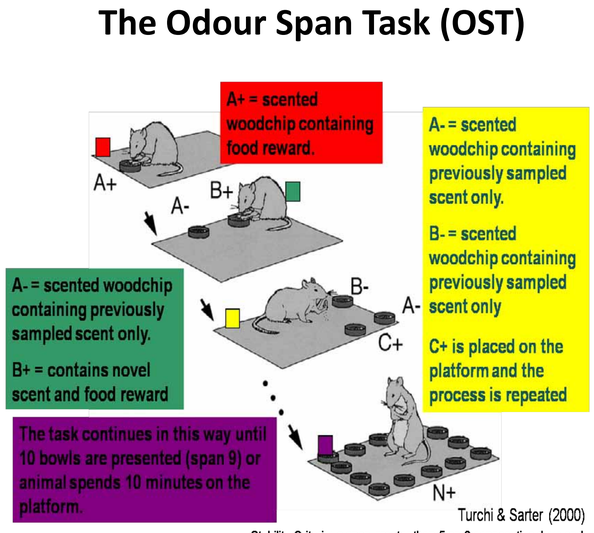
Introduction

- **Schizophrenia** is a debilitating disorder affecting ~1% of the population.
- **Cognitive deficits** are one of the core disabling features of the disease, yet remain inadequately treated by any pharmacological or behavioural therapy currently available.
- **Clozapine** is currently the most clinically-effective antipsychotic for treating cognitive deficits.
- **Nicotine** can improve cognitive performance in animal models and may be used as a form of self medication in schizophrenic patients since around 80% of this group are regular smokers
- **LY404039** is a novel mGluR2/3 agonist, shown to be as effective as olanzapine in phase II clinical trials (3).
- **PHA-543613** and **choline** are both agonists for the α7 nAChR receptor subtype which may mediate improvements in cognitive tasks.
- **5IA** and **metanicotine** are agonists for the α4β2 and α4β2, α6 nAChR receptor subtypes respectively and are also involved in cognition.
- **PNU120596** and **Compound 'X'** are positive allosteric modulators (PAM) for the α7 nAChR
- **Methyllycaconitine** (MLA) is an antagonist for the α7 nAChR

Aims

- To assess the effects of sub-chronic ketamine exposure on non-spatial working memory using the rodent Odour Span Task (OST) (1).
- To validate the significance of ketamine-induced deficits in the OST as a model of impaired cognition using the clinically-effective clozapine and LY404039, a novel generation antipsychotic.
- To assess the ability of nicotine, nicotinic agonists and nicotinic positive allosteric modulators (PAM's) to restore OST performance in rats treated with sub-chronic ketamine exposure.
- To assess the contribution on the α7 receptor to this restoration by antagonising the α 7 nAChr with methyllcaconitine.

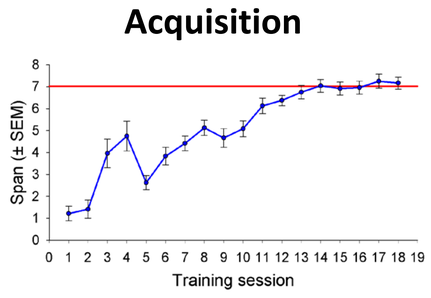
The Odour Span Task (OST)



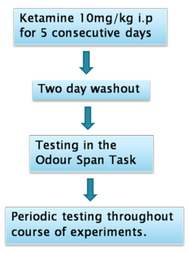
Turchi & Sarter (2000)

Stability Criteria: a span greater than 5 on 2 consecutive days and fluctuations no greater than 3 over 4 consecutive days

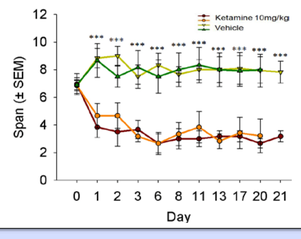
Acquisition



Dosing Regimen



Stability of Ketamine Deficits

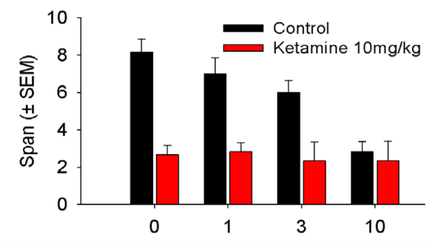


- Ketamine produced significant deficits, persistent over 21 days.
- Once performance was stable, animals were treated with nicotine, PHA-543613, clozapine or LY404039 to assess their ability to restore ketamine-induced deficits in OST perform to pre-ketamine baseline.

Anti-Psychotics

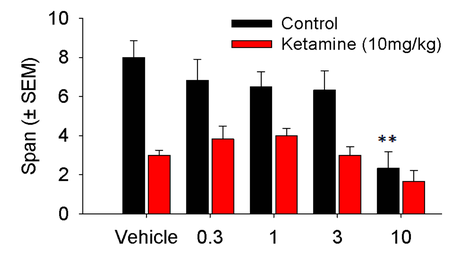
Clozapine (mg/kg)

- Clozapine did not reverse ketamine-induced deficits
- Clozapine caused a dose-dependent deficit in control animals

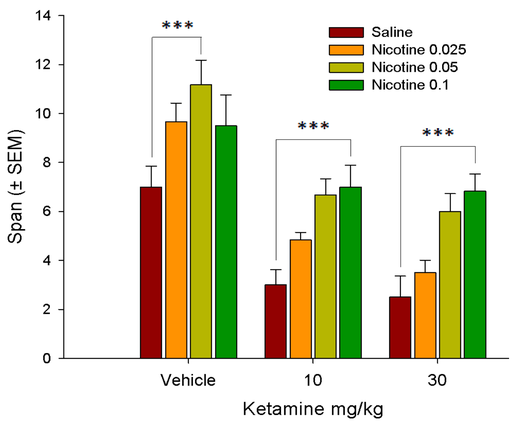


LY404039 (mg/kg)

- LY404039 did not reverse ketamine induced deficits
- LY404039 produced a robust deficit in control animals at the highest dose tested.



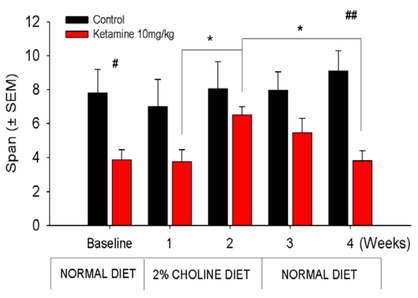
Nicotine



- Nicotine reversed ketamine-induced deficits in a dose-dependent manner.
- Nicotine also improved span length in control animals.

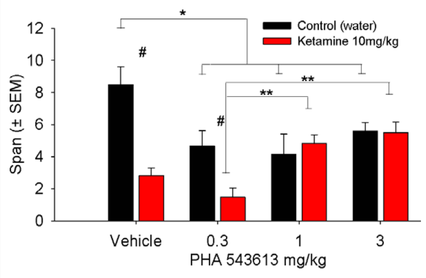
α7 Agonists

2% Dietary Choline



- Exposure to 2% dietary choline for at least 2 weeks was effective in restoring ketamine-induced deficits which were reversed upon choline withdrawal.
- Control animals were unaffected by the choline-supplemented diet.

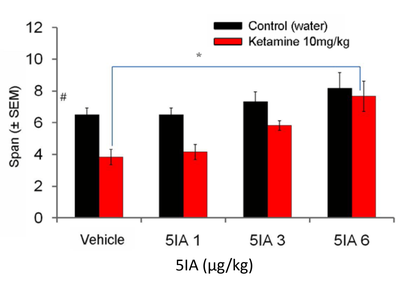
PHA-543612



- PHA-543613 dose-dependently improved ketamine-induced deficits.
- All doses of PHA -543613 significantly impaired performance of control animals.

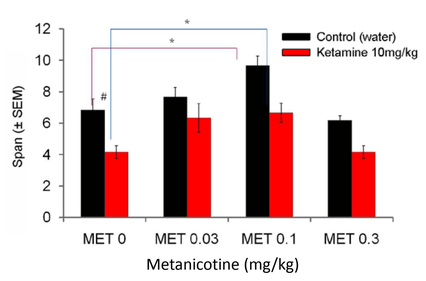
α4β2 Agonists

5-Iodo-A-85380 (5IA)



- 5IA dose-dependently improved ketamine-induced deficits.
- Control animals did not show any significant improvement in OST performance at any of the doses tested.

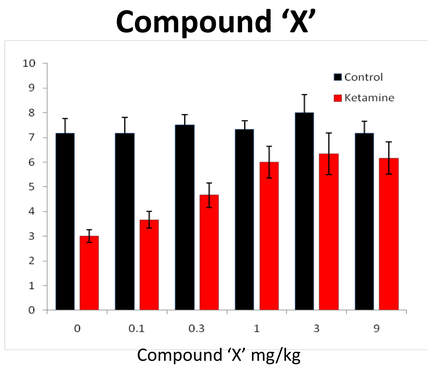
RJR-2403 (Metanicotine)



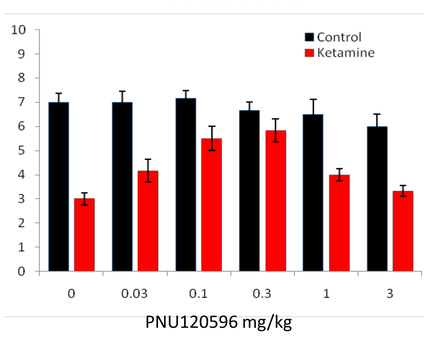
- Metanicotine dose-dependently improved ketamine-induced deficits.
- 0.1mg/kg metanicotine significantly also enhanced performance in control animals.
- The highest dose impaired control animal performance

α7 PAM

Compound 'X'



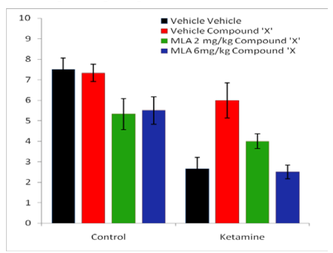
PNU120596



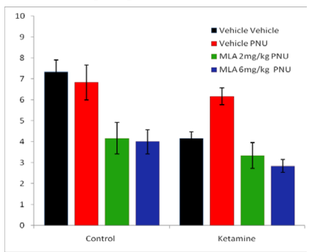
- Both the positive allosteric modulators, compound 'X' and PNU120596 were able to restore ketamine-induced deficits in a dose- dependent manner.
- Neither affected span length in control animals.

α7 Antagonist

Compound 'X' + Meythyllycaconitine



PNU120596 + Methyllycaconitine



- Pretreatment with MLA attenuated the ability of both compound 'X' and PNU120596 to restore ketamine-induced deficits in a dose-dependent manner. MLA also attenuated OST performance in controls subjects.

Gamma-frequency Network Oscillations

Introduction

- The neural code organized by **gamma oscillations** is essential for brain structures to communicate and therefore aberrant oscillations could affect normal cognitive functioning.
- Gamma frequency network oscillations are reduced in schizophrenia and the degree to which this reduction occurs, correlates to negative symptoms of the disease.(5)
- This means NMDA antagonists by reducing gamma oscillations could induce memory deficits similar to those in schizophrenia (6)
- This is supported by data demonstrating that acute application of ketamine can cause a significant reduction in gamma oscillations in the anterior cingulate, prelimbic and infralimbic regions of rat prefrontal cortex (7).
- Whether targeting nAChr's can enhance gamma-frequency network oscillations in these regions is still unknown.

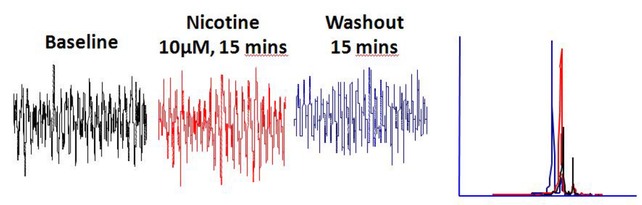
Aims

- To assess the effect of nicotine and nicotinic agonists on gamma oscillations in the prelimbic region of the PFC.
- To see if any positive effect can be blocked by nicotinic antagonist mecamylamine.

Methods

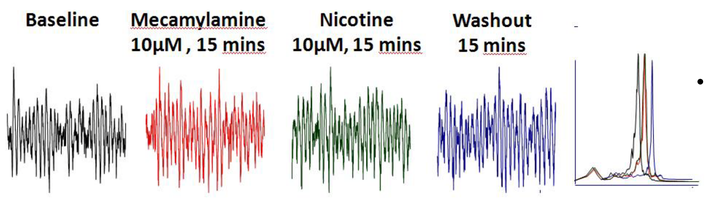
- **Male hooded Lister rats** weighing 200-300g were given an intramuscular injection of ketamine (100mg/kg) and xylazine (10mg/kg) following anesthesia with inhaled isoflurane.
- Upon termination of respiration, animals were **intracardially perfused** with 50ml of modified artificial cerebral spinal fluid (ACSF).
- The brain was then removed and submerged in ice-cold (4–5°C) ACSF and **450mm thick coronal PFC slices** were cut using a Leica vibroslice.
- Slices were held in a holding chamber and maintained at room temperature in **oxygenated (95% O_2, 5% CO_2) ACSF**.
- After one hour, slices were placed into the recording chamber and **maintained at 31-32°C** at the interface between a continuous stream (1.2 ml/min) of oxygenated, humidified ACSF.
- After a further 30 minutes in the recording chamber, gamma frequency oscillations were generated by simultaneous bath application of the cholinergic agonist **carbachol (10µM)** and the ionotropic glutamatergic agonist **kainate (200nM).**
- Gamma frequency activity was recorded extracellularly in the **prelimbic (prL)** regions of the PFC.
- Extracellular recording electrodes were pulled from borosilicate glass (Harvard Apparatus Ltd., Kent, UK) and filled with ACSF with resistance in the range of 2–5MΩ.
- **Power, peak frequency and amplitude** values of persistent gamma oscillations were taken from power spectra generated from a 60 second recording of PFC activity using Fourier analysis in the Axograph software package.
- Power was defined as the area under the peak in the power spectra between **15 and 48 Hz** for gamma frequency oscillations.

Nicotine



- Nicotine (10 µM) enhances area power of gamma frequency network oscillations by 65%.

Mecamylamine



- The effect of nicotine is blocked by broadspectrum nicotinic antagonist mecamylamine (10µM).

Conclusions

- **Ketamine induces significant long lasting and stable deficits in OST performance.**
- **This deficit was fully reversed with acute nicotine administration and also improved performance in the control subjects.**
- **The α7 and α4β2 nicotinic agonists improved performance in ketamine-treated animals but had differing effect in controls; PHA543613 impaired performance in control animals, both dietary choline and 5IA had no effect on control animals where as metanicotine significantly improved control animal performance.**
- **Both PAM's significantly improved OST performance; this effect was blocked by α7 antagonist MLA which also impaired performance in control subjects.**
- **These data suggest that the both the α7 and α4β2 nAChR subtype may have a role in mediating improvements in OST task performance.**
- **Bath application of nicotine increases gamma-frequency network oscillations in the pre-limbic area of the PFC in normal animals.**
- **This effect is blocked by broad spectrum nAChR antagonist mecamylamine.**

These data support the use of ketamine in the OST to model cognitive deficits in schizophrenia and indicates nicotine and nicotinic receptors as viable targets for the treatment of neuropsychiatric disorders.

References

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(6) Cunningham, M et al. (2006). *The Journal of Neuroscience*, 26 , pp.2767-2776

(7) Gilloughley and O'Donovan et al, unpublished data.