Ketamine-induced Behavioural and Neurochemical Effects: Simultaneous Measurements of Locomotor Activity, Extracellular Cortical Glutamate and Drug Levels in C57 Mice

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Introduction

Current treatments for schizophrenia do not possess complete therapeutic efficacy, nor are they devoid of unwanted side-effects. The need to create better and safer treatments is met by a need to develop animal models mimicking the core pathophysiological features of the disease. Psychostimulant-induced models of schizophrenia are widely accepted and utilised. Hyperlocomotor behaviour in rodents is a popular model as it resembles some components of the stereotyped tendencies seen in schizophrenic symptomatology. The glutamate hypothesis posits that the positive symptoms of schizophrenia stem from hypo-activity of glutamatergic NMDA receptors (Leite et al, 2008). NMDA receptor antagonists such as ketamine have also been shown to induce a behavioural syndrome in rodents that is characterised by not only locomotor hyperactivity but also head weaving, ataxia and stereotyped motor patterns. In addition to inducing hyperlocomotion, evidence suggests that blockade of NMDA receptors with ketamine elevates extracellular glutamate in the brain. One brain region thought to be subject to these neurochemical alterations is the medial prefrontal cortex (mPFC), a region which has been implicated in the pathophysiology of schizophrenia, both in mediating the positive and negative symptoms (Knable & Weinberger, 1997, Lorrain et al, 2003).

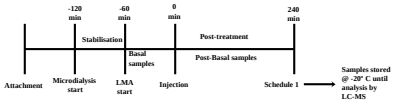
Whilst the behavioural and some of the neurochemical effects induced by NMDA antagonism are characterised to a certain extent, the present study attempts to refine this model by developing a method in freely moving mice in which both parameters can be recorded simultaneously in the same animal. The behavioural and neurochemical effects are compared to the pharmacokinetic profile of ketamine. The present study is the first, to our knowledge, which attempts to develop and validate an animal model whereby both the behavioural and neurochemical changes induced by NMDA antagonism and the drug pharmacokinetics are measured simultaneously in the same animal.

Methods

Animals and Apparatus: Male C57 mice, (Harlan, UK), weighing 20-35g at the start of the experiment were used. Procedures were conducted in accordance with the UK home office (Animals Scientific Procedures act, 1986) and all animals were subjected to a minimum of 1 week handling prior to experimentation. Food and water were provided *ad libitum* throughout. The experimental cages were made of clear perspex glass (25 X 19.5 X 34 cm), custom-built to fit both microdialysis and locomotor assay. Locomotor activity was recorded using LABORAS (Laboratory Animal Behaviour Observation, Registration and Analysis System, version 2.1.4, Metris B.V, Hoofddorp, Netherlands), an automated behaviour registration system. LABORAS recorded the behavioural profile of each mouse via a sensing platform on which its cage sits. The platforms transformed the mechanical vibrations caused by the mouse's movement into electrical signals which were amplified, filtered and processed. The computer programme classified the signals into behavioural categories based on the unique frequency/amplitude of the patterns recorded such as locomotion, immobility, distance travelled, velocity, climbing and grooming. The locomotor data generated using the LABORAS method was compared to automated visual tracking simultaneously (Ethovision 3.1).

Surgery: Under anaesthesia (3% isoflurane in 1% oxygen) mice were stereotaxically implanted with a guide cannula (Brain-Link, Groningen, Netherlands) to allow for the insertion of the microdialysis probe. Coordinates were: +2.0mm anterior-posterior from bregma; -0.7mm medio-lateral and -1.3mm dorso-ventral from dura at an angle of 10° from the midline (incision-bar at +0mm) (Franklin & Paxinos, 1997). A tethering peg (Instech Laboratories Inc., Plymouth Meeting, USA) was embedded in the ionomer to anchor the animals to the microdialysis system. Appropriate post-operative care was provided.

Experimental procedure: On the morning of the experiment, mice were attached to the microdialysis system. Microdialysis probes were inserted and flushed with artificial cerebrospinal fluid (aCSF: 147nM NaCl; 3mM KCl; 1.2mM CaCl2; and 1.2mM MgCl2) at a flow rate of 1.5µl per minute, 30 min interval. A 2-hour stabilisation period was given prior to collection of samples. Following the collection of 2 basal samples, 8 more fractions were collected after ketamine administration. Samples were analysed offline for glutamate and ketamine by LC-MS using a method developed inhouse. After the completion of experiments probe placement was validated. The microdialysis experiments were performed simultaneously with recording of locomotor activity.



Experimental protocol for combined LMA and microdialysis

Results

Figure 1. Effects of ketamine on extracellular glutamate levels in mPFC, profile superimposed with LMA

- LMA data (n=6) and Microdialysis data (vehicle: n=5-6) are expressed as mean ± SEM.
- Treatment occurred at 0 min.

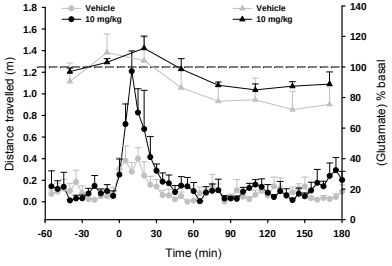


Figure 2. Ketamine levels mPFC dialysates

- Data (n=5-6) are expressed as mean ± SEM and corrected for delay (10 min) in sample recovery time to compensate the length of tubing between probe and collection vial. The in-vitro recovery of ketamine through the dialysis membrane is 7.37%. Ketamine administered at 0 min.
- A dose dependent increase in extracellular drug concentration was noted.

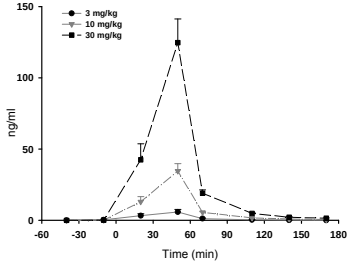


Figure 3. Effect of ketamine on grooming activity.

- Data (n=6) are expressed as mean ± SEM. Treatment occurred at 0 min.
- A significant time X treatment effect was seen ($F = 5.971$, $p<0.001$) for 30 mg/kg dose (two-way RM ANOVA -Holms Sidak's test).

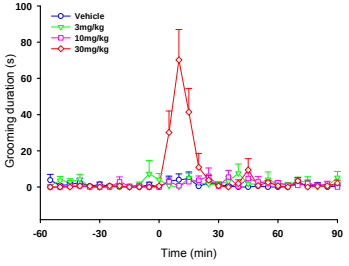
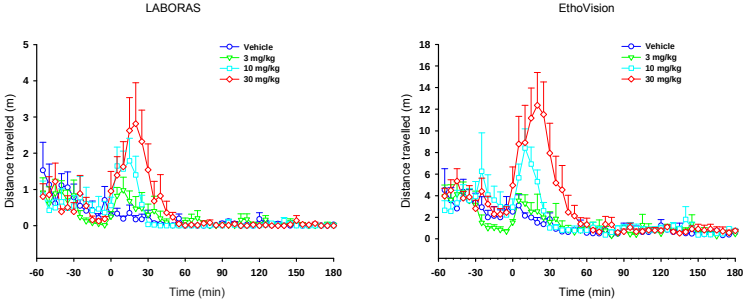
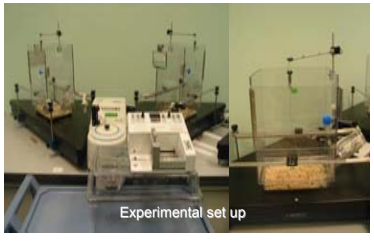
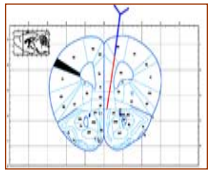


Figure 4. Effect of ketamine on LMA: a comparison of LABORAS and EthoVision systems.

- Data (n=6) are expressed as mean ± SEM. Animals were treated at 0 min.
- LABORAS: Dose dependent increase in LMA was observed. 10 and 30mg/kg differ significantly to control group (time X treatment, $F= 3.853$; $p<0.001$, two-way RM ANOVA).
- Although data for the two systems is quantitatively different, two-way RM ANOVA revealed similar time courses for each dose (Ethovision: $F = 2.69$; $p<0.001$, LABORAS: $F = 2.01$; $p < 0.001$).







Schematic representation and typical probe placement of the mouse mPFC

(adapted from Franklin & Paxinos, 1997)

Conclusions

- The present study has successfully developed a method to simultaneously measure neuro-transmitter levels and locomotor behaviour in mice.
- Acute ketamine administration induced hyperlocomotion in mice in a dose-dependent manner. A 30 mg/kg dose produced an immediate ataxic response (visual observation) and an increase in grooming activity.
- The presence of drug in the extracellular space in a concentration dependent manner confirms that the observed hyperactivity is indeed a drug-mediated response.
- The decrease in locomotor response despite increasing drug levels in the dialysates confirms that only a limited concentration of ketamine is required in the extracellular space to induce a maximal locomotor effect and any further blockade of NMDA receptors may lead to receptor and locomotor desensitisation and produce other effects. Therefore selecting the dose of ketamine to produce an optimal locomotor effect without causing any unwanted side-effects remains crucial in designing this animal model.
- A dose between 3 and 10 mg/kg range is optimal for inducing hyperactivity in mice without inducing pronounced side-effects.
- Despite discrepancies in the literature, NMDA antagonism increased glutamate levels in rat cortex and this has been correlated with activated locomotion (Moghaddam et al, 1997, Imre et al, 2006). However in the current study, ketamine did not increase extracellular glutamate levels in mPFC. In C57 mice measurement of cortical glutamate may not be a neurochemical marker for ketamine induced hyperactivity.
- The locomotor data of ketamine obtained from LABORAS and EthoVision are qualitatively similar. The quantitative difference can be attributed to tracking techniques. EthoVision is a more sensitive method of recording the locomotor behaviour of mice upon ketamine administration.

References

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