Measurement of the functional actions of ketamine in the rat brain using locomotor activity, microdialysis and phMRI imaging techniques

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Introduction The NMDA antagonist ketamine hydrochloride induces a psychotic-like state in man thought to be analogous to the positive, negative and disorganisation symptoms of schizophrenia (Andreasen *et al.*, 1995; Krystal *et al.*, 1994). NMDA receptor antagonists reliably increase locomotion and stereotypy in rodents at subanaesthetic doses (Moghaddam *et al.*, 1998), suggesting that ketamine challenge in rats may form a model of some of the symptoms of schizophrenia. To date, little research has been conducted into the neuroanatomical targets of subanaesthetic ketamine in rats (Burdett *et al.*, 1995; Duncan *et al.*, 1999; Duncan *et al.*, 1998). Thus, the current fMRI study aimed to measure spatial and temporal alterations in brain activity following an acute dose of ketamine (25 mg/kg s.c.) via localised changes in Blood Oxygen Level Dependent (BOLD) MR signal contrast. Microdialysis experiments using a separate group of rats examined temporal changes in dopamine concentrations in the nucleus accumbens (NAcc) following the same dose of ketamine was also performed.

Methods: Male Sprague Dawley rats were used and all procedures carried out in accordance with the UK Home Office Guidelines. In our initial studies we evaluated the effects of ketamine (2.5, 10, 25 and 50mg/kg) on locomotor activity, measured by light beam interruptions, thus creating an appropriate pharmacodynamic input funtion for subsequent correlation with MRI (Roberts et al., in press). Based on these data 25 mg/kg s.c. was selected for MRI and microdialysis studies.

MRI: Anaesthesia was induced with 3-4% isoflurane in 0.9 l/min medical air and 0.1l/min medical oxygen, and then maintained at 1.5-2% isoflurane. Animals were placed in a stereotaxic frame, and MRI measurements were performed on a 4.7 Tesla Varian Scanner. Using a multi echo gradient echo sequence (TE = 5, 10, 15 msec; TR = 940 msec, RF flip angle = 30^{o}) one whole brain volume was acquired every 60 seconds, taking 40 slices, with an isotropic voxel resolution of 0.5 x0.5 x0.5 mm. The acquisition matrix was 64 x 64 x 40 with a FOV of 4cm^2. The brain was imaged 180 times in the course of the experiment, with injection of the experimental drug or placebo at the 30th scan.

Post-processing of MRI data: All data was extensively post processed before analysis was carried out. Using the mean gradient echo data, images were coarse masked, realigned, fine masked, normalised and smoothed before whole brain group statistical parametric maps were constructed using SPM99, thresholded at $p<0.05$ and corrected for multiple comparisons. Covariates analysis identified changes in voxel intensity correlating with the observed changes in locomotor behaviour. Subsequent region of interest (ROI) analysis using MarsBar examined the time course of localised signal intensity changes.

Microdialysis experiments: Rats were anaesthesized in an induction chamber with 4% isoflurane delivered in 2 l/min medical oxygen. The animal was placed in a stereotaxic frame, while microdialysis probes were infused at 2 µl/min with artifical cerebrospinal fluid (aCSF). The probe was implanted into the NAcc shell using the following coordinates: +1.5, -0.8, -7.0 relative to bregma. Each rat was attached to a pump via tubing, left to recover for 90 minutes after implantation, with physiology still being monitored and isoflurane levels dropped to 1.5 -2 %. Samples were then taken for 4 hours, with injection of study drug or placebo at 2 hours.

Results: 25 mg/kg ketamine produced a significant increase in locomotion and stereotypy (area under the curve, T=-6.687, df(9), $p<0.001$) compared to vehicle (see Figure 2). MRI analyses, revealed significant increases in BOLD signal following ketamine challenge, most notably in subcortical structures (see Figure 1). ROI analyses confirmed increases in signal intensity of approximately 1.5% in the ketamine group compared to the vehicle group. Moreover, corresponding increases were seen in dopamine concentrations in the NAcc, an area known to be rich in dopaminergic fibres that are consistently activated by psychotomimetics (Kuczenski *et al.*, 1991) (see Figure 2).

Conclusions: 1. These results confirm previous observations that an acute dose of ketamine produces increases in locomotor behaviour and stereotypy in rats.

2. The behavioural effects of ketamine may be mediated by its actions in discrete brain regions including subcortical regions that were observed using fMRI.

3. MRI technology appears sensitive to the neural effects of ketamine, with changes in BOLD contrast correlating to the pharmcodynamic and initial changes in the neurochemical profile of the drug

4. In the future we plan to investigate the potential interaction of antipsychotics on the ketamine-induced effects. The current data suggest that MRI may form a powerful tool for future research into schizophrenia and development of new antipsychotic medications.

Figure 1: T distribution map of the main effect of ketamine($p<0.05$, $T>4.39$),n=8

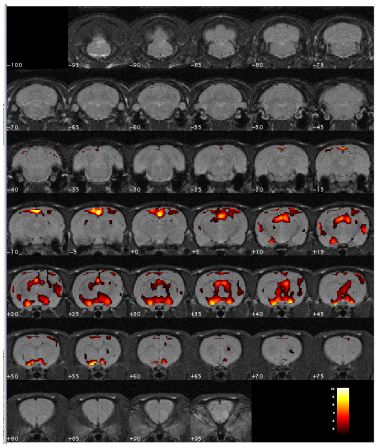
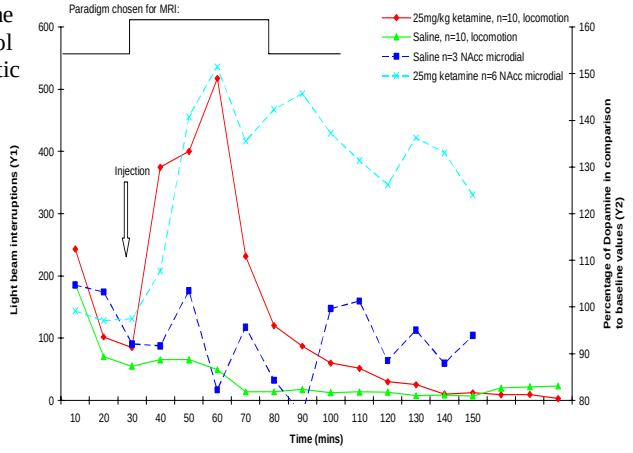


Figure 2:Timecourse of locomotion after 25mg/kg ketamine administration to freely moving SD rats (Y1) and Dopamine levels of the Nucleus Accumbens under continuous anaesthesia (2 % isoflurane) after 25mg/kg ketamine administration to SD rats (Y2)



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