CHARACTERISATION OF RAT MAM MODEL OF SCHIZOPHRENIA BY IN VIVO STRUCTURAL AND PHARMACOLOGICAL MRI

Diana Cash[1], Winfred Chege[1], Michel SB Mesquita[1], Nadia N Malik[2], Mark D Tricklebank[2], Michael J O'Neill[2], and Steven CR Williams[1]

[1]Neuroimaging, King's College London, London, United Kingdom, [2]Eli Lilly and Co., United Kingdom

Introduction Offspring of rats treated *in utero* with a DNA-alkylating agent MAM exhibit several cognitive and brain abnormalities typical of schizophrenia. The brains are overall smaller and ventricles enlarged, and there is an imbalance in the function of glutamatergic and dopaminergic systems, exemplified by hypersensitive behavioural responses to an NMDA antagonist phencyclidine and a dopamine agonist amphetamine[1]. The aim of this study was to test if the functional and structural brain abnormalities persist in the aged MAM rats.

Methods MAM or Sham (vehicle) treatment was administered to pregnant Sprague Dawley rat dams at E17 as previously described[2], resulting in a cohort of 16 MAM and 15 Sham male rats. Serial structural MRI (sMRI: 7T Varian scanner, FSE T2W images, TEeff 60ms, TR 4000ms, FOV 40x40mm, 256x128, 44 slices, 20') was conducted at 1, 3, 6 and 12 months of age. Measurements were made manually using JIM software (Xinapse Systems Ltd.) to delineate the brain and the dorsal hippocami (from -1.8 to -4.3 from Bregma), as well as to measure the cortical thickness of the prefrontal, parietal, visual and entorhinal cortices. Following final sMRI at 12 months, pharmacological MRI was undertaken (GE, multi TE 5,10,15ms [mean echo was analysed], TR 790ms, FOV 40x40mm, 44 slices, 0.6mm isotropic voxels, 72 volumes over time, 32" per volume) by imaging all rats continuously 15 minutes before and 45 minutes after NMDA antagonist ketamine (25mg/kg ip) injection. Data were pre-processed (realigned, normalised to template and Gaussian smoothed 2x in plane resolution) and statistically analysed (behavioural input function[3], GLM, 2nd order) using SPM8 software (Wellcome Trust Centre for Neuroimaging). Prior to the final MRI, at 12 months of age, the rats were also tested for locomotor activity (LMA) in response to ketamine (25mg/kg ip) by video recording their activity for 90' in 1300x1300x600mm activity chambers and computerised analysis (Ethovision) of the recordings, resulting in measure of distance travelled per minute. To avoid habituation, those rats that received ketamine in the LMA experiment received saline in MR scanner, and vice versa.

Results Body weights of MAM rats were significantly lower than Shams from 3 months of age, whereas MAMs' brains were significantly smaller at *all* time-points (1, 3, 6 and 12 months) by 11 – 14 % ($p<0.001$ throughout). Dorsal hippocampi were also smaller at all timepoints ($p<0.01$ at 1 and 3 mt, $p<0.001$ at 6 and 12 mt). During the early phase (1mt) there were significant differences in the thickness of the prefrontal, parietal and enthorhinal cortex, whereas at 12 months prefrontal and parietal cortex remained smaller, but entorhinal cortex normalised (Fig 1). Behavioural testing revealed an increased locomotor activity in response to ketamine in MAM rats ($P<0.01$ MAM saline vs. MAM ketamine), in comparison to shams in which any increase in LMA was probably masked by ketamine-induced stereotypies (Fig2). phMRI revealed widespread ketamine-induced BOLD changes in the sham rats, encompassing cingulate, prefrontal & retrosplenial cortex, striatum and thalamus, as we previously described[2]. SPM difference maps, particularly when displayed at the lower $p<0.05$ threshold (not shown here) revealed an attenuated BOLD response in the striatum and an increase in the hipocampus and retrosplenial cortex (Fig 3) in MAM vs. Sham rats response to ketamine.

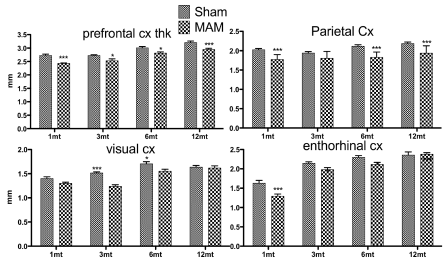

Fig 1 Cortical thickness measures in MAM and Sham rats. 2 way repeated measures ANOVA with Bonferroni post-tests, P<0.05*,0.01**, 0.001***.

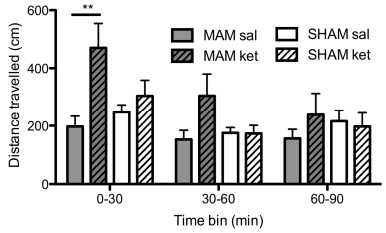

Fig 2 LMA activity: distance travelled (cm) is significantly increased in MAM rats during the first 30 minutes after ketamine. Similar, but not significant trend between 30 and 60 minutes. 2 way repeated measures ANOVA with Bonferroni post-tests, P<0.05*,0.01**, 0.001***.

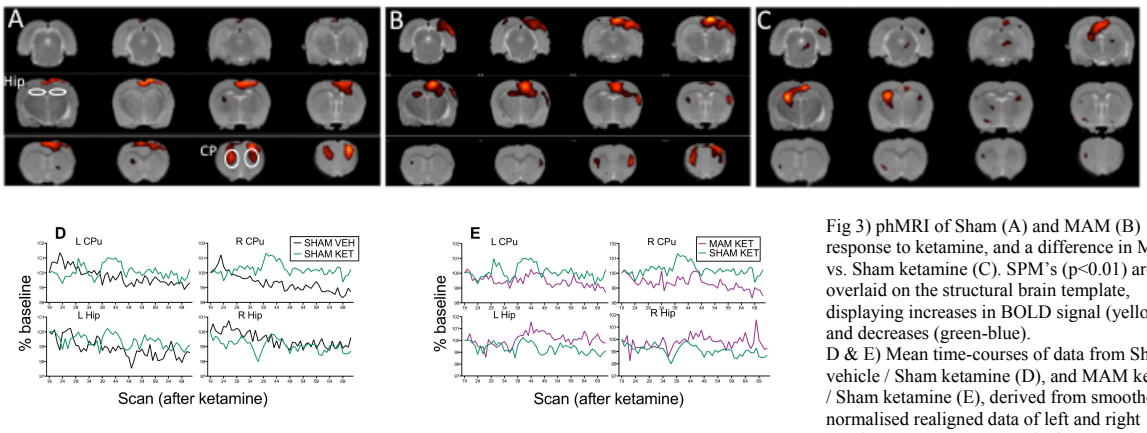

Fig 3) phMRI of Sham (A) and MAM (B) response to ketamine, and a difference in MAM vs. Sham ketamine (C). SPM's ($p<0.01$) are overlaid on the structural brain template, displaying increases in BOLD signal (yellow-red) and decreases (green-blue).
D & E) Mean time-courses of data from Sham vehicle / Sham ketamine (D), and MAM ketamine / Sham ketamine (E), derived from smoothed normalised realigned data of left and right Caudate Putamen (CPu) and Hippocampi (Hip) ROIs (drawn in A).

Conclusions Data reveal consistent and long-term structural changes in the brains of MAM rats, including shrinkage of the cortex as well as hippocampi, consistent with earlier reported results for younger rats[1]. The results also confirm that even in aged MAM rats, there is an enhanced behavioural response to ketamine. PhMRI results show a pattern of changes which likely reflect the underlying pattern of imbalance in glutamatergic and dopaminergic transmitter circuits. Increased ketamine-induced activation in the hippocampal areas may be indicative of the reduced glutamatergic activity in this model whereas a reduced caudate putamen activity could be linked to a dopaminergic increase, which has also been shown to attenuate BOLD response in this area. Structural and phMRI of MAM model could be used to characterise the efficacy of putative antipsychotic therapies.

References [1]Moore (2006) Biol. Psych. 60(3) 253, [2]Hradetzky et al, Neuropharmacol (2011) DOI. 2011 Sep 28. doi: 10.1038/npp.2011.219, [3]Littlewood et al. (2006) Neuroimage 32(4)1733, [4]Cash et al. (2003) J. Cereb. Blood Flow Metab. 23, S10.