Utility of MR-Spectroscopy in early drug discovery: characterization of dynamic temporal metabolic changes following psychoactive challenges in the rodent brain

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Target audience: *Psychopharmacologists, neuroimaging/drug discovery scientists*

INTRODUCTION: MR-Spectroscopy offers a unique opportunity to reveal the underlying mechanism of action of psycho-active compounds *in-vivo*. One of the principle factors in determining the biological substrates of psychosis and/or schizophrenia is derived from investigation of the N-methyl-D-aspartate (NMDA) receptors. A useful tool in this approach is to use the compounds that act as non-competitive NMDA antagonists, such as PCP, ketamine, MK-801, memantine etc. We used NMDA-mediated psychosis (memantine) model [1] and magnetic resonance spectroscopy, to understand the pathogenesis of disorders and the therapeutic efficacy of antipsychotic drugs, at a metabolite level. We have previously established the pharmacokinetic profile of acute & sub-chronic memantine (the NMDA antagonist) and its behavioural effects in locomotor activity (LMA) [1] and pre-pulse inhibition (PPI) [2], for hyperactivity and sensorimotor gating deficits, respectively in rats & have compared this with the effects of PCP, the classical NMDA-antagonist [1, 2]. The hippocampus has been implicated in the pathogenesis of schizophrenia and we have previously shown this region to be a target site for memantine actions, using combined phMRI, rs-fMRI & DKI [1]. Here, we extend these studies to accurately characterize the changes in neuronal metabolism *in-vivo*. We acquired single-voxel 1H-MRS at ultra high field (9.4T) to investigate the neurochemical changes in rat hippocampus, before and after acute & sub-chronic memantine challenge for an hour, documenting the dynamic temporal metabolite changes post challenge *in-vivo*, in particular changes in MR-observable metabolites involved in neurotransmission (glutamate, glutamine, glucose, glutamate, glutamine, GABA, glutathione, inositol, NAA & taurine). Furthermore, we examined the activity of a metabotropic glutamate receptor 2 (mGlu2) positive allosteric modulator (PAM), biphenylindanone A (BINA) on acute and sub-chronic memantine effects, to further exemplify the utility of the memantine model as well as the MRS approach.

EXPERIMENTAL PROTOCOL & DATA ANALYSIS: Male Wistar rats were used for this study (n=7 per MRS cohort). For acute studies: MRS dynamic temporal changes was acquired for 30 min before and 60 min after bolus administration of memantine (20 mg/kg, IP), metabotropic glutamate receptor 2 (mGlu2) positive allosteric modulator (PAM), biphenylindanone A (BINA, 32 mg/kg, IP) or saline. Furthermore, a BINA (32mg/kg, IP) pre-treated cohort, received an acute challenge dose of memantine (20mg/kg, IP), and were scanned for 60 mins. For sub-chronic studies: MRS was performed in three time points: pre treatment, post treatment and BINA pre-treatment. The treatment reflects the sub-chronic memantine administration for a period of 5 days (20 mg/kg, IP). In addition, a sub-chronically memantine treated cohort received BINA (32mg/kg, IP) pre-treatment 60 minutes prior to an acute challenge dose of memantine (20mg/kg, IP), & were scanned for 60 mins. Cohorts were scanned in a ultra high field 9.4T MRI (Bruker) scanner [TR/TE: 4000/13ms; number of averages 128; voxel size of 2.5×4×4 mm^3 in the hippocampus; acquisition time: 8 mins]. Water unsuppressed data was collected for scaling and eddy current compensation. Following global shimming, manual localized shimming on the ROI was conducted using FASTMAP prior to every data acquisition with line width of 9-12 Hz. Quanatative temporal profiles of several brain metabolites were generated using the LC Model. Datasets were normalized to phosphocreatine + creatine ratio. Amongst the various brain metabolites, we observed significant changes in several metabolites, although we only report on changes in glutamate and glutamine which reflected higher pronounced significance.

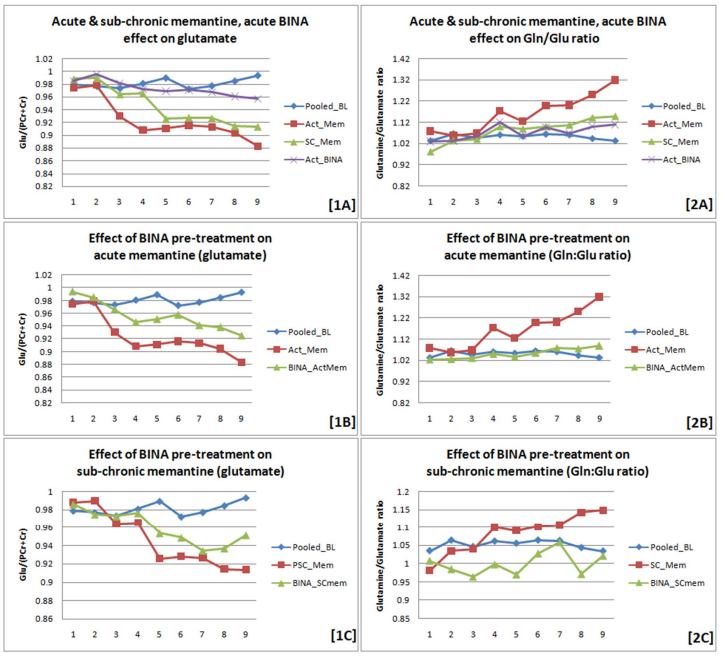

Fig 1A: Effect of acute and sub-chronic memantine on glutamate (Glu), along with effect of acute BINA on Glu;
Fig 1B, 1C: Effect of BINA pre-treatment followed by acute and sub-chronic memantine respectively on Glu;
Fig 2A: Effect of acute and sub-chronic memantine, acute BINA on glutamine/glutamate (Gln/Glu) ratio;
Fig 2B, 2C: Effect of BINA pre-treatment followed by acute and sub-chronic memantine resp. on Gln/Glu ratio.

RESULTS: Following acute memantine challenge, there was a significant reduction in glutamate concentration, whilst the glutamine/glutamate ratio was significantly increased [Fig 1A]; the effect of sub-chronic treatment were comparably pronounced [Fig 2A]. These neurochemical changes may support the observed behavioural effects reported earlier [1,2]. Furthermore, our results are similar to those observed in schizophrenic patients, and those following ketamine administration. Hence, this model demonstrates unique translational potential (against its scheduled counterpart, ketamine or banned PCP), and will be useful for screening novel drug candidates. This inference was exemplified by activity of a relatively novel mGlu2 PAM (BINA) pre-treatment, significantly inhibited memantine effects [Fig 1B, 1C; 2B, 2C].

DISCUSSION: During neurotransmission, glutamate is released by neurons in the extracellular space and taken up by astrocytes, where the conversion of glutamate to glutamine occurs via glutamine synthetase. Glutamine is subsequently transported back into neurons, whereby it is converted to glutamate. After a systemic i.p. injection of the NMDA receptor antagonist, memantine, we observed a concomitant decrease in glutamate concentration and increase in the glutamine to glutamate ratio. This decrease in glutamate concentration therefore appears to be compensated by the increase in the glutamine pool. This suggests that the NMDA receptor blockade influences the glutamate-glutamine cycle. It is hypothesized that the function of glutamine synthetase, maybe affected by oxidative stress, which has previously been shown to be associated with NMDA receptor blockade. Mechanism of action of BINA is still a subject of investigation; hypothetically BINA would potentiate pre-synaptic mGlu2 auto-receptors leading to decreased glutamatergic transmission, thereby inhibiting memantine effects.

CONCLUSION: These findings exemplify the utility of 1H-MRS in early drug discovery, allowing non-invasive investigation of dynamic metabolite changes in the brain, to support the behavioral effects of psychoactive compounds and their functional activations observed with other modalities (fMRI, rs-fMRI, electrophysiology, etc) [1-3].

REFERENCES: [1] Sekar S et al. Psychopharm 2013; [2] Sekar S et al. ADDC 2013 proceedings; [3] Sekar S et al., ISMRM 2010; [4] Brenner E et al. 2005; [5] Klamer D et al. 2005; [6] Fejgin K et al. 2008; [7] Sanacora G et al. 2008; [8] Iltis I et al. 2009; [9] Hackler EJ et al. 2010.