Antidepressant like effects of magic 'K' drug at subanaesthetic doses in CMS rat model of depression as detected by invivo 1H-MRS at 7T.

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Introduction: Stress is defined as any environmental change, whether endogenous or exogenous, that disturbs the maintenance of brain homeostasis. Chronic stress over a period of time may lead to depression, a complex psychiatric disorder characterized by anhedonia and feelings of sadness (Caspi, 2003). It has been shown that even a subanaesthetic dose of the magic 'K' drug ketamine has antidepressant action within hours of administration (Rowland et al., 2005). We wanted to assess the effect of ketamine (Ket) on Chronic Mild Stress (CMS) rat model of depression noninvasively. Proton MRS is nowadays the most frequently used method in neuro-spectroscopy and it is a non-invasive neuroimaging technique using which the integrity of neural tissue subsequent to any exposure or changes can be measured.

Aim: In this study we intend to identify the neuro-chemical profile and to assess the antidepressant activity of Ketamine in hippocampus on CMS rats using invivo proton magnetic resonance spectroscopy (^{1}H-MRS) at a magnetic field strength of 7T.

Methods: CMS animal model was developed by applying various unpredictable mild stressors for a period of 6 weeks and the model was validated using behavioural studies like sucrose consumption test, forced swim test and open field test. The rats were divided into four groups Control (Ctrl) CMS, Ctrl+Sal, Ctrl+Ket, CMS+Sal and CMS+Ket. 30 minutes prior to imaging, 15mg/kg body weight of Ketamine was injected (i.p) to the rats. Later, 1H-MRS was performed on all animals (n=8 each) in hippocampus region. Saline (Sal) was used as placebo. MRS was carried out on 7T Bruker biospec (AVANCE III) horizontal bore scanner. The rats were anesthetized with continuous inhalation of Isoflurane (2%) + oxygen mixture throughout the experiments. Spectra were acquired using Point Resolved Spectroscopy (PRESS) sequence with a voxel size of 2 x 4 x 3 mm^3 in Hippocampus with TR/TE of 2,500/20 ms and 512 scans were acquired and averaged. Water suppression was performed using variable power RF pulses with optimised relaxation delay (VAPOR). Quantitative assessment of the neurometabolites was done using LC model. The LC-model fit for metabolites was fixed with Crame´r–Rao lower bound (CRLB) of 20 % or less. Total creatine (Cr+PCr) spectral intensity was used as the internal reference for relative quantitation. Concentrations for N-acetyl-aspartate (NAA), Taurine (Tau), gamma amino butric acid (GABA), myo-inositol, unresolved glutamate and glutamine (Glx) and glutamate alone (Glu) were calculated for analysis.

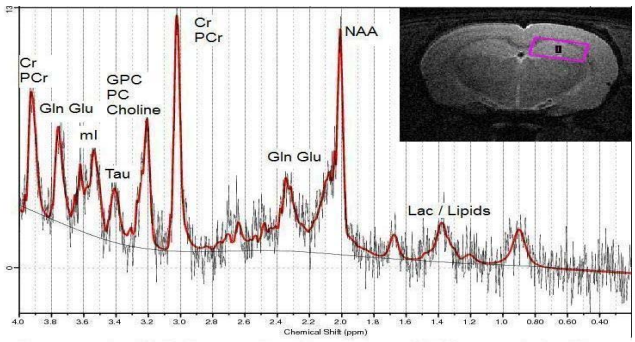

Figure 1: Representative 1H-MRS spectra acquired from Hippocampus of CMS rat at 7T.

Table 1: List of metabolite concentrations in control, CMS, Ctrl+Sal, Ctrl+Ket, CMS+Sal and CMS+Ket rats obtained from Hippocampus.

Metabolites	Hippocapmus					
	Control	CMS	Control+Sal	Control+Ket	CMS+Sal	CMS+Ket
GABA	0.259±0.07*	0.243±0.05*†	0.251±0.02	0.264±0.09	0.32±0.03	0.288±0.05†
Gln	0.469±0.03*	0.409±0.06*	0.493±0.06	0.381±0.02	0.45±0.05	0.345±0.10
Glu	1.004±0.15	1.001±0.11†	1.002±0.02	1.010±0.08	1.035±0.04	0.946±0.02†
Ins	0.776±0.05*	0.800±0.07*†	0.788±0.06	0.652±0.04	0.795±0.08	0.772±0.01†
NAA	0.804±0.04	0.780±0.05	0.797±0.08	0.858±0.07	0.848±0.05	0.834±0.03
Tau	0.706±0.04*	0.724±0.08*†	0.648±0.003	0.615±0.10	0.731±0.07	0.702±0.04†
Gua	0.49±0.19	0.371±0.06	0.455±0.25	0.403±0.08	0.305±0.06	0.459±0.04
GPC+PCH	0.172±0.02*	0.180±0.02*	0.153±0.01	0.163±0	0.164±0.01	0.165±0.01

Results and Discussion: The behavioural studies showed a decrease in sucrose intake in the CMS rats as compared to control rats on sucrose consumption test as an evidence of the onset of depression. The representative 1H-MRS spectra acquired from Hippocampus of CMS rat is shown in Figure 1. 1H-MRS results revealed a significant decrease of GABA, Glutamine (Gln) and GPC+PCH in hippocampus of CMS animals as compared to control rats and an elevated level of Myo Ionisitol (mI) and Taurine (Tau) was also observed as shown in Table 1. The spectra obtained after 30minutes of ketamine injection showed elevated level of GABA and decreased Glutamate (Glu), Ins and Tau levels. The GABA, Tau and mI alterations appear to normalize after successful treatment with ketamine. Tau and mI are known as osmoregulatory metabolites and mI is known as the glial marker, the elevated level observed in hippocampus suggests the possible role of neuroinflammation and glial physiology alteration in hippocampus on the early onset of depression later with ketamine treatment they were found to be decreased suggesting some antinflamatory mechanism taking place in brain. The overall study shows a rapid antidepressant like effect of ketamine in the hippocampus.

Conclusion: A localized alteration in neuro metabolites during the onset of depression reveals the metabolite fluctuations, altering the glial physiology by causing neuroinflamation in hippocampus in CMS animals. But those changes were found to decrease after successful treatment with acute dose of ketamine suggesting some repair process taking place in brain.

Reference: (1) Caspi A et. al., (2003), Science 301:386–389.
(2) Rowland LM et. al., (2005), Am J Psychiatry: 162(2):394–396.