Neuroinflamatory evidence during early onset of depression in CMS rats as detected by proton MRS at 7T.

Hemanth Kumar B.S. [1], Sushanta Kumar Mishra[1], Poonam Rana[1], Sadhana Singh[1], Rajendra P Tripathi[1], and Subash Khushu[1]
[1]*NMR Research Centre, Institute of Nuclear Medicine and Allied Sciences (INMAS), Newdelhi, Newdelhi, India*

Introduction: Day to day life stress has shown to play critical role in the etiology of numerous psychiatric illnesses, amongst which depression is a complex psychiatric disorder characterized by anhedonia and feelings of sadness and its etiology is not fully understood (Caspi, 2003). Chronic Mild Stress (CMS) seems to be a valuable animal model of depression, based on its resemblance with human depressive symptoms (Willner P, 2005). Proton MRS is nowadays the most frequently used method in neurospectroscopy 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: Identifying the neurochemical alterations in depression to enhance our understanding of the neurophysiology of depression. In this study we intend to assess the changes in neuro-metabolites in the onset of depression in prefrontal cortex (PFC) and hippocampus in 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. Proper day and night cycle was maintained and the food and water was supplied *ad libitum*. Later, 1H-MRS was performed in both control and CMS rats (n=10 each) on prefrontal cortex and hippocampus. MRS was carried out on 7T Bruker biospec (AVANCE III) horizontal bore scanner. The rats were anesthetized using Ketamine and xylazine mixture prior to MRS experiments. Spectra were acquired using Point Resolved Spectroscopy (PRESS) sequence with an voxel size of 2x3x3 mm^3 for frontal cortex and 2x4x3 mm^3 in the case of 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.

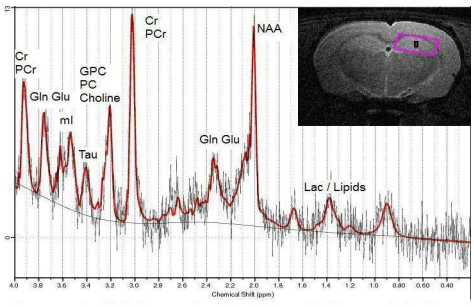

Figure 1: Representative 1H-MRS spectra acquuired from Hippocampus of CMS rat at 7T.

Table 1: List of metabolite concentrations in control and CMS rats obtained from PFC and Hippocampus.

Metabolite	Prefrontal cortex		Hippocampus	
	Control	CMS	Control	CMS
Glu	1.31±0.16	1.29±0.20	1.12±0.06	1.01±0.08*
Gln	0.78±0.12	0.59±0.07*	0.59±0.03	0.47±0.08*
Glu+Gln	1.96±0.33	1.52±0.31*	1.66±0.13	1.41±0.12*
mI	0.64±0.08	0.62±0.08	0.67±0.10	0.76±0.07**
GABA	0.38±0.01	0.30±0.00*	0.32±0.01	0.28±0.02*
NAA+ NAAG	0.88±0.16	0.79±0.07	1.01±0.03	0.90±0.09*
*0.01 Significant (<0.01), **0.05 Significant (<0.05)				

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 Hippocamus of CMS rat is shown in Figure 1. 1H-MRS results revealed a significant decrease of Glutamate (Glu), Glutamine (Gln), NAA+NAAG, Glu+Gln and GABA in both hippocampus and prefrontal cortex of CMS animals and an elevated level of Myo Ionisitol (mI) and Taurine (Tau) observed only in the case of Hippocampus as shown in Table 1. Glutamate is an excitatory neurotransmiter known to be a mediator of stress induced changes, whereas, GABA and Tau are inhibitory neurotransmitters. Cousins JP, et. al., have reported that, decrease in glutamate levels were shown to coincide with the transient experience suicidal depression. The decrease in NAA+NAAG levels suggests that there is decrease in the neuronal density occurring as an onset of depression. 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.

Conclusion: There might be localized alteration in neuro metabolites at the onset of depression. This study reveals the metabolite fluctuations, altering the glial physiology by causing neuroinflamation within prefrontal cortex and hippocampus in CMS model of depression. The overall finding suggests that, there might be a major contribution of the glia along with the neurons to play a major role in depression.

Reference: (1) Caspi A et. al., (2003), Science 301:386–389.
(2) Willner P et. al., (2005), Neuropsychobiology 52(2):90-110.
(3) Cousins JP (2000), J Am Acad Audiol. May;11(5):239-72.