Quantitation of Diffusion Indices in CMS rat model of depression – A DTI approach.

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Introduction: Depression is a commonly occurring, debilitating, and life threatening psychiatric disorder. According to World Health Organization, depression is the fourth most prevalent cause of loss in human disability adjusted life years worldwide (1). Diagnosis of depression remains subjective based on descriptive symptoms by depression patients. Out of many animal models available for depression, Chronic Mild Stress (CMS) seems to be a valuable model of depression, based on its resemblance with several human depressive symptoms (2). The recent development of neuroimaging technologies allows in-vivo characterization of the mood disorders. MR Imaging have showed that depressed individuals have reduced hippocampal volume with the magnitude of the atrophy (3) and decreased blood flow and metabolism was shown in several areas of prefrontal cortex in patients with depression, including dorsolateral prefrontal cortex. Water diffusion in the brain is influenced by the local microstructure of the tissue. Diffusion Tensor Imaging (DTI) is a quantitative non-invasive and objective method for assessing the integrity of major white matter tracts indirectly via measurement of the directionality of water diffusion (fractional anisotropy, FA) (4). The aim of our study is to assess the changes in DTI measures, in CMS rat brains by using quantitative DTI technique.

Materials and Methods: The study was approved by the animal ethics committee of our institute. Twenty healthy adult male Sprague–Dawley (SD) rats weighing 200–300 g (about 3–4 months of age) were included in this study. All animals were randomly divided into two main groups (Control and CMS), n = 10 each and were housed in a group of five, at an average room temperature of 23°C and humidity of 50%–60%, with illumination available from 8 AM to 8 PM daily. Food and water were given *ad libitum*. The CMS regime used in this study is described in detail elsewhere (5). CMS animal model was validated using behavioural studies i.e. sucrose consumption test, open-field test, and forced swim test.

All MRI experiments were carried out on a 7T Bruker Biospec USR 70/30, (AVANCE III) horizontal bore animal MRI scanner. In both controls and CMS rats, anaesthesia was induced by intra peritoneal injection of a mixture of xylazine (10mg/kg BW) and ketamine (80mg/kg BW). MRI protocol included turbo RARE T2-weighted and DTI sequence. DTI images were acquired using a multi-slice, multiple-shot spin echo EPI sequence with the following parameters: TR/ TE= 3800 ms/32 ms, number of gradient encoding directions = 46, and b= 700 s/mm^{-2}. The DTI data were processed as described in detail elsewhere (6). FA and mean diffusivity (MD) values were calculated by placing region of interests (ROIs) on prefrontal cortex (PFC), hippocampus, hypothalamus, cingulum (cng), sensory-motor cortex (SMC), thalamus, caudatoputamen, corpus callosum (CC) and cerebral peduncle (**Fig.1**), bilaterally.

Results: In both the group, no abnormality was observed on conventional MR images. In CMS group, a significantly decreased FA values were observed in right and left PFC, CC, right and left hypothalamus and right thalamus compared to controls (**Table**). Significantly increased MD values were observed in both right and left PFC of CMS group compared with controls. Though, an increasing trend in MD values was observed in CMS group compared with controls in hypothalamus region, it did not reach to the level of statistical significance.

Table: A summary of mean ± SD of DTI measures in brain parenchyma collected from controls and CMS rats.

Region		Fractional Anisotropy		Mean diffusivity (×10^{-3} mm^2/sec)		P value
		Control	CMS rats	Control	CMS rats	
PFC	Right	0.22±0.03	0.20±0.03	0.71±0.03	0.78±0.06	P_{FA}=0.05, P_{MD}= 0.01
	left	0.22±0.01	0.19±0.02	0.72±0.03	0.79±0.07	P_{FA}=0.02, P_{MD}=0.01
HT	Right	0.32±0.05	0.20±0.03	0.77±0.03	0.78±0.03	P_{FA} <0.001, P_{MD}=0.42
	left	0.29±0.06	0.19±0.02	0.76±0.03	0.79±0.02	P_{FA}<0.001, P_{MD}=0.08
CC		0.63±0.03	0.58±0.05	0.80±0.05	0.82±0.03	P_{FA}=0.03, P_{MD}=0.23

PFC=Prefrontal cortex, HT=Hypothalamus, CC=Corpus callosum

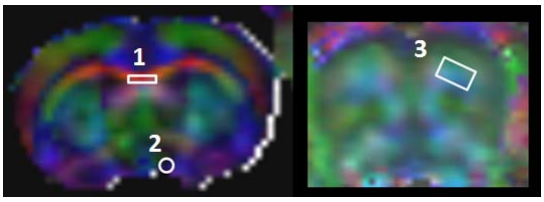

Fig.1: Demonstration of regions of interest placement in an age matched control rat. 1=CC, 2=hypothalamus and 3=PFC

Discussion: To the best of our knowledge this is the first non-invasive study showing unfavourable effects of CMS on brain architecture. Using DTI, we have documented the microstructural changes in PFC, hypothalamus and CC in CMS rat model of depression. Decreased blood flow and metabolism was shown in several areas of prefrontal cortex in patients with depression, including dorso-lateral prefrontal cortex (7). In a 1H-MRS study, decreased choline-containing compounds and increased myoinositol (mI) concentrations is shown in patients with major depressive disorder (8). mI is considered as a marker of glial proliferation, and an increase in mI resonance may be a proxy for increased inflammation in the brain. Our DTI findings also suggest that decrease FA values and increased MD values in CMS group compared to controls may represent an altered glial physiology and suggest that there might be some micro structual changes taking place in the brain parenchyma of CMS animals which in turn affects the diffusion pattern in the brain.

Conclusion: To conclude the changes in FA and the MD values obtained reveals that there might be structural abnormalities taking place in the brain regions which alters the micro-architecture of the tissue involved during the onset of depression.

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