Longitudinal MR based study of ipsilateral and contralateral hippocampus volume and cognitive assesment in traumatic brain injury rats

Bhanu Prakash KN[1], Sanjay K Verma[1], Sankar Seramani[1], Enci Mary Kan[2], Graham S[3], Childs C[4], Jia Lu[2], and S Sendhil Velan[1]

[1]*Laboratory of Molecular Imaging, Singapore Bioimaging Consortium, Singapore, Singapore,* [2]*Combat Protection and Performance Lab, Defence Medical and Environmental Research Institute, DSO National Laboratories, Singapore, Singapore,* [3]*Department of Psychology, National University of Singapore, Singapore, Singapore,* [4]*Alice Lee Centre for Nursing Studies, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore*

Objective: Traumatic Brain Injury (TBI) is a complex injury with a broad spectrum of symptoms and disabilities and is a major cause of morbidity and death in military and civilian populations. TBI costs the world more than $76 billion a year in US and more than 1.7 million people are affected by it every year[1]. There is a pressing demand to understand the effects (structural, biochemical, physical, psychological etc) of TBI on the people. The decrease in hippocampus volume due to neurodegeneration in TBI subjects is reported in Jorge et al[2]. In our study, we developed a non-invasive fluid percussion injury (FPI) technique on a rodent model to replicate the broad categories of injury severity: mild, moderate and severe TBI to explore the biochemical, cognitive and structural changes in the rat brain. Our study aims at exploring the longitudinal changes in the hippocampus volume (ipsilateral and contralateral) and cognitive assessment in different injury models.

Materials and Methods: Adult male Sprague Dawley rats (280 – 300g) were subjected to focal brain injury using the lateral fluid percussion device. Animals were subjected to either 1) Sham (4mm-diameter hole, in the skull), 2) Mild (22.75 ± 0.75 psi) or 3) Severe (64.04 ± 1.49 psi) injury at 2mm lateral and 3.8mm posterior to bregma. The study set had three shams, 6 mild and 6 severe rats. A 3D MPRAGE images of the rat brain were acquired on 7T Bruker Clinscan, along the transverse direction with TR/TE= 2000 ms/1.5 ms, IR = 800 ms, base resolution of 512 × 416 × 52, and voxel dimensions of 0.06836 x 0.06836 x 0.5 mm^3. MR imaging was performed on baseline (BL), day-1, 3, 7, 14 and 28 to study the ipsi- and contra- lateral hippocampus volume changes. Cognitive assessments were done at the same time points on a separate batch of rats with similar injury models. Animals were tested on the Rotarod (0 – 24 rpm in 60s) to assess for the motor skills. The segmentation of hippocampus and volume calculation was done semi-automatically using the ITK Snap[3]. Amyloid precursor protein and Fluro-jade staining were done to check the protein deposition and degeneration of neurons in the ipsilateral hippocampus.

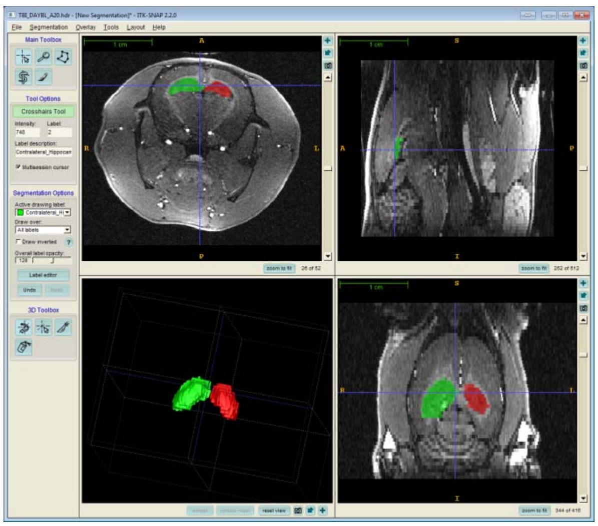

Fig. 1. Segmentation of hippocampus using ITK-Snap

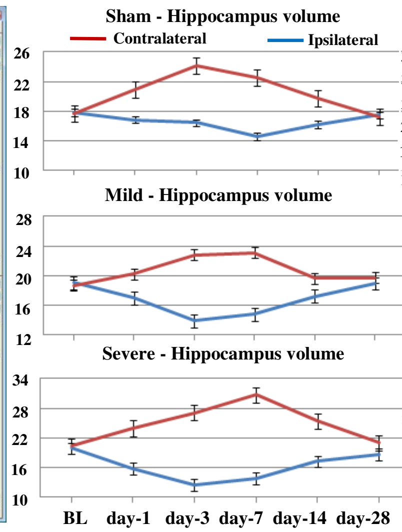

Fig.2. Plot of mean ipsi- and contralateral hippocampus volumes for different groups

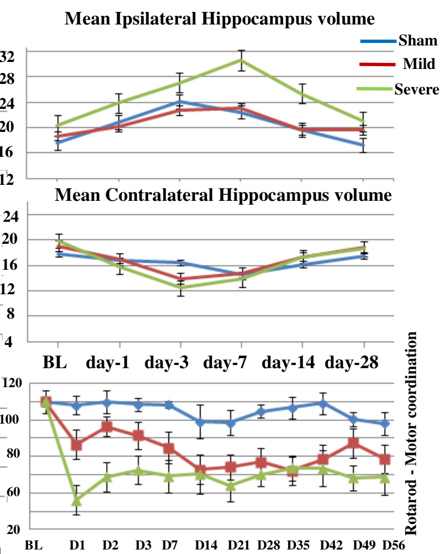

Fig.3. Comparison plots of mean volume and Rotarod cognitive assessment analysis for different groups

Results and Discussions: Fig.1 shows the three planes based segmentation tool used for hippocampus segmentation and volume quantification. The ipsilateral hippocampal volume decreased due to neuronal degeneration in all the injury models from day – 1 to day 3, increased from day-3 to day – 14, and reached the baseline volume by day 28. The contralateral hippocampal volume increased (due to neurogenesis) and decreased proportional to the ipsilateral volume during the same period as shown in Fig. 2. Sham rats had the least volume change and the severe models had the highest. The recovery rate of was least in severe when compared with the other groups. Fig.3 shows the mean volume change in ipsilateral and contralateral hippocampus on different days. The rotarod task based cognitive assessment showed injured animals were impaired in their balance and coordination immediately after injury on day-1 but showed progressive improvement by day 7, with the severe group having the least coordination. The Fluro-jade and Amyloid precursor protein staining (Fig.4) showed higher neurodegeneration and protein deposition in severe than in the mild and sham conforming the results of MR imaging.

Conclusions: Our study demonstrated good correlation of results obtained by MR image based analysis of hippocampal volume, histopathology and cognitive assessment. Our study results suggests both the rate of change and total volume of hippocampus due to neuro-degeneration (immediately after the injury) and the neurogenesis (during the recovery phase), depend on the type of injury. The changes in volume indicate reorganization of neurons could be taking place in the hippocampus after during the acute phase of injury and during the recovery phase. Reduction of hippocampus volume due to TBI suggests psychological and behavioral changes in the animal[2, 4]. Cognitive assessments also showed an improvement in motor coordination during the recovery phase (day-7 to day-14).

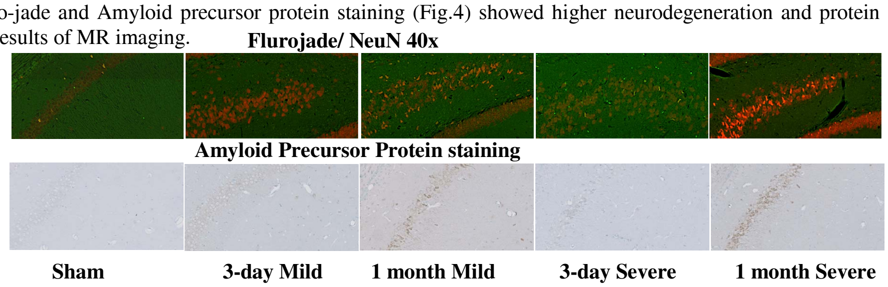

Fig.4. Flurojade /NeuN – 40x and Amyloid precursor protein staining – 20x of ipsilateral hippocampus

References: [1] http://www.cdc.gov/injury/about/focus-tbi.html - accessed on 5 Nov 2012 [2] Jorge RE *et al.* Biol Psychiatry. 2007;62:332–8. [3] Paul A *et al. Neuroimage* 2006 Jul 1; 31(3):1116-28. [4] Emanuel *et al.* 2011. *Undergraduate Honors Theses.* Paper 20.