

LONGITUDINAL BLAST INJURY CHARACTERIZATION USING MAGNETIC RESONANCE SPECTROSCOPY IN RAT HIPPOCAMPUS

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INTRODUCTION: Mechanisms underlying secondary neural insults due to Traumatic brain injury (TBI) are poorly understood. Recent advances in high field magnetic resonance spectroscopy (MRS) allow us to observe metabolic changes with increased spectral resolution in the brain after the injury. MRS offers a unique window to identify the severity of the injury, outcome of the injury and secondary insult. Alterations in major metabolites such as N-acetylaspartate (NAA), a biomarker of neuronal integrity; total choline (tCho), a marker of membrane metabolism; total creatine (tCr) levels are key to identify injury outcomes in-vivo. The objective of this study is to simulate the secondary injury, analogous to the blast injury of humans in the rat model and observe the metabolic changes in the rat brain due to injury. Blast related injury is very difficult to model since it is more diffused compared to a focal injury. Primary injury is the result of initial mechanical forces from brain trauma causing tissue distortion and destruction in the acute phase of injured period. Secondary injuries occur over time and are a result of the activation of bimolecular and physiological processes due to the primary injury. Secondary injury leads to alterations in cell function and propagation of injury through depolarization, excitotoxicity (primarily from glutamate release) alteration in calcium homeostasis, blood brain disruption, increase in intracranial pressure and oxygen damage (free radical generation) are the key component of secondary injury. Understanding these processes helps in the development of therapies for diffuse and secondary brain injuries.

METHODS: Blast Test Set-up 5 kg of 2, 4, 6-trinitrotoluene (TNT) with a penta-erythritol tetra-nitrate (PETN) booster was detonated at 1 m height in each blast. A metal cage along with the pressure transducer was set up at 3m from the blast source. The animals used in the study were Blast group (with no body protection) exposed to a single blast at ~180 kPa. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) prior to the experiments.

Imaging protocol: Single Voxel Spectroscopy (SVS) data was acquired before and after the injury with PRESS technique with a voxel size of $3.5 \times 2 \times 3.5 \text{ mm}^3$ in the hippocampus. Data were acquired with Bruker Clinscan 7 Tesla, with the TR/TE/number of averages 4000 ms/13 ms / 128 respectively. The total acquisition time was 8 mins. Water unsuppressed data (with eight averages) were also collected during the acquisition for scaling and eddy current compensation. Manual shimming was done prior to every data acquisition. Metabolite quantitation was performed with LC Model [1]. MRS data were collected on baseline (before the injury), Day 1, 3 & 5 after the injury.

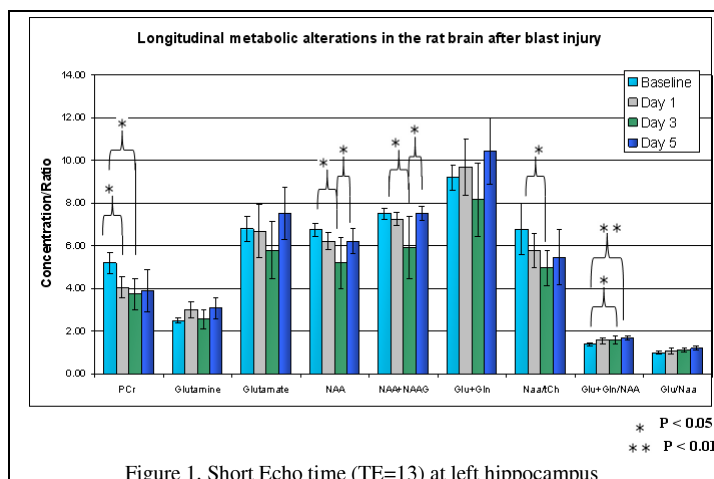


Figure 1. Short Echo time (TE=13) at left hippocampus

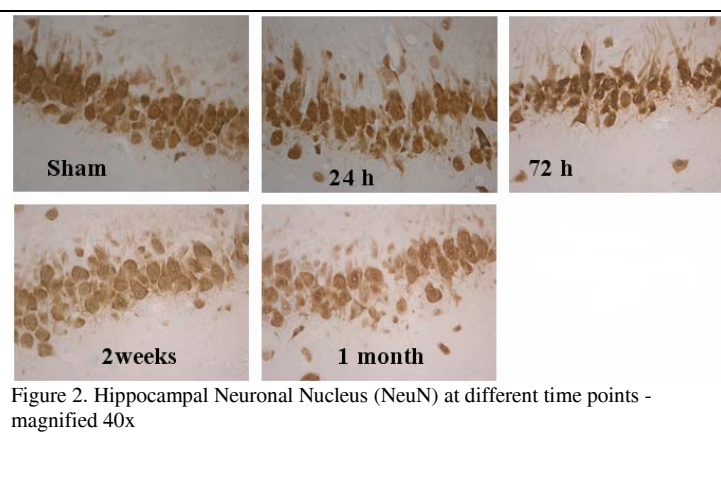


Figure 2. Hippocampal Neuronal Nucleus (NeuN) at different time points - magnified 40x

RESULTS AND DISCUSSIONS: The relative concentrations of the metabolites and their ratios (mean values) obtained by SVS are shown in Fig. 1. Hippocampal Neuronal Nucleus (NeuN) staining was performed at different time points (Fig. 2). The cell death process at 72 hrs after injury is high and recovered at 2 weeks. The same trend was observed in the NAA and NAA+NAAG MRS data. Significant reduction in the concentrations of the PCr, NAA, NAA+NAAG, NAA/tCho was observed on day 3 when compared to the baseline scans and there was a significant increase in [Glutamate (Glu) + Glutamine (Gln)] / NAA ratio on day3 and day 5. Although primary injury occurs at the cortex, hippocampus is more vulnerable to damage because of the presence of large number of glucocorticoid receptors [2]. The penumbra, damage that surrounds the initial site of injury is often attributed to apoptosis. The penumbra activates the glutamate to allow the calcium influx through the NMDA receptors. This excessive calcium influx into the neurons activates the apoptosis, which is referred to as excitotoxicity. Our histology results confirm the findings of MRS at 72 hrs after the injury.

CONCLUSIONS: We have investigated the metabolic changes by MRS in a rodent blast injury model and validated with histology. We have observed significant changes in different metabolites after injury including a reduction in NAA/(tCho) concentration in rodent model. Similar observation has been made in blast related injuries in human studies by Hetherington et al [3].

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References:[1]Provencher et al NMR Biomed. 2001; 14:260-264 [2] Sapolsky RM Prog Brain Res. 1990; 86:13-23 [3] Hetherington et al, Proc Intl Soc Mag Reson Med 20, 2012 [4] Giza et al, Journal of Athletic Training 2001; 36(3):228-235.