

# Longitudinal metabolic alterations in the cerebral cortex of rats after repetitive mild traumatic brain injury

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**Introduction:** Repetitive mild traumatic brain injury (rmTBI) may induce numerous metabolic alterations and can evolve into a range of neurodegenerative or neuropathological disorders [1, 2]. While conventional structural MRI failed to provide any neuropathological signature after mild TBI, <sup>1</sup>H magnetic resonance spectroscopy (MRS) and diffusion MRI have revealed significant metabolic and microstructural alteration after mild TBI [3, 4]. In the present study, we hypothesized that high field <sup>1</sup>H MRS can detect prominent metabolic alterations near the impact site of the cerebral cortex after rmTBI. Our longitudinal <sup>1</sup>H MRS examination of cerebral cortex before and after rmTBI have revealed significant metabolic alterations in comparison to the control level.

**Methods:** Fisher 344, male rats (n=5), of 12 weeks of age were employed in this study. Animal handling and experiment were performed as approved by national guidelines for animal research, Denmark (2016-15-0201-00877). The rmTBI protocol from Kane et al [2] was adopted and mild impacts were performed on day1, day 3 and day7. Rats were scanned using 9.4 T, MRI system before and immediately after each injury using the cryo-surface coil as described previously [5]. In-vivo <sup>1</sup>H MRS data was collected from the cerebral cortex of the right hemisphere of the brain using the PRESS sequence (TR/TE 5000/16 ms). <sup>1</sup>H MR spectra were processed using the LC model as previously described [5]. Results from the LC model analysis were then exported to Matlab and linear mixed model analysis was performed. The statistical F test was used to analyse the significant difference in comparison to the control.

**Results:** Here we presented only prominent changes in the ratio of metabolite level in the cerebral cortex (Figure 1), in comparison to control levels. GSH was significantly reduced after the first, second and third impact (TBI1-TBI3) (p<0.01) (Figure 2A). Phosphocholine increased significantly in all mild TBI groups (TBI1-TBI3) (p<0.01) (Figure 2B). Inositol was significantly reduced after the second (p<0.01) and third injury (p<0.05) (Figure 2C). NAA+NAAG also increased significantly after second and third impact (p<0.05) (Figure 2D). GABA was significantly increased only after first impact (TBI1) (p<0.05) (Figure 2E). Glutamate was slightly elevated after impact (TBI1-3), however, not significant (Figure 2F).

**Discussion:** Significantly reduced GSH level and higher NAA level suggest increased oxidative stress level in the cerebral cortex [5]. The significantly increased phosphocholine levels have also been observed previously after mild TBI and interpreted as indicative of membrane breakdown and/or repair [6]. Inositol is considered a glial marker and changes in its concentration suggests gliosis in the targeted tissue [7]. The observed increase in GABA and the apparent increase in glutamate suggest altered neurotransmission [8]. Collectively, these prominent metabolic alterations may be useful in the diagnosis of rmTBI cases, although the low number of animals used in this study is a major limitation.

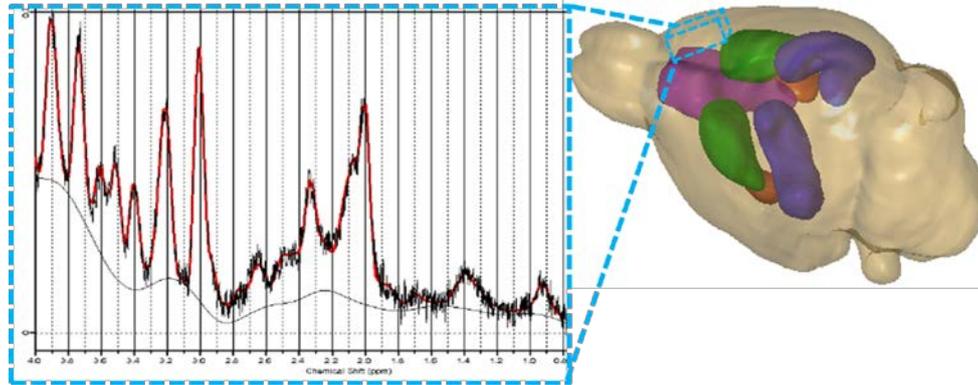
**Conclusion:** The metabolic alterations suggest oxidative stress and altered neurotransmission of cerebral cortex after mild TBI. Such high field MRI findings provide new insights into the pathophysiology of rmTBI at a time where this pathology is increasingly recognised as not sufficiently understood.

## References

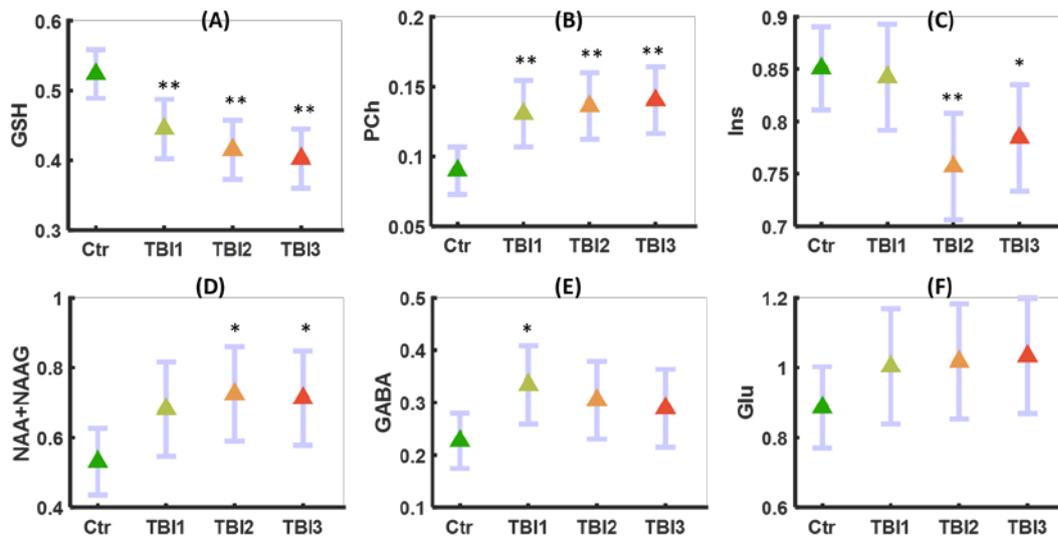
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**Figure 1:** A representation of voxel location in the cerebral cortex and a processed  $^1\text{H}$  MRS spectrum on LC model.



**Figure 2:** Metabolic normalized with total Cr as mean  $\pm$  confidence interval (CI) in control (Ctr: Green) and after first (TBI1: light green), second (TBI2: orange) and third (TBI3: red) head impact on day1, day 3 and day 7 respectively. Level of Glutathione (GSH) (A), Phosphocholine (PCh) (B), Inositol (Ins) (C), N-acetyl aspartate + N-acetyl aspartyl glutamate (NAA+NAAG) (D), Gamma amino buytyric acid (GABA), (E), and, Glutamate (Glu) (F). Level of significance(\* $p < 0.05$ , \*\* $p < 0.01$ ).