# Medullar and thalamic metabolic alterations following spinal cord injury (SCI): a preliminary mice study, combining early and longitudinal follow-ups using high-spatially resolved MRS and DTI at high field.

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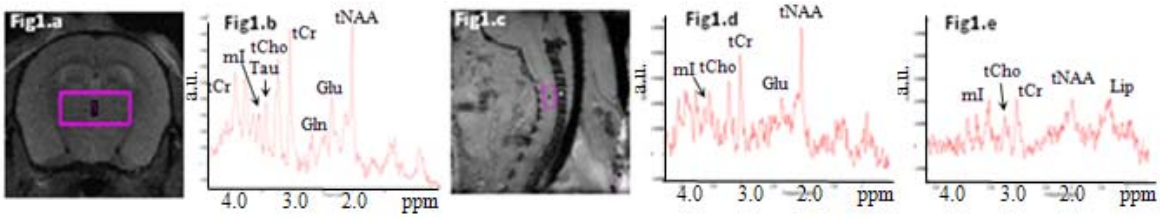
**Introduction:** Spinal Cord Injury (SCI) usually results in sensory and motor dysfunction and nervous system disturbance. It may induce spinal cord neuronal cell death, axonal degeneration and may lead to reorganization in the thalamus, which plays a central role in modulating the motor and pain transmission. To adequately and non invasively describe tissue metabolism alterations as well as morphological and structural impairments consecutive to the injury, MR methods including MR Spectroscopy (MRS) and Diffusion Tensor Imaging (DTI) have been optimized and used in various rodent studies. For instance, longitudinal MRS rat studies have been performed to study SCI-induced metabolic impairments, at the spinal cord level [1], eventually in combination with structural studies [2], or at the thalamic level [1,3]. However no mice studies have been reported so far.

In this preliminary study, we used high-spatially resolved 1H-MRS, in combination with refine adjustments and dedicated quantification, to examine medullar and thalamic metabolic alterations, from the very first days following the injury to six weeks after injury. Multi-slice DTI was concomitantly acquired at each time-point and immuno-histochemistry was finally performed to investigate correlation with both MRS and DTI data.

**Materials and methods:** Experiments were performed on injured anesthetized (1.5% isoflurane) C57BL/6J mice (n=2, age 10 weeks, 28 g). **SC Injury** (compression model) was induced by a smooth inflation of a balloon inserted into the epidural space at the C3 level. Following the compression, mice suffered from left fore-limb paralysis. **MR experiments**, including DTI and localized proton spectroscopy on both SC and thalamus were performed on an 11.75T vertical MR system (Bruker, AV 500WB) using a transmitter/receiver volume coil (∅ 2cm, L 3cm). **DTI** was acquired using an EPI-based Stejskal-Tanner sequence (2b-values, 6 directions, 100x100-$\mu m^2$ resolution, slice thickness 0.75mm, 5 slices) [4]. Localized spectra were acquired using Point RESolved Spectroscopy (PRESS) pulse sequence synchronized to the respiratory rate. First and 2nd order shims were optimized using manufacturer FASTMAP procedure. Based on localization gradient-echo images, voxels of interest (VOI) were placed in the SC (Fig1.c, VOI=2.0x1.8x1.1 $mm^3$~4µl) and in the thalamus (Fig1.a, VOI=1.5x4.5x1.5 $mm^3$~10 µl). Acquisition parameters were: TE/TR = 10/4000 ms, 512 averages, 512 samples, and a bandwidth of 10 ppm. The water signal was suppressed by using VAPOR sequence. Water navigator signals were additionally acquired at each repetition and used as reference signal and for $B_0$ drift and eddy current effect correction. Data were processed using jMRUI [5] and in-house-developed software running under Matlab. Metabolite spectra base was simulated using GAMMA [6]. Spectra quantification was performed with QUEST [7].

MR measurements and functional assessments (measurement of the developed fore-limb force) were performed at days 1, 2, 3, 5, 7, 14, 21, 28, and 42 post-injury (DPI). Mice were sacrificed at day 42 for immuno-histochemistry analysis.




**Results:** Figure 1 shows typical normalized spectra (relative to navigator signal intensity) obtained on healthy thalamus and SC (Fig1.b and Fig1.d), as well as on injured SC (Fig1.e), at 3 DPI, for a VOI centered in the lesion site and mainly containing gray matter (Fig3.a). Significant decreases of metabolite intensities combined with an enhancement of lipids intensities could be observed (Fig1.e). Figure 2 shows the temporal evolution of the main metabolites in the thalamus (Fig2.a) and in the SC (Fig2.b). Pathological variations were larger than the mean variability (previously obtained from MRS reproducibility tests performed on healthy mice (N=4), data not shown). For SC, a significant decrease was observed for all metabolites during the first days (<5 dpi), followed by a slow and progressive increase. tCr reached pre-injury level within two weeks (14dpi) and then continuously increased. tNAA recovered within four weeks (29 dpi) and tCho within few days. For the thalamus, metabolites were significantly altered during the first week (7 dpi) before recovering and remaining stable.

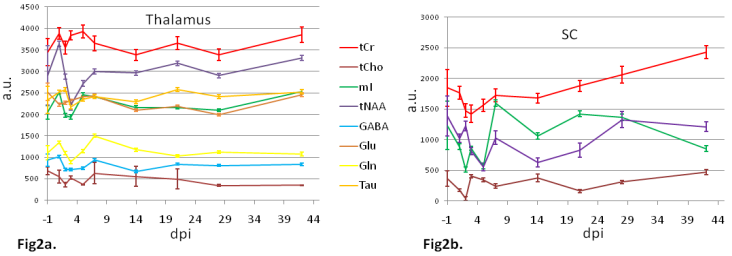
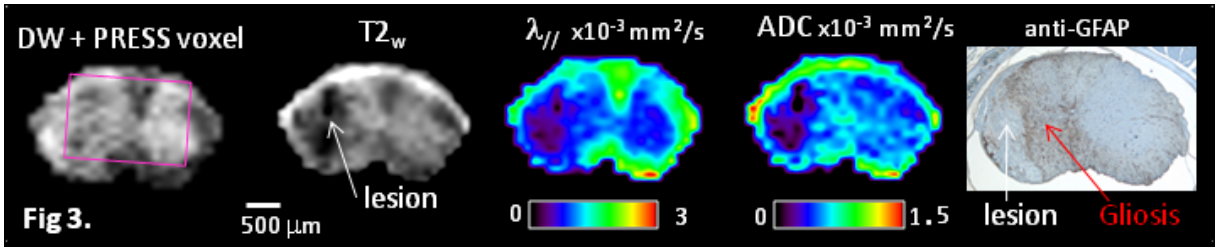



Fig2a. Fig2b.

Figure 3 shows DTI metrics maps (obtained at 7 dpi) for a slice acquired at the level of the lesion, which can clearly be seen on the $T_{2w}$ image (dark area). DTI metrics showed alterations on both white matter (strong decrease of $\lambda_{//}$) and gray matter (increase of ADC around the lesion area). ADC values in gray matter continuously increased until end-point (data not shown).




**Discussion:** Demyelination, axonal impairment and gliosis were demonstrated at the lesion site and adjacent slices through the use of DTI. The high-resolved MRS study additionally characterized the lesion and its thalamic repercussion by providing information on the axonal and neuronal alteration (NAA), membrane degradation and cellular turnover (Cho) and gliosis (Cr) [8], from the first day post-injury and during several weeks after the injury. This allowed following tissue alteration and potential spontaneous regeneration. We observed similar alteration patterns for the two mice and these preliminary results should be confirmed by further studies on larger cohort.

**Conclusion:** In this preliminary study, we successfully and non-invasively assessed to medullar and thalamic alterations following mouse SC injury, on both short and long-term, by combining two complementary MR techniques (MRS and DTI) at very high field. These findings could provide new criteria valuable for our understanding of the pathogenesis mechanisms and could offer new ways to assess the response to regenerative strategies.

**References:**

[1] Oberg et al., ISMRM proc. 2010 ; [2] Qian et al. J Neurotrauma 2010 ; [3] Likavcanova et al. MRM 2008 ; [4] Callot et al. NMR Biomed 2008 ; [5] Naressi et al. Comput. Biol. Med. 2001 ; [6] Smith et al. JMR 1994 ; [7] Ratiney et al. NMR Biomed 2005 ; [8] Salla et al. 2009