Multimodal spinal cord MRI for temporal characterization of posttraumatic vascular, metabolic and structural events in a mouse model of spinal cord injury.

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INTRODUCTION: Spinal cord (SC) rodent contusion models are widely used to study human SC trauma. Impact properties influence the pathological process, its progression and the injury outcome. Being able to assess, *in vivo* and non invasively, the temporal cascade of events consecutive to the injury would be beneficial to the pathological description and the identification of adequate repair strategies and optimal delivery time windows. In this context, an optimized multimodal MRI approach, including diffusion, perfusion and spectroscopic analyses, was applied in a follow-up study dedicated to the characterization of structural, vascular and metabolic posttraumatic events occurring in a moderate and a severe mouse SC contusion model. The relevant MR parameters were correlated with physiologic and immunohistochemistry analyses.

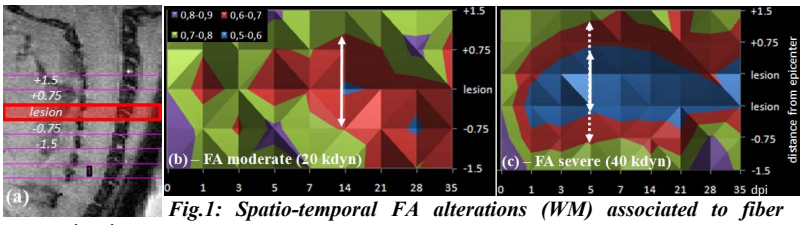

Fig.1: Spatio-temporal FA alterations (WM) associated to fiber disruption and demyelination, for moderate (b) and severe (c) lesion. (normal FA > 0.7).

MATERIALS AND METHODS: Experiments were performed on anesthetized C57BL/6 mice on an 11.7T MR system (Bruker), using a transmitter/receiver volume coil (∅ 2cm, L 3cm). Moderate (n=3) and severe (n=3) SC injury were induced at the C3-C4 level (left side) using a PSI Infinite Horizon impactor, (∅ tip 0.6-mm, dwell-time 5s, force 20 and 40 kdyn). Mice were scanned before (0) and 1, 3, 5, 7, 14, 21, 28, and 35 days post-injury (dpi) using diffusion tensor imaging (DTI-EPI, 2b-values, 12 directions, 6 slices [1]), perfusion imaging by arterial spin labeling (ASL) (presat-FAIR-QUIPSSII, 4 slices [2]) and monovoxel ^{1}H-spectroscopy (PRESS, TE 10 ms, VAPOR suppression, VOI 2.0x1.8x1.1 mm^3 [3]).

MR parameters (fractional anisotropy (FA), mean diffusivities ($\lambda_{//}$, $\lambda_{\perp}$), apparent diffusion coefficient (ADC), spinal cord blood flow (SCBF), normalized amplitude of total N-acetyl aspartate (tNAA), creatine (tCr), choline (tCho) and myo-inositol (mI)), as well as developed fore-limb force (grasping test, Bioseb apparatus), were examined at each time-point. At day 35, animals were sacrificed for SC immuno-histochemistry analysis.

RESULTS: The 0.6-mm ∅ tip contusion performed at 20 kdyn and 40 kdyn induced 1.5-mm and 2.5-mm widespread lesions respectively, in the rostro-caudal direction (as schematically represented by the spatio-temporal WM FA maps, fig.1b and 1c). As shown on fig.2, both lateral white matter (WM) and gray matter (GM) were altered. After SCI, mice suffered from left fore-limb paralysis and their developed force decreased by 39% (20 kdyn SCI) and 71% (40 kdyn) (fig.3a). In the acute phase (1dpi), the main MR features of the contusion models were: fiber disruption (FA↘), ischemia (SCBF↘, fig 3c), axonal ($\lambda_{//}$↘, fig 3b) and neuronal (NAA↘) losses and cellular impairment (tCr, tCho↘, fig.3d). In the early post-injury days (3<dpi<5), an increase of the ADC (↗ $\lambda_{//}$ and $\lambda_{\perp}$), attributed to vasogenic edema, was observed for the 20 kdyn lesion, whereas for the 40 kdyn lesion a decrease of ADC (↘ $\lambda_{//}$ and $\lambda_{\perp}$), mostly attributed to cytotoxic edema and debris, was noticed (fig.3b). A rapid increase in SCBF values (fig.3c), attributed to angiogenesis, in response to fuel demand for tissue regeneration and debris evacuation, was also observed in both ipsi and controlateral side (fig 2). The level of perfusion overreached the basal level for dpi>7. Perfusion in the adjacent slices (rostral and caudal) presented similar pattern for dpi>7 (data not shown). Moreover, for the same period (dpi>7), mI concentrations and mean GM diffusivities presented higher values than controls (data not shown), suggesting gliosis phenomenom. Five weeks after injury, mice presented an incomplete recovery (structural, vascular and metabolic parameters ≠ baseline), with defects and impairments confirmed by histology.

Finally, a canonical discriminant analysis (JMP software) was performed (fig.4) in order to give a better and synthetic representation of the posttraumatic events. All dpi could clearly be distinguished, which should permit to build specific diagrams showing pathogenesis of posttraumatic spinal cord injury, as in Hall's diagram [4].

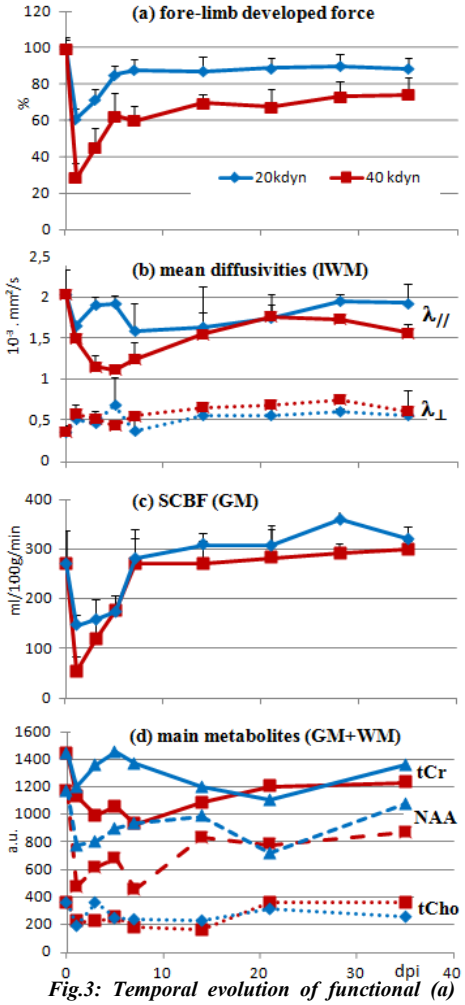

Fig.3: Temporal evolution of functional (a) and MR metrics measured in the lesion (b-d).

CONCLUSION: The multimodal MRI approach developed in this study allowed characterizing posttraumatic events following moderate and severe mouse SC injury. All MR parameters were modified, in correlation with histology and developed force, with an intensity time course and spatial extent dependant on the impact force. The sensitivity and specificity of the proposed multimodal MR approach should help to define the most relevant markers of disease and progression, and to identify posttraumatic diagrams.

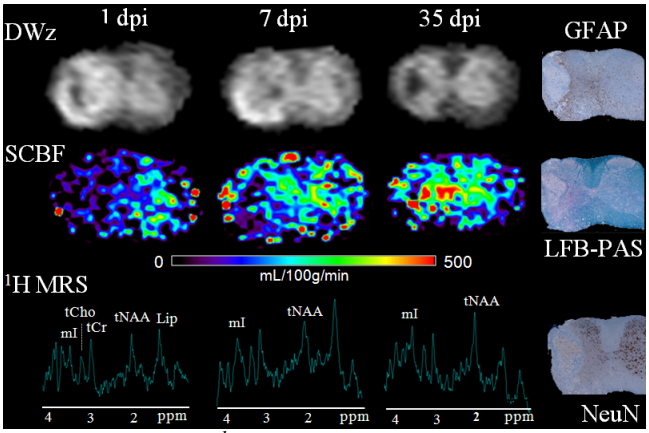

Fig.2: DWI, SCBF map, ^{1}H MRS and section staining for a 40-kdyn lesion.

References: [1] Callot et al. NMR Biomed 2008; [2] Duhamel et al. MRM 2011; [3] Tachrount et al. MRM submitted; [4] Hall et al., J Neurosurg 1986.

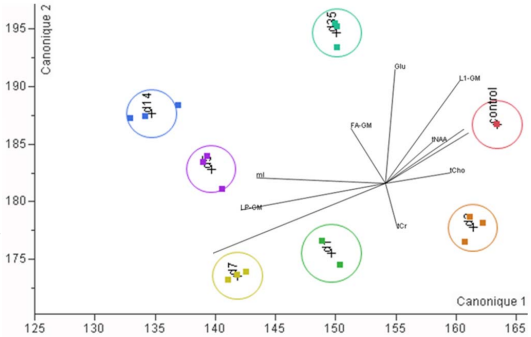

Fig.4: Canonical discriminant analysis for a moderate lesion using multimodal MR parameters.