Tract-based spatial statistics (TBSS) analysis reveals novel changes in lateral thalamic nuclei of kainic acid treated rats - comparison of DTI and histology

A. Sierra[1], K. Lehtimäki[1,2], T. Laitinen[1], L. Rieppo[3,4], A. Pitkänen[1,5], and O. Gröhn[1]

[1]Department of Neurobiology, A.I. Virtanen for Molecular Sciences, University of Kuopio, Kuopio, Finland, [2]Cerebricon Ldt., Kuopio, Finland, [3]Department of Physics, University of Kuopio, Kuopio, Finland, [4]Department of Anatomy, Institute of Biomedicine, University of Kuopio, Kuopio, Finland, [5]Department of Neurology, Kuopio University Hospital, Kuopio, Finland

Introduction

Diffusion tensor imaging (DTI) and tract-based spatial statistics (TBSS) analysis have been successful in identifying changes in several brain pathologies. However, the biological background of these changes should be investigated in more detail in animal models of neurodegenerative diseases. We aim to find new brain areas contributing to the epileptogenic process and the present work is focused on characterizing the interrelationship of histopathological and DTI changes in lateral thalamic nuclei, highlighted in TBSS after status epilepticus in rats.

Materials and methods

Status epilepticus (SE) was induced with kainic acid (KA) in male Wistar rats (n=6) and controls received saline (n=4). Six months after SE, animals were perfused intracardially using Timm fixation. DTI was carried out in a 9.4 T magnet using a 3D spin echo sequence (TR=1s, TE=60 ms, data matrix 192×64×64 zero padded to 192×128×128, FOV 23×15×15 mm^3). Six 3D images with diffusion weighting (diffusion time 17 ms, b-value 1000 s/mm^2) in six orthogonal directions and one image without diffusion weighting were obtained. Fractional anisotropy (FA) maps from individual subjects were co-registered to common space and further treated with conventional TBSS processing pipeline as described in the original TBSS-publications[1].

For histological analysis, 30 µm frozen sections were stained with Nissl staining to analyse the cytoarchitecture, and with gold chloride staining to visualize myelin in control and KA animals. Non-stained sections were analyzed in polarized light microscopy (PLM), and retardation and parallelism maps were calculated.

Results

The most significant changes in statistical images between control and KA-treated animals were observed in the dentate gyrus subfield of hippocampus, which were attributed to axonal reorganization in our previous study[2]. Furthermore, lateral thalamic nuclei were highlighted (Fig.1). Thalamic nuclei are part of the corticothalamic pathway in the brain, and also one of the targets in epilepsy[3]. Color-coded FA-maps of individual animals showed a relative increase in water diffusion laterally (green) (Fig. 2A and F). Increase in FA in lateral thalamic nuclei may be due to the combination of gliosis (Fig. 2B and G), shrinkage (double arrow in Fig. 2B and G) and demyelination (white arrow in Fig. 2H). Retardation maps are comparable with myelin stained sections and showed birefringent myelin content (Fig. 2D and I). Parallelism maps resembled FA maps and confirmed the organization of the fibers and the packing due to the shrinkage in these areas (Fig. 2E and J).

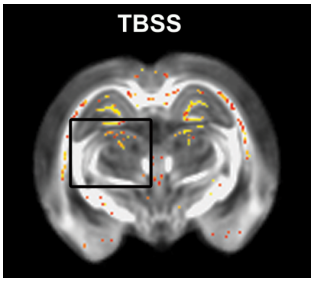

Figure 1. *Highlighted areas in TBSS analysis comparing control and KA animals (yellow increased FA). Black square indices the frame for Fig 2.*

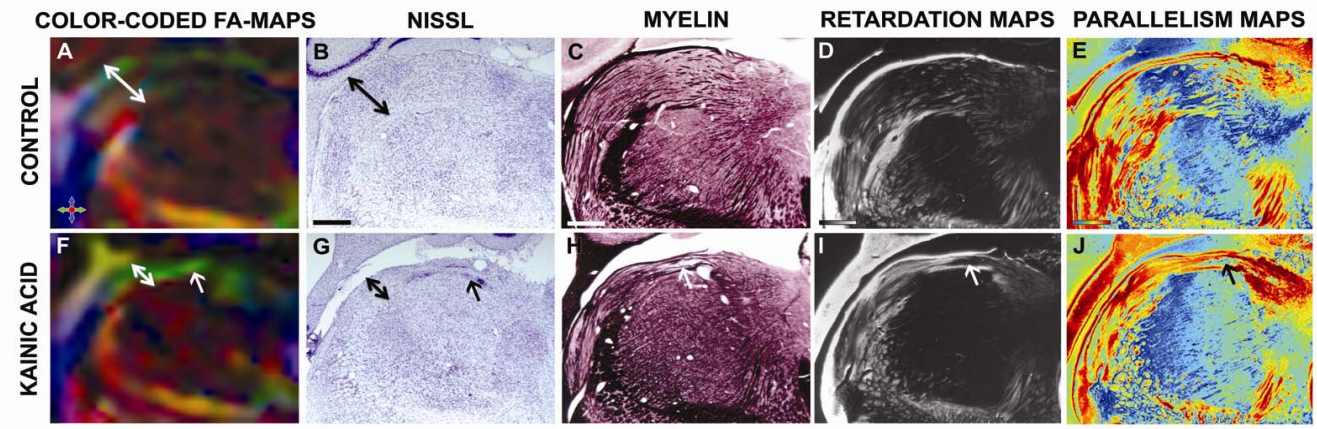

Figure 2. *Color-coded FA-maps from the left lateral thalamic nuclei in control (A) and kainic acid treated (F) rats. Directions for color-coding: red rostral-caudal, green left-right and blue dorsal-ventral. Photomicrographs are from the same area in Nissl staining (B, G), gold chloride staining for myelin (C, H), retardation maps (D, I), and parallelism maps (E, J). Control rats are presented in the upper row (A-E), and KA rats in the lower row (F-J) Arrows indicate increased FA (F), gliosis (G), demyelination (H, I and J), and double arrows shrinkage in lateral thalamic nuclei. Scale bar in Nissl and Myelin: 500 µm. Gray scale bar in retardation maps: 0-45 (black-white). Color bar in parallelism maps: 0-1 (blue-yellow-red).*

Conclusion-*Ex vivo* DTI in combination with TBSS provide valuable anatomical information about affected areas in a chronic epileptic model. A wide histological approach reveals possible microscopic causes of the increase in FA in lateral thalamic nuclei. We conclude that TBSS analysis of DTI data serves as a robust screening method to guide tedious histological analyses aimed to reveal cellular alterations of neuronal pathways.

References-[1]Smith S.M. et al., *NeuroImage* (2006)31:1487-1505; [2]Sierra A., et al., #3229 Proc. ISMRM 2009; [3]Hopkins K.J., et al., *Brain Res.*(2000)2;864(1):69-80.