DIFFUSION TENSOR IMAGE GUIDED HISTOLOGICAL ASSESSMENT OF A KAINIC ACID RAT EPILEPSY MODEL EX VIVO UTILIZING TRACT-BASED SPATIAL STATISTICS ANALYSIS

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Introduction

Epilepsy is a challenging disease for imaging due to heterogeneous, widespread and non-focal changes in the brain tissue. Diffusion tensor imaging (DTI) and tract-based spatial statistics (TBSS)[1] analysis has been successful to identify changes in patients with epilepsy[2]. However, histological analysis in experimental epilepsy models is necessary to understand the nature of these changes during epileptogenesis. The present study is focused on characterizing the interrelationship of histopathological changes and the highlighted areas in TBSS and eventually the aim is to find new brain areas contributing to epileptogenic process.

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 23×15×15mm^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. For the TBSS analysis, fractional anisotropy (FA) maps from individual subjects were determined. From the FA maps mean FA image was calculated and thinned to represent the mean FA skeleton onto which individual FA values were projected, and finally fed into the statistical group-analysis[3]. For histological analysis, 30 µm-frozen sections were stained with Timm staining in order to study mossy fiber sprouting in the hippocampus, with Nissl staining to analyse the cytoarchitecture, and with Gold chloride staining to visualize myelin in control and KA animals.

Results

The visual output of TBSS results highlights several brain areas with significant changes in FA-values between control and epileptic animals (Fig.1).

The axonal plasticity in the hippocampus (HC) is a well known phenomenon described previously in the literature[4]. Dentate gyrus (DG) showed increase in FA, consistent with mossy fiber sprouting[5] (Fig.2B,G). Color-coded FA-maps of this area presented orientational dependency of water diffusion and different pattern than in control animals (Fig.2A,B).

Entorhinal cortex (EC) is connected to DG via perforant pathway and after SE structural damage has been detected in this region[6]. In TBSS analysis, EC showed decrease in FA that could be due to axonal abnormalities showed in myelin staining (Fig.2C,H) and cell loss in Nissl staining (data not shown).

Thalamic nuclei (THA) are part of the corticothalamic pathway in brain, and also one of the targets in epilepsy[7]. They showed both increased (Fig.1) and decreased (data not shown) FA, and that could be a combination of gliosis (Fig.2D,I), cell loss and demyelination (Fig.2E,J) in those areas.

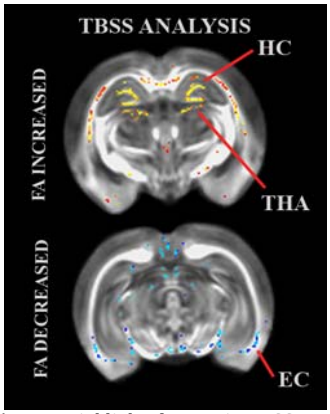

Figure1.*Highlighted areas in TBSS analysis comparing control and KA animals (red-yellow increased FA, blue decreased FA)*

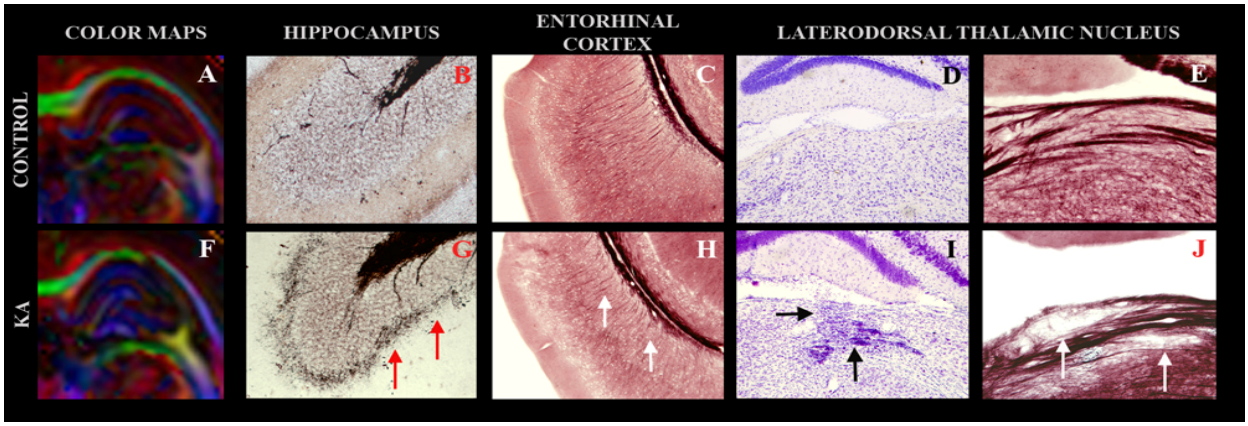

Figure 2. *Color-coded FA-maps from a control (A) and an epileptic (F) animal. Photomicrographs from hippocampus (B,F) stained with Timm staining (20x) [Red arrows: mossy fiber sprouting], entorhinal cortex (C,H) stained with gold chloride staining (20x) [White arrows: axonal abnormalities], and laterodorsal thalamic nucleus (D,E,I,J) stained with both Nissl staining (10x) [Black arrows: gliosis] and myelin staining (20x) [White arrows: demyelination]. Control animals are presented in the upper row (B-E), and KA animals in the lower row (G-J).*

Conclusion-*Ex vivo* DTI in combination with TBSS provides "anatomics" of the brain pathology during experimental epileptogenesis, revealing areas involved in the epileptogenic process in animal models. This technique has a great potential to serve as a robust screening method to guide tedious histological analysis aimed to reveal cellular alterations of neuronal pathways.

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