DIFFUSION TENSOR IMAGING OF INTACT AND INJURED RAT HIPPOCAMPUS– HISTOPATHOLOGICAL CORRELATES FOR ALTERATIONS CAUSED BY STATUS EPILEPTICUS AND TRAUMATIC BRAIN INJURY

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Introduction-Diffusion tensor imaging (DTI) has become a widely used technique to visualize intact white matter structures and changes caused by different pathologies in brain. While DTI of major myelinated tracts is relatively well characterized, the fact that DTI provides also high contrast in many anatomical areas outside the major myelinated tracts e.g. in sub-structures in hippocampus is getting more attention. The aim of this study was to investigate which cellular and tissue level features influence anisotropic diffusion of water in sub-regions of rat hippocampus and how this is altered after injury caused by status epilepticus (SE) or traumatic brain injury (TBI).

Materials and methods-SE was induced with pilocarpine (i.p., 320 mg/kg) in male Wistar rats (n=17 + 13 controls). In another group TBI was induced by lateral fluid percussion in male Sprague Dawley rats (n=8 + 7 controls). Six months (n=5 + 5) or one year (n=12 + 8) after SE, and 7 months after TBI animals were perfused intracardially. *Ex vivo* 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). For TBI animals: TR=1s, TE=60ms, data matrix 256×74×56 zero padded to 256×148×112, FOV 29.3×17×12.8mm^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 histological analysis, 30 µm-frozen sections were stained with Timm (mossy fiber sprouting), Nissl (cytoarquitecture, cell death, gliosis) and gold chloride (myelin) stainings.

Results-Color-coded fractional anisotropy (FA) maps showed in great detail the lamination[1] of the hippocampus (Fig. 1A-C). Structures as CA1 (dorsal-ventral (blue)), stratum lacunosum-moleculare (rostal-caudal (red)), dentate gyrus (dorsal-ventral (blue)), and CA3 (rostal-caudal (red)) are clearly delineated.

After injury, changes affected the direction of the principal eigenvector as indicated by color-coding in CA3a, to left-right (green/yellow) (Fig. 1B,C). This area showed loss of cells in the pyramidal cell layer in Nissl stained histological sections (Fig. 1E,F). In CA3bc, direction of the principal eigenvector changed dramatically to dorsal-ventral (blue) in both SE and TBI (Fig. 1B,C), and furthermore, a significant increase in FA (control: 0.23±0.04; SE: 0.32±0.07, P=0.0002; TBI: 0.35±0.06, P=0.008), mainly because of an increase in the axial diffusivity in both SE and TBI hippocampus (control: $0.96.10^{-3}$±$0.21.10^{-3}$ mm^2/s; SE: $1.12.10^{-3}$±$0.14.10^{-3}$ mm^2/s, P=0.006; TBI: $1.03.10^{-3}$±$0.09.10^{-3}$mm^2/s, P=0.008). Myelin staining revealed changes in the orientation of myelinated fibers in this area (Fig. 1H,I).

In stratum lacunosum-moleculare direction of the principle eigenvector changed after SE to dorsal-ventral (blue) (Fig. 1B,C) and axial and radial diffusivities were significantly increased (no changes in FA). This area contains fibers coming from the perforant pathway and in myelin staining an increase in myelinated fibers was seen after status (Fig. 1K). After TBI, this area showed also an increase in left-right diffusion (green) (Fig. 1C), but no changes in other DTI parameters. The stratum lacunosum-moleculare showed loss of myelinated fibers (Fig. 1L).

Even though dentate gyrus did not show changes in diffusion orientation after SE (dorsal-ventral), FA increased significantly (control: 0.36±0.05; SE: 0.45±0.05, P=0.0005) due to an increase of myelinated fibers (Fig. 1K) and mossy fiber sprouting (not shown)[2]. Hippocampus in TBI animals showed also similar orientation changes and a significant decrease in FA (TBI: 0.38±0.04, P=0.04) due to a decrease of myelinated fibers (Fig. 1L).

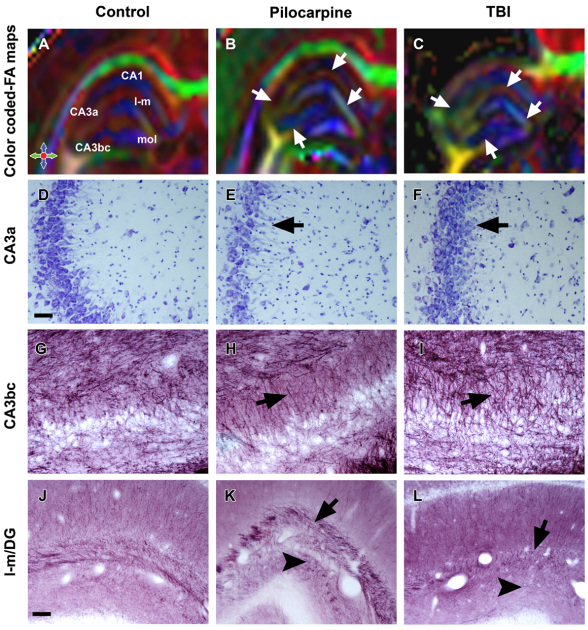

Fig. 1. Color-coded FA maps of hippocampus in a control (A), pilocarpine (B), and TBI (C). Directions for color-coding in the color-coded FA maps: *red* rostral-caudal, *green* left-right, and *blue* dorsal-ventral. Photomicrographs of CA3a in Nissl stained sections in a control (D), pilocarpine (E), and TBI (F) hippocampus. Black arrows indicate loss in pyramidal cell layer. Photomicrographs of CA3bc in gold chloride or myelin stained sections (G-I). Black arrows indicate changes in fiber orientation. Photomicrographs of stratum lacunosum-moleculare and molecular layer of dentate gyrus in myelin stained sections (J-L). Black arrows indicate changes in myelinated fiber density in stratum lacunosum-moleculare, and head arrows in molecular layer. Scale bars: 100 µm in panels D-I, 200 µm in panels J-L.

Conclusions-High resolution color-coded FA maps can detect tissue structure in hippocampus as also shown previously[3,4]. We were able to find histopathological correlates for DTI changes caused by SE or TBI. Our data indicate that diffusion anisotropy is influenced not only by major myelinated fiber bundles but also by other structures, e.g. neurons, in the hippocampus. These results shed light to cellular and tissue level alterations underlying diffusion anisotropy changes outside major white matter tracts, and furthermore, detection of detailed differences between etiologies sets the scene for even more wide use of DTI as a biomedical research tool and in diagnostics.

References-[1]Andersen et al. The hippocampus book. 2007 Oxford University Press. [2]Laitinen et al. Neuroimage. 2010 Jun;51(2):521-30. [3]Shepherd et al. Neuroimage. 2006 Oct 1;32(4):1499-509. [4]Zhang et al. Neuroimage. 2002 Apr;15(4):892-901.

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