Diffusion tensor imaging detects mossy fiber sprouting in rat hippocampus after status epilepticus

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Introduction

In acquired epiletogenesis, after the initial brain damaging insult several neurobiological alterations occur, including axonal sprouting, that eventually lead to spontaneous seizures. Previous studies suggest that these alterations can happen even before the occurrence of spontaneous seizures in some epilepsy models [1,2]. Axonal sprouting can be detected using manganese-enhanced MRI [3]. However, the use of manganese as a contrast agent is not clinically feasible because of its toxicity, and therefore, there is a need for completely non-invasive method for detecting the early markers of epilepsy. This study demonstrates the capability of diffusion tensor imaging (DTI) to detect changes in dentate gyrus (DG) sub region of rat hippocampus after kainic acid (KA) and pilocarpine -induced status epilepticus.

Methods

Six Male Wistar rats received KA (10 mg/kg, i.p.) and five pilocarpine (10 mg/kg i.p.) injections inducing status epilepticus and five age- and weight-matched animals served as controls. Animals were perfused transcardially with Na_2S (30 ml/min for 10 minutes) and 4% paraformaldehyde (30 ml/min for 10 minutes) six months after the status epilepticus and brains were dissected from the cranium. Prior to DTI, brains were immersed to perfluoro polyether to prevent signal from the solution. DTI experiments were carried out in a 9.4 T magnet interfaced to a Varian console using a quadrature volume RF-coil as transmitter and receiver. Data were acquired using a 3D spin echo sequence (TR=1s, TE=60ms, 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 1000s/mm^2) in six orthogonal directions and one image without diffusion weighting were obtained. From the measured images a diffusion tensor was determined and fractional anisotropy (FA) maps and color-coded FA maps were created. Timm-staining was used to quantify mossy fiber sprouting in the granule cell layer of dentate gurys.

Results

The results show a significantly increased DG volume ($p<0.01$) determined from 6 FA coronal maps covering dorsal dentate gyrus (Fig 1), in kainic acid and pilocarpine treated rats when compared to controls. Volumes were 0.44 ±0.04 mm^3, 0.70±0.12 mm^3 and 0.63± 0.11 mm^3 (mean ± SD) for control, KA treated and pilocarpine treated rats, respectively. Also a significant ($p<0.01$) FA increase was observed in dorsal dentate gyrus of KA and pilocarpine treated rats in comparison to controls. FA was also significantly ($p<0.05$) elevated in pilocarpine treated animals when compared to KA treated rats (Fig 2). The FA changes follow the increase in mossy fiber sprouting score, indicating the density of mossy fiber sprouting in histology sections corresponding to the slices used for FA analysis.

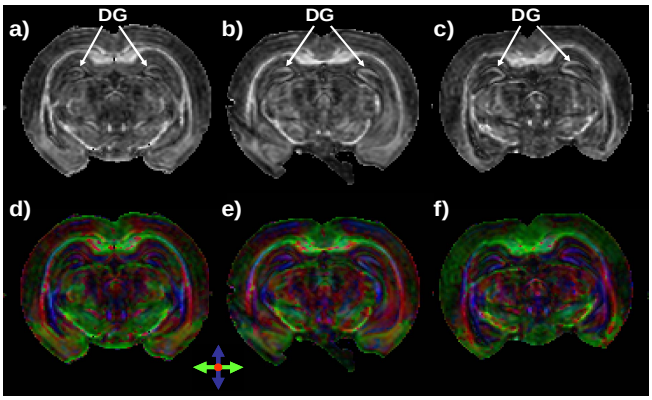

Figure 1. Fractional anisotropy (a-c) and color-coded FA maps (d-e) of a control (a,c), a kainic acid treated (b,e) and a pilocarpine treated rat (c,f). Dentate gyrus (DG) is marked in both hemispheres with white arrows in figures a-c.

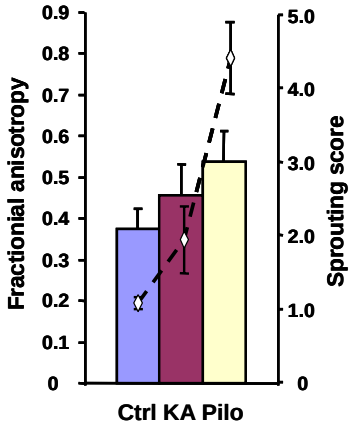

Figure 2. Mossy fiber sprouting score (◊) and fractional anisotropy (bars) of dorsal dentate gyrus.

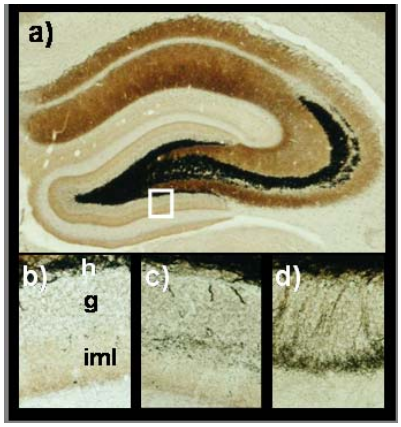

Figure 3. Histological sections from hippocampus of a control (a,b), a kainic acid (c) and a pilocarpine treated rat. **h** hilus, **g** granular cell layer, **iml** inner molecular layer

Conclusions

The increase in the volume and in the fractional anisotropy of dorsal dentate gyrus determined from FA maps suggests that mossy fiber sprouting influences water anisotropy and thus becomes detectable by DTI. As DTI is completely non-invasive technique and commonly used in clinical settings, our observations may have implications for detection of mossy fiber sprouting in patients at risk of epileptogenesis after brain trauma.

References

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