In vivo detection of axonal plasticity in rat hippocampus using DTI

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Introduction

After brain injury, several neurobiological alterations occur including structural plasticity of axons that has been found to be an important response to brain injury [1]. It has been shown that mossy fiber sprouting correlates with fractional anisotropy measured by diffusion tensor imaging (DTI) [2] and diffusion spectrum imaging [3], *ex vivo*. This study demonstrates the capability of *in vivo* DTI to detect changes in dentate gyrus sub region of rat hippocampus after kainic acid (KA) - induced status epilepticus.

Methods

Nine Male Wistar rats received KA (10 mg/kg, i.p.) injections inducing status epilepticus and five age- and weight-matched animals served as controls. 17 months later *in vivo* DTI was carried out in a 7 T magnet interfaced to a Varian console using a volume RF-coil as transmitter and a quadrature surface coil as receiver. For *in vivo* DTI, a single-slice segmented spin-echo EPI with a navigator echo for phase correction (TR=2 s, TE=27 ms, 16 segments, slice thickness 0.75 mm, axial slice, data matrix 256×256, FOV=30×30 mm^2, 32 averages for each k-space line) was used and six images with diffusion weighting (diffusion time 15 ms, b-value 1000 s/mm^2) in six directions and one image without diffusion weighting were obtained from septal dentate gyrus. After *in vivo* DTI the animals were perfused transcardially with Na_2S (30 ml/min for 10 minutes) and 4% paraformaldehyde (30 ml/min for 10 minutes) and brains were dissected from the cranium. To see whether *in vivo* DTI results correlate with *ex vivo* DTI the brains were scanned in a 9.4 T magnet interfaced to a Varian console using a quadrature volume RF-coil as transmitter and receiver. *Ex vivo* DTI was performed using a 3D spin echo sequence (TR=1s, TE=30ms, data matrix 256×72×60 zero padded to 256×144×120, FOV 29.3×16.5×13.7mm^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 data maps of fractional anisotropy (FA) were created. Timm-staining was used to assess mossy fiber sprouting and gold chloride staining to assess myelinated axons in the dentate gurys.

Results

The results show a significantly increased FA ($p<0.01$) in the dentate gyrus of KA-treated rats when compared to controls as observed *in vivo* and *ex vivo* (Figs 1 and 2). Also a good correlation was found between FA values obtained *in vivo* and *ex vivo* ($R=0.83$, $p<0.01$). The FA changes follow the increase in the density of mossy fiber sprouting and myelinated axons seen in histology sections (Fig. 3).

Conclusions

The increase in the fractional anisotropy due to axonal plasticity can also be detected using *in vivo* DTI. As DTI is completely non-invasive technique and commonly used in clinical settings, these results may have implications for the detection of axonal plasticity after several types of brain injury.

References
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[3] Kuo LW. et al. Neuroimage (2008) **41**:789-800

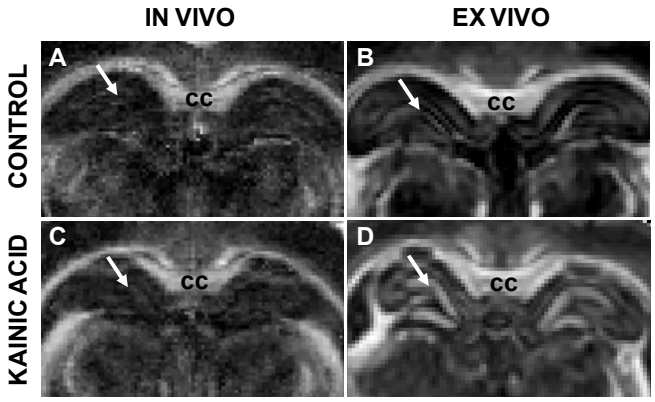

Figure 1. FA maps derived from *in vivo* (**A, C**) and *ex vivo* DTI (**B, D**) for a control rat and a KA-treated animal. The white arrows point to the left dentate gyrus. Abbreviations: *cc*: corpus callosum.

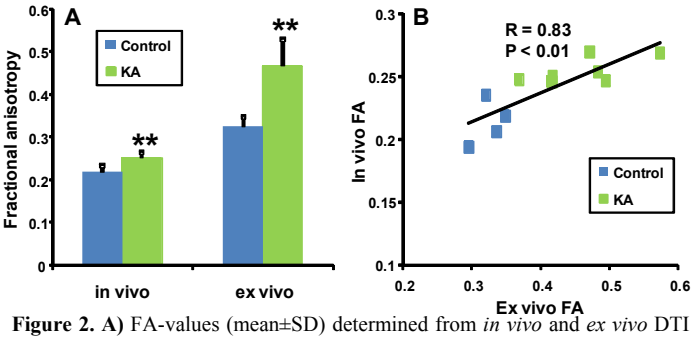

Figure 2. A) FA-values (mean±SD) determined from *in vivo* and *ex vivo* DTI for left septal dentate gyrus in control and KA-treated animals
B) Pearson correlation between FA values obtained using *in vivo* and *ex vivo* DTI from left septal dentate gyrus. **p<0.01 as compared to controls

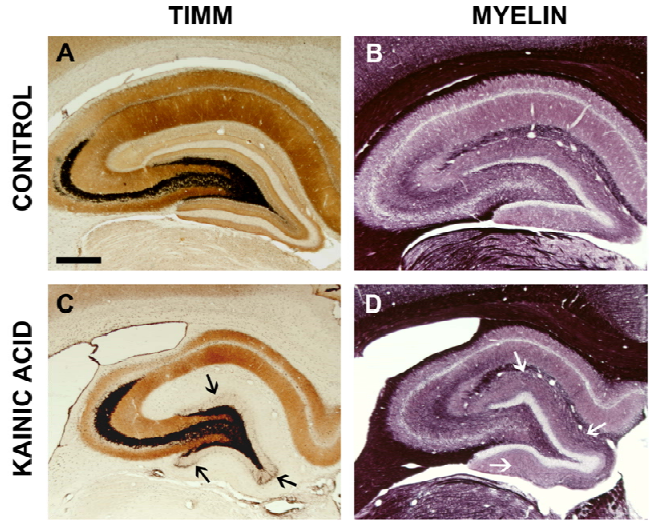

Figure 3. Photomicrographs of Timm stained sections (**A, C**) and myelin stained sections (**B, D**) of a control (**A, B**) and a kainic acid treated (**C, D**) animals. Black arrows indicate mossy fiber sprouting in the inner molecular layer of a kainic acid treated animal in Timm stained section (**C**). White arrows show increased density of myelinated fibers in outer molecular layer in myelin stained section (**D**). Scale bar: 500 µm.