Mapping Mossy Fiber Sprouting in Epileptic Rat Hippocampus With Diffusion Spectrum Magnetic Resonance Imaging

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Introduction

In order to improve the accuracy of localization for epilepsy surgery and reduce the need for invasive procedures, it is important to search for an accurate non-invasive neuroimaging method. Recent development of diffusion spectrum imaging (DSI) technique allows the mapping of 3D probability density profiles (PDF) of water molecular diffusion within each voxel [1]. With this ability, DSI is able to identify complex fiber structure in cortical gray matter and its result is consistent with cytoarchitecture study in histology [2]. This capability may be useful in the study of connectivity between the different gray matter regions within the hippocampus and provides a sensitive non-invasive method to detect microstructure changes of epileptic foci. In this study, DSI indices were used to correlate with the histological Timm's score which graded the degree of mossy fiber sprouting in the hippocampus. This study demonstrated the capability of DSI indices in differentiating normal and abnormal structures in rat hippocampus and its correlation with mossy fiber sprouting.

Materials and Methods

Male Wistar rats (180-300 gm) were injected with pilocarpine (300-380 mg/kg, intraparentally) to induce status epilepticus [3]. The behavioral seizures were evaluated according to Racine's score: stage 1, facial and body tremors; stage 2, myoclonic jerk of the whole body; stage 3, clonic convulsions of the whole body; stage 4, clonic-tonic convulsions with flexion of hind limbs; stage 5, clonic-tonic convulsions with extension of hind limbs [4]. Status epilepticus (SE) was defined with continuous convulsions with score 4 to 5 for at last one hour and terminated by pentobarbital (25-30 mg/kg, i.p). The animals were kept for more than one month and sacrificed to for MR scanning. In this study, a total of five normal rats and nine epileptic rats were used.

For MRI scanning, rat brains were dissected from the cranium and placed firmly in a plastic box filled with sucrose solution. The brain was cut transversely in front of the hippocampus region for marking the desired slices and matching the DSI results with histology. MR experiments were performed on a 3T Biospec MRI system (Bruker Biospin, Ettlingen, Germany) with an inserted micro-gradient system. Diffusion-weighted images of DSI were acquired using spin-echo pulsed-gradient sequence with 515 encoding gradients. These encodings are comprised of isotropic 3D grid points in the q-space contained within a spherical volume of 5 units. The pulsed-gradient spin-echo sequence used TR/TE = 1000/25.7 ms, number of excitation (NEX) = 2, and Δ/δ = 15/6.3 ms, yielding the maximum diffusion sensitivity (b-value) of 27000 s/mm^2. The slice thickness is 1 mm and single slice was acquired for each rat. Using Field-of-view (FOV) of 2.2 x 1.1 cm^2 and matrix size of 64 x 32, the in-plane resolution of 172 μm can be achieved by zero-filling the k-space data in both readout and phase encoding directions. Reconstruction of DSI data is based on the relationship that the echo signal $S(\mathbf{q})$ and diffusion probability density function (PDF) $P(\mathbf{r})$ is a Fourier pair, i.e., $S(\mathbf{q}) = \mathrm{FT}\{P(\mathbf{r})\}$ [1;5]. The integration of P(r) r^2 along each radial direction was used to calculate the orientation density function (ODF). To describe the mean diffusivity from DSI, mean squared length (MSL) of water molecular displacement was quantified as the second moment of the normalized PDF over the 3D space. To describe diffusion anisotropy from DSI (DA), standard deviation of the normalized ODF was computed [6]. After MR scanning, the rat brains were stained for mossy fibers with Timm stain and Timm's score was used to evaluate the mossy fiber sprouting: scores from 0 to 5 were used to define the severity [7]. To correlate the MRI results and histology, both the DSI index values and the Timm's scores of the same slice locations were evaluated in dentate gyrus (DG) and CA3 regions of both right and left hippocampus.

Results

Figure 1 showed the DA and MSL maps of one epileptic rat and the regions-of-interest (ROI) were used to derive the average values of both indices. As shown in the mapping, MSL and DA were equivalent to trace ADC and FA of the diffusion tensor. The Timm stains in CA3 and DG were also shown to provide the histology results. The correlation plots were shown in figure 2. For the correlation of DA and Timm's score, positive correlation in DG and negative correlation in CA3 were found. These results indicated that as Timm's score increased fiber architecture became more organized in DG and more disorganized in CA3. Among the results of the correlation of MSL and Timm's score, there was no significant correlation in DG but negative correlation in CA3 region, suggesting that disorganized fiber architecture in the CA3 area may restrict the water diffusivity.

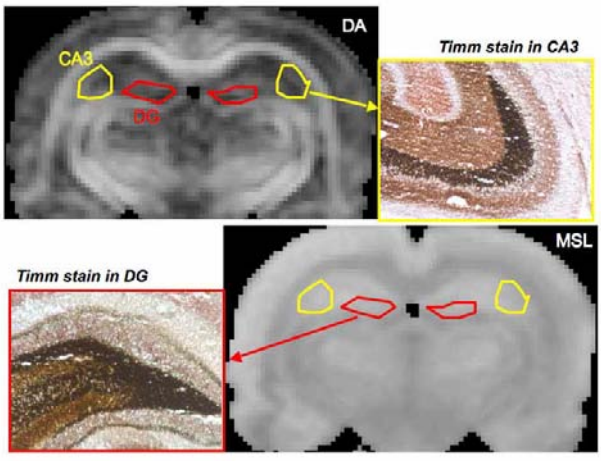

Figure 1. DA and MSL maps of an epileptic rat. The yellow and red ROI indicate CA3 and DG regions respectively.

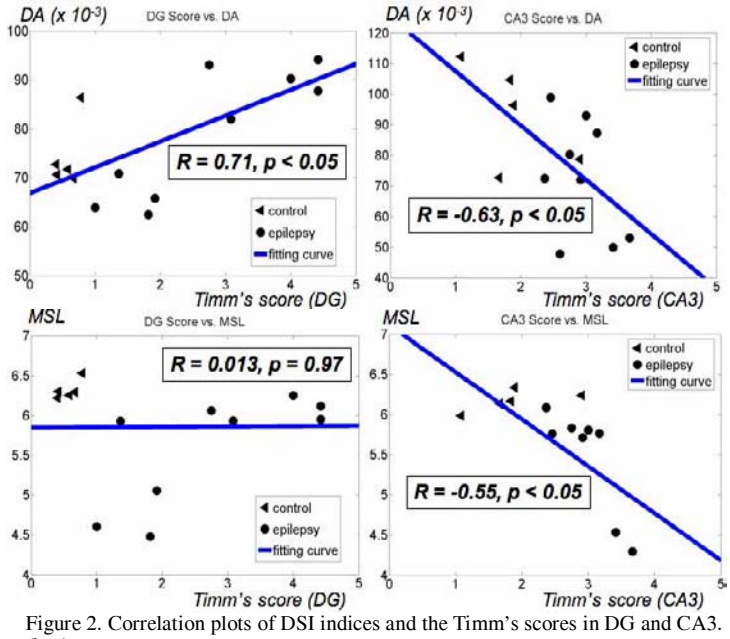

Figure 2. Correlation plots of DSI indices and the Timm's scores in DG and CA3.

Conclusions

This study demonstrated the capacity of DSI to reveal hippocampal changes in the SE rats. DA and MSL were shown to be able to correlate with the Timm's scores indicating the severity of mossy fiber sprouting. These data demonstrate that DSI can detect the microstructure changes of the epileptic foci and is a promising tool in prognosis prediction and decision making of pharmacological/ non-pharmacological treatment of epilepsy.

References

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