Whole brain diffusion tensor image analysis by tract-based spatial statistics (TBSS) in a kainic acid rat epilepsy model *ex vivo*.

K. K. Lehtimäki[1], T. P. Laitinen[2], A. Sierra[2], J. Nissinen[2], A. Pitkänen[2], and O. Gröhn[2]

[1]Department of Biotechnology and Molecular Medicine, A.I.Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, Finland, [2]Department of Neurobiology, A.I.Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, Finland

Introduction - Diffusion tensor imaging allows greatly improved differentiation of anatomical structures in the rodent brain and can detect many pathological changes[1,2]. Analysis by manual region of interest (ROI) selection is most often used to obtain quantitative data of changes in DTI parameters in the preselected anatomical regions. However, this approach has several inherent drawbacks such as user dependent selection of location and size of ROIs between different subjects. This is especially problematic when the results from different labs, which may have differing criteria for ROI selection, are compared. Furthermore, ROI based approach is crucially dependent on the prior knowledge of the brain areas affected by the disease and thus cannot easily be used in a search for new, potentially relevant brain changes. We have implemented a recently introduced whole brain voxelwise statistical analysis method, tract based spatial statistics (TBSS) [3,4], into the rat kainic acid epilepsy model for the black box statistical comparison of diffusion tensor imaging (DTI) parameters between control and kainic acid animals. While the method has previously been successful in human applications[5,6], as of today, no reports of TBSS analysis in the rodent brain exist.

Materials and Methods - Status epilepticus was induced in male Wistar rats (n=6) with kainic acid (10 mg/kg, i.p.). Controls received saline (n=4). Six months after status epilepticus, animals were perfused intracardially using Timm fixation. Prior to histological sectioning fixed brains were immersed in Fomblin and DTI was carried out in a 9.4 T vertical magnet interfaced to a Varian DirectDrive console. Data were acquired using a 3D spin echo sequence (TR = 1000 ms; TE = 60 ms; data matrix 192x64x64, zero padded to 192x128x128; FOV 23x15x15 mm^3). Six 3D data sets with diffusion weighting (diffusion time 17 ms, *b*-value 1000 s/mm^2) in six orthogonal directions and one reference data set without diffusion weighting were obtained.

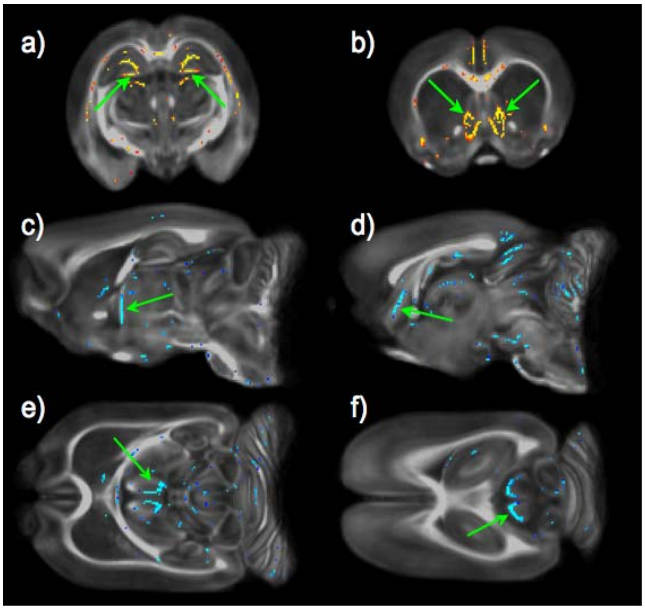

Figure 1. *FA changes in kainic acid animals as compared to healthy controls. Green arrows point to a region of interest; a) dentate gyrus, b) lateral septum, c) stria medullaris, d) medial septum, e) lateral habenular nucleus and f) superficial gray layer of superior colliculus. Red-yellow colormap indicates increased FA and blue-lightblue decreased FA in kainic acid animals as compared to healthy controls.*

Diffusion weighted data were corrected for eddy current distortions with affine (linear) alignment with *flirt*[7], which is included in the FSL software package[8] that provides all the tools used in the following analyses. The diffusion tensor was calculated with the *dtifit* program for whole brain volumes of each data set. After determination of FA-volumes we modified TBSS scripts to work with rat brain data; as an option for registration we applied free-search of best registration target for the whole data set to minimize the image warping required for other volumes. Calculated best target was used in subsequent scripts as a common template into which final transformations were done.

As described in the original papers[3,4] following registration, mean FA-image was created (background image in all panels in Fig. 1), thinned to represent mean FA-skeleton, individual animals FAs were projected onto this common skeleton and then fed into voxelwise cross-subject statistical analyses. Null distribution was built with an exhaustive permutation protocol and results are given here as $p < 0.05$, uncorrected, although we were able to reveal significant changes in the same anatomical regions with analysis as $t > 2.7$, $p < 0.05$, corrected. Initial analysis was conducted in a regular manner with group comparisons over kainic acid and healthy animals. As the rat brain, unlike the human brain, is highly symmetric and changes in the kainic acid model are known to be symmetrical, we created mirrored brain volumes for each animal and re-ran the analysis with now double the amount of data. All brain areas discussed here were highlighted in both analyses, but results were more profound while exploiting symmetry of the rat brain.

Results - Some of the findings from TBSS output are shown in Figure 1. Dentate gyrus a) has been reported to undergo mossy fiber sprouting after status epilepticus[9]; in this area we detected increased FA in kainic acid animals, and main changing tensor component was principal diffusion direction strongly supporting a sprouting hypothesis. Septal areas - lateral b) and medial septum d) - are known to have connections to hippocampal formation[10] and have indeed been used in modelling of epileptic changes after local intracerebral injection of kainic acid in that region[11]. Both the lateral habenular nucleus e) and superficial gray layer of superior colliculus f) are known to have a role in seizure control in epileptic models[12,13]. Additionally, we detected several brain areas that are not discussed in detail in the current epilepsy literature, such as the stria medullaris c).

Discussion - We have shown that the rodent *ex vivo* DTI can be combined with whole brain voxelwise statistical group-analysis by TBSS. This approach is particularly interesting in neurodegenerative animal models that may have widespread changes that are not highlighted by conventional MRI and are yet to be discovered. This is clearly indicated with our data from kainic acid models that revealed both brain regions that are known to be associated with epilepsy and anatomical regions that have not been earlier connected with epileptogenesis or epilepsy. A major strength of the animal models is that DTI findings can be readily verified with histological methods. Indeed, TBSS combined with animal models has great potential to serve as a robust screening method to guide tedious histological analysis to novel target areas in the brain.

References: *[1] Zhang J. et al., NeuroImage 15 (2002) 892-901, [2] Shepherd T.M. et al., NeuroImage 32 (2006) 1499-1509, [3] Smith S.M. et al., NeuroImage 31 (2006) 1487-1505, [4] Smith S.M. et al., Nature Protocols 2 (2007) 499-503, [5] Cader S. et al., NeuroImage 36 (2007) 19-27, [6] Douaud G. et al., Brain 130 (2007) 2375-2386, [7] Jenkinson M. et al., NeuroImage 17 (2002) 825-841, [8] http://www.fmrib.ox.ac.uk/fsl/, [9] Nairismagi J. et al., NeuroImage 39 (2006) 130-135, [10] Garrido Sanabria E.R. et al., Neuroscience 142 (2006) 871-883, [11] Venero J.L. and Hefti F. Brain Res 790 (1998) 270-277, [12] De Sarro G. et al., Brain Res 591 (1992) 209-222 , [13] Bressand K. et al., Brain Res 943 (2002) 93-100.*