Diffusion tensor imaging reveals nigrostriatal degeneration in the 6-Hydroxydopamine rat model for Parkinson's Disease

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Introduction

Parkinson's disease is a basal-ganglia related disorder characterised by neurodegeneration of the nigrostriatal connection resulting in dopamine depletion in the affected striatum. In contrast to other neurodegenerative disorders, such as Huntington's Disease, very subtle structural changes occur in the brain challenging the MRI researcher to find a specific imaging marker of PD-related neurodegeneration. Very recently Diffusion Tensor Imaging has been proven useful in characterising neurodegeneration by quantifying changes of anisotropy in both white and grey matter structures. In the present study we investigated whether diffusion tensor imaging could reveal changes in the well known model of PD, the 6hydroxydopamine rat model.

Material en methods

Animal model: Six rats were -6 weeks prior to imaging- injected with 6-hydroxidopamine (4µl, 5µg/µl dissolved in 3 µl of 0.9% sterile NaCl containing 0.1% ascorbinic acid) in the right striatum (AP 0.0mm, L 2.8 mm V5.5mm). This injection type is known to cause specifically retrograde leasioning of the nigrostriatal neurons (Figure 1). Imaging was performed successfully on all 6 PD (273.3±8.4g) and 4 control animals (188.3±8.7g).

Figure 1

MRI: High resolution (voxel size: 0.117µm x 0.117µm) DTI (SE DTI: TR/TE = 2200/43ms, 14 averages, b= 800 s^2/mm, diffusion sensitizing gradients along 7 directions) of the entire rat brain (coronal slices with slice thickness of 0.43mm) was performed on a 7T Bruker system under 1.5-2% isoflurane anesthesia. As the measurements took 8 hours, rats were intensively monitored and their temperature was kept constant at 37,2±0,1°C and breathing rate at 57±3 breaths per minute.

Data-analysis: DTI images were processed using in house developed Matlab routines to generate the eigenvalue and FA maps. First, region of interest analysis was performed by segmenting different grey (striatum, substantia nigra) and white matter structures (internal and external capsule, corpus callosum) on the different DTI maps using AMIRA software. Secondly, Voxel based statistics was performed using Matlab routines after accurate coregistration of DTI maps using non-linear warping allowing voxel-wise statistical mapping of tensor invariant differences between the control and PD groups. Visualisation of the significance maps (figure 3) was performed in AMIRA.

Results

Region of interest analysis showed no significant difference for any structure or for any parameter between control and PD rats. An example of the data in the substantia nigra is shown in figure 2. Within the control and PD group asymmetry ratios were calculated which were neither significantly different between both groups (data not shown).

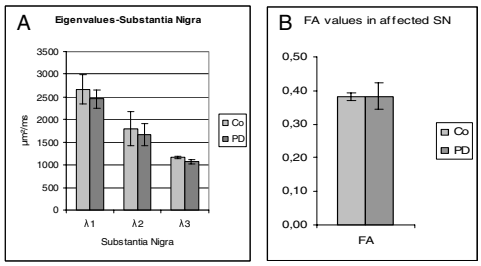
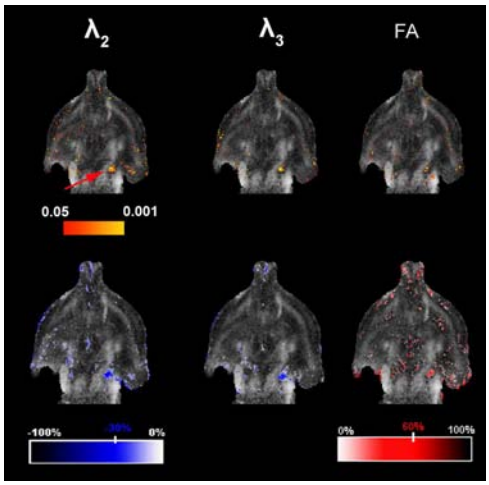


*Figure 2: (A) Eigenvalues (*λ1, λ2, λ3*) and (B) FA values measured in the affected and right SN of respectively PD and control animals. No significant differences were observed.*



Voxel Based Statistics (VBS) (figure 3, top row) showed however significant differences in the substantia nigra (red arrow) for λ2, λ3 and FA ($p<0.05$). The orange-yellow colored pixels display significant differences between control and PD group and are projected on FA maps of control animals.

To estimate how these parameters change within these voxels, relative difference maps were calculated ((PD group – Control group) / Control group *100%) displaying changes of the parameters relative to control animals (figure 3, bottom row). It was shown that the radial diffusivity λ2 and λ3 was decreased (blue) whilst FA was increased (red) (Figure 3, bottom row) in the affected SN.

Figure 3: Top row significance maps after VBS between control and PD rats. Bottom row: relative difference maps displaying the change of the parameters in the top row.

Discussion

We demonstrated for the first time significant changes in the SN of a rat model of PD using Voxel Based Statistic (VBS) on in vivo DTI data. These changes are the result of retrograde degeneration of the nigrostriatal neurons and not an artifact of the toxin injection as was reported in an earlier study that showed artefactual T2 changes after injection of 6OHDA in the SN itself (Kondoh et al Exp. Neurol. 2005). VBS actually allowed us to visualize changes in those structures that are too small to be accurately manually delineated as an ROI without creating effects of volume averaging. We observed a decrease in the radial diffusivity - λ2 and λ3 - and an increase in FA. These observations might be related to cytotoxic oedema as a result from the oxidative stress caused by the toxin. In the same animals also PET and behavioral studies were performed confirming degeneration of nigrostriatal neurons.

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