Quantitative Diffusion Tensor Imaging in a murine model of Parkinson's Disease

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Introduction. Nigrostriatal degeneration, a primary pathological hallmark of Parkinson's disease (PD) is reproduced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication. MPTP-treated animals show common behavioral, motor and pathological features of PD. Recent works from our laboratories indicate that immunization strategies can lead to viable therapeutic endpoints for disease. However, successful therapy requires early intervention before diagnosis and excessive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), the brain subregion most affected in PD. Thus, to improve diagnostic imaging for PD, we used diffusion tensor imaging (DTI) to measure fractional anisotropy (FA) and mean diffusivity (D_{av}) and co-register those measures with histopathology in an effort to evaluate the capacity for DTI to detect neuronal degeneration in MPTP-treated animals.

Methods. *Animals and MPTP intoxication.* Male C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and used at 6-10 weeks of age. Mice were administered 18 mg/kg MPTP (free base) (Sigma-Aldrich Chemical Co., St Louis, MO) i.p. in 250 µl of PBS every 2 hours for 4 total injections. Controls were administered PBS alone. Seven days post-treatment, mice were anesthetized by inhalation of 1% isoflurane in a nitrous oxide/oxygen mixture prior to MRI ,scanned by MRI, then sacrificed for histological examination. *Magnetic Resonance Imaging (MRI) and Diffusion Tensor Magnetic Resonance Imaging (DTI) Acquisition.* MRI data were obtained using a Bruker Avance 7 Tesla/21 cm system using actively decoupled 72 mm volume coil transmit and a laboratory built 1.25 x 1.5 cm receive surface coil. DTI data were acquired using a two-shot diffusion-weighted spin-echo echo-planar imaging (T_E=29 ms) with respiratory gating to prevent motion artifacts T_R=10-15 s, depending on respiratory rate, one shot per breath. EPI acquisition parameters included 14 slices, 200 KHz bandwidth, 96 X 96 in plane acquisition zero-filled to 256 X 256, and a 0.5 mm slice thickness. The diffusion encoding used is a balanced, rotationally-invariant and alternating polarity icosahedral scheme (12 directions)[1,2]. The encoding scheme was designed to reduce background-diffusion gradient couplings[3]. Diffusion weighting b-factor = 800 s mm^{-2}, δ=4ms, Δ=15ms, G_{dmax}= 40 G/cm, 200 µs rise time, 7 averages for b=0 acquisition, 3 averages for each b=800 encoding direction, for a total acquisition time of 15-30 min, depending upon respiration rate. *Data Analysis.* Analysis of the diffusion-weighted data was performed using custom programs written in IDL as previously described[1,4]. Analysis produced maps of the three primary eigenvectors of diffusivity (λ_1, λ_2, λ_3), average diffusivity (D_{av}) and fractional anisotropy (FA). *Immunohistochemistry and Quantitative Morphology.* Terminally anesthetized mice were transcardially perfused with PBS followed by 4% paraformaldehyde in PBS, brains excised, post-fixed overnight, cryopreserved and snap-frozen in 2-methylbutane. Frozen brains were mounted in OCT media, cut into 30 µm thick sections with a cryostat (Leica, Bannockburn, IL) and sections from the SN and striata collected as free floating tissues. Sections were concomitantly immunostained for expression of tyrosine hydroxylase (TH) with rabbit anti-TH antibody (1:2000; Calbiochem/EMD Biosciences, Inc., San Diego, CA). Anti-TH immunoglobulin was detected with biotinylated goat IgG anti-rabbit Ig (1:200 to 1:400) followed by streptavidin-conjugated horseradish peroxidase (HRP) (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). HRP was visualized with 3,3'-diaminobenzine (Vector Laboratories). Sections were mounted to microscope slides and nigral sections were stained for Nissl with thionin. All sections were dried and covered with glass coverslips. Numbers of TH+ and Nissl-stained neurons in SN were analyzed by unbiased stereological analysis using the optical fractionator module of Stereo Investigator software (MicroBrightfield, Williston, VT). *Statistical Analysis.* A multi-plane DTI viewer module was used to place regions of-interest in the striatum, corpus callosum and substantia nigra. Results were compared between PBS- and MPTP-treated groups using unpaired two-tailed t-test with SPSS 14 software (SPSS Inc, Chicago, IL).

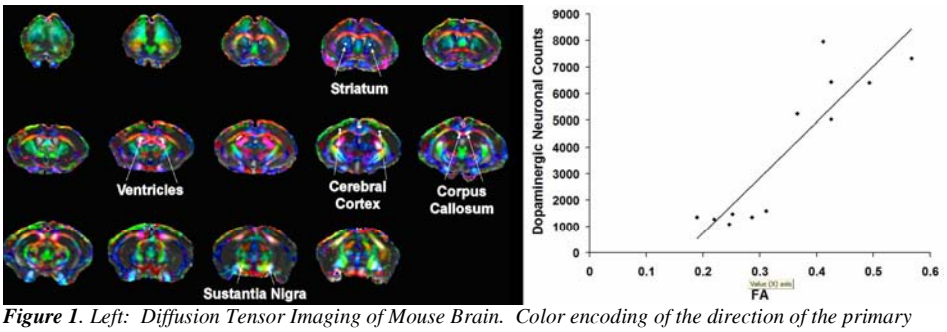

***Figure 1**. Left: Diffusion Tensor Imaging of Mouse Brain. Color encoding of the direction of the primary eigenvalue (λ_1) of the diffusion tensor is used to identify anatomical regions for analysis. Acquisition and analysis procedures are detailed in the text. Right: individual points and linear regression between fractional anisotropy in the substantia nigra and the number of dopaminergic neurons counted in each mouse studied. A significant correlation (R^2=0.80) was found between these measures in individual mice. No other correlations between either dopaminergic or glutaminergic neurons counted in the substantia nigra or density of tyrosine hydroxylase stain within the striatum were found in any other region versus either FA or D_{av}.*

Results. Quantitative DTI detects neuronal loss within the SN. The level is manifest by significant decreased fractional anisotropy (FA) (p=0.00018) for mice seven days post MPTP intoxication compared to PBS treated controls. This was accompanied by a loss of 70% of the dopaminergic neurons (p<0.0001) as measured by stereology. Moreover, FA levels in SN were directly correlated (r^2 = 80, p<.001) with numbers of surviving nigral dopaminergic neurons.(Figure 1). No differences in FA were found within the striatum, corpus callosum, cerebral cortex and ventricles.

Conclusion. Preliminary results indicate that the use of DTI for monitoring loss of dopaminergic neurons is feasible in a mouse model of PD and may provide an important non-invasive biomarker for assessing the kinetics and effects of experimental therapies in this disease model.

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