High Field MR Microscopy of Progressive Supranuclear Palsy in the *Ex Vivo* Human *Globus Pallidus*

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Introduction: The differential diagnosis of neuropathological conditions remains a significant challenge of clinical neurology and generally is confirmed only by postmortem histology. In this study, high resolution MR microscopy (MRM) of the postmortem human *globus pallidus* (GP) acquired at 21.1 T (900 MHz) was employed to distinguish between Progressive Supranuclear Palsy (PSP, Steele–Richardson–Olszewski syndrome) specimens and healthy brain tissue. PSP is a progressive parkinsonian disorder and the second most common cause of human neurodegeneration [1]. Pathologically, PSP is a sporadic four repeat tauopathy and associated with neuronal loss in both cortical and subcortical structures [2, 3]. With histological specimen, Magnetic Resonance Microscopy (MRM) at ultra-high magnetic fields provides the sensitivity required to visualize and measure potential biomarkers and relaxation parameters that may be related to neurodegeneration. As such, these investigations may provide insight not only into underlying pathology but also future diagnostic evaluations of PSP.

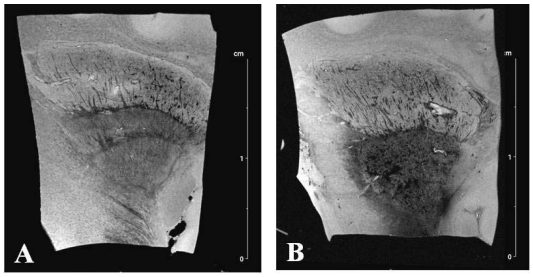

Fig 1. 3D FLASH MRI partitions (TE/TR=4/50 ms, isotropic resolution=50 μm, acquisition time = 4.3 hrs) of a (A) healthy GP section compared to (B) a PSP specimen acquired at 21.1 T.

Methods: Samples of the *globus pallidus* (GP) were harvested postmortem from patients diagnosed with PSP (n=8) for comparison to neurologically healthy controls (n=8). The specimens were immersion fixed using 4% paraformaldehyde (PFA) in phosphate-buffered saline (1xPBS), loaded into medical cassettes and maintained at 4 ˚C until evaluation. Prior to imaging, the samples were washed in 1xPBS and immersed in a fluorocarbon (FC-43, 3M Corp., Minneapolis, MN) at room temperature (21-23 ˚C) for 45 min to remove air bubbles.

All MR data was acquired using a 21.1-T, ultra-widebore (105-mm) vertical magnet equipped with a Bruker Avance III console and specially built, 1 T/m/A triple axis gradient system (RRI Inc., Billerica MA). Utilizing a 33-mm birdcage coil, three-dimensional Fast Low Angle Shot (FLASH) gradient recalled echo scans (TE = 4 ms; TR = 50 ms) were acquired at the isotropic resolution of 50 μm over 4.3 hours at 14 ˚C. In addition, T_2*-weighted multiple gradient recalled echo (GRE) and T_2-weighted spin-echo (SE) sequences were acquired over a range of echo times (TE = 3.5-45.4 ms and TE = 7.9-94.8 ms, respectively) to generate quantitative relaxation maps (Fig. 2A-D). Spatial resolution for these scans was 100x100x550 μm. Regions of interest (ROIs) included the entire GP *interna* (GPi), GP *externa* (GPe) and *putamen* (Put). MR findings were corroborated by histology using Prussian blue (PB) staining on 5-μm thick brain sections (Fig. 3).

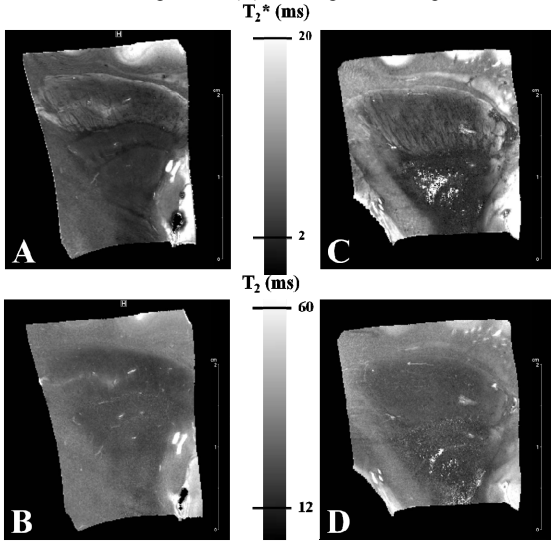

Fig 2. A-D: Relaxation maps visualizing T_2* (A & C) and T_2 (B & D) for the Control (A & B) and PSP (C & D) specimens.

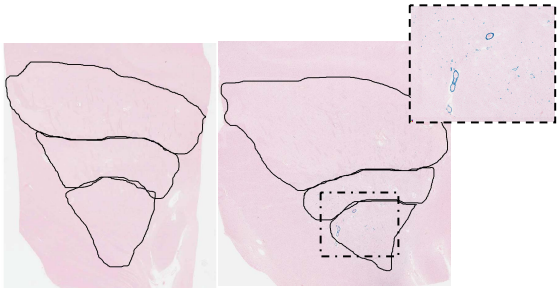

Fig 3. Prussian blue staining for iron burden in healthy (left) and severe PSP (right) specimens. Insert shows iron in the GPi.

Results & Discussion: Compared to surrounding tissue, high resolution 3D datasets (Fig. 1) and parametric relaxation maps (Fig. 2) clearly display a darker GP with shorter T_2 and T_2* relaxation in the PSP specimens than in the controls. Utilizing an independent samples t test ($p<0.05$), statistical significance was found between PSP specimen and controls for T_2* and T_2 times of the GPi and GPe as well as T_2* in the Put. In agreement with MRM, PB staining showed that the GPi displayed the largest difference between the PSP samples and controls, with the former showing a higher total iron burden. Previously, a wide range of postmortem biochemical studies have found increased iron content in the parkinsonian *GP* [4] when compared to healthy tissue. Furthermore, this iron accumulation has shown to be proportional to the presence and severity of the disease. For MRM, iron possesses paramagnetic properties that impact relaxation and induce localized susceptibility-based perturbations of the magnetic field. In the current study, the higher iron burden resulted in the loss of T_2- and T_2*-weighted signal and reduced relaxation both globally across the entire specimen and differentially in subregions of the GP. Non-hem iron in the brain, therefore, serves as a contrast enhancer and as a potential pathological biomarker in MRI studies, with the ability to distinguish PSP samples from healthy controls.

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