# Correlation of T2* and susceptibility mapping with histochemistry in the SN

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**Introduction** Death of neuromelanin containing dopaminergic neurones in the substantia nigra is a pathological hallmark of Parkinson's disease (PD). This cell death is accompanied by iron accumulation. Iron and neuromelanin are best identified histochemically in post mortem (PM) tissue. In this study we show that high resolution images and susceptibility maps acquired post mortem at 7T can be used to distinguish iron and neuromelanin in the SN.

**Methods** The post mortem brain stem of a 67 y.o. subject with no known neurological conditions was fixed in 10% formalin, washed in 0.09% saline for 24 hours before scanning and then placed in a Perspex sphere filled with saline. A 3D FFE HiRes scan was acquired for identification of anatomical details; TR/TE=46/15ms, FA=15°, FOV=90x90x40mm$^3$, 0.3mm isotropic resolution; Taq=10.13 hours. 3D FFE LoRes scans were acquired for susceptibility mapping; TR/TE=26/12ms, FA=12°, 0.5mm isotropic resolution, FOV =110x110x110mm$^3$, Taq=21.06min. This scan was repeated with the sample at 4 different orientations with respect to $B_0$ for robust estimation of susceptibility. The brain stem was then sectioned and Perls' stain (showing iron and neuromelanin) and TH stain (for dopaminergic cells) were applied to adjacent transverse slices. The histology stains were digitized into maps using a Hamamatsu NanoZoomer. A susceptibility map was calculated from the 4 LoRes images using a k-space threshold method [1] and registered to the HiRes scan using the transformation matrix obtained from registration of the LoRes data (linear, 12 DOF FLIRT (FSL)). The HiRes scan and TH map were registered to the Perls' map in MINC (using a linear, 7DOF, semi-automatic method based on manual landmarks, which compensated for deformation of the tissue during sectioning). This transformation was also applied to the susceptibility map. The TH map was converted to grey scale and the Perls' map was processed using colour deconvolution [2] to separate the blue contrast corresponding to iron from the brown contrast corresponding to neuromelanin. The maps were used to create binary masks of regions of high iron (Perl blue), neuromelanin (Perl brown) and dopaminergic cells (TH) by thresholding based on visual assessment. Since the histology maps showed details at much higher spatial resolution than the MR scans direct correlation of pixel values was not possible at this stage. Finally, a mask outlining the brain stem was created to remove the background, damaged tissue, small veins visible in the Perls' map, and an air bubble artefact in the susceptibility map. The masks were applied to the HiRes (T2* weighted signal, T2*w) and susceptibility images, and histograms were created of the MR values within the iron, neuromelanin and TH masks. Joint histograms of susceptibility against T2*w for the pixels within the different ROI were also created.

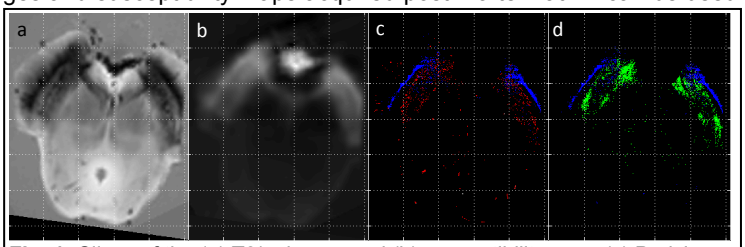


**Fig. 1:** Slices of the (a) T2*w image and (b) susceptibility map, (c) Perls' map masked for iron (blue) and neuromelanin (red) (d) masked TH map with neuromelanin with iron from Perls overlaid (green). Note that the dopaminergic cells (TH) are co-localized with the neuromelanin (Perl).

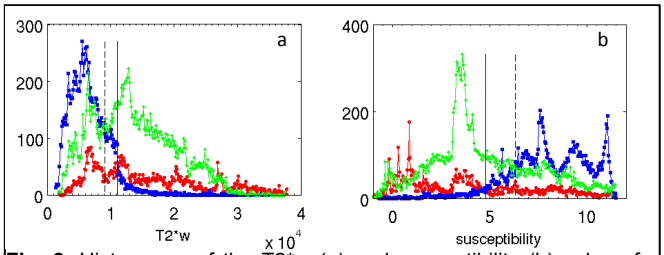


**Fig. 2**: Histograms of the T2*w (a) and susceptibility (b) values for iron (blue), neuromelanin (red) and TH (green) regions with visually selected thresholds separating iron and neuromelanin (solid line), iron and TH (dashed line).

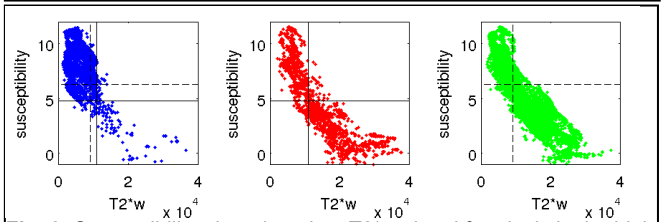


**Fig. 3**: Susceptibility plotted against T2*w signal for pixels in the high iron (blue), neuromelanin (red) and TH (green) ROIs. Lines indicate boundaries shown in Fig 2.

**Results** The binary masks showed that iron and neuromelanin are separated in the Perls' map (Fig 1c.), but that the location of the neuromelanin in the Perls' map corresponded to that of the dopaminergic cells in the TH map (Fig. 1c and 1d). Overlaying the masks on the HiRes T2*w image allowed the hypointense lateral 'black stripe' to be identified with high iron content and more medial 'grey patches' to be identified with neuromelanin and dopaminergic cells (Fig 1a) (mean signal as a fraction of white matter signal for the black stripe and grey patches was 0.3±0.2 and 0.7±0.4, respectively). Similarly susceptibility was higher in the high iron region (mean=8.0±2.1 ppm) and lower for the high neuromelanin region (mean=4.4±3.2 ppm) (with a similar value for dopaminergic region). Histograms of MR pixel values for the different masks showed the different distributions of T2*w signal (Fig 2a) and susceptibility (Fig. 2b) values within the iron, neuromelanin and TH regions. The scatter plots showed similar discrimination between ROI and that pixels with high susceptibility have low T2*w signal.

**Discussion** High resolution T2*w data and susceptibility maps obtained post mortem allow identification of regions of high iron and neuromelanin concentration that are also detected by Perls' staining. As neuromelanin containing neurons are considered to be catecholaminergic cells, the overlap between their location and the TH positive regions was expected. It is likely that the 'grey patches' correspond to the nigrosomes (subgroups of dopamine-containing neurons) [3], but additional calbindin $D_{28K}$ staining is needed to confirm this hypothesis. Better understanding of the relationship between MR data and histology will improve the analysis of in vivo MR scans, and may reduce the variability in the MR data on iron changes in the SN in PD which is found in the literature, by allowing consistent siting of ROIs in MR images of the SN. **References** [1] Wharton et al., MRM, 2010, 63:1292-1304; [2] Ruifrok & Johnston, Anal Quant Cytol Histol, 2001, 23: 291-299; [3] Damier et al., Brain, 1999, 122:1421-1436. *This work was funded by the Medical Research Council, UK.*