In vivo 7T imaging of nigrosome loss in Parkinson's disease

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Introduction Clinical diagnosis of Parkinson's disease (PD) shows limited accuracy. Functional imaging techniques such as the dopamine transporter scanning [1] have high sensitivity and specificity, but are expensive. Therefore, there is a need for a new cost effective and accurate diagnostic method. Changes in the substantia nigra (SN) occur in PD (including dopaminergic cell loss and increased iron concentration) particularly affect the nigrosomes, which are SN substructures that can be identified using immunostaining calbindin D_{28k} (CAL) [2]. **Aim**: To investigate whether: **(1)** nigrosomes can be visualised directly *in vivo* by comparing *in vivo* and *post mortem* (PM) high field MRI and histochemical data; **(2)** nigrosome visualization could provide a new diagnostic marker of PD.

Methods Two PM midbrains were scanned with a 3D FFE sequence: TE/TR=15/46ms, FA=15°, SENSE=1, FOV=90x90x40mm³, 0.3mm isotropic resolution, NSA=20, in 10:22 hours. Perls', TH and CAL stains of the adjacent transverse slices were digitized using a Hamamatsu NanoZoomer and co-registered with high resolution MR data using a semi-automatic method based on manual landmarks (9 DOF) to compensate for deformation of the tissue due to sectioning. The Perls' map was processed using colour deconvolution [3] to separate iron (blue contrast) from neuromelanin (NM, brown contrast). The resulting maps, CAL and TH stains were converted to grey scale and thresholded to obtain masks of the iron+, NM+, TH+ or CAL+ regions. After manually correcting for obvious staining artefacts, masks were overlaid on the high resolution T2*w images to compare the characteristics of the different regions of the SN (Fig 1). Ten PD patients and nine age-matched, healthy controls (HCs) were scanned using a 2D FFE sequence with TE/TR=16/385ms, FA=40°, 0.35x0.35x1.0mm³, FOV=180x160x16mm³, in a 10 min acquisition. Two blinded neuroradiologists classified T2*w datasets based on the presence/absence of the hyperintense structure within the dorsal/posterior hypointense region, meeting the criteria: visible on both sides of the SN, present on at least two adjacent axial slices, surrounded for more than half its boundary by hypointensity, not a part of perivascular space, extending below the red nucleus (RN).

Results *PM* Comparison of the stains revealed a CAL– region co-localised with dopaminergic cells (TH+) inside a larger CAL+ area (Fig 1, 1st row); a region with such staining, size and approximate location was previously defined as nigrosome 1 [2]. This area was also iron– and NM+ and corresponded to the hyperintense substructure visible in the PM T2*w data (Fig 1, 3rd, 4th, 6th row). The yellow mask representing iron+ regions on the Perls' stain overlapped with hypointense regions on the T2*w images in the lateral part of the SN (Fig 1, 6th row). Comparison of the Perl's iron (yellow), TH (red) and NM (purple) masks showed that the regions with high dopaminergic cell content do not overlap with the iron+ region, or the hypointense region on the T2*w images. *In vivo* A hyperintense substructure in the dorsal SN corresponding to nigrosome 1 could be detected *in vivo* in HCs on high resolution T2*w images (Fig 2). The neuroradiologists independently classified 7/8 HC and 10/10 PD correctly on the basis of presence/absence of nigrosome 1 (1 subject excluded due to motion): PD detection sensitivity=100%, specificity=88%, inter-observer agreement κ=1.

Discussion We previously showed that TH+ regions in the SN correspond to the NM+ regions in Perls' stain and iron+ in Perls' correspond to the hypointense regions in T2*w images [4]. Here we added CAL staining to confirm that the CAL–, iron–, NM+ region corresponds to the nigrosome 1. Importantly we also showed that absence of the nigrosome 1 on in vivo T2*w images can provide an accurate diagnostic test PD. These results agree with a recent publication which speculated that the T2*w hyperintense structure could correspond to a nigrosome within the SN and showed a change in PD [5].

References [1] Bajaj et al., *JNNP.* 2010, 81:1223-8; [2] Damier et al., *Brain* 1999, 122:1421-36; [3] Ruifrok et al., *Anal Quant Cytol Histol,* 2001, *23:291-9*; [4] Blazejewska et al., proc. ISMRM 2012; [5] Kwon et al., *Ann of Neurol*, 2012, 71:267-77.
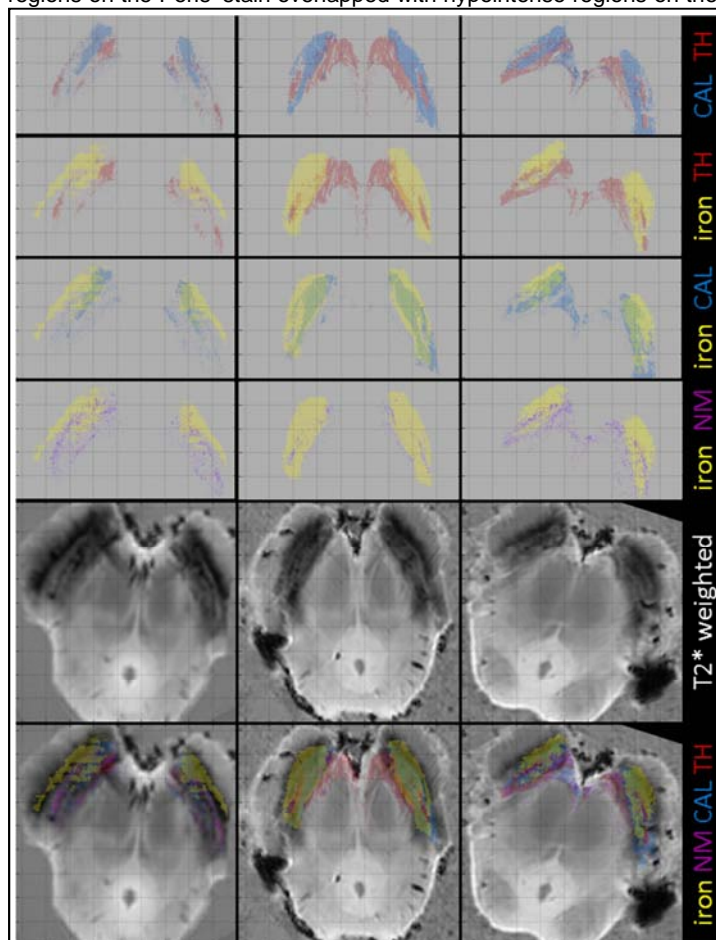
Fig 1. Three samples from 2 brain stems (middle & right same subject) cut through SN just below the RN. First 4 rows show superimposed stain masks: CALI & TH (1st), iron & TH (2nd), iron & CAL (3rd) and iron & NM (4th) (color of text on right is the key for the colors on the masks). Slice through the MR dataset (5th row), overlaid masks from stains (6th row).
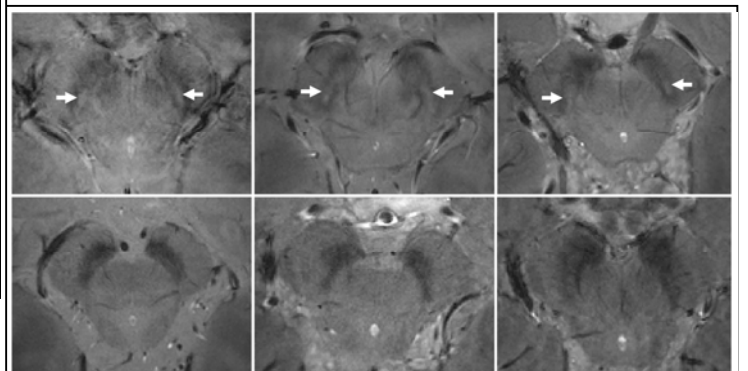

Fig 2. *In vivo* T2*w scans showing the SN in HC (top) and PD patients (bottom). Nigrosome 1 (white arrow) is visible in HCs, but not PD patients.