Iron, Ferritin, Myelin, and MR-Contrast: Proton-Induced X-Ray Emission (PIXE) Maps of Cortical Iron Content

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Introduction:
MR imaging at ultra-high field strengths has tremendously improved the visualization of human brain structures in recent years. In particular, strong differences in contrast are seen within the cerebral cortex that apparently reflect local myeloarchitecture. There is an ongoing controversy, however, regarding the relative importance of iron in myelinated tissue, as a partly independent source of tissue contrast. To explore histologically the possible role of iron as a source of MR cortical contrast, conventional standard staining procedures are used: Perls Prussian blue stain for trivalent and Turnbull blue stain for divalent iron. These techniques are known to be unreliable: some iron is masked and therefore not stained in the brain tissue, giving a biased representation of the true content. To circumvent this problem we have used a more powerful assay technique to map the true iron distribution, the so-called Proton-Induced X-Ray Emission (PIXE) [1]: a tightly focused beam of highly energetic protons is used to analyze a very thin slice of brain tissue [2]. The protons interact with the electrons and nuclei of the elements in the tissue slice, allowing spatially resolved quantification of elements, irrespective of their chemical state with a resolution down to 1 micrometer. This provides a gold standard for mapping the intracortical iron content. Combining this study with different indirect iron-staining methods, such as marking the iron-storage protein ferritin, gives a more comprehensive picture of the iron and myelin distribution within a post-mortem human brain section.

Methods:
We fixed a post-mortem human brain (female, 65 years, post-mortem delay = 24 hrs) in 4 % formalin at room temperature and cut out a tissue block from the central sulcus region (pre- and postcentral gyrus) which was scanned with a 7 Tesla MR scanner (MAGNETOM 7T, Siemens, Germany; MP2RAGE sequence; TR = 3000 ms, TE = 5.09 ms, 0.25 mm isotropic) [3]. The block was then embedded in paraffin and sectioned at 7 µm with a microtome. The sections were alternately stained for myelin (according to Gallyas) [4], cell bodies (according to Merker) [5], ferritin (immunhistochemical demonstration), and iron (according to Perls) [6]. Another adjacent section was left untreated for PIXE measurements, performed at the LIPSION laboratory. The sections were examined with a photomicroscope and an image analysis software package (AxioImager & AxioVision, Zeiss, Germany). Profiles were taken perpendicularly to the cortical layers from the pial surface to the grey/white matter boundary, standardized in length, and a mean optical density profile was computed for each staining modality. Pearson correlation coefficients were calculated for each profile pair with the Origin software package (OriginLab, Northampton, MA, USA).

Results and Discussion:
As seen in Figs. 1a, b and Table 1, the transcortical profile of the PIXE data, representing iron, correlates quite well with the optical density (OD) profiles of the Gallyas-myelin, Perls iron, and ferritin stained sections. Nevertheless the two iron stains cannot accurately reflect the true iron content: the Perls stain is a stain for trivalent iron, while the immunohistochemical stain of the iron-storage protein ferritin shows the distribution of this molecule, but does not show its iron content, which may well be below 100%. Furthermore, as seen in Fig. 2, high-resolution T1-images closely resemble both Gallyas-myelin and ferritin stained sections.

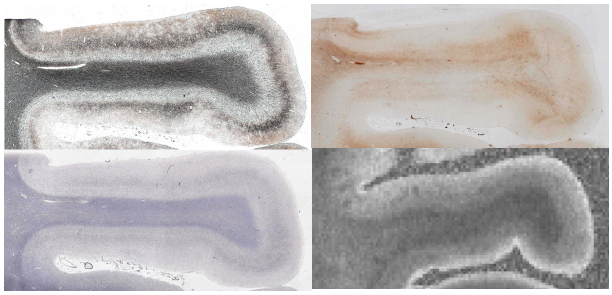
Conclusion:
This combination of applying conventional histological staining, immunohistochemistry, and modern x-ray spectra techniques of brain tissue allows a better understanding of the intracortical iron distribution, which helps to clarify the origin of MR-contrast. Further studies will include myelin and iron removal procedures and additional staining for iron-related molecules, as well as still higher resolution MRI.

References:
[1] Butz et al, Nucl Instrum Meth B 161: 323 ff (2000), [2] Morawski et al, Nucl Instrum Meth B 231: 224 ff (2005), [3] Marques et al, Neuroimage 49: 1271 ff (2010), [4] Gallyas, Neurol Res 1, 203 ff (1979), [5] Merker, J Neurosci Meth 9, 235 ff (1983), [6] Perls, Virchow Arch Path Anat 39, 42 ff (1867)

Table 1: Pearson correlation coefficient of stainings and PIXE data

	Gallyas	Perls	Ferritin	PIXE
Gallyas	1	0.75	0.85	0.64
Perls	0.75	1	0.94	0.68
Ferritin	0.85	0.94	1	0.7
PIXE	0.64	0.68	0.7	1

Fig. 2: Postcentral gyrus stained for myelin (Gallyas, top left), iron (Perls, top right), ferritin (bottom left), and scanned with 7T MRI (MP2RAGE sequence, bottom right)



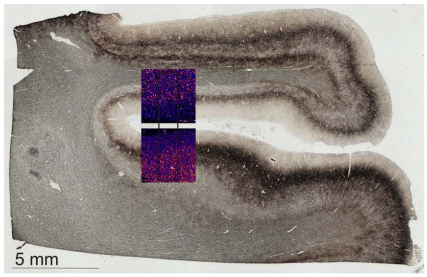


Fig. 1a: Myelin-stained section showing postcentral gyrus (top) and precentral gyrus (bottom). Superimposed are PIXE maps of iron distribution.

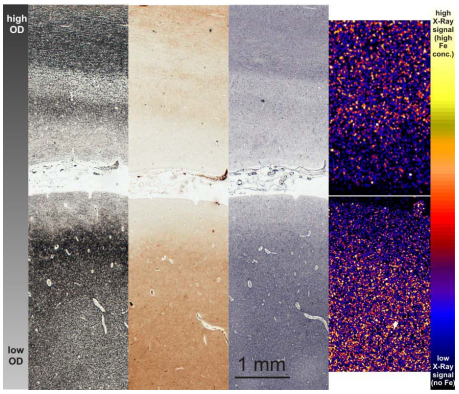


Fig. 1b: Higher-power photomicrographs of the postcentral gyrus (top row) and the precentral gyrus (bottom row) showing (from left to right) the distribution of myelin (Gallyas), iron (Perls), ferritin, and iron (PIXE maps).