Is iron the source of *post mortem* susceptibility contrast in the brain?

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INTRODUCTION – Quantitative information of the regional non-heme tissue iron distribution bears clinical potential in the context of various neurological and psychiatric disorders. The field perturbations induced by the spatial iron distribution are represented as contrast in gradient-echo (GRE) phase images. Approaches have been presented to quantify tissue iron based on its magnetic susceptibility using a technique referred to as quantitative magnetic susceptibility mapping[4,5] (QSM). The relative contribution of iron to the total bulk voxel susceptibility is, however, contentious, especially in the presence of significant portions of diamagnetic myelin[1,4]. Several authors have, thus, recently acquired phase images from *formalin fixed* brain tissue in order to investigate the sources of the susceptibility contrast[1-3], although it seems unclear whether fixation changes the magnetic tissue properties. The goal of the current study was to investigate and compare the relation between tissue iron concentration and tissue magnetic susceptibility of *in situ* and *fixed post mortem* brain tissue.

MATERIALS AND METHODS

MRI measurements: One deceased subject (male, age at death: 56y, cause of death: TBI, *post mortem* interval: 50h) underwent MRI at 3T (TimTrio, Siemens Medical Solutions) with a standard multi-echo GRE sequence ($TE_1/TE_6/\Delta TE/TR/FA$ = 4.9 ms/29.5 ms/4.9 ms/35 ms/15°, resolution = 1×1×2 mm^3) and head in normal scanning position (SAA; single angle acquisition). Core temperature at MRI was approx. 12°C. Subsequently, the brain was extracted, placed in 4% neutral buffered formalin, and kept refrigerated at 8°C (time in formalin: 2 month). This coronally oriented *fixed* brain was then imaged in three different rotations around the top-bottom axis (approx. -20°, 0°, and +20°; multi angle acquisition, MAA) with the same sequence ($TE_1/TE_5/\Delta TE/TR$ = 10 ms/50 ms/10 ms, resolution = 1x1x1 mm^3, temperature approx. 8°C).

Data Processing: Magnitude and phase images were reconstructed from all datasets. The phase images of the last echo were unwrapped and processed using the SHARP method[4]. All datasets were registered using FLIRT[6]. SA-susceptibility maps were computed from the *in situ* data and the 0° *fixed* brain data using regularized inversion[7] with a correction factor[4] of 0.74. MA-susceptibility maps were computed for the *fixed* brain data using a COSMOS[8]-like method[4]. Mean and standard deviation of the voxel susceptibility values were determined from all maps in 43 anatomical subregions. Pearson correlation was calculated and linear weighted rigorous least squares fitting was performed on the SA-values of the *fixed* and *in situ postmortem* brain.

Iron Quantification: After MRI, tissue specimens were dissected from the same regions as used for the susceptibility analysis, and their iron concentrations were determined with an inductively coupled plasma mass spectrometer (Agilent 7500ce, Agilent Technologies, Waldbronn, Germany) at m/z 56 in He-mode. Pearson correlation was calculated and linear least squares fitting (LLS) were performed on the obtained iron values and corresponding susceptibility values.

RESULTS – Figure 1 shows the *post mortem fixed* (first and second row) and *in situ* (bottom row) susceptibility maps computed from MAA (first row) and SAA data (second and bottom row). Contrast is similar in all maps with hyperintense deep gray matter nuclei and hypointense corticospinal tract and corpus callosum (see arrows in Fig. 1d,h). The most prominent difference between *in situ* and *fixed* maps is the presence of various hyperintense vessels in the *in situ* maps due to deoxygenated blood. The difference pattern between *fixed* SA- and MA-susceptibility maps (Fig. 1e,f) demonstrates streaking artifacts and some hypointense regions in the left internal capsule and right genu of corpus callosum (arrows in Fig. 1e). No further anatomical information is discernable in the difference patterns, indicating that SA-susceptibility maps are equivalent to MA-maps. Including all tissue samples, correlation between *fixed* susceptibilities and chemically determined iron concentration was high (Fig. 2a; R=0.91; p<0.0001) and linear regression yielded a slope of (0.0162 ± 0.0005) ppm·100g/mg (straight line in Fig. 2a). The formalin buffer solution appeared paramagnetic with respect to white matter brain tissue (white box in Fig. 2a). Correlation of average SA-susceptibilities of *in situ* and *fixed* maps (R=0.93; p<0.0001) yielded a slope of 0.97 ± 0.15. The determined regional iron concentrations were consistent with other *post mortem* studies[9,10].

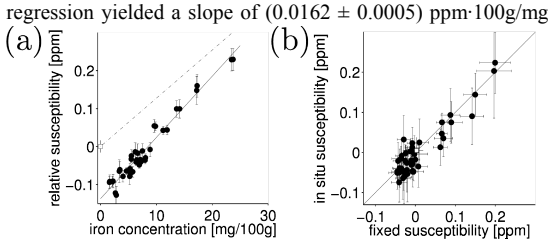

FIGURE 2. Magnetic MA-susceptibility (with respect to formalin buffer solution) of *fixed* brain plotted against tissue iron concentration (a) and *post mortem in situ* SA-susceptibilities plotted against *fixed* brain SA-susceptibilities (b). The dotted and straight lines in (a) represent the theoretical ferritin relation[12] and the LLS-fit, repectively. The line in (b) represents the line of identity. White squares mark the data points of formalin buffer solution and CSF, respectively.

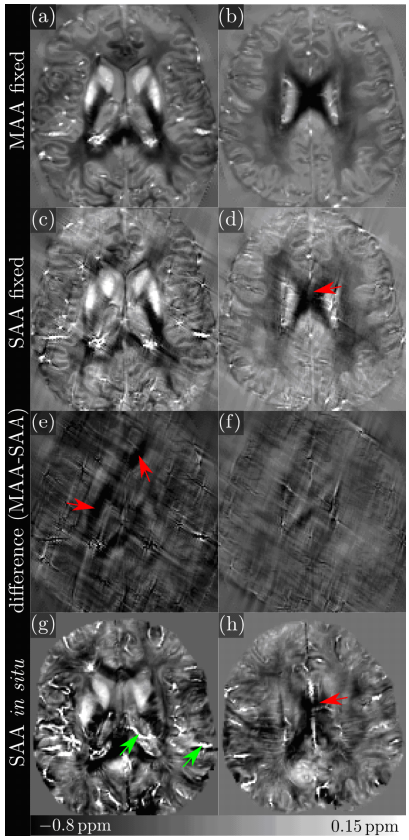

FIGURE 1. Two representative axial slices (left and right column) of the susceptibility maps of the *fixed* brain (a-d) and *post mortem in situ* brain (g and h). Differences between SA- (second row) and MA-maps (first row) of the *fixed* brain are shown in (e and f).

DISCUSSION AND CONCLUSIONS – In this study the correlation of magnetic susceptibility with brain tissue iron concentration was investigated. To this end, regional iron concentrations were measured chemically in several brain structures. This allows direct correlation of iron concentration with magnetic susceptibility, which is hampered in *in vivo* studies relying on iron concentrations from literature. The high correlation of susceptibilities measured in *in situ* and in *fixed* brain tissue (Fig. 2b) indicates that brain fixation does not significantly alter voxel susceptibility in regions with high iron concentrations. However, with the data available we were not able to identify diamagnetic contributions from myelin lipids, as discussed, e.g., by Duyn et al.[1] and Schweser et al.[4]. The small scatter in Fig. 2b (around 0 ppm) may be ascribed to fixation induced changes in myelin microstructure of white matter as well as to different scan temperatures[11]. The results highlight the necessity of investigating changes in white matter regions with high lipid content, e.g. the corticospinal tract or corpus callosum, separately from gray matter regions. The slope determined in the current study (0.0162 ppm·100g/mg) was significantly higher than theoretically estimated for tissue storage iron (ferritin) by Schenck et al.[12] (0.0127 ppm·100g/mg), which may be explained by the presence of free ferrous and ferric iron rather than iron bound to ferritin complexes. However, all results presented here may not be transferred directly to brain tissue *in vivo* since potential changes of myelin magnetic properties and iron oxidation states due to autolysis after death have not yet been analyzed thoroughly. Future studies will focus on investigating the impact of the *post mortem* interval and whether the scatter in Fig. 2b may be partially attributed to myelin. This will involve additional measurements of magnetization transfer and MAA QSM.

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