Multimodal MRI changes in cortical grey matter following formalin fixation

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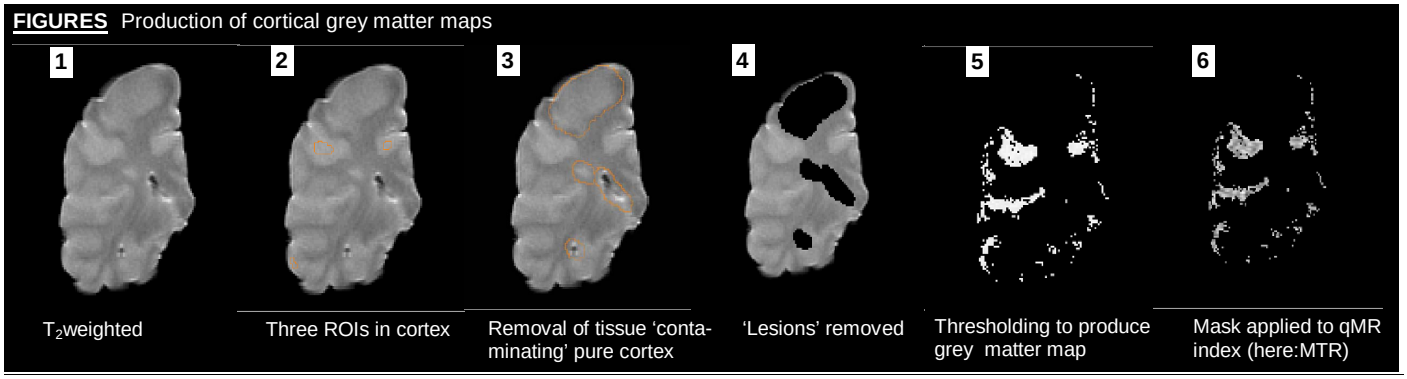
INTRODUCTION: *Post mortem* multiple sclerosis (MS) brain is being used to establish the pathological correlates of changes detected using MRI (1). Formalin fixation of the brain tissue introduces a potential confounder that may affect the inference of *in vivo* changes from MR/histology studies. Effects of fixation on quantitative MR indices in MS white matter (WM) have been reported (2). This study investigated changes following fixation of quantitative MRI indices in MS cortical grey matter (CGM). We also determined the relative proportion of CGM lesions (CGML) and healthy looking cortex (HLC) on histological sections of the same cases and estimated the contribution of these two tissue features towards MR indices.

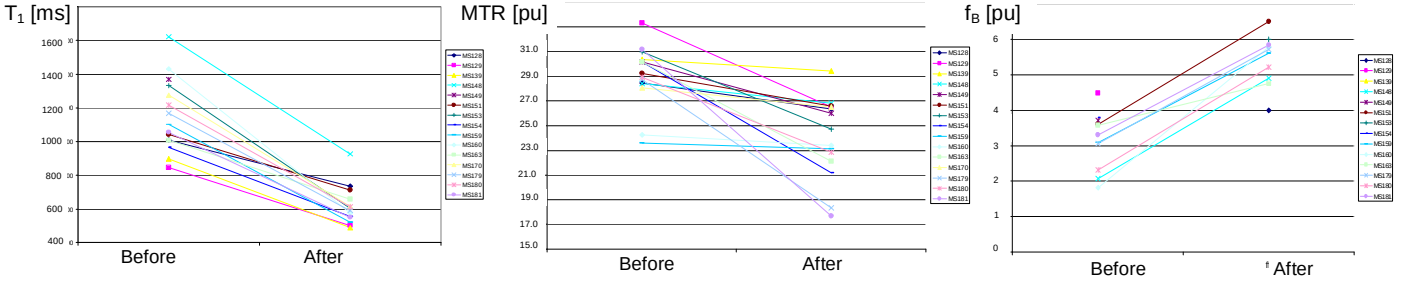
METHODS: Fifteen MS brain samples were studied unfixed 51 hours [standard deviation (SD) 28] post-mortem, and after 64 days [40] of fixation. Using a 1.5T GE scanner the following 2D datasets were acquired (1): (i) gradient echo (GRE) to calculate T_1, (ii) dual spin echo (SE) (with/-out MT pulse) to map magnetisation transfer (MT) ratio (MTR), (iii) spoiled GRE to map the 'fraction of macromolecular protons' from quantitative MT (f_B) (for details see ref 2) and (iv) SE T_2 weighted (T_2w) images. Starting with the unfixed cases, on T_2w SE images (fig 1) three regions of interest (ROI) were drawn in the cortical GM (fig 2), their mean and SD extracted and averaged. Regions of non-cortical brain tissue that potentially would have contaminated 'pure' CGM maps were removed (fig 3 & 4). Based on the three CGM ROI CGM masks were produced by thresholding the mean of the CGM ROI ± a multiple of their SD (fig 5). These masks were then applied to the quantitative MR maps of the each case to obtain an average 'CGM' map (fig 6). CGM maps for the fixed cases were acquired in the same way. Contrast between CGM and normal-appearing (NA) WM, and correlation between CGM and mean WM (NAWM + WM lesions)/2 values based on previously published data (2) were also investigated. Fourty-eight histological sections immunostained for myelin basic protein from the above 15 MS cases were used to identify CGML and HLC. CGML were classified into four lesion types (Fig 7) (3). The proportion of HLC and CGML was obtained using Image Pro Plus mounted on a PC that was connected to a Leica Axioskop microscope. Student's t test and regression models were used for analysis.

RESULTS: Differences between unfixed and fixed CGM were detected for T_1 (1156ms [SD 216] vs 617ms [114], p<0.01), MTR (29.1pu [2.5] vs 24.1pu [3.3], p<0.01) and f_B (3.2pu [2.3] vs 5.4pu [0.7], p<0.01). In 48 tissue blocks, 90 CGML (type 1: 16; type 2: 12; type 3: 59; type 4: 3) were detected. The proportion of CGML compared to HLC was highly variable ranging from zero to 33.8% (except for one outlier in which 84% of a small block was demyelinated). On average, 18.6% of the total CGM was demyelinated. Contrast between CGM and NAWM was not reduced for any of the obtained MR indices. T_1 (fixed: r=0.87, p<0.01) and MTR (unfixed: r=0.58, p=0.03 & fixed: r=0.81, p<0.01) in CGM correlated with respective WM values, whereas f_B did not. When comparing cases with high vs. low CGML load the proportion of CGML vs. HLGM did not siginificantly affect the qMR measures.

FIGURES Production of cortical grey matter maps



GRAPHS Changes of quantitative MRI indices following formalin fixation



CONCLUSION Formalin fixation results in a substantial drop of T_1, less so of MTR, and an increase of f_B in *post mortem* MS CGM, similar to changes observed in the WM (2). These changes are likely due to a combination of (i) direct formaldehyde effects (4) and (ii) intra- and intermolecular cross-linking of macromolecules (5). The proportion of CGML vs. HLC in this study is very similar to earlier reports (3). Comparing cases with high vs. low CGM lesion load the proportion of CGM vs. HLC did not siginificantly affect the qMR measures, which may have been due to (i) healthy cortex harbouring only ~10% of the amount of myelin present in the WM and (ii) less than 19% of the cortex in this study being demyelinated.

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