Phase Contrast in the Post Mortem Rat Brain: Comparison with T2* and Histology

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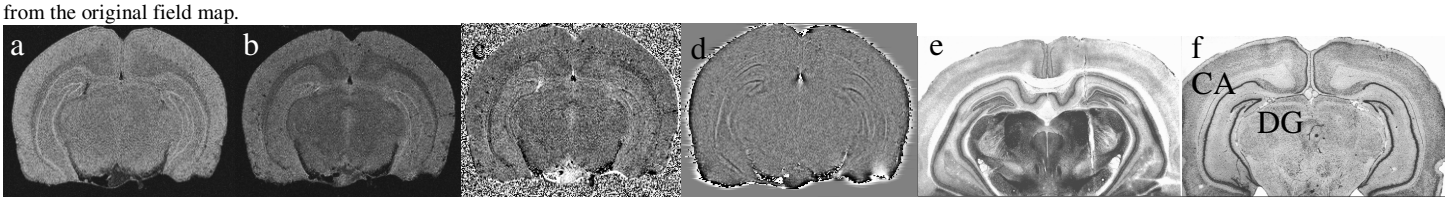
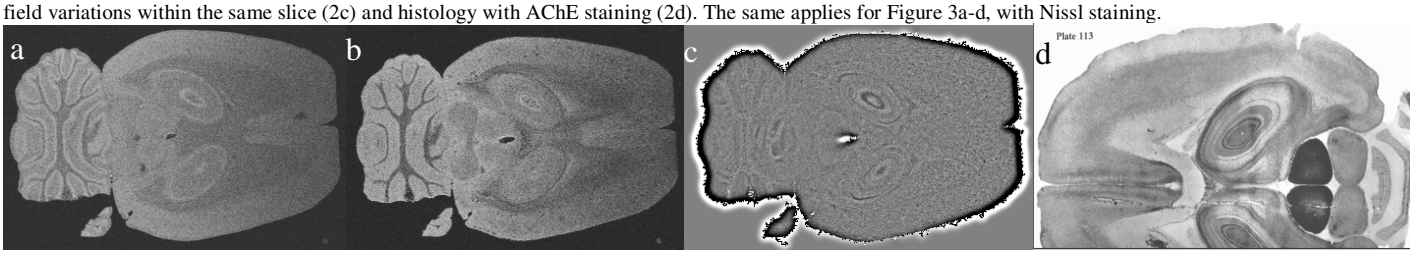
Introduction Measurement of the NMR frequency of water protons is one of the most sensitive ways to measure magnetic fields. Small susceptibility differences within tissue placed in a strong magnetic field give rise to effects which are well measurable by NMR and visualised in MR phase images. The information contained in the phase contrast between different tissue types is being increasingly used, for example to enhance visualisation of the vasculature in magnitude images [1], or to visualise subcortical structures with higher CNR than in magnitude images [2]. However, the microscopic origin of the susceptibility contrast between tissue types is not fully understood. Differences between distributions of capillaries, iron content, exchange with, and content of, macromolecules, and myelin content, have been proposed as contributors to the phase contrast between white and grey matter in the brain [2-4]. The quantitative interpretation of phase contrast is further complicated by geometrical effects and the fact that a well localised variation in susceptibility can give rise to much more extended variations in the magnetic field [5]. With the aim of contributing to the clarification of the origin of phase contrast, we investigated a post mortem rat brain with high-resolution (60µm isotropic) MRI. The phase contrast after formalin fixation was characterised in the whole brain and compared with T2* maps and cellular structure based on histology.

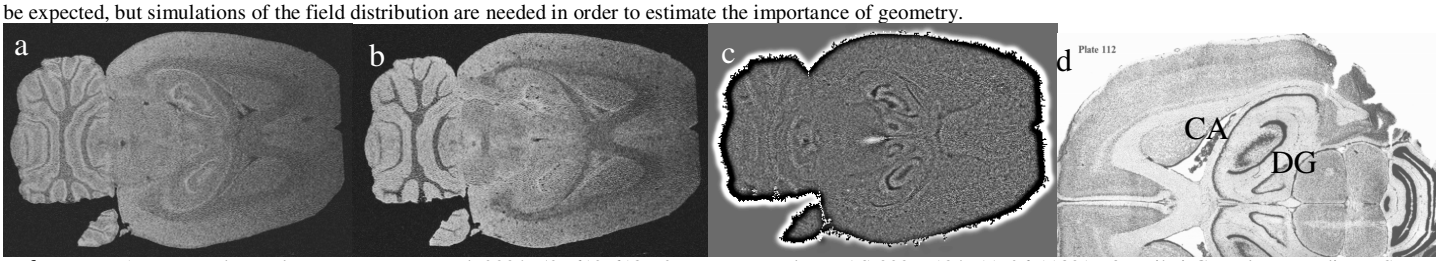
Methods MR imaging was performed on a home-built 7T animal scanner based on Siemens software and hardware and a 210mm bore superconducting magnet (Magnex) equipped with a gradient coil with maximum gradient strength of 400mT/m/axis and 170µs rise time. RF excitation and reception was performed with a surface coil of 3cm diameter. The brain of a male Wistar rat was investigated after storage in 6% formaldehide solution for 14 months. For the MRI examination, the brain was placed in a plastic container of 2cm diameter and 3cm height filled with Fomblin (perfluoro-polyether, Solvay Solexis). The parameters of the dual echo 3D gradient echo sequence used for imaging included: TR=80ms, TE=4.5ms and 14.5ms, alpha=50 deg, 2 averages, FOV=31mm x 25mm, matrix=512x416, slice thickness 60µm, 192 slices. Twenty separate scans were acquired over a period of 2 days and the complex data were averaged off-line using Matlab. After averaging, the phase images corresponding to each echo were unwrapped using PRELUDE (FSL) and the field map was calculated from a linear fit to the phase as a function of TE. The smooth contribution to the field variation was obtained by applying a Gaussian filter with FWHM of 24x20 pixels to the Fourier transform of the field map, slice-by-slice, and taking the inverse Fourier transform of the resulting matrix. The field maps discussed in the following were obtained by subtracting the smooth field variation from the original field map.



Results and Discussion As seen in Fig. 1a and 1b, the magnitude images obtained for the two different echo times show exquisite tissue contrast. At the resolution of 60µm isotropic, very fine hippocampal structures (cornu ammoni – with regions CA1, CA2, CA3 - and gyrus dentate - DG), are well visible. Careful comparison of Figs. 1a and 1b shows that at TE=4.5ms layers from both cornu ammoni and gyrus dentate are visible with equal intensity, but only layers belonging to the gyrus dentate remain visible at TE=14.5 ms, indicating different T2* properties for the two structures. This is reflected in the T2* map of the same slice displayed in Fig 1c (scale 0 to 20ms), where CA layers appear darker than those from gyrus dentate. In contrast, in the field map shown in Fig 1d, both structures are visible equally well. It is, however, clear that orientation effects are present, both in the field map and in the T2* map (Fig. 1d and 1c). The coronal slices were oriented parallel to the main magnetic field (B0 direction bottom to top). Figs. 1e and f show two histological slices selected from the rat brain atlas by Paxinos and Watson [6], and corresponding closely to the slice displayed in Fig1 a-d. Two different stainings were used: 1e acetylcholinesterase AChE staining (delineation of nuclei and fibre tracts; comprehensive but rather non-selective enzyme staining) and 1f cresyl violet (Nissl staining; sensitive to cell density). The MR contrast on the magnitude images seems to include factors visible in both stainings, e.g. contrast between corpus callosum and cortex (similar to 1e) and also high visibility of structures with increased cell density (similar to 1f). The substantia nigra, with a putativly high iron content, is present in this slice and visible on histology (1e), but is inconspicuous on any of the MR images. The same holds for the red nuclei (slice not shown). The lack of ferritin-induced signal decay from these regions might be due to the washout of iron from the brain over such prolonged formalin fixation. In Fig 2a-d, the magnitude images of an axial slice at different echo times (2a, 2b) are compared to the map of the local field variations within the same slice (2c) and histology with AChE staining (2d). The same applies for Figure 3a-d, with Nissl staining.



In conclusion, phase images acquired on a post mortem rat brain allow for a more complete visualisation and with higher contrast (factor 10-20) than the one in magnitude images of fine layers in the hippocampus. For the structures discussed here, myelin and iron content are not expected to be the main contrast-generating factor. Instead, the phase contrast seems more correlated with the cell density as described by the intensity of Nissl staining in histology. However, due to the fact that regions of similar cell density, for example in the cerebellum, produce smaller perturbations of the magnetic field, sensitivity of the details of phase contrast to the type of cells (e.g. pyramidal in CA vs granular in DG) and their content is very probable. Additional geometrical factors in the precise contrast of the phase images are also to be expected, but simulations of the field distribution are needed in order to estimate the importance of geometry.



References: [1] M. Haacke et al., Magn. Reson. Med. 2004, 52: 612-618; [2] J. Duyn et al., PNAS 2007, 104: 11796-11801; [3] Mihai G et al, proceedings ISMRM 2007; [4] Zhong K. et al., NeuroImage 2008; [5] Schaefer A, Gowland P and Bowtell RW, proceedings ISMRM 2008; [6] Paxinos G and Watson C, Academic Press Inc., 1986.