The preservation state of ethanol-fixated historic brain specimens revealed by quantitative MRI

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TARGET AUDIENCE - basic researchers on brain contrast mechanism, pathologists, museum curators, medical historicians
PURPOSE - to study the short term and long term effects of ethanol fixation on structural brain MRI
Fixation stabilizes soft tissue and prevents decay, but can severely alter MRI contrast. Cross-linking agents, like buffered formalin, largely preserve the protein structure and have replaced traditional methods, like immersion in alcohol, that denaturize proteins and lipid bilayers. Thus, there is little experience with MRI on ethanol fixated brains which can still be found in anatomical collections [1]. Digital asservation of such specimens is motivated by their historical, educational or scientific value [2], as much as by being threatened by slow decay.
METHODS -
Four brains of 'intelligent men' as prepared by the anatomist Wagner between 1855 and 1859 [3] were taken from the non-public part of medical collection of Göttingen University for photographic documentation and examined at 3T using 3D MP-RAGE (TI=900 ms, TR=2250ms, α=9°) at 0.8 mm and FLASH-based multi-parametric mapping at 0.6mm resolution. Two presumably untouched specimens were scanned in their original fixative, the other two in water. Eight in-phase gradient echo acquisitions (n*2.46 ms, 500 Hz/px) were averaged to increase SNR and reduce chemical shift and susceptibility artifacts[4]. From dual flip angles (α=6°, 20°, TR=23ms) and a magnetization transfer (MT) weighted volume (a=16°, TR=39ms; 500° Gaussian MT-pulse at 1.5 kHz offset), maps of R_1, signal amplitude and (MT) were calculated as in [5]. Bovine brain was obtained 24 hrs post mortem, fixated in graded solutions of ethanol (50, 70, 90 vol% for one week, kept in 93 vol%) followed up for currently four months. The formalin-fixated brain of a 57 year old male served as a gender and age-matched control.
Post-processing was performed using the tools of the FMRIB Software Library (FSL 4.1 www.fmrib.ok.ac.uk/fsl).
RESULTS -
MP-RAGE provided sufficient suppression of fixative and water (T1 ≈ 2.5 s) for masking and rendering the cortical surface, but lower signal in WM than in cortex as previously observed [1]. In all brains, high-resolution multi-parameter mapping revealed sub-millimeter tubular cavities in white matter (WM) (shown on the R1-map in Fig. 1), which strongly contributed to WM hypointensity on T1-w MRI. In contrast, the cortex appeared quite homogeneous. The typical rim of high R_1 in formalin-fixated brain was not seen (Fig 2). MT and proton density contrast were strongly reduced in the historical specimens (not shown), rendering R_1 maps as the prominent source of structural information. R_1 was lowest in one untouched brain (5 1/s in cortex) and markedly elevated (11 1/s in cortex) in a brain whose fixative had been replaced by formalin [1].
Ethanol fixation of a bovine brain showed a complex dynamic patter on MP-RAGE (Fig. 3), but neither the marked increase in R1, nor contrast inversion nor the tubular degeneration of WM was seen. The 35% loss of weight by extraction of cholesterol and water was comparable to that recorded on the jars (approx 30%).
DISCUSSION -
In ethanol fixation, rapid penetration of tissue and denaturization of proteins (and possibly membranes) is followed by slower extraction of cholesterol. By comparison to a recently fixated specimen, contrast inversion and macroscopic structural degeneration of WM could be related to long term storage, e.g. by a slow loss of lipids. This degeneration compromises the detection of WM lesions in the historical brains. One specimen, however, showed diffuse confluent hypointense WM regions on the R1 maps, which have been tentatively assigned to post mortem decay. Increase in R1 may have been exacerbated by frequent removal of the specimen, replacement of fixative, or higher ethanol concentration leading to "over-fixation". The loss of MT contrast is in line with the loss of cholesterol [6]. Additional cross-linking of denaturated proteins by formaldehyde further increased R_1 in white and gray matter, but did not result in better contrast. While the time scale of experimental fixation is limited, further information from original accounts and chemical analysis of fixative may provide crucial information.
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Fig.1: R_1 map and histogram of an ethanol-fixated brain

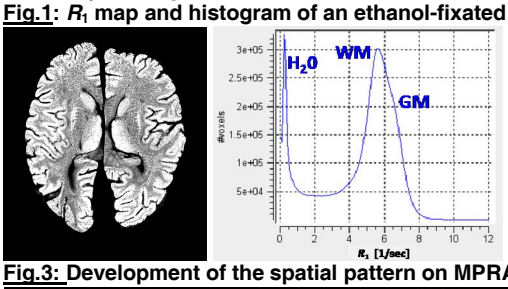


Fig. 2: High-resolution R_1 map of a formalin-fixated brain

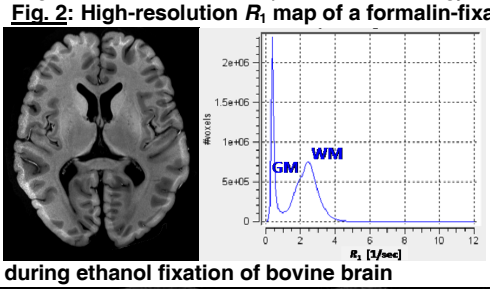
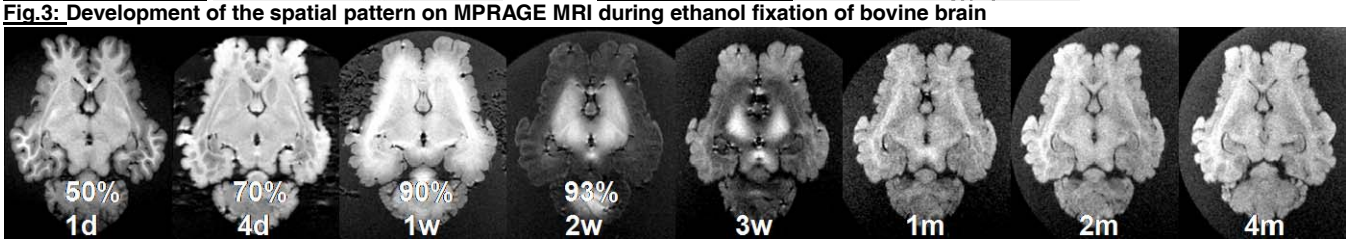


Fig.3: Development of the spatial pattern on MPRAGE MRI during ethanol fixation of bovine brain



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