Optimization of sample preparation for MRI of formaldehyde-fixed brains
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Context: MRI of post-mortem samples

Post-mortem MR imaging is useful for several applications:
- acquiring high quality anatomical reference images thanks to long scanning time;
- comparing MR images with gold standard histological imaging;
- studying autopsy samples;
- testing sequences on an anatomically realistic phantom.

However, post-mortem tissue needs to be fixed in order to prevent its degradation. **Fixation modifies the properties of the tissue (relaxivity, diffusion)** by inducing chemical changes (Tovi & Ericsson, 1992).

**Purpose and objective**

The change of tissue properties affects MR imaging: in particular, **$T_2$ is decreased by fixation, which is detrimental to SNR.**

Understanding the kinetics of the fixation and associated tissue changes is required to improve the preparation of tissue samples for MR imaging. Therefore, this study measures the evolution of several parameters relevant to MR imaging during post-fixation in formaldehyde over a period of months.

Moreover, it has been shown that soaking a fixed tissue sample in saline solution prior to imaging can partially restore the $T_2$ of the tissue and is beneficial to the SNR of acquired MR data. (D'Arceuil et al, 2007) Therefore, this study also measures the evolution of the same parameters during subsequent soaking in saline solution.

**Overall design of the study**

Two healthy ewes (adult two-year-old female sheep, Ovis aries) were used, in accordance with local animal regulation (authorization AT7091 of the French Ministry of Agriculture).

The following procedure was used for each animal:
- euthanasia using massive injection of barbiturate;
- immediate perfusion of the head with 4 L PFA at 4°C to prevent early tissue degradation;
- brain extraction;
- immersion of the brain in PFA.

**Imaging and analysis methods**

**High-resolution anatomical image**

During each preparation, an anatomical image was acquired for registration using a 3D turbospin echo sequence with variable flip angle, at 8 mm isotropic resolution (SPAIR, TR = 4000 ms, TE = 275 ms, $\lambda$GAPPA 3), turbo factor 10, field of view 256 mm, bandwidth 354 KHz, acquisition time 10 min 30 s.

In addition, one session was dedicated to acquiring an image at 3 mm isotropic resolution for anatomical references, using a 3D turbo spin echo sequence with variable flip angle (SPAIR, TR = 4000 ms, TE = 275 ms, turbo factor 14, echo train length 851 ms, bandwidth 163 KHz), acquisition time 1 h 24 min.

The longitudinal relaxation time $T_1$ was measured using a variable flip angle–actual flip angle imaging (VAPI) (Harley et al, 2012). The variable flip angle acquisition used a partially spoiled–steady state free precession sequence (SSFP) with 2 mm isotropic resolution, TR = 7 ms, TE = 16 ms, FA = 15°, $\lambda$F = 8, $\lambda$R = 8, $\lambda$GAPPA 3, $\lambda$GAPPA 1, TR = 3 ms, n = 10, FA = 15°, $\lambda$GAPPA 3, bandwidth 1650 Hz/Px. Total acquisition time was 12 min 7 s.

The transverse relaxation time $T_2$ was measured using a least-squares regression of a single exponential decay using a Levenberg-Marquardt algorithm implemented in PTK, an in-house software suite.

**Quantitative $T_2$ mapping**

The transverse relaxation $T_2$ was measured using a spin echo echo-planar imaging (EPI) with 1.7 mm isotropic resolution, TE = 3 ms, TR = 130 ms, n = 5, FA = 60°, $\lambda$GAPPA 3, bandwidth 1560 Hz/Px. Total acquisition time was 12 min 7 s.

The effective transverse relaxation $T_2^*$ was measured using multi-echo echo-planar imaging (EPI) with 3 mm isotropic resolution, TE = 3 ms, TR = 130 ms, n = 5, FA = 60°, $\lambda$GAPPA 3, bandwidth 1650 Hz/Px, acquisition time 11 min 45 s.

The signal decay across echoes was fitted with a least-squares regression of a single exponential decay using a Levenberg-Marquardt algorithm implemented in PTK, an in-house software suite.

**Quantitative $T_1$ mapping**

The apparent diffusion coefficient (ADC) and fractional anisotropy (FA) were extracted using a first-order tensor model, using the DSI software.

**Diffusion imaging**

Diffusion-weighted images were acquired using spin echo EPI with 2 mm isotropic resolution, 256 diffusion directions, b = 8000 s/mm$^2$, TR = 5000 ms, TE = 80 ms, $\lambda$GAPPA 3, bandwidth 1194 Hz/Px, acquisition time 39 min 18 s. The apparent diffusion coefficient (ADC) and fractional anisotropy (FA) were extracted using a first-order tensor model, using the DSI software.

**ROI (region of interest) analysis**

The parametric maps were analyzed using regions of interest (ROIs) manually defined on the high-resolution anatomical images, representing the white matter and caudate nucleus, respectively. The ROIs were transferred into the reference of each parameter within the different fixedation times.

A semi-automated method was used for the radiological interval duration and histological evaluation of the brain images. The method is described in detail in (D'Arceuil et al, 2007). The parametric maps were analyzed using regions of interest (ROIs) manually defined on the high-resolution anatomical image using FLIRT version 6.0 (Jenkinson et al, 2001, 2002) with a correlation ratio cost function. Voxels that contained partial volume were excluded from the analysis.

**Results**

The fixative solution, which is called PFA for short, is composed of 4% formaldehyde prepared by dissolving paraformaldehyde powder in phosphate-buffered saline (PBS).

Both brains were imaged repeatedly on a clinical 7 T MRI system over a period of 3 months during immersion in PFA. After that, one brain was imaged in isotonic saline solution for washing, and imaged repeatedly for 3 months. Initially two acquisitions per day were performed, then the frequency was decreased.

For the duration of each acquisition session the brains were transferred in Fluinertm, a fluorocarbon-based fluid that creates no signal and has a similar susceptibility to cerebrospinal fluid.

Maps of $T_1$, $T_2$, $T_2^*$, and diffusion were acquired during each session. Average values over white matter and grey matter were extracted in hand-delimited regions of interest (see details below).

**Discussion and conclusion**

- Seaking the tissue in saline had the intended effect of restoring higher $T_2$, $T_2^*$, and diffusivity values.
- The slow decrease of $T_2$ during fixation is consistent with previously published data (Tovi & Ericsson, 1992), as well as the decrease of diffusivity and stability of anisotropy (D'Arcueil et al, 2007).
- Good reproducibility is observed where data is available for both brains.
- The effective transverse relaxation $T_2^*$ is stable after 8 weeks.
- Soaking in saline solution achieves maximum recovery after 8 weeks.
- These values are expected to be longer for larger specimen such as human brains, which require longer penetration time.

**References**


