Optimization of sample preparation for MRI of formaldehyde-fixed brains
Yann Leprince, Benoît Schmitt, Élodie Chaillou, Christophe Destrieux, Laurent Barantin, Alexandre Vignaud, Denis Rivière, Cyril Poupon

To cite this version:
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 and Ericsson, 1992].

Overall design of the study

Two healthy eves (adult two-year-old female sheep, oure aries) were used, in accordance with local animal regulation (authorization A27801 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.

Results

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 imbibed 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 Fluorinet™, a fluorocontaining-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).

Diffusion imaging

Diffusion-weighted images were acquired using spin echo EPI with 2 mm isotropic resolution, 256 diffusion sequences, $b$-value 2000 s/mm$^2$, TR = 12000 ms, TE = 80 ms, GRAPPA 3, bandwidth 250 Hz/Px. The actual flip angle acquisition [Amadon et al, 2008] used 4 mm isotropic resolution, TR = 30 ms, GRAPPA 3, bandwidth 1950 Hz/Px, acquisition time 6 min 40 s.

The signal decay across echoes was fitted with a least-squares regression of a single exponential decay using a first-order tensor model, using the Diffusion software suite.

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 transformed into the reference of each parameter map. The time evolution of parameters during subsequent soaking in saline solution was estimated by rigid registration of the anatomical images onto the high-resolution anatomical images using FLIRT version 4.3 (Jakob et al, 2001, 2002) with a correlation ratio cost function. Voxels that contained partial volumes at the boundary of ROIs were excluded from the analysis.

Discussion and conclusion

- Seaking the tissue in saline had the intended effect of retarding higher $T_1$, $T_2$, and $T_2^*$ values.
- The slow decrease of $T_2$ during fixation is consistent with previously published data [Tovi and Ericsson, 1992], as well as the decrease of diffusivity and stability of anisotropy [D'Arcueil et al, 2007].
- The longitudinal relaxation time $T_1$ is stable after 8 weeks.
- 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.

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.

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).

Imaging and analysis methods

High-resolution anatomical image

During each imaging session, an anatomical image was acquired for registration using a 3D turbo spine echo sequence with variable flip angle, at 0.5 mm isotropic resolution (SPAIR, TR = 4000 ms, TE = 175 ms, GRAPPA 3, turbo factor 144, echo train length 851 ms, bandwidth 236 Hz/Px, acquisition time 1 h 24 min).

In addition, one session was devoted to acquiring an image at 0.3 mm isotropic resolution for anatomical reference images, using a 3D turbo spine echo sequence with variable flip angle (SPAIR, TR = 4000 ms, TE = 273 ms, turbo factor 144, echo train length 851 ms, bandwidth 163 Hz/Px, acquisition time 1 h 24 min).

The longitudinal relaxation time $T_1$ was mapped using variable flip angle-actual flip angle imaging (VAPI) (Harutyunyan et al, 2012). The variable flip angle acquisition used a partially-stacked steady-state free precession sequence (SPGR with 0.3 mm isotropic resolution, TR = 3 ms, TE = 14 ms, FA = 20°, FA = 30°, GRAPPA 3, bandwidth 310 Hz/Px, acquisition time 11 min 40 s).

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

Quantitative $T_1$ mapping

The transverse relaxation time $T_2$ was mapped using echo-planar imaging (EPI) with 1.7 mm isotropic resolution, 15 linearly spaced TE values between 15 ms and 90 ms, TR = 11 s, GRAPPA 5, bandwidth 1640 Hz/Px, acquisition time 11 min 40 s.

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

Diffusion imaging

Diffusion-weighted images were acquired using spin echo EPI with 2 mm isotropic resolution, 256 diffusion sequences, $b$-value 2000 s/mm$^2$, TR = 12000 ms, TE = 80 ms, GRAPPA 3, bandwidth 1354 Hz/Px, acquisition time 39 min 58 s. The apparent diffusion coefficient (ADC) and fractional anisotropy (FA) were extracted using a first-order tensor model, using the Diffusion software suite.

References


