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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 autolytic 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].

Purpose and objective

The change of tissue properties affects MR imaging: in particular, T2 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 T2 of the tissue and is beneficial to the SNR of acquired MR data. [D'Arceuil et al., 2007; Shepherd et al., 2009]

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 A37801 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 session, an anatomical image was acquired for registration using a 3D turbo spin echo sequence with variable flip angle, at 0.5 mm isotropic resolution (SPAIR, TR = 4000 ms, TE = 175 ms, GRAPPA 3, turbo factor 164, echo train length 604 ms, bandwidth 238 Hz/Px, acquisition time 1 h 36 min 10 s).

In addition, one session was dedicated to acquiring an image at 0.3 mm isotropic resolution for anatomical reference, using a 3D turbo spin 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 1 h 24 min).

Quantitative T2* mapping

The longitudinal relaxation time T1 was mapped using variable flip angle-actual flip angle imaging (VAFI) [Hurley et al., 2012]. The variable flip angle acquisition used a parallel-acquired steady-state free precession sequence (SPAIR) with 3 mm isotropic resolution, TR = 30 ms, TE = 14 ms, FA = 2°, FA = 5°, FA = 10°, GRAPPA 1, GRAPPA 2, GRAPPA 3, bandwidth 250 Hz/Px. The actual flip angle acquisition [Amadon et al., 2008] used 4 mm isotropic resolution, echo time 110 ms, TR = 600 ms, FA = 10°, GRAPPA 3, bandwidth 1650 Hz/Px. Total acquisition time was 12 min 7 s.

Quantitative T2 mapping

The transverse relaxation time T2 was mapped using echo-planar imaging (EPI) with 0.5 mm isotropic resolution, 30 linearly spaced TE values between 15 ms and 90 ms, TR = 11 s, GRAPPA 3, bandwidth 1640 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 T2* mapping

The transverse relaxation time T2* was mapped using echo-planar imaging (EPI) with 3 mm isotropic resolution, 30 linearly spaced TE values between 1.5 ms and 21.5 ms, TR = 25 ms, GRAPPA 3, bandwidth 1033 Hz/Px, acquisition time 14 min 46 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.

Diffusion imaging

Diffusion-weighted images were acquired using echo-planar imaging (EPI) with 3 mm isotropic resolution, 256 diffusion directions, b = 4000 s/mm², TR = 52 ms, TE = 600 ms, bandwidth 1534 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 DTI toolbox.

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 at each parameter time point. The inter-session transformation from the first to the second was computed using rigid registration of the anatomical images onto the high-resolution anatomical image using FLIRT version 4.5 (FSL) [Jenkinson et al., 2002] with a correlation ratio cost function. Voxel that contained partial volumes at the boundary of ROIs 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 immersed 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 Fluorinert™, a fluorocarbon-based fluid that creates no signal and has a similar susceptibility to cerebrospinal fluid.

Maps of T1, T2, T2*, 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

✓ Sealing the tissue in saline is intended to (a) prevent the tissue from shrinking and (b) preserve the tissue morphology.

✓ The slow decrease of T2 during fixation is consistent with previously published data [Tovi and Ericsson, 1992], which is a clear advantage of saline immersion.

✓ We can derive recommendations for optimal soaking times:
- fixation in PFA is stable after 8 weeks;
- soaking in saline solution achieves maximum recovery after 4 weeks;
- these values are expected to be longer for larger specimens such as human brains, which require longer penetration time.

References


