Staining Methods for Magnetic Resonance Microscopy of the Rat Embryo

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Introduction

Magnetic resonance histology (MRH) has become a valuable tool in assessing the developing rodent embryo (1). MRH provides a non-destructive method for generating three-dimensional images for quantitative assessment of organ morphology (2). To date, previous studies have been performed on formalin-fixed or fresh tissues with scan times > 7 hours (3). This study describes a staining (2) and fixation method for rat embryos by immersion designed to enhance the signal-to-noise ratio (SNR) and contrast-to-noise ratio with a paramagnetic contrast agent.

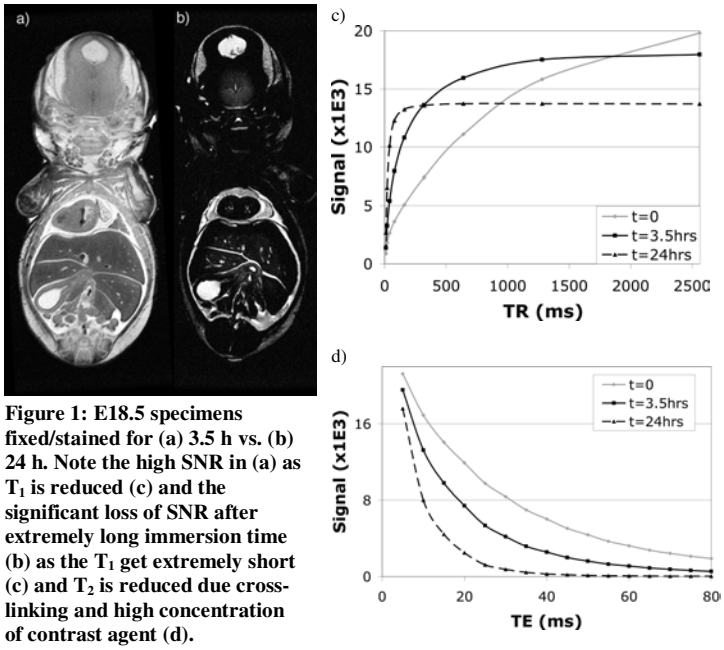

Figure 1: E18.5 specimens fixed/stained for (a) 3.5 h vs. (b) 24 h. Note the high SNR in (a) as T_1 is reduced (c) and the significant loss of SNR after extremely long immersion time (b) as the T_1 get extremely short (c) and T_2 is reduced due cross-linking and high concentration of contrast agent (d).

Methods

Gestational age 18.5 and 19.5 rat fetuses were immersion-fixed for different times (0 to 24 h) in Bouin's fixative containing different concentrations of gadolinium chelate (ProHance®, gadoteridol, Bracco Diagnostics, Inc., Princeton. NJ): 40:1, 20:1, and 10:1 (Bouin's:ProHance).

Results

The results showed a significant change in the SNR as a function of concentration of contrast agent and time of immersion. The process involved several competing phenomena that must be balanced for optimized imaging—as the contrast agent penetrates the tissues, T_1 is reduced as desired (typically from 2000 ms to 200 ms), which yields an increased SNR by a factor of 5; at the same time, T_2 was reduced by both the fixative (by protein cross-linking) and the high concentration of contrast agent. This reduction (from 28 to 7 ms) led to a dramatic decrease in SNR. It was therefore critical to find a storage solution that would stabilize the specimens in the long-term and conserve the image quality achieved. To avoid diffusion of the ProHance out of the tissues, and the dehydration caused by formalin, a mixture of phosphate buffered saline (PBS) and ProHance was used as the storage solution. The concentration that conserved T_1 (at 70 ms) and T_2 (at 15 ms) was found to be 160:1 PBS:ProHance.

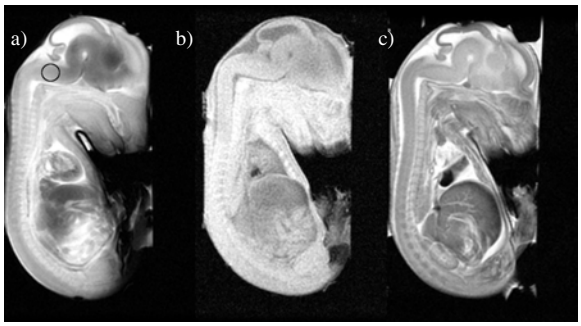

Figure 2: E18.5 specimens (a) before storage (right after 5-h staining), (b) after 4 weeks in PBS only, and (c) PBS:ProHance 160:1.

Conclusions

A systematic assessment of a staining protocol (concentration of contrast agent, fixation time, and storage conditions) has enabled us to increase the SNR by more than 5 times over that obtained in unstained (fixed without contrast agent) specimens. This staining protocol has allowed reduction in the scan time required for very high-resolution images (20 microns) from over 14 hours (for 40 microns) to only 3 hours, making MRH a routine tool for evaluating embryonic development.

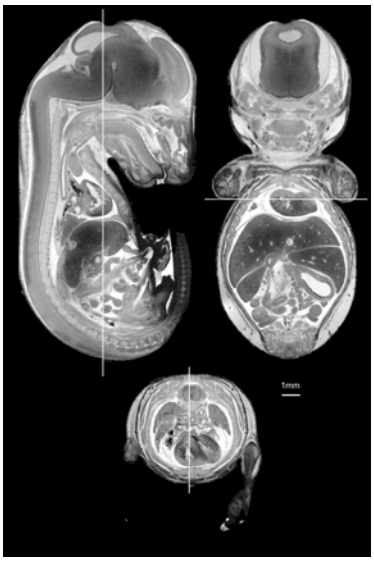

Figure 3: E18.5 at 20 microns, mid-sagittal, mid-coronal and axial slices.

References
1. Orita J, et al., Experimental Animals 45(2):171-174, 1996.
2. Johnson GA, et al. Radiology 222(3):789-793, 2002.
3. Dhenain M, et al. Developmental Biology 232(2):458-470, 2001.