

Preservation of manganese induced contrast in fixed rat brain

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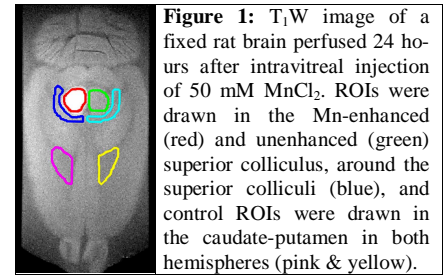
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Introduction

The paramagnetic properties of divalent manganese cations (Mn^{2+}) and their *in vivo* resemblance to calcium cations (Ca^{2+}) has led to their use in morphological (1) and neural pathway (2) imaging. Mn^{2+} is taken up through Ca^{2+} -channels during neural activity and, subsequently, undergo fast microtubuli-based anterograde axonal transport thereby making Mn^{2+} suitable for neuronal tract tracing(2). Aldehyde-fixation is frequently used to preserve tissue. If the Mn induced contrast could also be preserved in aldehyde-fixed tissue, morphological images devoid of flow and motion artifacts and of higher resolution could be acquired. Consequently, this study has investigated the preservation of the Mn signal in the fixed brain using an *in vivo* Mn-enhanced (ME)MRI optic tract tracing model (2) followed by standard arterial perfusion fixation or thermal fixation (3).

Materials and methods

Eighteen male Sprague-Dawley rats (300-400g) were used. $MnCl_2$ (3 μ l, 50 mM) was injected into the vitreous body of the animal's left eye under general anaesthesia. 24 hours after intravitreal injection, animals were anaesthetized and fixed with standard arterial perfusion fixation or thermal fixation methods(3). All animals were flushed with 400 ml isotonic saline. Subsequently, animals were fixed with 400 ml of either 1) 6% formaldehyde in 100 mM acetate-buffer (pH \approx 6); 2) 4% formaldehyde in 100 mM acetate-buffer (pH \approx 4.9); 3) 4% formaldehyde in 100 mM acetate-buffer (pH \approx 6); 4) 4% formaldehyde in isotonic saline (pH \approx 6); 5) 4% formaldehyde in 75 mM phosphate-buffer (pH \approx 7) and 6) isotonic saline (pH \approx 6) followed by microwaving the brain at 450 W in 20 ml isotonic saline for 40 seconds (thermal fixation). The brains were extracted from the skull and put in syringes containing the different solutions. Images were acquired using a Varian 4.7T animal scanner together with a home built volume and surface coil, starting approx. 1 hour after start of fixation. T₁W images were obtained using the acquisition parameters: TR=400ms, TE=16ms, NEX=17, slice thickness=1 mm, FOV=35x20mm, matrix 516x256. Thirty-seven scans were made with an acquisition time of approx. 30 minutes per scan. ROIs were drawn in the Mn-enhanced and unenhanced superior colliculus, around the superior colliculi to determine whether Mn diffuse after fixation, and as control, ROIs were drawn in the caudate-putamen in both hemispheres (figure 1). Ratios (in %) of the signal intensity for the three pairs of matching ROIs were calculated for each image and averaged for each of the six fixation-groups. The mean signal intensity ratio for the three matching pairs of ROIs from each fixation-group was plotted as a function of time. For the control ROIs the first scan was set to 100% (figure 2).



Results and discussion

The images for the individual brains showed that the fixed brains seem to swell in the first few hours post-fixation. The mean signal intensity ratio in the superior colliculus dropped in all six fixation-groups, suggesting that the paramagnetic enhancing effects of the Mn decreases with time (figure 2a). The Mn signal fades more rapidly when thermal fixation was used compared to chemical fixation. 4% formaldehyde in phosphate-buffer seems to preserve the Mn signal better than the other chemical fixation-solutions, despite that Mn and phosphate precipitates. Furthermore, pH seems to have an effect on preservation of the Mn signal since the contrast fades slightly faster, when the brains are fixed with the 4% formaldehyde in acetate-buffer (pH \approx 4.9) compared to the one with a pH \approx 6. In the control ROIs in the caudate-putamen, the mean signal intensity ratio remained steady throughout the study in all fixation-groups (figure 2c). The mean signal intensity ratio in the ROIs around the superior colliculus remained relatively constant, with a slight increase in the phosphate-buffer group and a slight drop in the thermal fixation group (figure 2b). The results, thus, suggest that the drop in the Mn contrast is due to changing local chemistry effects and not diffusion of Mn cations.

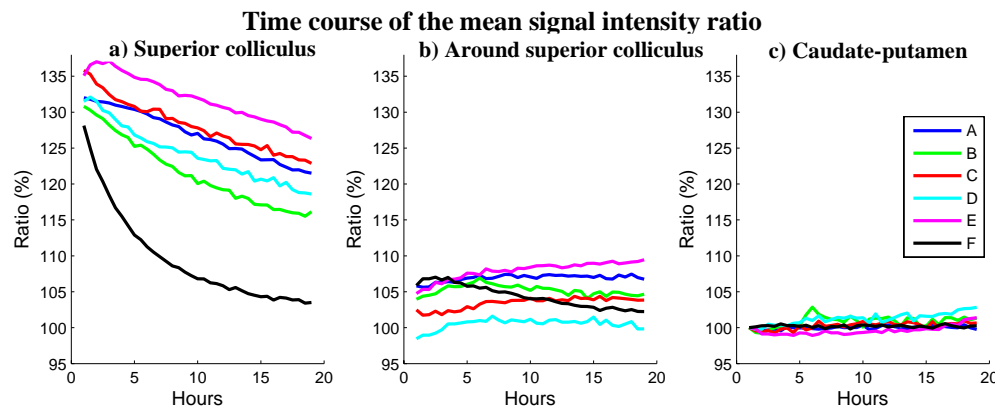


Figure 2: The mean signal intensity ratio for the three pairs of ROIs in the superior colliculus (a), around the superior colliculus (b) and the control area in the caudate-putamen (c) are plotted as a function of time for each of the different fixation groups (A-F). The mean signal intensity ratio in the first scan was set to 100% in the control ROI in all the fixation-groups. The standard deviation for all points was between 0.3-5.0% with a mean of 2.1%. The six groups were fixed in either:
A) 6% formaldehyde in acetate-buffer (pH \approx 6)
B) 4% formaldehyde in acetate-buffer (pH \approx 4.9)
C) 4% formaldehyde in acetate-buffer (pH \approx 6)
D) 4% formaldehyde in isotonic saline (pH \approx 6)
E) 4% formaldehyde in phosphate-buffer (pH \approx 7)
F) Thermal fixation

Conclusion

This study shows that chemical fixation is better at preserving the Mn induced contrast enhancement than thermal fixation. The use of acetate-buffer to try to stabilise Mn cations at lower pH and also avoiding phosphate able to precipitate Mn appeared to have little effect. Using the fixation methods described here, preservation of the original Mn contrast was possible for a limited period. After the initial fixation-related swelling, there appears to be some time to acquire high resolution MEMRI. However, methods for long-term preservation of Mn induced contrast still needs to be developed.

References

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- (3) Hopwood D. Cell and tissue fixation, 1972-1982. *Histochemical Journal* 1985; **17**:389-442.