Ex-vivo MRI of the brain: Longitudinal effects of formalin exposure on regional T1 relaxation times

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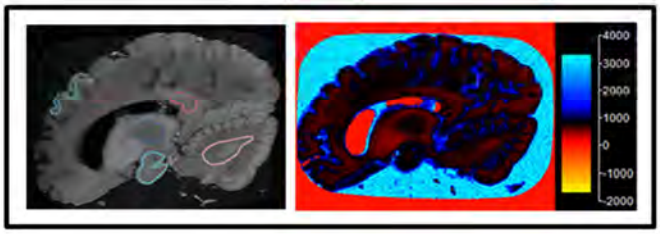
Target Audience Researchers interested in understanding the effects of tissue fixation over time on T1 relaxation times in *ex vivo* MRI of human brain tissue.

Purpose The purpose of this study is to measure the regional changes in T1 relaxation times of gray matter (GM) and white matter (WM) in *ex vivo* human brains over 6 months of fixation in 10% formalin. Relaxation times are useful as markers of tissue properties in imaging studies.(1) Also, clear distinction between GM and WM is necessary for tissue segmentation and volumetric measurements for studies of brain morphology. Formalin diffuses through the *ex vivo* brain, changing the apparent MR signal intensity of the tissue as it is fixed. Additionally, the ongoing process of tissue decay independently affects tissue properties. Established T1 relaxation times for living subjects will not be accurate for tissue that has been excised and stored in fixative for varying amounts of time. It has been previously reported that attenuation of T1 relaxation times occurs after fixation over three weeks.(2) In this study, we measured GM and WM T1 relaxation times in five *ex vivo* human brains in 10% formalin over 6 months. This allowed us to investigate the impact of fixation on *ex vivo* brain tissue over 6 months and map the attenuation of the T1 relaxation times. Our measurements were done in regions representing both GM and WM regions that are either on the brain surface, therefore directly exposed to formalin (GM in the frontal cortex, GM in the antero-medial temporal lobe, WM in the splenium of the corpus callosum), or exposed through diffusion of formalin into the tissue (GM in the thalamus, subcortical WM in the frontal lobe, WM in the cerebellum). Previous studies investigating effects of fixation over time have not reported values in these various regions.

Figure 1. ROIs shown on T1-weighted image (left) were used to measure mean T1 on T1 map (right) in 5 formalin fixed brains. ROIs represent GM and WM that are either directly exposed to formalin or exposed through diffusion of formalin.

ROI	Anatomy	Direct Formalin Exposure
GM	Frontal lobe cortex	Yes
GM	Thalamus	No
GM	Antero-medial temporal lobe	Yes
WM	Frontal lobe subcortical WM	No
WM	Corpus callosum (splenium)	Yes
WM	Cerebellar WM	No



Methods Using an Inversion Recovery Spin Echo sequence at 3T (GE Signa, v16, Milwaukee, WI), we acquired single-slice images of brain tissue at TI = 50 ms, 400 ms, 1100 ms, and 2500 ms. FOV 18 cm, matrix: 512x128, slice thickness 3mm, TR 2550 ms, TE 14 ms, band width of ±31.25 kHz. Scans were performed for five *ex vivo* brains that had been in 10% formalin for 10-12 days after autopsy. Scans for each brain were acquired every day for seven days, then at two weeks, 1 month, 3 months, and 6 months after the initial scan. Using fitting code from Barral J, et al (3) we calculated and mapped T1 relaxation times and measured mean T1 values from GM and WM ROIs that are directly exposed and not directly exposed to formalin **(Figure 1).**

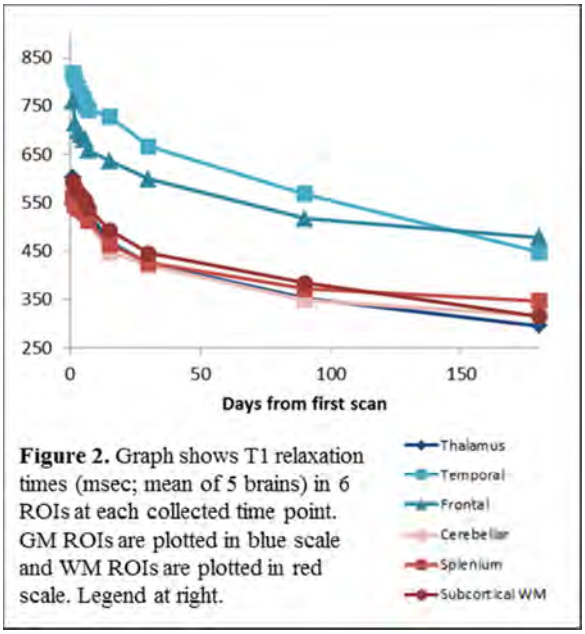


Figure 2. Graph shows T1 relaxation times (msec; mean of 5 brains) in 6 ROIs at each collected time point. GM ROIs are plotted in blue scale and WM ROIs are plotted in red scale. Legend at right.

Results T1 relaxation times shorten progressively over 6 months. **Figure 2** shows the mean T1 relaxation times of the five brains at each time point in each ROI. Shortening of T1 relaxation times seems to be more rapid within the first month of scanning, which is within 6 weeks after the start of fixation. However, by 6 months of fixation the attenuation of T1 relaxation times appears to plateau and stabilize. Consistent with *in vivo* studies, the cortical GM ROIs have longer T1 relaxation times than WM ROIs. However the thalamus, a dense subcortical GM region, has a T1 relaxation time and T1 attenuation rate similar to those of the WM ROIs; T1 relaxation times and attenuation rates in all WM ROIs are similar.

Conclusion Both GM and WM T1 relaxation times of fixed brain tissue attenuate over time with more rapid attenuation within the first 6 weeks after autopsy and approaching stabilization by 6 months. This information is crucial for determining the optimal time from the start of fixation and optimal sequence parameters for acquiring *ex vivo* brain MRI for GM and WM segmentation and volumetric analyses.

References

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3. http://www-mrsrl.stanford.edu/~jbarral/t1map.html