Ex-vivo MRI of the brain: Effects of long-term formalin exposure on T1 relaxation times

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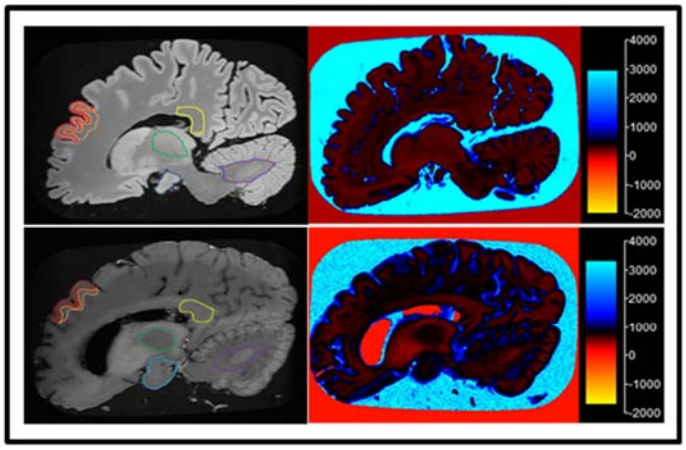
Target Audience Researchers interested in understanding the effects of tissue fixation on T1 (longitudinal) relaxation time in *ex vivo* MRI of human brain tissue. Relaxation times are useful as markers of tissue properties in imaging studies and determining tissue inversion times.(1)

Purpose To determine the effects of fixation on T1 relaxation of gray matter (GM) and white matter (WM) in *ex vivo* brain MRI. Tissue T1 relaxation times are critical for optimizing MPRAGE and Double Inversion Recovery (DIR) sequences. MPRAGE is commonly used in Alzheimer's disease research to measure GM atrophy. DIR is increasingly being used in multiple sclerosis (MS) research to suppress both fluid and either WM or GM for clearer visualization of MS lesions. (2) Optimized T1 relaxation times of GM and WM are required to calculate the appropriate inversion times for both sequences. Established T1 values for living subjects will not be accurate for tissue that has been excised and stored in fixative for varying amounts of time. We measured GM and WM T1 values in two ex-vivo brains; one at 7 days of fixation and the other at 24 months of fixation. We investigated the impact of long versus short fixation time periods on T1 values. Previous studies investigating effects of fixation time have only recorded effects on the order of weeks.(3)

Methods Using Inversion Recovery Spin Echo 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. Using fitting code from Barral J, et al (4) 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 1. ROIs shown on T1-weighted image (left) were used to measure mean T1 on T1 map (right) in (**A**) 24 month fixed brain and (**B**) 7-day fixed brain. ROIs represent GM and WM that are either exposed or not exposed to formalin.

ROI	Anatomy	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



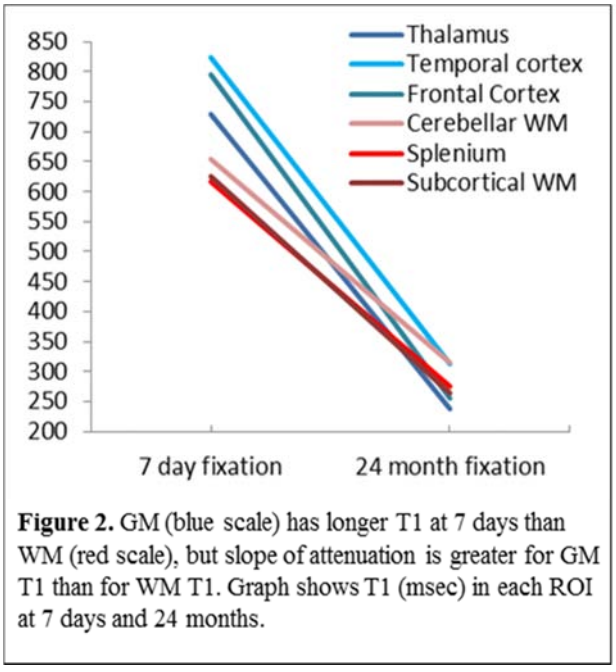


Figure 2. GM (blue scale) has longer T1 at 7 days than WM (red scale), but slope of attenuation is greater for GM T1 than for WM T1. Graph shows T1 (msec) in each ROI at 7 days and 24 months.

Results The 24 month fixed tissue has shorter T1 times than the 7 day fixed tissue. This is in agreement with studies that have shown attenuation of T1 over time after fixation over three weeks.(3) GM T1 is longer than WM T1 at 7 days post-fixation, similar to findings *in vivo*, but becomes shorter than WM T1 after ~ 2 years of fixation showing greater attenuation. The GM directly exposed to formalin in temporal and frontal cortices have longer T1s than the GM not directly exposed to formalin in the thalamus. Similarly, cerebellar WM that is not directly exposed to formalin has slightly longer T1 relaxation time than the splenium of the corpus callosum which is directly exposed to formalin. As shown in **Figure 2** the slope of T1 attenuation over time was greater for GM compared to WM suggesting that the effects of formalin exposure on relaxation time are greater in GM than in WM.

Conclusion T1 relaxation times of fixed brain tissue attenuate over time. Effects of formalin exposure on tissue relaxation time appear to be greater for GM than WM. This information is essential for optimizing inversion times for MPRAGE and DIR sequences used in ex-vivo brain MRI.

References

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