

## Real time changes of phase contrast of gray matter and white matter between *in vivo* and *in situ* postmortem rat brain

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**Purpose:** Phase images obtained by gradient echo MRI provide enhanced contrast of the brain anatomy [1]. The nature of this contrast is still a hot subject under investigation. One of the ways to understanding the biophysical origins of the phase contrast is by studying postmortem brain tissues. However, the phase contrast could change substantially between live and postmortem tissue e.g. [2] and between fresh postmortem and formalin fixed tissue e.g. [3], while the reasons for that are not known. In this pilot study, we measure the realtime changes of gray/white matter contrast in gradient echo MR signal in rat brain between *in vivo* and *in situ* postmortem states. We further confirm that the change in blood oxygenation level contribute little to gray/white matter contrast.

**Methods:** A female Sprague-Dawley rat was kept anesthetized by breathing 1.5% isoflurane (O<sub>2</sub> at 1 LPM) in the MR scanner. After 20 min of data acquisition, rat was euthanized by 0.3 ml Xylazine/Ketamine cocktail injected i.p. while isoflurane is turned off, leaving O<sub>2</sub> at 1 LPM. Scanning continued for another 20 min. The rat was kept warm by a pad with warm circulating water in the scanner throughout the process. Experiments were performed on a Varian DirectDrive<sup>TM</sup> 4.7T MR scanner using a 1.5 cm diameter surface transmit/receive RF coil. Data was collected using 2D multi-gradient echo sequence with 4 echoes, matrix 192 x 192, 3 slices; in plane resolution 0.167 x 0.167 mm<sup>2</sup>, slice thk 1mm, gap 1.2mm; TR = 163 ms, first TE = 10 ms, echo spacing = 12.8 ms, FA = 50 deg, SW = 30.5kHz, 4 averages, acquisition time = 2 min. Total of 20 acquisitions were collected (10 prior and 10 after euthanizing). R2\* and frequency maps were obtained by monoexponential fitting of magnitude data, and linear fitting of unwrapped phase images. Regional contrast changes were then analyzed based on ROI selection on postmortem T2\* weighted image – cortex represented as GM, corpus callosum as WM, and cortex excluding visible vessels as GMNV.

**Results:** Figure on the right shows R2\* maps (upper row) and unfiltered frequency maps (bottom row) of the rat brain live and postmortem respectively. Big blood vessels that run across the cortex become brighter on both R2\* and frequency maps due to the postmortem depletion of oxygen in the blood and brain tissue. Global shift of 7.3 Hz is observed in frequency maps between the live and the first postmortem data. This shift kept increasing and reached 14.8 Hz in the last postmortem frequency map. One of the explanations for this drift could be due to the temperature drop after blood circulation stops (- 0.01 ppm per K [4]). Plots shown on the right demonstrate changes of the contrast between gray matter and white matter over time. Each point represent 2 min period. The first 10 points represent live stage, which are stable with minor fluctuations. Immediately after euthanasia, the  $\Delta R2^*$  (GM-WM) went up by 8.0 s<sup>-1</sup>, whereas  $\Delta f$  (GM-WM) went up by 0.48 Hz. During the following 20 min,  $\Delta R2^*$  (GM-WM) continued to slightly increase while  $\Delta f$  (GM-WM) continued to decrease. On the other hand, when blood vessels are excluded from ROI selection of grey matter, the  $\Delta f$  (GMNV-WM) shows the same decrease during the last 20 min, without observable jump after euthanasia.

**Discussion:** At the transition point between live and postmortem stages, it is plausible to assume that the changes in  $\Delta R2^*$  (GM-WM) and  $\Delta f$  (GM-WM) can be attributed to the increase of blood deoxygenation level due to interrupted supply but continuous demand of oxygen. Further, the lack of change in  $\Delta f$  (GMNV-WM) at this point confirms with previous study that demonstrates deoxyhemoglobin has no significant contribution to gray/white matter phase contrast [5]. However the more curious phenomena are the gradual decrease in GM/WM contrast that happened throughout 20 min after the transition point. This cannot be attributed to inhomogeneous change of temperature since GM would be expected to cool faster than WM and would lead to the opposite  $\Delta f$  trend than shown in the plot. The frequency contrast decrease between GM and WM postmortem agrees with the observation in human study (compare figure 3c with figure 4c in [2]). One of the explanations can be proposed based on the generalized Lorantzian approach [6] that would predict substantial change in the phase contrast due to the changes in tissue integrity [7]. This observation should be kept in mind when trying to understand frequency contrast of grey/white matter in brain both *in vivo* and postmortem.

**Conclusion:** We have demonstrated for the first time changes in gray/white matter phase contrast and R2\* from live to postmortem conditions. The oxygen depletion alone could not explain the changes observed after death *in situ*. The decreasing GM/WM contrast calls for attention for current investigations in the origin of phase contrast *in vivo* based on only tissue chemical compositions. Further investigation is needed to clarify the mechanisms of the observed changes in the postmortem brain.

**References:** 1. Duyn, et al., PNAS, 2007, 104, p11796; 2. Walsh, et al., Radiology 2013, 267, p531; 3. Luo et al., MRM 2013, DOI:10.1002/mrm.24762; 4. Hindman, J. Chem. Phys, 1966, 44, p4582; 5. Lee, et al., NeuroImage, 2010, 49, p193. 6. He and Yablonskiy, PNAS 2009, 106, p13558; 7. Yablonskiy et al, PNAS 2012, 109, p14212.

