A Window for High-Resolution Post-Mortem DTI: Mapping Contrast Changes in Neural Degeneration

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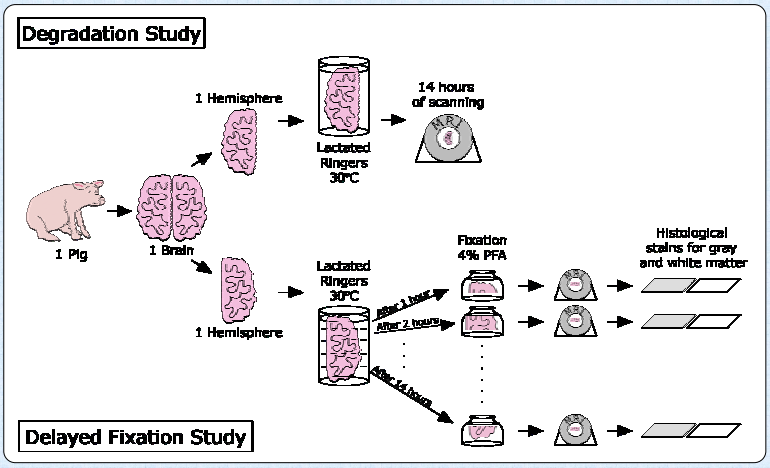
INTRODUCTION

- High-resolution, post-mortem MRI provides stunning structural images of cerebral anatomy that are not possible *in vivo* due to motion and rapid imaging artifacts.
- The reliability and accuracy of post-mortem diffusion tensor imaging (DTI) measurements are not well understood, in part due to the unknown effects of fixation on the brain's diffusion properties.
- In animal studies, peri-mortem perfusion fixation alters mean diffusivity (MD), but leaves fractional anisotropy (FA) relatively unaffected [1,2].
- In human studies, immersion fixation has lead to generally poor quality DTI contrasts [3,4].
- Routine post-mortem processing typically involves 24 to 48 hours post-mortem delay before fixation begins. Additionally, brain dead and cerebral ischemia patients are common donors, which may involve an additional 24 to 72 hours of pre-mortem cessation of cerebral vascular perfusion. Because neural tissue degrades relatively quickly *in situ* and *ex vivo* without fixation, DTI contrast is largely lost in these subjects. In cases of highly motivated donors – e.g., those with a terminal condition and a status of "do not resuscitate" and "do not intubate" – it is possible to acquire DTI data within a much shorter time period. **But how soon is "soon enough?"**

We use a porcine model to study the acute time course (<18 hours) of neural tissue degradation and response to immersion fixation to enable the design of rapid protocols for post-mortem DTI studies.



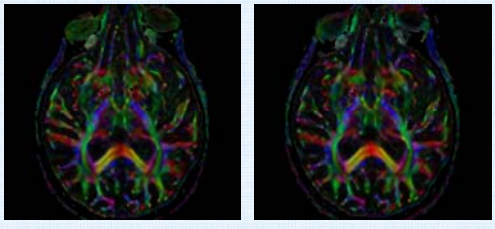
Methods

- Intact cerebra were removed from two porcine specimens (~25 kg, 3-4 months) ~1 hour post-mortem. The left frontal lobe from the first specimen was serially MR imaged without fixation or added fixatives (the "Degradation Study"). The right frontal lobe of the first specimen and both frontal lobes of the second specimen were immersed in lactated ringers solution in a water bath at 30 °C. At hourly intervals, a 5 mm section was cut from each lobe, immediately immersed in 4% paraformaldehyde (PFA), and refrigerated at 4 °C for 6 weeks, and then MR imaged (the "delayed fixation study"). All procedures were conducted in accordance with institutional animal care and use regulations. DTI was performed on a Bruker Biospin 11.7T MR scanner at a constant temperature of 30 °C and consisted of multi-slice, multiple spin echo RARE DTI sequences (four echoes TE= 24.41/34.67/44.93/55.19 ms, TR=2300 ms, 2 NSA, nominal 0.18x0.18x1 mm). Diffusion contrast was achieved with a six direction dual gradient diffusion weighting scheme with a b-value of 1000 s/mm^2 [5]. Each DTI sequence required approximately 65 minutes. Minimally weighted (b0) volumes were classified into gray matter (GM), white matter (WM), and cerebrospinal fluid/artifact categories with a semi-automated method.

Preview: A Rapid Protocol for *Post-Mortem* Human Imaging

DTI performed immediately *post mortem* (no fixation)

Philips 3T Intera, 6-channel SENSE head coil (factor 2).
In Vivo: 2.2 mm iso.
TE/TR:89/6000 ms
Post Mortem: 2.1 mm iso.
TE/TR:67/7295 ms.
Diffusion weighting applied along 32 independent directions with a b-value of 700 s/mm^2.



In Vivo *Post-Mortem (7 hrs)*

RESULTS

Degradation Study

(no fixation, between t=3 and 17.1 hrs)

- FA decreased by 0.05 [FA] (9.0%) in WM and 0.02 [FA] (6.2%) in GM.
- MD decreased by 0.067x10^{-3} mm^2/s (14.3%) and 0.004x10^{-3} mm^2/s (0.6%).

Delayed Fixation Study

(immersion fixation between 1 and 13 hrs)

Impacts of Fixation

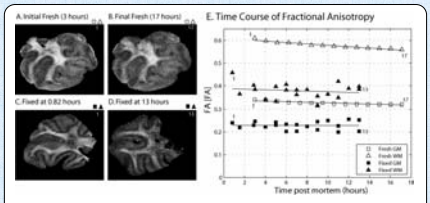
- Fixation resulted in a mean FA decrease of ~35% in WM and ~30% in GM and mean MD decrease of ~51% in WM and ~56% in GM relative to the scan of non-fixed tissue of the same post-mortem time point.

Impacts of Delayed Fixation

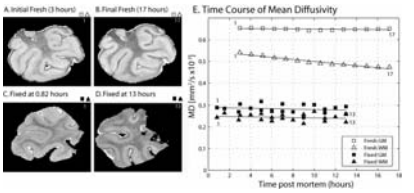
- FA decreased by 0.07 [FA] (16.0%) in WM and 0.04 [FA] (15.6%) in GM
- MD decreased by 0.022x10^{-3} mm2/s (9.0%) and 0.03x10^{-3} mm2/s (10.5%)

Summary

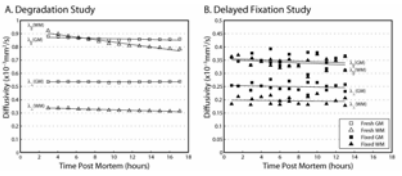
The within tissue compartment decrease in SNR with delayed fixation indicates that the changes in MD and FA are not uniform, which suggests increasingly uneven tissue degeneration over time, even with a short (13 hour) period. Delaying fixation negatively impacts the achievable FA contrast to noise ratio through (1) overall loss of diffusivity, (2) increased loss of diffusivity in WM relative to GM, and (3) increased intra-tissue compartment variability.



Fractional Anisotropy is lost more quickly in white matter than gray matter.



Mean Diffusivity shows a strong post mortem decrease, while gray matter is relatively stable.

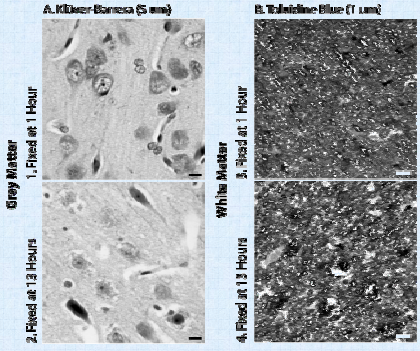


Diffusivity changes are principally due to alternations in $\lambda_{\perp}$ for both the degradation and delayed fixation studies.

CONCLUSION

- **DTI contrasts are well preserved during a "window" of 6-10 hours post-mortem.**
- ***During this period:***
  - **High resolution, post-mortem DTI will likely succeed without fixation.**
  - **The relationships between the parallel and perpendicular diffusivities of GM and WM are relatively intact.**
- **The observed dependence of DTI contrasts on *post mortem* delay could explain the variability of previous studies investigating *ex vivo* human imaging.**
- **In the acute time period, degeneration more severely impacted parallel diffusivity, which may be indicative of primary axonal degeneration with relative sparing of myelin integrity.**

Preview: Histological Changes in Tissue Degeneration

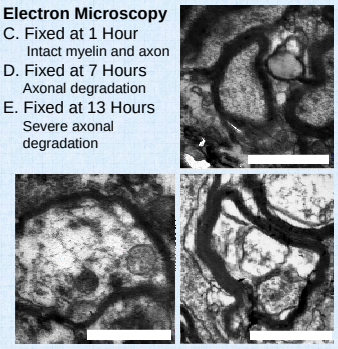


Electron Microscopy

C. Fixed at 1 Hour
Intact myelin and axon

D. Fixed at 7 Hours
Axonal degradation

E. Fixed at 13 Hours
Severe axonal degradation



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