Comparison of *in vivo* and *ex vivo* Diffusion Spectrum Imaging (DSI) of Rat Brain

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Introduction: Diffusion spectrum imaging (DSI) (1) allows for measurement of the three dimensional diffusion probability density functions (PDFs) in tissue without any assumptions as to the nature (e.g. Gaussian) of the diffusion curves. DSI tractography overcomes some of the drawbacks of 'conventional' DTI tractography (e.g. failure to resolve fiber crossings, and lack of information about cortex) but requires many more diffusion 'directions' (typically several hundred) and a much higher b-value. Multislice EPI DSI datasets can be acquired in humans in 20-30 minutes with 5mm isotropic resolution. In fixed tissue, higher spatial resolution is possible with longer scan times, which allows for histological validation of the MRI diffusion anisotropy data (2). The purpose of the current work was to develop an approach to acquiring *in vivo* and *ex vivo* high resolution DSI tractography data in the same individual brains to serve as a bridge between in vivo imaging and histological analysis of the tissue.

Methods: Adult male rats were prepared with a tracheostomy and ventilated at 43 bpm with 50/50 air oxygen and anesthesia was maintained with 2% Isoflurane. Arterial blood pressure, rectal temperature and pulse oxymetry parameters were measured continuously. Fluid (lactated Ringers) was given at 1ml/hr. This preparation maintained a stable physiological condition for more than 4 hours in the magnet. Animals were placed in a plastic stereotax / MRI probe assembly, with a close fitting elliptical transmit/receive surface coil on the head. Temperature was maintained with a warm water pad. We used a 4.7T Bruker Avance system equipped with actively shielded 40G/cm gradients. Our objective was to match the *in vivo* and *ex vivo* scans as closely as possible to facilitate a comparison of DSI contrast in various structures. In vivo scans were limited to 4 hours. Immediately after scanning, animals were removed from the magnet, euthanized and the brains removed and perfused fixed in 4% paraformaldehyde (PFA). After fixing, the brains were soaked for 3 days in a solution of 1mM GdDTPA in phosphate buffered saline (PBS). This concentration of GdDTPA was chosen so as to reduce the T_1 of the brain, allowing us to scan more rapidly, reaching the gradient duty cycle limit with minimal saturation of the signal. Brains were placed in a tube of susceptibility matching liquid (Fomblin LC/8) within a 2.5cm dia. birdcage coil. For all DSI scanning we used a 3D EPI diffusion sequence (2D EPI, Z phase encoded). A comparison of multislice 2D EPI and 3D EPI, running at the gradient duty cycle limit and acquiring a whole *live* brain volume in 28sec, resulted in a 2 fold SNR advantage for 3D EPI. DSI scans used a 514 point Q-space trajectory covering a 3D Cartesian grid. Relevant imaging parameters are:

Brain	T_2 (ms)	T_2* (ms)	T_1 (ms)	TE (ms)	TR (ms)	Resol. (μm)	δ (ms)	Δ (ms)	b_{max} (smm^{-1})	NEX	Acq time	Peak SNR
in vivo	65	55	1500	39	875	400	12.75	22.41	30000	1	4:00	60:1
ex vivo	43	30	200	46	450	265	7.6	19.3	10000	3	25:00	60:1

Raw data were Fourier transformed offline with our own software. Q-space data for each voxel was Fourier transformed to generate the 3D diffusion probability density functions, which were used by the tracking algorithm to calculate all possible tracts in the brain.

Results: Soaking the rat brains in PBS after fixation in PFA increases the T_2 and T_1 (to ~35 and 400ms) compared to the values in PFA. The use of GdDTPA reduces the T_1 significantly, with only slight effect on T_2. Using the same pulse sequence, similar cortical SNR was achieved in the b=0 images in spite of the 3 fold smaller voxel volume. Figure 1 compares DSI fiber tracts from the same rat brain scanned alive and dead. For simplicity, only fibers passing through the axial slice in (A) are displayed While the higher resolution in the *ex vivo* scan shows substantially more fibers, the same structures are clearly visible in both scans.

Conclusion: Our results show that diffusion anisotropy and apparent fiber structure in the brain are substantially maintained after death and fixation. The excellent correspondance between fiber structures seen *in vivo* and *ex vivo* supports the use of *ex vivo* DSI (and subsequent histology) as a means of understanding the source of both normal and pathological image contrast on the *in vivo* scans.

References: (1) Wedeen et al., ISMRM, 2000, p. 38. (2) Tseng et al., JMRI 17(1), 203, 31-42.

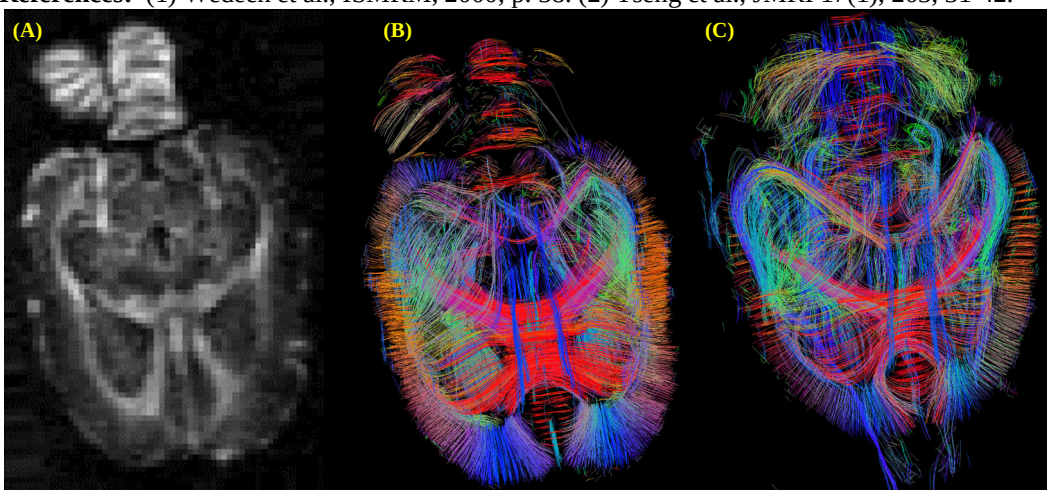

Figure 1: *In vivo* and *ex vivo* images of the same rat brain:

(A) Heavy diffusion weighted image (not FA) showing the axial anatomical level.
(B) *Ex vivo* brain: 3D rendering of the DSI fibers passing through the axial slice shown in A.
(C) *In vivo* brain: 3D rendering of fibers passing through a similar axial slice.

Structures seen in both *in vivo* and *ex vivo* data include the radial and some horizontal cortical fibers, the fimbria, the external and internal capsule, and the cingulum bundle.