Reproducibility and Variation in Diffusion Measures of the In Vivo and Ex Vivo Squirrel Monkey Brain

Kurt Schilling[1], Yurui Gao[1], Iwona Stepniewska[2], Ann S Choe[1], Bennett A Landman[3], and Adam W Anderson[1]

[1]*VUIIS, Vanderbilt University, Nashville, TN, United States,* [2]*Psychology, Vanderbilt University, Nasvhille, United States,* [3]*Electrical Engineering, Vanderbilt University, Nashville, TN, United States*

PURPOSE The purpose of this study is to characterize the diffusion properties of the common squirrel monkey brain, and determine whether the squirrel monkey is a suitable model for studying diffusion MRI (dMRI). The use of non-human primates allows *in vivo* as well as *ex vivo* acquisitions, which have several experimental advantages including longer scan times and absence of motion. Another advantage afforded by *ex vivo* dMRI is the ability to compare the diffusion data directly to histology, which serves as a gold standard for microstructural measurements. The aims of this study are threefold: (1) assess the reproducibility of *in vivo* DTI measures both within and between subjects; 2) evaluate the agreement between *in vivo* and *ex vivo* DTI data acquired from the same specimen and 3) determine normal diffusion values and their variation in white matter (WM) and gray matter (GM) regions of interest (ROIs).

METHODS The data in this experiment were acquired from three healthy squirrel monkeys, each imaged twice *in vivo* and once *ex vivo*. Each monkey was anaesthetized and scanned in two sessions (with 20-day interval) on a 9.4T scanner (PGSE EPI sequence, TR=5.5s, TE=44ms, gradient directions=32, b≈1000s/mm^2, voxel size=630×630×630μm^3, data matrix=64×64×65). The monkey was then sacrificed, the brain perfusion fixed, and e*x vivo* diffusion MR images were acquired at 300um isotropic resolution using a PGSE multi-shot spinwarp sequence on a 9.4 T (TR=4.6s, TE=42ms, gradient directions=32, b≈1000s/mm^2, voxel size=300×300×300μm^3, data matrix=115x128x192). For comparisons both within and between monkeys, a template image was created using an iterative registration procedure similar to that used in [1]. All datasets were registered to a "common space" defined by the template. The brain was frozen and cut to 50um thick histological sections, which were used to define ROIs as described previously[2].

RESULTS Reproducibility: reproducibility of *in vivo* scans was assessed through calculation of the intra-subject and inter-subject coefficient of variation (CV) for both FA and MD. The calculated CVs for both intra-subject and inter-subject MD were all below 10% for both WM and GM ROIs, while the CV results for FA had a wider range (<20%). The CVs reported here align well with the literature of the human brain[3,4]. *In Vivo/Ex Vivo*: Figure 1 shows comparisons of *in vivo* and *ex vivo* MD and FA in both WM and GM ROIs. MD values in the fixed *ex vivo* tissue are much lower than those of the living monkey (30%-50% decrease), while FA values increased in both WM and GM ROIs (30-39% increase). The decreased MD is attributed to a reduced temperature as well as the effects of formalin fixation on membrane permeability[5], while the increased FA may be due to the smaller voxel size resulting in less partial volume effects. Finally, the orientation of the primary eigenvectors (PEV) of the *in vivo* and *ex vivo* scan sessions were compared, resulting in a mean angular difference of 22 degrees, while over 75% of voxels agreed to within 25 degrees. A closer inspection of the data shows good agreement in areas of high FA, and poor agreement in regions with crossing fibers and lower FA (Figure 2).

CONCLUSION Here, we characterize the diffusion properties of the squirrel monkey brain. We show the reproducibility of MD, FA, and PEV is comparable to that of human DTI studies. Second, we determine the relationship between *in vivo* and *ex vivo* diffusion properties. Finally, we establish normal values of diffusion indices in both WM and GM regions. Together, this serves as the basis for using the squirrel monkey brain for diffusion MRI studies.

REFERENCES [1] Fonov et al. 2011, *NeuroImage*. [2] Gao et al. 2013. *PlosOne*. [3] Papinutto et al. 2013. *MRI*. [4] Heim et al. 2004. *Magn Reson Med*. [5] Thelwall et al. 2006. *Magn Reson Med*.

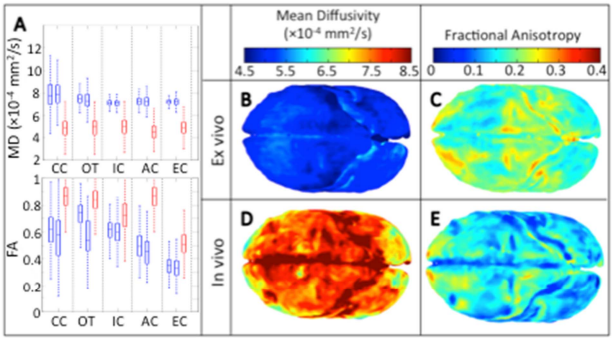

Figure 1. Comparison of *in vivo* and *ex vivo* diffusion properties. MD (A, top) and FA (A, bottom) over five WM ROIs. *Ex vivo* MD (B) and FA (C) across the cortex, and *in vivo* MD (B) and FA (C) across the cortex.

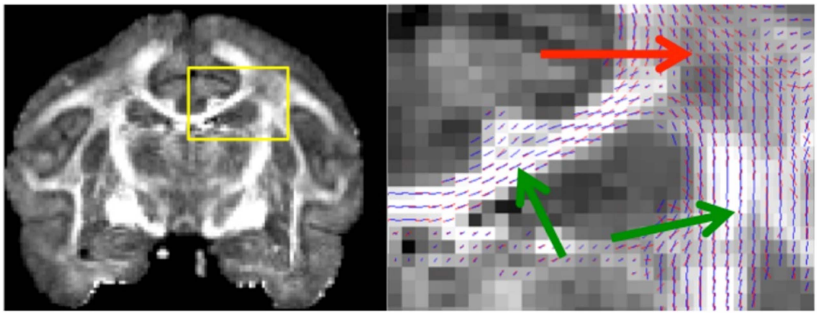

Figure 2. Voxel-wise comparison of *in vivo* and *ex vivo* PEV orientation. Coronal *ex vivo* FA map (left) shows location of white matter region (right) which includes lines of principal eigenvector of *ex vivo* (red) and *in vivo* (blue) sessions of a single monkey. Green arrow points towards regions of high agreement (corpus collosum, corticospinal tract), and red arrow points towards region of crossing fibers and low