Comparison of in vivo and ex vivo DTI cortical connectivity measurements in the squirrel monkey brain

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INTRODUCTION

In a previous study[1], we validated DTI-tractography-derived measures of primary motor area (M1) corticocortical (CC) connectivity by comparing the *ex vivo* DTI connectivity with the histological ground truth. *Ex vivo* acquisitions typically provide higher image quality than *in vivo* experiments, since scan times can be much longer and motion is usually not an issue. The goal of the present study was to get a more realistic understanding of the limitations of *in vivo* DTI measures of CC connectivity by comparing the reliability of *in vivo* and *ex vivo* DTI data acquired from the same animal.

MATERIALS AND METHODS

Data acquisition: A live squirrel monkey was anaesthetized and scanned on a 9.4T Agilent scanner (PGSE EPI, TR=5.5s, TE=44ms, number of gradient directions=20, b≈1000s/mm^2, voxel size=0.63mm×0.63mm×0.63mm, data matrix=64×64×80). A month later, the bidirectional neural tracer biotinylated dextran amine (BDA) was injected into 8 sites covering the forelimb movement representation area in the left M1 cortex of the monkey. After two weeks, the monkey was sacrificed and the brain was immediately extracted, fixed and scanned (PGSE multishot spinwarp imaging, TR=4.6s, TE=42ms, number of gradient directions=31, b≈1020s/mm^2, voxel size=0.3mm×0.3mm×0.3mm, data matrix=128×128×192). Then the brain was sectioned coronally at 50μm thickness on a freezing microtome and the blockface was photographed. Every sixth section was reacted for BDA (each adjacent section was stained for Nissl) and photographed under a light microscope (0.5X objective for registration and 4X for fiber segmentation).

Data preprocessing: Locations of BDA-stained fibers crossing the white-gray matter interface were identified by computer-assisted morphological segmentation on 4X micrographs. Using computer-assisted gridding and counting, the 2D density distribution maps (DDMs, 256×256 pixels per DDM) of BDA-stained interface-crossing fibers were produced. The 2D BDA DDMs were transferred from the micrograph space to the DTI space using deformation fields calculated via a modified multi-step registration procedure[2][3]. Finally, the intensities of DDMs were projected onto the 3D white-gray matter interface which was segmented using a variational level set approach [4] on the registered T2w images. In addition, the BDA injection region and different cortical projection regions were manually segmented based on architectonics of BDA-stained and Nissl-stained neurons[5].

DTIStudio[6][7] was used to estimate tensors and perform deterministic fiber tracking (parameters: start FA=0.1, stop FA=0.2, stop angle=70°) with the transformed BDA injection region as the seed region. The DTI fiber DDM was produced by counting the DTI fibers passing through each DTI voxel located at the gray-white matter interface. Seed regions that extend to two depths in the white matter (denoted by '*dw*', =0mm and 0.6mm) were used. The above processing pipeline was performed on both the *in vivo* and *ex vivo* DTI data.

Data analysis: We calculated the average fractional anisotropy (FA) and average mean diffusivity (MD) over the white matter (WM) and gray matter (GM) based on the *in vivo* and *ex vivo* DTI data. We also calculated Pearson's (and Spearman's rank) correlation coefficients r_p (and r_s) with p values between numbers of *in vivo* (and *ex vivo*) DTI-tractography-derived and BDA-stained fibers across all the projection regions.

RESULTS

Table 1 shows the average FA and MD over the WM and GM on DTI data sets. Fig.1 displays the DDMs of BDA, *in vivo* DTI and *ex vivo* DTI fibers rendering on the white-gray matter interface. Table 2 displays the Pearson correlation (r_p) and Spearman rank correlation (r_s) coefficients (with p values) of numbers of tractographic-histological fibers across all the projection regions.

(Abbreviation: i-ipsilateral; c-contralateral; PF-prefrontal cortex; PM-premotor cortex; PA-anterior parietal cortex; PP-posterior parietal cortex; SMA-supplementary motor area; AC-anterior cingulate cortex; IR-injection region)

CONCLUSION AND DISCUSSION

Compared with *ex vivo* data, the average FA (MD) over both WM and GM on *in vivo* data decreased (increased) significantly, as shown in Table 1. Comparison of DTI DDMs in Fig.1 revealed that the *in vivo* DTI fibers propagated less far (i.e. do not reach iPF, iPA and iPP regions) than *ex vivo* fibers using the same tractography parameters. Fig.1 also illustrates that the distribution pattern within each projection region of the *in vivo* DDM was quite different from the BDA and *ex vivo* DDM patterns. Both Pearson and Spearman correlations in Table 2 indicated that the *in vivo* tractography-derived CC connectivity was less associated with histological connectivity compared with *ex vivo* tractographic connectivity. After reducing the values of the start FA and stop FA for tractography on *in vivo* data, the DTI fibers were able to propagate farther as predicted, even to reach all the projection regions, but the correlation coefficients of tractographic-histological CC connectivity did not show a significant increase.

REFERENCES

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	In vivo	*Ex vivo*
FA_{WM}	0.49±0.16	0.76±0.16
FA_{GM}	0.20±0.12	0.40±0.17
MD_{WM}	0.73±0.11	0.29±0.08
MD_{GM}	0.81±0.23	0.33±0.10

Table 1. Average fractional anisotropy (FA) and mean diffusivity (MD, mm^2/ ms) over the white matter (WM) and gray matter (GM) on the *ex vivo* and *in vivo* DTI data of the same monkey brain.

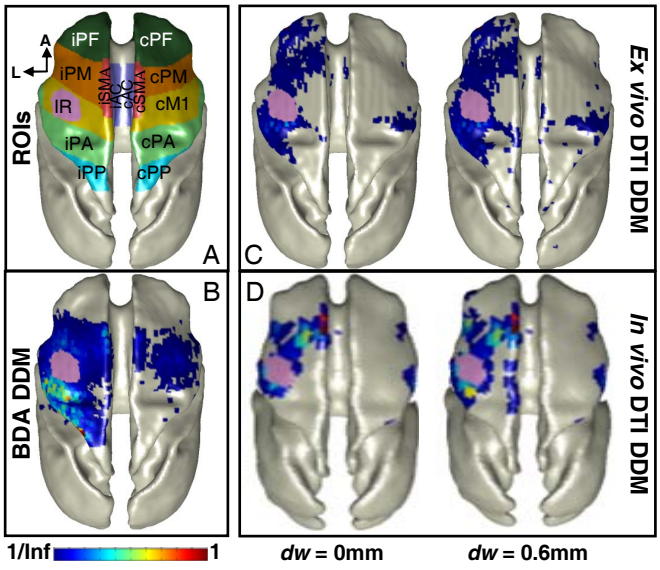

Fig.1. Dorsal view of normalized 3D DDMs rendering on the white-gray matter interface of the monkey brain. (A) shows the territories of all the projection regions and BDA injection region (IR). (B) shows the BDA fiber DDM and (C-D) show the DTI fiber DDMs calculated from *ex vivo* and *in vivo* DTI data (in rows) using seed region with different *dw* (in columns).

	dw = 0mm		*dw* = 0.6mm	
	In vivo	*Ex vivo*	*In vivo*	*Ex vivo*
r_p (p)	0.30 (0.25)	0.75 (0.00)	0.50 (0.04)	0.86 (0.00)
r_s (p)	0.37 (0.14)	0.30 (0.24)	0.37 (0.15)	0.45 (0.07)

Table 2. Pearson correlation (r_p) and Spearman rank correlation (r_s) coefficients (with p values) of numbers of tractographic-histological fibers across all the projection regions.