Discrepancies Between DTI Measures and Histology in Spinal Cord and Brain in Experimental Animal Models

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Introduction

Fractional anisotropy (FA), based on diffusion tensor imaging (DTI), provides important information about tissue integrity. The value of FA depends on a number of factors that include degree of myelination and axonal morphometry and thus lacks pathologic specificity. There is some literature, mainly based on animal studies, that the individual diffusivities, (longitudinal (λ_l) and radial (λ_t)) improve pathologic specificity over FA[1,2]. For example, in a mouse models of white matter injury Song et al[2] interpreted a decrease in λ_l to be associated with axonal injury and dysfunction while increased λ_t to be associated with myelin damage. Herrera et al[3], in experimental spinal cord injury, demonstrated inconsistent correlations between the individual diffusivities and the histology [3]. Similar inconsistencies in spinal cord injury were also reported by DeBoy, et al[4]. In this study we performed DTI and histology in both spinal cord and brain in experimental animal models. These studies do not demonstrate a direct correlation between the individual diffusivities and myelin and neurofilament.

Materials and Methods

These studies were performed on Sprague Dawley rats weighing between 350-450g. The experimental models include 1) spinal cord and brain tissues in spinal cord injury and 2) brain tissue with chronic cocaine treatment. SCI animals were divided into two groups of six each: 4 and 8 week post-injury. Six cocaine and six saline treated animals were also included in this study. All the MRI studies were performed at eight weeks post injury for brain scans and multiple time point for spinal cord scans. The spinal cord was injured under controlled conditions at T7 level. Heart and respiratory rates and rectal temperature were monitored during the entire MRI scan (7T on a Bruker horizontal bore scanner). Diffusion weighted images were acquired using a four-shot spin echo EPI readout sequence with a rotationally invariant and balanced icosahedral scheme with 21 alternating positive and negative encoding directions (Ne = 42). Acquisition parameters: TR of 3000 msec, TE = 38 msec and 20 slices of 1 mm thickness. Diffusion weighted images were registered to the rat brain in stereotaxic coordinates (Paxinos and Watson). The images were then imported into DTI Studio (Jiang H 2006) software to calculate the DTI parameters such as fractional anisotropy (FA), mean diffusivity (MD), and eigenvalue maps and fiber tracking. Following the terminal MRI scans spinal cord and brain sections were processed and were incubated and stained for myelin basic protein (MBP), neurofilament-heavy protein (NF-H), GAP-43 and glial fibrillary acidic protein (GFAP).

Results

(A) ***Spinal cord:*** Our results from histologic analysis of rostral and caudal sections indicated correlations between individual diffusivity measure λ_t and myelin in both the dorsal and ventral white matter regions and no correlation in the lateral white matter. Axonal integrity was also examined and based on our histological analysis we observed correlations between λ_l in the dorsal and ventral rostral regions of the injured spinal cord. No correlations were observed in either the lateral or caudal regions to the epicenter. (B) Represents a spin echo image showing the placement of the ROI used and the corresponding immunoflourescent image labeling neurofilament.

(C) ***Brain:*** Similar analysis was performed on brain regions demonstrating an increase in individual diffusivities and histology. We observed no correlation between λ_l and λ_t diffusivities compared with neurofilament and myelin. We did however observe other populations that may contribute to both λ_l and λ_t diffusivities (see Table 2).

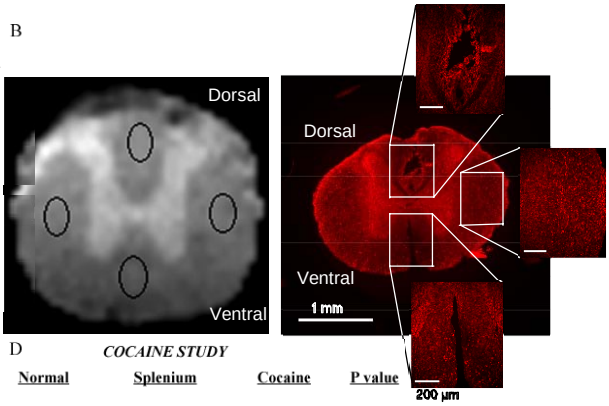
(D) ***Cocaine Study:*** A major observation made in this study was the demyelination in the splenium of cocaine treated subjects, which is evident by the reduced FA values. Also there is a significant increase in the value of λ_t, which is consistent with the decreased MBP count, however, the NF-H value does not correlate with the λ_l measures.

A

SPINAL CORD

SCI Region 4 weeks injured			SCI Region 8 weeks injured	
Rostral			**Rostral**	
Ventral	0.70 ± 0.085	*FA*	Ventral	0.65 ± 0.097
Ventral	2.11 ± 0.180	λl	Ventral	2.22 ± 0.154
Ventral	0.52 ± 0.031	λt	Ventral	0.65 ± 0.014
Ventral	13.89 ± 3.04	*Percent NF-H*	Ventral	4.24 ± 0.441
Ventral	6.80 ± 1.623	*Percent MBP*	Ventral	3.25 ± 1.959
Rostra Lateral			**Rostra Lateral**	
Lateral	0.65 ± 0.045	*FA*	Lateral	0.68 ± 0.047
Lateral	2.37 ± 0.102	λl	Lateral	2.24 ± 0.211
Lateral	0.68 ± 0.046	λt	Lateral	0.61 ± 0.049
Lateral	14.02 ± 2.36	*Percent NF-H*	Lateral	3.66 ± 0.469
Lateral	4.89 ± 0.778	*Percent MBP*	Lateral	0.31 ± 0.187

B



C

BRAIN

Normal	Internal Capsule	6 weeks injured	P value
328 ± 7.992	Fiber counts	664 ± 81.33	0.0022
0.5338 ± 0.005	FA	0.6333 ± 0.006	0.0022
0.00117 ± 0.000002	λl	0.00118 ± 0.000003	0.7169
0.00039 ± 0.000007	λt	0.00038 ± 0.000001	0.1063
72.83 ± 2.826	Percent NF-H	50.22 ± 4.040	<0.0001
35.12 ± 2.490	Percent MBP	13.69 ± 1.568	<0.0001
0.6602 ± 0.0552	Percent GAP-43	1.112 ± 0.0754	<0.0001
1.662 ± 0.0579	Percent GFAP	4.169 ± 0.1192	<0.0001

D

COCAINE STUDY

Normal	Splenium	Cocaine	P value
0.56 ± 0.04	FA	0.48 ± 0.02	0.0051
0.0013 ± 0.00001	λl	0.0012 ± 0.0001	0.03
0.00044 ± 0.00007	λt	0.0005 ± 0.00006	0.021
20.8 ± 2.46	Percent NF-H	28.1 ± 3.39	0.165
37.1 ± 3.82	Percent MBP	21.5 ± 2.59	0.0378

Discussion: Our studies on both brain and spinal cord tissue demonstrate that in some circumstances there is an apparent correlation between individual diffusivities and histology. Yet our studies also demonstrate discrepancies between λ_l and λ_t diffusivities and the results of neurofilament and myelin. We observed more discrepancies with λ_l a proposed indicator of axonal integrity than λ_t. Other studies have demonstrated similar discrepancies between individual diffusivities [4] and have indicated that other cell populations might play a role in determining the individual diffusivities [5]. These studies demonstrate that care should be exercised in inferring the pathologic substrate from the measured diffusion metrics.

Reference:

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