Brain DTI of spinal cord injured rats

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Introduction

Recent fMRI studies have identified activation of multiple areas in both cortical and deep gray matter structures in response to electrical stimulation of the forepaw in spinal cord injured animals, indicating extensive brain plasticity [1]. However, the reasons for the activation of multiple sites is not known. We hypothesize that the spontaneous generation of new fibers and/or reorganization of existing fibers could be responsible for the observed activation. We further hypothesize that this reorganization would occur in the corticospinal tract (CST), the largest fiber bundle travelling between the spinal cord and the brain. In this study we used diffusion tensor imaging (DTI) along with the fiber tracking and histology to investigate fiber tract plasticity in experimental SCI.

Materials and Methods

These studies were performed on Sprague Dawley rats weighing between 350-450g. They were divided into two groups of six each: normal-uninjured and injured. All the MRI studies were performed at six weeks post injury. The spinal cord received a moderately severe injury at the T7 level under controlled conditions. Anesthesia was maintained with α-chloralose (100 mg/kg, single i.p. injection). Heart and respiratory rates and rectal temperature were monitored during the entire MRI scan. MR studies were performed on a 7T 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. These images were then imported into DTI Studio software[2] to calculate the DTI parameters such as fractional anisotropy (FA), mean diffusivity (MD), and eigenvalue maps and fiber tracking. The t-maps, based on the two-tailed t-test, representing significant group differences in the FA values between the normal and injured groups, were generated using SPM99.

Following the terminal MRI scans, animals were transcardially perfused with saline followed by 4% paraformaldehyde (PFA) and brains were removed for immunohistochemical analysis. Brain sections were then processed for GAP-43 labeling, a marker for axonal plasticity.

Results

The t-maps shown in Fig. 1a (indicated by green) show significant increase in FA in the internal capsule (ic) and cerebral peduncle (cp) in injured animals relative to controls. This is also confirmed by the measured FA values along the CST that indicate significant differences between the control and injured groups in the -3 - -4 mm region posterior to Bregma that correspond to ic and cp (indicated by circle in Fig. 1d). Additionally, as shown in Figs. b and c, in injured animals more fibers extend towards cortex terminating in the regions that were activated in fMRI (Ramu 2006). As summarized in the Table 1, quantitatively there was a significant increase in the fiber counts in the 6 weeks post-injured group relative to the uninjured group in both ic and cp, consistent with the visual inspection. Also an increase in the GAP43 expression was observed in ic in the injured group.

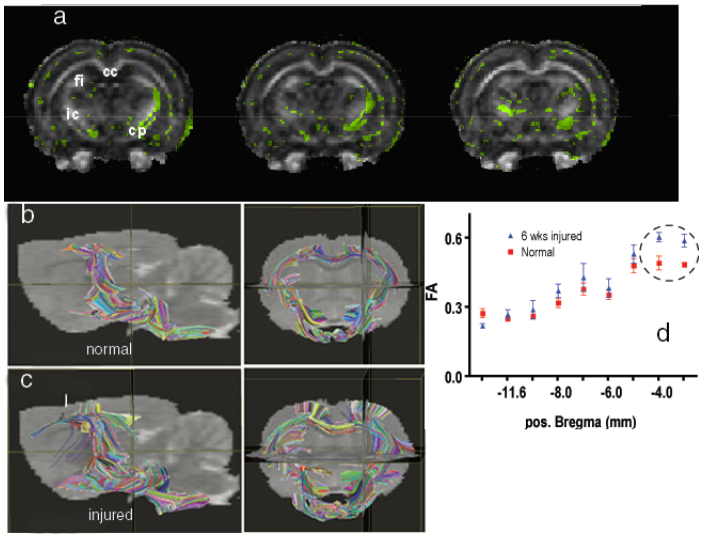

Figure 1. a) t-maps shown in green, superimposed on FA maps, b) and c) fiber tractography in normal and injured brains, and d) FA values along the length of CST. The circle in d) indicates the regions that correspond to inernal capsule and cerebral peduncle

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
0.6602 ± 0.0552	Percent GAP-43	1.112 ± 0.0754	<0.0001
	Cerebral Peduncle		
234 ± 13.56	Fiber counts	319 ± 26.42	0.0022
0.4155 ± 0.105	FA	0.5229 ± 0.0167	0.0152
0.2780 ± 0.0575	Percent GAP-43	0.3035 ± 0.0652	0.8633

Table 1. DTI and GAP43 results in normal and injured groups

Discussion

A major observation made in this study was the fiber plasticity in ic and cp. The plasticity includes both strengthening of the connectivity of the existing fibers, as inferred by the increased FA values, and increased number of fibers, as inferred from the tractography. The increased number of fibers possibly explains the observed increase in FA values and to the multiple sites of activation observed in the earlier fMRI study [1]. Both ic and cp contain multiple fiber tracts including the corticopontine, corticospinal, corticobulbar, corticofugal, and spinothalamic[3] that are involved with the planning and initiating of movements, coordinating fine motor movements, and conveying sensory information. The increased expression of GAP43 in ic is consistent with the increased fiber count observed in DTI. However, it is not clear why a concomitant increase in the GAP43 expression is not observed in cp. In conclusion, this study demonstrates that extensive activation in fMRI is, at least in part, a result of fiber plasticity in ic and cp.

References

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