High resolution diffusion tensor imaging of post-stroke white matter integrity in ex vivo rat brain

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Introduction: Functional recovery after stroke has been associated with structural changes in neuronal connectivity[1,2,3]. A growing amount of data demonstrates that Diffusion Tensor Imaging (DTI) may inform on alterations in brain connectivity associated with neuroplasticity. Structural repair or remodeling of neuronal projections, associated with increased density of myelinated fibers, may give rise to higher fractional anisotropy (FA). Recently, *in vivo* DTI studies on neuronal repair after experimental cerebral ischemia have shown an increase in FA in perilesional white matter at chronic time-points that correlated with high density of axons and myelin[4,5]. However, *in vivo* DTI is subjected to motion sensitivity due to respiration and heart rate, and restricted time to scan anesthetized animals, which constrains signal-to-noise ratio and spatial resolution. In the present study we applied high resolution DTI with isotropic voxels on *ex vivo* rat brains for detailed assessment of alterations in whole brain white matter integrity chronically after unilateral stroke.

Methods: Experimental stroke was induced by transient (90 minutes) intraluminal occlusion of the right middle cerebral artery (tMCA-O) in male Wistar rats (n = 5). Ischemic lesion size and location was assessed at 3 days after stroke on a 4.7T Varian MR scanner with T_2-weighted MRI (multiple spin echo; TR/TE = 3000/17.5 ms; echo train length = 8; FOV = 32 x 32 mm; matrix = 128 x 128; 19 coronal slices; slice thickness = 1 mm). Rats had large subcortical (n = 1) or subcortical and cortical lesions (n = 4). At 10 weeks after tMCA-O, when recovery of sensorimotor function had plateaud, as was confirmed by adhesive removal test[3] and the neurological deficiency score[3], rats were euthanized and transcardially perfused with PBS and 4% paraformaldehyde (PFA). Four age-matched healthy rats underwent the same fixation procedure and served as controls. After one year of storage in 4% PFA, *ex vivo* brains were fixated in a syringe filled with perfluoro polyether (Fomblin®, Solvay Solexis) to prevent susceptibility artifacts at the borders of the brain. High resolution DTI was conducted on a 9.4T Varian MR scanner (eight-shot EPI; TR/TE = 6000/32 ms; FOV = 25 x 25 mm; matrix = 128 x 128; zero-filled to 256 x 256; 91 coronal slices with slice thickness of 0.2 mm; isotropic voxel resolution = 0.2 x 0.2 x 0.2 mm^3; 4 images without diffusion-weighting; diffusion-weighted images in 60 directions with b = 2871.50 s/mm^2 and b= -2871.50 s/mm^2 (Δ/δ=13/6 ms); total acquisition time = 13.25 h). The diffusion tensor for each voxel was calculated based on the eigenvectors and eigenvalues using multivariate fitting and diagonalization. Derived FA maps were further analyzed using unbiased whole brain tract-based spatial statistics (TBSS)[6]. Image-based registration was performed with Elastix (http://elastix.isi.uu.nl). FA maps of all animals were first aligned to a 3D reconstruction of the Paxinos & Watson rat brain atlas[7] using non-linear registration with limited degrees of freedom preceded by affine-only registration. A skeleton of white matter tracts shared across subjects was obtained by thresholding the mean FA map at 0.2. Subject FA maps were registered with a perpendicular search algorithm starting from the skeleton towards individual tracts, and subsequently stacked into a sparse skeletonized 4D image. Permutation tests with threshold-free cluster enhancement[8] were conducted for each point at the mean FA skeleton to assess statistically significant differences between stroke and control groups.

Results: Figure 1A shows a 3D rendered overview of the results of the TBSS whole brain analysis on the mean FA skeleton. White matter is shown in green voxels. Voxels in blue and red indicate a significant decrease and increase in FA with respect to the control group, respectively. Reduced FA was evident in the right ipsilesional internal (ic) and external capsule (ec) and lateral corpus callosum (cc) in the stroke brains. Significant reduction in FA was also found in the ipsi- and contralesionsl anterior commissure (ac). A significant increase of FA was detected in a corticospinal tract area at the border of the ischemic lesion. Interestingly, ipsi- and contralesional medial cc just below the primary and secondary motor cortex (M1 and M2, displayed in red in figure 1A) also showed a significant elevation of FA. Figure 1B shows the voxels with significantly increased (red) or decreased (blue) FA on the mean FA skeleton in different slices and orientations.

Discussion: This high resolution *ex vivo* DTI study demonstrates significant reductions and increases of FA in ipsi- and contralesional white matter regions in PFA-fixated brains at a chronic stage after stroke as compared to control brains. The combination with TBSS whole brain analysis avoids biased regions-of-interest analyses. The reduced FA in the ipsilesional ic and ec, lateral cc and ac may be explained by pathological degenerative processes, such as axonal degeneration and demyelination. The reduced FA in the contralesional part of the ac may be a consequence of secondary degeneration. The increase of FA in the corticospinal tract area at the border of the ischemic lesion could point toward remyelination processes as has been shown recently[4,5]. However, histological examination should be performed to evaluate the influence of perilesional reactive gliosis. Speculatively, the elevations of FA in ipsi- and contralesional medial cc might be related to axonal sprouting of bilateral M1 and M2[1,2]. These results demonstrate that isotropic high resolution DTI in combination with unbiased TBSS-based analysis can provide a valuable tool for whole brain studies on structural plasticity after stroke.

References:

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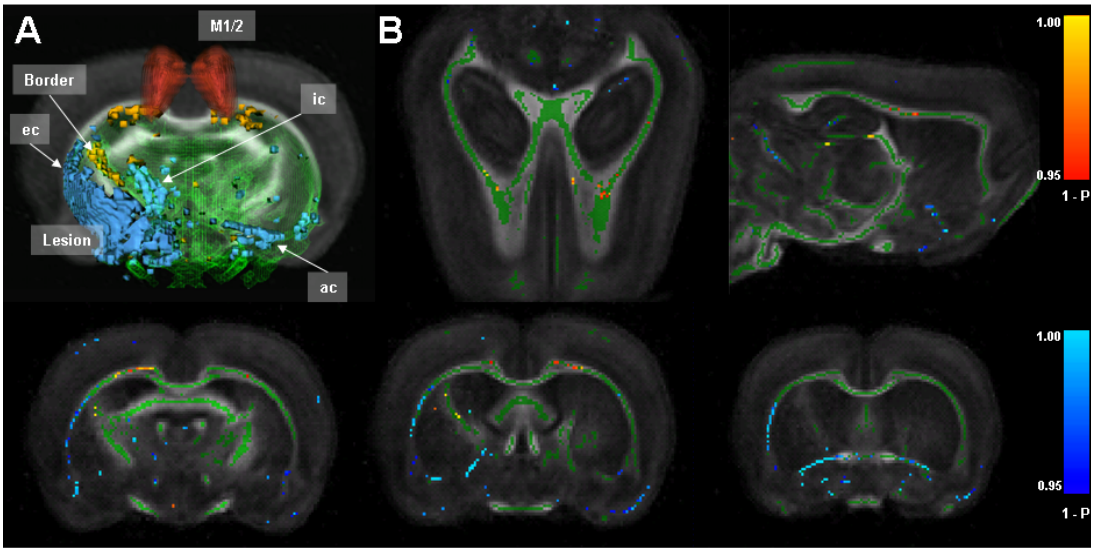

Figure (1A). 3D rendered overview of the mean FA skeleton with statistically significant positive (yellow voxels) and negative FA changes (blue voxels) in stroke brains (n=5) with respect to control brains (n=4). (1B). Selection of 2D slices with overlays of the mean FA skeleton (green) in different orientations: transversal (top, middle), sagittal (top, right) and coronal (bottom (3x)), showing the same statistically significant increased or decreased FA voxels in stroke brain. Border: lesion border; M1/2: primary/secondary motor cortex; ic: internal capsule; ac: anterior commissure; ec: external capsule