Structural brain plasticity visualized with diffusion MRI following a learning and memory task

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Introduction

Recently MRI has been used to follow brain plasticity following the acquisition of new cognitive skills in healthy adults. Such studies report on increase in cortical thickness and increase in white matter organization (using voxel-based morphometry of T1 weighted images and tract-based spatial statistics of fractional anisotropy maps) (Draganski and May, 2008; Scholtz and Johansen-Berg, 2009).While the histological micro-structural and regional characteristics of neuro-plasticity are well studied (neurogenesis, synaptogensis), their correlations with the observed MRI changes are poorly understood. Moreover, while histological studies report that neuro-plasticity is more significant in the hippocampus than other brain regions, MRI studies of neuro-plasticity points to additional regions that undergo significant changes.
In this study we utilized diffusion tensor imaging (DTI) to follow on micro-structural changes that occur following a spatial memory task. Rats were scanned by MRI before and after a water maze task. Significant changes in the various DTI parameters (mainly apparent diffusion coefficient (ADC) and fractional anisotropy (FA)) were found in several domains, including the limbic system (hippocampus, dentate gyrus and cingulate cortex), white matter regions, motor system (sensory areas, thalamus and striatum), and reward system (ventral striatum). The MRI results were compared with a multitude of histological cellular markers to define the origin of the observations.

Methods

24 male Wistar rats (ages 4 months) were examined in this study and underwent two MRI scans (7T MRI system. Bruker, Germany). Between the scans the rats underwent a behavioral learning and memory test (Morris water maze). In the test the rats need to learn the location of a hidden platform in a pool in a period of 6 days, the latency to reach the platform was recorded. Additional group of rats (n=16) underwent the exact same protocol without any behavioral manipulation (control group). The MRI protocol included a DTI acquisition with diffusion-weighted spin-echo echo-planar-imaging (EPI) pulse sequence with the following parameters: TR/TE = 4000/25ms, Δ/δ=10/4.5ms, 4 EPI segments and 16 non-collinear gradient directions with b of 1000s/mm^2.12 slices resolution of 0.2x0.2x1.2mm^3.
Image analysis included DTI analysis using Matlab in-house software of the DWI-EPIs to produce for each rat FA, ADC, radial and axial diffusivity maps. In addition, we used geometrical measures of the diffusion tensor (spherical, linear and planar components) (Westin et al., 2002). For statistical comparison between rats we used a voxel-wise approach where each rat brain volume was co-registered and normalized with Paxisons and Watson stereotactic atlas and included a registered template b0 and FA images. Following normalization, a paired t-test was performed on a pixel-by-pixel basis for each of the groups between the first and second MRI examinations.
Five brains were selected for histological analysis from the water maze and control groups. Sections were stained with markers for the following cellular components: dendrites (with anti microtubule-associated protein 2, MAP2); synapses (with anti synaptophysin, SYP); myelin (with anti myelin basic protein, MBP); astrocyte (with anti glial fibrillary acidic protein, GFAP); and neurons (with anti neuronal nuclei, NeuN).

Results:

The learning and memory group showed significant improvement each day of the water maze test. The paired statistical parametric maps revealed a highly significant FA decrease in several gray matter regions and an increase in white matter regions, ADC decreases in a multitude of brain regions that included both gray and white matter. The principal diffusivities (radial and axial) effect did not differ much from the ADC and FA maps. Figure 1 shows regions where both ADC and FA declined (The hilus of the hippocampus and the dentate gyrus). In these regions, the spherical component of the diffusion tensor increased at the expense of the linear and planar components (Figure 1A&B).Morphological change found by immuno-histochemistry analysis which showed increase in the intensity of synaptophysin, immuno-reactivity, NeuN (in the cell layer of the dentate gyrus) and MAP2 (slight increase in the hilus of the hippocampus) (Figure 1C). In addition, there was a significant increase in the immuno-reactivity of GFAP, manifested by more intense staining and change in the shape of the astrocytes (Figure 1C). The geometrical changes in the astrocyte GFAP staining are best visualized in a 3D confocal microscope (Figure 1D), where both change in the number of astrocyte processes as well as staining intensity is observed.
The neo-cortex regions showed an increase in ADC and a decrease in FA indices (data not shown) this pattern was located mainly in the cingulate cortex and was correlated with a decrease in the immuno-reactivity of GFAP.

Discussions & Conclusions:

One of the DTI changes patterns after water maze test consisted of a reduction in both FA and ADC, and was attributed to an increase in the spherical component of tensor combined with a decrease in its linear and planar components (Figure 1). The immuno-histchemistry disclosed morphological micro-structural differences between the two groups of rats that appear to be in line with the observed geometrical tensor changes. Although changes were found in synaptic density, dendrite staining and neuronal marker staining, the most significant observation that can account for the diffusion MRI is the astrocyte staining, it is reasonable to assume that

diffusion MRI changes might be significantly influenced by glial morphology. Other types of patterns were observed in different regions (gray matter and white matter).
The ability to follow, in vivo and non-invasively, dynamic morphological tissue changes induced by cognitive experience or training has far-reaching implications for neuroscience. As a complement to functional measurements, the use of DTI to study structural plasticity can provide deeper insight into the affected regions, the structural processes, and the timing of the effects. Finally, as MRI is a true translational methodology, research should be conducted in humans to verify whether the phenomena viewed in tissues also occur in the human brain, and their regional pattern and time dependency.
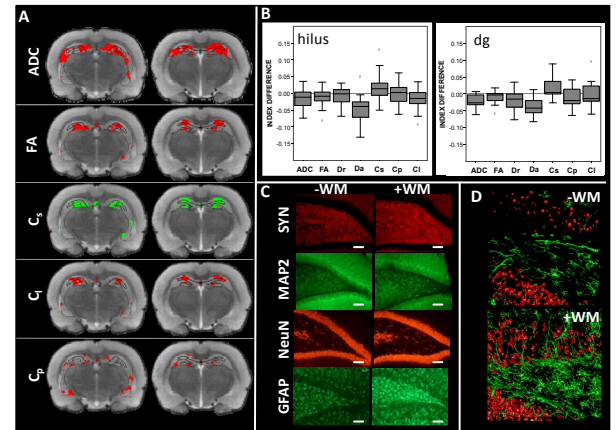

Figure 1: **Geometrical and histological analysis of the dentate gyrus and hippocampus:**

(A) Changes in the different DTI indices (FA, ADC, spherical component of the tensor (Cs), linear component of the tensor (Cl) and planar component of the tensor (Cp)) showed only for the hippocampus and dentate gyrus regions. Red indicates decrease in the index following water maze while green indicates increase.

(B) Box-whiskers graphs of the information given in (A) for two selected region of interests: on the left is the hilus of the hippocampus and on the right the dentate gyrus (dg).

(C) Immuno-histochemical staining (magnification x10) of the cell layer of the dentate gyrus and hilus of the hippocampus for the following markers: SYN (marker of synapses), MAP2 (marker of dendrites), NeuN (marker of neurons) and GFAP (marker of astrocytes). The white line in the right corner is 100µm scale.

(D) 3D reconstruction of confocal images of NeuN (red) and GFAP (green) immuno-reactivity of two rats (one from the control, -WM, and one from the water maze group, +WM) at the region of the dentate gyrus and hilus of the hippocampus.

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