Diffusion MRI measurement of Axon Diameter alterations induced by White Matter Plasticity

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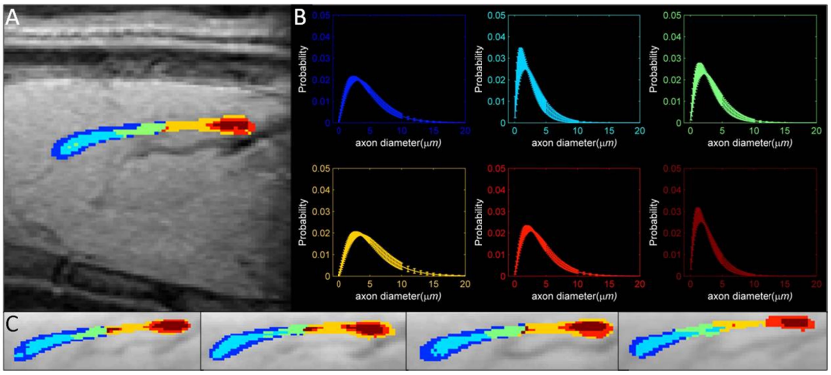
Introduction

Brain Plasticity has been one of the most widely explored processes in recent years. A multitude of invasive and non-invasive studies have shed light on an array of mechanisms that once were an enigma for neuroscientists. Neurogenesis, dendrogenesis, and synaptogenesis have been established as processes that accompany plasticity, but the involvement of myelinated axons remained vague. Recent studies linked between neuronal activity, plasticity, and myelination[1,3], suggesting that white matter also changes in response to continuous stimulus. Imaging studies[2,3] showed changes in white matter regions as a result of learning tasks, but the nature of these changes is still unknown. A key feature of white matter morphology is the axon diameter distribution (ADD). The axon diameter is in correlation with conduction velocity and other physiological measures of signal transmission within nerves[6]. In this study we focus on the Corpus Callosum (CC), the largest and most ordered white matter tract in the brain, to observe changes in axon diameter distribution as a result of a spatial learning task. We used AxCaliber, an advanced diffusion MRI method[4] to extract morphological parameters of the tissue, such as the ADD and axonal density.

Methods

37 male Wistar rats (ages 4 months) divided into 3 groups were examined in this study and underwent two MRI scans a week apart (7T MRI system. Bruker, Germany) under ~1.5% isofluorane anesthesia. Between the scans the first group of rats (n=15) underwent a behavioral learning and memory test (Morris Water Maze). In the maze the rats had to learn the location of a hidden platform in a pool, based on spatial cues, in a period of 5 days. The latency of each swim to the platform was recorded. The second group (n=15) was placed in the pool and allowed to swim freely, without a platform and without being exposed to spatial cues. The third group did not undergo any behavioral manipulation in the period between the scans.

The experimental protocol consisted of a series of diffusion-weighted stimulated-echo echo-planar-imaging acquisitions with the following parameters: TR/TE = 1500/22ms, δ =3.2ms, 16 diffusion gradient increments (linearly from 0 to 282 mT/m) and 12 averages (NEX). Gradients were applied only along the x-direction, which is perpendicular to the CC fiber axes in the mid-sagittal plane. The experiment was repeated for 5 different diffusion times: 11, 20, 30, 60 and 100ms. Images were acquired in the sagittal plane with 5 slices of 1.8mm thickness and in-plane resolution of 0.125x0.125mm2; only the mid-sagittal slice was analyzed. The total acquisition time was about 2.5 hours per rat. Voxel-by-voxel analysis was performed on the acquired scans using the AxCaliber framework. Total number of free parameters was 11 – perpendicular intra-axonal and hindered diffusion coefficients, fractional volume of restricted (FR) and CSF (FCSF) compartments, α and β parameters of the gamma-function representing the ADD, and 5 initial magnetization coefficients for each delta acquisition. FR and FCSF, together with α and β, were used as an input to a clustering algorithm (k-means). The number of clusters was set to 6 based on previous research. For each of the 6 clusters the signal decay was averaged over the cluster, and analysis was performed on the averaged signal. 2-way ANOVA tests were performed on FR, α and β parameters.



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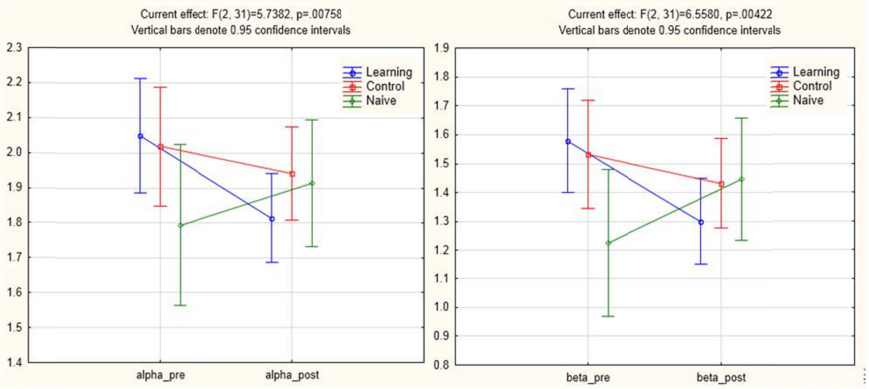
Results & Discussion

The learning and memory group showed significant improvement each day of the water maze test. For each individual scan the clustering procedure yielded a result corresponding to histologically retrieved ADD of the rat CC[4]. The first cluster represents surrounding voxels with a high percentage of fractional volume of non-restricted compartments, cluster 2 the genu, 3 and 4 represent the body , 5 and 6 represent the splenium. The result was consistent over pre and post scans and over the entire cohort. This establishes the method as robust enough for longitudinal research. Statistical analysis yielded a significant interaction in cluster 2 between on α and β parameters representing the ADD. The reduction in α and β parameters accounts for a decrease in mean axon diameter and narrowing of the distribution. Also a noticeable difference of trend was observed on most clusters between both active groups and the naïve group. The difference between both active groups and the naive group cold be attributed to several processes other than spatial learning, such as learning a new motor skill and stress response[5]. The interaction in cluster 2 could be attributed to the learning process itself.



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Conclusions

This study establishes AxCaliber as a powerful method for analysis of white matter tracts. The applications are many, from subtle changes induced by cognitive tests, to profound effects of pathological conditions and normal development. The current study exhibits changes in ADD in the CC as a result of a behavioral manipulation. The results further support the involvement of white matter in plasticity, and a reduction of mean axon diameter is observed in response to behavioral manipulation. Further studies and histological analysis are required to establish the precise microstructural process accountable for these findings.

References

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