In-vivo Measurement of the Axon Diameter Distribution in the Rat's Corpus Callosum

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Introduction

The axon diameter distribution (ADD) is an important anatomical feature of nerve fascicles both in normal and abnormal development. It is well known that the nerve conduction velocity is directly proportional to axon diameter in myelinated axons[1]. Generally, large-diameter axons arise in pathways that require short conduction times while smaller axons are found in pathways that can tolerate longer conduction delays. The corpus callosum (CC) is the largest pathway in the brain, and is conserved among different mammals [2]. The fiber composition of the CC is homogenous within different regions: but fibers that connect the somato-sensory areas have a much larger axon diameter than fibers that connect to frontal and temporal areas [2]. It has been suspected that the fiber composition of the CC is altered in Autism [3] and is known to change in rats that were exposed to alcohol [4].

Despite its importance, the ADD within nerve fascicles has not been measurable *in vivo*, and currently can only be assessed by invasive histological means. In this work, we use AxCaliber [5]- a new diffusion MRI based framework to extract the ADD within the CC of the rat, *in vivo* and non-invasively. By altering the diffusion time and diffusion weighting, morphological parameters of the tissue, such as the ADD and axonal density, can be estimated or inferred on a voxel-by-voxel basis.

Methods

Three Wistar male rats (4-6 months old) were scanned in a 7T/30 spectrometer (Bruker) under ~1.5% isofluorane anesthesia. The experimental protocol consisted of a series of diffusion-weighted stimulated-echo echo-planar-imaging acquisitions with the following parameters: TR/TE = 1500/23ms, 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 7 slices of 1.8mm thickness and in-plane resolution of 0.125x0.125mm^2; only the mid-sagittal slice was analyzed. The total acquisition time was about 2 hours per rat.

The ADD was computed on a voxel-by-voxel basis according to the AxCaliber framework[5], which fits the signal decays at different diffusion times simultaneously to a function that employs contributions from an extra-axonal extracellular matrix exhibiting hindered Gaussian diffusion (modeled by the Stejskal-Tanner relation[6]) and intra-axonal space exhibiting restricted diffusion (modeled by Callaghan's formula for restricted diffusion within cylinders[7]). The intra-axonal component was weighted for the ADD by a gamma-function. The total number of free parameters was only 4: The volume fraction of the hindered compartment, the diffusivity of the hindered compartment and α and β parameters of the gamma-function. The intra-axonal diffusivity was set to 1μm^2/ms. The extracted parameters were used as an input to a clustering algorithm (k-means). The number of clusters (k) was incremented until no additional information was observed.

Results & Discussion

Using AxCaliber we were able to segment the CC to at least 5 distinct regions (Fig. 1) corresponding to the genu (cluster 1), body (clusters 2 and 3) and splenium (clusters 4 and 5). The most anterior part of the corpus callosum (the genu) exhibited a narrow ADD with a small mean diameter (green). The cluster that represents the body of the CC, which corresponds to fibers that connect the somato-sensory cortex of the rat (cluster 3, red) exhibited the broadest ADD of all measured clusters. More posterior parts (cluster 5) exhibited a narrower ADD than the body. This arrangement of ADDs within the CC is in-line with the histological studies. It should be noted that AxCalibers estimated ADD produce distributions with larger mean diameters than those found histology. For example, the ADD cluster 3 which correspond to the body of the CC, peaks at around 6 μm. Histological studies of this region indicates that this region ADD should peak around 3 μm. This may be a result of two factors: The first is due to limitations on the maximum q values that can be applied (due to noise) and minimum diffusion times that can be achieved (due to gradients) ; The second is due to shrinkage of the tissue when it is prepared histologically.

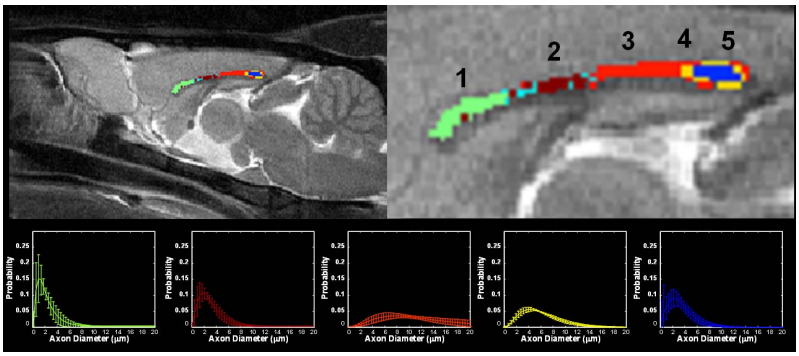

Figure 1: Cluster analysis of the ADD in the rat CC measured *in-vivo* estimated from AxCaliber. The different colors represents distinct clusters found by the k-means algorithm with the corresponding the ADD shown in the graphs.

Conclusions

This work presents, for the first time, *in-vivo* measurements of an important histological feature, the axon-diameter distribution that had been measured previously only by electron microscopy. AxCaliber was able to reconstruct the diameter distribution within the rat's corpus callosum and that ADD corresponds to known morphometry in the CC. Applications of AxCaliber are expected in longitudinal studies designed to follow nerve growth in normal and abnormal development, as well as in diagnosing disorders and diseases affecting specific populations of axons in the CNS and PNS.

References

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