Hippocampal Anisotropy is Associated with Dendritic Quantity

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Introduction

Due to the radial orientation of dendrites in the hippocampus, diffusion tensor imaging (DTI) may be able to detect differences in the amount of hippocampal dendritic material. Previous research from our lab demonstrated that DTI-derived fractional anisotropy (FA) values in Tg2576 Alzheimer's Disease model mice were stable between 12 and 24 weeks of age while FA values for wildtype controls (B6/SJL) increased, suggesting that wildtype, but not transgenic, mice added new dendritic material [1]. The present study seeks to understand the structural correlates of hippocampal anisotropy. We hypothesized that higher hippocampal FA values would be associated with relatively more hippocampal dendritic material. The hippocampi of wildtype B6/SJL mice were first characterized with DTI and then via stereologically correct light microscopy. Results supported our hypothesis.

Methods

A total of 11 B6/SJL mice (Taconic, German, NY) at 3 or 5 months of age were used. Mice were transcardially perfused with PBS followed by a 4% paraformaldehyde/1% glutaraldehyde fixative. Brains were excised and post-fixed for two weeks prior to DTI. DTI was conducted at 26°C on a Bruker vertical bore MR imager operating at a proton frequency of 600 MHz. Diffusion-weighted spin-echo images were acquired using TR = 3000 ms, TE = 27 ms, time between diffusion gradient pulses, Δ, = 14 ms, duration of diffusion gradient, δ, = 7 ms, field of view = 1.5 cm, and matrix size = 256 x 256. Diffusion sensitizing gradients were applied along six directions: [Gx,Gy,Gz] = [1,1,0], [1,0,1], [0,1,1], [-1,1,0], [1,0,-1], [0,-1,1]. Six *b* values, [200, 500, 1000, 1500, 2500 and 3500 s/mm^2], were used along each diffusion gradient direction. A total of ten 500 µm thick slices in the coronal orientation were imaged with the five to six slices containing the hippocampus situated in the middle of the slice package. Average FA was calculated separately for the apical dendritic layer (stratum radiatum) of CA3 and CA1 using software developed in-house. The scalar metric fractional anisotropy (FA) was calculated from the eigenvalues on a pixel-by-pixel basis.

After DTI was completed, brains were embedded in Spurr's resin and sectioned on a Leica Ultracut ultramicrotome with section thickness = 0.5 µm. Sections were stained with methlyne blue/azure II and observed under an 100x oil-submersion objective on an Olympus BX60 microscope. Images of the hippocampus were captured from the stratum radiatum in areas CA3 and CA1 with a Qimaging Micropublisher 3.3 megapixel digital camera. Percent of the neuropil occupied by various tissue components was quantified by using a point-count technique with the software package Image-Pro Express 6.0. Different tissue components were identified as follows: dendrite, myelinated axon, soma, blood vessel and "other" (brain tissue components that are unidentifiable due to the resolution limits of the light microscope). As shown in Figure 1, care was taken to ensure that the sections evaluated for microscopy matched the DTI slices.

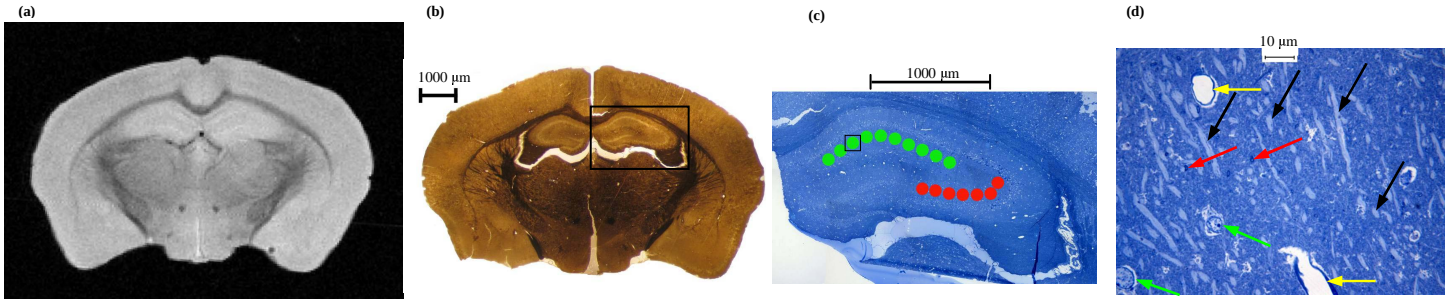

Figure 1: (a) A representative diffusion-weighted spin-echo image acquired for the present experiment. (b) The histological section corresponding to the MR image in (a) with the right hippocampus denoted by the black rectangle. (c) A low-magnification image of the right hippocampus seen in (b), stained with methlyne blue/azure II. Circles represent the location of the high-magnification images used for microscopic quantification of the different hippocampal tissue components. Red and green denote areas CA3 and CA1 of the hippocampus, respectively. (d) A high-magnification image from area CA1 of the hippocampus. The location of the image is denoted by the black square in (c). Arrows pointing to the different tissue components are as follows: Black = dendrites; red = myelinated axons; yellow = blood vessels; green = soma.

Results and Discussion

As shown in figures 2a and 2b, respectively, FA and percent of neuropil occupied by dendrites increased in CA1 between 3 and 5 months of age. No FA differences were observed between age groups in CA3 (data not shown). No differences were observed in the percent of the neuropil occupied by non-dendritic tissue components (i.e. myelinated axons, soma, blood vessels and other unresolvable components; data not shown).

The findings of the present experiment support the hypothesis that relatively higher FA values in CA1 of the hippocampus are associated with relatively more dendritic material in CA1. The increase in FA is most likely due to a greater number of barriers to diffusion created by a greater number of dendrites in CA1 at 5 months of age. Future applications of DTI-derived hippocampal anisotropy as an index of the quantity of hippocampal dendrites could prove useful in a wide range research and clinical areas related to normal and abnormal brain function such as brain development and neurodegenerative disease.

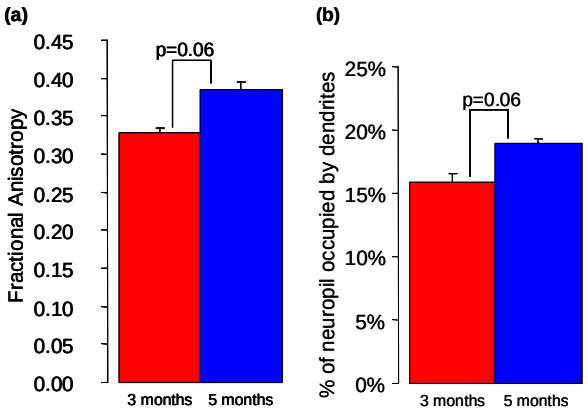

Figure 2. Fractional anisotropy (a) and percent of neuropil occupied by dendrites (b) in area CA1 of the hippocampus increased between 3 and 5 months of age.

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1 Venkatasubramanian PN, Faulkner J, Tom B & Wyrwicz AM. Proc Intl Soc Mag Reson Med. 13 (2005).