Detection of Microstructural Alterations in the Hippocampus of APP Transgenic Mice Using DTI

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Introduction

The integrity of hippocampus is crucial for memory and learning. Medial temporal lobe, comprising of hippocampus and adjacent cortical areas, is targeted by Alzheimer's disease (AD) pathology [1]. The end stage AD brain is characterized by atrophy of hippocampal formation. Structural changes such as atrophy, however, are not the primary events in the course of the disease. The early cognitive decline that occurs prior to neuronal losses or plaque deposition might involve alterations to a specific subregion of hippocampus and not the entire structure. In order to detect the earliest changes critical to cognitive decline, we have examined the microstructure of hippocampus in Tg2576 transgenic mice using diffusion tensor imaging. The Tg2576 mouse that overexpresses the Swedish mutation of human APP_{695} has shown potential as a model for AD as the mouse develops age-dependent behavioral, biochemical and pathological changes resembling those in AD [2]. Our results reveal that significant reductions in fractional anisotropy begin to appear in specific subfields within the hippocampus of young transgenic mice long before amyloid deposition occurs.

Experimental

Twelve and 24-week old heterozygous female Tg2576 and littermate wildtype mice (Taconic, Germantown, NY) were used in the investigation. Mice were anesthetized and transcardially perfused with 4% paraformaldehyde. Excised, fixed brains were imaged at 18°C on an imaging spectrometer operating at a proton frequency of 400 MHz. A multislice, spin-echo imaging pulse sequence, modified by adding a pair of Stejskal-Tanner diffusion sensitizing gradients, was employed for acquisition of DTI data. Diffusion-weighted images of 0.7mm thick axial slices containing dorsal and ventral hippocampus were acquired using TR 1.8s, TE 40ms, Δ 20ms, δ 6ms, FOV 14mm, and matrix size 128x128. Diffusion sensitizing gradients were applied along six directions. Six *b* values in the range 0-3700s/mm^2 were used along each diffusion gradient direction. Diffusion tensor data was analyzed using software written in house based on published methods [3]. Three eigenvalues, λ_1, λ_2, λ_3, sorted from largest to smallest were calculated by matrix diagonalization. The scalar metric fractional anisotropy (FA) was calculated from the eigenvalues on a pixel-by-pixel basis.

$$FA = \sqrt{\frac{(\lambda_1-\lambda_2)^2+(\lambda_1-\lambda_3)^2+(\lambda_2-\lambda_3)^2}{2(\lambda_1^2+\lambda_2^2+\lambda_3^2)}}$$

Regional measure of FA was calculated by averaging 6-10 pixels in CA1 and CA3 subdivisions of the hippocampus and dentate gyrus (DG).

Results and Discussion

A comparison of FA in the subdivisions of the hippocampal region in 12- and 24-week old mice is presented in Table 1.

Table 1. Fractional Anisotropy in the subdivisions of the hippocampal region in Tg2576 and wildtype control mice (*n=3*)

Genotype	12-Week Old			24-Week Old		
	CA1	CA3	DG	CA1	CA3	DG
Dorsal Hippocampus						
WT	0.33±0.01	0.24±0.01	0.27±0.01	0.33±0.04	0.32±0.06	0.44±0.06
Tg	0.33±0.01	0.24±0.01	0.28±0.01	0.30±0.04	0.22±0.06**	0.27±0.06*
Ventral Hippocampus						
WT	0.29±0.01	0.23±0.01	0.23±0.02	0.22±0.05	0.20±0.03	0.24±0.04
Tg	0.32±0.02	0.21±0.01	0.23±0.02	0.29±0.06	0.17±0.02	0.21±0.05

**p<0.05; **p<0.08.*

At 12-weeks, WT and Tg mice had nearly identical FA in each of the hippocampal subfields compared. This similarity in anisotropy suggests that very young wildtype and APP transgenic mice have the same cellular microstructure in CA1, CA3 and DG subfields, and rules out neurodevelopmental abnormalities in the hippocampus of Tg2576 mice. At 24-weeks of age, on the other hand, region-specific differences between the two groups of mice were detected in diffusion anisotropy. Dentate gyrus and CA3 fields of dorsal hippocampus in 24-week old Tg mice had significantly lower FA and CA1 had slightly lower, but not statistically significant, FA than in WT mice. Differences in FA did not reach significance in any ventral hippocampal subfield. The two groups of mice had the same high FA (~0.65) in corpus callosum, a fiber-rich structure, indicating that the observed anisotropy differences were specific to the hippocampus. Reduced anisotropy in the hippocampus of 24-week old Tg2576 mice most likely represents subtle microstructural changes at the cellular level that occur several months before amyloid plaques appear. Absence of plaques in the brains used for DTI was verified by immunohistological staining using biotinylated 4G8, a monoclonal antibody for detecting amyloid plaques. Further, previous studies have confirmed that Tg2576 mice develop amyloid plaques only when they are 9 months or older [2].

The occurrence of changes in hippocampal anisotropy at 24-weeks coincides with the age of onset of cognitive decline and changes in amyloid properties in Tg2576 mice. Impairment in spatial reference memory was found to begin at 6 months and coincided with the appearance of detergent-insoluble, neurotoxic Aβ [4]. These observations strongly suggest that DTI-detected microstructural changes could have relevance to critical functional deficits and biochemical alterations in APP transgenic mice. The early changes in CA3 and DG microstructure reported here parallel the previously reported ultrastructural changes such as the reduction in CA1 dendritic spine density in 4.5 months old Tg2576 mice [5] and the reduction in dendrite length of dentate gyrus granule cells in 3 month old PDAPP mice [6].

Literature Cited

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