

## Mitochondrial Fission Arrest Phenotype in Brain Tissue of Patients and Animal Models of Familial Alzheimer's Disease Revealed with 3D EM

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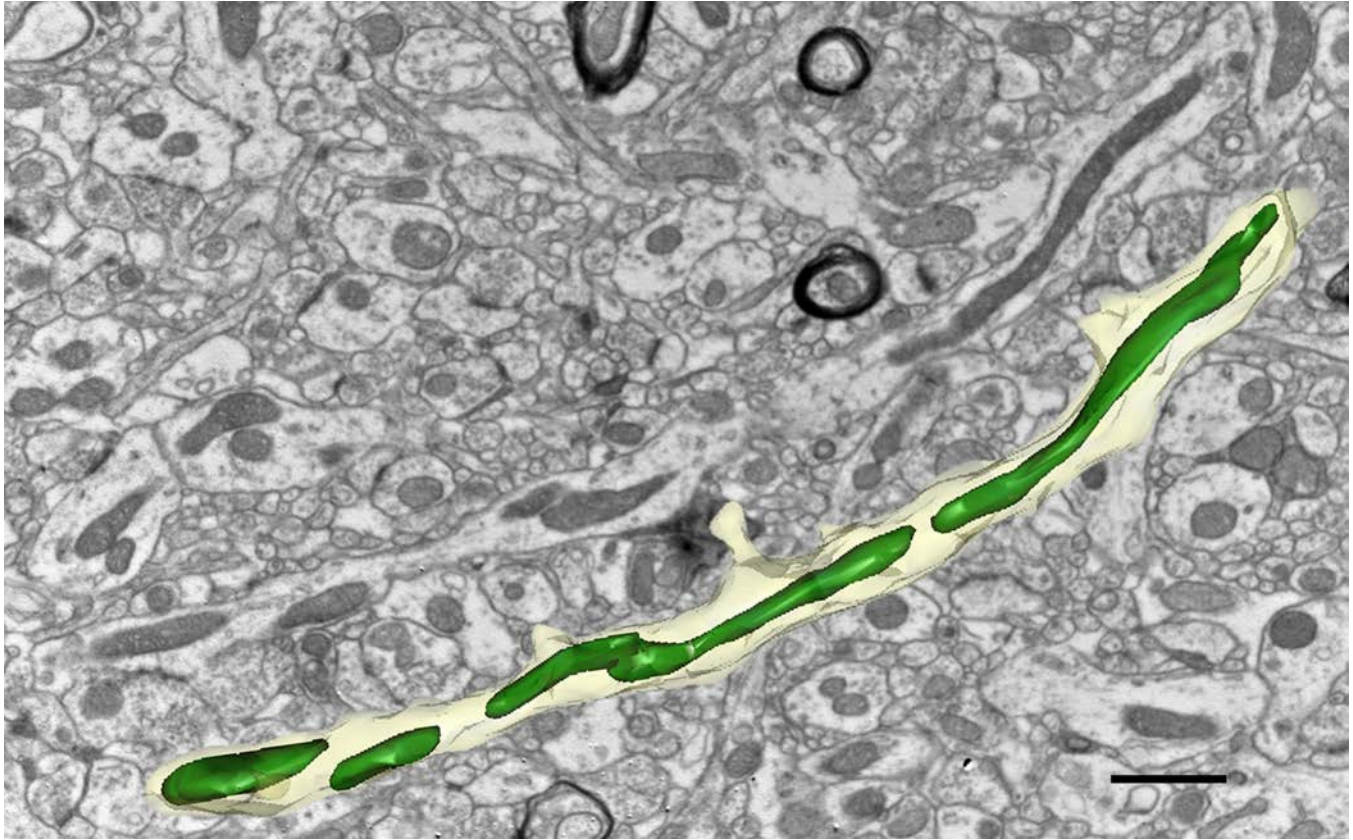
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Alzheimer's Disease (AD) is a devastating neurodegenerative disorder characterized by progressive cognitive decline that affects the aging population. Hallmarks of AD include deposition of extracellular amyloid plaques, the accumulation of intraneuronal neurofibrillary tangles comprised of hyperphosphorylated tau protein, loss of synapses, memory deterioration and neuronal cell death. Recent data generated in animal models of AD and in AD patients suggest that altered mitochondrial dynamics contribute to the onset and progression of the disease.

Mitochondria are dynamic organelles that constantly move within neurites in both anterograde and retrograde directions. Mitochondria function in local energy production to meet cellular demands, while the number and quality of the organelles is determined in part by cycles of fission and fusion. Mitochondrial fission is required for their proper distribution, movement, and quality control. Conversely, the merging or fusion of mitochondria allows the exchange of contents facilitating repair and the increase in size or functional volume of the organelle. The fidelity of fission and fusion machinery depends on a number of recently identified proteins, including mitochondrial fission factor (Mff), mitofusin-1 and 2 (Mfn1, Mfn2), optical atrophy 1 (Opa1), mitochondrial fission protein 1 (Fis1) and dynamin-related protein 1 (Drp1). Alterations in mitochondrial fission and fusion have been shown to be critical to the development of neurodegeneration in Charcot-Marie-Tooth disease type 2A, autosomal dominant optical atrophy, Alzheimer's, Huntington's and Parkinson's diseases. The importance of fission in mitochondrial function was highlighted when deletion of Drp1 was shown to lead to mitochondrial elongation and to an increase in oxidative damage, loss of respiratory function, and neurodegeneration. These observations suggest that mitochondrial division serves as a quality control mechanism and plays an essential role in neuronal health. Nonetheless, there is a delicate balance between accumulation and disposal of defective mitochondria. Loss of those organelles that can still contribute to a positive energy balance may ultimately be detrimental to cellular health and survival. Thus, better understanding of mitochondrial dynamics with respect to the progression of neurodegenerative diseases is needed in order to develop therapeutic strategies to protect neurons from damage.

Using transmission electron microscopy, we examined mitochondria in hippocampal tissue from five transgenic mouse models carrying familial AD human mutations for presenilin 1 (PS1, M146L); amyloid precursor protein (APP, K670N, M671L); double transgenic mutant APP/PS1; triple transgenic mutant (3xTg) overexpressing tau (P301L), APP (K670N, M671L) and PS1 (M146V); and tau (P301L). Reconstruction of 3-dimensional renditions from serial thin-sections (3D EM) revealed changes in mitochondrial morphology in AD that was otherwise obscured or difficult to appreciate in individual thin sections. Using this technique, we identified a novel mitochondrial fission arrest phenotype that increased with disease progression and was more pronounced in animals with multiple FAD mutations. We established experimental conditions that mimic the mitochondrial fission arrest phenotype in wild-

type disease-free mice. These conditions and brain tissue from FAD animals were used, together with Western blot analysis, fax analysis and immunofluorescence, to characterize the abundance and activity of proteins that play a direct role in mitochondrial fission including Mff, Mfn1, Mfn2, Opa1, Fis1 and Drp1. Taken together our observations suggest that fission arrest occurs in AD in response to altered brain energetics.



The figure shows an electron micrograph from a series of serial thin-sections of hippocampal brain tissue from a wild-type non-transgenic mouse. Superimposed on the electron micrograph is 3D reconstruction of mitochondria (green) within a neuropil. Here, the mitochondria have a regular tubular appearance (approximately 1/3 micron in diameter and up to several microns in length). Bar = 1 micron.