

Supplementary Data

Amyloid- β and the Failure to Form Mitochondrial Cristae: A Biomimetic Study Involving Artificial Membranes

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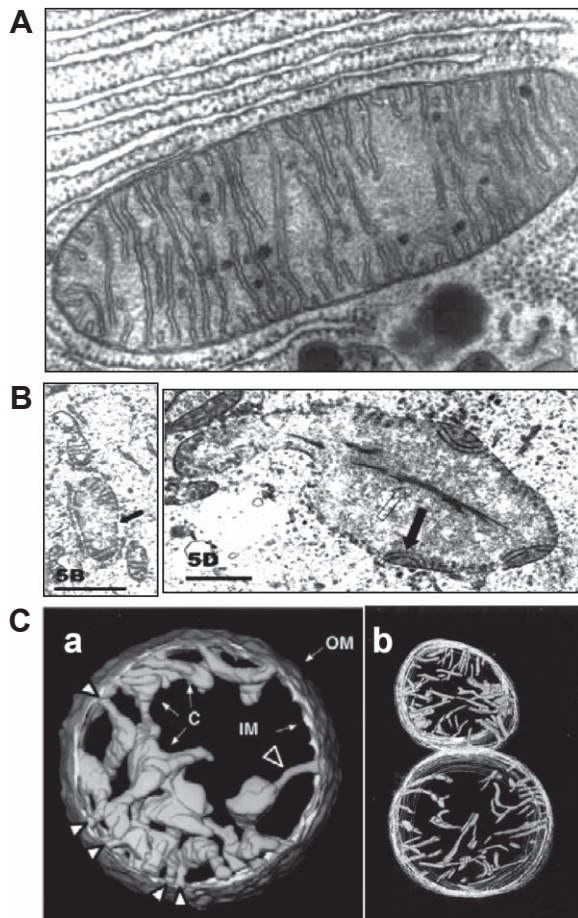
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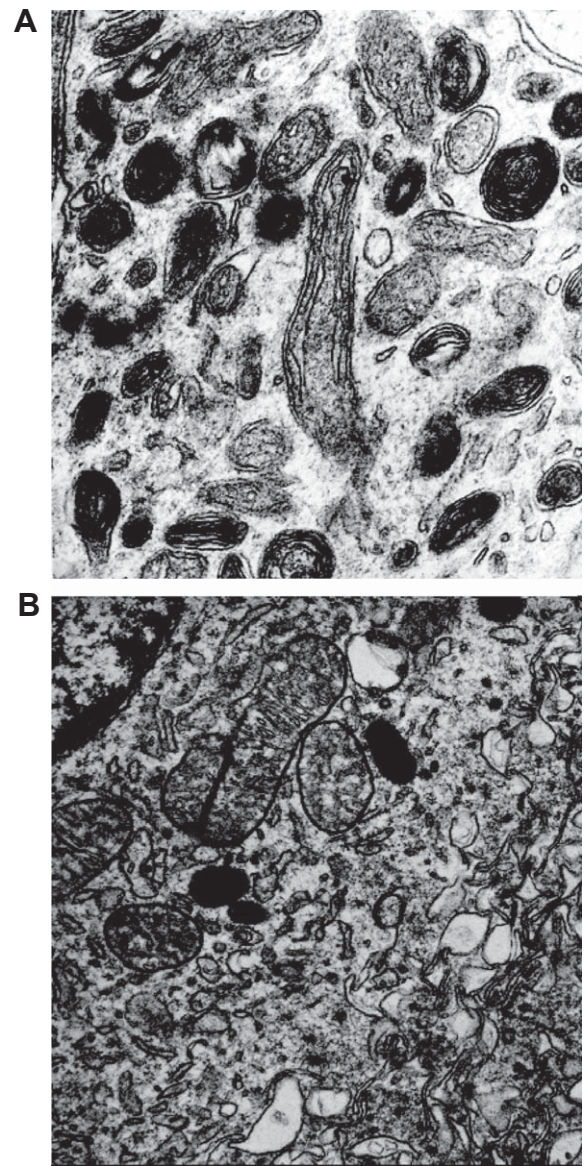
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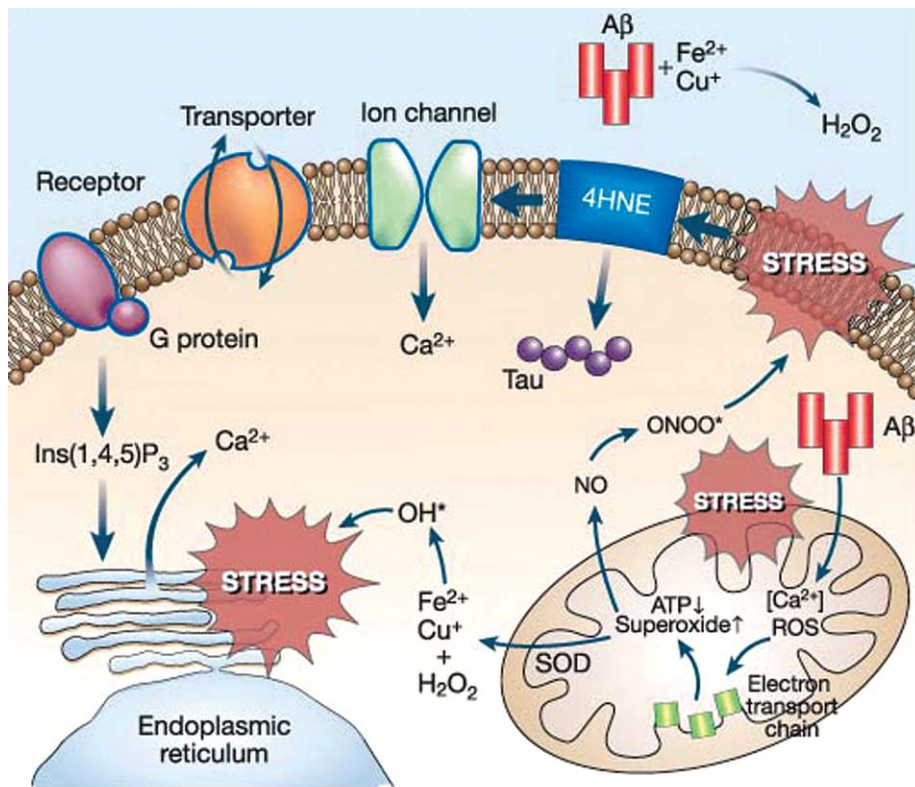
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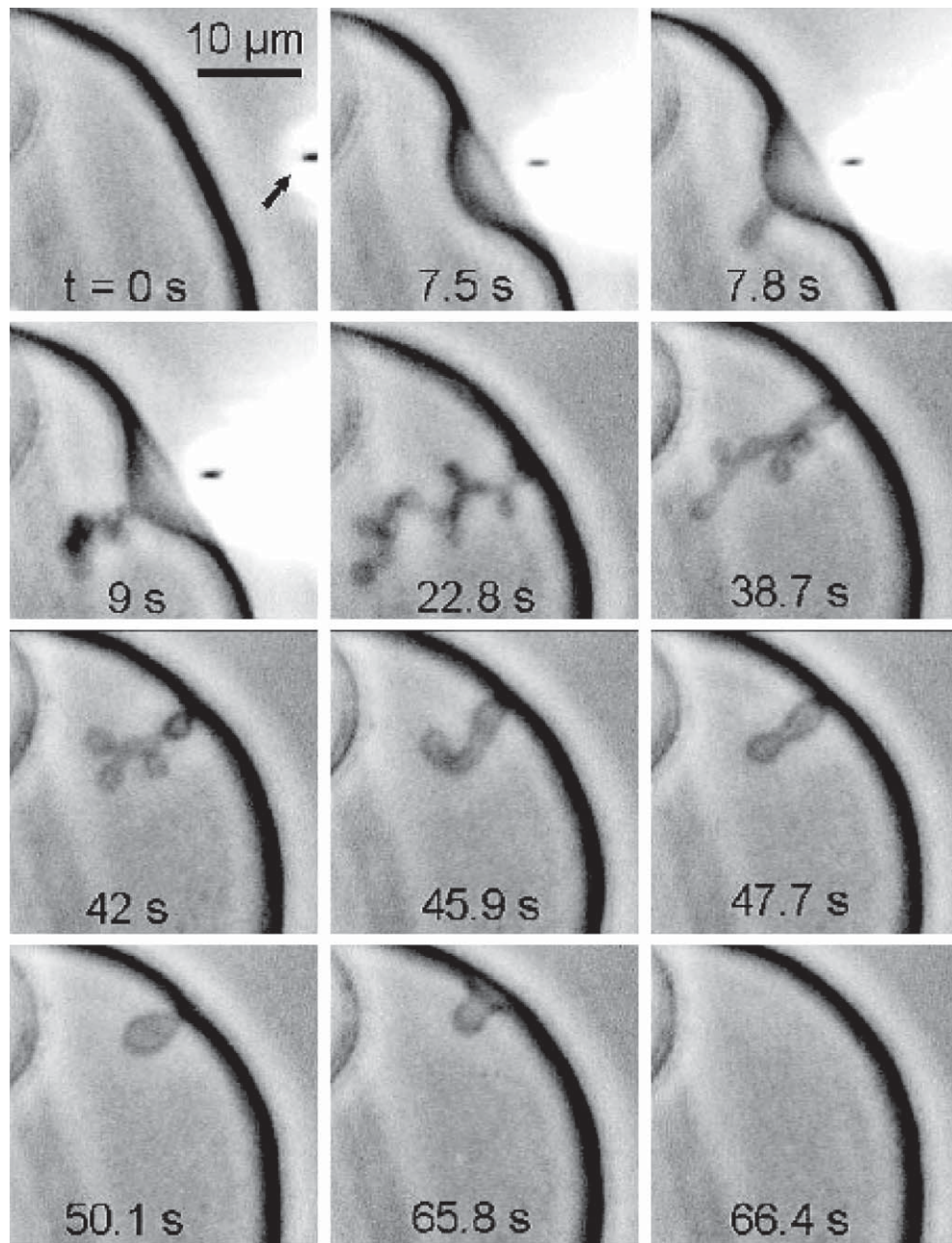
Supplementary Figure 1. A) Conventional transmission electron microscopy (TEM) of a normal (healthy) mitochondrion (a typical text book image). This electron micrograph, taken from Fawcett, *A Textbook of Histology*, Chapman and Hall, 12th edition, 1994, <http://cellbio.utmb.edu/cellbio/mitoch1.htm>, shows the organization of the two membranes. B) TEM micrograph of Alzheimer's disease (AD) mitochondria. AD cybrid cells contained increased percentage of enlarged or swollen mitochondria, which had a pale matrix and only a few remaining cristae. Bar = 1 μm . Reprinted from [1] Copyright (2000) with permission of Elsevier by Copyright Clearance Center. C) Electron microscopy tomography images of a normal ("healthy") mitochondria. In living cells, mitochondria are usually tubular (1 to 2 μm long and 0.1 to 0.5 μm large) and interact with other cellular components, especially with the cytoskeleton and endoplasmic reticulum [2, 3]. Normally functioning mitochondria oscillate between (a) condensed and (b) orthodox state morphology, depending on their respiratory rate. Mitochondria performing maximum respiratory rate (in excess ADP and respiratory substrate) present condensed (matrix contracted) morphology (a). Their cristae are swollen cisterns or sacs connected to the peripheral part to the inner membrane (IM) by narrow tubular segments (cristae junctions), Fig. 1C,a (the large white arrow). The cristae in orthodox (matrix expanded) morphology are narrow, flattened, or almost tubular (b). OM diameter in (a) is 1.5 μm , in (b, lower), 1.2 μm . (a) reprinted from [4] Copyright (1998) with permission of IOS Press. (b) reprinted from [5] Copyright (1994) with permission of John Wiley & Sons, Inc. by Copyright Clearance Center.



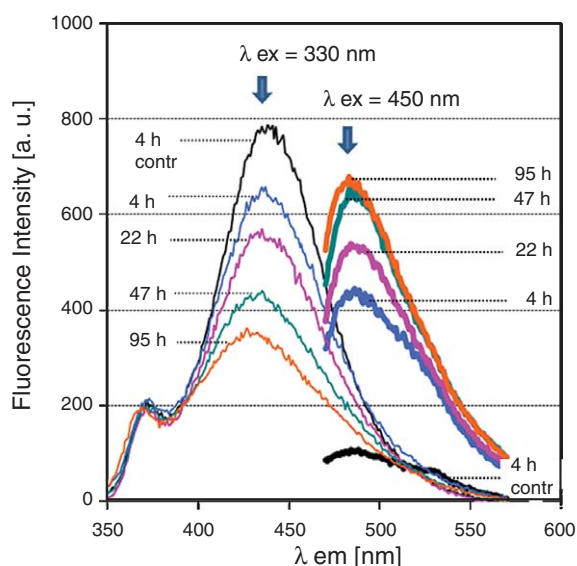
Supplementary Figure 2. Electron micrographs of mitochondria from a patient with AD and from a normal control patient. (a) Polymorphism of mitochondria in a dendritic profile of a neuron of the globus pallidus in a patient with AD aged 62 years; the majority of AD patient mitochondria were small and round or elongated; a substantial number of them showed disruption of the cristae. (b) Mitochondria in the soma of a neuron of the globus pallidus in a control patient, aged 62 years. Reprinted from [6] Copyright (2004) with permission of Sage Publications by Copyright Clearance Center.



Supplementary Figure 3. The neurotoxic action of amyloid- β ($A\beta$) involves generation of reactive oxygen species and disruption of cellular calcium homeostasis. Interactions of $A\beta$ oligomers and Fe^{2+} or Cu^{+} generates H_2O_2 . When $A\beta$ aggregation occurs at the cell membrane, membrane-associated oxidative stress results in lipid peroxidation and the consequent generation of 4-hydroxynonenal (4HNE), a neurotoxic aldehyde that covalently modifies proteins on cysteine, lysine and histidine residues. Some of the proteins oxidatively modified by this $A\beta$ -induced oxidative stress include membrane transporters (ion-motive ATPases, a glucose transporter and a glutamate transporter), receptors, GTP-binding proteins ("G proteins") and ion channels (VDCC, voltage-dependent chloride channel; NMDAR, *N*-methyl-d-aspartate receptor). Oxidative modifications of tau by 4HNE and other reactive oxygen species can promote its aggregation and may thereby induce the formation of neurofibrillary tangles. $A\beta$ can also cause mitochondrial oxidative stress and deregulation of Ca^{2+} homeostasis, resulting in impairment of the electron transport chain, increased production of superoxide anion radical, and decreased production of ATP. Superoxide is converted to H_2O_2 by the activity of superoxide dismutases (SOD) and superoxide can also interact with nitric oxide (NO) via nitric oxide synthase (NOS) to produce peroxynitrite ($ONOO^*$). Interaction of H_2O_2 with Fe^{2+} or Cu^{+} generates the hydroxyl radical (OH^\bullet), a highly reactive oxyradical and potent inducer of membrane-associated oxidative stress that contributes to the dysfunction of the ER. Reprinted from [7] Copyright (2004) with permission of Nature Publishing Group by Copyright Clearance Center.



Supplementary Figure 4. Design of a minimal model membrane system exhibiting dynamic cristae-like morphologies (a “healthy” GUV). See also supplementary Movie 1. Modulation of local pH gradient at membrane level of a cardiolipin containing vesicle induces dynamic cristae-like membrane invaginations. GUV is made of PC/PE/CL (60 : 30 : 10 mol/mol) in buffer at pH 8. The local delivery of HCl (100 mM pH 1.6 in the micropipette), which lowers the local pH down to about 5 to 4 at the membrane, is carried out by a micropipette (its position is pointed by the arrow in frame $t=0$ s). The induced membrane invagination (frame 22.8 s) is completely reversible (frames 38.7 to 66.4 s) as soon as the acid delivery is stopped. Deflated GUVs yield cristae-like morphology with large tubes and vesicular shape segments. Reprinted from [8] Copyright (2008) with permission of Elsevier by Copyright Clearance Center.



Supplementary Figure 5. Spectra of Thioflavin T (ThT) total fluorescence for A β ₄₂ buffer solution at different times after solution preparation. Aliquots of peptide buffer solution were taken and mixed with aliquots of ThT solution (resulting sample: 35 μ M ThT, 11 μ M A β ₄₂ peptide, 5 vol % DMSO, 0.5 mM HEPES, 0.5 mM EDTA, pH 7.4) at 4 h, 22 h, 47 h, and 95 h, respectively. $T = 25^\circ\text{C}$. The ThT fluorescence in the control solution is shown only for 4 h (4 h-control) as it was not changing significantly during the time of our experiment. It was as well practically the same as for the ThT fluorescence in the just buffer (no DMSO), data not shown, both being very low regarding the characteristic band for ThT linked to A β fibrils ($\lambda_{ex}/\lambda_{em}$ 450/482 nm). The two vertical arrows indicate the two bands of ThT characteristic fluorescence corresponding respectively to the two states of ThT fluorophores eventually presented in the sample: the free ThT ($\lambda_{ex}/\lambda_{em}$ 330/445 nm) and, the ThT linked to A β fibrils ($\lambda_{ex}/\lambda_{em}$ 450/482 nm).

REFERENCES

- [1] Trimmer PA, Swerdlow RH, Parks JK, Keeney P, Bennett JP, Miller SW, Davis RE, Parker WD (2000) Abnormal mitochondrial morphology in sporadic Parkinson's and alzheimer's disease cybrid cell lines. *Exp Neurol* **162**, 37-50.
- [2] Boldogh IR, Pon LA (2007) Mitochondria on the move. *Trends Cell Biol* **17**, 502-510.
- [3] Pizzo P, Pozzan T (2007) Mitochondria-endoplasmic reticulum choreography: Structure and signaling dynamics. *Trends Cell Biol* **17**, 511-517.
- [4] Mannella CA, Buttle K, Rath BK, Marko M (1998) Electron microscopic tomography of rat-liver mitochondria and their interactions with the endoplasmic reticulum. *BioFactors* **8**, 225-228.
- [5] Mannella CA, Marko M, Penczek P, Barnard D, Frank J (1994) The internal compartmentation of rat-liver mitochondria: Tomographic study using the high-voltage transmission electron microscope. *Microsc Res Tech* **27**, 278-283.
- [6] Baloyannis SJ, Costa V, Michmizos D (2004) Mitochondrial alterations in Alzheimer's disease. *Am J Alzheimers Dis Other Dement* **19**, 89-93.
- [7] Mattson MP (2004) Pathways towards and away from Alzheimer's disease. *Nature* **430**, 631-639.
- [8] Khalifat N, Puff N, Bonneau S, Fournier JB, Angelova MI (2008) Membrane deformation under local pH gradient: Mimicking mitochondrial cristae dynamics. *Biophys J* **95**, 4924-4933.