

Supplementary Data

FIB/SEM Technology and Alzheimer's Disease: Three-Dimensional Analysis of Human Cortical Synapses

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SUPPLEMENTARY VIDEOS

Supplementary Video 1. Sequence of 100 serial photomicrographs taken from a plaque-free region of layer IV of the frontal cortex of Alzheimer's disease (AD) patient P2. Pictures were taken using a dual-beam electron microscope (FIB/SEM). Synapses are clearly identifiable mainly by the heavily electrondense postsynaptic densities and by the accumulation of synaptic vesicles in the vicinity of the presynaptic membrane. No pathological structures such as dystrophic neurites or amyloid- β (A β) deposits are visible. Field width 15 μ m, section thickness 20 nm. The original series comprised 352 images that are available upon request.

Supplementary Video 2. Sequence of 100 serial photomicrographs taken from a plaque located in layer IV of the frontal cortex of AD patient P2. The dominant feature of this region of the plaque is the presence of dystrophic neurites of different sizes and appearances. Synapses can be identified in the portions of the tissue not occupied by dystrophic neurites. Field width 17 μ m, section thickness 20 nm. The complete FIB/SEM series comprised 499 frames that are available upon request.

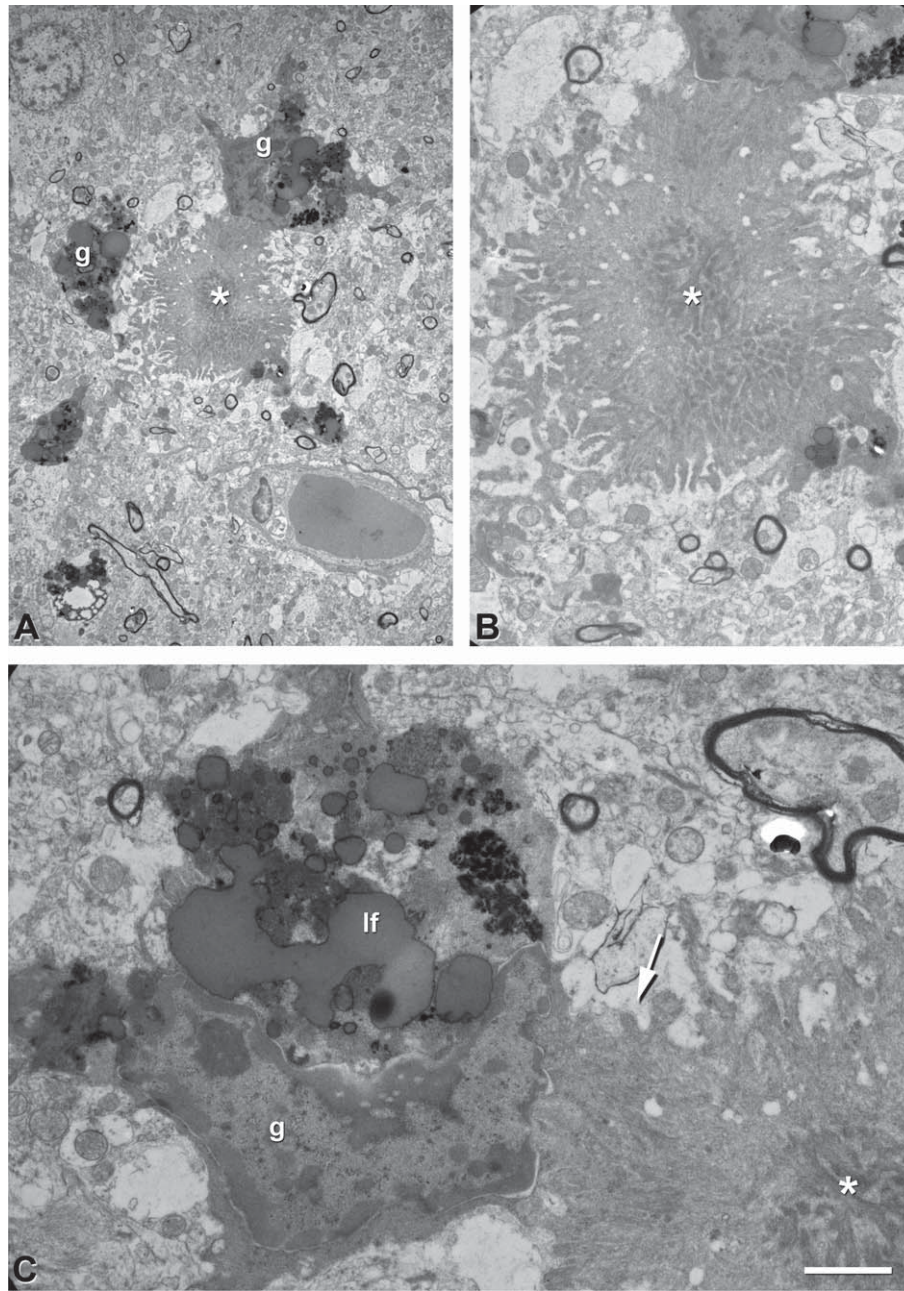
Supplementary Video 3. Sequence of 100 consecutive photomicrographs taken from a plaque located in layer IV of the frontal cortex of AD patient P2. In this region of the plaque the most salient feature is the accumulation of A β deposits (especially at the top left corner of the images). Dystrophic neurites are also present. Synapses can be seen in the areas devoid of A β or dystrophic neurites. Field width 17 μ m, section thickness 20 nm. The original FIB/SEM series comprised 625 photomicrographs that are available upon request.

Supplementary Video 4. Three-dimensional reconstruction of the synaptic profiles that can be found in the stack of serial sections obtained with the FIB/SEM from the plaque-free region shown in Supplementary Video 1. Green objects represent asymmetric synaptic junctions, red objects are symmetric synaptic junctions.

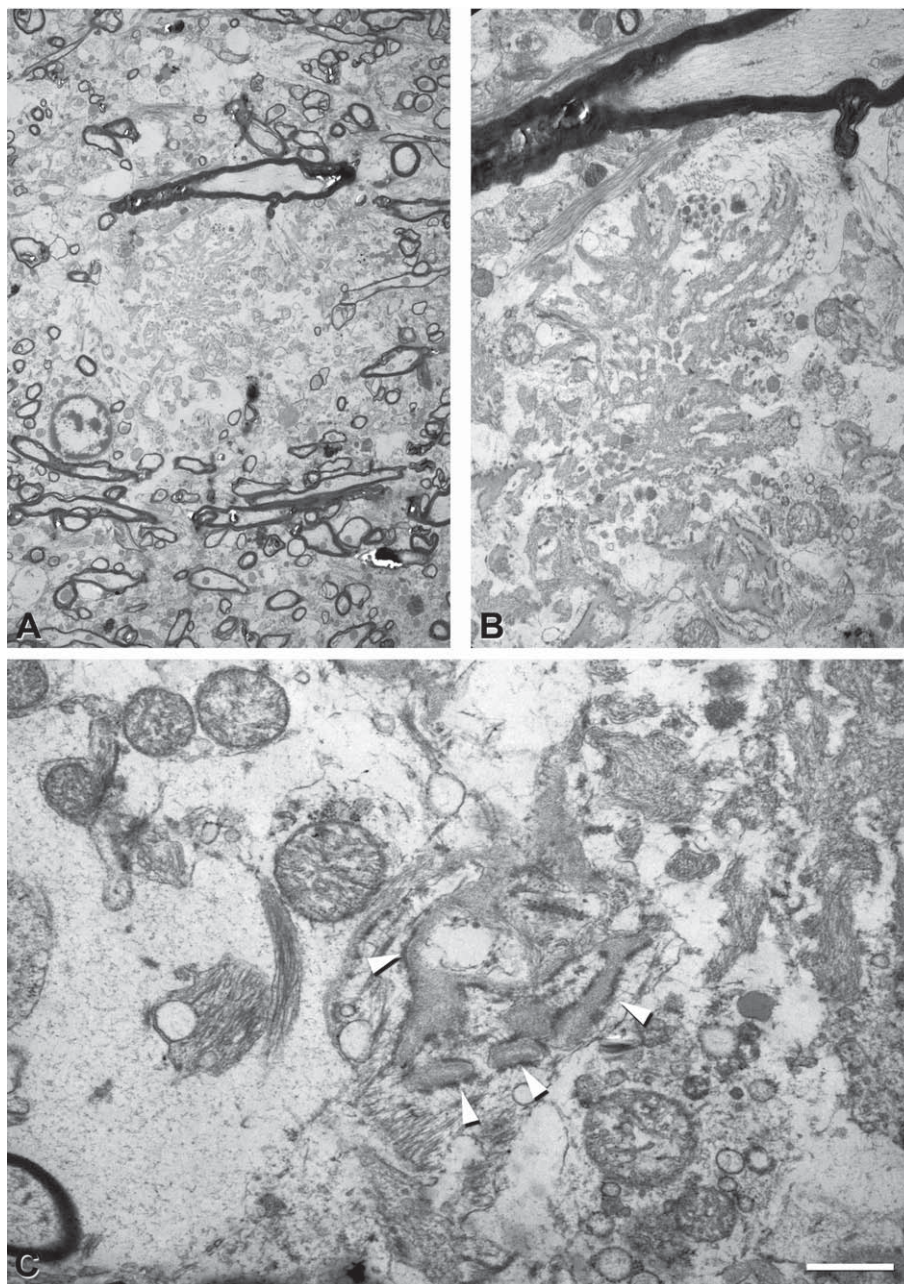
Supplementary Video 5. Three-dimensional reconstruction of the neuron and glial somata, dystrophic neurites and synaptic junctions present in the stack of serial sections obtained with the FIB/SEM of the region shown in Supplementary Video 2. Green objects represent asymmetric synaptic profiles, red objects are symmetric synaptic profiles, the blue object is a neuronal soma, the purple object is a glial soma, brown objects are dystrophic neurites.

Supplementary Video 6. Three-dimensional reconstruction of the extracellular A β peptide, dystrophic neurites, and synaptic profiles found in the serial sections obtained with the FIB/SEM of the region shown in Supplementary Video 3. Green objects, asymmetric synaptic junctions; red objects, symmetric synaptic junctions; brown objects, dystrophic neurites; yellow objects, extracellular A β deposits.

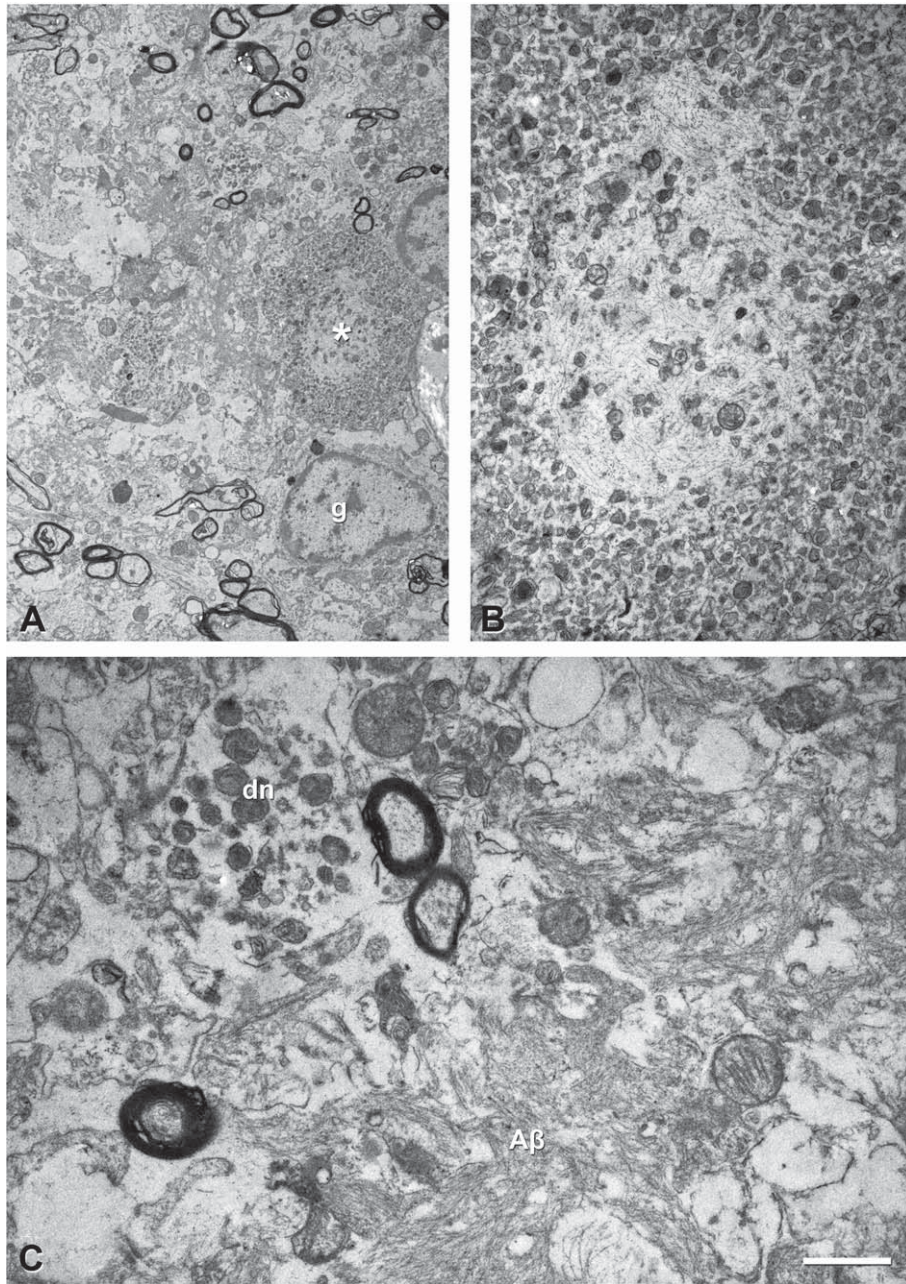
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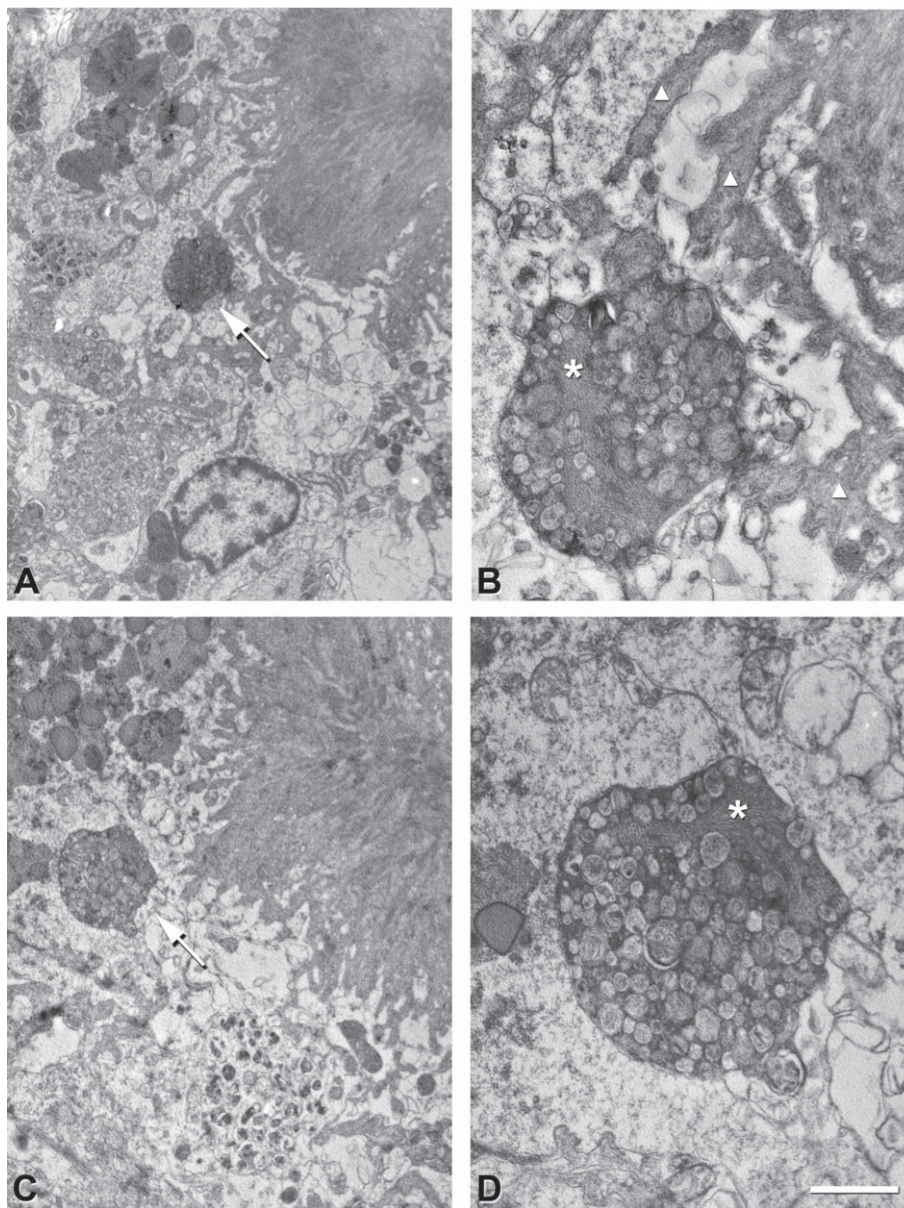
Supplementary Figure 1. A β -cored plaque in the temporal cortex (Brodmann area 21) from patient P9. A) Low-power TEM micrograph of a plaque similar to that shown in Fig. 2, with a central core (asterisk), but with few or no dystrophic neurites. B, C) Higher magnification of A showing the central core (B) and glial cells (g) loaded with lipofuscin bodies (lf). Arrow indicates A β fibrils arising from the core. Scale bar (in C): A, 3.2 μ m; B, 1.4 μ m; C, 0.9 μ m.



Supplementary Figure 2. A β -diffuse plaque in the white matter of the frontal cortex (Brodmann area 10) from patient P9. A) Low-power TEM micrograph showing the plaque where A β is present as lax bundles with relatively few dystrophic neurites displayed. B) Higher magnification of A. C) High-power micrograph of the plaque showing some of its components. Note the membranous processes around A β fibrils (arrowheads). Scale bar (in C): A, 3.2 μ m; B, 1.1 μ m; C, 0.4 μ m.



Supplementary Figure 3. A β -diffuse plaque in the white matter of the frontal cortex (Brodmann area 10) from patient P9. A) Low-power TEM micrograph of a plaque similar to that shown in Supplementary Figure 2, but with numerous dystrophic neurites, some of them of giant size (asterisk). Note that the size of the dystrophic component is similar to the soma of an adjacent glial cell (g). B) Higher magnification of the large dystrophic element shown in A. C) Higher magnification of the plaque showing A β fibrils and a dystrophic neurite (dn). Scale bar (in C): A, 2.1 μ m; B, 0.8 μ m; C, 0.4 μ m.



Supplementary Figure 4. TEM micrographs (from the frontal cortex; Brodmann area 10) showing dystrophic neurites containing filamentous material in patient P2. A, C) Low power magnification illustrating two dystrophic neurites near the core of A β plaques. B, D) Higher magnification of dystrophic neurites in A and C (arrows), respectively. Note the presence of filamentous material (asterisks) that resembles the typical extracellular fibrillar aggregates of A β (triangles) of the plaques. Scale bar (in D): A, 2.3 μ m; C, 1.8 μ m; B, D, 0.7 μ m.