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Observation of morphological relationships between angiopathic blood vessels and degenerative neurites in Alzheimer's disease

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Summary. Two main techniques are used to stain the three types of brain lesions characteristic of Alzheimer's disease: Neurofibrillary tangles (NFT), senile plaques (SP) and congophilic angiopathy. Thioflavine-S is an histochemical marker of the amyloid substance located essentially in the central core of senile plaques and in the walls of the pathological blood vessels. Specific antibodies against Paired Helical Filaments (PHF), the ultrastructural elements of NFT, reveal neuron cell bodies with NFT and numerous dystrophic neurites, mostly around neuritic plaques. Using simultaneous histochemical and immunohistochemical labellings on the same tissue sections of Alzheimer cortex (association cortex and hippocampus), the different lesions were stained with great sensivity and specificity. Moreover, an unusual morphological relationship between two types of lesions was detected in two Alzheimer brains with prominent congophilic angiopathy: we observed a well marked concentration of dystrophic neurites, immunolabelled with anti-PHF, around blood vessels with Thioflavine-S stained amyloid angiopathy. These lesions were distributed like a sleeve around 1/10 of dyshoric or congophilic blood vessels. The significance of such lesions is unknown but they probably represent a step of the pathogenesis of Alzheimer brain lesions and may explain the general mechanism of lesion formation in Alzheimer's disease.

Key words: Alzheimer's disease – Paired helical filaments – Senile plaque – Congophilic angiopathy – Amyloid

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Introduction

Alzheimer's disease is a neurodegenerative disorder characterized by the presence of three cortical lesions: neurofibrillary tangles, senile plaques and congophilic angiopathy whose a etiopathogenesis is still unknown. Neurofibrillary tangles (NFT) are intracellular accumulations of 10 nm filaments regularly twisted in a helix, named Paired Helical Filaments (PHF). The bundles of PHF are found in the perikaryon of neurons, which are mostly pyramidal cells, and in degenerating neurites. Senile plaques (SP) are globular, spherical structures ranging between 20 μm and 200 μm in diameter, characterized by three major components: – dystrophic, degenerating and regenerating neurites, – reactive astroglia and microglial cells, and – amyloid deposits. Senile plaques range in form from discrete, cotton wool-like spherical areas to a dense core of amyloid (burned-out plaque) (Wisniewski and Merz 1983). Congophilic angiopathy (CA) is manifested by the deposit of an amyloid substance in the wall of cortical and leptomeningeal vessels, sometimes extending out into the neuroparenchyma (dyshoric angiopathy: DA) (Glenner 1980, 1983).

To date, degenerating neurites have been visualized with silver impregnation techniques (Bodian for example) and the amyloid substance is stained with Thioflavine-S or Congo Red. The elaboration of specific antibodies against PHF now allows a more precise comparison of the distribution of immunolabelled tangles with that of thioflavine-S stained amyloid deposits in SP and CA. Using both techniques simultaneously, we were able to observe a remarkable histological relationship between

blood vessels with amyloid deposits and degenerating neurites concentrated around these structures. In this report, we present these lesions which were found in the association cortex of two Alzheimer brains.

Material and methods

The diagnosis of Alzheimer's disease was established according to NINCDS-ADRDA classification (Khann et al. 1984), after clinical and anatomopathological investigations. The last one was performed by a count of Alzheimer lesions using silver impregnation, immunolabelling by anti-PHF, Congo Red and Thioflavine-S staining of the amyloid substance and electronmicroscopic observations.

Two Alzheimer brains with a severe congophilic angiopathy are described here. The first, COR, is a 72 year old woman who died after 8 years of illness. The second, MAR, died at the age of 80, after a 5 year illness. The autopsies were carried out 6 h after death.

The polyclonal antiserum against Paired Helical Filaments was prepared by Persuy et al. (1985) and reports on the immunological properties of this antiserum at the lightmicroscopic level (Persuy et al. 1985; Defossez et al. 1986) and at the electronmicroscopic level (Delacourte and Defossez 1986a; Foncin and El Hachimi 1986; Defossez et al. 1987) were described. The polyclonal antiserum against Glial Fibrillary Acidic Protein (GFAP), the protein subunit of glial filaments, was raised against GFAP isolated from Ox spinal cord. This antiserum specifically labels mammalian astrocytes on cryostat and paraffin sections.

The immunohistochemistry was performed as previously described (Delacourte and Defossez 1986b) on paraffin embedded sections for case COR (Carnoy fixation) and on cryostat sections for case MAR (formalin fixation). The blocks were cut in 5 μm-thick sections. Immunoperoxidase reactions were performed according to the indirect method (Persuy et al. 1985). The anti-PHF was used at a dilution of 1/500 and the conjugated sheep anti-rabbit immunoglobulins at 1/50 (Pasteur Production, Paris, France). For visualization of peroxidase, the sections were stained with diaminobenzidine tetrahydrochloride or 4-Chloro-1-Naphtol (20 mg/100 ml of a 0.1 M Tris buffer, pH 7.6, and 0.001% H_2O_2)

For Thioflavine-S staining sections were incubated for 8 minutes in a 1% thioflavine-S (Sigma product) aqueous solution and washed in 80% alcohol.

The sections stained immunohistochemically were counterstained with Thioflavine-S. They were either observed and exposed to bright-field optics (specific visualisation of NFT), to fluorescence optics (visualisation of Thioflavine-stained structures) or to fluorescence optics and low-intensity bright-field optics simultaneously to compare the distribution of NFT with the Thioflavine-stained material.

The simultaneous technique consisting of Thioflavine-S staining associated with PHF immunolabelling was analyzed in order to determine if there was a possible interference between both stainings: the Thioflavine-S staining was performed before and after the immunolabelling and compared to the staining obtained on adjacent sections. The intensity of the Thioflavine-S staining was identical, whatever the procedure. In the same way, the immunolabelling was performed before and after the Thioflavine-S staining and compared with the immunolabelling performed in parallel on adjacent tissue sections. This revealed that Thioflavine-S staining of the amyloid substance did not modify the immunolabelling of NFT.

Results

The anti-PHF, as described previously (Persuy et al. 1985; Defossez et al. 1986, 1987; Delacourte and Defossez 1986a, b) labelled NFT, SP and degenerating neurites on COR occipital sections and MAR temporal sections. NFT were found essentially in the pyramidal cells of the IIIrd and Vth layers whereas SP were observed in all layers but mainly in the IInd, IIIrd and IVth. Moreover, degenerating neurites were particularly numerous around blood vessels (10 to 20 in a centimeter square area) and were found in the IInd and IIIrd layers of the occipital cortex, as represented in Fig. 1. These immunolabelled neurites constitute a sleeve in the nervous tissue surrounding the vessels which were 20–80 μm diameter (Fig. 2a–c and Fig. 3a). Neither the central amyloid cores of senile plaques nor the wall of the blood vessels with congophilic angiopathy were stained by anti-PHF.

Thioflavine-S staining revealed some NFT and the different types of plaques, from cotton wool-like plaques with a homogeneous network of fibrils to neuritic plaques with a dense central core of amyloid substance and burned-out plaques (only a dense core of amyloid substance without neurites at the periphery). These plaques were observed mainly in the IInd, IIIrd and IVth layers (as represented in Fig. 1). In the same way, Thioflavine-S stained pathological vessels: vessel walls (CA) or vessel walls and a trellis of fibrillary material (Fig. 2d and Fig. 3b) in the nervous tissue surrounding the vessel (DA). Congophilic angiopathy

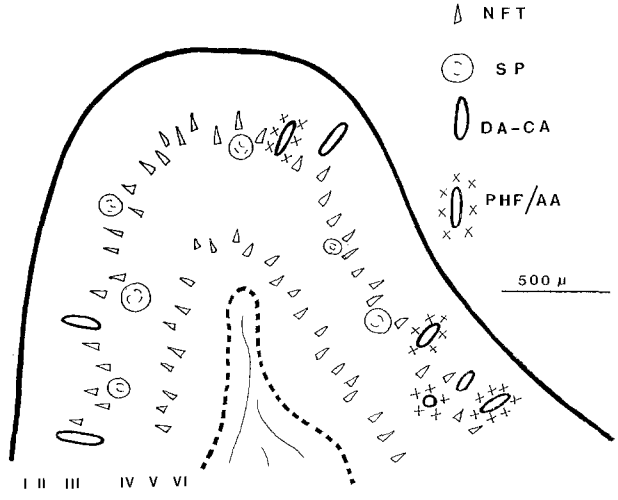

Fig. 1. Topographical distribution of the different Alzheimer lesions in a COR occipital section (taken as an example). I, II, III, IV, V, VI: the different layers of the cerebral cortex. *NFT:* Neurofibrillary tangles; *SP:* Senile plaques; *DA* and *CA:* Dyshoric angiopathy and congophilic angiopathy; *PHF/AA:* Paired Helical Filaments around amyloid angiopathy

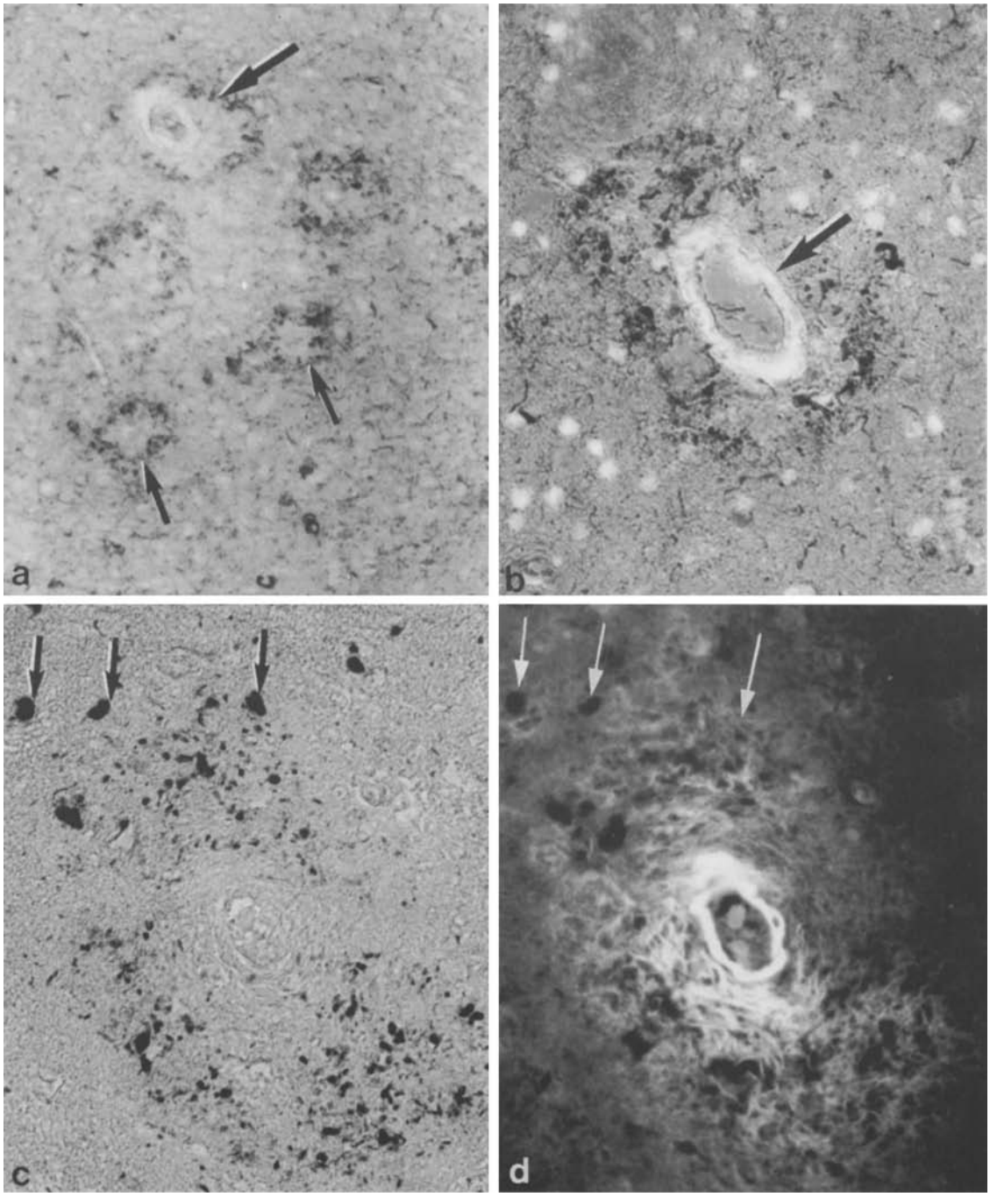

Fig. 2. Paraffin sections of COR occipital cortex immunolabelled with the anti-PHF serum and counterstained with Thioflavine-S. **a** (×250): Bright-field optics observation of the immunolabelling. Many vessels are surrounded by degenerating neurites (*arrows*). **b** (×500): Simultaneous observation with bright-field and fluorescent optics of the congophilic angiopathy: the wall of the blood vessel is fluorescent and is surrounded by immunolabelled neurites. **c, d** (×500): Successive observation with bright field optics (**c**) and fluorescent optics (**d**) of a dyshoric angiopathy: immunolabelled neurites visualized at c are intimely intricated with amyloid fibrils at the periphery of the vessel (**d**). Three immunolabelled cell bodies with tangles are noted (*arrows*)

was found in 1/3 of the vessels with a 30 μm to 80 μm diameter and located in layers I to III, whereas dyshoric angiopathy was localized in the same layers and found in 1/3 of the vessels with a 20 μm to 50 μm diameter.

In order to determine the relationships between Thioflavine-S stained amyloid blood vessels and the immunolabelled degenerative neurites around these vessels, two visualization techniques were used successively or simultaneously on the same

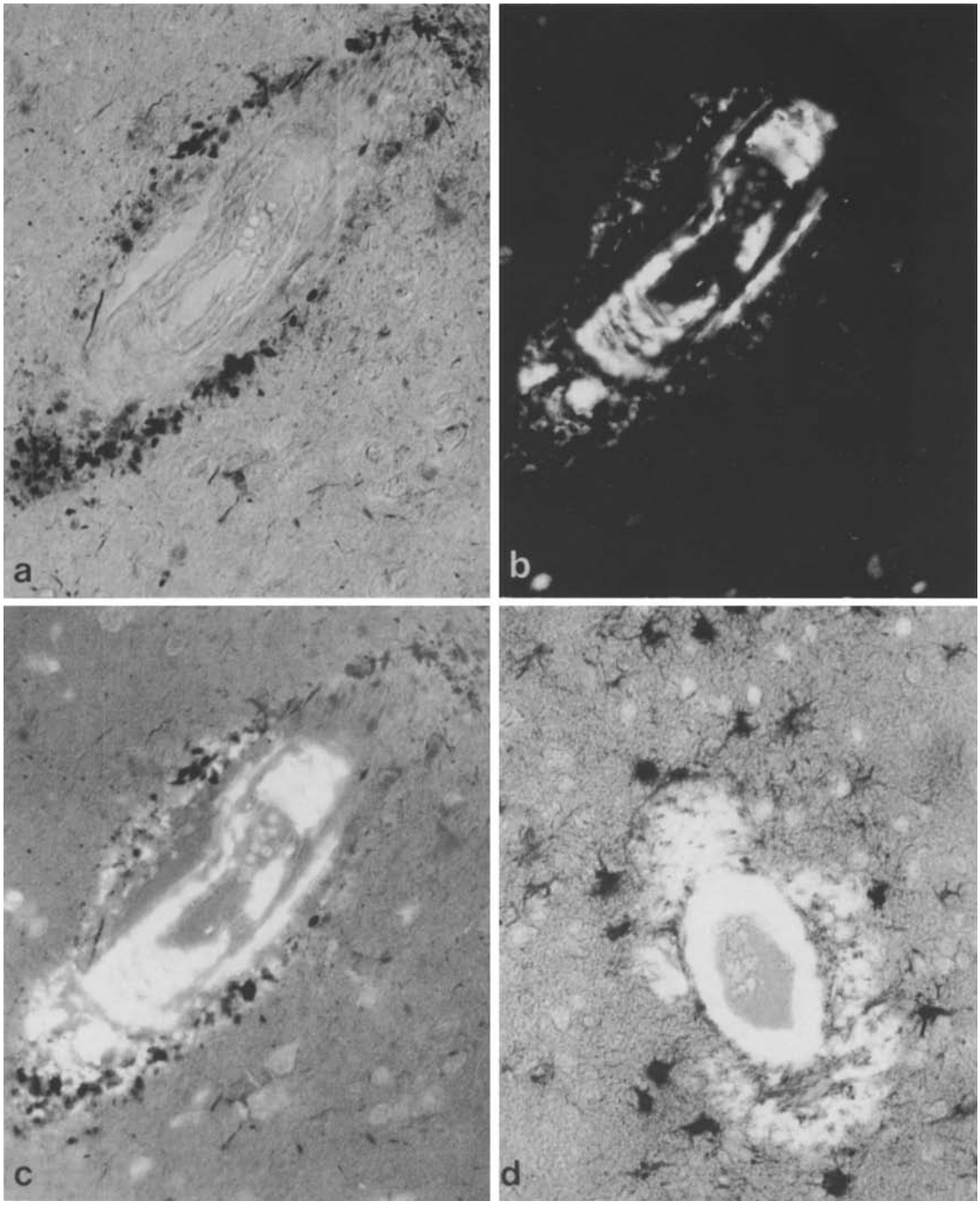

Fig. 3. Cryostat sections of MAR temporal cortex. **a–c** (× 500): Immunolabelling with the anti-PHF serum and counterstaining with Thioflavine-S: Successive observation of a dyshoric angiopathy with bright-field optics (**a**), fluorescent optics (**b**) and both optics (**c**). Note the intimate intricacy of the immunolabelled neurites (**a**) with Thioflavine-S stained amyloid fibrils (**b**) and compared in (**c**); **d** (× 500): Immunolabelling by anti-GFAP immunserum and counterstaining with Thioflavine-S. Note the dyshoric angiopathy (fluorescence) and the distribution of astrocytes

tissue sections: bright-field optics for the visualization of the immunological labelling and fluorescence optics for the Thioflavine-S staining. 1/10 blood vessels with CA, which were observed simultaneously with both optics, showed an amyloid deposit in the blood vessel walls (fluorescence) surrounded by degenerating neurites (immunolabelling) (Fig. 2b). In the same way, 1/10 blood vessels with DA visualized successively with bright-field optics and fluorescence optics showed that degen-

erative neurites are in close contact with amyloid fibrils surrounding the blood vessel (Fig. 2c, d and Fig. 3a, b). Using both optics simultaneously, as presented in Fig. 2d and 3c, the amyloid fibrils and degenerating neurites were intimately mixed but they have different morphological structures. These lesions were termed PHF/AA lesions (Paired Helical Filaments/Amyloid Angiopathy).

Thus, about 10 PHF/AA to 20 PHF/AA lesions were found in a centimeter square area on the association cortex sections of both patients. At last, an immunological reaction with anti-GFAP was carried out in order to determine if astrocytes were involved in the formation of PHF/AA lesions. The distribution of glial cells was thus stated precisely (Fig. 3d).

Discussion and conclusion

The Alzheimer lesions were visualized with two techniques: the first one, Thioflavine-S, is very suitable for the staining of the amyloid core of senile plaques and the amyloid of angiopathic blood vessels. Our experience showed that this staining was, by far, more sensitive than Congo Red. The second one is the immunolabelling of NFT at the optical level or of their ultrastructural elements which are PHF. These antibodies are a new tool for neuropathologists, more specific and more sensitive than silver impregnation (Persuy et al. 1985; Defossez et al. 1986). Moreover, by using the immunolabelling and the Thioflavine-S staining simultaneously, we were able to compare the distribution of NFT, SP, CA and DA on the same tissue section.

We observed a morphological relationship between two different Alzheimer lesions: degenerating neurites and vascular amyloid deposit. Degenerating dystrophic neurites were found around some cortical vessels with CA and in close contact with the amyloid fibrils outside the vessel of DA. Since anti-PHF specifically labels tangles at the optical level and PHF at the electronmicroscopic level (Delacourte and Defossez 1986a; Foncin and El Hachimi 1986; Defossez et al. 1987), we can ascertain that the degenerating neurites concentrated around pathological vessels contain bundles of PHF. Therefore, these lesions were termed PHF/Amyloid Angiopathy lesions (PHF/AA).

The relation between NFT, SP, CA and DA is a matter for extensive discussion. Two hypotheses have been proposed:

Firstly, NFT precursor proteins from neuronal origin might be deposited in the extracellular space to crystallize within the center of a plaque or migrate and accumulate around and within the walls of small blood vessels (Masters et al. 1985).

Secondly, an abnormal serum protein is processed by endothelial cells to produce amyloid fibrils. After, this chronic vascular amyloidosis causes a breakdown of the blood brain barrier and permits an egress of nervous tissue by a neurotoxic substance leading to the appearance of PHF (Glenner 1983; Wisniewski and Merz 1983).

The observation of PHF/AA lesions shows that degenerating neurites are closely distributed around and along some vessels with angiopathy. Furthermore, concentration of degenerating neurites around normal blood vessels has never been observed. Thus, if PHF are at the origin of the formation of the amyloid angiopathy, these PHF should be distributed at random around all vessels. This was not the case. On the contrary, the presence of an accumulation of PHF forming a sleeve only around some, always angiopathic vessels, indicates that PHF formation is a consequence of the primary vascular pathology, whose a etiopathogenesis is unknown. The formation of PHF/AA lesions is not a major phenomenon compared with the other lesions. However, this observation is interesting since it might explain the formation of certain neuritic plaques, the mechanism of which is not yet determined. We suggest that the presence of degenerating neurites around the central core of neuritic plaques, which are always in the vicinity of capillaries (Miyakawa et al. 1982, 1985), might be comparable to those around angiopathic blood vessels.

In conclusion, it is likely that Alzheimer's disease is characterized by the presence of different types of amyloid substances, different in origin, maturation and degradation. For example, amyloid angiopathy is probably followed by the formation of PHF bundles which are also beta pleated sheet conformations with amyloid properties (Kirschner et al. 1986). Thus, more information on the biochemistry of the different Alzheimer amyloid substances are needed to confirm or disprove our proposition on the etiopathogenesis of PHF/AA lesions and neuritic plaques.

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