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# Cortical angiopathy in Alzheimer's disease: the formation of dystrophic perivascular neurites is related to the exudation of amyloid fibrils from the pathological vessels

**M.C. Peers [1], M.B. Lenders [2], A. Défossez [1], A. Delacourte [3], and M. Mazzuca [1]**

[1] Laboratoire d'Histologie, INSERM U 156, Faculté de Médecine, 1 Place de Verdun, 59045 Lille Cedex, France
[2] Service de Neurologie C (Pr H. Petit) – Hôpital B CHU de Lille, Lille, France
[3] Laboratoire de Neurosciences, INSERM U 16, Faculté de Médecine, Lille, France

**Summary.** We studied the organization of dystrophic neurites around pathological vessels in Alzheimer cortex. Two techniques were used simultaneously on serial sections: thioflavine staining of amyloid substance and immunohistochemistry with immune sera against Paired Helical Filaments (anti-PHF) and native Tau proteins (anti-Tau). We observed different distributions of dystrophic neurites (immunolabelled with anti-PHF or anti-Tau) around thioflavine-stained angiopathic arterioles. The wall of the vessels with large diameter (>100 μm) presented a congophilic angiopathy without neuropil reaction. In vessels with lesser diameter (<100 μm), dystrophic neurites constituted a discontinuous sleeve around vessels, always in close contact with amyloid substance outside the wall (dyshoric angiopathy). We observed structures similar to senile plaques around capillaries (diameter: 10–15 μm). The sleeve of dystrophic neurites with aggregated Tau proteins were always observed in the close vicinity of the amyloid substance which exuded from the pathological blood vessels. Thus, the exudation of these amyloid fibrils seems to induce the formation of dystrophic neurites (neuritic reaction).

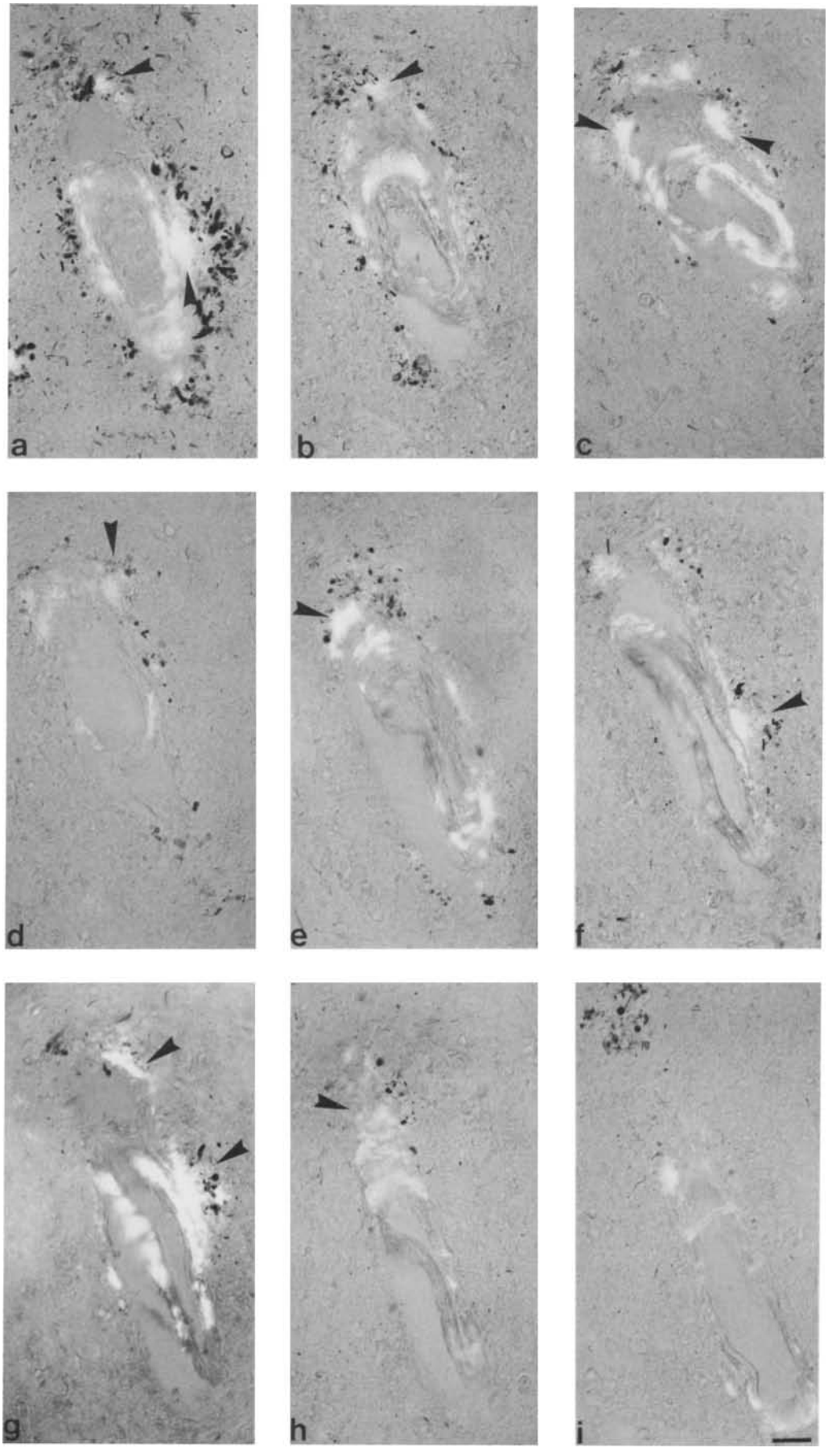
**Key words:** Alzheimer's disease – Dystrophic neurites – Congophilic and dyshoric angiopathy – Amyloid substance – Senile plaques – PHF

## Introduction

Alzheimer's disease is characterized by the accumulation of two types of fibrillar structures in the brain: paired helical filaments (PHF) and amyloid filaments (AF). PHF are 10 nm double-stranded twisted filaments which are accumulated in the perikaryon of degenerating neurons (neurofibrillary degeneration: NFD) (Kidd 1963) and in neurites, scattered in the parenchyma (Braak et al. 1986), concentrated at the periphery of senile plaques or sometimes around pathological blood vessels (Delacourte et al. 1987). AF are extra-cellular 6–9 nm straight filaments which are found in the senile plaque cores (Terry 1985) and in the wall of leptomeningeal and cortical arterioles (congophilic angiopathy: CA) (Pantelakis 1956), sometimes extending out into the neural parenchyma (dyshoric angiopathy: DA) (Surbeck 1961).

Dystrophic neurites around pathological vessels and around senile plaques have been observed with silver impregnation (Morel 1946). Recently, it has been shown that Tau proteins are the major components of PHF (Grundke-Iqbal et al. 1986; Delacourte and Défossez 1986). With polyclonal antibodies against PHF and native Tau proteins, we have observed a neuritic reaction around cortical vessels with amyloid angiopathy, demonstrating the presence of PHF with aggregated Tau proteins in these dystrophic neurites (Delacourte et al. 1987; Défossez and Delacourte 1987). Neurites were distributed like a sleeve around 10% pathological blood vessels; we have hypothesized that Tau aggregation in neurites was due to an intoxication of the nerve endings in the vicinity of pathological vessels. In this paper, we have analysed more precisely the distribution of this neuritic sleeve with PHF around vessels on serial sections and its relationship with CA or DA. The study was performed using simultaneous Thioflavine-S staining and anti-PHF or anti-Tau immunolabelling with polyclonal antibodies, enabling us to distinguish dystrophic neurites and amyloid fibrils.

*Offprints requests to:* M.C. Peers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## Materials and methods

The observations of dyshoric and congophilic angiopathies were performed on tissue sections of temporal and occipital cortices of three patients, respectively 72 (A), 75 (B), 80 (C) years old. The autopsies were carried out within 6 h after death. The diagnosis of Alzheimer's disease was established according to NINCDS-ADRDA classification (Khann et al. 1984) after clinical and pathological investigations.

For two patients (A and B), Carnoy fixation (alcohol, benzene, chloroforme, 3/3/1) and paraffin embedding of temporal and occipital cortex pieces were performed and cut in 6 µm-thick sections. For one patient (C), after fixation in picric-acid-paraformaldehyde and 24 h of impregnation in a 10% sucrose solution in 0.1 M veronal buffer, pH 7.4 at 4° C, blocks of temporal cortex were frozen in liquid nitrogen and cryostat sections (12 µm thick) were prepared.

The polyclonal antiserum against PHF was prepared and characterized as previously published (Persuy et al. 1985; Delacourte and Défossez 1986; Défossez et al. 1988). The anti-Tau was raised against Tau proteins isolated from a bovine brain (Delacourte and Défossez 1986).

Tissue sections were stained for 8 min in a 1% thioflavine-S aqueous solution and were washed in 80% alcohol. Then, immunoperoxidase reactions were performed according to the indirect method. The anti-PHF was used at a dilution of 1/200, the anti-Tau at 1/10000, and the peroxidase conjugated sheep anti-rabbit immunoglobulins at 1/100 (Pasteur Production, Paris). For visualization of peroxidase, the sections were stained with a solution of 20 mg of 4-chloro-1-Naphtol in 0.25 ml dimethyl formamide /50 ml of 0.1 M Tris buffer, pH 7.6, 0.001% $H_2O_2$.

Sections were observed simultaneously in fluorescence (visualization of thioflavine-stained structures) and bright-field optics (visualization of immunolabelling).

Different pathological vessels were first visualized on serial sections and photographed. The diameter was measured with a micrometric ocular, the length was approximately calculated on the thickness and the number of serial observed sections. Secondly, a schematic three-dimensional reconstruction was performed using photographs. We have illustrated one vessel of the temporal cortex of patient C in particular.

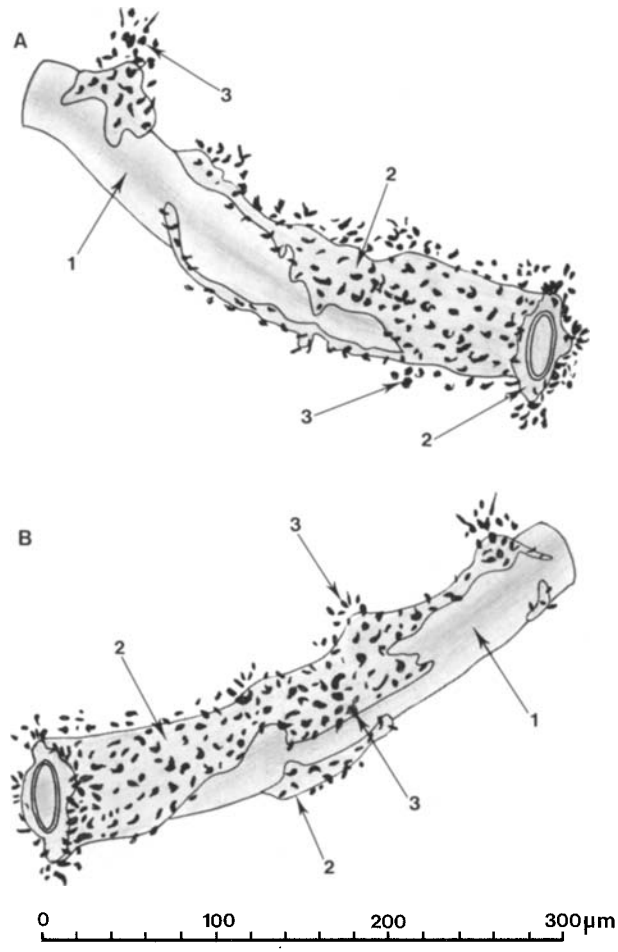


**Fig. 2.** Three-dimensional scheme of the same arteriole. **(A)** representation of its left side; **(B)** representation of its right side. Note the irregularity of the distribution of amyloid deposits (2) outside the vessel wall (1), and the systematic presence of dystrophic neurites (3) in their close vicinity

## Results

In the observed cortices, we distinguished three kinds of pathological vessels: larger arterioles (diameter >100 µm), smaller arterioles (diameter: 15–100 µm) and capillaries (diameter: 10–15 µm).

In large arterioles, amyloid substance was only present in the vessel walls (congophilic angiopathy: CA) and never invaded the neuropil.

For smaller arterioles, amyloid fibrils exuded from the vessel wall into the neuropil (dyshoric angiopathy: DA) in close contact with dystrophic neurites (immunolabelled with anti-PHF or anti-Tau). Different arterioles were observed on the whole length (300–1200 µm). For practical reasons, we illustrate a small arteriole (300 µm in length, 40 µm in diameter) some serial sections from which are illustrated in Fig. 1. A schematic three-dimensional reconstruction is presented in Fig. 2. Amyloid exudation was distributed like an irregular sleeve, always associated with dystrophic neurites that reacted with the anti-PHF or the anti-Tau.

For capillaries (10–15 µm), neurites were wrapped round these vessels without discontinuity; this structure was indistinguishable from SP

**Fig. 1.** Cryostat serial sections (**a b c d e f g h i**) of the same arteriole in Alzheimer's temporal cortex. Immunolabelling with anti-PHF and counterstaining with Thioflavine-S. Observation with both bright-field optics and fluorescence. Note the intimate intricacy (*arrowheads*) of dystrophic neurites (immunolabelled with anti-PHF) and amyloid fibrils (fluorescence) outside the vessel walls (*scale bar:* 20 µm, reduced to 85%)

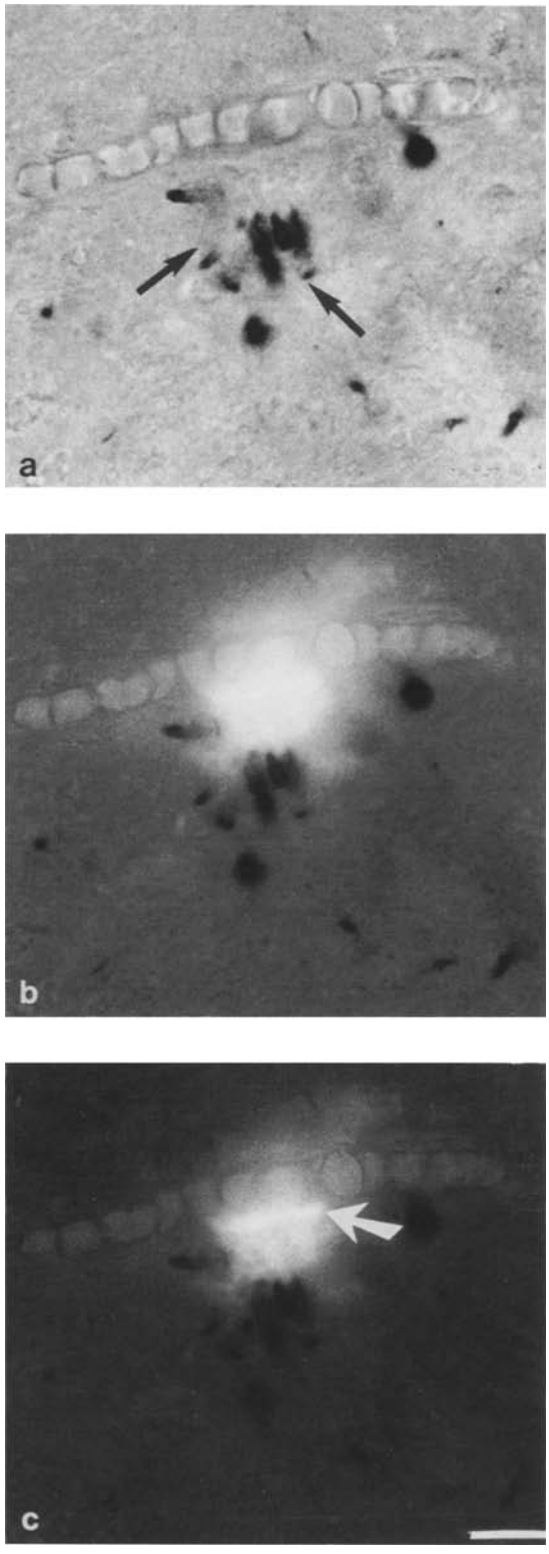


**Fig. 3.** Capillary observed with bright field optics **(a)**, with both fluorescence and low intensity brigth-field optics **(b)**, with fluorescence **(c)**. Immunolabelling with anti-Tau and counterstaining with Thioflavine-S. Note dystrophic neurites (*black arrows*) around the capillary and the thickening of the wall on the same side (*white arrows*) (scale bar: 10 μm)

(Fig. 3). We never observed dystrophic neurites around normal blood vessels.

## Discussion

We have used two staining methods on serial sections of Alzheimer brain tissue simultaneously, namely thioflavine and immunolabelling with anti-PHF or anti-Tau. Thioflavine-S electively stains all amyloid substances. The anti-PHF immune serum has the properties of an anti-Tau immune serum (Delacourte and Défossez 1986; Delacourte et Défossez 1988; Parent et al. 1988) and these two immune sera specifically stained neurofibrillary degeneration (NFD) at the optic microscope level and paired helical filaments (PHF) at the electron microscope level (Défossez et al. 1988). Anti-PHF and anti-Tau labelled neither the amyloid central core of senile plaques, nor the amyloid angiopathy (AA).

Concentrations of dystrophic neurites with PHF bundles were observed in close contact of cortical vessels with AA in several Alzheimer brains (Delacourte et al. 1987). The analysis of pathological vessels with a neuritic reaction on serial sections and the three-dimensional reconstruction showed that dystrophic neurites, stained with anti-PHF and anti-Tau, were always in close contact with the amyloid fibrils exuding from the vessel wall. The neuritic reaction was observed neither at the periphery of normal vessels nor around pathological vessels without dyshoric angiopathy (DA). Indeed, like Constantinidis and Richard (1985), we have observed that in large arterioles no amyloid substance went through the thicker wall to spread out in the parenchyma. No neuritic reaction was observed around these large vessels.

In small vessels, amyloid fibrils damaged the entire wall thickness, provoking DA. These vessels were often surrounded by a neuritic reaction. Thus the diameter and the thickness of the vessel seemed to be determinant for the development of these lesions.

As far as capillaries were concerned, the analysis was more difficult for technical reasons: their walls only consist of endothelial cells and the amyloid substance spreading out from these cells into parenchyma has only been observed at the electron microscope level (Miyakawa et al. 1982; Miyakawa 1988). Furthermore, the relationship between capillaries and amyloid fibrils was difficult to analyze at the optical level because they are small and, since the study had to be performed on serial sections, a large deposit of amyloid substance might be spreading out from a capillary which was not

necessarily in the same section. However, we were able to study capillaries (diameter: 10–15 μm) which were also surrounded by a neuritic reaction.

Since the NFD with Tau aggregation is a non-specific consequence of numerous and different aetiologies (Bancher et al. 1987; Pollock et al. 1987), it is very likely that the neuritic reaction observed here is a direct consequence of the exudation of amyloid fibrils or of toxic factors accompagning these fibers.

Due to its constant spherical shape, most of the authors agree that there is a point source that leads to the formation of senile plaques (SP). Different hypothesis have been raised for the formation of SP: a localized deposit of alumino-silicate (Crapper McLachlan 1986), a microglial cell in the center (Probst et al. 1987) and a pathological capillary in the center (Miyakawa et al. 1986). Our results show that the different steps of degeneration around small vessels are very similar to those observed during the formation of SP: firstly, a localized deposit of amyloid substance in the vessel wall, secondly, a spreading of the amyloid deposits, then a neuritic reaction near the amyloid substance. Therefore, from our observations, we favour the third hypothesis and we think that the deposit of amyloid fibrils around a pathological capillary might be the first event of the SP formation. Finally, we have examined the question of the significance of the neuritic reaction around pathological vessels that we named PHF/AA lesions (Delacourte et al. 1987). Could PHF/AA lesions be a characteristic feature of Alzheimer's disease (AD)? In our experience, they have been found in large amounts in 3 AD brains out of 50. Therefore, these lesions are rare but not exceptional since they have been described by other authors (reviewed by Constantinidis and Richard 1985). They were often associated with a well developped AA but the presence of AA is not a constant feature of Alzheimer's disease. The formation of PHF/AA seems to result of the addition of two factors: a well developped AA and a long course of the disease. Although these lesions cannot be considered to be characteristic of Alzheimer's disease, they show a mechanism of PHF formation around pathological vessels with AA. They might be considered as a model for the study of the aetiopathogenesis of SP.

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