Acta Neuropathol (Berl) (1982) 57:75–79

Acta Neuropathologica

Short Original Communication

Senile Dementia of Alzheimer Type: Astroglial Reaction to Extracellular Neurofibrillary Tangles in the Hippocampus

An Immunocytochemical and Electron-microscopic Study

A. Probst, J. Ulrich, and Ph. U. Heitz

Dept. of Pathology of the University of Basel, Schönbeinstr. 40, CH-4056 Basel, Switzerland

Summary. Two types of Alzheimer neurofibrillary tangles may be found in the hippocampus in senile dementia of the Alzheimer type. Besides classical flame-shaped intraneuronal tangles, there are less compact tangles representing extracellular remnants of destroyed neurons with neurofibrillary change. Strong immunoreactivity for glial fibrillary acidic protein (GFA) was found in the second type of tangles, which was due to penetration of fine processes of fibrous astrocytes into bundles of paired helical filaments (PHF). PHF appear to be a strong stimulus for astrocytic reaction when they are not segregated from the neuropil by the neuronal cell membrane.

Key words: Senile dementia of Alzheimer type – Neurofibrillary tangles – Fibrous astrocytes – Immunocytochemistry

Introduction

In senile dementia of the Alzheimer type (SDAT) two types of Alzheimer neurofibrillary tangles can be found in silver-impregnated sections of the hippocampus. One type is the classical flame-shaped intracellular tangle. The second type displays a less compact structure, is less argyrophilic and not accompanied by a neuronal nucleus. It is thought to represent remnants of destroyed neurons containing Alzheimer neurofibrillary tangles (Alzheimer 1907a, b).

During an immunocytochemical investigation of the brain of patients suffering from SDAT we found peculiar glial fibrillary acidic protein (GFA)-reactive structures in the hippocampus resembling the second type of tangles. As the presence of GFA in tangles is very puzzling, we decided to carry out a more detailed analysis, using immunocytochemistry and electron microscopy.

Offprint requests to: A. Probst, MD (address see above)

Material and Methods

Seven brains of patients with a severe senile dementia and typical lesions of the Alzheimer type were obtained 3–12 h postmortem and fixed in formaldehyde (4%) in phosphate-buffered saline (PBS; pH 7.2). The total time of fixation did not exceed 10 days. Tissue blocks were taken from the middle third of the dentate-hippocampal complex after histological confirmation of the diagnosis of SDAT.

Deparaffinized sections (5 µm) were stained with the Holmes technique for the demonstration of Alzheimer neurofibrillary tangles and nerve fibers. Further sections were stained with alcaline Congo red (Puchtler et al. 1962) and viewed under polarized light for identification of amyloid.

The unlabeled antibody enzyme method (Sternberger 1979) was applied to further deparaffinized sections of the hippocampus (5 µm), using a serum raised in the rabbit against GFA protein from degraded bovine spinal cord (Eng 1980). The primary antibody was diluted 1/2000 with PBS (pH 7.2) containing 0.25% human serum albumin and 0.1% sodium azide. Sheep anti-rabbit IgG and rabbit peroxidase-anti-peroxidase complexes were diluted 1/30. The histochemical reaction for peroxidase was carried out using 3'3'-diaminobenzidine-tetrahydrochloride (0.05%) and hydrogen peroxide (0.01%) in 0.05 M Tris-HCl-buffer (pH 7.6). After post-fixation (5 min) with osmium tetroxide (1%), the sections were counterstained with thionin, dehydrated, and mounted.

Controls

Controls were (1) omission of GFA-antibody or use of non-immune rabbit serum as the first layer and (2) omission of hydrogen peroxide from the incubation medium of the peroxidase reaction.

Further small tissue blocks were taken from the hippocampal cortex (sector CA 1, subiculum, presubiculum), immersed in glutaraldehyde (2.5%) in PBS (pH 7.4), postfixed in 1% osmium tetroxide, dehydrated, and embedded in araldite. One section (1.5 µm) of each block was stained with toluidine blue. Serial sections (1.5 µm) were then immunostained for GFA using the unlabeled antibody enzyme method. The araldite was removed by alcoholic sodium hydroxide (Lane and Europa 1965; Baskin et al. 1979a). The sections were then bleached by an aqueous solution of periodate (5%; Baskin et al. 1979b). All buffers used contained 0.5 M NaCl to avoid non-immunologic binding of immunoglobulins to the plastic-embedded sections (Grube 1980). Some of the sections were counterstained

0001-6322/82/0057/0075/$1.00

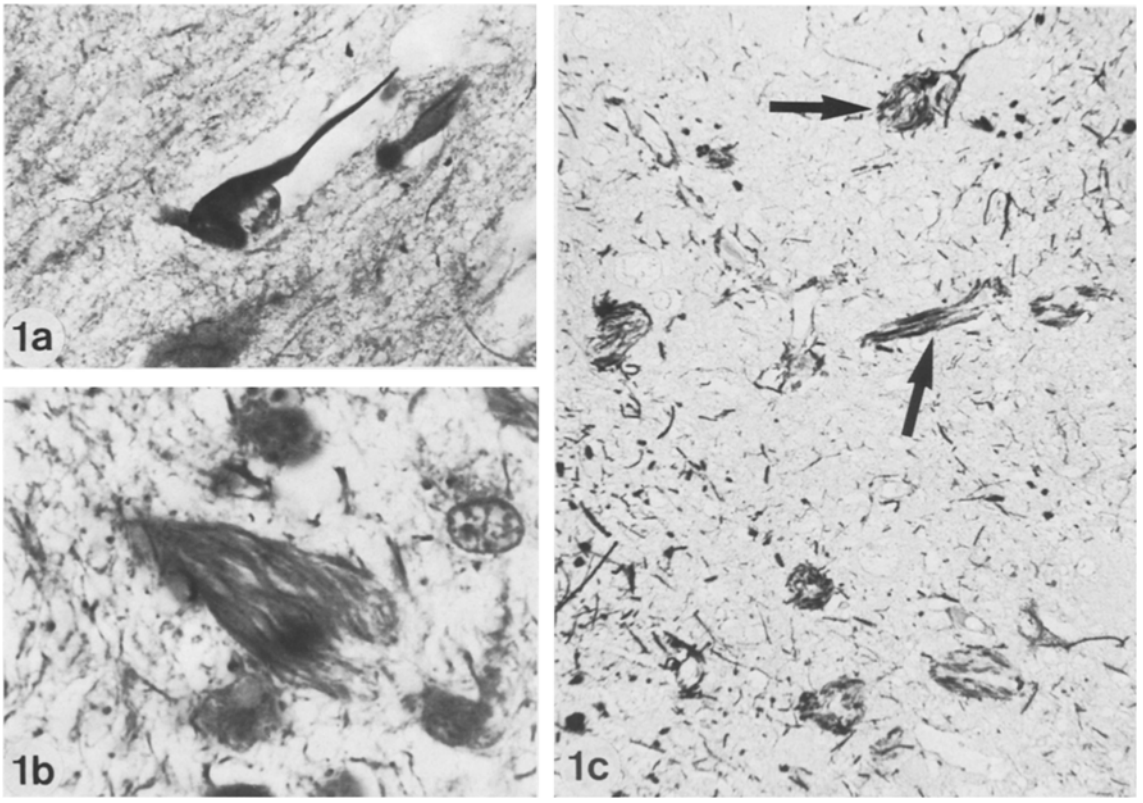

Fig. 1. a Classical flame-shaped neurofibrillary tangle in the hippocampus. Holmes technique, × 800. **b** Tangle of type B. Note absence of a nucleus, lesser argyrophilia and loose texture as compared to the classical type. Holmes technique, × 1,200. **c** CA 1 sector of the hippocampus Tangles of type B displaying immunoreactivity for glial fibrillary acidic protein (GFA) (*arrows*). Semithin plastic section, × 550

with thionin. Differential interference contrast optics (Nomarski) were used for evaluation.

Ultrathin sections were cut from the CA 1 sector of the hippocampal cortex, stained with uranyl acetate and lead citrate, and viewed with a Zeiss 9 A or Philips EM 300 electron microscope.

Results

In sections stained with Holmes method, two types of Alzheimer's neurofibrillary tangles could be distinguished, i.e. (1) the well-known flame-shaped intracellular tangles (Fig. 1 a) (type A tangles) and (2) type B tangles displaying a comparable size, shape, and orientation, but staining usually gray instead of black and devoid of a visible nucleus (Fig. 1 b). Type B tangles often displayed a loose texture, whereas type A tangles were in general compact. Both types were congophilic, producing a characteristic green birefringence in polarized light.

In the GFA-immunostain tangles of type A were consistently negative (Fig. 2 b) whereas type B tangles showed a strong reaction (Fig. 1 c, 2 a). Intensely staining tangles could sometimes be shown to be continuous with processes of fibrous astrocytes (Fig. 2 c). These processes split into multiple thin, hair-like fibers forming a kind of "painting brush" in the shape of the type B tangles. In semithin sections some thicker fibrils not reacting to GFA antibody were visible between the fine GFA-containing processes. By electron microscopy, a small number of characteristic type B tangles consisting of parallel cellular processes and containing filaments of the intermediate type (approximately 10 nm in width) and glycogen granules could be identified (Fig. 3). Between the cellular processes, rectilinear fibrils (maximum width 25 nm) with sometimes a periodic variation of their diameter and a medium electron density were present (Fig. 3, inset). The fibrillary material was separated from the intermediate filaments by a unique cellular membrane. Its position was therefore obviously extracellular.

Discussion

The type B tangles were shown to contain astrocytic glial fibrils. These tangles are likely to represent bundles of intracellular intermediate filaments seen in the electron microscope. The extracellular GFA-negative material between the astrocytic fibrils shows a periodic variation of the fibrillary thickness. It therefore consists

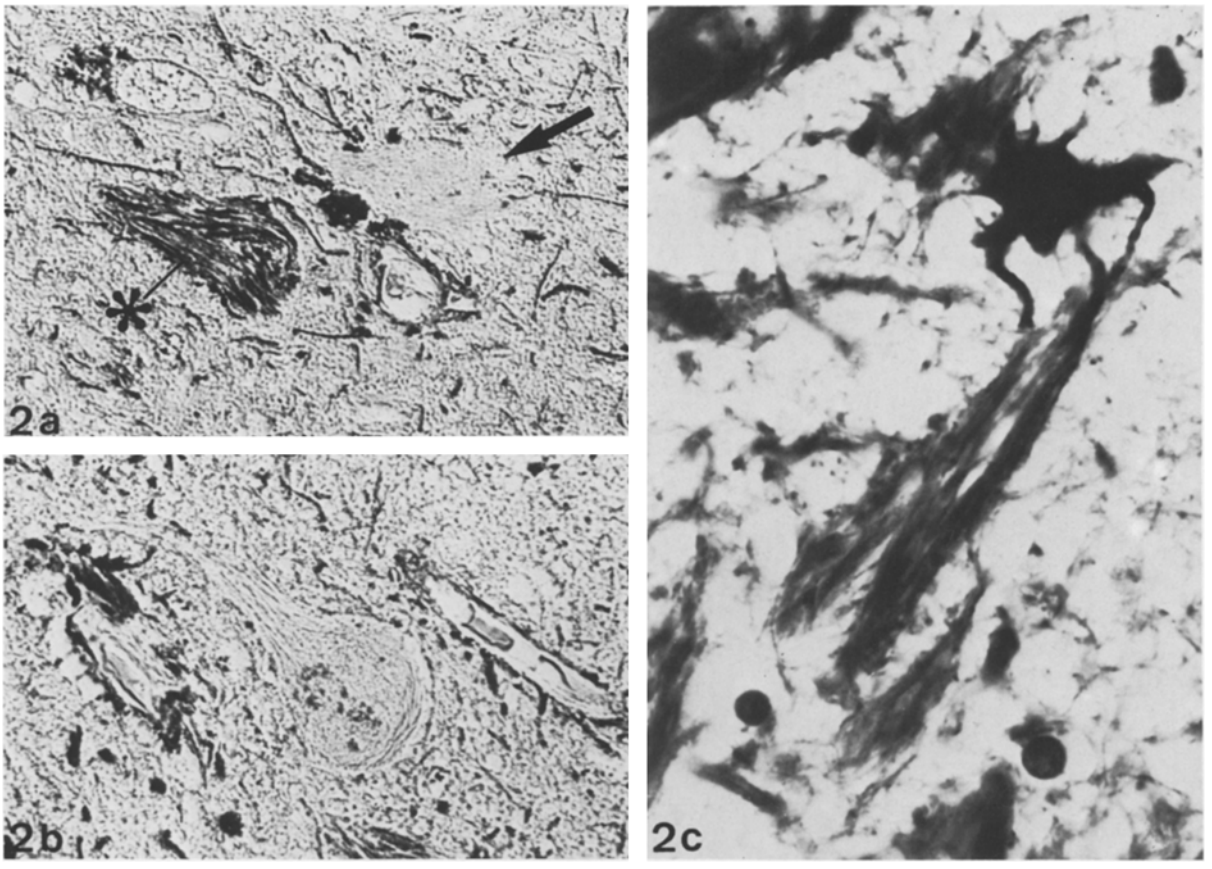

Fig. 2a–c (see also Fig. 1c). Immunostaining for glial fibrillary acidic protein. Unlabeled antibody enzyme method. **a** CA1 sector of the hippocampus. Strongly reactive tangles of type B (*) in the vicinity of a non-reactive neurofibrillary tangle (*arrow*). Note numerous astrocytic processes. Semithin plastic section. Differential interference contrast optics (Nomarski), ×750. **b** Non-reactive pyramidal cell of the hippocampus containing neurofibrillary tangle. Dark intracytoplasmic granules correspond to lipofuscin. Semithin section. Nomarski optics, ×750. **c** Immunreactive tangle of type B continuous with three thick cell processes of a fibrous astrocyte. Paraffin section, ×1,500

probably of slightly altered paired helical filaments (PHF) (Kidd 1963; Terry 1963). The green birefringence of type B tangles after staining with Congo red is in agreement with this finding (Wisniewski and Iqbal 1980; Glenner 1980).

We interprete type B tangles as remnants of destroyed neurons presenting the neurofibrillary change. Astrocytes react by circumventing and penetrating them with fine cellular processes. The intensity of this reaction indicates that paired helical filaments constitute a strong attractant for invasion by astrocytes once they are extracellular.

Structural stability of PHF in the exctracellular space is likely to be a prerequisite for the astrocytic reaction to type B tangles. It has recently been proposed (Glenner 1980; Wisniewski and Iqbal 1980) that PHF constitute one type of amyloid or β-pleated sheet fibrils in the brain. A β-pleated sheet configuration of PHF would explain their resistance to proteolytic digestion (Glenner 1980) and their persistence in the extracellular space after neuronal death.

Acknowledgements. We thank Dr. Lawrence F. Eng, Palo Alto, for kindly providing the GFA protein antiserum. Moreover, we are grateful to Miss Marlis Kasper and Mrs. Ruth Kohler for immunohistochemical, Miss Vera Basler and Miss Mia Nebiker for neurohistologic and photographic work.

References

Alzheimer A (1907a) Über eine eigenartige Erkrankung der Hirnrinde. Allg Z Psychiat 64:146–148

Alzheimer A (1907b) Über eine eigenartige Erkrankung der Hirnrinde. Zentralbl Ges Neurol Psychiat 18:177–179

Baskin DG, Erlandsen SL, Parsons JA (1979a) Immunocytochemistry with osmium-fixed tissue. I. Light-microscopic localization of growth hormone and prolactin with the unlabeled antibody-enzyme method. J Histochem Cytochem 27:867–872

Baskin DG, Erlandsen SL, Parsons JA (1979b) Influence of hydrogen peroxide of alcoholic sodium hydroxide on the immunocytochemical detection of growth hormone and prolactine after osmium fixation. J Histochem Cytochem 27:1290–1292

Eng LF (1980) The glial fibrillary acidic (GFA) protein. In: Bradshow R, Schneider D (eds) Proteins of the nervous system, 2nd ed. Raven Press, New York, pp 85–117

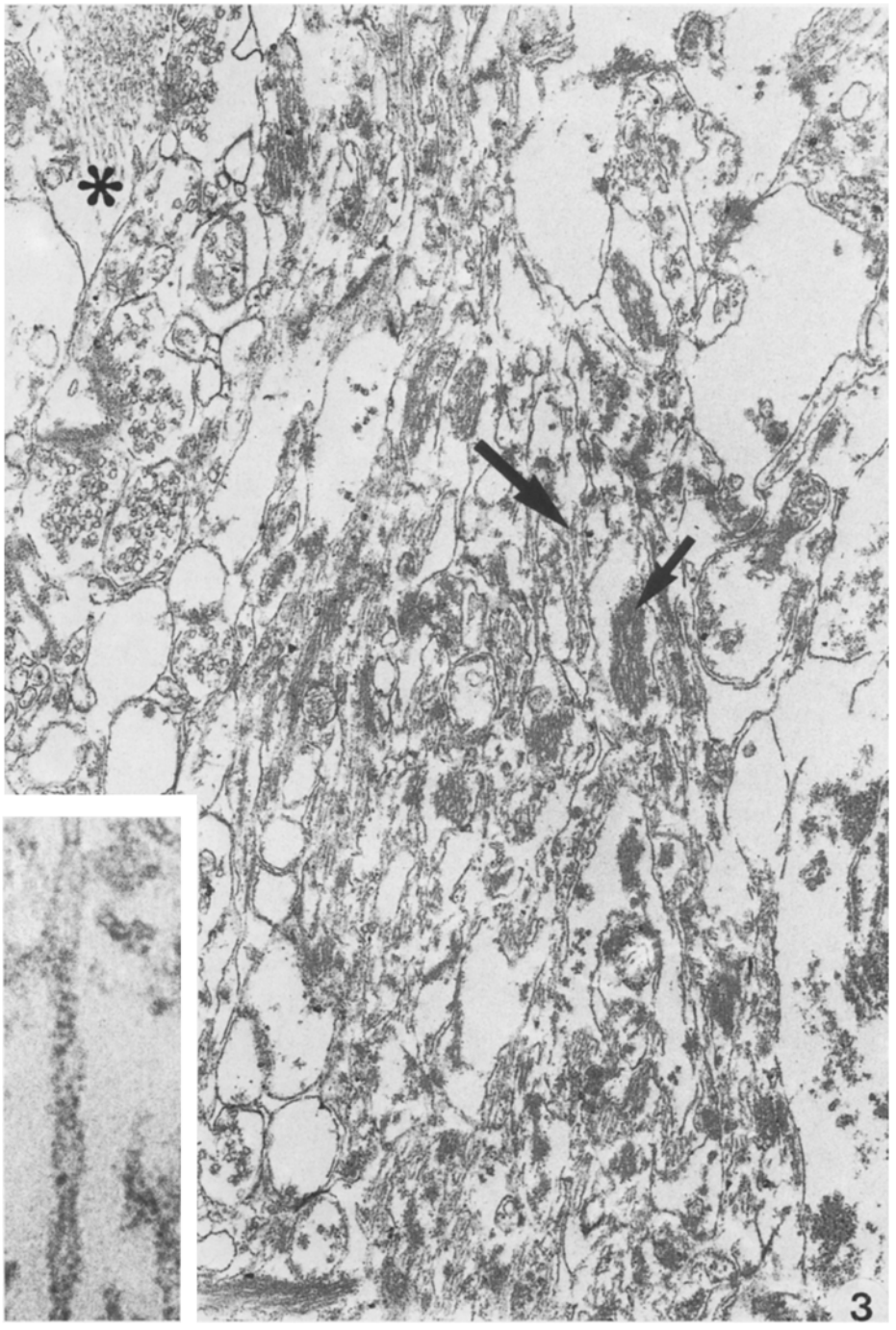

Fig. 3. Electron micrograph of a type B tangle showing cellular processes (*short arrow*) separated by extracellular thick fibrils (*long arrow*) and containing dark bundles of intermediate filaments. A neuronal process (*), probably a dendrite, contains paired helical filaments. Uranyl acetate and lead citrate. × 24,000. *Inset:* High magnification of an extracellular fibril showing periodic variation of its diameter (maximum width 25 nm), × 160,000

Glenner GG (1980) Amyloid deposits and amyloidosis: The β-fibrillosis. New Engl J Med 302:1283–1292, 1333–1343

Grube D (1980) Immunoreactivities of Gastrin (G-) cells. II. Non-specific binding of immunoglobulins to G-cells by ionic interactions. Histochemistry 66:149–167

Kidd (1963) Paired helical filaments in electron microscopy of Alzheimer's disease. Nature 197:192–193

Lane BP, Europa DL (1965) Differntial staining of ultrathin sections of Epon-embedded tissues for light microscopy. J Histochem Cytochem 13:579–582

Puchtler H, Sweat F, Levine M (1962) On the binding of Congo red by amyloid. J Histochem Cytochem 10:355–364

Sternberger LA (1979) Immunocytochemistry, 2nd edn. Wiley, New York Chichester Brisbane Toronto

Terry RD (1963) The fine structure of neurofibrillary tangles in Alzheimer's disease. J Neuropathol Exp Neurol 22:629–642

Wisniewski HM, Iqbal K (1980) Ageing of the brain and dementia. TINS 3:226–228

Received January 25, 1982/Accepted February 2, 1982