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Curly fibers are tau-positive strands in the pre- and post-synaptic neurites, consisting of paired helical filaments: observations by the freeze-etch and replica method

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Summary. The ultrastructure of the curly fibers was examined by the transmission and immunoelectron microscopy as well as by the rapid-freeze, deep-etch and replica method. The curly fibers consisted mainly of paired helical filaments (PHF) in the neuropils, both pre- and post-synaptic. On the deep-etch replicas, PHF in the neurites had similar dimensions to those of Alzheimer's neurofibrillary tangles in the nerve cell somata, having the width of 28 to 36 nm. The two component filaments, 14 to 18 nm in width, were twisted left-handedly with about 70- to 90-nm intervals. There were also cross-bridging fibrils of about 6 nm in diameter connecting the neighboring PHF.

Key words: Curly fibers – Anti-tau antibody – Immunoelectron microscopy – Rapid-freeze and deep-etch method – Neuritic paired helical filaments

Alzheimer's neurofibrillary tangles (NFT) and senile (neuritic) plaques (SP) distributed in the cerebral neocortex are the well-known histopathological, diagnostic features of Alzheimer's disease (AD) and senile dementia of Alzheimer type (SDAT) [18]. Furthermore, it has been reported that curly fibers in the neocortex, the third location of tau-positive protein or paired helical filaments (PHF), are found more widely in the brains of demented patients and not limited in the NFT or SP [7, 8]. The curly fibers were first described as the argyrophilic neuropil threads presenting as PHF by electron microscopy and immunostain with anti-PHF serum [2]. The similar structure had been demonstrated by Golgi preparation [14], by electron microscopy [19] and by immunostaining with anti-PHF antibodies [5]. Kosik et al. [7] later found fine neurites stained with anti-tau antibody in the temporal cortex of the AD brain. Recently, Ihara [4] has immunohistochemically shown that the curly fibers sprout from somata and dendrites of pyramidal cells of the layer III and V of the neocortex affected by AD.

Kosik et al. [7] and Wolozin and Davies [21] have suggested that these degenerating neuropil threads represent more extensive abnormality than SP and NFT do in SDAT. To clarify more precise localization and structure of curly fibers, we studied the SDAT brains by conventional electron microscopy, immunoelectron microscopy and the rapid-freeze, deep-etch and replica method.

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Materials and methods

The right inferior temporal and parahippocampal gyri from ten brains obtained at autopsy were submitted for the study (Table 1). For the immunohistochemical screening for curly fibers, an antibody to phosphorylated tau protein [11] was used as primary antibody. Formalin-fixed, paraffin-embedded specimens were deparaffinized and incubated with the diluted antibody overnight at room temperature, followed by peroxidase-antiperoxidase (PAP) reaction as previously reported [9].

Immunoelectron microscopy was done by preembedding reaction. Formalin-fixed tissues were cut into 40-μm-thick Vibratome sections and stained with the anti-tau antibody-PAP as above, post-fixed with 2% OsO_4 in 0.1 M cacodilate buffer at 4°C, dehydrated and embedded in epoxy resin. Ultrathin sections were observed without contrast staining with H-600 (Hitachi) electron microscope. The portions of the tissues were fixed with 4% paraformaldehyde in 0.1 M cacodilate buffer at 4°C and processed for transmission electron microscopic observations.

For rapid-freezing and deep-etching, the extirpated tissues were treated as previously described in detail [12]. Briefly, they were rinsed with calcium-free saline and fixed with 1% formaldehyde and rapidly frozen by metal contact method using liquid helium in a quick-freezing system (SLAMMER/QF5000, Meiwa Co., Oosaka, Japan). The deep-etching was performed in a freeze-etching device (CFE-40, Cressington Inc., GB) at −100°C and at or below 1×10^{-7} mbar. Platinum-carbon was applied at an angle of 25° followed by carbon coating at the angle of 90° to the rotating specimens. The replicas were observed in a JEM-2000EX transmission electron microscope at an accelerating voltage of 200 kV. Photographs are of reverse images with the platinum deposits appearing white.

Table 1. Presence of curly fibers, neurofibrillary tangles (NFT) and senile plaques (SP) in the ten examined cases

Case no.	Age and sex	Clinical[a] dementia	Pathological diagnosis	Curly fibers[b]		NFT	SP
				IHC	TEM		
1	75 F	++++	AD	+	+++	++	++
2	87 F	++	SDAT	+	+++	++	++
3	100 F	++	SDAT	+	+	++	++
4	90 M	+++	SDAT, Parkinson	+	−	+	++
5	73 M	+++	Parkinson	−	−	−	−
6	82 F	−	Parkinson	+	NE[c]	±	++
7	82 M	+	Infarction	−	−	−	−
8	88 M	−	Infarction	−	−	±	±
9	94 F	+	Pons bleeding	+	−	++	++
10	102 F	−		−	−	+	±

[a] Graded by one of the authors (S. K.)
[b] Semiquantitatively observed curly fibers in the inferior temporal gyrus (IHC: imunohistochemistry) and in the parahippocampal gyrus (TEM: transmission electronmicroscopy), together with NFT and SP in the inferior temporal gyrus stained with modified Bielshowsky method
[c] Not examined

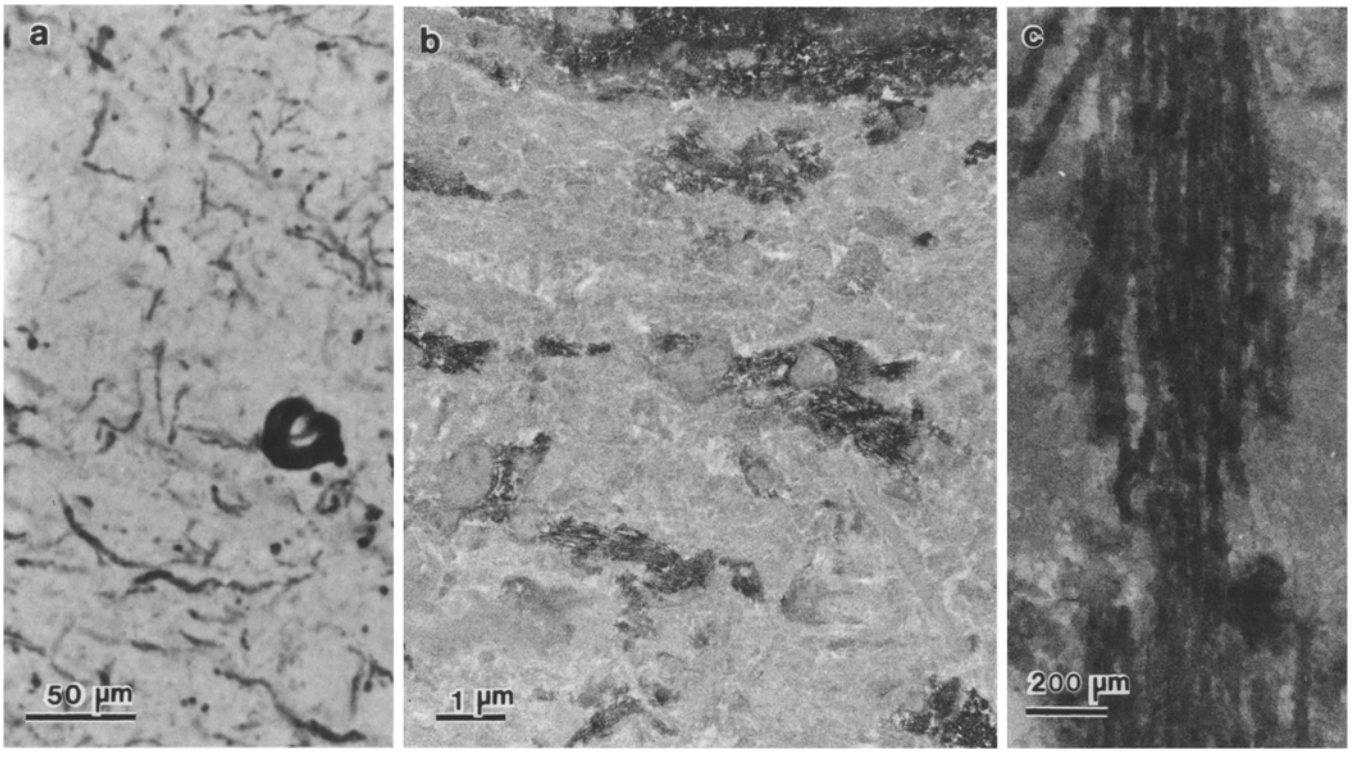

Fig. 1. Immunohistochemistry (**a**) and immunoelectron microscopy (**b** and **c**) of curly fibers stained with anti-tau antibody. Formalin-fixed Vibratome section (**a**) shows numerous curly fibers and a nerve cell with positively stained neurofibrillary tangles (NFT). A low-magnification electron micrograph (**b**) shows an NFT as coarsely positive staining in the cytoplasm (*upper part* of the figure). Patchy and fibrillary stainings below correspond to curly fibers. Gray filamentous structures are proliferated glia fibers. Higher magnification (**c**) revealed intensely peroxidase-antiperoxidase-decorated helical structure of the filaments

Results

Immunohistochemical staining with anti-tau serum was performed in all ten cases and tau-immunoreactive curly fibers as well as NFT were revealed in six of them (Table 1, Fig. 1 a). Immunoelectron microscopy with anti-tau antibody showed coarsely positive materials in the cytoplasm of the pyramidal cells and patchy materials in the neurites corresponding to the intracytoplasmic NFT and intraneuritic curly fibers in the histochemical preparations, respectively (Fig. 1 b, c). Both the post-synaptic and presynaptic neurites and, rarely, myelinated axons contained PHF. In the cortical neuropils, PHF were absent in five non-demented and one demented cases on electron micrographs (Table 1). SP from SDAT patients contained abundant PHF in the degenerated neuritic processes, while those from non-demented patients contained sparse PHF.

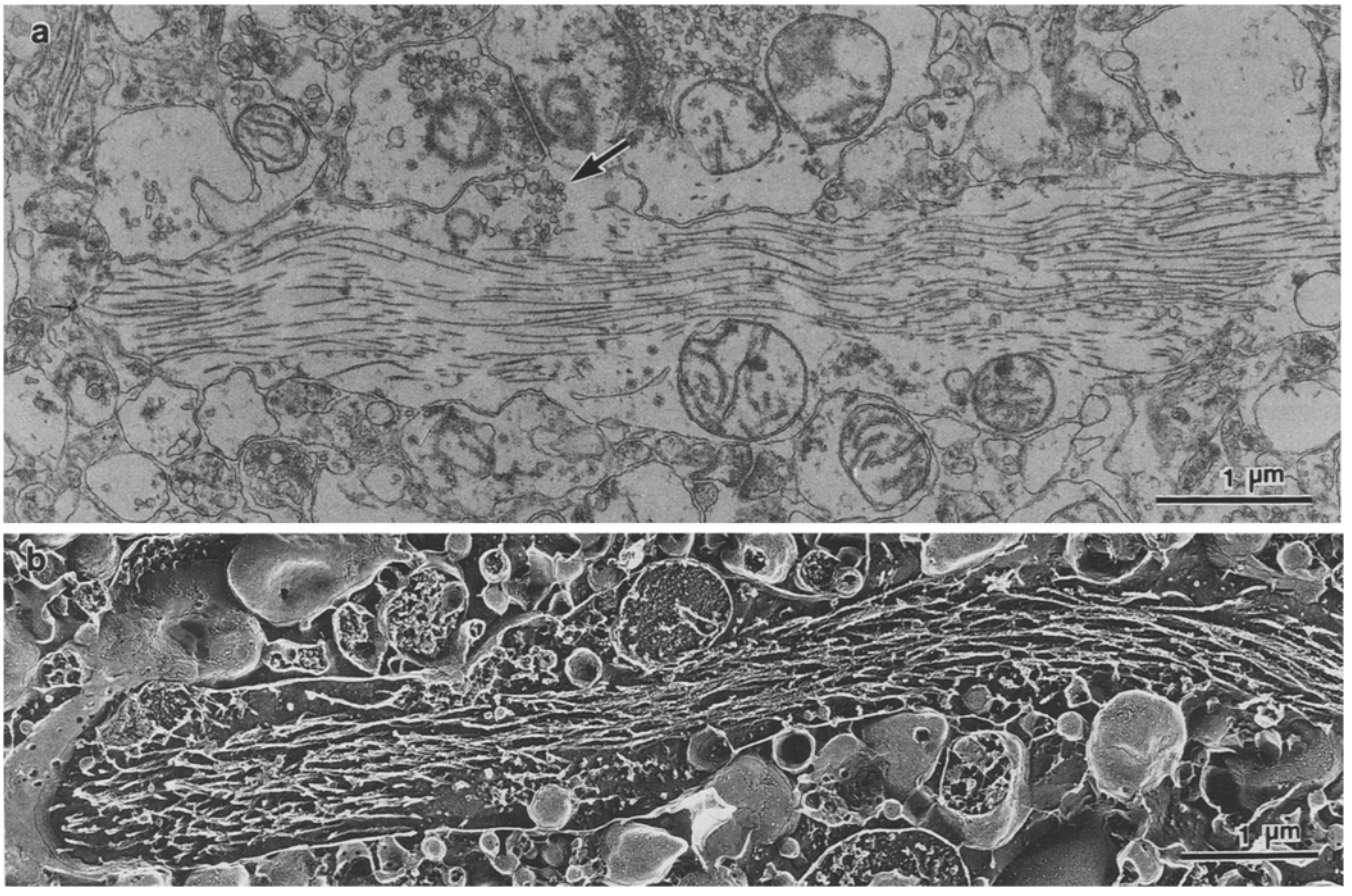

Fig. 2. Low-magnification view of a freeze-etch replica of a longitudinally cut neurite (**b**) containing loosely arranged paired helical filaments (PHF) and some swollen mitochondria. A similar view of transmission electron microscopy (**a**) shows accumulation of synaptic vesicles in the neuropil (*arrow*) indicating that it is presynaptic

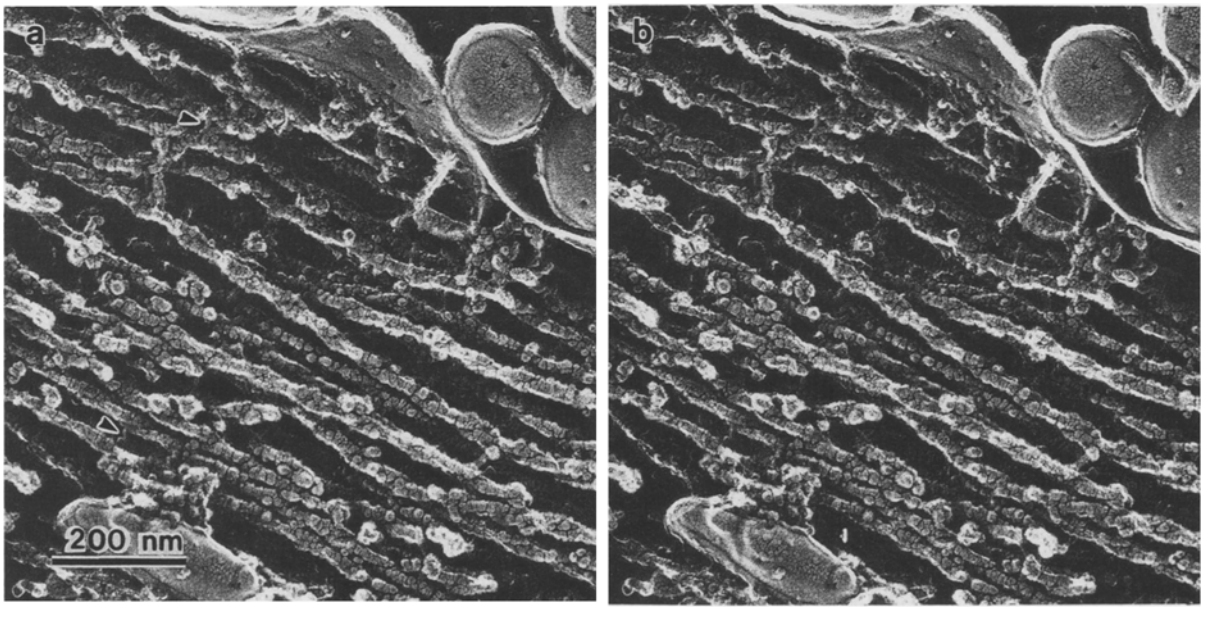

Fig. 3. Stereo-pair photographs, with tilting angles at $\pm 10°$, of intraneuritic PHF showing helical structure and bridging filaments (*arrowheads*)

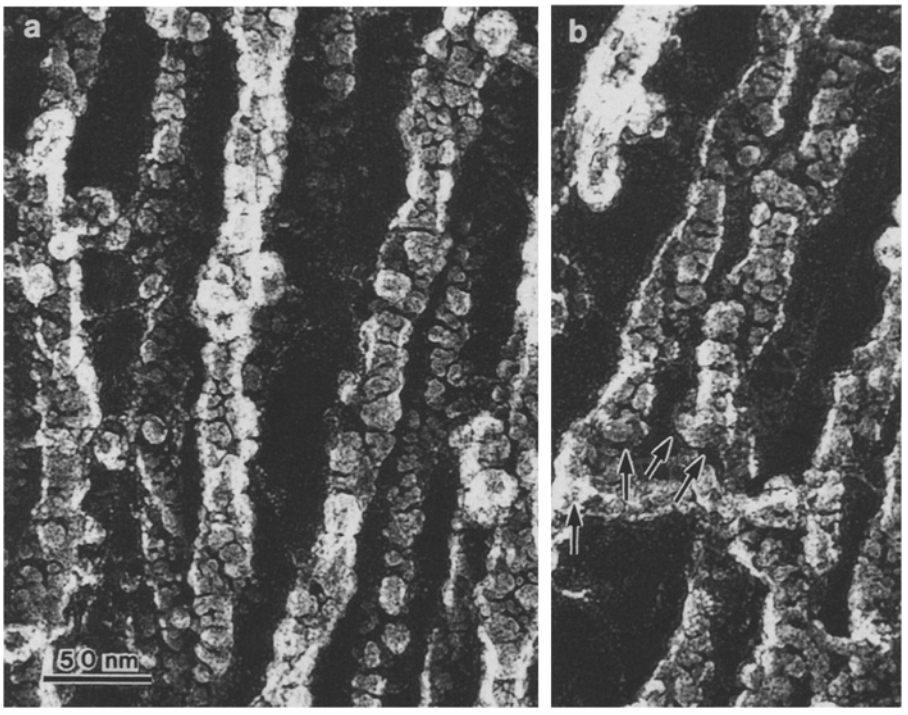

Fig. 4. High magnification of typically wound, left-handed helices of paired filaments which seem to consist of globular or granular subunits. Fractured ends (*paired arrows* in **b**) of PHF are clearly visible

Using the deep-etch method, PHF in neurites were encountered in only one case of SDAT (case 3 in Table 1). A longitudinal view of a swollen neurite revealed loosely arranged parallel fibers which had characteristics similar to those of PHF of NFT in the neuronal cytoplasm (Fig. 2b). In higher magnifications (Figs. 3, 4), they were left-handed helices of 28 to 36 nm in width which consisted of a pair of filaments of 14 to 18 nm in width. The component filaments appeared to be composed of globular or granular subunits (Fig. 4). By stereoscopic observations intervals of a complete twisting were about 90 nm (Fig. 3). Straight type filaments of 15 nm in width were rarely seen in the neurites [12].

Discussion

From our immunohistochemical and immunoelectron microscopic studies, both cytoplasmic NFT and curly fibers were confirmed to be PHF, as they were stained with an anti-tau antibody (Fig. 1). PHF located in the neuritic fibers were clearly shown in both pre- and postsynapses in the present study and in that of Wisniewski et al. [20]. Deep-etch replica studies revealed, as in the previous report [12], that most of PHF showed parallel arrangement with typical helical structure. Better resolution of the replicas than before [12] has been obtained by adopting a new type of filaments and improving the vacuum grade of the freeze-etch device, enabling the demonstration of the granular or globular appearance of each filament of PHF (Fig. 4). Whether this appearance represents the surface view of subunit structure [19] remains to be clarified. The cross-bridging filaments between PHF [12] were also shown (Figs. 3, 4). No electron microscopic evidences for dendritic or neuronal sprouting [4] were observed in our present study.

Our present findings as well as previous ones [12] apparently contradict the twisted tubule model of Terry [17] and, recently, of Miyakawa et al. [10] that the fibrils of NFT consist of hollow tubules with periodical constrictions, and rather support the idea of Kidd [6] and many others [2] that they are helical filaments. Straight fibers [8] or straight tubules [13] have been described, although we rarely observed straight fibers [12].

The neuritic dystrophy with curly fibers and NFT may have a closer relationship to the clinical symptoms of dementia. In the present study, all but one brains of alleged SDAT cases showed PHF in cortical neurites (Table 1). Dickson et al. [3] observed extensive neuritic degeneration in SDAT brains, but not in the brains from non-demented elderly with SP or from non-SDAT dementia cases. According to Probst et al. [15], there were numerous neuropil threads and SP neurites immunoreactive to anti-PHF and anti-tau in the presence of NFT, whereas PHF- and tau-immunoreactive structures were not found in the absence of NFT. More recently Barcikowska et al. [1] demonstrated that the dystrophic neurites of SP contained PHF only when cortical neuron cells in the area formed NFT. Thus, the curly fibers may be one of the most characteristic pathological changes in AD/SDAT brains, especially in the cerebral cortex as Selkoe [16] suggested.

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