Paired Twisted Filaments in Human Pinealocytes

Akio Hasegawa[1,2], Hiroyuki Shimada[3], Naotaka Izumiyama[2], and Kohichiro Ohtsubo[2]

Paired twisted filaments (PTF) have been confirmed and described ultrastructurally in the normal human pineal gland for the first time. The PTF showed a peculiar double helical structure, measuring 12 to 25 nm in maximal helix width with a half-periodicity of 30 to 35 nm (periodicity of the constrictions). Each filament was about 10 nm wide. The PTF formed parallel aggregates in the perikaryotic cytoplasm of the pinealocytes. In 8 of 12 autopsied middle-aged to elderly individuals, the PTF were present in a small proportion of pinealocytes. The identity of the PTF remains unclear in terms of their apparent similarity to the paired helical filaments observed previously in aged rats. However, the age distribution of individuals with PTF suggests that the intermediate filaments of human pinealocytes are more susceptible to early deterioration during aging than those of other cerebral neurons. Acta Pathol Jpn 41 : 265-269, 1991.

Key words: Pinealocytes, Ultrastructure, Paired twisted filaments, Human, Aging

INTRODUCTION

The ultrastructure of the pineal gland in aged humans has recently been investigated in our laboratories. Our studies have demonstrated many synaptic ribbons and ribbon fields, microtubular sheaves and other organelles in the pineal parenchymal cells (1) similar to those described in the pineals of other vertebrates (2). Subsequent examinations also revealed paired twisted filaments (PTF), resembling Alzheimer's paired helical filaments (PHF), in pinealocytes of aged individuals. These PTF were first reported in human pinealomas by Hassoun *et al.* (3) and similar structures have been described only exceptionally in spinal ganglia (4) and cerebral neurons (5) of aged rats.

Until now there has been a remarkable paucity of data on PTF in human pinealocytes. In this paper, we describe the ultrastructural features and distribution patterns of PTF in human pinealocytes in normal pineals obtained from middle-aged to elderly humans at autopsy, and briefly discuss the implications of these findings.

MATERIALS AND METHODS

Cases

Twelve pineal glands were obtained at autopsy from 8 aged (aged 68-100 yr) and 4 middle-aged (aged 36-52 yr) individuals. The specimens were taken within 5 h of death (mean 2.5 h) at three Departments of Pathology, those of Tokyo Metropolitan Geriatric Hospital, Tokyo University School of Medicine, and Odawara Municipal Hospital. Table 1 shows the case profiles including the final patho-anatomical diagnoses.

Transmission electron microscopy

After removal from the brain at autopsy as soon as possible, each pineal gland was immediately fixed by immersion in ice-cold 2.0% paraformaldehyde-2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.3). About 15 min later, the gland was weighed on a direct reading balance (Mettler H 20), and its external diameters were measured. Then the gland was divided longitudinally into two halves with a clean blade, one half for light microscopy (hematoxylin and eosin, Watanabe's silver impregnation, Azan-Mallory and Congo red) and the other half for transmission electron microscopy.

Received September 27, 1990.
Accepted for publication December 3, 1990.
[1]Department of Pathology and Laboratory Medicine, Odawara Municipal Hospital, Odawara. [2]Department of Clinical Pathology, Tokyo Metropolitan Institute of Gerontology, Tokyo. [3]Division of Pathology, Tokyo Metropolitan Geriatric Hospital, Tokyo.
Mailing address: Akio Hasegawa, M.D., Ph.D. (長谷川章雄), Department of Pathology and Laboratory Medicine, Odawara Municipal Hospital, 46 Kuno, Odawara 250, Japan.
Part of this study was presented at the 78th Congress of the Japanese Society of Pathology, Kyoto, Japan, on March 30, 1989.

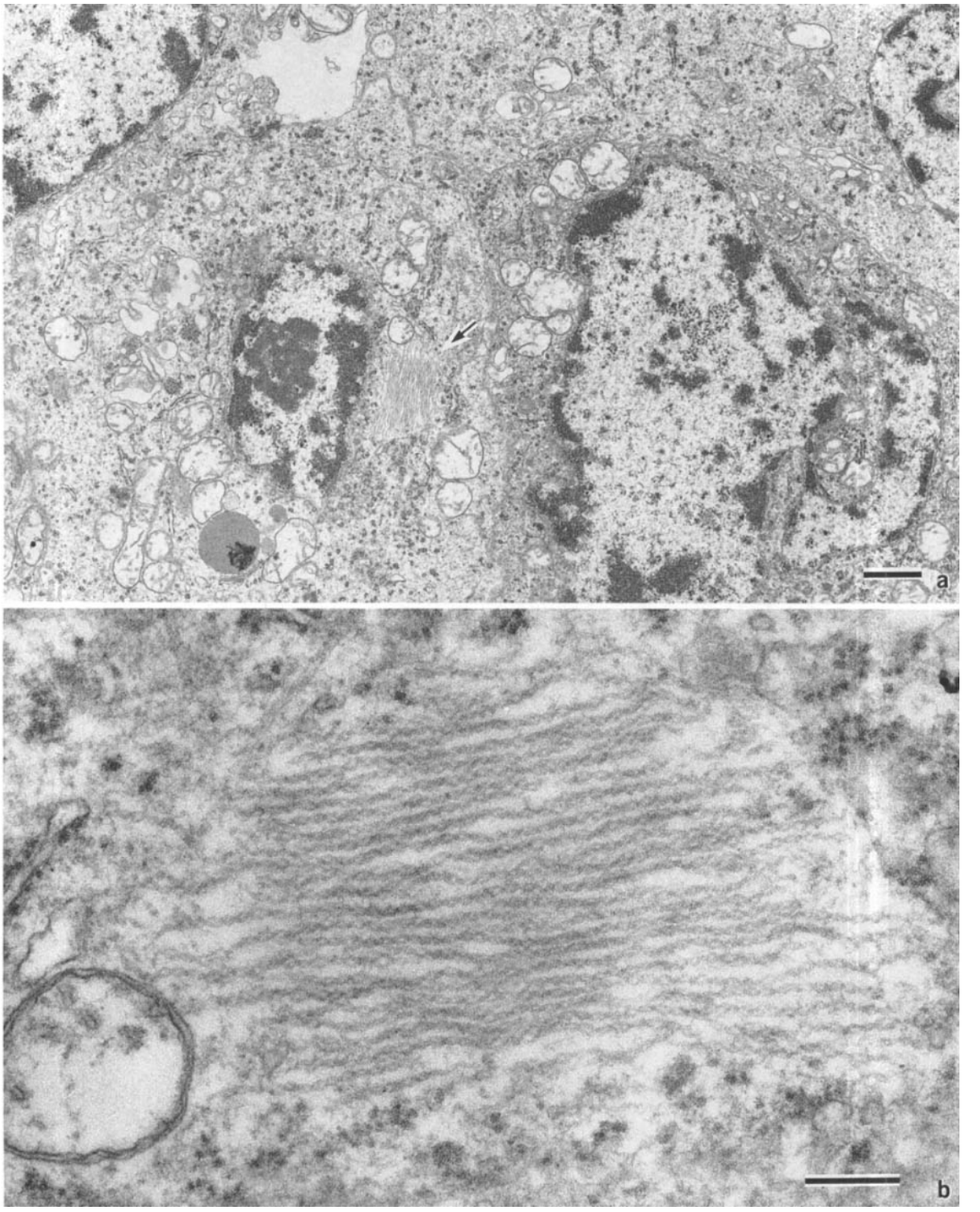

Figure 1. **a**: Paired twisted filaments (PTF, arrow) in a pineal parenchymal cell (×9,300) from a 73-year-old male. Bar=1 μm. **b**: Enlarged longitudinal section of the PTF, illustrating double helical structures (×74,000). Bar=200 nm.

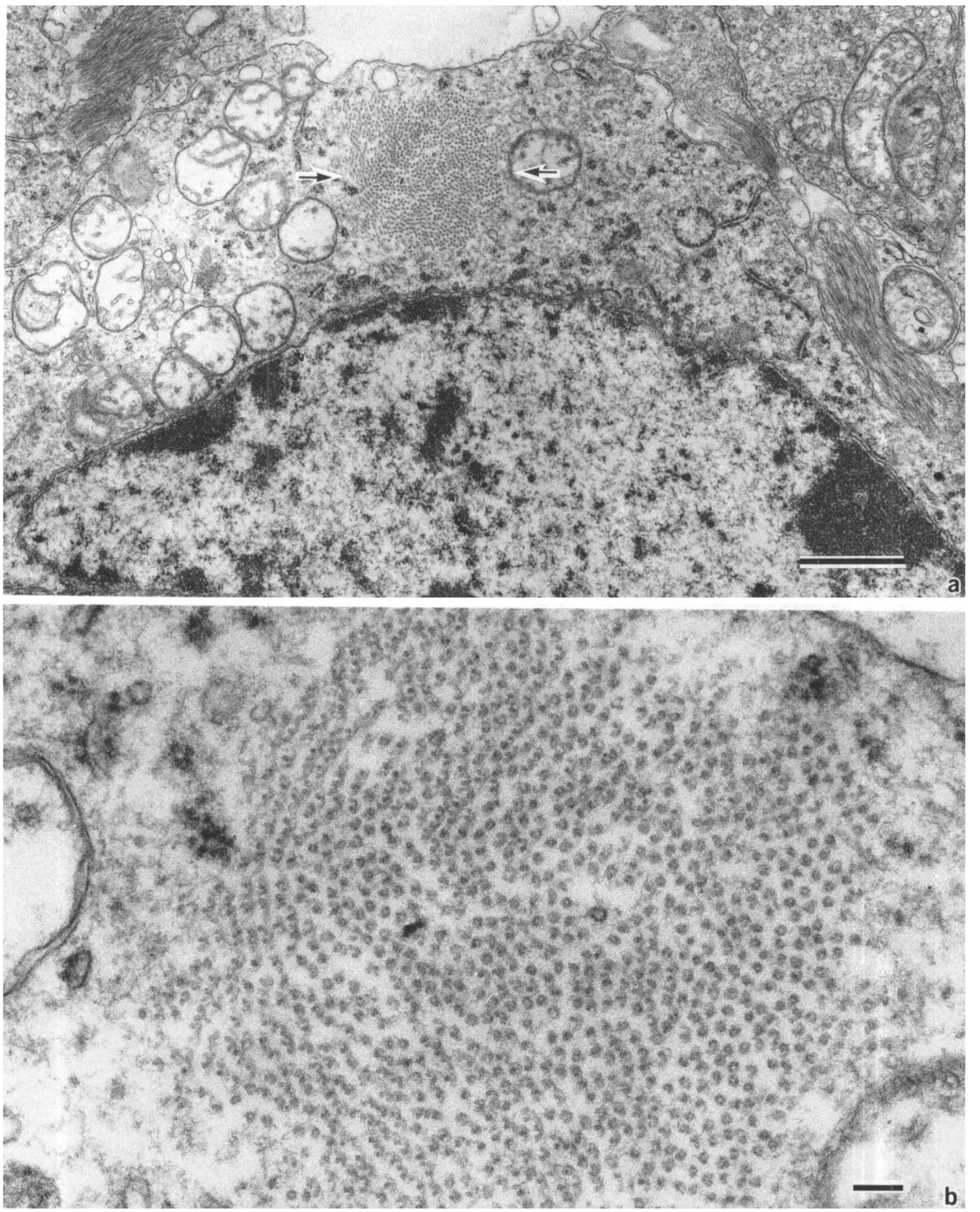

Figure 2. **a**: PTF (arrows) in a pineal parenchymal cell (×17,500) from a 73-year-old male. Bar=1 μm. **b**: Enlarged cross-sectional view of the PTF (×79,400). Bar=100 nm.

After being minced into 1×1×1-mm cubes and placed in a container filled with the above fixative, the tissue was washed with 0.1 M Na-cacodylate-buffer (pH 7.3), post-fixed in 2.0% osmium tetroxide for 1.5 h and embedded in a single Poly/Bed 812 block. Each block was finally trimmed down to a selected area corresponding to one light microscopic field of view for a ×20 objective lens. Ultrathin sections, cut at a thickness of 50-70 nm and stained with uranyl acetate and lead citrate, were photographed with a H-7000 (Hitachi Ltd.) and/or JEM 1200EX transmission electron microscope (JEOL Ltd.) operated at 80 kV.

RESULTS

Figures 1 and 2 show the ultrastructure of the paired twisted filaments (PTF) of human pinealocytes. They were sparsely distributed in ultrathin sections and aggregated focally within the perikarya of the pineal parenchymal cells, arranged compactly in parallel bundles and covering an area up to about 3 μm in diameter. They did not displace the normal organelles to any noticeable extent. When observed parallel to the plane of section, each pair appeared as a double helical structure, measuring 12-25 nm in greatest helix width with a longitudinal periodicity of 30-35 nm (half period of the helix, Fig. 1b). Each filament was about 10 nm wide. Cross-sections of the PTF showed C-shaped, U-shaped, or O-shaped forms (two curved segments) (Fig. 2b). The morphological appearance of the PTF in the human pinealocytes was basically the same as that reported by Hassoun in human pinealomas (3) and different from that of Alzheimer's paired helical filaments (6-8). They appeared relatively resistant to autolysis. No spatial associations between the PTF and other organelles such as Golgi apparatus, endoplasmic reticulum, synaptic ribbons or microtubular sheaves were seen. The PTF could not be found in the cell processes of pinealocytes, nor in the fibrous astrocytes (interstitial cells) located between them.

Table 1. Characteristics of Patients at Autopsy and Presence of PTF Revealed by Electron Microscopy

Patient No.	Age (yr)	Sex	Post Mortem (h)	Pathological Diagnosis	PTF
1	100	F	3.0	Pneumonia	+
2	83	F	0.5	Pneumonia	−
3	80	F	1.0	Cancer of pancreas	+
4	76	M	1.0	Seminoma	−
5	73	M	1.5	Hepatoma	+
6	72	M	0.5	Cancer of colon	−
7	71	F	2.0	Cancer of colon	+
8	68	F	1.5	Myocardial infarction	+
9	52	M	5.0	Hepatoma	−
10	52	F	5.0	Cancer of breast	+

Table 1 refers to the distribution of the PTF in the 12 pineals analyzed: they were distinctly present in 8 (aged 36-100 yr; 5 aged and 3 middle-aged) out of the 12 individuals, without any significant age distribution. Even in these cases, the PTF were apparently sparcely distributed, each ultrathin section yielding at most several pinealocytes possessing a typical PTF focus. Almost all of the involved cells had only a single compact bundle of PTF within the cytoplasm.

DISCUSSION

The present study has demonstrated ultrastructurally that PTF are present in pinealocytes of middle-aged to elderly subjects. In our previous ultrastructural study of pineal glands from aged humans (1), we noticed characteristic but sparce filamentous inclusions composed of double helices in the pinealocytes (unreported). At the light microscopy level, no cytoplasmic inclusion-like bodies corresponding to these were evident (9). To elucidate the nature of the PTF, we re-examined ultrathin sections of normal human pineals including those from middle-aged individuals in the light of these new findings. To our knowledge this is the first report confirming the presense of PTF in the normal human pineal gland.

The morphological appearance of the PTF was identical to those reported in the literature (3), although in our study the maximal width of the helix and the periodicity were rather greater. This difference is probably due to the post-mortem interval and artefacts produced by tissue processing. In the present series the PTF were distributed over a wide age range (36-100 yr) and the configuration did not vary from case to case. Moreover, it was indicated that the PTF were located exclusively inside the pinealocytes, and not in the glial component. It is difficult at the present time to speculate on the nature of these structures; they may arise through degeneration of neurofilaments (intermediate filaments) or microtubules of pinealocytes during regressive changes after puberty, or may be physiologically func-

filaments with a half-periodicity of 26.63 nm are possibly specific to pinealocytes. In spite of comprehensive investigations of the pineal gland, the PTF have never been described in other vertebrates (2). In this context, the PTF in human pinealocytes cannot be regarded as merely a rudimentary evolutionary remnant of pineal structures in lower vertebrates. In neurons from patients with senile dementia of the Alzheimer type, and also from normal aged patients, paired helical filaments (PHF) show an average constriction periodicity of 65 nm (8) to 80 nm (6, 10). The ultrastructure of the PTF in human pinealocytes is similar to these PHF, but differs in certain aspects (3). However, cytoplasmic inclusions very similar to the PTF have been reported; the filaments in the dendritic processes of neurons of the cerebral cortex in a 27-month-old Wistar-Kyoto rat were 10 nm in diameter with a half periodicity of about 34 nm (5), and those in the spinal ganglion neurons of elderly rats aged between 24 and 32 months were 8-9 nm in diameter with a half periodicity of 40 nm (periodicity of the constrictions) (4). It was noteworthy that these tangles were observed only in the oldest rats. Although the possibility still exists that the PTF are a specific marker of human pinealocytes and tumors arising from pineal parenchymal cells, it can also be postulated that they represent a normal aging phenomenon of neuronal cells, which in the human pineal occurs from middle age in only a small proportion of pinealocytes. No detailed chemical analysis of the PTF has yet been done, but they seem to originate from intermediate filaments (neurofilaments) about 10 nm in diameter which form a three-dimensional structural lattice in neuronal cells (11). It would be interesting to determine whether the PTF react with anti-tau (τ) (12, 13) or anti-neurofilament antibodies (14). It is also possible that the PTF may correspond to one of the pineal-associated proteins or enzymatic activities (15) already detected biochemically.

Considering their sparce distribution in ultrathin sections of pinealocytes, the PTF might not be of significant value in routine laboratory diagnositics including pineal pathology. However, they may prove to be a focus of renewed scientific attention in relation to the role of the human pineal in the aging of the neuroendocrine system.

Acknowledgements: The authors thank Mr. Y. Suganuma and Mr. T. Iwasaki of the Pathology Department at Odawara Municipal Hospital for their cooperation with photographic services. The present research was supported in part by a Grant from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Hasegawa A, Ohtsubo K, Izumiyama N, and Shimada H. Ultrastructural study of the human pineal gland in aged patients including a centenarian. Acta Pathol Jpn 40: 30-40, 1990.
2. Vollrath L. The pineal organ. Springer-Verlag, Berlin, 1981.
3. Hassoun J, Devictor B, Gambarelli D, Peragut JC, and Toga M. Paired twisted filaments: A new ultrastructural marker of human pinealomas? Acta Neuropathol (Berl) 65: 163-165, 1984.
4. van den Bosch de Aguilar Ph and Goemaere-Vanneste J. Paired helical filaments in spinal ganglion neurons of elderly rats. Virchows Arch [Cell Pathol] 47: 217-222, 1984.
5. Knox CA, Yates RD, and Chen I-I. Brain aging in normotensive and hypertensive strains of rats. II. Ultrastructural changes in neurons and glia. Acta Neuropathol (Berl) 52: 7-15, 1980.
6. Kidd M. Paired helical filaments in electron microscopy of Alzheimer's disease. Nature 197: 192-193, 1963.
7. Terry RD. The fine structure of neurofibrillary tangles in Alzheimer's disease. J Neuropathol Exp Neurol 22: 629-642, 1963.
8. Wiśniewski HM, Narang HK, and Terry RD. Neurofibrillary tangles of paired helical filaments. J Neurol Sci 27: 173-181, 1976.
9. Hasegawa A, Ohtsubo K, and Mori W. Pineal gland in old age: Quantitative and qualitative morphological study of 168 human autopsy cases. Brain Res 409: 343-349, 1987.
10. Constantinides P. Ultrastructural pathobiology. Elsevier, Amsterdam, 1984: 325-326.
11. Lazarides E. Intermediate filaments as mechanical integrators of cellular space. Nature 283: 249-256, 1980.
12. Ihara Y, Abraham C, and Selkoe DJ. Antibodies to paired helical filaments in Alzheimer's disease do not recognize normal brain proteins. Nature 304: 727-730, 1983.
13. Kosik KS, Joachim CL, and Selkoe DJ. Microtubule-associated protein tau (τ) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci USA 83: 4044-4048, 1986.
14. Anderton BH, Breinburg D, Downes MJ, *et al.* Monoclonal antibodies show that neurofibrillary tangles and neurofilaments share antigenic determinants. Nature 298: 84-86, 1982.
15. Vaughan MK. Pineal peptides: An overview. *In* Reiter RJ, ed. The pineal gland (Comprehensive endocrinology), Raven, New York, 1984: 39-82.