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Reappraisal of neurofibrillary tangles*

Immunohistochemical, ultrastructural, and immunoelectron microscopical studies

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Summary. We have studied the immunohistochemical reactivity and ultrastructure of both neurofibrillary tangles (NFTs) occurring with severe neurofibrillary diseases, and Pick bodies (PBs) associated with Pick's disease. The NFTs and PBs did not react immunohistochemically with the anti-nonphosphorylated neurofilament monoclonal antibody irrespective of whether they were pretreated with alkaline phosphatase. In granular neurons of the dentate fascia of Ammon's horn in cases of dementia of the Alzheimer type (DAT), NFTs either resembled PB-like inclusion bodies (Horoupian's inclusion bodies) in form, or had a perinuclear structure. Immunohistochemically and ultrastructurally, the NFTs in the dentate fascia in cases of DAT, including Horoupian's inclusion bodies, were similar to the NFTs in the pyramidal neurons of Ammon's horn, which are found most frequently in association with severe neurofibrillary diseases. Under a light microscope, Horoupian's inclusion bodies and PBs could not be differentiated and appeared to be argyrophilic round cytoplasmic inclusions in granular neurons of the dentate fascia. There were, however, ultrastructural differences. Horoupian's inclusion bodies consisted of bundles made up of straight tubules (STs), each about 15 nm in diameter. These bundles were intermixed with a few paired helical filaments which occurred at intervals of about 80 nm. On the other hand, PBs were composed of randomly distributed 15-nm-wide STs, intermixed with a very few fibrillary structures. These fibrils had a periodicity of about 160 nm, and ranged in width from about 15 nm to 30 nm. Horoupian's inclusion bodies associated with DAT and PBs associated with Pick's disease are different in this neuropathological aspect. The NFTs, including Horoupian's inclusion bodies in the dentate fascia in cases of DAT, are considered to be a manifestation of neurofibrillary degeneration.

Key words: Neurofibrillary tangles – Alzheimer's disease – Pick bodies – Immunohistochemistry – Ultrastructure

The neurofibrillary tangle (NFT) is a neuropathological feature observed most frequently in patients with dementia of the Alzheimer type (DAT) or with a severe neurofibrillary disease, such as in Down's syndrome (DS) when age is above 31 years [22], progressive supranuclear palsy (PSP), and amyloid angiopathy (AA). In man, neurofibrillary degeneration most commonly occurs in the pyramidal neurons of the cerebral cortex and hippocampus, as well as in the neurons of the basal forebrain, hypothalamus and certain nuclei of the brain stem [5, 8]. Neurons of the lateral geniculate nucleus, Purkinje neurons, and many of the small neurons including the granular neurons of the dentate fascia, do not generally undergo neurofibrillary changes [5]. With Pick's disease, it is common for many of the neurons in the dentate fascia to become highly altered and, characteristically, to develop Pick bodies (PBs) [2, 3, 5]. Similarly, Dickson et al. [5] have reported the existence of argyrophilic round inclusion bodies (Horoupian's inclusion bodies, HIB), that resemble PBs, in the cytoplasm of granular neurons of the dentate fascia affected by DAT. We carried out immunohistochemical, ultrastructural and immunoelectron microscopical studies on NFTs, HIBs, PBs and Pick cells occurring in conjunction with several neurofibrillary diseases, and endeavored to more accurately describe these neuropathological features.

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Table 1. Immunohistochemical evaluation

	Anti-npNF Ab subunit				Anti-PHF Ab	Bielschowsky stain
	68	145	160	200		
	(kDa)					
NFT in pyramidal neurons of Ammon's horn	−	−	−	−	+	+
	AP−	AP−	AP−	AP−	AP+	
Argyrophilic inclusion body in granular neurons of Ammon's horn in patients with DAT	−	−	−	−	+	+
	AP−	AP−	AP−	AP−	AP+	
NFT in the locus ceruleus affected by PSP	−	−	−	−	+	+
	AP−	AP−	AP−	AP−	AP+	
PB in granular and pyramidal neurons of Ammon's horn	−	−	−	−	+	+
	AP−	AP−	AP−	AP−	AP+	
PB in small neurons of the cingulate gyrus	−	−	−	−	+	−
	AP−	AP−	AP−	AP−	AP+	
Pick cell in the cingulate gyrus	+	+	+	+	−	−
	AP+	AP+	AP+	AP+	AP−	
Cytoplasm of normal neurons	+	+	+	+	−	−
	AP+	AP+	AP+	AP+	AP−	

Anti-npNF Ab, anti-nonphosphorylated neurofilament monoclonal antibody; Anti-PHF Ab, anti-paired helical filament polyclonal antibody; AP, alkaline phosphatase; DAT, dementia of the Alzheimer type; NFT, neurofibrillary tangle; PB, Pick body; PSP, progressive supranuclear palsy

−, negative; +, positive; AP−, negative after pretreatment with AP; AP+, positive after pretreatment with AP

Materials and methods

NFTs and argyrophilic inclusions were examined in seven patients with DAT, two patients with DS (33 and 35 years old), one patient with PSP and two patients with AA, and PBs and Pick cells in five patients with Pick's disease.

Immunohistochemical examination

Specimens were fixed in either 70% ethanol or 2% paraformaldehyde supplemented with 0.1 M cacodylate buffer (pH 7.3). Next they were embedded in paraffin and cut into sections 5 μm thick, and then studied by light microscopy.

Sections to be examined under a light microscope were stained with hematoxylin and eosin, Klüver-Barrera, Bodian, and Bielschowsky stains. For immunohistochemical studies, the primary antibodies used were: 200 kDa, 145 kDa and 68 kDa subunits (Oncogene Science, Mineola, NY; derived from human tissue); and 160 kDa subunit (Biomarkor, Rehovot, Israel; derived from porcine tissue) of anti-nonphosphorylated neurofilament (NF) monoclonal antibodies (anti-npNF Abs), and anti-paired helical filament (PHF) polyclonal antibody (anti-PHF Ab) (ICN, Lisle, Ill; derived from human tissue). The ABC system (Vectastain ABC kits, Vector, Calif) was used for staining in combination with the antibodies. Reactivity of the antibodies was studied in specimens, both with and without pretreatment with alkaline phosphatase (AP), according to the method of Dickson et al. [6] using AP (*E. coli*, type III, Sigma, St. Louis, Mo). Six concentrations of AP were used (10, 20, 50, 100, 250 and 500 IU/ml), and specimens were treated for 24 h at 37°C.

Ultrastructural examination

Specimens were taken from the hippocampi of subjects with DAT, Pick's disease, DS and AA; the cingulate gyrus in those with Pick's disease; and the locus ceruleus in the case of PSP. These specimens were fixed in formalin. After rinsing, tissue samples were re-fixed in 5% buffered glutaraldehyde for 1 h at 4°C, washed, and then post-fixed in 1% buffered osmium tetraoxide for 2 h at room temperature, processed by conventional methods and embedded in epoxy resin (Epok 812). Sections were cut at the thickness of 1 μm, stained with toluidine blue, and examined under a light microscope. Selected areas were identified, trimmed and ultrathin-sectioned with a diamond knife in a Sorvall MT2-B ultramicrotome. The ultrathin sections were contrast-stained with uranyl acetate and lead citrate, and then examined under a Hitachi H-300 electron microscope.

Electron microscopic immunohistochemical examination

Formalin-fixed specimens from the hippocampi in cases of DAT were subjected to electron microscopic immunohistochemical analysis, using the anti-PHF Ab. A modification of the technique of Lassmann et al. was employed [12]. The tissues of the hippocampus were sectioned at 30 μm with a Lancer PL-1000 vibratome. After incubation with normal goat serum for 48 h at 4°C, the sections were allowed to react for 72 h at 4°C with the anti-PHF Ab diluted to 1:200. Next, the sections were incubated with biotinylated goat anti-rabbit IgG immunoglobulin for 72 h at 4°C, and then with avidin-biotin complex for 1 h at 37°C. Subsequent preincubation in darkness with 0.05% 3,3′-diaminobenzidine in 0.05 M Tris-HCl buffer (pH 7.6) for 30 min, was followed by a 30-min incubation in 0.05% 3,3′-diaminobenzidine with 0.03% H_2O_2 in darkness at room temperature. Samples were post-fixed in 1% buffered osmium tetraoxide for 24 h at room temperature, washed, stained en bloc with 0.5% uranyl acetate for 1 h at room temperature in darkness, and then dehydrated in ethanol prior to being embedded in epoxy resin.

Results

The immunohistochemical behavior of NFTs after treatment with the anti-npNF Abs and anti-PHF Ab

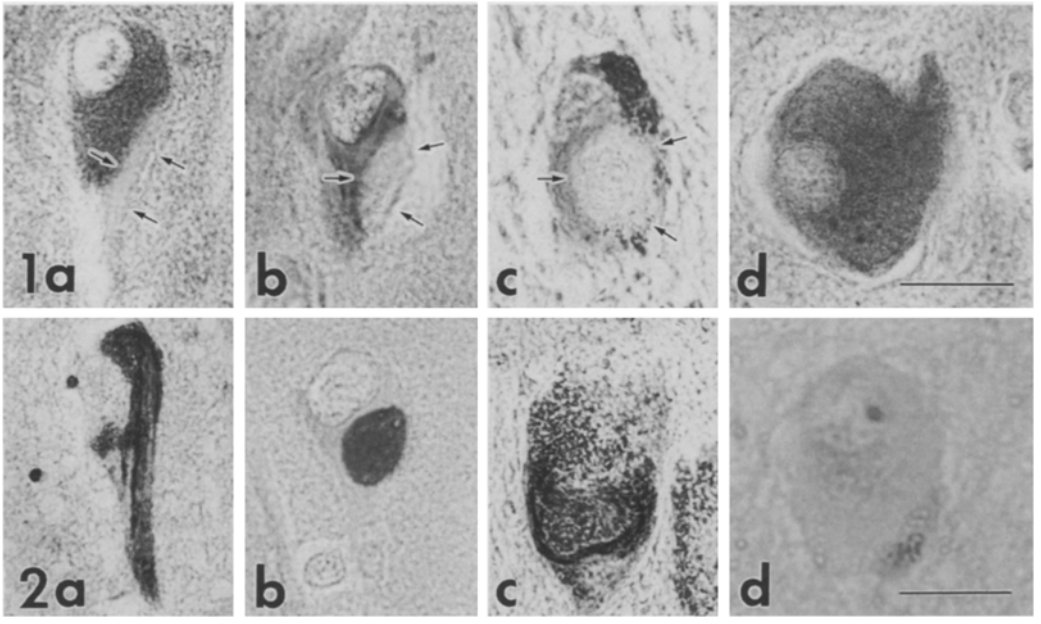


Fig. 1a–d. Immunostaining of anti-200 kDa subunit of nonphosphorylated neurofilament monoclonal antibody (anti-npNF Ab). **a** Anti-npNF Ab recognizes the cytoplasm, but not the neurofibrillary tangles (NFTs) of a pyramidal neuron of Ammon's horn with dementia of the Alzheimer type (DAT) (*arrows*). **b** Pick body (PB) is recognized as negative halo in a pyramidal neuron of Ammon's horn with Pick's disease (*arrows*). **c** Here, NFTs in a neuron of the locus ceruleus with progressive supranuclear palsy (PSP) also do not react with anti-npNF Ab (*arrows*). **d** Anti-npNF Ab diffusely stains the cytoplasm of a Pick cell in the cingulate gyrus in Pick's disease. Bar = 20 μm

Fig. 2a–d. Immunostaining using anti-paired helical filament polyclonal antibody (anti-PHF Ab). **a** Anti-PHF Ab recognizes NFTs with DAT. **b** PB is also recognized by anti-PHF Ab. **c** NFTs associated with PSP react with anti-PHF Ab. **d** Anti-PHF Ab does not stain a Pick cell associated with Pick's disease. Bar = 20 μm

is summarized in Table 1. NFTs in pyramidal neurons affected by severe neurofibrillary disease, argyrophilic inclusions (HIBs) in granular neurons of Ammon's horn from patients with DAT, NFTs in the locus ceruleus from a patient with PSP, and PBs associated with Pick's disease did not react with any of the subunits of anti-npNF Abs (68 kDa, 145 kDa, 160 kDa and 200 kDa); Pick cells, on the other hand, did (Fig. 1a–d). Cytoplasm and dendrites of normal neurons reacted with each subunit of the anti-npNF Abs, but axon was not stained. In the case of anti-PHF Ab, a positive reaction was induced in NFTs and PBs, but not in Pick cells (Fig. 2a–d). The reactivities of NFTs and PBs against anti-npNF Abs and anti-PHF Ab did not change after pretreatment of the samples with AP varying in concentration from 10 to 500 IU/ml.

In pyramidal neurons of Ammon's horn in subjects with severe neurofibrillary diseases such as DAT, DS and AA, the NFTs were mainly recognized as flame-like configurations in the cytoplasm, as picked out by Bielschowsky stain and anti-PHF immunostain (Fig. 3a). Ultrastructurally, some NFTs consisted of bundles of PHFs constricted at about 80 nm intervals, but most NFTs were composed of bundles made up of straight tubules (STs) about 15 nm in diameter; in many cases these bundles were intermixed with a few PHFs (Fig. 3b). HIBs in granular neurons of the dentate fascia of Ammon's horn were stained with Bielschowsky stain and anti-PHF immunostain in sections from all seven patients with DAT. Some of these NFTs (HIBs) had forms resembling PB-like cytoplasmic inclusion bodies (Fig. 4a, c) as has previously been reported by Dickson et al. [5], but others had perinuclear forms, which did not develop into inclusion bodies (Fig. 4b, d). When NFTs observed as inclusions by light microscopy, were seen under an electron microscope, they were recognized as globular structures well demarcated from other cytoplasmic structures, but with no limiting membranes. Components of the inclusions included fibrillary structures, a few mitochondria, and osmiophilic granular and vesicular structures (Fig. 5a). The fibrillary structures occurred as bundles comprised of STs with a 15-nm approximate diameter, and sometimes had intermixtures of PHFs with constrictions spaced about 80 nm apart (Fig. 5c). Perinuclear NFTs (seen to surround the nucleus when observed by light microscopy) also had ultrastructures consisting of bundles made up of about 15-nm-wide STs, interspersed with a few PHFs constricted at about

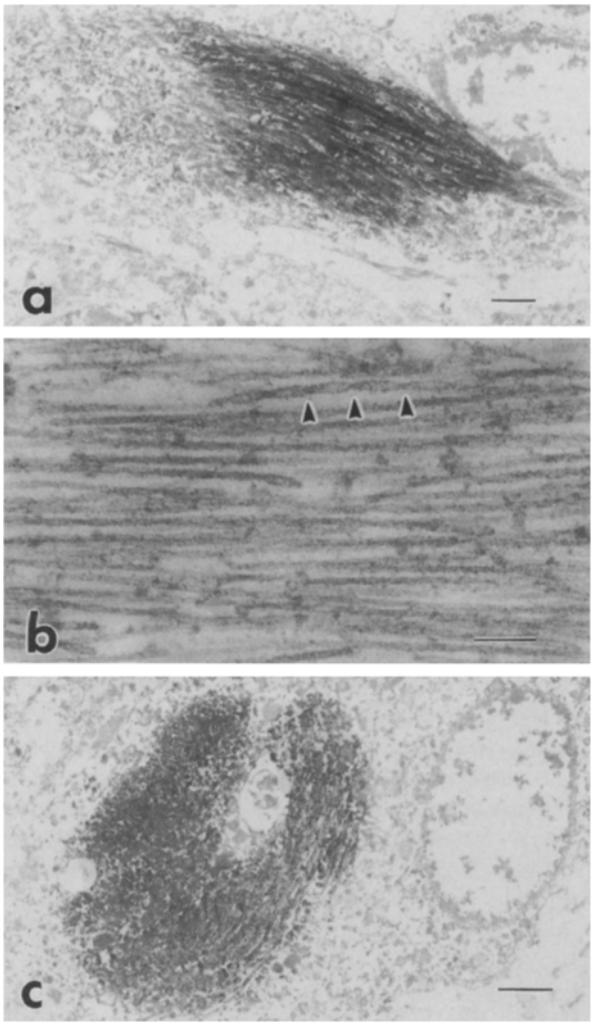


Fig. 3. a Demonstration by immunoelectron microscopy (using anti-PHF Ab) of NFTs in a pyramidal neuron of Ammon's horn with DAT. NFTs immunostained with anti-PHF Ab are arranged in bundles. **b** Ultrastructure of NFTs in a pyramidal neuron of Ammon's horn with DAT is revealed here. Most NFTs consist of bundles of straight, approximately 15-nm-wide tubules (STs), intermingled with a few PHFs with a periodicity of about 80 nm (*arrowheads*). **c** In this section, immunoelectron microscopy was used to identify a PB-like inclusion body immunostained with anti-PHF Ab in a pyramidal neuron of Ammon's horn with DAT. NFTs were recognized within a distinct intracytoplasmic inclusion body which was globular and electron dense. Electron-dense immune products are located on NFTs that are arranged in bundles. Bars **a, c** = 2 μm; **b** = 100 nm

80-nm intervals (Fig. 5b, c). Thus, these perinuclear NFTs exhibited the same ultrastructural features as the HIBs, but differed in that their fibrillary bundles were found around the periphery of the nucleus and did not develop into inclusions. In samples from patients with DAT, the neurons of Ammon's horn had very few NFTs that were similar in form to PB-like inclusion bodies (Fig. 3c); most NFTs were of the flame type. Under an electron microscope, bundles of 15-nm-wide STs, intermixed with a few PHFs with a periodicity of about 80 nm, were observed both in NFTs of the flame type in pyramidal neurons of Ammon's horn and in HIB.

In neurons of the dentate fascia of Ammon's horn affected by Pick's disease, all PBs were seen as cytoplasmic inclusion bodies when observed by light microscopy. With respect to morphology, the PBs had globular or reniform shapes and did not have perinuclear NFTs observed in cases of DAT. Electron microscopy revealed that the PBs were globular intracytoplasmic inclusion bodies without limiting membranes, but were, nevertheless, distinct from other cytoplasmic structures. Components of the PBs included fibrillary elements, and osmiophilic granular and vesicular structures. The fibrillary structures consisted of STs (diameter about 15 nm), which appeared to be randomly distributed, but which frequently formed a complicated meshwork pattern (Fig. 6a, c). Periodically constricted fibrillary elements that differed from the PHFs observed in association with such severe neurofibrillary diseases as DAT, were also found within the PBs. These filaments with constrictions in PBs were regularly wound in a helical fashion crossing at about 160-nm intervals, and were approximately 30 nm wide at their widest point midway between the constrictions and about 15 nm wide at the point of constriction (Fig. 6c).

The PBs in pyramidal neurons of Ammon's horn showed the same light and electron microscopic features as the PBs found in granular neurons of the dentate fascia. With the presence of Pick's disease, argyrophilic inclusions in small neurons of the cingulate gyrus were rarely stained by Bielschowsky and Bodian stains. Anti-PHF immunostain high-lighted PBs as globular inclusion bodies mainly in small neurons of the 2nd and 4th layers of the cingulate gyrus. Electron microscopy showed that PBs were globular intracytoplasmic inclusion bodies, which were distinct from other cytoplasmic structures despite the lack of limiting membranes. Both fibrillary and granular structures were present within these PBs. The fibrillary structures were oriented in a streaming pattern without tight bundles (Fig. 6b), and were composed of STs with an approximate 15-nm diameter, and very scarce, periodically constricted filaments occurring at about 160-nm intervals. These latter had diameters varying from about 15 nm at the constricted points to about 30 nm at their widest parts (Fig. 6d). In some PBs in the cingulate gyrus, straight filaments about 15 nm in diameter were undergoing transformation into fibrillary structures with constrictions at about 160-nm intervals (Fig. 6e).

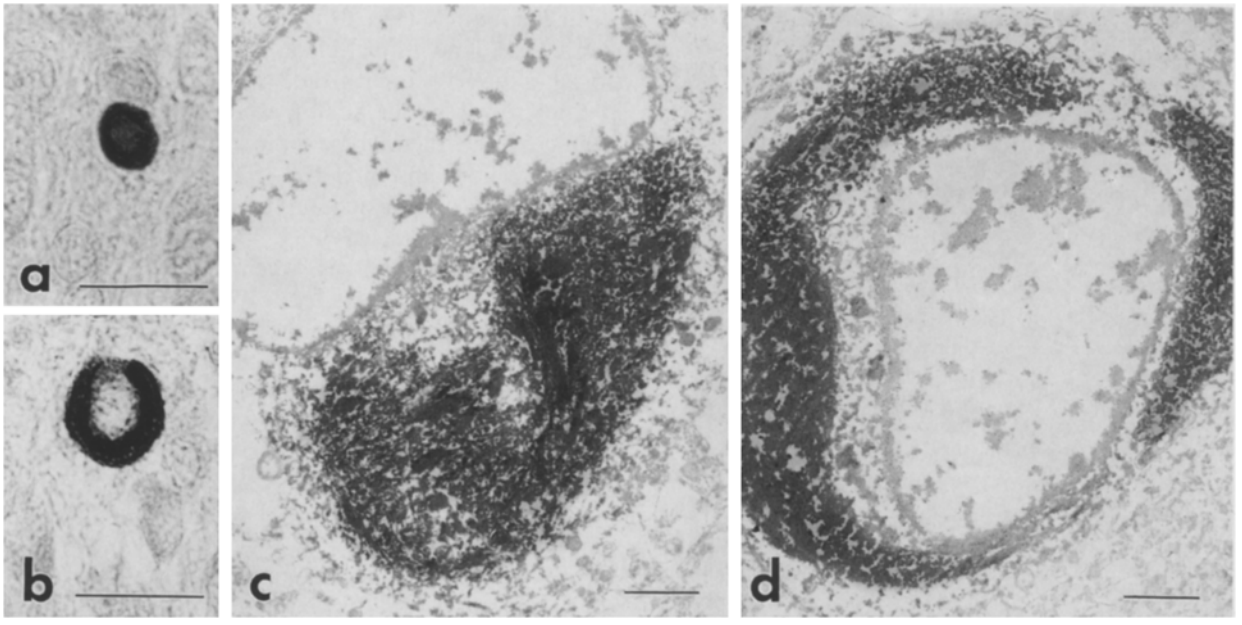


Fig. 4a–d. Sections for immunohistochemical and immunoelectron microscopical analysis show anti-PHF Ab immunostaining of NFTs in granular neurons of the dentate fascia of Ammon's horn with DAT. **a** Light micrograph of a PB-like inclusion body (Horoupian's inclusion body). **b** Light micrograph of NFTs that surround a nucleus, which do not form an inclusion body. **c** Immunoelectron micrograph of a Horoupian's inclusion body (HIB). Electron-dense immune products are located on NFTs that are arranged in bundles. **d** Immunoelectron micrograph of NFTs that surround the nucleus. NFTs immunostained with anti-PHF Ab are arranged in bundles. Bars **a, b** = 20 μm; **c, d** = 2 μm

An electron micrograph of Pick cells taken from the cingulate gyrus of a subject with Pick's disease generally demonstrated compression of the nucleus toward the cell periphery, and presence of electron-lucent cytoplasm. The cytoplasm had a relative paucity of granular and fibrillary components, and did not contain inclusion-like structures (Fig. 7a). Fibrillary elements in the cytoplasm were made up of NFs which were about 10 nm in diameter and possessed side arms (Fig. 7b). Accompanying them, there were rare, periodically constricted filaments with a periodicity of about 160 nm and approximate diameter ranging from 15 nm at the constrictions to 30 nm otherwise, as observed in PBs (Fig. 7c).

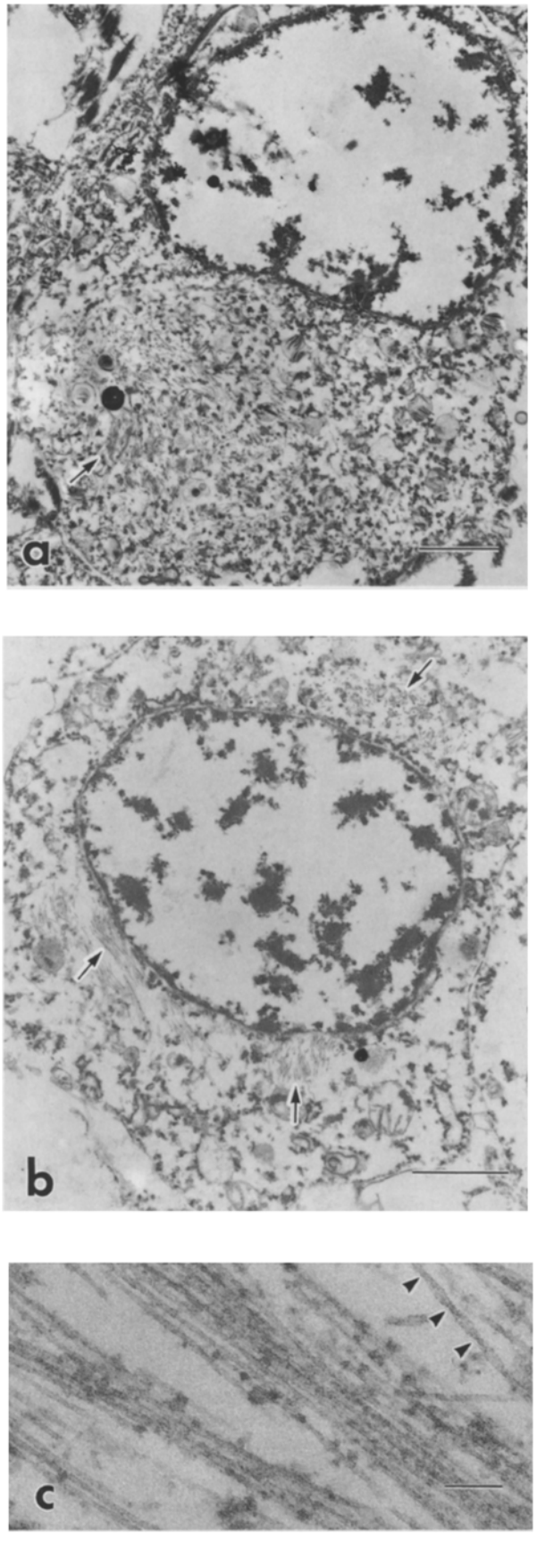
In the locus ceruleus affected by PSP, electron microscopy revealed that NFTs were formed of bundles of STs (diameter about 15 nm) intermixed with very occasional PHFs with a periodicity of about 80 nm (Fig. 8).

Discussion

NFTs observed by electron microscopy are found to mainly consist of STs about 15 nm wide and PHFs with a periodicity of about 80 nm, and are not present in normal neurons [11, 17–19, 23]. Immunohistochemical staining of NFTs became possible after anti-PHF Ab was prepared by Ihara et al. [9]. The anti-PHF Ab has an affinity not only for phosphorylated tau protein integrated into PHFs [10, 14, 15] but also for STs [1, 4], while anti-phosphorylated NF Ab (anti-pNF Ab) recognizes PHFs [7, 21]. When NFTs are treated with AP, the anti-pNF Ab either does not react with the dephosphorylated NFTs, or shows a reduction in reactivity [7, 21]. In other words, NFTs are supposed to be derived from aberrantly phosphorylated NFs [7, 21]. However, Nukina et al. [16] suggested that the anti-pNF Ab has an affinity for NFTs as a result of crossreaction of the Ab with phosphorylated tau protein integrated into PHFs.

Our results were consistent with those of Sternberger and Sternberger [20] in that the anti-npNF Ab recognized the cytoplasm and dendrites of neurons, but not the axon. In the present study, the anti-npNF Ab generally did not react with NFTs and PBs irrespective of whether or not there was AP pretreatment, while the anti-PHF Ab reacted with NFTs and PBs both with and without AP pretreatment. When NFTs and PBs were dephosphorylated by AP, their immunoreactive sites were not in common with those of nonphosphorylated NFs. These facts suggest that the NFs are not present as major components of the core proteins of NFTs and PBs.

The HIB observed in patients with DAT could not be discriminated immunohistochemically from the PB in granular neurons of the dentate fascia resulting



from Pick's disease. However, in cases of DAT, although some NFTs in granular neurons of the dentate fascia had the form of inclusion bodies, others were perinuclear and did not develop into inclusions. NFTs in pyramidal neurons of Ammon's horn were mainly of the flame type, but occasionally developed into inclusion-like structures. HIBs and inclusion bodies in pyramidal neurons of Ammon's horn observed in cases of DAT, had similarities in ultrastructure of perinuclear NFTs in the granular neurons of the dentate fascia and NFTs of the flame type in pyramidal neurons of Ammon's horn. Specifically, it was common to find bundles made up of STs (about 15 nm in diameter) and intermixed with PHFs in all of these NFTs. The anti-PHF Ab-positive fibrils (STs intermixed with PHFs), composing HIBs and the perinuclear NFTs in granular neurons, and composing the NFTs of the flame type and the inclusion bodies in pyramidal neurons, were immunoelectron-microscopically arranged in bundles. On the other hand, Pick's disease had PBs in granular neurons of the dentate fascia and pyramidal neurons of Ammon's horn, but it did not have the flame form as observed in the case of DAT. PBs in granular neurons of the dentate fascia were different from HIBs on an ultrastructural level, in that their STs were randomly distributed and not arranged in bundles, and periodically constricted filaments were present at about 160-nm intervals. Thus, after immunohistochemical, ultrastructural, and immunoelectron microscopical investigations, the HIB is considered to be a manifestation of neurofibrillary degeneration. Further, although it is generally accepted that granular neurons of the dentate fascia are resistant to neurofibrillary changes associated with DAT [2, 3, 5], when neurofibrillary degeneration is advanced, even these neurons may develop NFTs with some frequency. It remains to be clarified, however, how some NFTs in the dentate fascia develop into such inclusions as HIBs.

In specimens taken from patients with Pick's disease, PBs in small neurons of the cingulate gyrus were slightly different from PBs in granular neurons of the

Fig. 5a–c. Ultrastructure of NFTs in granular neurons of the dentate fascia of Ammon's horn with DAT. **a** This shows NFTs developing a HIB. The intracytoplasmic inclusion body is globular and distinct from other cytoplasmic structures. Fibrillary structures making up the inclusion body consist of STs about 15 nm wide arranged in bundles (*arrow*). **b** In this figure, NFTs surround the nucleus. Bundles of STs with the diameter of about 15 nm surround the periphery of the nucleus and are not developing into an inclusion (*arrows*). **c** In both HIB and perinuclear NFTs, PHFs with a periodicity of about 80 nm (*arrowheads*) are intermingled with bundles of 15-nm-wide STs. Bars **a**, **b** = 2 μm; **c** = 100 nm

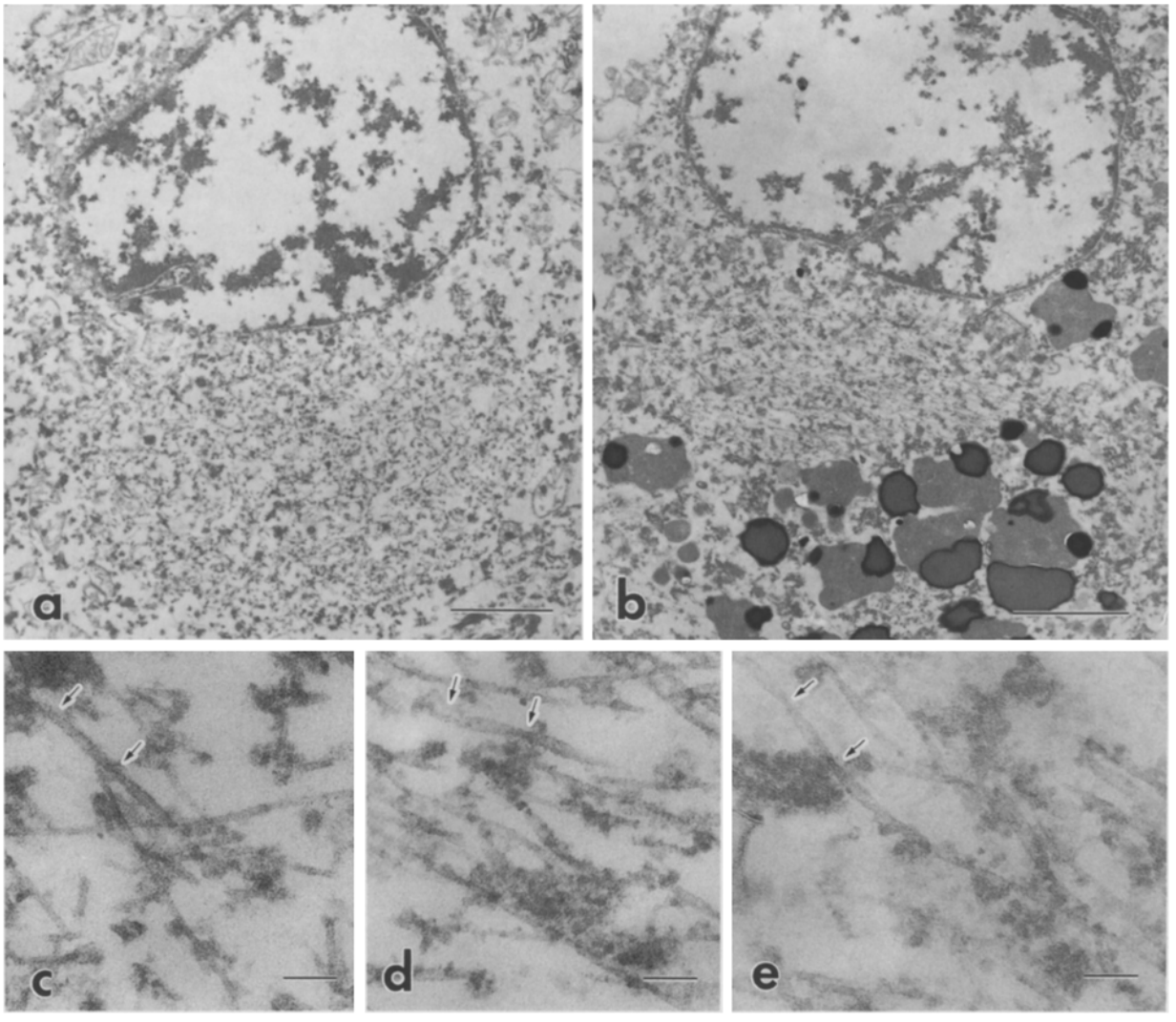


Fig. 6a–e. Ultrastructure of PBs. **a** Electron micrograph of a PB in the dentate fascia of Ammon's horn. The PB is a globular intracytoplasmic inclusion body without a limiting membrane, and is distinct from other cytoplasmic structures. Fibrillary structures forming the PB are mostly composed of STs (about 15 nm wide) that are distributed randomly. **b** Electron micrograph of a PB in a small neuron of the cingulate gyrus. The inclusion body is globular and distinct from other cytoplasmic structures. Fibrillary structures making up the PB are mostly composed of STs (about 15 nm in diameter) that are oriented in a streaming pattern from the *upper left* to the *lower right* of the electron micrograph. No tight bundles are evident. **c** This is a high magnification view of a PB in the dentate fascia. STs of about 15-nm width are randomly dispersed. There is one filament with constrictions (*arrows*), which has a width ranging from about 15 nm to 30 nm. These latter filaments are wound in a helical fashion crossing at about 160-nm intervals. **d** In this high magnification view of a PB in the cingulate gyrus, fibrillary structures which are the same as those observed in the PB in the dentate fascia (**c**), and which have constrictions spaced about 160 nm apart (*arrows*), are seen to be intermingled with STs of approximately 15-nm width. **e** Here, in a PB in the cingulate gyrus, a straight filament (about 15 nm wide) is undergoing transformation into a periodically constricted filament. Such a constricted filament occurs with a periodicity of about 160 nm (*arrows*). Bars **a, b** = 2 μm; **c–e** = 100 nm

dentate fascia, in that their STs were oriented in a streaming pattern and were not disordered. A specific-ordered pattern has previously been reported in an atypical type of PB, whose filamentous constituents were clustered in a geometric arrangement [13]. In the present study, periodically constricted filaments with a periodicity of about 160 nm and which differed from the usual PHFs found associated with DAT, were present in PBs both in the cingulate gyrus and in the dentate fascia. Therefore, PBs in small neurons of the cingulate gyrus are considered to be essentially identical to PBs in granular neurons of the dentate fascia and in pyramidal neurons of Ammon's horn.

On the other hand, Pick cells observed in the cingulate gyrus differed immunohistochemically from PBs. Ultrastructure of PBs and Pick cells was also different,

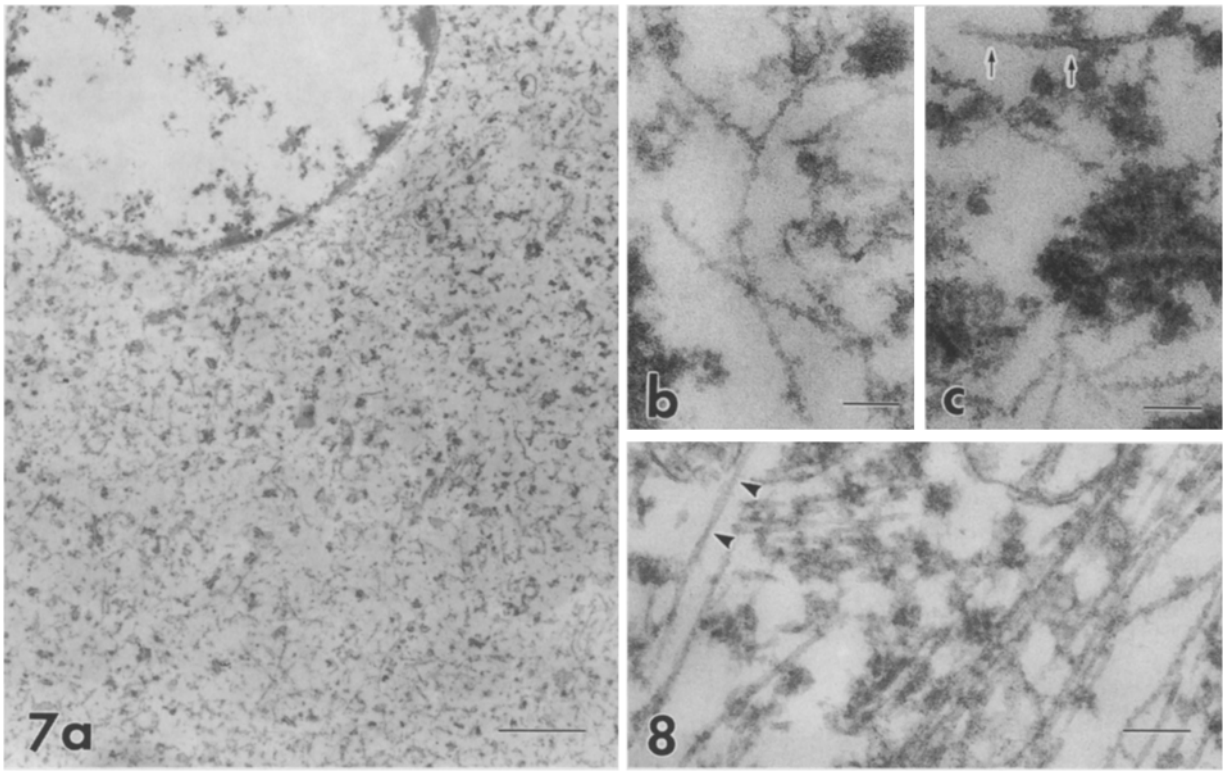


Fig. 7a–c. Ultrastructure of a Pick cell in the cingulate gyrus with Pick's disease. **a** The nucleus of the Pick cell is compressed toward the periphery. Cytoplasm is electron lucent, contains no inclusion-like structures, and has a relative paucity of filaments and granules. **b** The major fibrillary components in the cytoplasm are neurofilaments (of about 10-nm diameter) with side arms. **c** A filament with constrictions and with a periodicity of about 160 nm (*arrows*) is presented here and resembles those observed in PBs. Bars **a** = 2 μm; **b, c** = 100 nm

Fig. 8. Ultrastructure of NFTs in the locus ceruleus with PSP. The NFTs associated with PSP consist of bundles of STs (about 15 nm in width), intermingled with a PHF with a periodicity of about 80 nm (*arrowheads*). Bar = 100 nm

since the major fibrillary structures in PBs were STs, while in Pick cells there were mainly NFs. It is quite probable that Pick cells are neuropathologically independent of PBs, or, in other words, that Pick cells and PBs are not expressions of the same neuropathological processes. In Pick cells as in PBs, however, we observed filaments with constrictions and with a periodicity of about 160 nm, so there remains the possibility that Pick cells and PBs are different manifestations of the same neuropathological processes.

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