Acta Neuropathol (1996) 92 : 588–596

REGULAR PAPER

A. Probst · M. Tolnay · D. Langui · M. Goedert
M. G. Spillantini

Pick's disease: hyperphosphorylated tau protein segregates to the somatoaxonal compartment

Received: 7 March 1996 / Revised, accepted: 22 May 1996

Abstract Pick bodies and ballooned cells of Pick's disease and the neurofibrillary lesions of Alzheimer's disease are characterized by the presence of hyperphosphorylated microtubule-associated protein tau. Little is known about the mechanisms underlying tau hyperphosphorylation in Pick's disease and the distribution of abnormal tau in affected neurons. We have used a panel of phosphorylation-dependent (AT270, AT8, AT180, 12E8, PHF-1, AT10 and Tau-1) and phosphorylation-independent anti-tau antibodies (N-tau 5 and 134) to stain brain tissue sections from subjects with Pick's disease and Alzheimer's disease. These antibodies labeled Pick bodies and neurofibrillary lesions in a similar way, with the exception of antibody 12E8, which stained a subset of neurofibrillary tangles, but no Pick bodies. Moreover, abundant AT8- and PHF-1-positive neuritic profiles were observed in cortical areas rich in Pick bodies, even in the complete absence of neurofibrillary lesions. Unlike the Gallyas-positive neuropil threads of Alzheimer's disease, which were of variable diameter and covered by spiny appendages, neuritic profiles of Pick's disease showed a regular diameter, appeared smooth and were Gallyas-negative. In contrast to Alzheimer's disease, dendritic branches of neurons containing Pick bodies were not labeled by anti-tau antibodies. In the hippocampus, numerous tau-positive axon terminals were found along dendrites of the polymorphic layer of the dentate gyrus. Our results indicate that tau proteins in Pick's disease and Alzheimer's disease share similar phosphorylated residues, with the exception of serine 262, which is phosphorylated in Alzheimer tangles but not in Pick bodies or neuritic profiles. Furthermore, we show that hyperphosphorylated tau segregates to different neuronal compartments in the two diseases, with a somatoaxonal distribution in Pick's disease and a somatodendritic distribution in Alzheimer's disease.

Key words Pick's disease · Tau protein · Axons

A. Probst · M. Tolnay · D. Langui
Institute of Pathology, Division of Neuropathology,
Schönbeinstrasse 40, CH-4003 Basle, Switzerland
Tel.: +41 61 265 2895; Fax: +41 61 265 3194

M. Goedert · M. G. Spillantini
MRC Laboratory of Molecular Biology, Cambridge, UK

Introduction

Pick's disease (PD) is a rare form of progressive dementing disorder which occurs in middle-late life. Its most distinctive pathological feature is a circumscribed cortical atrophy, mainly of the frontal and anterior temporal lobes. The main histological features of PD consist of a severe neuronal loss, ballooned neurons and pathognomonic spherical argyrophilic inclusions called Pick bodies; these are located in neocortical neurons of layers II–III and VI [13], dentate gyrus granule cells, pyramidal cells of hippocampus, subiculum and entorhinal cortex and in some subcortical nuclei. Ultrastructurally, the Pick body is composed of 15-nm, randomly oriented straight filaments [15, 17, 20] intermingled with a few thicker twisted filaments, with a periodicity of 120 nm [20] or 160 nm [15].

Pick bodies have been shown to share antigenic determinants with the neurofibrillary lesions of Alzheimer's disease (AD) [16, 17, 19, 20, 23, 24, 28, 32]. The main protein constituent of both Pick bodies and neurofibrillary lesions is hyperphosphorylated tau protein [17, 20]. At present, nothing is known about the mechanisms underlying tau hyperphosphorylation in PD and only very little about which sites are hyperphosphorylated and the intracellular distribution of abnormal tau within affected neurons. Neuritic pathology has been stressed in AD but has not been fully documented in PD, despite an earlier suggestion that a primary axonal pathology could account for the neuronal changes observed in PD, particularly the neuronal ballooning [30]. Dendritic swellings of degenerating neurons have been observed by Golgi impregnation of biopsy samples from patients with PD [7] and decreased numbers or lack of dendritic spines and poor dendritic branching have been documented in neocortical pyramidal neurons [5, 29]. Murayama et al. [20] men-

tioned "small structures," probably neurites, reacting with an antibody to phosphorylated tau, in the neuropil of PD cases, but did not characterize them further. More recently, great numbers of PHF-1-immunoreactive (IR) neuropil threads have been reported in a case of severe panencephalic PD [6].

In the present study, we have investigated the distribution of tau protein epitopes in brain sections of PD cases using a panel of well-characterized phosphorylation-dependent anti-tau antibodies. Particular attention was paid to the distribution of tau protein reactivity in neurites and to differences from tau pathology in AD.

Materials and methods

Brain material

Brains from three cases of PD [one female, aged 76 and two males, aged 67 and 65; postmortem delays (PMDs) 6, 7 and 39 h], two patients with clinically and neuropathologically documented AD (63 and 74-year-old males; PMDs 5.5 and 7 h) and two intellectually normal subjects (one female, aged 74, and one male, aged 69; PMDs 1.5 and 6 h) were investigated in this study. Severe progressive dementia was documented in all three PD patients. Neuropathological examination revealed a severe atrophy of circumscribed parts of the brain, mainly of the temporal lobes and of the basal aspects of the frontal lobes, together with some atrophy of the caudate nucleus. All brains were immersed in 4% phosphate buffered formaldehyde (pH 7.2) for about 10 days. Several tissue blocks were taken from various parts of the brain after completion of fixation and embedded in paraffin. Deparaffinized 4-μm-thick sections were stained with hematoxylin and eosin, Holmes-Luxol (HL) and Gallyas. For the present study we used the anterior hippocampal region containing the cornu ammonis, the dentate gyrus, the entorhinal cortex and parts of the temporo-basal neocortex near the rhinal sulcus.

Antibodies

Anti-tau antibodies are listed in Table 1 together with antibody specificities and dilutions. Most of the listed antibodies (AT270, AT8, AT180, 12E8, PHF-1 and AT10) recognize tau only when it is phosphorylated at specific amino acids. Antibody Tau-1 is directed to the same region of the human tau molecule as the phosphorylation-dependent anti-tau antibody AT8, but needs this region to be unphosphorylated. Antibody 134 is directed against the C-terminus of tau, whereas antibody N-tau 5 recognizes the N-terminus of tau. Other antibodies included monoclonal antibody to synaptophysin (clone SY38, Progen; dilution 1/200), an antibody to the N-terminal amyloid precursor protein (APP; Boehringer Mannheim; dilution1/20), SMI 32 (Sternberger Monoclonals; dilution 1/1000) which reacts with non-phosphorylated epitope on neurofilament H and a monoclonal antibody to MAP 2 (Boehringer; dilution 1/600).

Immunohistochemistry

Deparaffinized sections of hippocampus and neocortex were treated with normal goat serum (prior to use of polyclonal antibodies) or normal horse serum (prior to use of monoclonal antibodies) to block non-specific sites. This was followed by overnight incubation with the primary antibody at 4° C. Bound antigens were detected using avidin-biotin-peroxidase (Vectastain, Elite kit; Vector Laboratories). Peroxidase acitivity was revealed with a substrate solution containing 1% diaminobenzidine and 0.3% H_2O_2. Sections were slightly counterstained with hematoxylin. Tau-1 and AT8 immunoreactivities were examined on sections with and without prior alkaline phosphatase treatment which involved incubation of sections with 1000 U/ml alkaline phosphatase in 0.1 *M* TRIS-HCl pH 8.0 at 37° C for 1 h.

Tau-immunolabeled neuritic profiles (NP) were drawn, in one selected case of PD, using a drawing tube attached to a microscope (Zeiss Axioplan), under an oil immersion × 100 objective.

Morphometric analysis of neuritic profiles and threads

AT8-immunostained (non-counterstained) paraffin sections (4 mm) of the hippocampus from two PD cases without associated Alzheimer changes (without neurofibrillary tangles as assessed by Gallyas staining) and one AD case were used to measure the thickness of immunostained NP in PD and threads in AD. Analysis was performed in ten contiguous fields of the CA1 sector of the hippocampus and the subicular area in each case. The mean diameters of immunostained neurites were determined using a computer-assisted image analysis system combining a microscope (Zeiss Axioplan), a high sensitivity camera (LH-4036, Lhesa Electronic), a microcomputer (COMPAC Deskpro 386/20) and a software system (SAMBA 2005) developed by TITN (Alcatel, Grenoble, France).

Table 1 Tau antibodies used on tissue sections. The numbering of the phosphorylated residues corresponds to that of the longest human brain tau isoform [8] (*mAb* monoclonal antibody, *AS* antiserum, *Ser* serine, *Thr* threonine)

Antibody	Specificity	Reference(s)	Dilution
N-Tau 5 (AS)	N-terminus	Goetz et al. 1995 [11]	1:1000
AT270 (mAb)	Phosphorylated Thr-181	Goedert et al. 1994 [9]	1:500
AT8 (mAb)	Phosphorylated Ser-202 and Thr-205	Goedert et al. 1995 [10]	1:1000
Tau-1 (mAb)	Unphosphorylated 189–206	Binder et al. 1985 [2] Szendrei et al. 1993 [27]	1:2000
AT180 (mAb)	Phosphorylated Thr-231 and Ser-235	Goedert et al. 1994 [9]	1:1000
12E8 (mAb)	Phosphorylated Ser-262 and/or Ser-356	Seubert et al. 1995 [25]	1:10000
PHF-1 (mAb)	Phosphorylated Ser-396 and Ser-404	Greenberg et al. 1992 [12] Otvos et al. 1994 [22]	1:500
At10 (mAb)	Phosphorylated residue(s) of unknown location	Merken et al. 1992 [18]	1:500
134 (AS)	C-terminus	Goedert et al. 1989 [8]	1:1000

Table 2 Staining reactions with anti-tau antibodies in Pick's disease and Alzheimer's disease. (–, (+), +, ++, +++ no, very weak, weak, medium or strong reactivity)

Antibody	Pick's disease			Alzheimer's disease	
	Pick bodies	Ballooned cells	Filiform profiles	Neurofibrillary tangles	Threads
N-Tau 5	++	+++	–/(+)	++/+++	++
134	+/++	–/++	–/(+)	+	+
AT180	++	(+)	(+)	+++	+++
AT10	+++	+	++	+++	+++
AT8	+++	++	+++	+++	+++
			Smooth surface		Spiny appendages
AT270	++	+	+	++	+
PHF-1	+++	++	+++	+++	+++
12E8	–	–	–	++	++
					Only few threads

Results

Histology

Microscopic examination of PD cases revealed high densities of Pick bodies in hippocampus, subiculum, entorhinal cortex as well as in frontal and temporal cortices, mainly in layers II and VI. Pick bodies were found in virtually all granule cells of the dentate gyrus, in most pyramidal cells of CA1 and CA2 and in scattered neurons of the CA4 subfield. Gliosis, neuronal loss and particularly ballooned neurons were mainly features of the neocortex. Pick bodies, and to a lesser degree ballooned cells in the neocortex, were stained with HL but not with Gallyas. As expected, Gallyas stained numerous neurofibrillary tangles in the hippocampus and entorhinal cortex of AD cases. Few neurofibrillary tangles were encountered in CA1 and in the entorhinal cortex in one PD case. No intra- or extracellular Gallyas-positive neurofibrillary tangles were observed in the other two cases (these cases were used for morphometrical analysis of tau-immunostained neurites in cortical neuropil, see below).

Immunohistochemistry

The results obtained with antisera N-tau 5 and 134 and with phosphorylation-dependent anti-tau antibodies in PD and AD cases are summarised in Table 2. A comparison of the labeling of Pick bodies obtained with various anti-tau antibodies is shown in Fig. 1 A–F. Most intense labeling of Pick bodies was obtained with monoclonal antibodies AT8 (Figs. 2 A, 3 A, 6 A), PHF-1 and AT10 (Fig. 1 E). Slightly less intense staining was obtained with AT270 and AT180 (Fig. 1 B, C) and the non-phosphodependent anti-tau antibodies N-Tau 5 and 134 (Fig. 1 A, F). Pick bodies remained consistently unstained with antibody 12E8 (Fig. 1 D). However, few neurofibrillary tangles in CA1 and entorhinal cortex of one PD case were stained with 12E8, as was a large subset of neurofibrillary tangles in AD. Fewer neuropil threads were labeled with 12E8 than with AT8 or PHF-1. Pick bodies were negative with Tau-1 without previous alkaline phosphatase treatment. However, strong Tau-1 labeling was observed following

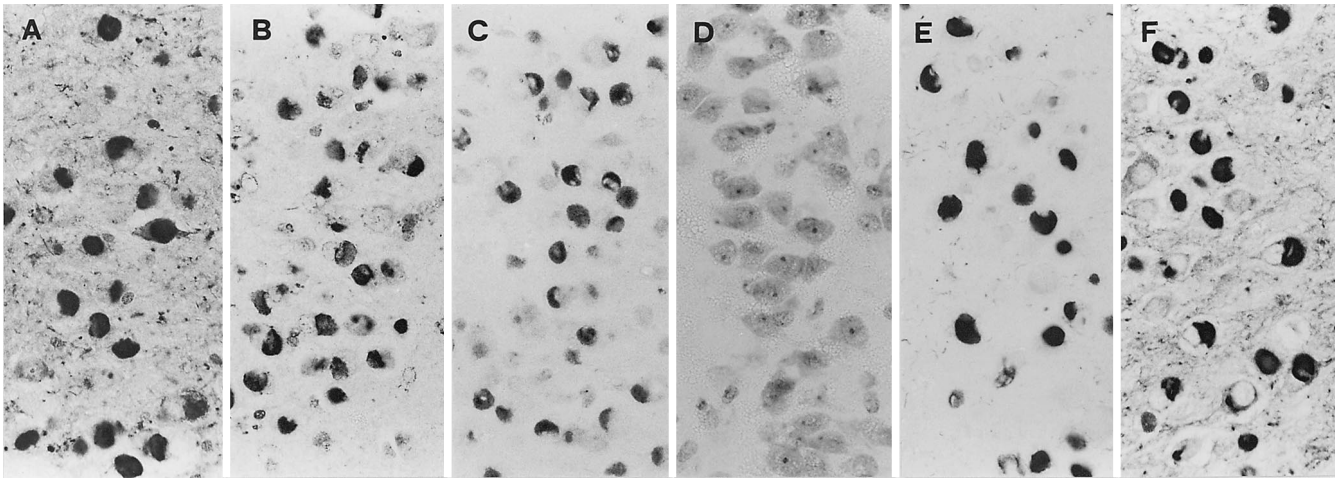


Fig. 1 A–F Stratum granulosum of the dentate gyrus from a case with PD. Staining of Pick bodies with anti-tau antibodies: N-Tau 5 (**A**), AT270 (**B**), AT180 (**C**), 12E8 (**D**), AT10 (**E**) 134 (**F**). Note absence of labeling of Pick bodies when using antibody 12E8 (**D**). **A–F** Weak counterstain with hematoxylin, × 300

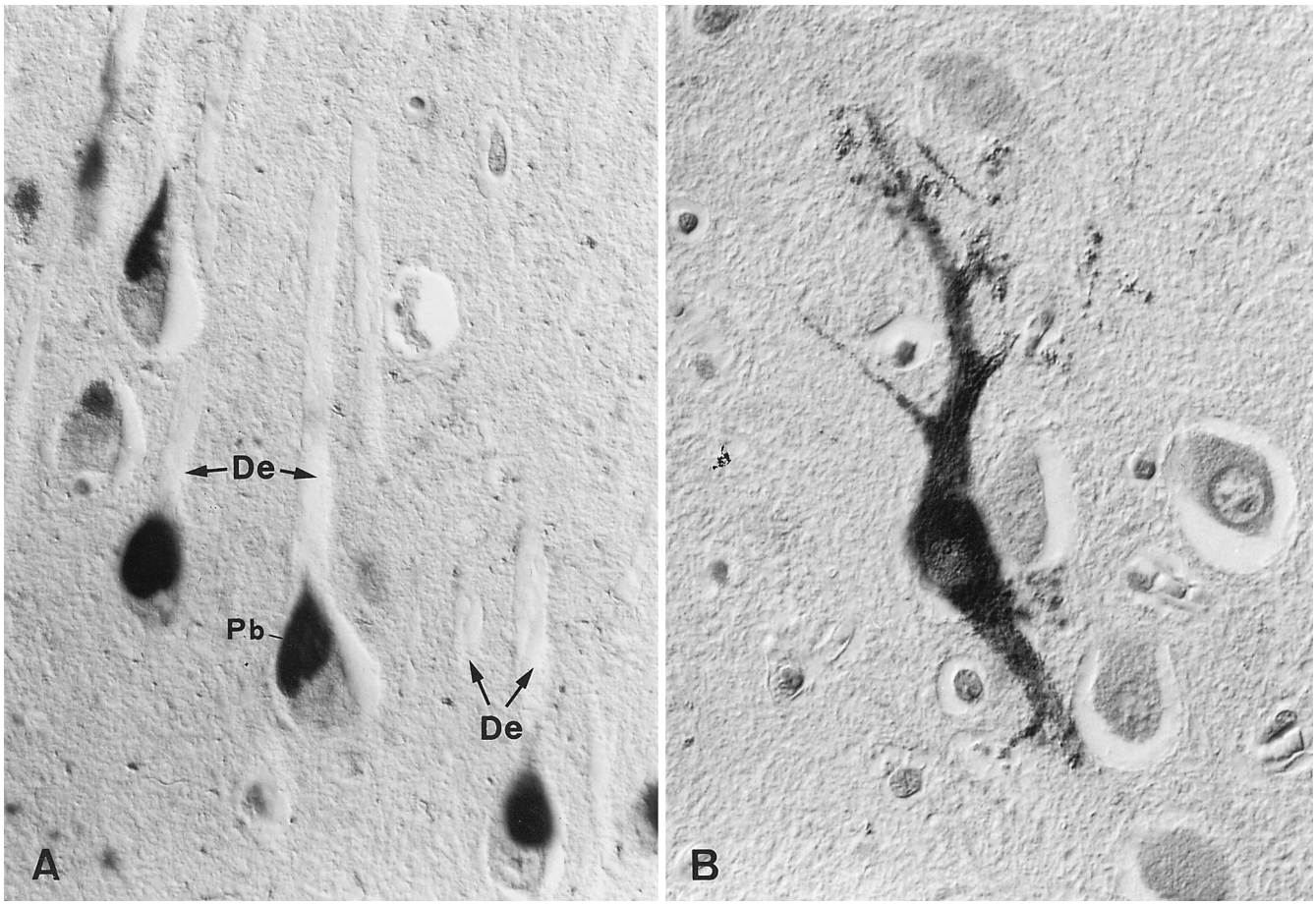


Fig. 2 A Pick's disease. Pyramidal cells of the CA1 sector stained with AT8 antibody. Strongly stained Pick bodies *(Pb)* located at the apical pole of the cell soma. The conus-like inclusion extends into the very proximal part of the apical dendrite *(De)*, but not any further. **B** Alzheimer's disease. Neuron of the hilus of the dentate gyrus, stained with AT8. All parts of the cell, including the soma and the dendrites are labeled **A, B** Nomarski optics. Slight counterstain with hematoxylin, × 600

dephosphorylation of the sections. The MAP-2 and SMI 32 antibodies stained the somatodendritic cytoplasm of pyramidal cells in CA1–4 subfields and subiculum, but did not stain Pick bodies (not shown). In 70–80% of affected pyramidal cells, Pick bodies were surrounded by less intensely stained PHF-1/AT8-positive granular material which filled the cell soma partly or entirely but which did not extend into the dendrites (Fig. 2 A). In contrast, PHF-1 and AT8 staining of pyramidal cells in AD cases extended far into the dendritic tree (Fig. 2 B). In one case of PD, about 50% of Pick bodies of the granule cell layer of the dentate gyrus were extracellular (ghost Pick bodies). These were less intensely stained with HL or tau antibodies than their intracellular counterparts and were often surrounded by APP 474-, tau- and synaptophysin-IR small neurites (not shown).

In pyramidal cells of the hippocampus, Pick bodies consisted of large spherical or horseshoe-shaped inclusions that were located most often at the apical pole of the cell soma. In some pyramidal cells a more elongated conus-like inclusion was seen that extended into the very proximal part of the apical dendrite, but not any further (Fig. 2 A). Pick bodies were not labeled by the synaptophysin antibody, thus contrasting with the heavily stained surrounding neuropil. Ballooned cells of the deep neocortical cell layers showed weak cytoplasmic reactivity with the antibody to MAP2, with SMI 32 and with the C-terminal tau antibody 134, and strong immunoreactivity with AT8 (not shown). Next to ballooned cells and neurons containing Pick bodies, we found few glial cells, presumably astrocytes, containing abnormally phosphorylated tau. These cells were Gallyas-negative and showed very short tapering tau-positive processes.

Tau-positive neuritic profiles

Immunostaining with phosphodependent antibodies AT8, PHF-1 and Tau-1 (after dephosphorylation) revealed a network of intensely labeled NP in areas rich in neurons with Pick bodies (Fig. 3 A, B). When using AT180, AT270 or non-phosphodependent anti-tau antibodies (Table 2), NP were not or only weakly stained. NP were slightly curvilinear and showed regular diameter. Some of them could be followed over long distances. No focal enlargements or "dystrophic" changes could be seen along NP (Fig. 3 A, B). Their surface was smooth, contrasting with threads of AD, which often presented small spine-like ap-

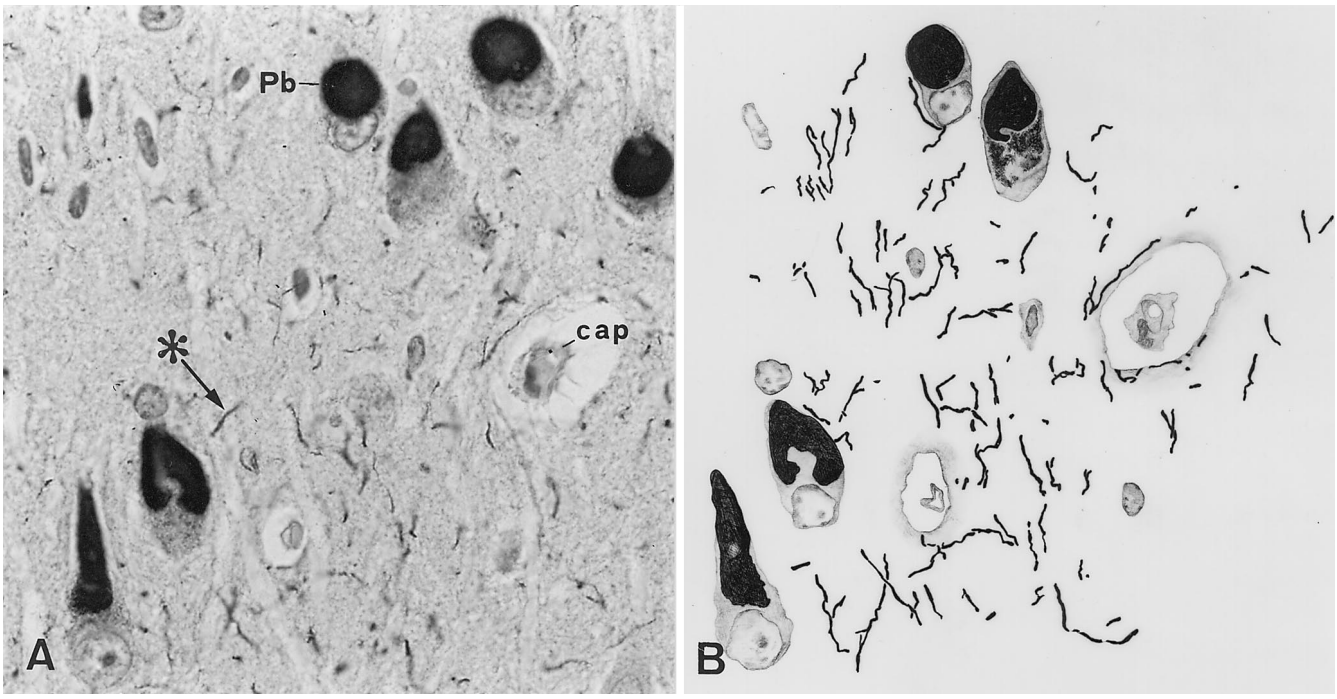
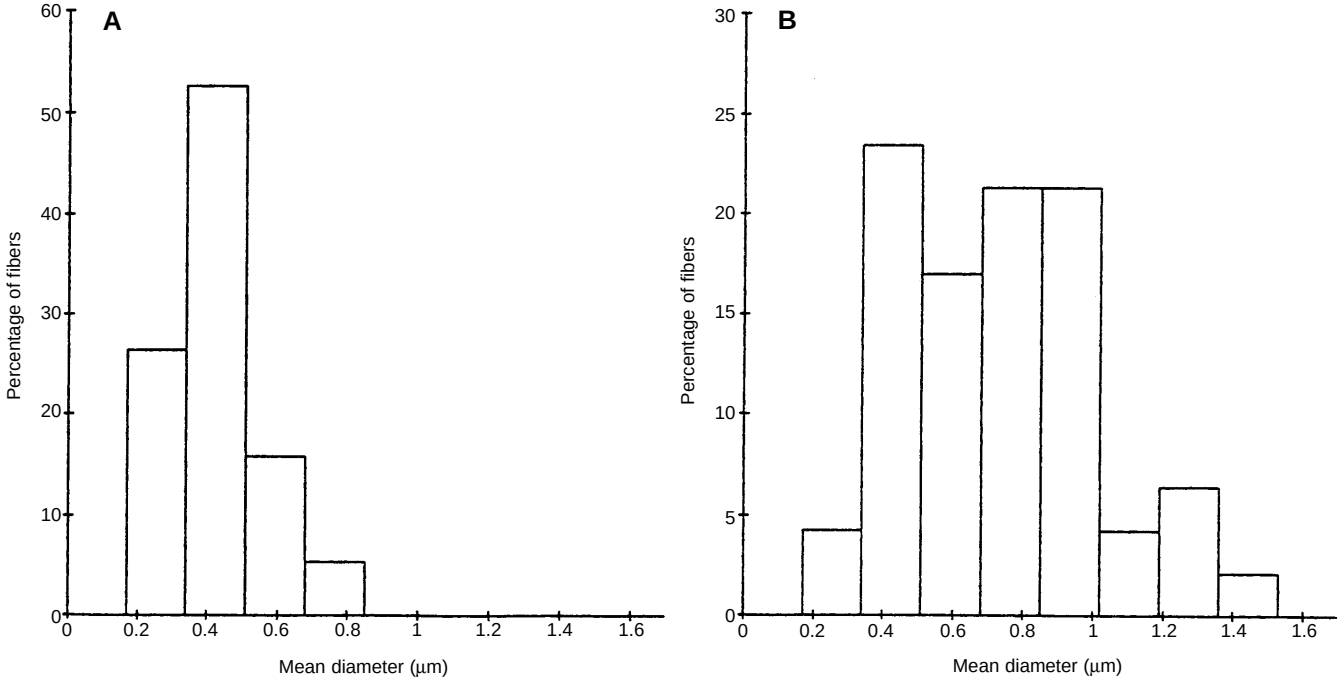


Fig. 3 A, B Pick's disease. Subiculum. **A** Phosphorylation-dependent antibody PHF-1 labeled neuritic profiles *(arrow with asterisk)* in the cortical neuropil in areas rich in Pick bodies *(Pb)*. Counterstain with hematoxylin and eosin, × 600. **B** Camera lucida drawing of PHF-1-positive Pick bodies and neurites in the same area as shown in **A** (*cap* capillaries)

pendages on their surface when immunostained with AT8 (not shown). When compared with NP, neuropil threads were of more irregular thickness. NP could generally not be traced back to neuronal cell bodies with Pick bodies. The cerebral white matter, particularly the white matter tracts in the hippocampus, contained great numbers of NP, which were generally thicker than the NP encountered in the cortical neuropil.

Morphometric analysis of AT8-positive NP in CA1 sector of the hippocampus in PD cases revealed a relatively narrow range of diameter distribution [X (SD) = 0.41 (0.11) μm]. In AD, tau-IR neuropil threads, when analyzed in the same area as in PD, were of larger average

Fig. 4 A, B Histograms of diameter distribution of AT8-labeled neurites in the CA1 subfield of the hippocampus in a case with Pick's disease (**A**) and in Alzheimer's disease (**B**)



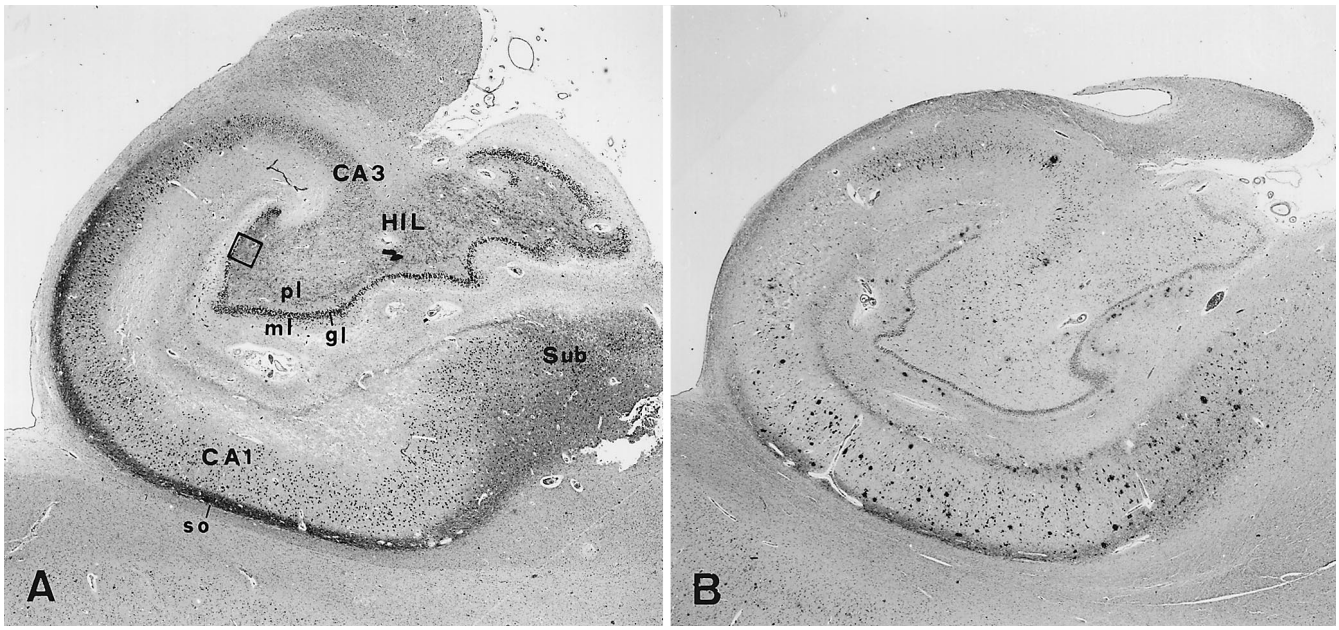


Fig. 5 A, B Middle third of the hippocampus stained with AT8. In Pick's disease (**A**), diffuse background staining of the neuropil of the Ammon's horn, including the hilus *(HIL)* of the dentate gyrus where AT8 staining is slightly more intense in the polymorphic layer *(pl)*. The granular layer *(gl)* of the dentate gyrus is strongly stained due to the presence of numerous Pick bodies. Note also the strong labeling of the stratum oriens *(so)* of the Ammon's horn. The *frame* in the polymorphic layer contains details shown in Fig. 7. In Alzheimer's disease (**B**), there is no background staining of the neuropil of the Ammon's horn. Dark dots in CA1 and CA2 sectors correspond to neurofibrillay tangles. **A, B** Counterstain with hematoxylin, × 8 (*ml* molecular layer of the dentate gyrus, *Sub* subiculum)

thickness with a broader diameter distribution [*X* (SD) = 0.73 (0.27) μm; Fig. 4 A, B).

Axonal projections in the polymorphic layer of the dentate gyrus

In PD a great number of tau-positive neurites was found throughout Ammon's horn, including the hilus and mainly the polymorphic layer of the dentate gyrus, contrasting with the paucity of threads in this area in AD (Fig. 5 A, B). In contrast, only few immunostained neurites were seen in the molecular layer of the dentate gyrus and in the granule cell layer itself. Numerous fine AT8-, PHF-1- and AT10-positive neurites, probably axon terminals, with intensely stained knob-like endings were found along dendrites of the polymorphic layer of the dentate gyrus (Fig. 7 A, B).

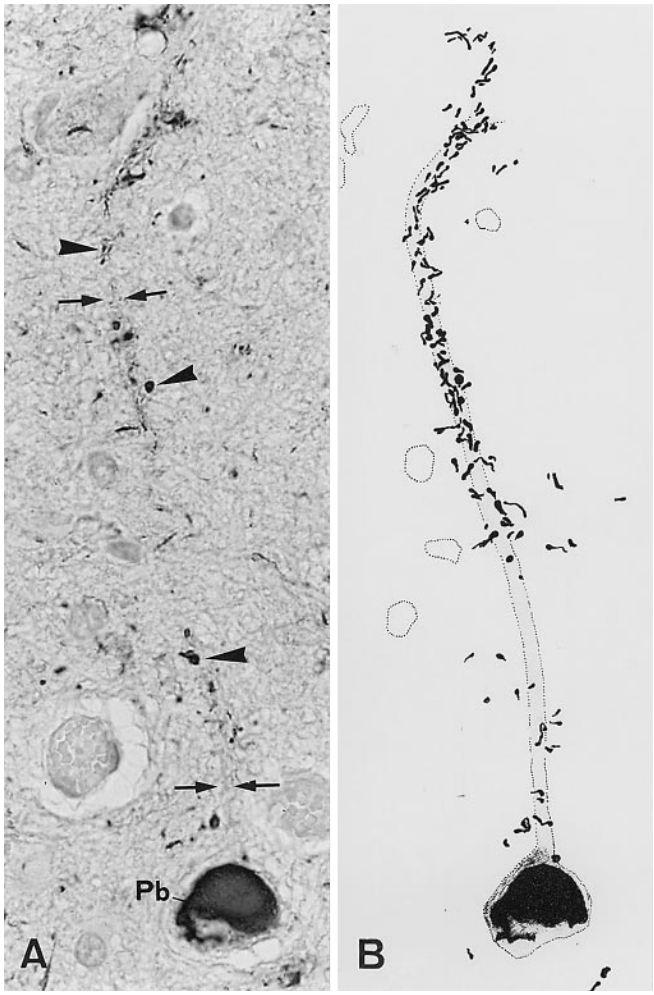


Fig. 6 A, B Pick's disease. **A** Immunostaining with AT8 antibody. Large neuron of the hilus of the dentate gyrus containing AT8-immunoreactive Pick body *(Pb)* in its soma. The dendrite shown in the figure (between *arrows*) is not stained by itself but is decorated by numerous AT8-positive fibers and boutons *(arrowheads)*. Paraffin section. × 600. **B** Camera lucida drawing of the same neuron as shown in **A** to demonstrate details of AT8-immunostained terminals and boutons at the dendrite surface

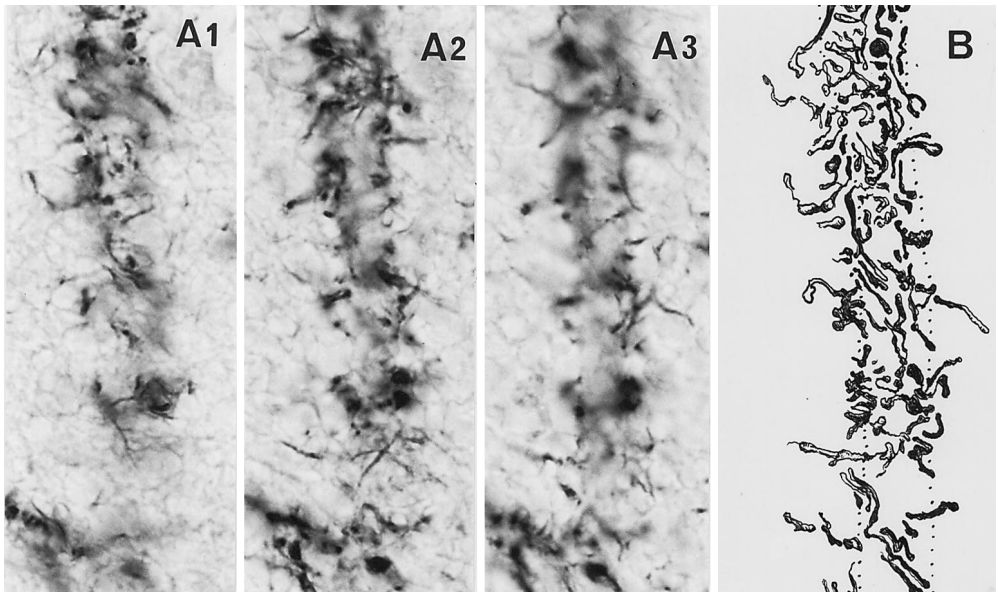


Fig. 7 A Pick's disease. Area of the polymorphic layer of the dentate gyrus corresponding to the frame in Fig. 5 A. Short dendritic segment of a neuron, stained with AT8. Only surface structures, probably axonal terminals, but not the dendrite itself, are stained. The dendrite was photographed at three consecutive (**A1–3**) levels in the paraffin section. × 1000. **B** Camera lucida drawing of the same dendrite segment as in figure **A1–3**. The *dotted line* indicates the contour of the dendrite

Few dendrites covered with tau-positive axon terminals were also found in deeper parts of the hilus of the dentate gyrus (Fig. 6 A, B). The dendrites of hilar neurons, like their counterparts in the CA1 sector, did not stain for tau protein. Only a few labeled neurites or boutons were found on cell bodies of hilus neurons of along the proximal portions of their dendrites. Rows of AT8/PHF-1-positive boutons were also seen along dendritic branches of neurons in the stratum oriens of the cornu ammonis.

Discussion

Our results demonstrate the presence of a network of abnormal tau-IR NP, in addition to the pathognomonic Pick bodies in nerve cell somata. With few exceptions, NP could not be traced back to neuronal perikarya with Pick bodies. However, that NP constitute an integral part of the pathology of PD is made likely by their increased density in cortical areas rich in neurons with Pick bodies. As shown by Hof et al. [13], neurofibrillary lesions of the Alzheimer type are more commonly encountered in PD than previously appreciated. However, an admixture of AD changes in the shape of neuropil threads is unlikely to account for NP in our cases, since lesions of the Alzheimer type were scarce in one case and absent in the other two cases. Furthermore, threads of AD are usually Gallyas-positive [34], thicker and of more irregular diameter than Gallyas-negative, tau-IR NP found in PD.

Morphometric data, including the small and regular diameter of NP and the absence of tau immunoreactivity in proximal dendritic segments, indicate that axons and not dendrites probably account for the bulk of tau immunoreactivity in NP. Furthermore, the surface of the NP was smooth, thus contrasting with the presence of spine-like appendages on the surface of neuropil threads. Indirect evidence for NP being mainly axonal in origin comes from anatomical observations in the hippocampus. For instance, tau-IR fiber projections in the polymorphic layer of the dentate gyrus are unlikely to correspond to dendrites, considering the almost complete absence of neurons containing Pick bodies in this area. A large number of NP corresponded to axon terminals ending on dendrites of neurons of the polymorphic layer, as suggested by the presence of parallel rows of tau-positive boutons. The cellular origin of tau-IR fibers in the CA4 field of the hippocampus cannot be clearly established, although granule cells of the dentate gyrus constitute a likely source of these fibers. It also remains to be established which type(s) of neurons filiform profiles are contacting in the polymorphic layer.

We found small numbers of tau-positive glial cells in the cortex of our PD cases, thus confirming earlier reports by Iwatsubo et al. [14] and Yashuhara et al. [32]. It could therefore be hypothesized that the tau-positive fibers described in our PD cases correspond to glial cell processes. However, we consider this an unlikely possibility. The small numbers of tau-positive glial cells could hardly account for the extensive distribution of tau-positive fibers detected in our PD cases. The tau-containing glial cell processes were rather short and tapering, whereas the tau-labeled neuropil fibers showed regular diameters and could often be followed over long distances. Moreover, tau-IR fibers were never disposed radially around a center and did not form pericapillary end-feet as would be expected from glial cell processes.

The Gallyas stain, which labels neurofibrillary lesions in AD, did not stain any pathological structures in PD. Tau proteins in AD (possibly as a consequence of hyperphosphorylation) are thought to self-assemble into paired helical filaments (PHFs) which are recognized by the Gallyas stain only when compacted into neurofibrillary tangles. Thus, next to neurons containing well-defined neurofibrillary tangles, various numbers of cells are encountered with hyperphosphorylated tau but without

demonstrable neurofibrillary tangles ("stage 0 tangles" of Bancher et al. [1] and Gallyas-negative "group 1 neurons" of Braak and Braak [3]. In these cells abnormal tau is in a poorly compacted state, as indicated by the presence of only scarce and dispersed PHFs [1]. Immunoelectron microscopic studies of Pick bodies have revealed loosely arranged straight filaments and a few twisted filaments decorated by phosphorylation-dependent anti-tau antibodies [15, 17, 20]. Thus, Gallyas negativity in Pick bodies might be explained, as in stage 0 tangles, by the scarcity of twisted filaments and their lack of compaction into fibrillary bundles. One characteristic of PD is that abnormal tau in Pick bodies and NP, in contrast to tau in stage 0 tangles, does not evolve into more aggregated, Gallyas-positive stages.

Our immunohistochemical results show that tau proteins in Pick bodies and neurofibrillary lesions of AD share a number of phosphorylated sites, such as Thr 181, Ser 202, Thr 205, Thr 231, Ser 235, Ser 396 and Ser 404. These results confirm and extend results obtained in recent studies showing staining of Pick bodies by phosphorylation-dependent anti-tau antibodies M4, C5 and PHF-1, indicative of phosphorylation at Thr 231, Ser 396 and Ser 404 [6, 14]. By contrast, antibody 12E8 stained some neurofibrillay lesions of AD, but failed to stain Pick bodies or NP, indicating that phosphorylation of Ser-262 may be a late event, that occurs only in highly aggregated and compacted neurofibrillary lesions.

An essential somato-axonal distribution of hyperphosphorylated tau was observed in PD. AT8/PHF-1-IR was strong in the Pick bodies and the axon, weaker in parts of the cell soma surrounding the Pick bodies, and absent from the dendritic tree. In the axon, tau is probably hyperphosphorylated only distally, as suggested by the labeling of axon terminals in the hippocampus and by our failure to encounter contacts between IR axons and the parent cells.

Our observations agree with recent findings by Nakamura et al. [21] who reported the presence of clathrin in Pick bodies and suggested that this was due to impaired axonal transport. The suggestion that hyperphosphorylation of tau might result in an impaired axonal flow in PD is supported by our immunocytochemical findings.

The intraneuronal distribution of hyperphosphorylated tau protein we describe in PD differs from that observed in AD. "Group 1 neurons" of the transentorhinal/entorhinal region have been considered a possible precursor stage of neurons with neurofibrillary tangles [3]. In such neurons diffuse granular AT8 immunoreactivity is distributed to all compartments, including soma, dendrites and axon. At later stages, highly aggregated Gallyas-positive tau accumulates mainly as neurofibrillary tangles and in the distal portions of dendrites as neuropil threads [3, 31]. In PD, we failed to observe neurons with a distribution of AT8 immunoreactivity suggestive of the presence of "group 1 neurons". Early phosphorylation of tau in AD has been reported to occur at Ser-202 and Thr-205 and to be preferentially located within neurites [26]. We cannot exclude that a similar sequence of events occurs in PD. However, AT8-positive NP were constantly associated with the presence of Pick bodies, suggesting that hyperphosphorylation of tau within axons may occur after the formation of Pick bodies.

Taken together, the present results which demonstrate a somatoaxonal distribution of hyperphosphorylated tau in PD indicate that different primary mechanisms may lead to the cytoskeletal lesions characteristic of PD and AD.

Acknowledgements We thank Dr. E. Vanmechelen (Innogenetics) for AT8, AT10, AT180 and AT270, Dr. P. Seubert (Athena Neurosciences) for 12E8, Dr. P. Frey (Sandoz) for N-Tau 5, and Dr. S. Greenberg for PHF-1. We also thank Dr. C. Bouras, P. G. Valet and M. Surini, Department of Psychiatry, University of Geneva, for computer-assisted microscopy of AT8-IR neurites and threads.

References

1. Bancher C, Brunner C, Lassmann H, Budka H, Jellinger K, Wiche G, Seitelberger F, Grundke-Iqbal I, Iqbal K, Wisniewski HM (1989) Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. Brain Res 477:90–99
2. Binder LI, Frankfurter A, Rebhun LI (1985) The distribution of tau in the mammalian central nervous system. J Cell Biol 101:1371–1378
3. Braak E, Braak H (1994) A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. Acta Neuropathol 87:554–567
4. Braak H, Braak E, Grundke-Iqbal I, Iqbal K (1986) Occurrence of neuropil threads in the senile human brain and in Alzheimer's disease: a third location of paired helical filaments outside of neurofibrillary tangles and neuritic plaques. Neurosci Lett 65:351–355
5. Catala I, Ferrer I, Calofré E, Fabregues I (1988) Decreased numbers of dendritic spines on cortical pyramidal neurons in dementia. A quantitative Golgi study on biopsy samples. Human Neurobiol 6:255–259
6. Cochran EJ, Fox JH, Mufson EJ (1994) Severe panencephalic Pick's disease with Alzheimer's disease like neuropil threads and synaptophysin immunoreactivity. Acta Neuropathol 88:479–484
7. Ferrer I, Guionnet N, Cruz-Sanchez F, Tunon T (1990) Neuronal alterations in patients with dementia: a Golgi study on biopsy samples. Neurosci Lett 114:11–16
8. Goedert M, Spillantini MG, Jakes R, Rutherford D, Crowther RA (1989) Multiple isoforms of human microtubule associated protein tau: sequences and location in neurofibrillary tangles of Alzheimer's disease. Neuron 2:519–526
9. Goedert M, Jakes R, Crowther RA, Cohen P, Vanmechelen E, Vandermeeren P, Cras P (1994) Epitope mapping of monoclonal antibodies to the paired helical filaments of Alzheimer's disease: identification of phosphorylation sites in tau protein. Biochem J 301:872–877
10. Goedert M, Jakes R, Vanmechelen E (1995) Monoclonal antibody AT8 recognizes tau protein phosphorylated at both serine 202 and threonine 205. Neurosci Lett 189:167–170
11. Goetz J, Probst A, Spillantini MG, Schäfer Th, Jakes R, Bürki K, Goedert M (1995) Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform. EMBO J 14:1304–1313
12. Greenberg SG, Davies P, Schein JD, Binder LI (1992) Hydrofluoric acid-treated tau PHF proteins display the same biochemical properties as normal tau. J Biol Chem 267:564–569
13. Hof PR, Bouras C, Perl P, Morrison JH (1994) Quantitative neuropathologic analysis of Pick's disease cases: cortical distribution of Pick bodies and coexistence with Alzheimer's disease. Acta Neuropathol 87:115–124

14. Iwatsubo T, Hasegawa M, Ihara Y (1994) Neuronal and glial tau-positive inclusions in diverse neurologic diseases share common phosphorylation characteristics. Acta Neuropathol 88: 129–136
15. Kato S, Nakamura H (1990) Presence of two different fibril subtypes in Pick bodies: an immunoelectron microscopic study. Acta Neuropathol 81: 125–129
16. Love S, Saitoh T, Quij S, Cole GM, Terry RD (1988) Alz 50, ubiquitin and tau immunoreactivity of neurofibrillary tangles, Pick bodies and Lewy bodies. J Neuropathol Exp Neurol 47: 393–405
17. Love S, Burrola P, Terry RD, Wiley CA (1989) Immunoelectron microscopy of Alzheimer and Pick brain tissue labelled with the monoclonal antibody Alz 50. Neuropathol Appl Neurobiol 15: 223–231
18. Mercken M, Vandermeeren M, Lübke U, Six J, Boons J, Vaan de Voorde A, Martin JJ, Gheuens J (1992) Monoclonal antibodies with selective specificity for Alzheimer tau are directed against phosphatase-sensitive epitopes. Acta Neuropathol 84: 265–272
19. Munoz-Garcia D, Ludwin SK (1984) Classic and generalized variants of Pick's disease: a clinicopathological, ultrastructural and immunochemical comparative study. Ann Neurol 16: 467–480
20. Murayama S, Mori H, Ihara Y, Tomonaga M (1990) Immunocytochemical and ultrastructural studies of Pick's disease. Ann Neurol 27: 394–405
21. Nakamura Y, Takeda M, Yoshimi K, Hattori H, Hariguchi S, Hashimoto S, Nishimura T (1994) Involvement of clathrin light chains in the pathology of Pick's disease. Neurosci Lett 180: 25–28
22. Otvos L, Feiner L, Lang E, Szendrei G, Goedert M, Lee VM-Y (1994) Monoclonal antibody PHF-1 recognizes tau protein phosphorylated at serine residues 396 and 404. J Neurosci Res 39: 669–673
23. Perry G, Stewart D, Friedmann R, Manetto V, Autilio-Gambetti L, Gambetti P (1987) Filaments of Pick's body contain altered cytoskeletal elements. Am J Pathol 127: 559–567
24. Probst A, Anderton B, Ulrich J, Kohler R, Kahan J, Heitz PU (1983) Pick's disease: An immunocytochemical study of neuronal changes. Monoclonal antibodies show that Pick bodies share antigenic determinants with neurofibrillary tangles and neurofilaments. Acta Neuropathol (Berl) 60: 175–182
25. Seubert P, Mawal-Dewan M, Barbour R, Jakes R, Goedert M, Johnson GVW, Literski JM, Schenk D, Lieberburg I, Trojanowski JQ, Lee VM-Y (1995) Detection of phosphorylated Ser 262 in fetal tau, adult tau and paired helical filament-tau. J Biol Chem 270: 18917–18922
26. Su JH, Cummings BJ, Cotman CW (1994) Early phosphorylation of tau in Alzheimer's disease occurs at Ser-202 and is perferentially within neurites. Neuro Report 5: 2358–2362
27. Szendrei GI, Lee VM-Y, Otvos L (1993) Recognition of the minimal epitope of monoclonal antibody tau-1 depends on the presence of a phosphate group but not on its location. J Neurosci Res 34: 243–249
28. Ulrich J, Haugh M, Anderton BH, Probst A, Lautenschlager C, His B (1987) Alzheimer dementia and Pick's disease: neurofibrillary tangles and Pick bodies are associated with identical phosphorylated neurofilament epitopes. Acta Neuropathol 73: 240–246
29. Wechsler AF, Berity MA, Rosenschein S, Friede I, Scheibel AB (1982) Pick's disease. A clinical, computed tomographic and histological study with Golgi impregnation observations. Arch Neurology 39: 287–290
30. Williams HW (1935) The peculiar cells of Pick's disease. Their pathogenesis and distribution in disease. Arch Neurol Psychiatry 34: 508–519
31. Yamagushi H, Nakazato Y, Shoji M, Ihara Y, Hirai S (1990) Ultrastructure of the neuropil threads in the Alzheimer brain: their dendritic origin and the accumulation in the senile plaques. Acta Neuropathol 80: 368–374
32. Yashuhara O, Matsuo A, Tooyama I, Kimura H, McGeer EG, McGeer PL (1994) Pick's disease immunohistochemistry: new alterations and Alzheimer's disease comparison. Acta Neuropathol 89: 322–330