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REGULAR PAPER

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Tau accumulation in astrocytes in progressive supranuclear palsy is a degenerative rather than a reactive process

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Abstract Tau-immunoreactive astrocytes in progressive supranuclear palsy (PSP) have a distinctive morphology and are referred to as tufted astrocytes (TA). We hypothesized that TA may be a degenerative change in reactive astrocytes. To test this hypothesis we examined the relationship of TA to gliosis in PSP. We first examined the distribution of gliosis [glial fibrillary acid protein (GFAP)-positive astrocytes], TA, neurofibrillary tangles (NFT) and pretangles in brain sections of neuropathologically pure PSP cases. Second, we examined PSP cases complicated by infarcts or Alzheimer's disease, since these cases would have reactive astrocytes associated with lesions. We used double immunostaining for GFAP and tau for cases with vascular lesions, and triple immunostaining for GFAP, tau and β-amyloid protein for sections with senile plaques. There was no correlation between the distribution of gliosis and TA, with gliosis prominent in globus pallidus and subthalamic nucleus, and TA prominent in motor cortex and striatum. On the other hand, gliosis paralleled the distribution of NFT, but not the distribution of pretangles, suggesting that NFT contributes to gliosis in PSP. Although reactive astrocytes were present around infarcts and senile plaques, TA were not associated with these lesions. Tau accumulation in astrocytes in PSP was not preferential to (and was actually independent of) reactive astrocytes. This is consistent with the notion that tau accumulation in astrocytes is a degenerative rather than reactive process. Unlike NFT, astrocytic degeneration does not seem to contribute to gliosis or neuronal loss in PSP, and its clinical significance remains unclear.

Keywords Progressive supranuclear palsy · Tufted astrocytes · Gliosis · Neurofibrillary tangles

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Introduction

Progressive supranuclear palsy (PSP) is clinically characterized by parkinsonism, vertical eye movement abnormalities and subcortical dementia [18], and is the second most frequent cause of degenerative parkinsonism after Lewy body parkinsonism [4]. First reported by Steele and coworkers [21], the pathology of PSP includes neuronal loss and gliosis in subcortical nuclei, particularly the substantia nigra and basal ganglia. Recent studies using tau immunostaining or the Gallyas silver stain have revealed pathological features not previously suspected in PSP and indicative of widespread neuronal and glial tau pathology. Tau protein accumulates in both neurons and glia in PSP, in contrast to Alzheimer's disease, where tau inclusions are mainly neuronal. Neurofibrillary tangles (NFT) in PSP often have a globose appearance and tangles in astrocytes also have a distinctive morphology. Tau-immunoreactive astrocytes in PSP have been referred to as tufted astrocytes (TA) [13, 22, 23]. It remains unknown if there is any clinical significance to glial lesions in PSP.

Tau pathology is central to several other neurodegenerative conditions [20] and its presence may reflect not only a degenerative process, but also a reactive process. Examples of tau-positive lesions that are considered to be reactive include dystrophic neurites in senile plaques [9] and so-called thorn-shaped astrocytes [16] found frequently in subpial and perivascular locations at the base of the brain. In the present study, we examined the relationship of TA to reactive glial changes to test the hypothesis that TA represented tau accumulation in reactive astrocytes.

Materials and methods

The present study had two parts. We first examined the distribution and density of TA, NFT, pretangles and gliosis, as reflected by glial fibrillary acid protein (GFAP)-positive astrocytes [10], in brain sections of neuropathologically pure PSP cases. Secondly, we examined PSP cases complicated by infarcts or AD, since these cases would have reactive astrocytes associated with lesions. We used double immunostaining for GFAP and tau for cases with vascular

lesions, and triple immunostaining for GFAP, tau and Aβ for cases with senile plaques.

Brain sections from 21 patients with pathologically confirmed PSP [14] were used, including 10 cases with pure PSP [2 men, 8 women; age at death (mean ± SD) 69.5±7.1 years], 6 cases with concomitant vascular pathology such as lacunar infarcts or hemorrhages (4 men, 2 women; age at death 77.3±8.2 years) and 5 patients with concomitant Alzheimer pathology (1 men, 4 women; age at death 81.4±5.3 years). Blocks of tissue containing precentral gyrus, putamen, globus pallidus, thalamus, subthalamic nucleus, midbrain, pons, medulla, cerebellar dentate nucleus and samples of the focal brain lesions were dissected from formalin-fixed brain and embedded in paraffin. Sections were cut at a thickness of 5 μm and used for this study.

The deparaffinized and rehydrated sections were microwaved in distilled water at high-power setting for 7 min, and then incubated in 0.01 M phosphate saline (PBS; pH 7.4) containing 0.3% hydrogen peroxide (H_2O_2) for 20 min. To examine the density and distribution of TA, NFT, pretangles and gliosis, the sections of pure PSP cases were treated with 5% normal goat/horse serum for 20 min and incubated overnight with two anti-tau antibodies {CP13; anti-phosphorylated tau (S202) antibody, mouse IgG1, 1:100, from Dr. P. Davies; Ab39; anti-NFT antibody, mouse IgG1, 1:10, from Dr. S.-H. Yen [24]} and an anti-GFAP antibody (rabbit polyclonal, 1:200, BioGenex; San Ramon, Calif.). For staining with Ab39, sections were treated with 0.1 mg/ml proteinase K for 10 min in 37°C before incubation with primary antibody. After incubation with primary antibody, sections were next treated with biotinylated anti-mouse/rabbit IgG secondary antibody (1:200, Vector Labs, Burlingame, Calif.) for 2 h followed by incubation in the avidin-biotinylated horseradish peroxidase (HRP) complex (ABC Elite; 1:200, Vector Labs) for 2 h. Peroxidase labeling was detected with 0.3 mg/ml 3,3'-diaminobenzidine (DAB) and 0.03% H_2O_2. After single immunostaining, sections were immersed in hematoxylin briefly for counterstaining.

To examine the relationship between TA and gliosis, double immunostaining for GFAP and tau (CP13) in the sections with vascular lesions was carried out. For the double immunostaining, sections were treated for 20 min with 0.1 M glycine pH 2.5, and 20 min with 0.3% H_20_2 solution in PBS after the DAB reaction of the first immunohistochemical cycle with CP13. The second immunohistochemical cycle for GFAP was carried out similarly, except that the HRP complex was detected by Vector SG (1:50, Vector Labs).

For triple immunostaining of GFAP, tau and Aβ, the sections were pretreated with 98% formic acid for 30 min in addition to above-mentioned treatment with microwave, H_2O_2 and normal goat/horse serum. The sections were incubated overnight with a cocktail of anti-GFAP antibody (mouse IgG, 1:100, BioGenex), anti-tau antibody (Alz50; tau conformation-dependent antibody, mouse IgM, 1:5, from Dr. P. Davies) and anti-Aβ antibody (AB5306; Aβ37-42, rabbit polyclonal, 1: 250, Chemicon; Temecula, Calif.). After incubation with the primary antibodies, sections were treated with biotinylated anti-mouse IgM secondary antibody for 2 h followed by incubation with a cocktail of anti-avidin cy2, anti-rabbit IgG cy3 and anti-mouse IgG cy5 (cyanine dyes, 1:100; Jackson Immunoresearch, Pa.) for 2 h, and observed with fluorescence microscopy.

The density of TA, NFT, pretangles and gliosis in each region was estimated semiquantitatively for the sections stained with CP13, Ab39 and GFAP and graded as 0 (absent or rare), 1 (low density: 1–3 per ×40 field), 2 (high density: 4–6 per ×40 field) or 3 (very high density: 7 or more per ×40 field). TA were defined as star-like tufts of tau-positive abnormal fibers [13, 22, 23] and pretangles were defined as neuronal staining with a granular, rather than fibrillary morphology [2, 5]. TA and pretangles were estimated in sections stained with CP13. Multiple linear regression was used to examine the relative contribution of TA, NFT and pretangles to gliosis with respect to brain regions (Sigma Stat for Windows Ver. 2.03, Jandel Scientific).

Results

Two antibodies for neurofibrillary pathology, CP13 and Ab39, labeled essentially the same structures in astrocytes in PSP. In all 21 PSP cases examined, at least some TA were detected. TA appeared predominantly in the precentral gyrus, caudate/putamen, ventral thalamus, midbrain tectum, red nucleus and subthalamic nucleus (see Fig. 2A). On the other hand, gliosis was predominant in the globus pallidus, subthalamic nucleus, substantia nigra, ventral thalamus, red nucleus and midbrain tegmentum. There was no apparent relationship between the density of TA and gliosis (see Fig. 2A). For example, TA were numerous in regions that lacked significant gliosis, such as the precentral gyrus (Fig. 1A, B), and low in spite of severe gliosis in other regions, such as the subthalamic nucleus (Fig. 1C, D). The distribution of TA and gliosis were almost the same in all cases, whereas the relative severity of the density of the two lesions varied from case to case.

The phospho-tau antibody, CP13, was a sensitive marker for both NFT and pretangles, whereas Ab39 stained only NFT. Pretangles were not immunoreactive for Ab39, as expected from previous studies [24]. Both NFT and pretangles (CP13) were detected in all cases, and they were most numerous in the subthalamic nucleus, basal nucleus, pontine base, substantia nigra, hypothalamus and locus coeruleus (data not shown). On the other hand, NFT (Ab39), but less so for pretangles, were predominant in the locus coeruleus, subthalamic nucleus, basal nucleus, substantia nigra, pontine base and globus pallidus (Fig. 2B). The distributions of NFT and pretangles were almost the same in all cases, but the relative densities varied. Gliosis paralleled the distribution and density of NFT (Fig. 2B). Multiple linear regression as well as Spearman rank order correlation analysis demonstrated that gliosis could be predicted based upon anatomical region ($P<0.001$) and NFT ($P=0.008$), but not on TA ($P=0.306$) and pretangles ($P=0.458$).

In PSP cases with vascular or Alzheimer-type pathology double-immunostained with CP13 and anti-GFAP, TA were not prominent in the vicinity of cortical or basal ganglia infarcts in spite of severe gliosis associated with the infarcts (Fig. 1E). Similarly, in sections with senile plaques triple-immunostained with anti-GFAP, Alz50 and anti-Aβ37–42 antibody, TA were not prominent around senile plaques, even for plaques in the motor cortex, where TA were numerous and despite the presence of reactive astrocytes associated with senile plaques (Fig. 1F). Although the morphology of TA observed with fluorescent microscopy was not typical, they were confirmed to be TA in adjacent tau-immunostained sections. Morphological diversity of TA by different staining methods was described previously [17].

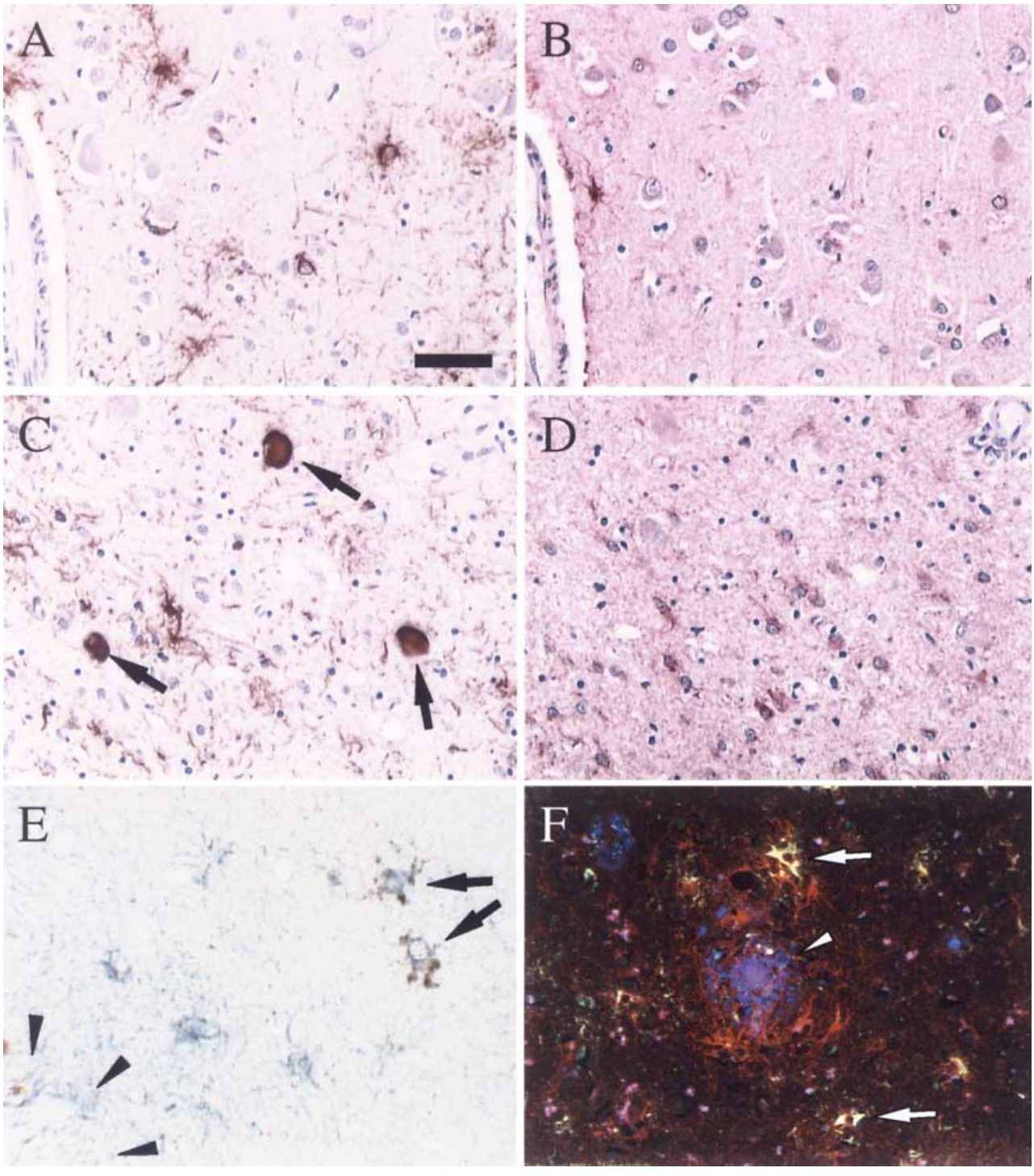

Fig. 1 **A**, **B** Adjacent sections of precentral gyrus in PSP immunostained with CP13 (**A**) and anti-GFAP (**B**). A number of TA are present (**A**), but only rare GFAP-positive astrocytes (**B**). **C**, **D** Adjacent sections subthalamic nucleus immunostained with CP13 (**C**) and anti-GFAP (**D**). No TA are seen (**C**) despite many GFAP-positive astrocytes (**D**) (globose NFT, *arrows*). Double immunostaining with CP13 (*brown*) and anti-GFAP (*blue*) in PSP with a striatal lacunar infarct. **E** Reactive astrocytes, but not TA (*arrows*), are present around the infarct (*arrowheads*). **F** Triple immunostaining with anti-GFAP (*red*), Alz50 (*green*) and anti-$A\beta_{37–42}$ (*blue*) in PSP with concurrent AD. Reactive astrocytes (*red*), but not TA (*yellow*, *arrow*), are associated with the senile plaque (*blue*, *arrowhead*) (*PSP* progressive supranuclear palsy, *TA* tufted astrocytes, *NFT* neurofibrillary tangles, *GFAP* glial fibrillary acidic protein)

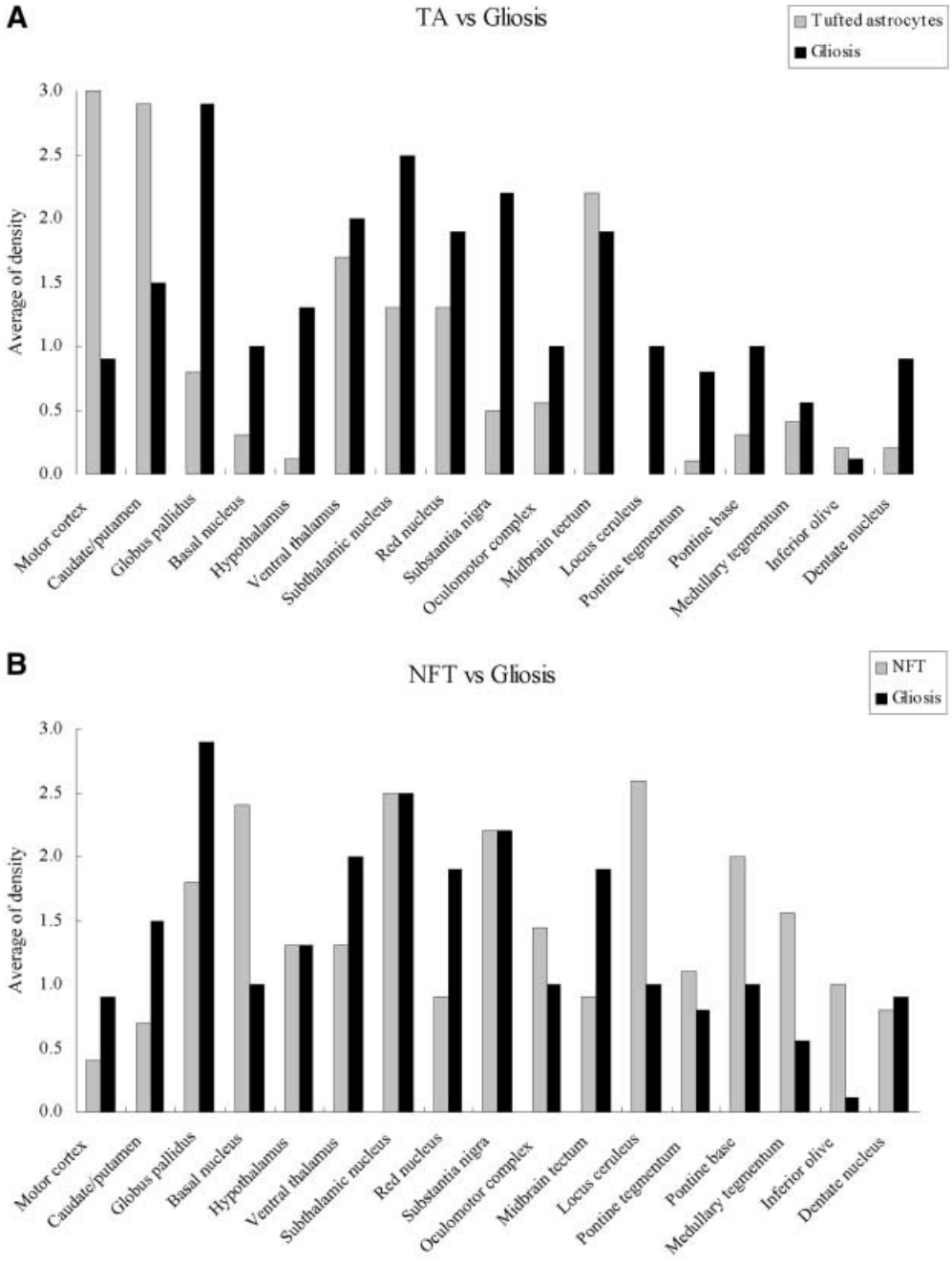

Fig. 2A, B The density of TA, NFT and gliosis in each region of ten cases with pure PSP was estimated semiquantitatively for sections stained with CP13 (phosphorylated tau), Ab39 (fibrillary tangles) and GFAP (astrocytes) and graded as *0* (absent or rare), *1* (low density: 1–3 per ×40 field), 2 (high density: 4–6 per ×40 field) or 3 (very high density: 7 or more per ×40 field). The density of pretangles was also estimated for the sections stained with CP13. The density of TA does not correlate with that of reactive astrocytes (**A**), but parallels NFT (**B**)

Discussion

Tau-positive astrocytes in PSP were first described by Hauw et al. [13]. Subsequently, Yamada et al. [22, 23] proved their astrocytic origin. Tau-positive astrocytes in PSP are commonly referred to as TA. A series of studies using immunohistochemistry have also revealed other types of tau-immunoreactive astrocytes. To date, at least three distinctive types of tau-positive astrocytes are recognized: TA, astrocytic plaques [11], and thorn-shaped astrocytes [16]. Of these TA of PSP and astrocytic plaques of corticobasal degeneration are considered to be diagnostically significant lesions in that they rarely coexist [17]. The diagnostic value of TA in PSP brains, as shown in previous publications [14, 19], was bolstered by the present study. We detected TA in all PSP cases examined. There have been several reports suggesting the neuropathological and clinical heterogeneity of PSP [7, 8, 12, 14], and atypical PSP cases are occasionally found during routine neuropathological examinations. Although it may be difficult to classify all of such cases at the moment, the present results suggest that TA may not only be specific for PSP, but also necessary for neuropathological diagnosis of typical PSP.

In this study, we speculated that tau-positive astrocytes in PSP might be a reactive phenomenon; however, we failed to show a consistent relationship between tau-posi-

tive astrocytes and reactive astrocytes in PSP. Tau accumulates as a secondary or reactive process in the dystrophic neurites of senile plaques [9], but also as a primary degenerative process in cases of frontotemporal dementia with mutations in the tau gene [15]. Similarly, tau accumulates in astrocytes in the subpial and perivascular space at the base of the brain of elderly individuals presumably as a reactive age-related change. These astrocytes are referred to as thorn-shaped astrocyte [16]. In the present study, we attempted to determine if TA in PSP brains were reactive secondary lesions or primary degenerative changes in astrocytes. We found that tau accumulation in TA in PSP was not preferential to reactive astrocytes and was actually independent of reactive gliosis. This is consistent with the notion that tau pathology in astrocytes in PSP is a degenerative rather than a reactive process.

Another outcome of the present study was the observation that reactive astrocytes do not parallel the distribution of TA, but rather NFT. Reactive gliosis is common to many neuropathological disorders and is especially common in disorders associated with neuronal degeneration and neuronal loss. Not surprisingly, therefore, we found that gliosis paralleled the distribution and density of NFT in PSP. This suggests that NFT, neuronal loss and gliosis are anatomically specific and likely major contributors to clinical symptoms in PSP. This fits with increasing evidence from studies of aging and AD where signs and symptoms have been shown to correlate well with NFT density and distribution [1, 3, 6].

The clinical significance of tau accumulation in astrocytes is not clear at present, but the present results suggest that it is an inherent part of the degenerative process of PSP and, furthermore, that it is independent of gliosis.

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