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Progressive supranuclear palsy with hypertrophy of the olives*

An immunocytochemical study of the cytoskeleton of argyrophilic neurons

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Summary. In a patient with progressive supranuclear palsy (PSP) and hypertrophy of the olives, neurons with different forms of argyrophilic degeneration were detected by means of Bodian's silver staining method, i.e., neurofibrillary tangle-bearing neurons in the basal ganglia and brain stem, ballooned argyrophilic neurons in the brain stem, and hypertrophied neurons in the olives. In these cells, the cytoskeleton was investigated to ascertain whether neurons with different cytoskeletal changes contained phosphorylated neurofilaments (P-Nf) in the perikaryon. This study, carried out using two monoclonal antibodies that recognize phosphorylated epitopes of the neurofilament high molecular weight subunits, showed that hypertrophied olivary neurons, most ballooned neurons and a small aliquot of tangle-bearing neurons were labelled. The immunostaining of hypertrophied and ballooned neurons was localized in the whole perikaryon and dendrites, whereas that of tangle-bearing neurons was confined to the tangle. These findings were reproduced in five additional patients (one with hypertrophy of the olives, four with PSP) and demonstrated that, in PSP, the mechanism responsible for tangle formation does not affect the ability of neurons to accumulate P-Nf. This fact suggested that perikaryonal P-Nf accumulation is likely to be part of the cell reaction to abnormal conditions affecting the neuronal cytoskeleton.

Key words: Progressive supranuclear palsy – Hypertrophy of the olives – Neurofilaments – Immunocytochemistry

In healthy neurons, phosphorylated neurofilaments (P-Nf) are confined in the axon [26]. The pathology of the cytoskeleton often induces P-Nf accumulation in the perikaryon of the nerve cell. Perikaryonal immunoreactivity with antibodies to P-Nf has been demonstrated, in fact, in neurofibrillary bundles of amyotrophic lateral sclerosis [18] and aluminum-induced encephalomyelopathy [3, 30], in Lewy bodies of Parkinson's disease [10], and in neurons after axotomy [22]. In Alzheimer's disease, immunoreactivity of neurofibrillary tangles with antibodies to P-Nf has been reported [6, 12, 28, 31], likely to be due, however, to cross reactivity with phosphorylated epitopes of tau proteins [15, 19]. Progressive supranuclear palsy (PSP) is characterized by two types of nerve cell degeneration involving subcortical neurons: neurofibrillary tangles (NFT) with curvilinear bodies in the perikaryon [25], and ballooned degeneration with perikaryonal homogeneous argyrophilia [5]. PSP tangles are due to the accumulation of 15-nm straight filaments [29]. According to a report based on five patients [1], PSP tangles are negative for P-Nf, whereas they are immunoreactive with anti-tau antibodies. Here, we report that in PSP both NFT-bearing neurons and ballooned neurons contain, although in a quite different proportion, perikaryonal epitopes reacting with antibodies to P-Nf. This study was carried out in a patient with a rare association between PSP and hypertrophy of the olives. Hypertrophied olivary neurons, which contain abnormal amounts of perikaryonal neurofilaments [2, 13], were regarded as the immunocytochemistry-matched control for NFT-bearing neurons and ballooned neurons. As to the immunocytochemical properties of the argyrophilic (NFT-bearing, ballooned, and hypertrophied olivary) neurons, this patient was compared with five additional patients, one with hypertrophy of the olives and four with PSP.

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Case report

Personality changes, undue anger reactions, forgetfulness and sudden awakening at night were the first symptoms of the disease that appeared when the patient was 45. Three years later, incontinence, impotence and difficulties with balance and walking, mostly ataxic gait and unsteadiness when turning, also developed. At that time, voluntary eye movements were normal, whereas facial expression was vacant, blinking poor, pupils small and minimally reacting to light, lacrimation unexpectedly abundant. Slight axial rigidity with limitation of armswing appeared 1 year later. According to his wife, the patient had blurred vision ("He sometimes behaves as if he is unable to see various objects. He often has to be told what is on his plate because he does not comprehend, or does not see, exactly what it is, or where, he is eating. If he happens to drop something, he appears to look for it, but does not seem to see it"). Later, dysphagia and dysarthria also appeared and slowness of movements became extreme. At the same time, voluntary conjugate vertical eye movements were absent, while the corresponding horizontal movements were slow and limited. Palatal myoclonus, which is commonly related to the hypertrophy of the olives [9], was never observed. The patient died at 52. Necropsy was performed 3 h after death.

Neuropathological study

For this study, 10 μm-thick sections from formaldehyde-fixed, paraplast-embedded blocks of the cerebral and cerebellar hemispheres and brain stem, stained with routine (H&E, cresyl violet, Heidenhain-Woelke, Holzer's) and Bodian's methods, were used. The immunocytochemical study was conducted using two monoclonal antibodies (Mab) anti-P-Nf, SMI31 (Sternberger-Meyer Immunocytochemicals) and BM200 (Boehringer-Mannheim), anti-glial fibrillary acidic protein (GFAP) monoclonal and polyclonal antibodies, and Mab raised against cytokeratin (Labsystem), followed by a standard peroxidase-anti peroxidase (PAP) method [27]. Mab SMI31 is known to recognize phosphorylated epitopes of the neurofilament high molecular weight subunits (Sternberger-Meyer Immunocytochemical, Catalog 1984), and most closely to resemble Mab 06-17 (LA Sternberger, personal communication), which reacts with phosphorylated epitopes of axonal neurofilaments [26] and of neurofilament bundle-bearing neurons of experimental aluminum-induced encephalomyelopathy [3, 30]. A cross reaction of SMI31 with phosphorylated epitopes of tau proteins has been observed on immunoblots [19]. BM200 recognizes phosphorylated epitopes of the neurofilament 200 kDa subunit, whereas does not cross react with tau proteins [15, 19].

In our experience, that partly confirmed previous reports [17, 19, 20], Mab SMI31 and BM200 recognized axons, not nerve cell bodies or dendrites, in normal human, rabbit, and rat formaldehyde-fixed brain tissue. Furthermore, they did recognize axonal torpedoes of Purkinje cells in several pathological conditions and neurofilament bundles of experimental aluminum-induced encephalomyelopathy, but not NFT of Alzheimer's disease.

Results

Neuropathological findings

Routine staining methods disclosed neurofibrillary tangles, nerve cell loss, simple and pigmentary atrophy of neurons, astrocytosis and fibrillary gliosis. Such lesions mainly involved brain stem, pallidum and basal forebrain, substantia nigra, and cerebellar dentate. Bulbar olives were slightly enlarged and presented with a marked astrocytosis. In preparations stained with Bodian's, most neurofibrillary tangles were of the globose type and presented with either whorls of curvilinear, discontinuous structures (also evidenced by H&E, Fig. 1a) or round, homogeneously argyrophilic bodies (Fig. 2a). Most NFT-bearing neurons were found in subthalamic nucleus, upper reticular formation and raphe nuclei. Periaqueductal gray and pontine tegmentum con-

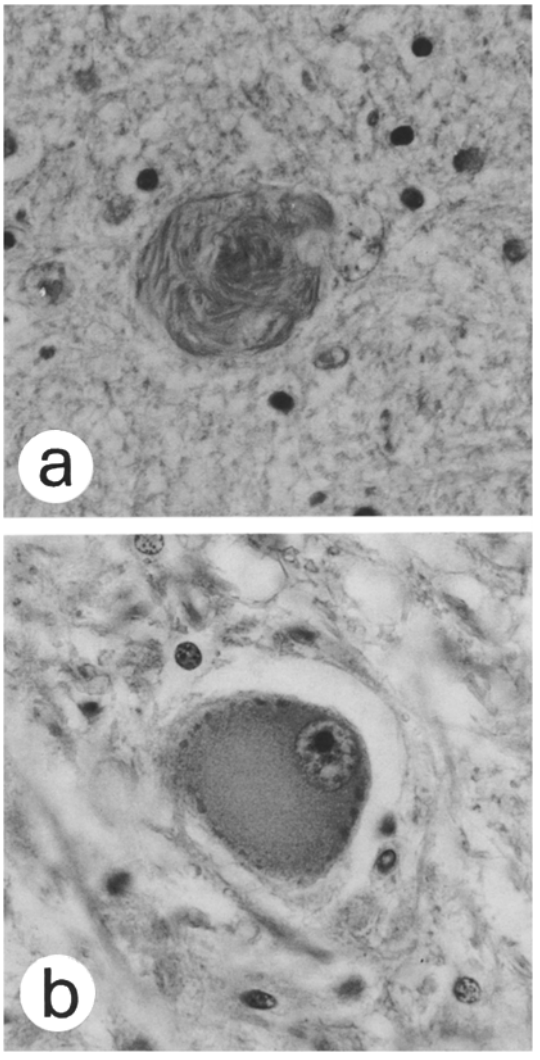

Fig. 1a, b. A NFT-bearing neuron (**a**) compared with a ballooned neuron (**b**), both observed in the reticular nucleus of the pontine tegmentum. The perikaryon of the NFT-bearing neuron contains whorls of curvilinear bodies, whereas the perikaryon of the ballooned neuron contains uniformly distributed, eosinophilic material. H&E, ×680

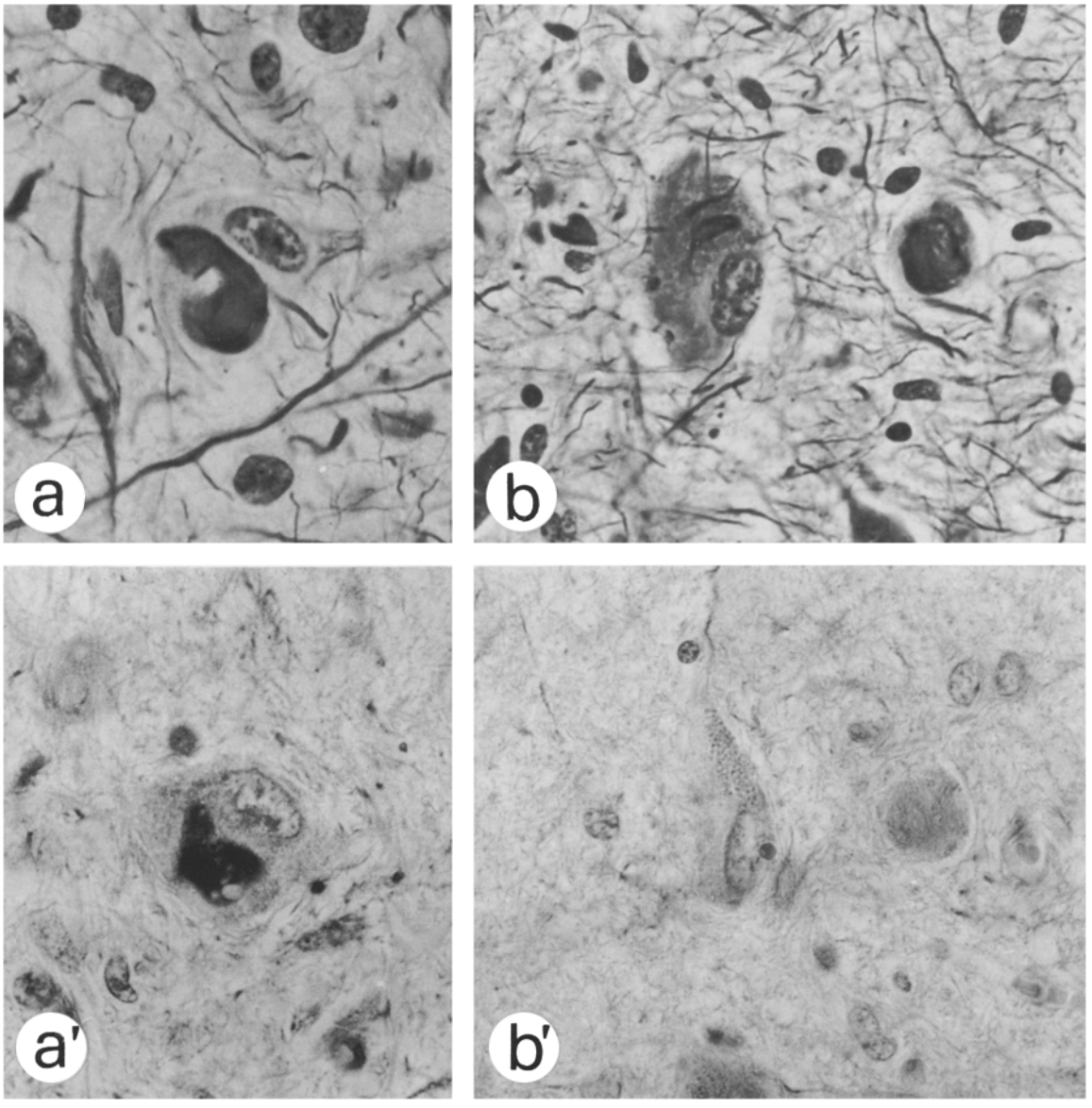

Fig. 2a, b, a′, b′. NFT-bearing neurons. Adjacent sections show that some NFT react with Mab SMI31 (**a, a′**), while others do not (**b, b′**). Only one out of 20 NFT-bearing neurons was labelled by SMI31. **a, b** Bodian's, **a′, b′** Mab SMI31; **a, a′**, ×680; **b, b′**, ×1200

tained not only NFT-bearing neurons, but also ballooned neurons, which differed from the former in having argyrophilic material diffusely distributed within the cell body (Fig. 4a) in an amount large enough to displace the nucleus toward the cell membrane (Fig. 1b). In bulbar olives, neurons were either hypertrophied, vacuolated (Fig. 5a) and provided with enlarged dendrites (Fig. 5b), or replaced by argyrophilic bodies composed of large, curved cell processes. In the absence of focal lesions in the dentato-olivary pathway, olivary changes were regarded as secondary to nerve cell loss in the dentate nuclei. Cell loss was more severe in the ventrolateral part of the cerebellar dentate and, according to the somatotopic organization of the dentato-olivary pathway [16], hypertrophy was prominent in the dorsal portion of the olives.

Immunocytochemical findings

Mab SMI31 (1:1000 to 1:40000) and BM200 (1:10 to 1:100) labelled NFT-bearing neurons, ballooned neurons and hypertrophied olivary neurons. In hypertrophied and ballooned neurons, immuno-

reactivity was diffusely distributed over the perikaryon and dendrites (Figs. 4b; 5c, d). In NFT-bearing neurons, immunoreactivity, when present, was restricted to the tangle (Figs. 2a′; 3). Anti-GFAP, monoclonal (1:100) and polyclonal (1:800), disclosed hypertrophied astrocytes in bulbar olives. A semiquantitative evaluation, Bodian's vs SMI31 (counts were performed on six adjacent sections, 1, 3, 5 stained with Bodian's, 2, 4, 6 with SMI31), revealed 375 NFT, of which 18 labelled by SMI31, at the mesencephalic (nucleus supratrochlearis) level, 60 and 3, respectively, at the pontine (nn. pontis centralis caudalis, gigantocellularis, vestibularis lateralis) level, and 51 and 3, respectively, at the bulbar (nn. raphe pallidus, gigantocellularis, paragigantocellularis lateralis, dorsalis motorius nervi vagi) level. Figure 2 shows two NFT (a, b), one labelled (a′), the other not labelled (b′) by SMI31. In the same structures, the ratio of argyrophilic to SMI31-labelled ballooned neurons was 5 to 4. Counts, replicated with BM200 in the same nuclei, yielded identical ratios of argyrophilic to BM200-labelled neurons.

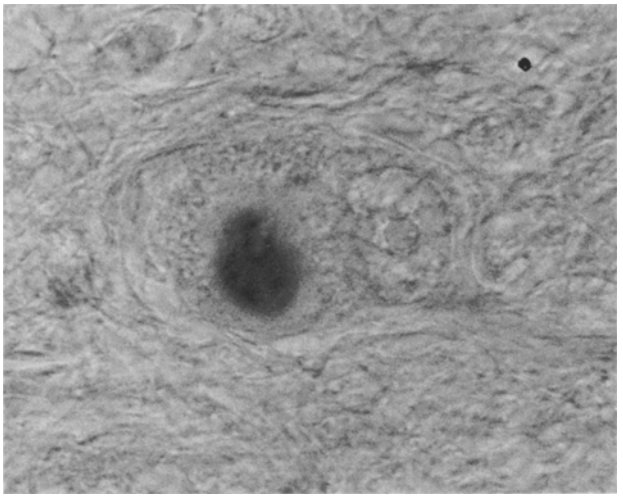

Fig. 3. PSP NFT-bearing neuron stained with Mab BM200. The immunoreactivity is strictly confined to the tangle. As SMI31, BM200 labelled only one out of 20 tangles. ×1450

Controls

Pretreatment with trypsin followed by calf intestinal phosphatase abolished immunoreactivity to SMI31 and BM200, whereas pretreatment with either trypsin or phosphatase was ineffective. Omission of the primary antibody or exposure to Mab against cytokeratin (1:100) resulted in no staining. In a patient with focal, unilateral olivary hypertrophy due to a small softening of the opposite cerebellar dentate, SMI31 and BM200 immunoreactivity was restricted to the hypertrophied olivary neurons. In four additional PSP patients, one out of 20 NFT was labelled by SMI31 and BM200; in these patients, the olivary neurons were not immunoreactive.

Discussion

In this patient with PSP and hypertrophy of the olives, the immunocytochemical study of different argyrophilic neurons (tangle-bearing, tegmental ballooned and olivary hypertrophied) carried out with

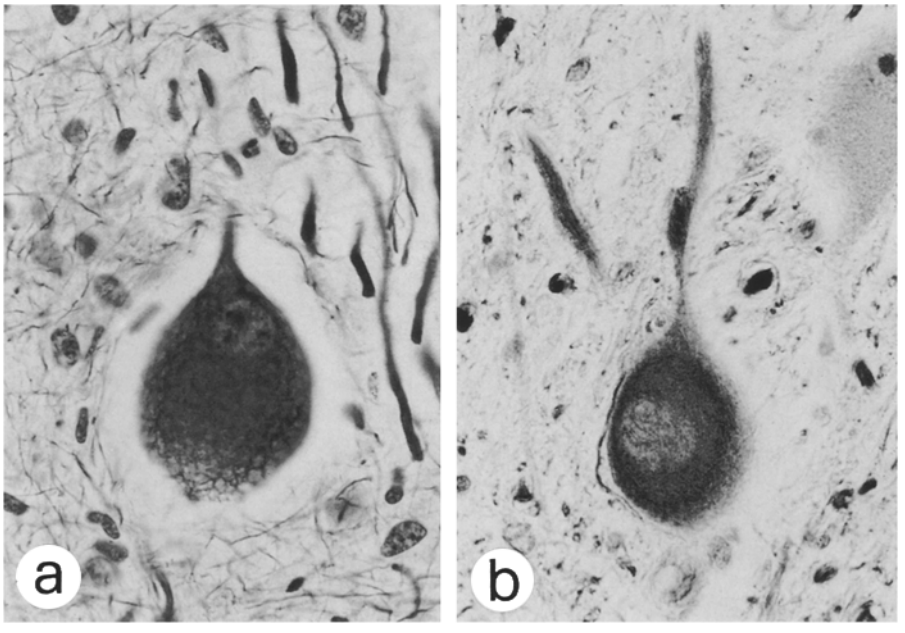

Fig. 4a, b. The perikaryon of ballooned neurons is filled with argyrophilic, diffusely distributed material (**a**) that, in four out of five cells, reacts with Mab SMI31 (**b**). ×610

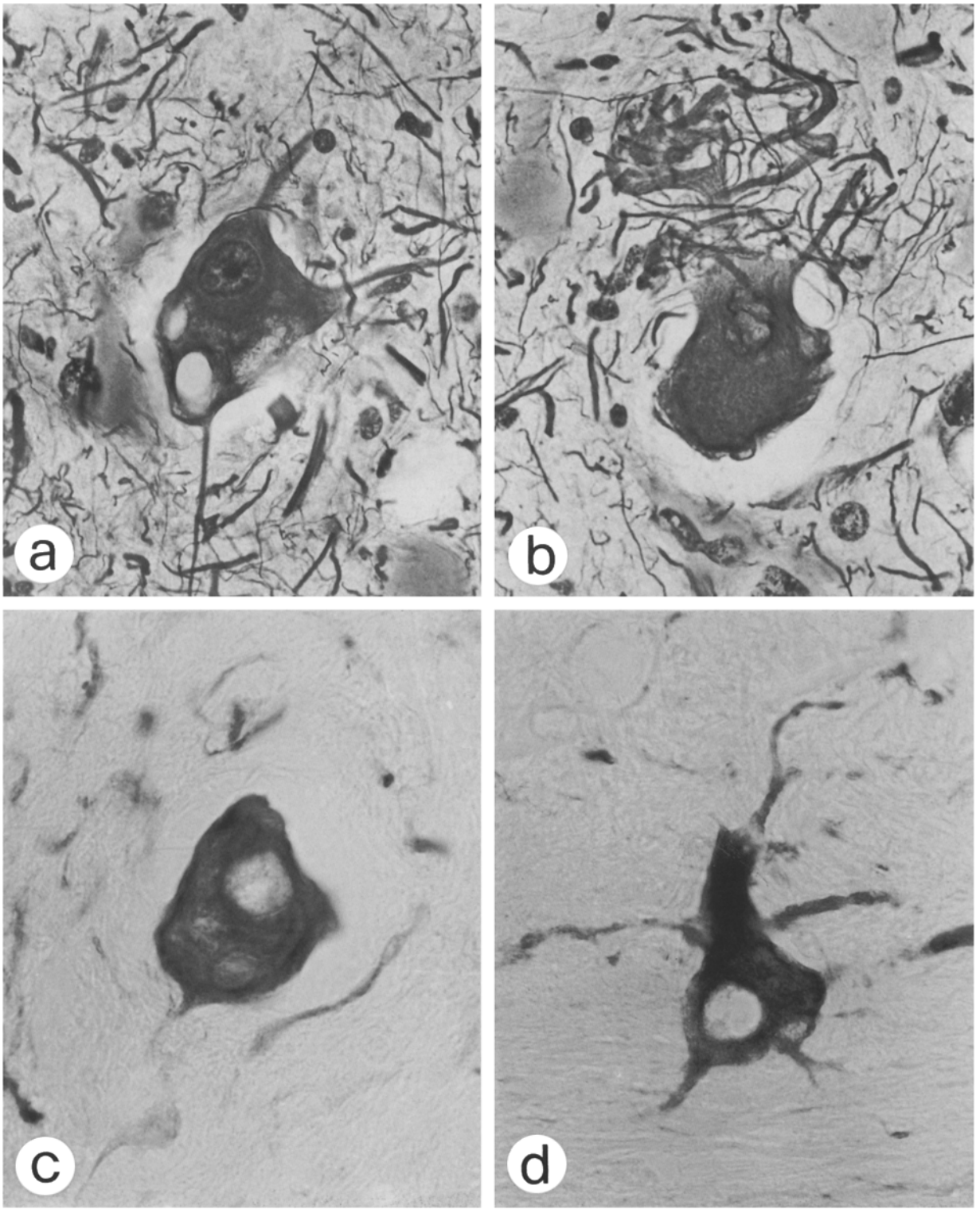

Fig. 5. a, b Hypertrophied olivary neurons appear as enlarged, vacuolated cells provided with thickened, convoluted dendrites and surrounded by hypertrophied astrocytes. **c, d** The immunoreactivity for phosphorylated epitopes of neurofilaments is uniformly distributed over both cell perikaryon and processes. **a, b** Bodian's; **c, d** Mab SMI31. ×680

two monoclonal antibodies to P-Nf, one of which did not cross react with tau proteins, gave identical results. A small aliquots of tangles were immunoreactive, while a large majority were not, and hypertrophied olivary neurons and most tegmental ballooned neurons were diffusely labelled. These findings do not fit the view that the accumulation of P-Nf in the perikaryon is strictly related to peculiar cytoskeletal changes like tangles, and raise the question of the relationship of tangle-bearing neurons to hypertrophied neurons and ballooned neurons.

The hypertrophy of olivary neurons has been related to deafferentation [16]. It is quite uncommon in PSP, being observed in a single patient [21] out of 75 whose neuropathological reports have been reviewed [4]. Its hallmarks are enlargement of nerve cell bodies and dendrites with 10-nm neurofilament accumulation, cytoplasmic vacuoles and astrocytosis [2, 11, 13, 14, 16, 24]. According to our present findings, hypertrophied olivary neurons contain P-Nf, a fact also shown in one illustration of a previous report [23]. In the olivary neurons of our PSP patient, a P-Nf to tangle relationship was unlikely, since perikaryonal immunoreactivity of olivary neurons for P-Nf was also present in a non-PSP control with, but absent in several PSP controls without, hypertrophy of the olives. These findings indicated, therefore, that in olivary neurons the presence of P-Nf depends on the reaction of the olives to deafferentation and that in PSP the involvement of the neuronal cytoskeleton specifically leading to the tangle does not affect the ability of neurons to accumulate neurofilaments.

Ballooned neurons, rather common in PSP [5], at the light microscopy level (such cells have not so far been investigated electron microscopically) differed from tangle-bearing neurons in that the argyrophilic material was homogeneously distributed in the perikaryon and, in four out of every five such cells, reacted uniformly with the anti-P-Nf antibodies. According to their morphological and immunocytochemical properties, ballooned neurons resembled hypertrophied olivary neurons more closely than tangle-bearing neurons. However, they were unlikely to be due to deafferentation, since this does not induce neuronal hypertrophy in structures other than the olives [7]. Also, they resembled neurons undergoing central chromatolysis after axotomy [22], or cortical neurons with perikaryonal P-Nf occurring in neurodegenerative diseases [8], for which axonal involvement has been postulated. In PSP, however, there is no evidence for ballooned degeneration of tegmental neurons due to axotomy.

Since a large majority of tangle-bearing neurons was not labelled by antibodies to P-Nf, a relationship between ballooned neurons and tangle-bearing neurons was unlikely. However, serial sections, alternately investigated for argyrophilic structures and P-Nf, showed that one tangle out of 20 was labelled by SMI31 and BM200. Since BM200 was shown not to cross react with tau proteins [15, 19], we had evidence that in PSP a small aliquot of tangles contain P-Nf. The relationship between such few tangles and the large majority that did not contain P-Nf remains to be elucidated. It is tempting to speculate, however, that tangles decorated by anti-P-Nf antibodies were the least mature among tangles and might have issued from ballooned neurons. Accordingly, it is noteworthy that ballooned neurons could be observed only in tegmental nuclei with numerous tangle-bearing neurons, while there is evidence that perikaryonal P-Nf accumulation following experimental axonal injury is time-related [22]. Further studies are needed to evaluate such hypotheses. Our present findings are likely to confirm, however, that accumulation of P-Nf in the perikaryon is part of a basic mechanism of nerve cell reaction to different abnormal conditions.

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