Ultrastructural study of Betz cells in the primary motor cortex of the human brain

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ABSTRACT

The ultrastructure of Betz cells in the 5th layer of the primary motor cortex of 17 neurologically and psychiatrically normal control individuals was studied. Normal-appearing Betz cells showed a wide range of features including novel electron-dense inclusion bodies (Bunina-like bodies) resembling Bunina bodies characteristic of amyotrophic lateral sclerosis (ALS), accumulations of neurofilaments (10 nm in diameter), bundles of filaments (20–25 nm in diameter) thicker than neurofilaments, lamellar structures, lamellar bodies and structures similar to Hirano bodies. Among these 'abnormal' features, the presence of Bunina-like bodies may be an age-related nonspecific degenerative change, since they appeared more frequently in elderly individuals. The presence of these abnormal features—particularly the Bunina-like bodies—in the Betz cells of normal human brains must be considered in the assessment of the pathognomonic significance of such structures in ALS and other neurological diseases that affect the motor cortex.

Key words: Motor cortex; Betz cell; Bunina-like body; ultrastructure; human brain; amyotrophic lateral sclerosis.

INTRODUCTION

Ultrastructural studies of Betz cells of the primary motor cortex have recently been conducted in patients with neurological diseases such as amyotrophic lateral sclerosis (ALS) (Murayama et al. 1992; Sasaki & Maruyama, 1994) and in normal monkeys (Tigges, 1992). However, to the best of our knowledge, little is known about the ultrastructural features of Betz cells in normal human controls (Sasaki & Maruyama, 1994). We have therefore used electron microscopy to examine Betz cells in the fifth layer of the cerebral motor cortex from control individuals without any neurological or psychiatric disease.

MATERIALS AND METHODS

Tissue samples were obtained from 17 individuals without any known neurological or psychiatric disease, as established from their clinical charts and through consultation with their attending physicians (Table 1). In an effort to exclude the influence of changes in the general condition of the patients and side effects of anticancer agents or other medications, we included the brains of 6 healthy individuals whose deaths were accidental (cases 6, 8, 9, 14, 15, 17). The subjects ranged in age from 44 to 83 y (average 65.1 y) (Table 1). Autopsies were performed within 6 h of death in all individuals. Tissue blocks were obtained from approximately the same level of the left motor cortex corresponding to the legs (sagittal sections 1 cm lateral from the interhemispheric fissure).

Tissue was fixed in 2% glutaraldehyde in phosphate buffer (pH 7.40) at the autopsy. After fixation, the motor cortex was cut into small pieces approximately 1 mm thick including layer V, postfixed in 1% osmium tetroxide for 2 h, dehydrated, and then embedded in epoxy resin. Each embedded tissue block was subsequently cut into serial semi-thin ($\sim$1 μm thick) sections and stained with toluidine blue.

Layer V of the human primary motor cortex comprises pyramidal neurons ranging in size from small to giant. Giant pyramids are conventionally called Betz cells, but no size criterion has been agreed

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Table 1. *Summary of ultrastructural findings of Betz cells*

Case	Age (y)	Sex	Pathological diagnosis	Number of Betz cells examined	Number of Betz cells with Bunina-like bodies	Other ultrastructural findings
1	44	M	Banti syndrome	15	0	Accumulation of neurofilaments
2	48	F	Aortic vulve insufficiency	15	0	
3	52	M	Idiopathic cardiomyopathy	20	0	
4	54	F	Diabetes mellitus, sepsis	15	0	Bundle of thick filaments lamellar structure
5	56	M	Cirrhosis of the liver	15	0	
6	60	M	Myocardial infarction	10	0	
7	61	M	Thyroid cancer	10	1	
8	62	M	Myocardial infarction	10	0	
9	65	M	Myocardial infarction	10	0	
10	65	M	Liver cancer	10	0	
11	70	F	Pancreas cancer	10	0	
12	74	M	Renal cancer	10	1	Bundle of thick filaments structure similar to Hirano body lamellar structure
13	75	F	Stomach cancer	10	0	
14	78	M	Trauma	10	1	
15	80	M	Ruptured abdominal aneurysm	15	4	Lamellar structure, lamellar body annulate lamellae accumulation of neurofilaments bundle of thick filaments
16	80	F	Acute heart and lung failure	15	2	
17	83	M	trauma	10	0	

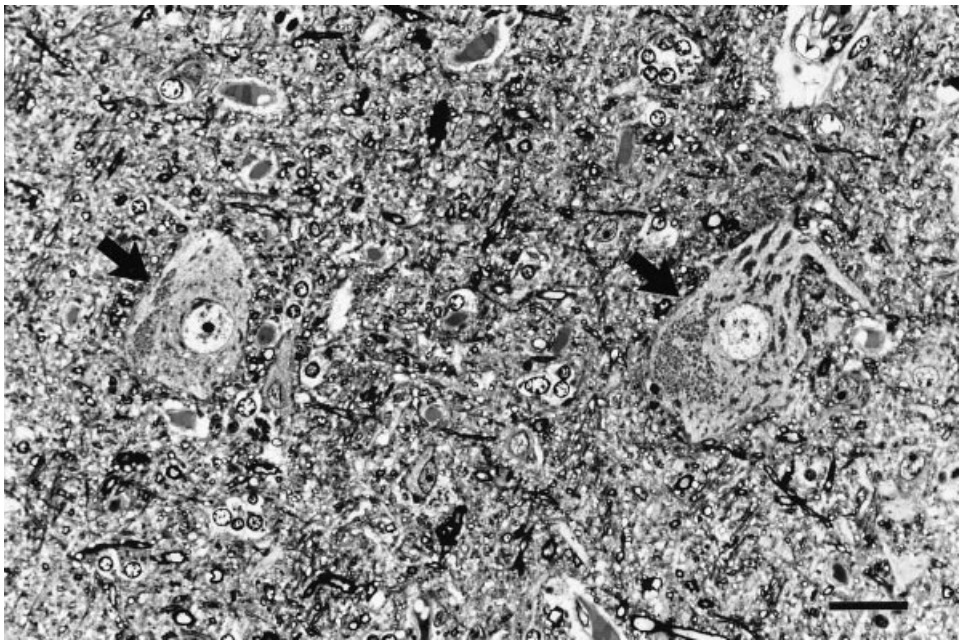

Fig. 1. Betz cells with a perikaryal area greater than 1,000 μm² in the V layer of the motor cortex (arrows) (plastic section, toluidine blue staining). Bar, 30 μm.

upon to differentiate them from large pyramids. Braak & Braak (1976) proposed that the occurrence of large and profuse lipofuscin granules be used as a criterion to identify Betz cells. In the present study, we defined Betz cells as layer V large pyramidal neurons having somal areas greater than 1000 μm² with abundant lipofuscin granules and Nissl granules in the cytoplasm (Fig. 1). After light microscopic identi-

Table 2. *Incidence of Bunina-like bodies in Betz cells*

Group	Number of Betz cells with Bunina-like bodies	Number of Betz cells without Bunina-like bodies	
> 65 y	1	99	$P = 0.02$*
⩽ 65 y	9	91	

* Fisher's exact probability test.

fication of the Betz cells, ultrathin sections were cut from appropriate portions of the specimens, stained with uranyl acetate and lead citrate and examined with an electron microscope. We observed 200 normal-appearing Betz cells in total, and statistically analysed the frequency or number of the 'abnormal' structures.

For convenience, we divided the individuals into 2 age groups, 65 y and older (n = 9), and younger than 65 y (n = 8). The data on electron-dense inclusion bodies (Bunina-like bodies) were analysed by Fisher's exact probability test (Table 2).

RESULTS

Betz cells were easily recognised in serial ultrathin sections by their size and position. They were either situated in a line or loosely clustered. Betz cells occasionally contained one or more inclusion bodies in the cytoplasm, and these varied in size from small to relatively large (Fig. 2). Ultrastructurally, the cytoplasm of Betz cells almost always contained abundant lipofuscin granules. The surface of the Betz cells was covered by several synaptic boutons. Where the surface of the Betz cells was not occupied by axon terminals, either astrocytic processes, unmyelinated axons or myelinated fibres were adjacent to the surface.

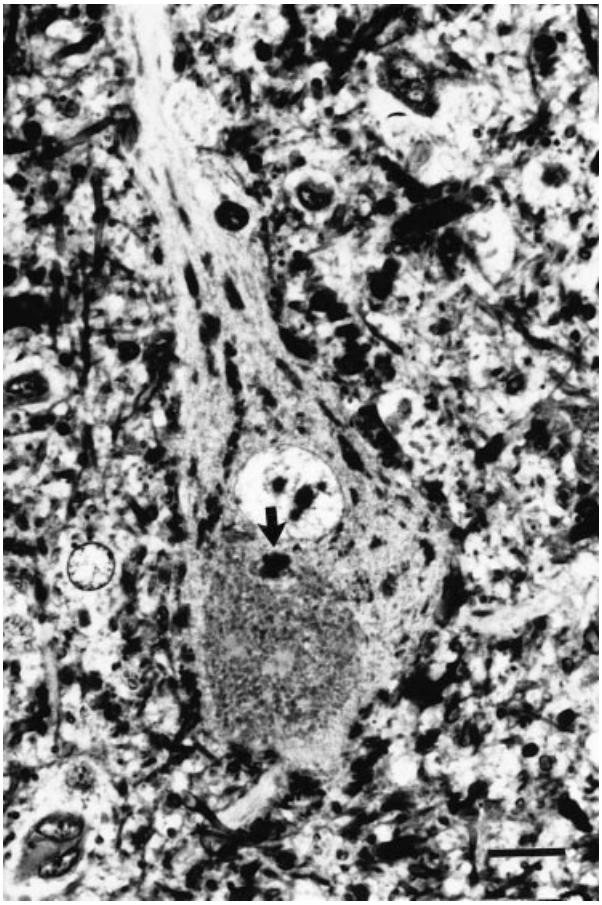

Fig. 2. A Betz cell containing an inclusion body (arrow) in the perikaryon. Bar, 15 μm.

The ultrastructural findings are summarised in Table 1. Various kinds of electron-dense inclusion bodies (Bunina-like bodies) resembling Bunina bodies were found in the cytoplasm of Betz cells in 5 individuals (cases 7, 12, 14–16); small electron-dense inclusions with vesicles and tubular structures were observed in 1 individual (case 7) (Fig. 3); tiny portions of electron-dense material with vesicles of various sizes were seen in 2 individuals (cases 13, 15) (Fig. 4); and large electron-dense inclusions were found in 2 individuals (cases 14, 16) (Fig. 5). The large electron-dense inclusion bodies were several microns in size, and usually occurred one to a cell profile, though occasionally several such bodies were seen in a single cell profile. At the periphery of the inclusions, a number of small cytoplasmic organelles, such as vesicles and rough endoplasmic reticulum, were found. Unlike Bunina bodies, these Bunina-like bodies did not contain neurofilaments or other cytoplasmic organelles. They were observed more frequently in the group of older patients than in the group of younger patients ($P < 0.02$) (Table 2).

In 2 individuals (cases 1, 15), there were occasional focal accumulations of neurofilaments (10 nm in diameter) and mitochondria in the centre or periphery of perikarya of Betz cells (Fig. 6). These neurofilaments did not run in parallel but were interwoven. Bundles of filaments thicker than neurofilaments were

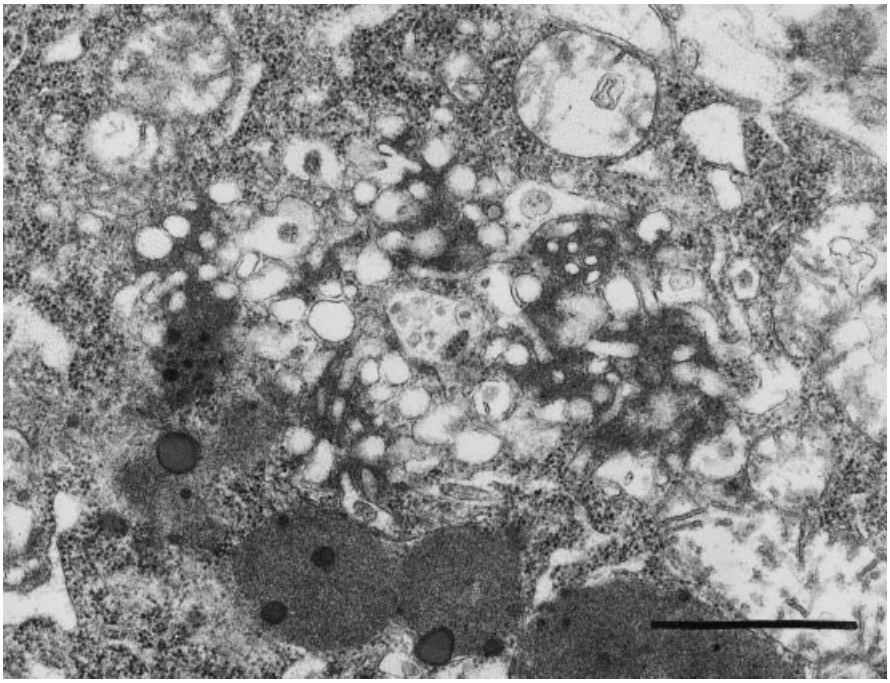

Fig. 3. Electron-dense material with vesicles and tubular structures resembling Bunina bodies in the cytoplasm of a Betz cell. Bar, 1 μm.

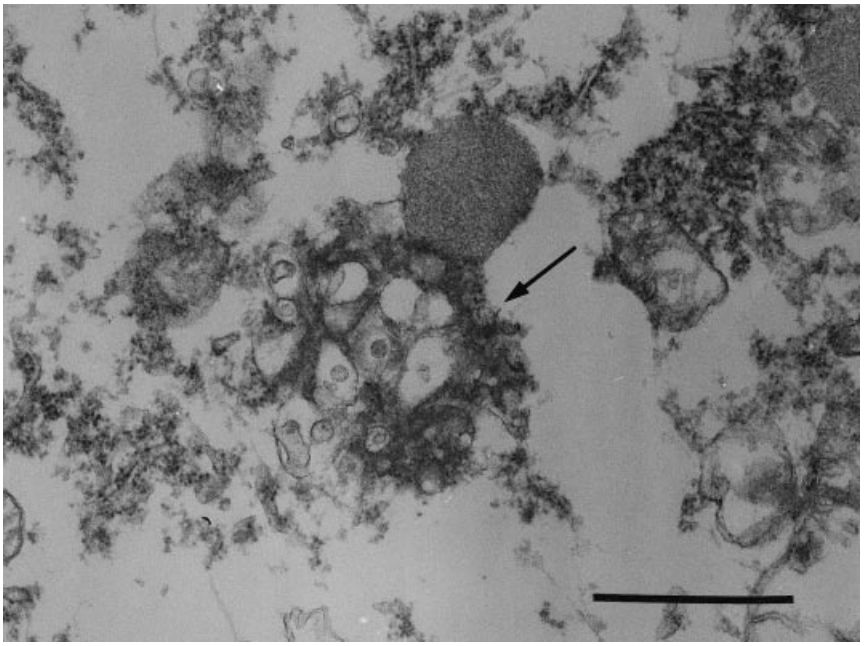

Fig. 4. Tiny electron-dense inclusion with vesicles of various sizes resembling Bunina bodies (arrow). Bar, 1 μm.

seen in the perikarya of Betz cells in 2 individuals (cases 12, 15) (Fig. 7). The thickest section of these filaments measured ~20–25 nm in diameter. The filaments appeared tubular on transverse section, and some were composed of bundles that had constrictions at about 40 nm intervals. In 3 individuals (cases 4, 12, 15), lamellar structures were present in the soma of the Betz cells (Fig. 8); these structures had a tubular profile on transverse section and measured ~70 nm in diameter. Lamellar bodies were seen in the cytoplasm of Betz cells in 1 individual (case 15) (Fig. 9). They consisted of short stacks of membranous cisterns, and ribosomes were present at the periphery. Annulate lamellae were also observed in the same individual (case 15) (Fig. 10). The annulate lamella is similar in structure to the lamellar body, but the membranes of adjacent lamellae fuse to form pores. Structures similar to paracrystalline arrays (Hirano

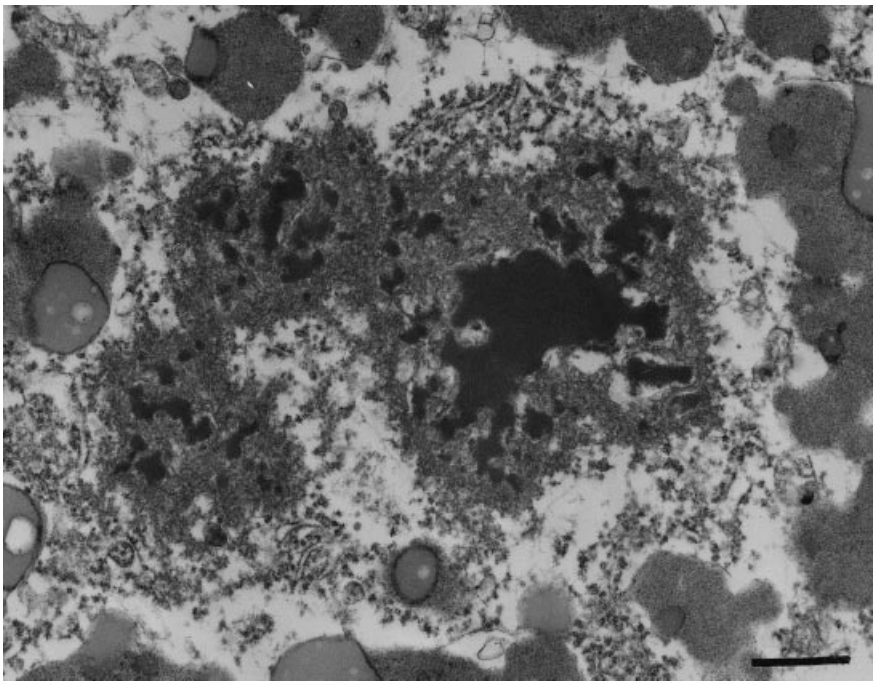

Fig. 5. Large electron-dense inclusion resembling a Bunina body. Many small electron-dense inclusions are observed at the periphery of the large electron-dense inclusion. Bar, 1 μm.

bodies) were observed in one individual (case 12) (Fig. 11). These structures occasionally contained intermediate filaments or vesicles in the interior. There was no significant difference in frequency of any of the above abnormal structures except Bunina-like bodies between the 2 age groups.

DISCUSSION

There have been several reports on the occurrence of somal inclusion bodies in Betz cells in animals (Braak & Braak, 1976; Tigges, 1992) and humans with neurological disease (Sasaki & Maruyama, 1994). Electron-dense materials with vesicles and tubular structures (Bunina bodies), skein-like inclusions and Lewy body-like inclusions, all of which are characteristic of ALS, have been reported in the somata of Betz cells in patients with ALS (Sasaki & Maruyama, 1994). Bunina bodies are small, 1–2 μm eosinophilic granules, ultrastructurally consisting of irregularly shaped, dense, granular materials (Sasaki & Maruyama, 1993*a*, *b*). The ill defined border seems to be associated with nearby organelles including elements of the endoplasmic reticulum, vesicles and mitochondria. The interior sometimes contains small islands of scattered fragments of filaments such as neurofilaments and filaments (20–25 nm in diameter) (Sasaki & Maruyama, 1993*a*, *b*).

Novel inclusion bodies have also been observed in Betz cells of cortical area 4 of aged rhesus monkeys (Tigges, 1992). The occurrence and number of these inclusion bodies may be age-related, since they appeared only in monkeys in mid- to late adulthood and were most numerous in the eldest monkey (Tigges, 1992). The present study reports, for the first time, various kinds of electron-dense inclusions, ranging in size from small to large, in Betz cells of individuals without any neurological or psychiatric disease. These Bunina-like bodies are different from ALS Bunina bodies in that they do not contain neurofilaments or other cytoplasmic organelles. They were more frequently seen in the elderly individuals. These findings suggest that electron-dense inclusions (Bunina-like bodies) may be a fairly common feature in the Betz cells of the human brain, and that, judging from the high frequency of the inclusions in the group of older patients, they probably represent an age-related degenerative change as in rhesus monkeys (Tigges, 1992).

The accumulation of 10 nm neurofilaments in the somata is a very common finding in anterior horn neurons of the spinal cord in ALS patients (Hirano et al. 1984; Sobue et al. 1990). In the motor cortex, however, accumulated neurofilaments in the cytoplasm of Betz cells are a rather unusual finding in both controls and ALS patients (Murayama et al. 1992; Sasaki & Maruyama, 1994). We previously reported observing many positively immunostained Betz cells that had been stained by anti-phosphorylated neurofilament antibody in 1 out of 12 controls (Sasaki &

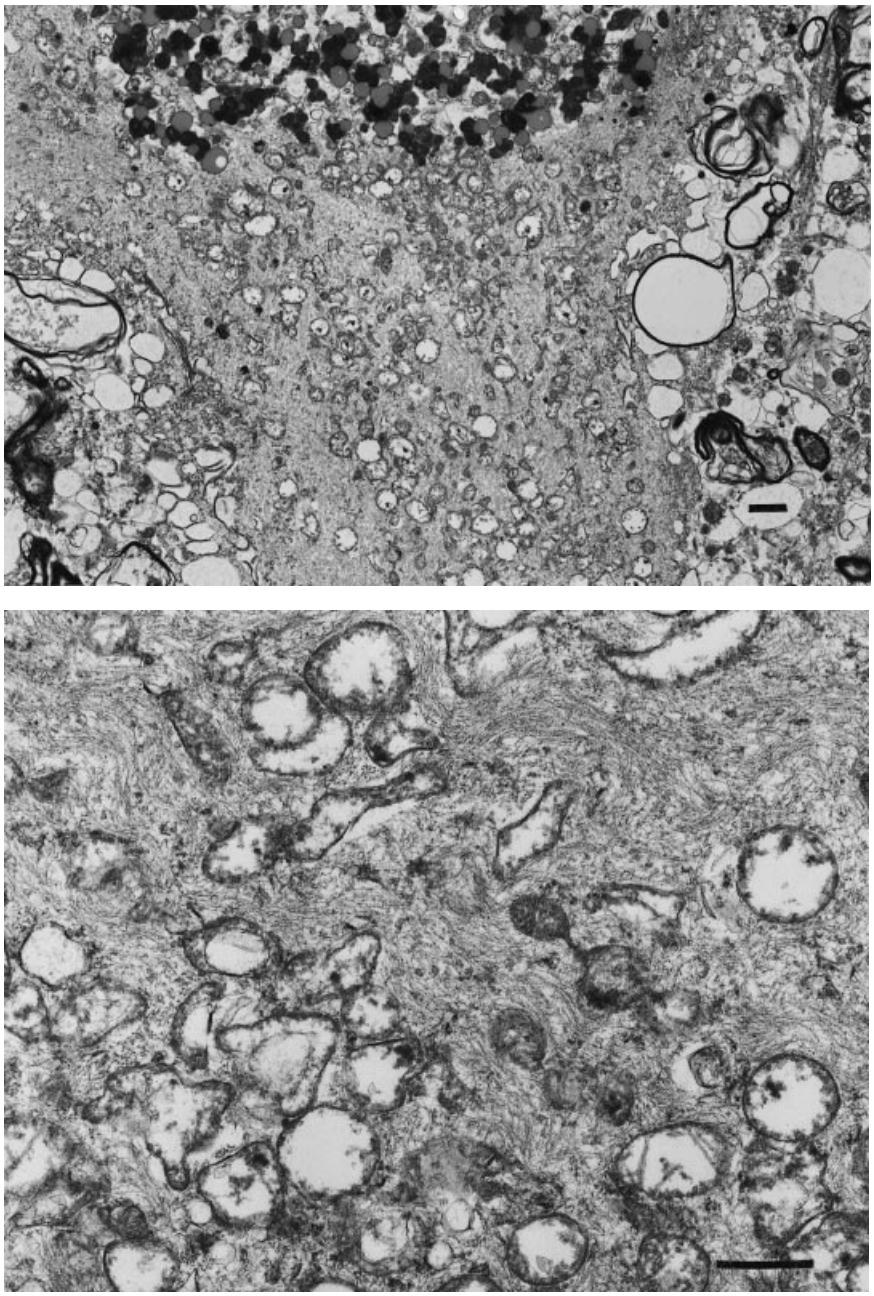

Fig. 6. (*a*) Accumulation of neurofilaments in the soma of a Betz cell. (*b*) High-power view of *a*. Accumulation of interwoven neurofilaments and mitochondria are found in the cytoplasm of a Betz cell. Bars, 1 μm.

Maruyama, 1994). The present study showed that accumulation of neurofilaments in Betz cells can occur even in controls, although such accumulations appear to be rare. The accumulation of neurofilaments may be caused by an overproduction of neurofilaments in the somata or impairment of slow axonal transport in the proximal axons, as has previously been reported in the anterior horn neurons of ALS patients (Carpenter, 1968; Sasaki et al. 1989); the etiology of this impairment is unknown.

Another novel finding in Betz cells is the presence of bundles of filaments (20–25 nm in diameter) thicker than neurofilaments. In a previous study, we found such bundles in 1 elderly patient in a group of 12 control patients (Sasaki & Maruyama, 1994). In the present study, we observed this structure in 3 of 17 individuals. The filaments closely resembled those found in Bunina bodies (Sasaki & Maruyama, 1993*a*). Some were constricted and the others straight, and the constricted filaments were similar to the paired helical filaments previously seen in the spinal ganglion neurons of senescent rats (Van den Bosch de Aguilar & Goemaere-Vanneste, 1984). They were also similar to the paired filaments found in the dendritic terminals

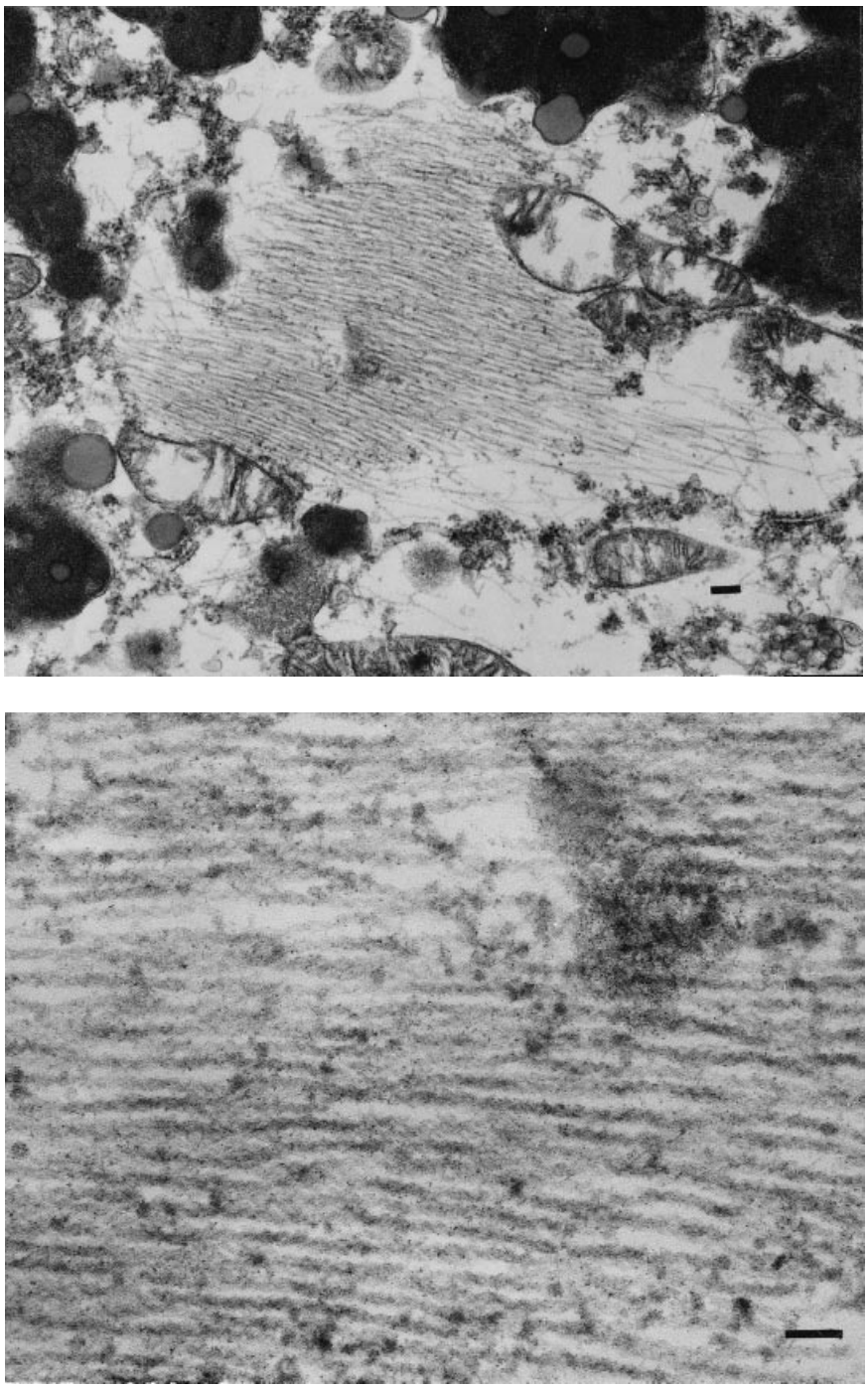

Fig. 7. (*a*) Bundles of filaments thicker than neurofilaments are shown in the perikaryon of a Betz cell. Bar, 0.2 μm. (*b*) High magnification view of *a*. The filaments are between 20 and 25 nm in diameter and some are constricted. Bar, 0.1 μm.

of the cerebral cortex in aged rhesus monkeys (Wisniewski et al. 1973). However, they differed from the paired helical filaments (PHF) seen in patients with Alzheimer's disease, in which the fibrils show regular constrictions at about 80 nm intervals, and were approximately 25 nm wide at their widest point midway between the constrictions (Terry, 1963).

Lamellar structures have been reported in the neurons of the Onuf nucleus (Sasaki & Maruyama, 1993*b*) and in Betz cells (Sasaki & Maruyama, 1994) of ALS patients. In the present study, lamellar structures were not uncommon in the perikarya of Betz cells in control individuals. Herndon (1964) reported lamellar bodies predominantly in cerebellar Purkinje cells, presumably as a result of poor preservation or other unknown causes. These structures have only rarely been found in other neurons under certain conditions (Hirano, 1981). Annulate lamellae are normal constituents of a variety of neuronal and nonneuronal cells such as oocytes,

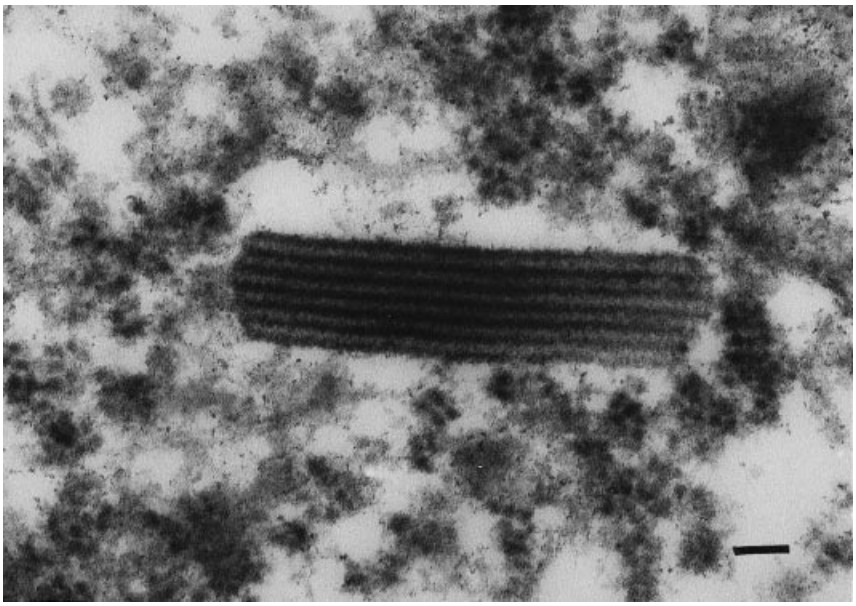

Fig. 8. Lamellar structure measuring about 70 nm in diameter. Bar, 0.1 μm.

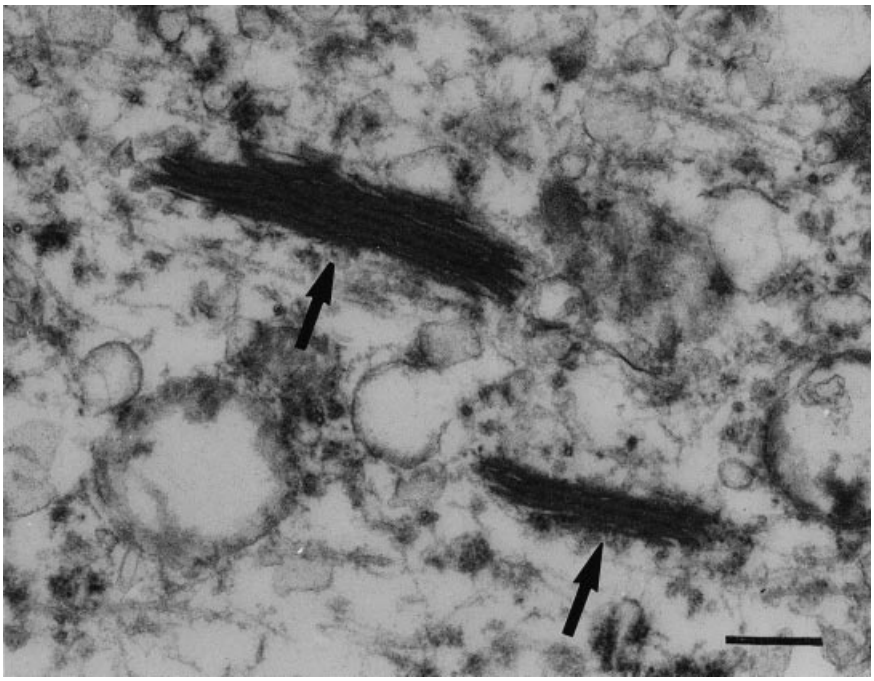

Fig. 9. Lamellar body consisting of short stacks of membranous cisterns (arrows). Bar, 0.3 μm.

and are normally observed in neurons of the lateral geniculate body and dorsal root ganglia in at least some species (Hirano, 1978). They are also common in developing cells and can be found in the pituitary adenomas and in germinomas, among other neoplasms; in addition, they have been described in anterior horn cells undergoing retrograde degeneration in the cat and in humans with ALS (Hirano, 1978). To our knowledge, however, neither lamellar bodies nor annulate lamellae have yet been reported in the Betz cells of the human motor cortex. In addition, the present study is also the first to describe the presence of a structure showing partly paracrystalline arrays in the human motor cortex. This structure is reminiscent of, but not entirely similar to, a Hirano body consisting of highly organised, crystalloid arrays of interlacing filaments displaying either a lattice-like or 'herring-bone' configuration (Hirano et al. 1968).

The detection of these abnormal structures—particularly the Bunina-like bodies—in the Betz cells of normal human brains may contribute to the

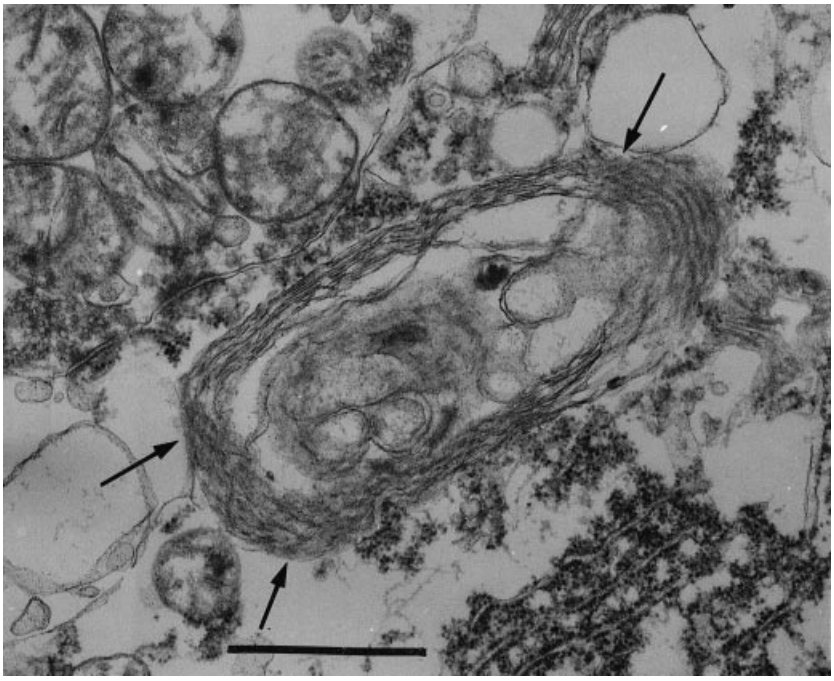

Fig. 10. Annulate lamellae are composed of fusion of membranes of adjacent lamellae (arrows). Bar, 1 μm.

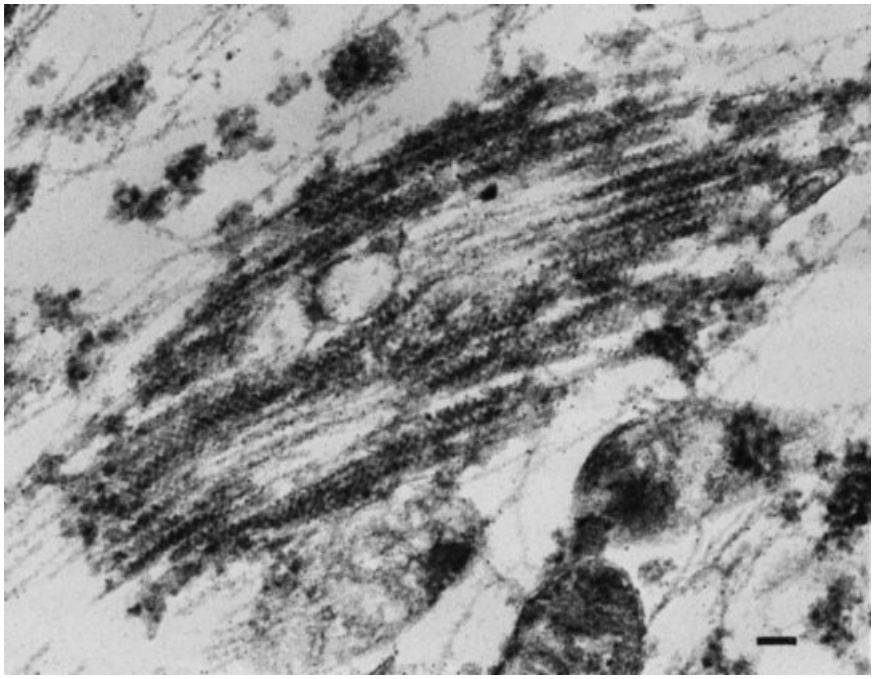

Fig. 11. A structure similar to a Hirano body is seen in the soma of a Betz cell. Some of the filaments contain intermediate filaments in the interior. Bar, 0.1 μm.

assessment of the pathognomonic significance of such structures in ALS and other neurological diseases that affect the motor cortex.

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REFERENCES

BRAAK H, BRAAK E (1976) The pyramidal cells of Betz within the cingulate and precentral gigantopyramidal field in the human brain. *Cell and Tissue Research* **172**, 103–119.

CARPENTER S (1968) Proximal axonal enlargement in motor neuron disease. *Neurology* **18**, 841–851.

HERNDON RM (1964) Lamellar bodies, an unusual arrangement of the granular endoplasmic reticulum. *Journal of Cell Biology* **20**, 338–342.

HIRANO A (1978) Changes of the neuronal endoplasmic reticulum in the peripheral nervous system in mutant hamsters with hind leg paralysis and normal controls. *Journal of Neuropathology and Experimental Neurology* **37**, 75–84.

HIRANO A (1981) *A Guide to Neuropathology*. Tokyo: Igaku-Shoin.

HIRANO A, DEMBITZER HM, KURLAND LT, ZIMMERMANN HM (1968) The fine structure of some intraganglionic alterations. Neurofibrillary tangles, granulovacuolar bodies and 'rod-like' structures as seen in Guam amyotrophic lateral sclerosis and parkinsonism-dementia complex. *Journal of Neuropathology and Experimental Neurology* **27**, 167–182.

HIRANO A, DONNENFELD H, SASAKI S, NAKANO I (1984) Fine structural observations of neurofilamentous changes in amyotrophic lateral sclerosis. *Journal of Neuropathology and Experimental Neurology* **43**, 461–470.

MURAYAMA S, BOULDIN TW, SUZUKI K (1992) Immunocytochemical and ultrastructural studies of upper motor neurons in amyotrophic lateral sclerosis. *Acta Neuropathologica* **83**, 518–524.

SASAKI S, MARUYAMA S (1993*a*) Ultrastructural study of Bunina bodies in the anterior horn neurons of patients with amyotrophic lateral sclerosis. *Neuroscience Letters* **154**, 117–120.

SASAKI S, MARUYAMA S (1993*b*) A fine structural study of Onuf's nucleus in sporadic amyotrophic lateral sclerosis. *Journal of the Neurological Sciences* **119**, 28–37.

SASAKI S, MARUYAMA S (1994) Immunocytochemical and ultrastructural studies of the motor cortex in amyotrophic lateral sclerosis. *Acta Neuropathologica* **87**, 578–585.

SASAKI S, MARUYAMA S, YAMANE K, SAKUMA H, TAKEISHI M (1989) Swellings of proximal axons in a case of motor neuron disease. *Annals of Neurology* **25**, 520–522.

SOBUE G, HASHIZUME Y, YASUDA T, MUKAI E, KUMAGAI T, MITSUMA T et al. (1990) Phosphorylated high molecular weight neurofilament protein in lower motor neurons in amyotrophic lateral sclerosis and other neurodegenerative diseases involving ventral horn cells. *Acta Neuropathologica* **179**, 402–408.

TERRY RD (1963) The fine structure of neurofibrillary tangles in Alzheimer's disease. *Journal of Neuropathology and Experimental Neurology* **2**, 629–642.

TIGGES J (1992) Novel inclusion bodies in Betz cells of cortical area 4 of aged rhesus monkeys. *Anatomical Record* **233**, 162–168.

VAN DEN BOSCH DE AGUILAR PH, GOEMAERE-VANNESTE J (1984) Paired helical filaments in spinal ganglion neurons of elderly rats. *Virchows Archiv* **47**, 217–222.

WISNIEWSKI HM, GHETTE B, TERRY RD (1973) Neuritic (senile) plaques and filamentous changes in aged rhesus monkeys. *Journal of Neuropathology and Experimental Neurology* **32**, 566–584.