THE FINE STRUCTURE OF NEUROFIBRILLARY TANGLES
IN ALZHEIMER'S DISEASE* †‡

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The neurofibrillary tangle of Alzheimer is one of the most distinctive cyto-
pathologic changes that occurs in the central nervous system. This communi-
cation presents the ultrastructure of neurofibrillary tangles as they were seen in
brain biopsies of two patients with advanced Alzheimer's disease. The relation
between these abnormal formations and certain other cell structures will be
discussed.

MATERIAL AND METHODS

The biopsies§ are removed from the cerebral cortex at craniotomy. A wide region of bone
is elevated and a dural flap turned on the non-dominant side. A gyrus is selected which is
free of major vessels, and the leptomeninges are peeled off from a small area. A segment of
cortex about 1 cm. square is quickly outlined with a pointed scalpel. This piece is scooped
out, and only then are hemostatic measures applied. The biopsy, lying on a chilled poly-
ethylene plate, is divided into three parts. The first slice is 1 to 2 mm. thick and is placed
in formalin for light microscopy. The second portion is about 1 mm. thick and is flooded
with buffered osmic acid (1) to which sucrose (2) has been added. The remainder (200 to
500 mg.) of the biopsy is chilled for metabolic and chemical analyses (3). The tissue for
electron microscopy is divided into blocks less than 1 mm. on each side, and fixation is con-
tinued for 90 minutes at 4°C. The blocks are then dehydrated in graded ethanol and em-
bedded in methacrylate or Epon (4). The polymerized blocks are sectioned on a Porter-
Blum microtome with glass or diamond knives, and the sections are mounted on Formvar
or parlodion-covered copper grids. The contrast is enhanced by a lead hydrosilicate stain
modified by Winkler from the technic of Millonig (5). A carbon film is then evaporated
over the methacrylate sections. The electron micrographs are taken with a Siemens
Elmiskop I.

CASE REPORTS

Case 1: This 62 year old woman entered the Neurological Service of the Bronx Municipal
Hospital Center with a 7 year history of progressive dementia. During the 2 years prior to
admission she became unable to care for herself, and was severely deteriorated.

The past history was otherwise irrelevant, and the family history was unknown.

The physical examination was unremarkable. The patient was agitated and displayed
plucking motions of her hands, but was emotionally depressed. She was poorly oriented
as to time and place, and was unable to recite the alphabet or to count. Language was
markedly disrupted. Urinary incontinence was present. The cranial nerves functioned nor-

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cooperation in this study.
nally. The gait was shuffling, slow and hesitant. Myoclonic jerks were apparent in the upper extremities. The reflexes were normal.

The spinal fluid pressure was 101 mm H₂O. It contained no leukocytes. The protein was 46.5 mg per cent and the sugar was 78 mg per cent. The electroencephalogram was diffusely abnormal.

A right sided parieto-occipital brain biopsy was performed. The postoperative course was characterized by steady deterioration of her mental status. Her increased aggressiveness caused her to be transferred to another hospital where she died several months after operation. Autopsy confirmed the biopsy diagnosis of Alzheimer's presenile dementia.

**Case 2:** This 63 year old woman was admitted to the Neurological Service of the Bronx Municipal Hospital Center with a 1½ year history of progressive mental deterioration which was first noted when she became unable to sign her name. Shortly thereafter, memory loss became apparent. During the 18 months prior to admission, the patient frequently became lost in the street. She was unable to do her housework and had increasing difficulty in finding words.

The past history and family history were not informative.

The general physical and neurologic examination was normal. The patient did not know her name, her home address, the season of the year, or her location. Her recent memory was very poor, and she was not able to perform simple calculations or to recite the alphabet. Myoclonic jerks of the head, arms and trunk first appeared during hospitalization.

Severe cerebral atrophy was noted in a pneumoencephalogram. The electroencephalogram recorded frequent synchronous bursts of 3 to 4 per second activity. Spinal fluid protein was 27 mg per cent, sugar was 40 mg per cent and there were 5 leukocytes.

A biopsy was made of the second right frontal convolution. The patient tolerated the operative procedure well and was later transferred to a state mental hospital.

**GROSS EXAMINATION AND LIGHT MICROSCOPY**

**Case 1:** Although the gyrus was slightly narrower than usual, the cortical ribbon was not abnormally thinned. The tissue was firm to the knife. Microscopically, the cortical architecture was well preserved although there was mild gliosis. Neuronal loss was very slight. Bodian stains revealed many neurofibrillary tangles and a moderate number of senile plaques (fig. 1). The former were made up of rather delicate intraneuronal fibers. The plaques did not have the typical central core of amorphous material, but were composed of loosely organized aggregates of irregular clubs and thick fibers lying in a clear matrix which appeared to be extracellular space.

**Case 2:** In this biopsy, the cortex was seen to be moderately thinner than normal. Microscopic examination demonstrated a mild loss of neurons. The layers of the cortex were normally distinct. Bodian preparations showed numerous neurofibrillary tangles similar to those of the first case. Senile plaques were not found.

**ELECTRON MICROSCOPY**

The over-all image of cortical organization was unremarkable. The neuropil was close-packed and made up of great numbers of cell processes of varying diameter and density. Extracellular space was generally limited to the gap between apposed cell membranes, and measured about 200 Å in width. A few

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Symbols used on electron micrographs: $G$, Golgi apparatus; $GF$, glial fibrils; $L$, lysosome; $LA$, lipid aggregate; $LP$, lipofuscin; $M$, mitochondrion; $N$, Nissl substance; $NF$, neurofilaments; $S$, synapse.

Fig. 1. Bodian preparation of cortex in Case I. The neurofibrillary tangles and the senile plaques are displayed. $\times$ 310.

Larger and more irregular intercellular spaces sometimes occurred between the corners of adjacent processes. There was no recognizable material, either fibrillar or particulate, in these extracellular regions.

All plasma membranes were distinct and of the usual thickness. There was no evidence of any abnormal deposits on either their internal or external surfaces.

Most neurons were normal (figs. 2, 3). Their nucleoplasm was finely divided and quite regularly dispersed, although there was a slight tendency to flocculation of the nuclear granules toward the periphery. The nucleoli were compact, but not entirely solid, aggregates of somewhat coarser granules. The nuclear membrane was double. Not infrequently the two membrane profiles were seen to join and form a single, thin layer which for a short distance was the only separation between nucleoplasm and cytoplasm. These were the nuclear pores. Less commonly, the outer nuclear membrane was visibly continuous with the granulated endoplasmic reticulum.

The concentration of ergastoplasm varied very considerably from one neuron to another. Clusters and rosettes of 150 Å granules were prominent among the flat saes of ergastoplasm. Golgi apparatus was an important component of the neuronal cytoplasm and consisted of clusters of vesicles and flattened saes. The Golgi aggregates were found most often close to the nucleus, but also in occasional sites throughout the perikaryon and in the major dendrites.
Fig. 2. Part of an essentially normal neuron from Case 2. The paranuclear Nissl body is clear because there is little other basophilic material nearby. Sparse neurofilaments, mitochondria and a few lysosomes are present. Nuclear pores are evident at the arrows. The neuropil is compact. Adjacent myelin is artefactiously ruffled; × 18,000.
Fig. 3. An essentially normal neuron from Case 2. Here the Nissl bodies are poorly demarcated. Golgi apparatus, mitochondria and lysosomes are abundant. A few neurofibrils (arrow) are present in the hyaloplasm. Several lipofuscin bodies are visible; × 9,200.
Lipofuscin granules were quite common and had a characteristic, compound appearance. They were rounded but irregular, and were closely surrounded by a single membrane. Most of the lipid body was very dense, but there was usually a smoothly contoured region of lesser density and remarkable homogeneity. Occasional coarse granules and short membranes could sometimes be distinguished in the denser zone. Lysosome-like dense bodies were also found scattered at random. These organelles measured about 0.34 \( \mu \) in diameter in these human neurons, were generally round, and were bounded by a single membrane. They were filled by fine, evenly dispersed granules of moderate density. These bodies were not apparently altered in the diseased neurons except for being displaced. However, among some aggregates of lipofuscin, there were moderately large and irregular organelles with the density of the lysosome-like bodies and the shape of lipofuscin.

Mitochondria were distributed at random in the cytoplasm. In the normal neurons their matrix was usually moderately dense, but in general it was not as dark as the mitochondrial matrix found in immediately adjacent neuropil elements. The mitochondria in neurons marked by neurofibrillary change were usually swollen and watery. They were in sharp contrast to normally dense mitochondria of other cells which were only a fraction of one micron distant.

Many neurons displayed abnormally prominent neurofibrils. These were distributed in the neuronal cytoplasm in 3 ways. The most common arrangement was as small bundles, 0.5 to 1.0 \( \mu \) wide, of loosely aggregated, roughly aligned fibrils (figs. 4, 5). Sometimes these bundles were considerably wider and occupied much of the perikaryon (figs. 6, 7). The least common distribution was as a more uniform and less close-packed dispersal of neurofilaments throughout much of the cytoplasm (fig. 8). Among these latter fibrils were normal organelles. Intermediate in frequency was a very dense, broad group of oriented fibrils which completely filled one end of a cell. Here the normal organelles and hyaloplasm were almost entirely displaced (fig. 9).

The individual neurofibrils were essentially similar in all of these situations. Their true length was not measurable because of the thinness of the sections, but was in the order of several microns. Their width was found to vary around a mean of 100 Å. The narrowest were 70 Å wide and the thickest about 125 Å. They displayed a tubule-like triple density, with pale center and dense borders. The central zone was usually slightly wider than the edge. The density of the fibrils was somewhat less than that of cell membranes or of ribosomes. The neurofilaments did not branch, nor did they anastomose with one another (fig. 10).

The fibrils were not spatially related to other intraneuronal cytoplasmic organelles in any regular way. Continuity between endoplasmic reticulum and neurofibrils was not demonstrated. Some neurons with large concentrations of lipofuscin granules showed severe neurofibrillary change, while in other cells tangles of filaments were prominent and there was no lipofuscin. Large, close-packed groups of fibrils often displaced other organelles, but there was some tendency to spare small groups of Golgi saes and vesicles.
Fig. 4. A moderately affected neuron from Case 1. A small satellite cell with dense cytoplasm lies below the large neuron. Between the lipofuscin bodies there are many bundles of neurofibrils (arrows). × 6,800.

Fig. 5. A higher magnification of part of the previous picture emphasizes the neurofibrils. The single membrane bounding lipofuscin is visible; × 20,000.
Fig. 6. A more severely diseased neuron from Case 2. Wide bundles of neurofilaments fill much of the cytoplasm. The neuronal mitochondria are swollen and watery compared with those outside. A so-called spine apparatus is present in the perikaryon at the arrow; × 8,600.

Fig. 7. A higher magnification of part of Figure 6; × 29,000.
Fig. 8. A neuron from Case 2 with evenly dispersed neurofibrils running out into a dendrite. Lipid material distends part of the dendrite to form a torpedo; × 14,000. The inset shows the triple density of the neurofilaments; × 42,000.
Fig. 9. One end of a neuron from Case 2. This portion of the perikaryon is completely filled by neurofilaments. All organelles are displaced except some of the Golgi apparatus; × 22,000.

Fig. 10. Perikaryon neurofilaments of Case 2. Their tubular character is apparent; × 57,000.

A variable and moderate degree of gliosis was apparent in the cortex. This was evidenced by long cell processes with relatively clear hyaloplasm containing many loosely arranged, solid fibrils which were 60 to 70 Å wide. These glial fibrils were never seen without surrounding cytoplasm and plasma membrane.
Fig. 11. A hypertrophied, fibrous astrocyte from the cortex in Case 1. Its cytoplasm contains many delicate fibrils. The mitochondria are poor in cristae. Endoplasmic reticulum is sparse. The neuropil is compact; × 15,000.

Mitochondria were often present next to the fibrils in these cellular extensions. These fibril-containing cells correspond to the astrocytes of classical neurocytology. The cell bodies of these glia were enlarged and often rounded (fig. 11). The hyaloplasm was light and surrounded large numbers of delicate fibrils...
similar to those in the more distant glial processes. Ergastoplasm and Golgi apparatus were sparse, while the mitochondria were moderately numerous. These had dense, granular matrix and few cristae.

DISCUSSION

Although many descriptions of the ultrastructure of axonal and dendritic neurofibrils have been published, there is a remarkable paucity of detail concerning these elements in the perikaryon. Several times their very existence at the fine structure level has been denied, or has been suggested to be artefactual (6–8). Palay and Palade (9), however, did note occasional bundles of fibrils, 60 to 100 Å wide, coursing through the cytoplasm between clumps of Nissl substance. Roizin and Dmochowski (10) saw individual fibrils but did not measure them, while Schultz, Maynard and Pease (11) mentioned filaments 100 to 200 Å wide. The hypertrophic sensory ganglion cells of the lizard contain numerous 60 to 70 Å wide solid fibrils (12). Filaments in the perikaryon or in the axon have been described as solid, while dendritic neurofibrils measure 200 Å, and have been repeatedly found to be tubular. More recently Palay has noted that the 100 Å wide fibrils within the neuronal cell bodies are also hollow (13). The abnormally prominent neurofibrils of Alzheimer’s disease are thus apparently not different in structure from those of the normal cell body.

At most, neurofilaments are very sparse in the normal neuronal perikaryon. A clear difference in fibril concentration exists between the normal neurons described elsewhere and many of the neurons from the two biopsies reported here. But there is a spectrum of fibril concentration from a normal number to a complete replacement of cytoplasm in the diseased cortex.

Artefacts of hydration, such as expanded ergastoplasmic sacs, are almost inevitable, to at least a minor degree, in tissue taken under such complex circumstances as those which obtain at human craniotomy, and fixed by immersion rather than by perfusion. Although fibril formation as the result of this sort of artefact is unknown, mitochondrial swelling is very common in all sorts of tissue. Furthermore, neuronal mitochondria are often less dense than those seen elsewhere. But the distribution of swollen mitochondria in these biopsies is strikingly confined to diseased neurons while mitochondria in adjacent glia, less than one micron distant, are of normal size and density as are those in normal neurons. Specific swelling of neuronal mitochondria is not unique for Alzheimer’s disease in that a similar discrete phenomenon has been noted in Jakob-Creutzfeldt disease (14). This alteration of neuronal mitochondria seems to be more than a simple artefact. It may reflect a lessened reserve in the diseased neurons of some very rapidly utilized substrate such as glucose or oxygen.

The extracellular space in these cortical biopsies, is limited to a gap of 100 to 200 Å, which is within normal limits. Syneresis has been evoked as a causal process in this disease, but evidence for it is lacking in the current study.

The absence in these tissues of extracellular neurofibrillary tangles has several possible explanations: (1) extracellular tangles do not exist except as
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1. Electron microscopic studies of two cortical biopsies in Alzheimer's disease are reported.

2. Neurofibrillary tangles were made of bundles of 100 Å hollow fibrils occupying the neuronal cytoplasm and displacing the normal organelles.

3. In the affected neurons the mitochondria were swollen and watery.

4. The neuropil was normal save for moderate gliosis. The long glial processes contained solid 60 Å fibrils.

5. Neurofibrillary tangles and glial fibrils were never seen except inside cells.

6. The hypothetical neurofibril diagram of Schmitt and Davison is noted to be remarkably similar to the electron micrographs of filaments in both normal and diseased human neurons.

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