

# In Alzheimer's Disease the Golgi Apparatus of a Population of Neurons without Neurofibrillary Tangles Is Fragmented and Atrophic

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**Recent immunocytochemical and morphometric studies in amyotrophic lateral sclerosis, Alzheimer's disease (AD), and aging indicate that the neuronal Golgi apparatus is a reliable index of activity or degeneration. To further evaluate a possible role of the Golgi apparatus in the pathogenesis of AD, we examined by double labeling the neuronal Golgi apparatus, neurofibrillary tangles (NFTs), and senile plaques (SPs) in the hippocampus of six cases of AD, and in 13 controls including three cases of a rare form of dementia lacking distinctive histopathological features. The Golgi apparatus was visualized with a polyclonal antiserum against MG-160, a membrane sialoglycoprotein of the organelle, and NFTs and SPs were visualized with biotinylated basic fibroblast growth factor (bFGF). Only a rare SP contained a few small immunostained elements of the Golgi apparatus. Neurons with intracellular NFTs, labeled with biotinylated bFGF, contained intensely labeled but deformed Golgi apparatus, which was displaced by the NFTs and coalesced into larger irregular granules. In contrast, a population of neurons without NFTs displayed fragmentation of the Golgi apparatus, ie, the organelle appeared in the form of small round, disconnected, and dispersed elements instead of the normal perinuclear network of irregular or linear profiles which often extended into the proximal segments of dendrites. In addition, the fragmented neuronal Golgi apparatus was atrophic as the percentage of the cell surface area occupied by the organelle was  $4.4 \pm 0.6\%$  SD, whereas in neurons with a normal Golgi apparatus the percentage of**

**the cell surface area occupied by the organelle was  $10.3 \pm 0.3\%$  SD. The results of this study suggest that in AD the Golgi apparatus of a population of neurons without NFTs is involved in the pathogenesis of the disease. Considering the role of the Golgi apparatus in the processing of polypeptides destined for fast axoplasmic transports, the fragmentation of the organelle may be associated with functional and structural impairments of axons and presynaptic terminals. (Am J Pathol 1996, 148:415–426)**

Numerous studies have established the central role of the Golgi apparatus in important physiological posttranslational modifications, transport, and targeting of a variety of proteins destined for secretion, the plasma membrane, and lysosomes.<sup>1,2</sup> In neurons all newly synthesized proteins destined for fast axoplasmic transport are processed through the Golgi apparatus.<sup>3</sup> The Golgi apparatus has been implicated in the pathogenesis of a variety of human diseases associated with defects of intracellular trafficking of proteins.<sup>4</sup>

It has been suggested that the two principal morphological hallmarks of Alzheimer's disease (AD), the neuronal neurofibrillary tangles (NFTs) and the amyloid deposits in senile plaques, are associated with the etiology or pathogenesis of this dementia.<sup>5,6</sup> However, a large part of NFTs and most of the amyloid fibrils in the senile plaque are extracellular. Therefore, it might be argued that NFTs and amyloid deposits represent terminal events rather than causes of neuronal degeneration. In the absence of reproducible animal models for AD that would dis-

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play not only the histopathological lesions but also the cognitive changes characteristic of the disorder, and because of the heterogeneity of gene defects or genetic linkages associated with a small number of familial AD, it is difficult to determine with certainty the role of NFTs and amyloid deposits in the etiology and/or pathogenesis of the disease.<sup>7,8</sup>

Emerging evidence suggests that the size of the Golgi apparatus is a reliable index of neuronal activity and that the organelle is involved in the pathogenesis of AD and of amyotrophic lateral sclerosis (ALS). In these two diseases the Golgi apparatus of neurons, examined by immunocytochemistry with highly specific antibodies against MG-160, an intrinsic membrane sialoglycoprotein of the organelle, showed atrophy and dispersion or fragmentation of the immunostained elements of the organelle.<sup>9-18</sup> Fragmentation of the Golgi apparatus has also been observed in a case of multiple myeloma with chronic inflammatory demyelination and in a case of chronic lymphocytic leukemia with leukemic infiltrates in the leptomeninges and Virchow-Robin spaces. In these two cases the patients were treated with a variety of drugs and, therefore, a causal relationship between the disease *per se* and the fragmentation of the Golgi apparatus cannot be established.<sup>19</sup>

In AD, a recent morphometric study based on serial sections stained with silver for NFTs and senile plaques (SPs), and by immunocytochemistry for the Golgi apparatus, showed that a population of neurons without NFTs showed atrophy of the Golgi apparatus.<sup>20</sup> In the present study we examined the reciprocal relation between accumulation of NFTs and a lesion of the Golgi apparatus by double labeling NFTs and the Golgi apparatus in the same section. The Golgi apparatus was labeled with an organelle-specific antibody against MG-160, and biotinylated basic fibroblast growth factor (bFGF) was used to label intracellular and extracellular NFTs and SPs. The results of this study are consistent with the conclusion of the previous study performed with a different method, namely, that in AD a population of neurons without NFTs contain fragmented and atrophic Golgi apparatus.<sup>20</sup> The properties of MG-160, and the rationale for the use of bFGF to label NFTs and SPs will be summarized.

MG-160 is a conserved and well characterized protein of the Golgi apparatus.<sup>21</sup> The gene for MG-160, named *GLG1*, has been assigned to chromosome 16, 16q22-q23, by fluorescence *in situ* hybridization.<sup>22</sup> The protein is a primordial constituent of the Golgi apparatus, appearing ubiquitously only in the organelle and very early during the development of chicken embryos.<sup>23</sup> In cells treated with brefeldin

A, MG-160 behaves in a fashion typical of an intrinsic protein of the Golgi apparatus.<sup>24</sup> MG-160 binds to E-selectin and to several FGFs.<sup>25-28</sup> In immunocytochemical studies both from this laboratory and confirmed by other laboratories, antibodies against MG-160 have been localized exclusively in the Golgi apparatus of neurons and other cells.<sup>13,21,29-31</sup> Furthermore, in optimally fixed tissues of transgenic mice with a Cu,Zn, superoxide dismutase mutation, we observed the characteristic fragmentation of the Golgi apparatus found in autopsy tissues from patients with sporadic ALS.<sup>30</sup> This finding excludes the possibility that the fragmentation of the Golgi apparatus is due to a postmortem degradation of the protein or organelle.<sup>30</sup>

The use of biotinylated bFGF to label NFTs and SPs is justified by a number of studies suggesting that bFGF is involved in the pathogenesis of AD. In brief, in AD, bFGF binds to SPs and to extracellular NFTs.<sup>32,33</sup> Consistent with this observation is the finding of increased levels of bFGF in AD brains.<sup>34</sup> Furthermore, in AD, heparan sulfate proteoglycans (HSPG), which bind FGFs, are present in the neuritic plaques and in the congophilic angiopathy.<sup>35-37</sup> Recently, Snow et al<sup>38</sup> reported that infusions into rat brains of a synthetic  $\beta$ -amyloid protein ( $A\beta$  1-40) and perlecan, a specific HSPG, induced the formation of extracellular amyloid fibrils similar to those seen in AD. Also, Snow et al<sup>39</sup> have indicated that HSPGs are essential for the formation of amyloid. The finding of immunoreactive bFGF within neuritic plaques and NFTs has raised the question whether the factor might be involved in the pathogenesis of AD. According to Stopa et al,<sup>34</sup> bFGF might be implicated in the pathogenesis of AD by stimulating the production and secretion of the amyloid B/A4 protein, which, together with HSPG, may bind and "sequester" bFGF from its physiological targets. An alternative hypothesis, advanced by Cummings et al,<sup>40</sup> proposes that bFGF is upregulated in AD and deposits of bFGF in SPs may attract neuritic sprouts.

The present double labeling study of the Golgi apparatus and intracellular NFTs has shown that in AD an NFT-negative population of neurons in the subiculum-entorhinal cortex display atrophy, decreased immunoreactivity of the bFGF binding Golgi protein MG-160, and fragmentation of the neuronal Golgi apparatus. Thus, an NFT-independent fragmentation of the neuronal Golgi apparatus and neuronal atrophy may be important events in the pathogenesis of AD.

## **Materials and Methods**

### *Double Staining of the Golgi Apparatus, NFTs, and SPs*

Sections 5- $\mu\text{m}$  thick from blocks of hippocampus, routinely fixed in neutral buffered formalin and embedded in paraffin, were mounted on slides coated with Superfrost-plus (Fisher Scientific, Philadelphia, PA) or poly-L-lysine and deparaffinized by immersion in ascending solutions of ethanol and a brief wash in xylene. To "retrieve" immunoreactive antigen, the slides were boiled for 6 minutes in a microwave oven in 1% zinc sulfate in water.<sup>11,41</sup> Subsequently, the sections were permeabilized and the background blocked by immersion for 30 minutes in phosphate-buffered physiological saline, pH 7.3 (PBS), containing 0.5% Triton X-100 (PBS-T) and 2% fish gelatin. The sections were then incubated overnight in a 1:250 to 1:2000 dilution of rabbit antiserum raised against MG-160, purified by immunoaffinity chromatography, combined with 0.05–0.4  $\mu\text{g}/\text{ml}$  biotinylated bFGF (Boehringer-Mannheim, Indianapolis, IN) in buffered physiological saline containing 1% Triton-X (PBS-T).<sup>29</sup> The next day, sections were washed in PBS-T and incubated for 4 hours in 22  $\mu\text{g}/\text{ml}$  of PBS-T goat anti-rabbit immunoglobulin G coupled with alkaline phosphatase (Cappel, Durham, NC) and combined with avidin-biotin-horse-radish peroxidase (HRP) conjugate according to the instructions of the vendor (Vector Laboratories, Burlingame, CA). After washing in PBS, peroxidase was developed with the Vector VIP kit, and alkaline phosphatase with the Vector alkaline phosphatase substrate Kit III, according to the manufacturer's directions. Slides were coverslipped with permount or crystal mount (Fisher Scientific).

### *Controls with Biotinylated Epidermal Growth Factor or with Biotinylated bFGF in the Presence of an Excess of Non-Biotinylated FGF*

Blocks of hippocampus were routinely fixed in neutral buffered formalin, infiltrated in 10% sucrose in PBS, and frozen in isopentane cooled in liquid nitrogen. Frozen sections 10  $\mu\text{m}$  thick were picked up on Superfrost-plus slides (Fisher Scientific) and microwaved, permeabilized, and blocked as previously described. Sections were then incubated overnight either with 22 nmol/L biotinylated bFGF, or 250 nmol/L biotinylated epidermal growth factor (EGF; Boehringer-Mannheim). Additional sections were incubated for 4 hours in 100-fold excess of bFGF

before incubations with biotinylated bFGF were carried out. The next day, slides were incubated with avidin-biotin-HRP and stained for HRP as described above.

### *Experiments with HSPG*

Sections were deparaffinized and boiled in a microwave oven as above. Sections were then preincubated for 4 hours at 37°C in HSPG obtained from Collaborative Biomedical Products (Bedford, MA), at concentrations of 1 or 100  $\mu\text{g}/\text{ml}$  in 0.1 mol/L Tris, pH 7.6 containing 10 mmol/L  $\text{CaCl}_2$ . Sections were washed in Tris before overnight incubations with biotinylated bFGF at 0.4 ng/ml in 0.1 mol/L Tris. Control sections were incubated in biotinylated bFGF without a previous incubation in HSPG. The next day all sections were washed and incubated for 4 hours in avidin-biotin-HRP, and stained for HRP as described previously.

### *Morphometric Studies*

Fields from the end plate (CA4), and the subiculum-entorhinal cortex from all cases were examined blindly by one of us, and neurons with fragmented or normal Golgi apparatus were counted. In order to determine the total cell area and the area occupied by the immunostained Golgi apparatus, photomicrographs of individual cells were enlarged and printed at magnifications of 2800 or 3100. Subsequently, the photomicrographs were perforated by a grid with points 1 cm apart, and points over the entire cell and the labeled Golgi apparatus were counted. A total of 56 neurons with fragmented Golgi apparatus and 448 neurons with normal Golgi apparatus were analyzed. For calculation of standard deviations, one cell was treated as one sample. Analysis of the neuronal size in the end plate and subiculum-entorhinal cortex were conducted with the use of a CUE-2 image analyzer using the planimorphometry program (Olympus, Lake Success, NY).<sup>10</sup>

## **Results**

The neuronal Golgi apparatus from the end plate (CA4) and from the subiculum-entorhinal area of hippocampus was examined qualitatively and by morphometry in 10 controls, in 6 cases of AD, and in 3 cases of dementia lacking distinctive histological features (DLDH) or mesolimbocortical dementia, a rare type of dementia without NFTs, SPs, Lewy bodies, Pick's bodies, or lesions characteristic of

**Table 1.** *Patients with AD, DLDH, Controls*

	Age at death (years)	Sex	Death/Autopsy (hours)	Duration of illness (years)	Cause of death
AD					
88-18	84	M	24	>5	
89-41	84	F	16	4	
89-24	90	F	24	>1	
89-48	83	M	29	5	
OSC	78	F	24	10	
94-4					
92-53	75	M	6	5-7	
DLDH					
94-94	48	F	24	5	
93-233	81	F	13.5	4	
90-93	67	M	19	8	
Controls					
90-45	70	F	2		ALS
88-187	90	F	14		Aspiration pneumonia
93-204	47	F	5		Sepsis secondary to liver transplantation
94-11	70	M	18		Adenocarcinoma of the lung
93-217	65	M	16		Pleural mesothelioma
93-221	80	M	47.5		Adenocarcinoma of the cecum
93-215	52	M	14		End stage alcoholic liver disease/Pulmonary hypertension
94-96	62	M	7		Sepsis secondary to total esophagectomy for squamous cell carcinoma of the esophagus
93-211	71	M	17		Left ventricular failure secondary to coronary artery bypass surgery
93-230	79	F	10		Cerebral toxoplasmosis/Small lymphocytic malignant lymphoma with plasmacytic differentiation

Creutzfeldt-Jakob disease.<sup>42,43</sup> These areas of the hippocampus were selected for analysis because in AD, the CA4 area is relatively spared, and might constitute an internal control, whereas the subiculum-entorhinal zones are maximally involved.<sup>44-46</sup>

In Table 1, the 19 cases used in this study are summarized. All autopsies were performed at the Hospital of the University of Pennsylvania during the last 6 years. In the cases with AD or DLDH, the intervals between death and autopsy varied from 6 to 29 hours. In all cases the diagnosis of AD or DLDH was established on the basis of clinical and autopsy findings. Detailed clinical, neuroimaging, and neuropathological findings in two out of the three cases of DLDH will be reported elsewhere. The brains were routinely fixed in 10% buffered neutral formalin for 2 to 4 weeks before brain cutting. For counts of SPs and NFTs, sections of the middle frontal gyrus; caudate nucleus; globus pallidus; putamen, amygdala, hippocampus, temporal-

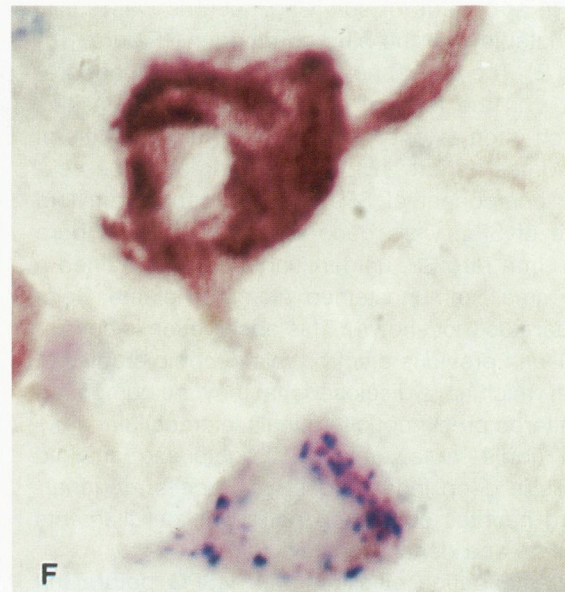
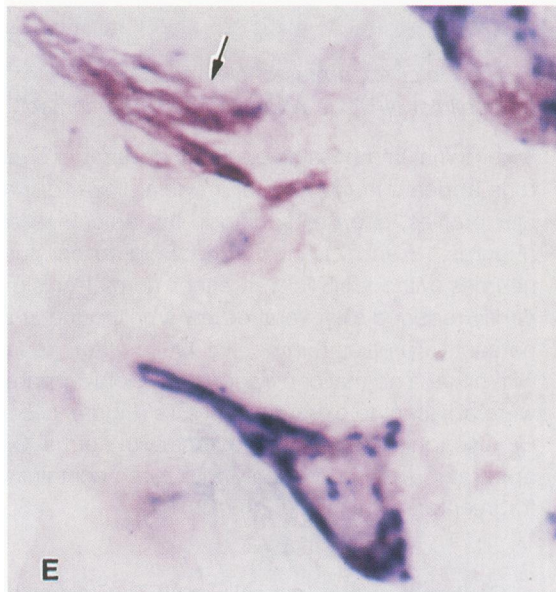
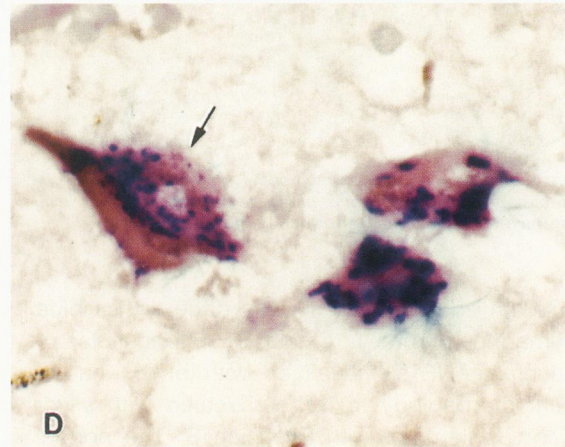
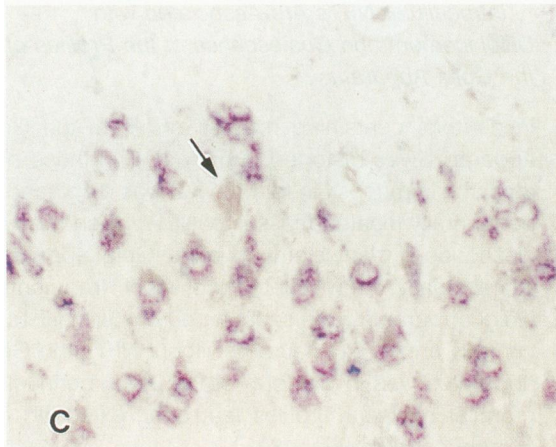
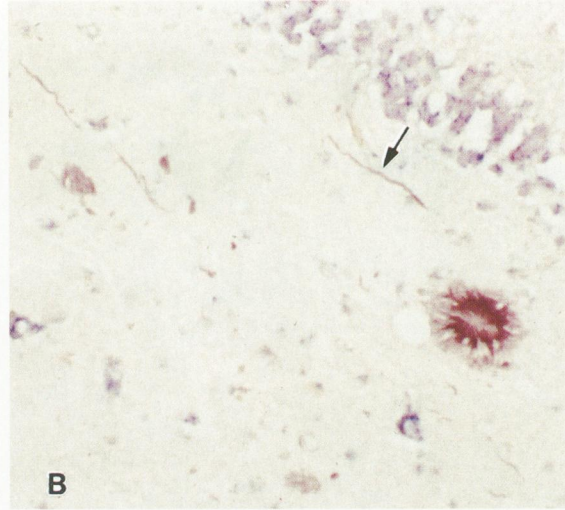
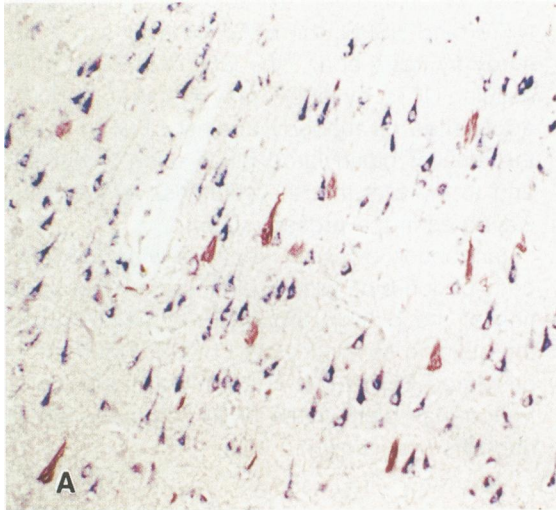
parietal, and occipital lobes; and substantia nigra were stained with thioflavin S and examined under a fluorescent microscope. The diagnosis of AD was established from the clinical evaluation and course, and quantitation of SPs and tangles.<sup>47</sup> Case 90-45 was a control with ALS and no dementia. Case 88-187 was a 90-year-old nondemented control. Four of the six patients with AD had focal lacunar infarcts in the cerebrum and basal ganglia, but no multiple cortical infarcts. None of the AD patients had a family history of the disease.

### *Immunocytochemical Observations*

#### *bFGF Binds Specifically to Intracellular and Extracellular NFTs, to SPs Including both Neurites and the Amyloid Core, and to Neuropil Threads*

The demonstration of biotinylated bFGF was done with a red chromagen, and the binding of the

**Figure 1.** *In all photomicrographs, the red stain represents the binding sites of biotinylated bFGF, and the blue stain represents elements of the Golgi apparatus that have been immunostained with a polyclonal antiserum against MG-160. (A) This photomicrograph, from the entorhinal cortex of a case of AD, shows the Golgi apparatus of pyramidal cell neurons (blue), and NFTs (red). × 110. (B) End plate from a case of AD: an SP (middle of right margin), and neuropil thread indicated by arrow, decorated by bFGF. × 220. (C) Preincubation of section bearing NFTs and SPs with a 100-fold concentration of bFGF eliminates subsequent binding of biotinylated bFGF. Arrowhead shows a pale stain over NFTs. × 220. (D) Section from a case of AD. Arrow points to a pyramidal cell neuron with an intracellular NFT (red), which displaces the Golgi apparatus (blue). × 1000. (E) Two pyramidal neurons from a case of AD with a normal Golgi apparatus in the perikaryon and proximal dendrite (lower neuron). Arrow points to extracellular NFTs. × 1000. (F) Neuron with an atrophic and fragmented Golgi apparatus is adjacent to a mass of extracellular NFTs. Note that neuron does not contain intracellular NFTs, and extracellular NFTs do not contain elements of the Golgi apparatus. Compare with D showing a neuron with intracellular NFTs and a fairly intact Golgi apparatus. × 1000.*





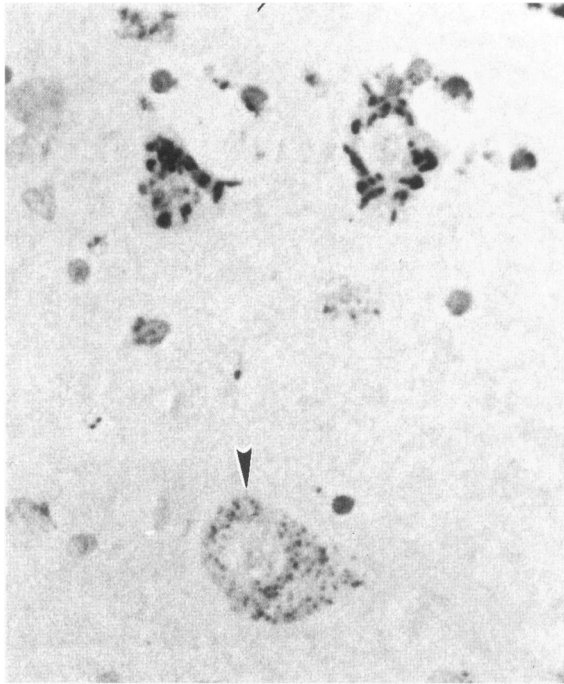


Figure 2. Two neurons with intensely stained irregular profiles of the immunostained Golgi apparatus are quite close to a neuron with fragmented Golgi apparatus (arrowhead).  $\times 800$ .

antiserum against MG-160 was done with a blue chromagen (Figure 1, A and B). Extracellular or intracellular NFTs, visualized with bFGF, had a similar morphology to NFTs revealed with silver stains (Bodian), or with thioflavin S. The normal Golgi apparatus of surviving neurons was easily detectable with the blue chromagen. (Figure 1, A and E). The typical normal neuronal Golgi apparatus is in the form of perinuclear linear densities which often extend into the proximal segments of dendrites (Figure 1E). Neuropil threads, the neuritic component of SPs and the central amyloid core of SPs, were decorated with bFGF (Figure 2B). In a rare SP, unidentifiable cells contained a few small immunostained elements of the Golgi apparatus (not shown). This observation is consistent with previous electron microscopic studies of SPs, which have disclosed that they are composed of altered presynaptic terminals, extracellular amyloid fibrils, a few microglia containing amyloid fibrils in deep indentations within cells, degenerating neurites containing paired helical filaments (PHFs), and dense bodies.<sup>5,48-50</sup>

Preincubation of slides with HSPG before the application of biotinylated bFGF did not alter the intensities of the staining of intracellular and extracellular NFTs, SPs, and neuropil threads with bFGF. In part this result is consistent with the

observation that both extracellular and intracellular NFTs bind HSPG, the bFGF binding site.<sup>36</sup> In the study by Perry et al<sup>36</sup> the lack of staining of intracellular NFTs by bFGF may be attributed to the application of antibody against bFGF, instead of the smaller biotinylated bFGF used in our study, and to the fact that the sections were not micro-waved as in the present study.

Incubation of tissue sections with 100-fold excess of non-biotinylated bFGF before the application of the biotinylated bFGF virtually eliminated the staining by biotinylated bFGF (Figure 1C). Biotinylated EGF, used in a 10-fold higher concentration than biotinylated bFGF, did not stain NFTs and SPs (not shown).

#### *Intracellular NFTs Are Associated with Displacement and Coalescence of the Profiles of the Golgi Apparatus*

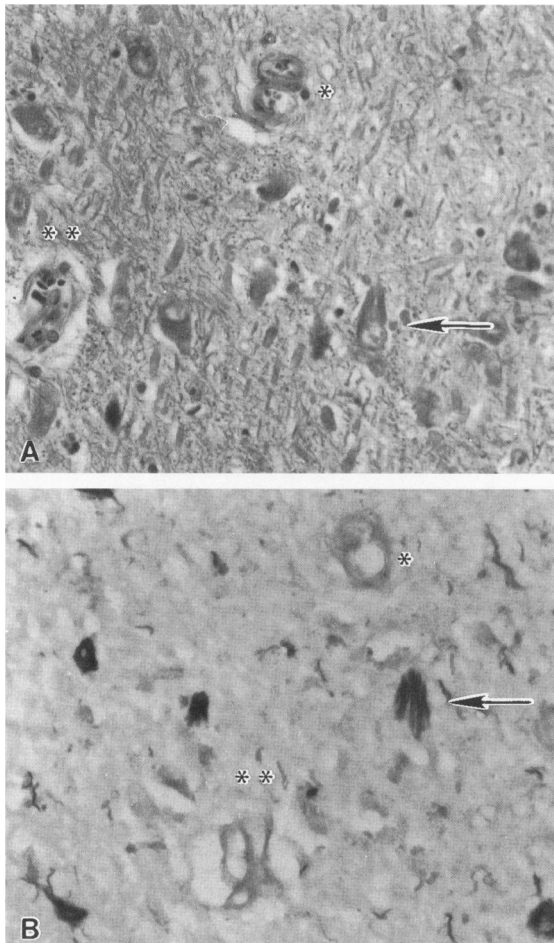
In neurons containing masses of NFTs, the Golgi apparatus was distorted (Figure 1D). In those neurons, the immunostain for MG-160 was displaced by peripherally located masses of NFTs, or showed coalescence of many elements of the Golgi apparatus. Occasionally, a few isolated profiles of "fragmented" Golgi apparatus were seen in neurons with NFTs. In controls and in AD neurons without NFTs the Golgi apparatus was in the form of the usual perinuclear granular or linear profiles that often extended into apical dendrites (Figure 1E).

#### *The Golgi Apparatus of Neurons Lacking Intracellular NFTs Is Atrophic and "Fragmented"*

The normal immunostained Golgi apparatus of neurons appears in the form of irregular linear or granular profiles, often extending in the apical dendrite (Figures 1E and 2). In AD, the Golgi apparatus of neurons without NFTs was either normal or significantly reduced to several or few small round immunoreactive profiles (Figure 1F). Quite often, neurons with either a normal or a fragmented Golgi apparatus were adjacent to extracellular NFTs (Figure 1, E and F). Also, quite frequently, neurons with normal Golgi apparatus were in proximity with those containing a fragmented organelle (Figure 2).

#### *Confirmation that Intracellular NFTs Stain with bFGF*

Because NFTs are classically defined by a silver impregnation stain, we stained consecutive 5  $\mu\text{m}$ -



**Figure 3.** Subiculum from a case with AD. Consecutive sections 5  $\mu\text{m}$  thick stained with Bielchowski (A) or with bFGF (B). Arrows indicate identical cell. Note similar shape of intracellular NFTs stained with Bielchowski's method and with bFGF. Single or double stars mark the two blood vessels that appear in these two consecutive sections.  $\times 450$ .

thick sections with Bielchowski's stain and with bFGF. In several sections we were able to trace the same neuron stained with these two methods. These experiments show that in microwave-treated sections it is possible to demonstrate that intracellular NFTs, stained by the Bielchowski method, can be visualized with bFGF (Figure 3). In Figure 3, the orientation of the blood vessels and the V-like shape of the NFTs indicate that they belong to one and the same neuron. Because of the thickness of the two consecutive sections, in one of the two sections the nucleus is not included, creating the false impression that the NFTs are extracellular.

#### **Failure to Detect Endogenous bFGF in Tissue Sections**

Four different sources of antibodies against bFGF were used to detect endogenous bFGF by immuno-

histochemistry, and none gave a positive reaction, even after prolonged incubations of antibodies with sections at 37°C. The binding of one of these antibodies to bFGF was checked in sections preincubated with exogenous bFGF (0.2  $\mu\text{g}/\text{ml}$  in buffered physiological saline containing 0.5% Triton X-100), before the application of the antiserum against bFGF. This experimental procedure resulted in a strong staining of both NFTs and SPs and validated the ability of the antibody to bind exogenous bFGF (not shown). Therefore, we concluded that the failure to detect endogenous bFGF by immunohistochemistry was not due to low titers or affinities of the antibodies used. However, these negative results may reflect the choice of antibodies used and the treatment of tissues and, therefore, do not preclude the existence of bFGF in AD brains.

#### **Morphometric Studies**

The end plate and subiculum-entorhinal cortex areas were examined at a magnification of  $\times 400$  from the six patients with AD. Three categories of structures were identified: 1) neurons containing Golgi apparatus but no NFTs, 2) neurons containing both stained Golgi apparatus and bFGF including intracellular NFTs, and 3) apparently extracellular NFTs. Because of the degree of neuronal loss, the numbers of presumably viable and functional neurons counted varied from 115 to 243 per case in the end plate, and from 171 to 502 in the entorhinal cortex. The ratios between extracellular NFTs and neurons with or without intracellular NFTs varied considerably among the cases studied. For example, in the subiculum-entorhinal cortex of case 88-18, 304 extracellular NFTs and 198 presumably viable neurons were counted (ratio 1.5:1), whereas in case OSC-94-4, there were 87 extracellular NFTs and 112 presumably viable neurons (ratio 0.8:1). Also, in both end plate and entorhinal cortex, the percentage of identifiable and presumably viable neurons, with intracellular NFTs divided by the total neurons counted, varied considerably from 0 to 10%.

To evaluate the frequency of neurons with a fragmented Golgi apparatus, sections from the hippocampus of all cases were screened blindly and randomly by one of us. Table 2 shows that in the subiculum-entorhinal zones, the numbers of neurons with a fragmented-atrophic Golgi apparatus were at least twice as numerous in AD as in controls and in DLDH; in the end plate (CA4), there were no significant differences in the percentages of neurons with fragmented Golgi apparatus among the three groups studied (not shown). To further evaluate the

**Table 2.** Percent of Cells with Fragmented Golgi Apparatus  $\pm$  SEM

	Endplate	ER Cortex
AD	3.6 $\pm$ 2.8	5.9 $\pm$ 1.1*
Control	0.4 $\pm$ 0.2	1.2 $\pm$ 0.5
DLDH	0.3 $\pm$ 0.3	2.0 $\pm$ 1.5

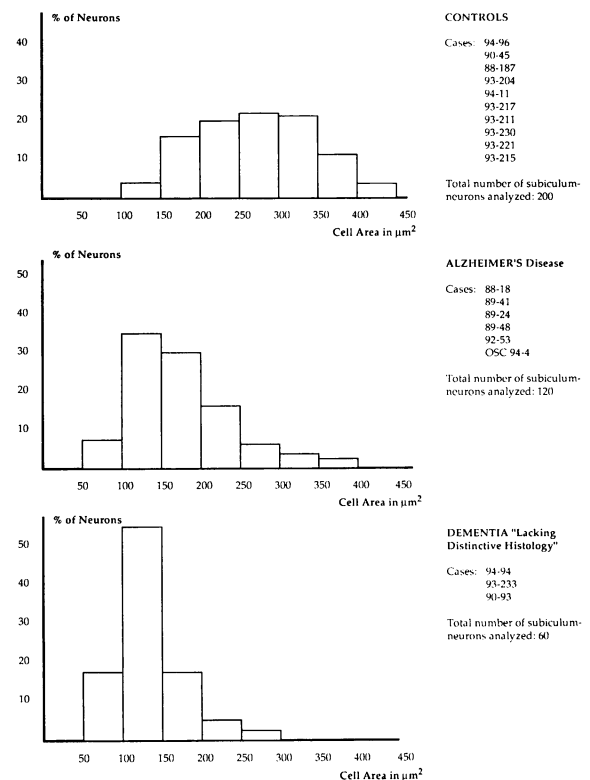
\*Significantly different from controls and DLDH by Student's *t* test; *P* < .02. Total number of cells counted: 4200.

degree of atrophy of the fragmented Golgi apparatus, morphometric studies were carried out that showed that the percentage of the cell surface area occupied by "fragmented" Golgi apparatus was reduced to ~50% of the normal value. Specifically, in sections of 56 neurons with fragmented Golgi apparatus, the percentage of the cell surface area occupied by the organelle was 4.4  $\pm$  0.6, whereas in 448 neurons the percentage of the cell surface area occupied by an apparently normal organelle was 10.3  $\pm$  0.3. Because these studies suggest that in AD neuronal atrophy may be a significant parameter, histograms of neuronal size were prepared from randomly sampled neurons. In the end plate, there were no significant differences among the three groups of patients (not shown). However, in AD and in DLDH in the subiculum-entorhinal cortex, the histograms showed a clear predominance of smaller neurons (Figure 4).

### Discussion

We have examined by a double labeling method the neuronal Golgi apparatus, NFTs and SPs in AD and DLDH, and controls. Extracellular and intracellular NFTs and SPs were visualized with biotinylated bFGF, while the Golgi apparatus was immunostained with an antiserum against MG-160, an intrinsic membrane sialoglycoprotein of the organelle.<sup>21,28</sup> In AD, a population of neurons without NFTs was atrophic with a fragmented and atrophic Golgi apparatus.

The extensive binding of bFGF in AD may be relevant to the pathogenesis of the disorder because FGFs are present and exert potent trophic or proliferative effects on a variety of cells, including neurons and astrocytes.<sup>35,51-55</sup> Immunocytochemical and *in situ* hybridization studies have shown that neurons produce and transport FGFs.<sup>35,53,55</sup> In rats, bFGF prevents the death of cholinergic neurons after lesions in the entorhinal area and transection of the fimbria-fornix.<sup>56,57</sup> Also in rats, bFGF prevents the retrograde degeneration of thalamic neurons after the ablation of the somatosensory cortex, while increased levels of FGF receptor were detected in the



**Figure 4.** Histograms of neuron areas in the subiculum-entorhinal cortex of controls, AD, and DLDH patients.

lesioned somatosensory cortex.<sup>58,59</sup> Spinal cord neuroblasts proliferate in response to bFGF.<sup>60</sup> Several neuronal populations, including the septohippocampal nucleus, the cingulate cortex, and a CA2 subfield of the hippocampus, contain neurons immunoreactive to bFGF.<sup>57,61</sup> High affinity receptors to both acidic and bFGFs have been detected in membrane preparations from adult brain but not from certain other tissues.<sup>62</sup>

MG-160 has two intriguing properties. 1) The protein binds bFGF and shows a striking sequence similarity to CFR, an FGF receptor in chicken. 2) MG-160 is homologous to a ligand to E-selectin, an adhesion molecule.<sup>25,27,28</sup> It is not known whether the function of MG-160 in the Golgi apparatus is related to bFGF and E-selectin binding. However, as discussed elsewhere, MG-160 may be involved in the regulation of secretion of certain FGFs.<sup>63,28</sup> Therefore, the finding of a fragmented and atrophic Golgi apparatus in certain AD neurons (Figures 1F and 2) may have significant functional implications considering the evidence for a trophic role of FGFs in the central nervous system.<sup>40,51,55,56,61</sup> Furthermore, as the Golgi apparatus is involved in the processing of molecules undergoing fast axoplasmic transport, a possible functional deficiency of the fragmented



organelle may account for the abnormalities of presynaptic terminals seen in AD.<sup>3,5,48-50,64</sup> The presence of HSPGs in NFTs and SPs, known to bind bFGF, may promote the *in vivo* binding of bFGF in these structures.<sup>36-39,65</sup> However, we were not able to demonstrate endogenous bFGF in NFTs and SPs. This negative result may be attributed to tissue processing, antibodies used and other factors, and should not invalidate the attractive hypothesis according to which the probable *in vivo* "sequestration" of bFGF in SPs may deprive adjacent neurons from the trophic effects of this potent trophic factor.<sup>34</sup>

We were not able to show an association between fragmentation and atrophy of the GA and accumulation of intracellular NFTs, although we demonstrated the presence of intensely immunoreactive, albeit distorted and occasionally fragmented, Golgi apparatus in neurons with NFTs as previously shown by electron microscopic reconstructions by Ellisman et al<sup>66</sup> (compare Figure 1D with 1F). Also, we failed to detect any correlation between masses of extracellular NFTs and abnormalities of the Golgi apparatus in adjacent neurons (compare Figure 1E with 1F).

In the electron microscope, NFTs appear as PHFs, which are composed of "hyperphosphorylated" tau, a microtubule-associated protein.<sup>5,67,68</sup> Recently, the hyperphosphorylation of tau in NFTs and PHFs has been attributed to defective activities of phosphatases rather than to pathological hyperphosphorylations, whereas another report claims that there is no abnormal phosphorylation of tau in intracellular tangles.<sup>68,69</sup>

The recent immunocytochemical detection in both NFTs and SPs of two Maillard reaction products, pyrroline and pentosidine, suggests that alternative mechanisms may be operational to account for presumed enzymatic deficiencies.<sup>70</sup> Specifically, according to Smith et al,<sup>70</sup> during aging certain post-translational modifications of "long-lived proteins" of the type associated with Maillard reaction products induce the formation of crosslinks between proteins, resulting in decreased solubility and resistance to digestion by proteases. Therefore, an alternative mechanism for the accumulation of hyperphosphorylated tau in NFTs, or of other nondegradable products in SPs, may be based on the resistance of modified substrates to enzyme digestions rather than to enzyme deficiencies *per se*.

Several studies have explored the distribution of PHFs or NFTs within AD neurons (quoted and summarized by Ellisman et al<sup>66</sup>). In brief, NFTs are found in neuronal perikarya, proximal dendrites, and processes within senile or neuritic plaques. Ellisman and collaborators,<sup>66</sup> using computer-assisted recon-

structions from serial thin and thick sections viewed under conventional and high voltage electron microscopy, concluded that in comparison with neurons without NFTs, the Golgi apparatus of NFT-bearing neurons was displaced by NFTs and appeared more globular and less extended.<sup>66</sup> In the present double labeling study, we have confirmed that the Golgi apparatus of NFT-bearing neurons was often displaced by NFTs, and that the organelle lost its usual network configuration and it coalesced into larger globules (Figure 1, D and E). However, NFT-bearing neurons did not show the severe fragmentation and atrophy of the organelle seen in neurons without NFTs (Figure 1F); furthermore, with the exception of the previous study by Salehi et al,<sup>20</sup> we are not aware of another study showing that in AD the Golgi apparatus of neurons without NFTs is atrophic (Figures 1F and 2). These findings are consistent with the conclusion that in AD, in a certain population of neurons in the subiculum-entorhinal cortex, the fragmentation of the Golgi apparatus is unrelated to NFT accumulation. In part, this conclusion is consistent with a recent report on the lack of an obligatory correlation between apoptosis and NFT-bearing neurons. Specifically, it has been demonstrated in hippocampi and entorhinal cortices from AD brains, that many NFT but also many non-NFT-bearing neurons displayed the distinct apoptotic features.<sup>71</sup> Taken together, these results suggest that in AD degeneration, atrophy and possible death of a certain population of neurons is not necessarily linked with NFT accumulation.<sup>72,73</sup>

The fragmentation of the Golgi apparatus in AD is similar to a change noted in motor neurons in ALS and in cultured cells treated with drugs that depolymerize microtubules.<sup>74,75</sup> In certain cases, the experimentally induced fragmentation or dispersion of the Golgi apparatus by microtubule disrupting agents is associated with a severe decrease in secretion.<sup>76,77</sup> Therefore, it is likely that neurons with fragmented Golgi apparatus (Figure 1F and 2) do not display a physiological processing and secretion of proteins.

In view of the interdependence between the "cytoskeleton" and the Golgi apparatus, it is difficult to envision a lesion of the Golgi apparatus occurring in the absence of changes in the cytoskeleton. For example, the observed fragmentation of the organelle may be caused by a paucity of neuronal microtubules and/or by abnormal microtubule-associated proteins, including tau. It is also possible that the fragmentation of the neuronal Golgi apparatus is caused by primary subtle but specific abnormalities of proteins linking the membranes of the organelle with the cytoskeleton, which may predate gross changes of the cytoskeleton

and the appearance of NFTs, which may occur at later stages of neuronal degeneration.

In summary, the results of this study support the hypothesis that the fragmentation and atrophy of the Golgi apparatus in a population of neurons of patients with AD contributes to the pathogenesis of the disorder. This hypothesis is reasonable, considering 1) the importance of the Golgi apparatus in secretion, plasma membrane biosynthesis, and targeting of lysosomal enzymes; 2) the specific role of MG-160 in bFGF binding; 3) the proposal that bFGF is involved in the pathogenesis of AD; and 4) the previous studies of the Golgi apparatus in ALS, aging, and AD showing that the size of the organelle is a reliable index of neuronal activity or degeneration.

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