Diagnostic Utility of Quantitating Neurofilament-immunoreactive Alzheimer's Disease Lesions

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The diagnosis of Alzheimer's disease (AD) neurodegeneration is based on histopathological detection of paired helical filament-associated lesions. Silver stains are routinely used but the results are fraught with intra- and interinstitutional variability. This study employed monoclonal antibodies to middle and high molecular weight neurofilament subunits in an immunohistochemical assay to assess the extent of paired helical filament-associated lesions in brains with AD, Down's syndrome plus AD lesions (AD + DN), Parkinson's disease dementia (PD), AD + PD, and normal aging changes.

The densities of neurofilament-immunoreactive (NFI) cortical neurofibrillary tangles and plaques were significantly higher in AD and AD + DN than in PD and aged control brains (p<0.001), and NFI neurofibrillary tangles and plaques were more abundant in AD and AD + DN compared with AD + PD and PD, yet all patients with AD, AD + PD, or PD died with end-stage dementia. In contrast, the densities of NFI dystrophic neurites (primarily dendrites) in cortical Layer 2 were similar among the AD, AD + DN, AD + PD, and PD groups, and all were significantly higher than control (p<0.005). Stepwise multivariate regression analysis demonstrated significant correlations between AD diagnosis and high densities of NFI neurofibrillary tangles and plaques (p<0.001) and between end-stage AD-type dementia and high densities of NFI dystrophic neurites (p<0.001). This study demonstrates that the histopathological lesions correlated with AD dementia can be readily detected and quantified by immunostaining with monoclonal antibodies to phosphorylated and non-phosphorylated neurofilaments. Moreover, the findings suggest that NFI neurite pathology may be an important feature contributing to the clinically manifested AD-type dementia in individuals with Parkinson's disease. (J Histochem Cytochem 42:1625-1634, 1994)

KEY WORDS: Neurofilament immunoreactivity; Alzheimer's disease; Parkinson's disease; Down's syndrome; Neurofibrillary tangle; Paired helical filaments; Dystrophic neurites and dendrites.

Introduction

Neurofibrillary tangles, neurofil threads, dystrophic neurites, and neuritic plaques are the major histopathological lesions correlated with dementia of Alzheimer's disease (1-11) and share in common the accumulation of straight and paired helical filaments (12-16). The complete composition of the filamentous material may not be known because of its high degree of insolubility (17,18). However, the associated immunoreactivities for phosphorylated neurofilament (4,19-28), tau (24,29-34), and microtubule-associated protein 2 (35-37) suggest that cytoskeletal proteins are major constituents of paired helical filaments. Since phosphorylated cytoskeletal proteins are normally confined to axons (38), their accumulation in conjunction with neurofibrillary tangles and dystrophic neurites may reflect abnormal translocation or processing (hyperphosphorylation) of the molecules within neuronal perikarya and dendrites (20,23,25).

Neurofilament immunoreactivity in neurofibrillary tangles and dystrophic neurites is due to the accumulation of phosphorylated high (200 KD) and middle (153-168 KD) molecular weight subunits of the protein (20,39,40). Controversy has been raised by reports that monoclonal antibodies (MAbs) to neurofilament crossreact with phosphorylated tau in neurofibrillary tangles (32,41). However, more recent findings demonstrated distinct neurofilament epitopes on paired helical filaments (42) and low affinity of neurofilament antibodies for tau (43), thereby reinstating the likelihood that phosphorylated neurofilament is indeed an integral component of paired helical and straight filaments in Alzheimer's disease. As an empirical finding, we observed that all of the structural lesions that corre-

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late with dementia in Alzheimer’s disease could be detected simultaneously by immunoassaying with a cocktail of the SMI 31, 32, and 34 MAbs to the high and middle molecular weight subunits of phosphorylated (SMI 31 and 34) and non-phosphorylated (SMI 32) neurofilament protein (Sternberger-Meyer; Washington, DC) (38). The present study was designed to characterize and quantify the regional distribution of neurofilament-immunoreactive (NFI) neurofibrillary tangles, cortical dystrophic neurites, and neuritic plaques in Alzheimer’s disease and to determine whether this type of assessment could be used to effectively distinguish Alzheimer’s disease and Parkinson’s disease dementia from normal aging.

Materials and Methods

Source of Tissue. Post-mortem brain tissue was obtained from demented individuals with Alzheimer’s disease (AD), Parkinson’s disease (PD), or Alzheimer’s plus Parkinson’s disease (AD+PD), who had been enrolled in the Massachusetts General Hospital (MGH) Alzheimer’s Disease Research Center (ADRC), individuals with Down’s syndrome plus AD lesions (AD+DN) who had been residents at the Fernald State School (Waltham, MA), and from aged control patients who had no clinically recognized neurological or neurological demented or who had been regularly evaluated at the MGH. The brain tissue was harvested within 18 hr of death according to the MGH ADRC protocol described previously (44). Histopathological diagnoses were established with paraffin-embedded tissue sections stained with luxol fast blue, hematoxylin and eosin, Bielschowsky silver impregnation, and Congo red staining. The diagnosis of AD was rendered using threshold criteria reported by the NIA NINCDS group (45). In addition, all brains diagnosed with AD, including those with AD+DN and AD+PD, had neocortical neurofibrillary tangles. The diagnosis of PD was based on neuron loss, gliosis, and intraneuronal Lewy body inclusions in the substantia nigra, locus ceruleus, and dorsal motor nucleus of the vagus nerve. However, the brains that were classified as PD dementia also contained plaques and neurofibrillary tangles, but the densities of these lesions were insufficient to render the diagnosis of AD+PD. The diagnosis of AD+PD was rendered when histopathological criteria for both diseases were present in the same brain. In both the PD and AD+PD cases, the diagnosis of diffuse Lewy body disease was excluded by the absence of widespread Lewy bodies in sections of cerebral cortex immunostained with polyclonal antibodies to ubiquitin (Biomedica; Foster City, CA) and with monoclonal antibodies to neurofilament.

Population Profile. The brain tissue included in this study was obtained from individuals with AD (n = 4), AD+DN (n = 4), AD+PD (n = 5), or PD (n = 6) and from non-demented aged controls (n = 6). The groups were similar with respect to mean age and gender, except that the mean age of the AD+DN group was 7–14 years less than the other groups (p< 0.001), and all patients with AD group were female, whereas most of the patients in the other groups were male (p< 0.001) (Table 1).

Tissue blocks no larger than 2 x 2 x 0.5 cm were fixed in 10% buffered formalin for 4–6 days and embedded in paraffin by a standardized protocol. The brains included in this study were devoid of significant axonic/isochronic encephalopathy, infarction, or other neuropathological lesions unrelated to the primary diagnosis. In addition, specimens were deemed suitable for immunohistochemical studies based on clear positive immunoreactivity for glial fibrillary acidic protein in sections adjacent and identical to those used to evaluate neurofilament immunoreactivity. In total, 153 blocks of tissue (30 AD; 30 AD+DN; 12 AD+PD; 14 PD; and 65 control) were examined for neurofilament immunoreactivity using a cocktail of the SMI MAbs. Tissue blocks from Brodmann Areas 21 and 24, and the entorhinal cortex were available from all patients. For Areas 4, 11, 40, and 17, tissue blocks were available only from the AD, AD+DN, and control groups. In addition, for the control group, duplicate blocks from both hemispheres were studied in most instances. Blocks including the CA1 + CA2 regions of the hippocampal formation were available only from the AD and AD+DN groups. Several blocks were missing from the AD+PD and PD cases because they had been exhausted by previous studies. Despite this limitation, sufficient data were obtained by studying the same three regions of brain from all cases to appreciate regional variability in the distribution of lesions in AD and PD dementias, as well as in aged controls.

<table>
<thead>
<tr>
<th>Table 1. Demographic profile</th>
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<tr>
<td><strong>Diagnosis</strong></td>
</tr>
<tr>
<td>AD</td>
</tr>
<tr>
<td>AD + DN</td>
</tr>
<tr>
<td>AD + PD</td>
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<tr>
<td>PD</td>
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<td>Controls</td>
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AD, Alzheimer’s disease; AD + DN, Down’s syndrome with AD lesions; AD + PD, AD plus Parkinson’s disease dementia; PD, Parkinson’s disease dementia; Controls were neurologically and neuropathologically intact.

For the control group, replicate blocks from both hemispheres were studied in most instances. Blocks including the CA1 + CA2 regions of the hippocampal formation were available only from the AD and AD+DN groups. Several blocks were missing from the AD+PD and PD cases because they had been exhausted by previous studies. Despite this limitation, sufficient data were obtained by studying the same three regions of brain from all cases to appreciate regional variability in the distribution of lesions in AD and PD dementias, as well as in aged controls.

Immunohistochemical Studies. Formalin-fixed, paraffin-embedded sections (8 µm thick) from Brodmann Areas 24, 11, and 4 in the frontal lobe, Area 21, the entorhinal cortex, and hippocampal formation (Ammon’s horn) in the temporal lobe, Area 40 in the parietal lobe, and Area 17 in the occipital lobe were immunostained using a cocktail of the SMI MAbs to neurofilament (SMI 31, SMI 32, and SMI 34) with each diluted 1:3000 (combined final dilution 1:10000). Of note is that SMI 31 and SMI 34 are not immunoreactive with tau (46). The sections were dewaxed in xylens and hydrated through graded alcohol solutions. Nonspecific binding was blocked by incubating the sections with 1% normal horse serum for 30 min. The sections were incubated overnight at room temperature with the cocktail of diluted SMI MAbs. Endogenous peroxidase activity was blocked by treating the sections with 0.6% H2O2 in methanol for 30 min. Immunoreactivity was detected by the avidin–biotin–horseradish peroxidase method (Vector ABC Elite Kit; Vector Laboratories, Burlingame, CA) following the manufacturer’s protocol, and with 3,3'-diaminobenzidine as the chromogen. The diaminobenzidine was co-precipitated with 0.04% NiCl2 to enhance the immunoreactive signal. The sections were counterstained lightly with hematoxylin, dehydrated in graded alcohol solutions, cleared in xylens, and preserved under coverglass with Permoun (Fisher Scientific; Fair Lawn, NJ). Adjacent sections were immunostained with MAbs to glial fibrillary acidic protein as a positive control and with MAbs to Dengue virus as a negative control. Also as a negative control, sections were immunostained with the primary antibody omitted.

Analysis of Immunoreactivity. The slides were coded and interpreted without knowledge of the official diagnosis. The codes were broken at the time of data analysis. Each histological section was evaluated with respect to the densities of neurofilament-immunoreactive (NFI) neurons, neurofibrillary tangles, dystrophic neurites, and plaques contained within 40 adjacent microscopic fields confined to cerebral cortex. To accomplish this, the full thickness of cortex was surveyed using an ocular grid and mechanical microscope stage, beginning the enumeration in Layer 1 at the crest of a gyrus and proceeding systematically toward Layer 6, then across, upwards toward Layer 1, across, then downwards again, and so on, with unidirectional horizontal progression. Neuronal perikarya, neurofibrillary tangle, and dystrophic neurite labeling were assessed at a magnification of 10 x 40, and plaques were counted at a magnification of 10 x 20. All sections contained a large excess of 40 x 400 and x 200 microscopic fields for examination. NFI dystrophic neurites were enumerated in a 400-mm² area...
within Layer 2 of the cerebral cortex. This was accomplished by projecting the microscopic field image through a video display device to record the positions of all NFI dystrophic neurites onto a transparent overlay. The recording and enumeration of NFI dystrophic neurites were performed under code by a trained assistant, and checked (still under code) by a neuropathologist (SMD). Layer 2 was selected for analysis because NFI dystrophic neurites were most abundant in this cortical lamina. In addition, the specific fields selected for evaluation (by the neuropathologist) were those that contained the highest densities of NFI dystrophic neurites. Coarse irregular axons accompanied by attrition of normal fibers in white matter were noted as present or absent. White matter fiber attrition was associated with a lack of uniform fiber staining, together with the presence of clear spaces among sparse, well-stained NFI fibers.

Statistical Analysis. The groups were compared with respect to the densities of NFI neurons, neurofibrillary tangles, dystrophic neurites, and neuritic plaques. The data were analyzed by analysis of variance (ANOVA) with post-hoc Duncan and Fisher least significant difference (LSD) tests for significance, cross-tabulation chi-square contingency table analysis, and stepwise multivariate regression using the Number Cruncher Statistical System, version 5.01 (Dr. Jerry L. Hintze, Kaysville, Utah) interfaced with an IBM-compatible personal computer. F-ratios reflect degrees of variance, and “df” refers to degrees of freedom in the ANOVA tests. ANOVA results indicate presence or absence of significant differences among the groups. The post-hoc Duncan and Fisher LSD tests were used to identify which groups had significantly different mean values.

Results

NFI Neurons

Cortical neurons devoid of neurofibrillary tangles often exhibited granular neurofilament immunoreactivity throughout the perikarya, as described previously (20, 26, 47) (Figures 1A and 1B). The densities of NFI neurons differed significantly among the groups (F-ratio 5.85, 4/149 df, p<0.001). Post-hoc testing revealed significantly higher overall (all regions combined) densities of NFI neurons in AD compared with AD+PD (p<0.05), PD (p<0.01), and control (p<0.005) brains, and in AD+DN compared with control (p<0.05) brains (Table 2).

With regard to specific regions of the brain, in AD the mean densities of NFI neurons in Brodmann Areas 24, 4, and 21 were significantly greater than control (p<0.001) (Figure 2). In Brodmann Area 11 and the entorhinal cortex, NFI neurons were also more abundant in AD compared with control brains, but the standard errors were too large to achieve statistical significance. In the CA1+CA2 region of the hippocampal formation the mean density of NFI neurons in AD (69.7 ± 5.8) was similar to the findings in Area 11, but corresponding control hippocampal sections were not available in sufficient quantities for statistical comparison. In contrast, NFI neurons in Areas 40 and 17 were not significantly more abundant in AD relative to control. In AD+DN, the mean densities of NFI neurons in Brodmann Areas 24, 4, 40, and 21 were also significantly higher than control (p<0.001) (Figure 2). With respect to Area 11, Area 17, and the entorhinal cortex, the density of NFI neurons in AD+DN was not significantly different from control. The CA1+CA2 region of the AD+DN hippocampal formation had a mean density of 37.5 ± 15.7 NFI neurons, similar to several regions of control brains. For Area 17, the mean density of NFI neurons in AD+DN was also not significantly different from control. The AD+PD and PD brains exhibited significantly higher mean densities of NFI neurons relative to control in Brodmann Area 24 (p<0.001) but not in Area 21 or the entorhinal cortex. AD+PD and PD brain sections from Areas 4, 11, 40, and 17 were not available for study.

NFI Neurofibrillary Tangles

NFI neurofibrillary tangles exhibited linear, smooth, often flame-shaped fibrillar immunoreactivity in neuronal perikarya (Figures 1C–1E). Some neurons with NFI neurofibrillary tangles also contained granular NFI immunoreactivity. The overall mean densities (all regions combined) of NFI neurofibrillary tangles differed significantly among the groups (F-ratio 22.3, 4/149 df, p<0.001) (Table 2). In AD, NFI neurofibrillary tangles were significantly more abundant than in AD+PD (p<0.005), PD (p<0.001), and control (p<0.001) brains. In AD+DN, NFI neurofibrillary tangles were significantly more abundant than in all other groups (p<0.001). In control brains, NFI neurofibrillary tangles were either rare or not detected. In AD+PD and PD, NFI neurofibrillary tangles were slightly more numerous than in control brains, but the differences were not statistically significant (Table 2).

For all regions examined except Brodmann Area 17, NFI neurofibrillary tangles were significantly more abundant in AD and AD+DN than in control brains (all p<0.005) (Figure 2). In AD, the highest densities of NFI neurofibrillary tangles were observed in Brodmann Areas 11, 21, and 40, and in the CA1+CA2 region of the hippocampus (148 ± 71). Similarly, in AD+DN, significantly increased densities of NFI neurofibrillary tangles were observed in Brodmann Areas 24, 11, and 21, the entorhinal cortex, and the CA1+CA2 region of the hippocampal formation (339 ± 93) relative to control (p<0.001). Significant differences between AD and AD+DN were also observed in Area 24, Area 40, Area 17, and the entorhinal cortex. In all except Area 40, the mean densities of NFI neurofibrillary tangles were higher in AD+DN than in AD. In AD+PD, the regional mean densities of NFI neurofibrillary tangles were consistently higher than control (p<0.01), but below AD and AD+DN (both p<0.005). All but one of the five PD brains either lacked or exhibited only rare NFI neurofibrillary tangles, similar to control. In the remaining PD brain, NFI neurofibrillary tangles were as abundant as observed in AD+PD.

NFI Plaques

NFI plaques were characterized by discrete aggregates of swollen, irregular neurites, often with a cleared central area (Figures 1G and 1H). Significant intergroup differences were observed with respect to the overall mean density of cortical NFI plaques (F-ratio 130.1, 4/149 df, p<0.001) (Table 2). In AD, the overall NFI plaque density was significantly greater than in AD+PD (p<0.05), PD (p<0.001), and control (p<0.001) brains. AD+DN brains exhibited 2.5- to fourfold higher NFI plaque densities than in AD, and five- to 20-fold higher plaque densities than in AD+PD, PD, and control brains (all p<0.001) (Figure 3). In addition, NFI plaques were on average twice as abundant in AD+PD as in PD and twice as abundant in PD compared with control brains; however, these differences were not statistically significant because of large standard errors.
Since the within-group regional variability of plaque density and NFI dystrophic neurites (see below) was small, only the results corresponding to Area 24, Area 21, and the entorhinal cortex, in which comparison data from all five groups were available, are depicted graphically (Figure 3). In AD, the mean density of NFI plaques in Area 21 was significantly greater than in AD+PD (p<0.01), PD (p<0.005), and control (p<0.001) brains. In addition, in the entorhinal cortex the NFI plaque density in AD was significantly higher than in all regions of control brains (p<0.05). In AD+DN, the mean densities of NFI plaques in Area 24, Area 21, and the entorhinal cortex were all significantly higher than in the other four groups (p<0.001). In contrast, in AD+PD and PD the mean NFI plaque densities in all three regions were not significantly different from control.

**NFI Dystrophic Neurites (Dendrites)**

NFI dystrophic neurites consisting of scattered (non-aggregated) swollen, irregular, coarse, short fibers (Figure 1F) were distributed throughout the neuropil in AD, AD+DN, AD+PD, and PD. The density of NFI dystrophic neurites was assessed in cortical Layer 2, where they were most abundant. The overall mean density of NFI dystrophic neurites was similarly high in all four of the dementia groups, whereas in control brains NFI dystrophic neurites...
NF Expression in AD and PD

were seldom detected (F-ratio 14.0, 4/149 df, p < 0.001), regardless of cortical layer (Table 2). Correspondingly, the mean densities of NFI dystrophic neurites in Area 24, Area 21, and the entorhinal cortex were all significantly greater in AD (p < 0.001), AD + PD (p < 0.001), and PD (p < 0.005) than in control brains (Figure 3). For the AD + DN group, a significant difference from control was obtained only for Area 21 (p < 0.005). For Area 24 and the entorhinal cortex, the standard errors of the means overlapped with those of the other four groups, thereby rendering the differences not statistically significant.

NFI Irregular Axons

Coarse, irregular NFI axons associated with attrition of normal fibers were observed in virtually all sections of central cerebral white matter from AD (97%) and AD + DN (100%) brains (Figure 1; Table 2). Similar but significantly less common abnormalities were also detected in AD + PD (50%), PD (29%), and control (9%) brains (χ² 101.9, 4 df, p < 0.001). In addition, the white matter fiber loss was more severe in AD and AD + DN than in the other groups (compare Figures 1I and 1J). White matter fiber attrition could not be quantitated; however, its presence was noted by the absence of a coarse meshwork of uniformly distributed NFI fibers (compare backgrounds in Figures 1I and 1J). The axonal irregularity and fiber loss described herein is indistinguishable from that described previously in silver impregnated sections of AD (9, 13) and AD + DN (11) brains.

Discussion

This study demonstrates that the mean densities of NFI neurons, neurofibrillary tangles, dystrophic neurites (predominantly dendrites), and neuritic plaques are significantly higher in brains with AD and AD + DN than in brains from aged control patients. In addition, AD and AD + DN brains more frequently contained coarse irregular axons in central cerebral white matter compared with aged...
controls. Using stepwise multivariate regression analysis, the best predictors of an AD diagnosis were high densities of NFI dystrophic neurites \((r = 0.77, p<0.001)\), neurofibrillary tangles \((r = 0.69, p<0.01)\), and neuritic plaques \((r = 0.81, p<0.001)\). Therefore, by quantitating NFI paired helical filament-associated lesions immunostained with a cocktail of MAbs to phosphorylated and non-phosphorylated neurofilament, one could readily distinguish between AD and changes due to aging. Although the densities of NFI neurofibrillary tangles and plaques in AD+PD and PD were significantly lower than in AD or AD+DN, these four groups shared in common similarly high densities of NFI dystrophic neurites, which were seven- to 20-fold more abundant than in control brains. This suggests that the single feature that consistently distinguished the dementia groups from the control brains was the presence of highly abundant NFI dystrophic neurites in Layer 2 of the cerebral cortex.

Granular neurofilament immunoreactivity in neuronal perikarya that were devoid of neurofibrillary tangles has been reported previously \((20,26,47)\) and attributed to one of the following processes: (a) perikaryal accumulation of non-phosphorylated neurofilament in neurons destined to undergo neuronal degeneration, including neurofibrillary tangle formation \((47)\); (b) a normal distribution of non-phosphorylated neurofilament proteins in neuronal perikarya \((20)\); or (c) accumulation of phosphorylated neurofilament, perhaps secondary to terminal nonspecific neuronal injury \((26)\). Our own observation of increased densities of neurons with this type of labeling in brains with AD lesions is consistent with the first of these hypotheses. On the other hand, we also found that neurons located in the immediate vicinity of recent or resolving incidental microinfarcts in control tissue sections also exhibited increased granular perikaryal immunoreactivity for neurofilament.

### Table 2. Overall distribution of neurofilament immunoreactivity

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of blocks</th>
<th>Neurons*</th>
<th>Neurofibrillary tangles*</th>
<th>Plaques*</th>
<th>Neurites*</th>
<th>Irregular white matter axons*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>33</td>
<td>87.8 ± 9.3</td>
<td>93.8 ± 13.7</td>
<td>30.6 ± 4.0</td>
<td>151.9 ± 19.3</td>
<td>100%</td>
</tr>
<tr>
<td>AD + DN</td>
<td>30</td>
<td>65.4 ± 9.8</td>
<td>148.1 ± 14.4</td>
<td>90.4 ± 3.7</td>
<td>100.5 ± 25.4</td>
<td>100%</td>
</tr>
<tr>
<td>AD + PD</td>
<td>15</td>
<td>49.9 ± 15.5</td>
<td>14.8 ± 22.8e</td>
<td>13.3 ± 5.5e</td>
<td>133.3 ± 24.3</td>
<td>53%e</td>
</tr>
<tr>
<td>PD</td>
<td>18</td>
<td>39.4 ± 14.3e</td>
<td>3.0 ± 21.1e</td>
<td>6.8 ± 3.3e</td>
<td>134.7 ± 23.4</td>
<td>28%e</td>
</tr>
<tr>
<td>Aged controls</td>
<td>58</td>
<td>35.4 ± 6.6e</td>
<td>0.6 ± 9.8e</td>
<td>3.3 ± 3.2e</td>
<td>7.3 ± 13.9f</td>
<td>10%f</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

* Frequency (% of blocks).

* Significantly less than in AD and AD + DN.

Granular neurofilament immunoreactivity in neuronal perikarya that were devoid of neurofibrillary tangles has been reported previously \((20,26,47)\) and attributed to one of the following processes: (a) perikaryal accumulation of non-phosphorylated neurofilament in neurons destined to undergo neuronal degeneration, including neurofibrillary tangle formation \((47)\); (b) a normal distribution of non-phosphorylated neurofilament proteins in neuronal perikarya \((20)\); or (c) accumulation of phosphorylated neurofilament, perhaps secondary to terminal nonspecific neuronal injury \((26)\). Our own observation of increased densities of neurons with this type of labeling in brains with AD lesions is consistent with the first of these hypotheses. On the other hand, we also found that neurons located in the immediate vicinity of recent or resolving incidental microinfarcts in control tissue sections also exhibited increased granular perikaryal immunoreactivity for neurofilament.
whereas more distant neurons lacked or exhibited only faint neurofilament immunoreactivity. This observation would be more consistent with the third hypothesis.

Experimentally, injury that results in impaired axonal transport has been associated with neurofilament accumulation in neuronal perikarya (48). Nerve fiber transection itself causes enhanced phosphorylation of neurofilament in the perikarya (49), as well as increased NFI in swollen axons (50). Moreover, a number of neurodegenerative diseases that affect neurofilament structure exhibit neurofilament phosphorylation in neuronal perikarya, as well as in dendrites (20,28,39,40,51–54). Increased granular immunoreactivity for other phosphorylated cytoskeletal proteins in neuronal perikarya devoid of neurofibrillary tangles has also been described in AD (55–57). Overall, the findings suggest that phosphorylation of neurofilament and tau precedes the development of neurofibrillary tangles in AD, as well as in other diseases in which dystrophic neurites and neurofibrillary tangles are integral histopathological components (55–58). Hyperphosphorylation of neurofilament and tau is also a feature of PD (13,28,31,59,60), Pick's disease (61), and neuronal achromasia (54), in which insoluble filamentous material accumulates as neuronal perikaryal inclusions. The increased density of such neurons in AD and AD+DN observed herein may represent either a stage in the evolution of neuronal degeneration and neurofibrillary tangle formation, or increased vulnerability of cortical neurons to nonspecific injury imposed by terminal metabolic dysfunction.

High densities of NFI neurofibrillary tangles were significantly correlated with AD diagnosis (r = 0.62, p<0.001) and clearly distinguished between AD and aged control brains. However, the AD+PD and PD brains also had end-stage AD-type dementia, yet the densities of NFI neurofibrillary tangles were significantly lower than in AD, and in PD the densities were similar to control. Similar results were obtained by Bielschowsky silver staining. Therefore, although classical AD dementia was associated with high densities of NFI or Bielschowsky-stained neurofibrillary tangles, cognitive impairment in the AD+PD and PD groups could not be explained on this basis and must therefore be attributed to another pathological process or lesion. The occurrence of AD-type dementia in the context of low densities of neurofibrillary tangles has been described and previously attributed to abundant senile plaques (11,45).

Corresponding with the clinical manifestation, in AD, high densities of NFI neurofibrillary tangles were detected in the CA1+CA2 region of the hippocampus, orbital frontal region, temporal lobe neocortex, and Area 40 of the parietal lobe compared with Area 17 of the primary visual cortex. Similarly, in AD+DN, significantly higher densities of NFI neurofibrillary tangles were observed in the cingulate gyrus, orbital frontal gyrus, entorhinal cortex, and temporal lobe neocortex compared with primary motor and visual cortex.

The densities of NFI plaques observed in the AD and control brains were similar to those reported by others using tau immunostaining (1). NFI- or tau-immunoreactive plaques were rarely identified in aged control brains, and ostensibly no overlap was observed between AD and normal aging. In contrast, with Bielschowsky silver impregnation or by immunostaining for amyloid β-peptide (1), there was considerable overlap between the AD and control groups. Therefore, despite the greater sensitivity of Bielschowsky and amyloid stains, quantitation of neurofilament- or tau-immunoreactive plaques may provide a more specific and reliable means of discriminating between AD and normal aging. Although AD-type dementia has been attributed to the presence of abundant senile plaques in up to 30% of cases (11), this would be an unlikely explanation for patients in the AD+PD and PD groups, because their densities of NFI (or Bielschowsky stained) plaques were not significantly greater than control.

Abundant and widespread NFI dystrophic neurites in Layer 2 of the cerebral cortex was the third feature that distinguished AD from aged control brains. Unlike the findings for NFI neurofibrillary tangles and NFI plaques, the densities of NFI dystrophic neurites were equally high in AD, AD+DN, AP+PD, and PD. Moreover, a high density of NFI neurites was the single best independent predictor of AD-type dementia (r = 0.5, p<0.001).
other words, widespread abundant NFI neurites comprised the major immunohistopathological lesion correlated with dementia in AD, AD+PD, and PD, whereas NFI neurofibrillary tangles and plaques did not uniformly predict dementia in all three groups. Dystrophic neurites are a well-recognized neuropathological feature of AD, as previously demonstrated by silver impregnation stains (9,62) and immunohistochemical staining for neurofilament, tau, or Aβ8 protein (using Alz-50 antibody) (7,8,24,55,63). In addition, recent observations suggest that neurite pathology may be a fundamental and early feature of AD neuronal degeneration and that higher densities of dystrophic neurites are correlated with greater severity of dementia in AD (6). Dystrophic neurites in AD appear to result largely from dendritic pathology characterized by degeneration, loss, swelling, and "lawless growth" or sprouting of dendritic spines (9).

White matter NFI lesions were also more prominent in AD than control brains. The principal findings included axonal swellings, dystrophic neurites, and attrition of NFI fibers. These lesions were indistinguishable from those described previously in AD (44,64,65) and in trauma-induced axon injury (50). The pathogenesis of the axon loss and fiber swellings is unknown but may result from abnormal phosphorylation of neurofilament and other cytoskeletal proteins or from impaired axonal transport. Alternatively, ischemic atrophy secondary to hyperfusion of central white matter has been proposed to be the underlying basis of axon degeneration in Alzheimer's disease (64).

In conclusion, NFI neurofibrillary tangles, neuritic plaques, dystrophic neurites, and irregular, swollen white matter axons could be detected simultaneously using a cocktail of MAb's to phosphorylated and non-phosphorylated forms of the high and mid-molecular weight subunits of neurofilament protein. With this method it was possible to delineate all of the typical CNS structural lesions correlated with dementia in AD and, with quantification, to better characterize or classify AD+PD and PD dementia within the spectrum of AD neurodegenerative pathology. It is unlikely that the findings in this study resulted from cerebral atrophy alone, since previous morphometric analysis studies of gross cerebral slices revealed markedly different and distinct patterns of atrophy in AD, AD+DN, AD+PD, and PD (44,65,66). The findings in this study underscore the concept that neurite dystrophy with widespread interneuronal synaptic disconnection constitutes a fundamental lesion in AD-type dementia (6,67–70). Moreover, neurite degeneration superimposed on subcortical degeneration (66) may contribute to the clinical manifestations of AD-type dementia in individuals with Parkinson's disease.

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