Systematic Regional Variations in the Loss of Cortical Cholinergic Fibers in Alzheimer’s Disease

The loss of cortical cholinergic fibers in Alzheimer’s disease was investigated using choline acetyltransferase immunohistochemistry and acetylcholinesterase histochemistry. Within both the normal and Alzheimer’s cerebral cortex, the two methods revealed an identical pattern of fiber staining. In the normal brain, cholinergic fiber density was highest in limbic and paralimbic cortical zones, intermediate in most sensory-motor and association zones, and lowest within the primary visual and visual association areas of the occipital lobe. In general, supragranular cortical layers contained a higher density of cholinergic fibers, and most of these were oriented vertically. In Alzheimer’s disease, an overall 55% loss of cortical cholinergic fibers was detected. There was, however, marked regional variations in the extent of this loss in different cortical areas. Cortical areas within the temporal lobe, particularly the temporal association areas, displayed a dramatic loss of cholinergic fibers. By contrast, the anterior cingulate cortex, primary visual, primary somatosensory, and primary motor cortex displayed a relative preservation of cholinergic fibers. As a whole, greater loss of cholinergic fibers was detected in supragranular layers and in fibers oriented vertical to the cortical surface. These results indicate that cholinomimetic therapies are likely to have different effects on cholinergic transmission in various cortical areas. The precise mechanisms that lead to the regional variations in cortical cholinergic degeneration in Alzheimer’s disease remain to be elucidated.

The human cerebral cortex contains a complex and extensive network of cholinergic axons (Geula and Mesulam, 1990; Mesulam et al., 1992; Mesulam and Geula, 1994). Virtually all of these axons originate from the cholinergic neurons of the basal forebrain (Ch1–Ch4), which are rich in the enzymes choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) (Mesulam et al., 1983, 1986; Mesulam and Geula, 1988a). Within the human cerebral cortex, AChE and ChAT staining reveal a completely overlapping pattern of axons (Mesulam and Geula, 1992).

A marked loss of cortical cholinergic innervation was the first neurotransmitter abnormality detected in the brains of patients suffering from Alzheimer’s disease (AD). In 1976, Davies and Maloney (1976) and Bowen et al. (1976) reported a dramatic loss in levels of biochemically determined ChAT activity in postmortem tissue from cerebral cortex of AD patients. Since that time, a severe loss of cortical ChAT activity (up to 95%) has become established as one of the most consistent findings in AD (Perry et al., 1977; Bowen et al., 1979; Aranu et al., 1986; Reinikainen et al., 1988). Our earlier observations using AChE histochemistry of three cortical areas showed considerable variation in the loss of cholinergic fibers in different cortical areas (Geula and Mesulam, 1989). Here, we report marked regional variations in the loss of cortical cholinergic fibers using AChE histochemistry and ChAT immunohistochemistry combined with a survey of a large number of cortical areas.

Materials and Methods

Tissue Preparation and Pathological Observations

The observations described in this report were made in 10 brains from normal-aged individuals with no prior history of neurologic or psychiatric disorders and 10 brains from patients with a history of dementia of the Alzheimer type. The characteristics of the subjects are summarized in Table 1. Each brain was cut into 1–2 cm hemispheric coronal slabs and examined for the presence of atrophy, ventricular enlargement, and other gross abnormalities. The slabs of tissue were placed in cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24–30 hr, then into graded concentrations of sucrose (10–40% in 0.1 M phosphate buffer, at 4°C) for cryoprotection. The slabs were then sectioned at 40 μm on a freezing microtome into 0.1 M phosphate buffer and stored at 4°C until used. Representative sections from each tissue block were stained with hematoxylin-eosin and thioflavins-S for neuropathological observations and with cresyl echt violet for delineation of cytoarchitectonic boundaries. In most of the brains (six normal and six AD cases), tissue was available from the whole extent of the hemisphere and all cortical areas. In the rest, tissue was available from temporal, anterior parietal, and posterior frontal areas, and in some of these from the occipital and anterior frontal cortex as well.

Only brains with no gross or microscopic abnormalities and no or very few cortical plaques and tangles, consistent with normal aging (Khachaturian, 1985), were designated as normal (cases 1–10, Table 1). The brains from the demented individuals (cases 10–20) contained numerous cortical plaques and tangles in a density and distribution consistent with the neuropathological diagnosis of AD (Khachaturian, 1985). Brains that displayed other gross or microscopic neuropathological abnormalities were not used in this study.

Acetylcholinesterase Histochemistry

Acetylcholinesterase activity within cortical axons and perikarya was visualized in a representative series of sections from each brain with the help of a new and highly sensitive histochemical method. The principles of this method (incubation in a dilute Karnovsky-Roost medium followed by metal ion-diaminobenzidine intensification) have been described by Hanks et al. (1973) and Tago et al. (1986). We have introduced a number of changes in this method as described elsewhere (Geula and Mesulam, 1989; Mesulam and Geula, 1994).

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Assessment of Cholinergic Fiber Density

To inhibit butyrylcholinesterase (BChE, nonspecific cholinesterase), 2 × 10^{-4} M ethopropazine (MW 348.9) or 10^{-5} M Iso-OMPA (MW 342.4, Sigma Chemical Company, St. Louis, MO) were used in the incubation medium. The specific AChE inhibitor BW284C51 (MW 556.4, Sigma Chemical Company) was added (10^{-4} M) to demonstrate the specificity of the AChE staining.

Choline Acetyltransferase Immunohistochemistry

Representative series of sections from three normal and three AD cases were processed for ChAT immunohistochemistry using a well-characterized polyclonal antibody (generously provided by Dr. L. B. Hersh, University of Kentucky, Lexington, KY) raised in the rabbit against human placental ChAT (German et al., 1985). The antibody was used at a dilution of 1:500 to 1:700 in an avidin–biotin–peroxidase (ABC) immunohistochemical procedure employing the Vecta-stain Elite ABC kit (Vector Laboratories, Burlingame, CA). The final immunohistochemical reaction product was intensified according to the method described by Kitt et al. (1988).

Two types of control procedures were used. In one set of control sections, an irrelevant IgG was substituted for the primary antibody.

Assessment of Cholinergic Fiber Density

All sections processed for AChE histochemistry and ChAT immunohistochemistry were subjected to a qualitative survey for the assessment of regional variations in fiber density in the normal brains and in fiber loss in AD brains. In normal (N = 6) and AD (N = 6) brains from which whole hemispheric sections were available, an intersect analysis was used (Geula and Mesulam, 1989) to obtain a quantitative estimate of fiber density in 28 cytoarchitectonically and functionally distinct cortical areas (Tables 2, 3). For this purpose, tissue sections processed for AChE histochemistry were viewed at 200× magnification through a square 10 × 10 grid (5 × 5 mm actual dimensions) placed in the ocular of a Nikon Compound microscope (Fig. 1). At the above magnification, the grid contained a square of tissue 250 × 250 μm. The grid was adjusted such that one side of it was parallel to the cortical surface. The number of fibers intersecting the 10 lines parallel (for determination of vertically oriented fibers) and perpendicular (for determination of horizontally oriented fibers) to the cortical surface were counted and recorded in lower lamina III (lamina IIIC and upper lamina V of each cortical area examined. In areas with more primitive lamination, such as the hippocampus and the cingulate cortex, counting was performed in a superficial and a deep layer. To ensure that intersects from all stained fibers within the full 40 μm thickness of each section were counted, the plain of focus on the microscope was systematically varied while counting. The best stained areas within each cytoarchitectonic region were chosen for this analysis. The counts obtained from the lines of the grid were then added to obtain an estimate of fiber density (Tables 2, 3).

These counts were subjected to analysis of variance for repeated measures with Newman–Keuls post hoc tests to determine significant differences.

Results

General Staining Characteristics

Immunohistochemistry with the ChAT antibody revealed very thin cholinergic fibers and varicosities throughout the cerebral cortex (Fig. 2). No staining was observed when nonspecific IgG was substituted for the antibody or when the antibody was adsorbed with purified ChAT. No specific ChAT staining was observed in cortical neurons.

The AChE histochemical procedure also revealed a dense plexus of fibers throughout the cerebral cortex. When compared with the ChAT-positive fibers, AChE-positive fibers appeared thicker and more distinctly stained (Fig. 2). The histochemical procedure used also revealed many AChE-positive cortical neurons (see Figs. 4, 5). The great majority of these neurons were pyramidal in shape and were distributed in lamina III and V of many cortical areas. We have previously described the staining characteristics and distribution of these neurons.
neurons in both the normal and AD cortex (Mesulam and Geula, 1988b; Mesulam and Geula, 1991; Heckers et al., 1992) and therefore will not dwell on them in this report. The AChE activity in both fibers and neurons was reliably and completely inhibited by 10^{-4} M of the specific AChE inhibitor BW28c51 but was unaffected by an equal concentration of the specific BChE inhibitor iso-OMPA.

Within the brains that were processed for ChAT immunohistochemistry and AChE histochemistry, the two procedures revealed an identical regional and laminar pattern and density of cortical fibers. In both the normal and AD brains, areas that showed a high density of ChAT-positive fibers also displayed a high density of AChE-positive fibers, and areas with a low density of ChAT-positive fibers also displayed a low density of AChE-positive fibers (Fig. 2). This matched pattern of ChAT and AChE stained fibers was observed in all cortical areas examined for this reason, and because the very thin and varicose ChAT-positive fibers did not lend themselves to counting, we carried out our quantitative assessments of fiber density using AChE stained material.

### Cortical Cholinergic Fibers in the Normal Brain

A prominent plexus of cholinergic fibers was present in all cortical areas of the normal brains. The density of these fibers, however, displayed considerable regional variations (Table 2).

### Table 3

<table>
<thead>
<tr>
<th>Cortical regions</th>
<th>Normal</th>
<th>Alzheimer’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Layer III</td>
<td>Layer V</td>
</tr>
<tr>
<td>Prefrontal association (9*)</td>
<td>157 ± 18</td>
<td>109 ± 11</td>
</tr>
<tr>
<td>Frontal pole (10)</td>
<td>145 ± 34</td>
<td>117 ± 47</td>
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<tr>
<td>Frontal operculum (44)</td>
<td>163 ± 36</td>
<td>138 ± 26</td>
</tr>
<tr>
<td>Prefrontal association (10)</td>
<td>160 ± 30</td>
<td>127 ± 32</td>
</tr>
<tr>
<td>Temporal visual association (21)</td>
<td>153 ± 17</td>
<td>135 ± 22</td>
</tr>
<tr>
<td>Temporal parietal association (20)</td>
<td>189 ± 26</td>
<td>142 ± 33</td>
</tr>
<tr>
<td>Inferior parietal lobule (39-40)</td>
<td>154 ± 25</td>
<td>126 ± 25</td>
</tr>
<tr>
<td>Superior parietal lobule (17)</td>
<td>138 ± 18</td>
<td>93 ± 13</td>
</tr>
<tr>
<td>Unimodal association areas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premotor association (6)</td>
<td>158 ± 32</td>
<td>110 ± 17</td>
</tr>
<tr>
<td>Auditory association (22)</td>
<td>182 ± 47</td>
<td>146 ± 29</td>
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<tr>
<td>Somatosensory association (5)</td>
<td>142 ± 18</td>
<td>111 ± 21</td>
</tr>
<tr>
<td>Visual association (18)</td>
<td>102 ± 31</td>
<td>81 ± 26</td>
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<tr>
<td>Visual association (19)</td>
<td>115 ± 33</td>
<td>88 ± 28</td>
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<tr>
<td>Primary sensory and motor areas</td>
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<td></td>
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<tr>
<td>Primary auditory (41-43)</td>
<td>214 ± 33</td>
<td>185 ± 37</td>
</tr>
<tr>
<td>Primary motor (4)</td>
<td>181 ± 35</td>
<td>145 ± 23</td>
</tr>
<tr>
<td>Primary somatosensory (3, 1, 2)</td>
<td>169 ± 29</td>
<td>146 ± 29</td>
</tr>
<tr>
<td>Primary visual (17)</td>
<td>111 ± 20</td>
<td>97 ± 27</td>
</tr>
<tr>
<td>Limbic and paralimbic areas</td>
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<td></td>
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<tr>
<td>Anterior cingulate (24)</td>
<td>306 ± 60</td>
<td>284 ± 40</td>
</tr>
<tr>
<td>Anterior cingulate (23)</td>
<td>278 ± 31</td>
<td>268 ± 50</td>
</tr>
<tr>
<td>Parolfactory area (25)</td>
<td>384 ± 104</td>
<td>357 ± 140</td>
</tr>
<tr>
<td>Posterior cingulate (23)</td>
<td>268 ± 40</td>
<td>219 ± 43</td>
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<tr>
<td>Granular temporal pole (30)</td>
<td>251 ± 29</td>
<td>189 ± 47</td>
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<tr>
<td>Granular insula</td>
<td>360 ± 92</td>
<td>223 ± 31</td>
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<tr>
<td>Temporal pole (30)</td>
<td>337 ± 64</td>
<td>330 ± 33</td>
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<tr>
<td>CAT Sector of hippocampus</td>
<td>471 ± 144</td>
<td>465 ± 131</td>
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<tr>
<td>Subiculum</td>
<td>367 ± 98</td>
<td>324 ± 101</td>
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<tr>
<td>Dlgysynaptic orbital frontal (11-12)</td>
<td>374 ± 77</td>
<td>257 ± 72</td>
</tr>
<tr>
<td>Granular orbital frontal (11-12)</td>
<td>231 ± 94</td>
<td>169 ± 27</td>
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</tbody>
</table>

Each value represents the sum of counts for fibers with vertical or horizontal orientation in layer II or V averaged across subjects ± standard deviation. Repeated measures ANOVA indicated significant effects for variables of cortical area (F = 71.69, p < 0.0001), disease state (F = 97.20, p < 0.0001), cortical layer (F = 114.33, p < 0.0001), and fiber orientation (F = 85.52, p < 0.0001). The probability values referred to in the text indicate results of Newman-Keuls pairwise comparisons.

*Numbers in parentheses refer to cortical areas according to Brodmann’s classification.

Among the areas examined, the hippocampal formation displayed the highest density of fibers (p < 0.001). Paralimbic cortical areas such as the cingulate, entorhinal, orbitofrontal cortex, and the temporal pole and insula displayed the next highest density of cholinergic fibers (p < 0.025). The primary auditory, somatosensory, and motor cortex displayed an intermediate density of fibers and tended to contain slightly more fibers than most association cortical areas, while the primary visual and visual association areas displayed the lowest density of cholinergic fibers. The later differences, however, were not statistically significant (p > 0.05).

In all cortical areas, fibers travelled vertically, horizontally, and tangentially. Overall, a greater number of fibers were oriented vertically as compared with horizontally oriented fibers. This pattern of fiber orientation was a regularly encountered characteristic of extragranular (superficial) layers. Our counts in layer III showed this difference to be significant in 50% of the areas examined (p < 0.05) and to be a consistent trend in the rest (Table 3). Within the deep layers, horizontally oriented fibers were encountered almost as frequently as vertical fibers (p > 0.05). The overall density of fibers was greater in superficial layers as compared with the deep cortical laminae. Quantitative comparison of fiber density in layer III versus V revealed consistently higher fiber counts in layer III, but this difference did not reach significance (p > 0.05).

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Loss of Cortical Cholinergic Fibers in Alzheimer's Disease

All cortical areas examined in AD brains displayed some degree of cholinergic fiber loss when compared with matching areas in normal brains (Table 2). Our quantitative estimate revealed on average a 55% loss of cortical cholinergic fibers in AD. Comparison of individual cortical areas, however, revealed marked and systematic variations in the extent of this loss. These variations were apparent on gross examination of ChAT and AChE stained whole hemispheric sections (Fig. 3). In general, cortical areas within the ventral aspects of the hemisphere displayed a greater loss of fibers when compared with cortical regions within the dorsal portions of the hemisphere. Cholinergic fibers displayed a major depletion in the temporal neocortical areas, most of which were virtually empty of fibers (Fig. 4). By contrast, the cholinergic fibers in the premotor cortex, the cingulate gyrus (Fig. 5), the sensorimotor cortex, and in some of the frontal association areas seemed to be relatively well preserved.

The areas with the greatest loss (> 80% reduction) of cholinergic innervation were all in the temporal lobe and included the primary auditory cortex, superior temporal gyrus and inferior temporal sulcus. These areas showed a 70% loss of fibers. The anterior cingulate gyrus, primary motor, primary somatosensory, and primary visual cortex displayed less than 40% loss of cholinergic fibers (Table 2, Fig. 5). In the primary visual area, anterior cingulate (Brodmann areas 24, 25, and 32) and the primary motor cortex of AD brains, the loss of cholinergic fibers was not statistically significant (p > 0.05). Within the hippocampal formation, fiber density appeared to be reduced in all sectors (Fig. 3), although the very high density of fibers in the CA1 sector of Ammon's horn and a 59% loss in the subiculum of AD brains.

No consistent pattern was observed in the loss of cholinergic axons in functionally similar cortical areas. Some trends, however, were observed. Primary motor and sensory areas, except the primary auditory cortex, displayed among the lowest percentages of loss in AD (18–39%). Consistent with its anatomical location within the temporal lobe, the primary auditory cortex displayed a relatively high degree (69%) of cholinergic fiber loss. In general, paralimbic cortical areas displayed a more marked loss of cholinergic fibers than most neocortical areas. This trend was particularly apparent within the entorhinal cortex, temporal pole, and insula, which displayed relatively high percentages (73–80%) of loss. The cingulate paralimbic areas, on the other hand, displayed among the lowest loss of fibers (26–42%). All association cortical areas except those within the temporal lobe displayed an intermediate reduction in the density of cholinergic fibers. The association cortical areas within the temporal lobe (Brodmann areas 20, 21, and 22) displayed the highest percentage of cholinergic fiber loss (84–85%) amongst all areas examined.

The superficial cortical laminae tended to display a consistently greater magnitude of cholinergic fiber loss than deep laminae. In most cortical areas, there was also a greater loss of vertically oriented fibers as compared with fibers with a horizontal orientation. These differences, however, were not statistically significant (p > 0.05), except in a few cortical areas such as the visual association cortex (area 18, p < 0.05).

Discussion

The histochemical methods used in this experiment revealed a dense plexus of AChE- and ChAT-positive fibers throughout the cerebral cortex within the normal brain, and a dramatically reduced density of these fibers in AD. Consistent with our earlier observations (Mesulam and Geula, 1992), which were based on tissue stained separately as well as concurrently for ChAT and AChE, we found a completely overlapping pattern and apparent density of cortical AChE- and ChAT-positive fibers in the normal brain. We further observed an overlap between these fibers in the AD cortex. Thus, within both the normal and AD cerebral cortex, an AChE-positive pattern of fiber staining is a good marker for cholinergic axons (See Mesulam et al., 1984; Geula and Mesulam, 1989, for review).

Within the normal brain, we found variations in the regional pattern of cortical cholinergic fiber distribution as well as in fiber lamination and orientation. The core limbic regions displayed the highest density of cholinergic fibers closely by paralimbic areas. The majority of primary sensory and association areas displayed an intermediate fiber density. The primary visual cortex and the visual association areas within the occipital lobe displayed the lowest density of cholinergic fibers. In the majority of areas, fiber density tended to be higher in the superficial layers within which vertically oriented fibers predominated. These observations are consistent with our recent investigations based on AChE histochemistry and ChAT immunohistochemistry in the human brain (Mesulam and Geula, 1988a, 1992; Geula and Mesulam, 1989, 1990). Several studies of biochemically determined ChAT and AChE activities have shown a similar distribution pattern of cholinergic markers in the human cerebral cortex (Perry et al., 1977; Davies, 1979; Rossor et al., 1982a; Reimkainen et al., 1988; Javoy-Agid et al., 1989).

Cortical Cholinergic Fibers in Alzheimer's Disease

In the AD cerebral cortex, we found very substantial but regionally variable loss of cortical cholinergic fibers. The severity of this depletion was greatest (80–85%) in the temporal lobe, including its paralimbic and association components. The visual association areas within the temporal lobe (areas 20, 21, and 22) were virtually emptied of cholinergic fibers.
Figure 2. Photomicrographs of ChAT (A, C, and E) and AChE-positive (B, D, and F) fibers in the CA1 sector of the hippocampus (A and B), anterior cingulate cortex (area 24, C and D), and granular insula (E and F) from an AD case. Note the virtually identical pattern and density of ChAT- and AChE-positive fibers in each area. The two enzymes revealed the same pattern of fiber staining in all cortical areas within both normal and AD brains. Magnification: A and B, 176.8×; C–F, 110.5×.
Figure 3. Whole hemisphere sections of anterior (A and B), middle (C and D), and posterior (E and F) aspects of the brain in normal (A, C, and E) and AD (B, D, and F) cases stained for AChE. The numbers refer to cortical regions according to the classification of Brodmann (see tables for functional affiliations of various areas). Note the relative preservation of cortical AChE staining (mostly of fiber type) within the dorsal and medial aspects of the hemisphere and the marked loss of staining within the ventral cortical areas in the AD brain. AChE-positive fibers within the anterior cingulate cortex (areas 32 and 24) and the motor cortex (area 4) are well preserved, while the neocortical zones within the temporal lobe (areas 20, 21, and 22) are virtually empty of fibers. The AChE-positive bands seen within some cortical areas (arrowheads) are collections of AChE-positive cortical pyramidal neurons. Magnification: A and B, 1.26×; C-F, 0.84×.
By contrast, cholinergic fibers within the anterior cingulate areas (areas 24, 25, and 32), the primary visual, primary somatosensory, and primary motor cortex were relatively well preserved (18–39% loss). The remaining cortical areas displayed an intermediate degree of loss (42–74%).

Among the functionally similar cortical areas, the greatest loss of cholinergic fibers was consistently associated with cortical areas located within the ventral aspects of the hemisphere, while cortical areas located within the dorsal portions of the hemisphere showed relative preservation of fibers. For example, among the primary sensorimotor areas, the primary auditory cortex, which is located within the temporal lobe, displayed a relatively large percentage of loss (69%) while the other primary areas, which are located dorsally, showed some of the lowest percentages of fiber loss (18–39%). A similar pattern of loss was observed amongst the paralimbic and association areas of cortex. Thus, the major determinant of the severity of cholinergic fiber loss in AD cortex appears to be anatomical location rather than functional affiliation, with cortical areas situated within the temporal lobe displaying the most dramatic fiber loss and cortical areas situated within the dorsal and particularly the dorsomedial aspects of the hemisphere displaying the least pronounced loss.

Our observations on the regional variability of cortical cholinergic innervation in AD are in agreement with previous histochemical studies (Henke and Lang, 1983; Mesulam and Geula, 1983; Geula and Mesulam, 1989). In an earlier study of three cortical areas (Geula and Mesulam, 1989), we found a greater loss of AChE-positive fibers in the cingulate cortex than that described in the present report. This is most likely due to slightly different methods of counting and a more severe overall pathology of the cholinergic system in the AD sample used in the former study. It should be pointed out, however, that the regional pattern of AD-related fiber loss displayed by the three areas investigated in our earlier study is identical to that reported here. Biochemical studies have also shown the greatest decrement in cholinergic enzymes in AD to occur in cortical structures within the temporal lobe (Rossor et al., 1980, 1982a; Wilcock et al., 1982; Reinkainen et al., 1988) while cingulate cortex (areas 24, 25, and 32) and cortical areas within the occipital lobe, particularly the primary visual cortex (area 17) have been shown to display the least depletion of cholinergic enzymes (Davies, 1979; Rossor et al., 1980, 1982a; Henke and Lang, 1983). Biochemical studies, however, include many inconsistencies with regards to the extent of cholinergic loss in various cortical regions (Davies and Maloney, 1976; Perry et al., 1977; Davies, 1979; Araujo et al., 1988; Reinkainen et al., 1988). One likely reason for these inconsistencies is the difficulty in precise dissection of various cortical areas. The failure by some investigators to observe regional variations in the depletion of AChE activity (Davies and Maloney, 1976; Davies, 1979; Reinkainen et al., 1988), may also result from the inability of biochemical measurements to distinguish the AChE activity within cholinergic fibers from that present within AChE-positive (but ChAT-negative) cortical neurons. Observations based on histochemical methods are relatively immune from these limitations.

Although the loss of cortical cholinergic markers is found in all cortical laminae, a greater loss has been demonstrated within the superficial laminae (II–III) by both histochemical (Geula and Mesulam, 1989) and biochemical (Henke and Lang, 1983; Perry et al., 1984; DeKosky et al., 1985) studies. Our results indicate a similar trend and further demonstrate a tendency for greater loss of vertically oriented fibers as compared with fibers with a horizontal orientation.

The regional variations in the loss of cortical cholinergic fibers in AD could reflect one of two mechanisms. (1) The effect of AD upon cholinergic innervation could vary from
Figure 4. Examples of cortical areas with the most severe depletion of cholinergic fibers in AD. The entorhinal cortex (A and B) and the auditory association cortex (C and D) of AD (B and D) brains showed a marked loss of their AChE-positive fibers when compared to the same areas in the normal brain (A and C). Magnification, 262×.
Figure 5. Examples of cortical areas with relatively well-preserved cholinergic fibers in AD. The anterior cingulate cortex (area 24, A and B) and the primary visual cortex (C and D) of AD brains (B and D) showed only a minor and nonsignificant loss of their cholinergic fibers when compared with the same areas of the normal brains (A and C). Magnification: A and B, 262×; C and D, 174.7×.
one cortical area to another, or (2) all areas could loose an equal number (or percentage) of their cholinergic fibers but the residual density could vary because of regional differences in premorbid density. Our observations tend to argue against the latter possibility since many cortical areas, which in the normal brain contain comparable densities of cholinergic fibers, showed different degrees of fiber loss in AD. For example, the primary visual cortex and the superior parietal association cortex normally display low densities of cholinergic fibers in the cerebral cortex. In AD, however, the superior parietal association cortex displayed a much more pronounced loss of cholinergic fibers than primary visual cortex (68% vs 39%). Furthermore, the anterior cingulate area, which normally contains quite high densities of cholinergic fibers, displayed a modest depletion (26-33%) when compared to the severe depletion (59-80%) seen in other paralimbic areas (e.g., temporal pole, insula and entorhinal cortex) with a comparable baseline density of cholinergic fibers.

**Relationship to Loss of Cholinergic Neurons of the Basal Forebrain and to Cortical Pathology**

A large body of evidence has indicated a consistent loss of basal forebrain cholinergic neurons in AD brains, ranging in magnitude from 30% to 95% (Perry et al., 1982; Whitehouse et al., 1982; Arendt et al., 1983; Wilcock et al., 1983; Mann et al., 1984; Rinne et al., 1987; Kobayashi et al., 1991). Biochemical investigations have also reported a significant loss of ChAT activity (30-90%) in the nucleus basalis of Meynert (nbM, Ch4) of AD patients (Perry et al., 1982; Rosser et al., 1982b; Bird et al., 1983; Henke and Lang, 1983; Etienne et al., 1986; Koshimura et al., 1987; Rinne et al., 1987).

The magnitude of cholinergic neuronal loss within the basal forebrain of AD patients seems to display regional variations. For example, studies that have compared the sectors of Ch4 (nbM) (Arendt et al., 1985; Etienne et al., 1986; Doucette and Ball, 1987; Mesulam and Geula, 1988a; Wilcock et al., 1988; Mufson et al., 1989; Vogels et al., 1990; Iraizoz et al., 1991; Lehericy et al., 1993) have demonstrated that posterior (Ch4p) and anterolateral (Ch4al) sectors of this cell group show the greatest and most consistent loss, followed by the intermediate (Ch4i) and anteromedial (Ch4am) sectors. This finding is consistent with the greater loss of cholinergic innervation within at least some of the cortical areas that are innervated predominantly by the Ch4p and Ch4al neurons, namely, the temporal cortex and the amygdala (Emre et al., 1993). The few studies that have compared Ch4am to Ch4al (Arendt et al., 1985; Wilcock et al., 1988; Drachman and Leavitt, 1974) have reported the former subsector to be less affected than the latter. This is consistent with the greater preservation of the cholinergic innervation of the cingulate gyri, which is derived predominantly from Ch4am when compared to that of the amygdala, which is derived predominantly from Ch4al.

It should be noted that correspondence between cholinergic loss within specific Ch1-Ch4 sectors and the cortical areas to which they project is not a universal finding. Although a significant correlation has been reported between the depletion of cortical ChAT activity and reduction of nucleus basalis neurons (or basal forebrain ChAT activity) by some investigators (Etienne et al., 1986; Koshimura et al., 1987), insignificant or inconsistent correlation has been found by others (Perry et al., 1982; Wilcock et al., 1983, 1988; Rinne et al., 1987). Furthermore, a number of investigations report that the magnitude of cortical ChAT depletion is much larger than the magnitude of cholinergic neuronal loss in the basal forebrain (Perry et al. 1982; Wilcock et al., 1983, 1988; Etienne et al., 1986; Rinne et al., 1987). If substantiated, this could favor the suggestion that the cholinergic pathology originates in the axonal projections within cortex rather than in the Ch1-Ch4.

ChAT activity in many cortical areas of AD brains has been found to show a significant negative correlation with the density of plaques (Perry et al., 1978, 1981a; Mountjoy et al., 1984; Zubenko et al., 1989). The size of this correlation, however, is quite variable, ranging from -0.34 to -0.82. Moreover, some studies show no significant relationship between the loss of cholinergic enzymes and plaque density (Wilcock et al., 1982; Brashear et al., 1988; DeKosky et al., 1992). Some investigators have also found a small but significant negative correlation (-0.38 to -0.58) between residual cortical ChAT levels and density of tangles (Wilcock et al., 1982; Mountjoy et al., 1984), while others have found no such correlation (Zubenko et al., 1989, Ransmayr et al., 1992). Almost all of these studies have investigated the correlation of plaques and tangles with the residual density of cholinergic input rather than its loss. This is an important distinction because equivalent residual densities could result from greatly different magnitude of loss, depending on premorbid density.

**Relationship between Cholinergic Loss and Cognitive Deficits in Alzheimer's Disease**

The relationship of central cholinergic systems to learning and memory has been the subject of extensive research. Lesions of the Ch4 cholinergic cells deplete the cortex of its cholinergic innervation and impair learning and memory in a number of animal species (Flicker et al., 1983; Aigner et al., 1987; Irl and Markowitsch, 1987; Miyamoto et al., 1987; Peterzel et al., 1988; Ridley et al., 1992). The cholinergic basis of this deficit was demonstrated through the reversibility of memory loss by cholinergic agonists (Dokla and Thal, 1988; Tilson et al., 1988; Ueki and Miyashi, 1989). In humans, cholinergic agonists such as scopolamine have been shown to interfere with learning in young volunteers and to cause a memory deficit reminiscent of that which arises in the course of normal aging (Drachman and Leavitt, 1974).

In view of these relationships, considerable interest has been generated by the possibility that the cognitive deficits in AD are caused by the cholinergic denervation of the cerebral cortex. A significant negative correlation has been reported between cortical ChAT activity and the degree of dementia, as determined by neuropsychological tests (Perry et al., 1978, 1981a; Wilcock et al., 1982; Ruberg et al., 1990; DeKosky et al., 1992), whereas cortical levels of other neurotransmitters, such as NE do not appear to show a significant relationship with dementia (Perry et al., 1987b). The extent of Ch4 neuronal loss has also been shown to be correlated with the degree of dementia (Lehericy et al., 1993).

The possible involvement of the cholinergic system in the processes of learning and memory, the relationship between cholinergic loss and cognitive deficits in AD, and the observation that the loss of cortical cholinergic innervation in this disorder is more severe and occurs earlier than the loss of other cortically projecting neurotransmitter systems (Mann et al., 1980; Perry et al., 1981a, b; Bowen et al., 1983; Cross et al., 1984; D'Amato et al., 1987; Palmer et al., 1987a; Zweig et al., 1988; Aletrino et al., 1992) have provided the basis for cholinergic replacement therapy in AD. Although many trials of AChE inhibitors, cholinergic precursor substances, and receptor agonists have been undertaken (Etienne et al., 1981; Moos et al., 1985; Gwodon et al., 1986; Mouradian et al., 1988; Weinstein et al., 1991), only a few based on AChE inhibition have shown minor and mostly transient improvements (Gustafson et al., 1987; Stern et al., 1987; Francis and Bowen, 1989).

The pattern of residual cholinergic innervation in AD (Table 2) suggests that cholinergic therapies based on the inhibi
tion of AChE are likely to have regionally variable consequences. For example, the inhibition of AChE is unlikely to have much effect in temporal neocortical areas that are virtually empty of cholinergic fibers, specially late in the course of the disease. Even late in the disease, however, entorhinal cortex, hippocampus, and amygdala still have a substantial residual density of cholinergic fibers whose activity could be enhanced by the administration of AChE inhibitors or ACh precursors.

Notes

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