Reduction of Choline Acetyltransferase Activity in Primary Visual Cortex in Mild to Moderate Alzheimer’s Disease

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Background: Cholinergic deficits in the primary visual cortex (PVC) may underlie some of the abnormalities in visual processing and global cognitive performance in Alzheimer’s disease (AD).

Objective: To correlate measures of general cognition (Mini-Mental State Examination and Global Cognitive Score) and visuospatial function with choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities, and nerve growth factor protein levels in the PVC.

Design: The ChAT and AChE enzyme assays and a nerve growth factor protein enzyme-linked immunosorbent assay were performed on PVC tissue samples from subjects clinically diagnosed as having mild cognitive impairment (MCI), AD, or no cognitive impairment (NCI).

Setting and Patients: Nuns, priests and brothers enrolled in the Religious Order Study, with annual premortem records of neuropsychological testing.

Results: Significant differences in ChAT activity, but not in AChE activity or nerve growth factor protein levels, were found among diagnostic groups ($P = .049$). The ChAT activity was lower in AD than in MCI or NCI ($P < .01$); MCI was not different from NCI. The PVC ChAT activity correlated with measures of overall cognitive function (Mini-Mental State Examination and Global Cognitive Score), but less so with a composite measure of visuospatial function.

Conclusions: The reduction in ChAT activity in the PVC of mild to moderate AD, but not in MCI, might serve to distinguish between clinical and preclinical forms of the disease. It appears that this change relates to generalized cognitive abnormalities but not specifically to visuospatial function.

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Methods

Subjects

Subjects were 54 individuals (Table 1) from a longitudinal clinicopathological study of aging and AD of older Catholic clergy (Religious Orders Study) categorized as having NCI, MCI, or AD. The investigators were blinded to the selection process. The PVC samples were obtained from the same Religious Orders Study cases previously showing stable association cortex ChAT activity and NGF protein levels in MCI and mild to moderate AD. No PVC tissue...
samples were available from 1 AD and 3 NCI subjects studied in these earlier reports. The AD group included 1 severe case (Mini-Mental State Examination [MMSE] score = 7) in the analysis of ChAT and AChE activities, and 1 severe case (MMSE score = 5) in NGF protein level analysis; excluding these cases constrained the AD group to the mild to moderate AD category (MMSE score range, 12-25). The Rush University Medical Center (Chicago, Ill) and University of Pittsburgh (Pittsburgh, Pa) Human Investigations Committees approved the study.

CLINICAL EVALUATION

Descriptions of the clinical evaluation have been published.14

The score on each neuropsychological test was standardized to that of a reference population (the first 82 deceased Religious Orders Study cases), then averaged across 20 tests to derive a Global Cognitive Score (GCS), a composite z score.14 A GCS of 0 indicated overall cognitive function similar to the reference population average. A GCS of +1 (or –1) indicated one’s overall cognitive function was 1 SD above (or below) the reference population average. Subjects were classified as having NCI (mean GCS = 0.5 ± 0.3) (Table 2), MCI (GCS = 0.2 ± 0.3), or AD (GCS = –1.0 ± 0.6). A composite z score specific to visuospatial ability included the Standard Progressive Matrices (SCPMAT) and Standard Line Orientation (SCLOPAIR) tests.15

Diagnosis of AD dementia was made using standard criteria.16

Mild cognitive impairment was defined as impairment on neuropsychological testing but without a diagnosis of dementia by the examining neuropsychologist,14 criteria similar to those describing patients who were not cognitively intact but nonetheless did not meet the criteria for dementia.17–20 A postmortem interview with family or caregivers determined that no other medical conditions occurred after the last clinical evaluation (within 12 months prior to death). A consensus conference of neurologists and neuropsychologists reviewed all the clinical data, the postmortem interview, medical records, and neuroimaging studies and assigned final clinical diagnoses.

PATHOLOGICAL EVALUATION AND TISSUE PREPARATION

Brains were processed as described previously.12,13 Blocks of tissue containing the PVC (Brodmann area 17) were dissected from one hemisphere and fresh frozen. Cases were excluded if they showed non-AD pathology. A board-certified neuropathologist blinded to the clinical diagnosis determined the Braak neuropathological staging score of the NFT pathology21 and the National Institute on Aging–Reagan Institute diagnosis criteria.22 Cases were coded, and all assays were performed in triplicate by a technician blinded to the diagnosis.

ChAT AND AChE ACTIVITY ASSAYS

Frozen PVC gray matter samples were processed as described previously.12,13 The ChAT activity assay used radioactive carbon 14–labeled acetyl coenzyme A (New England Nuclear, Boston, Mass). Protein assay kits (Pierce, Rockford, Ill) were used to determine the protein content of the samples. The ChAT activity was expressed as micromoles per hour per gram of protein, and the AChE activity as millimoles per hour per gram of protein.

NGF PROTEIN ASSAY

The NGF protein assay was performed on frozen tissue samples.13 A sandwich enzyme immunoassay used mouse anti-β (2.3S, 7S)
NGF as the capture antibody and mouse anti-β (2.55,75) NGF β-galactosidase as the detection antibody (Roche Diagnostics, Mannheim, Germany). Protein determination was done using a BCA Protein Assay Kit (Pierce). Results were expressed as picograms of NGF per milligram of protein.

**STATISTICAL ANALYSES**

Summary statistics were presented as mean ± SD, range, frequency, or percentage. The PVC ChAT and AChE activities and the NGF protein level data were log-transformed to approximate a normal distribution. For the comparison of clinical, demographic, and neuropathological characteristics among diagnostic groups, 1-way analysis of variance, Fisher exact test, and the nonparametric Kruskal-Wallis test were used, as appropriate. Cognitive functions and biomarker activity were compared among diagnostic groups with the use of analysis of variance. Post hoc pair-wise comparisons were performed, adjusting for multiple comparisons by Tukey studentized range test. Spearman rank correlation and the Kruskal-Wallis test determined the relationship between clinical, demographic, and neuropathological variables vs and ChAT and AChE activities and NGF protein levels. The age- and education-adjusted analyses were performed by regression models. Statistical significance was set at .01 (2-sided).

**RESULTS**

The 3 Religious Orders Study diagnostic groups (NCI, MCI, and AD) were similar in age, sex, years of education, presence of APOE ε4 allele, and postmortem interval (Table 1). Significant NFT pathology was observed in all of the groups. Sixty-five percent of NCI, 89% of MCI, and 92% of AD cases were diagnosed as having Braak stage III or higher. The AD cohort performed worse than the NCI or MCI cohort on the MMSE and GCS (P<.0001 in both); the NCI and MCI were not significantly different (Table 2). The clinical groups were also different on the composite measure of visuospatial function (P<.0001) and on the individual SCPMAT and SCLOPAIR tests (Table 2).

The PVC ChAT activity was reduced significantly in those with AD compared with those with NCI and MCI (P = .0049, Table 3, Figure 1); the latter 2 groups were not different. When ChAT activity analysis excluded the only severe case (MMSE score = 7), a significant difference was achieved among the 3 groups (P = .015), with reduced PVC ChAT activity in the mild to moderate AD group (mean MMSE score = 19.4 ± 4.7) compared with the NCI group, but not when compared with the MCI group. The PVC AChE activity and NGF protein levels were not significantly different among the 3 groups (Table 3, Figure 1). Across the entire cohort, there were no correlations between PVC ChAT and AChE activities and the NGF protein level. Small sample size precluded analyses within the diagnostic groups.

Associations between PVC ChAT, AChE activity, or NGF protein levels and clinical variables were assessed on all subjects combined. Only PVC ChAT activity, but not AChE activity or NGF protein level, showed consistent association with MMSE scores (Spearman rank correlation, r = 0.35, P = .017) and GCS (r = 0.39, P = .0081; Figure 2). The data were suggestive of a possible correlation between the PVC ChAT activity and the composite z score for visuospatial ability (r = 0.26, P = .083) and the SCPMAT test score (r = 0.25, P = .099). In addition, there was a correlation between PVC AChE activity and SCPMAT test scores (r = 0.35, P = .023). Results remained unchanged after adjusting for age and education, except for a notably stronger association between

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**Table 2. Cognitive Function by Clinical Diagnosis Category**

<table>
<thead>
<tr>
<th></th>
<th>NCI (n = 23)</th>
<th>MCI (n = 18)</th>
<th>AD (n = 13)</th>
<th>Total (N = 54)</th>
<th>Comparison by Diagnosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE Mean ± SD</td>
<td>27.9 ± 1.6</td>
<td>26.1 ± 2.5</td>
<td>17.3 ± 6.6</td>
<td>24.7 ± 5.6</td>
<td>F(2,51) = 36.6; P &lt; .0001†</td>
</tr>
<tr>
<td>Range (25 to 30)</td>
<td>27.9 ± 1.6</td>
<td>26.1 ± 2.5</td>
<td>17.3 ± 6.6</td>
<td>24.7 ± 5.6</td>
<td>F(2,51) = 36.6; P &lt; .0001†</td>
</tr>
<tr>
<td>GCS Mean ± SD</td>
<td>0.5 ± 0.3</td>
<td>0.2 ± 0.3</td>
<td>-1.0 ± 0.6</td>
<td>0.0 ± 0.7</td>
<td>F(2,49) = 59.5; P &lt; .0001‡</td>
</tr>
<tr>
<td>Range (-0.1 to 1.1)</td>
<td>0.5 ± 0.3</td>
<td>0.2 ± 0.3</td>
<td>-1.0 ± 0.6</td>
<td>0.0 ± 0.7</td>
<td>F(2,49) = 59.5; P &lt; .0001‡</td>
</tr>
<tr>
<td>SCPMAT Mean ± SD</td>
<td>9.0 ± 2.5</td>
<td>8.6 ± 2.4</td>
<td>5.5 ± 2.7</td>
<td>8.0 ± 2.9</td>
<td>F(2,49) = 59.5; P &lt; .0001‡</td>
</tr>
<tr>
<td>Range (5 ± 15)</td>
<td>9.0 ± 2.5</td>
<td>8.6 ± 2.4</td>
<td>5.5 ± 2.7</td>
<td>8.0 ± 2.9</td>
<td>F(2,49) = 59.5; P &lt; .0001‡</td>
</tr>
<tr>
<td>SCLOPAIR Mean ± SD</td>
<td>10.5 ± 2.9</td>
<td>7.6 ± 2.9</td>
<td>5.7 ± 3.8</td>
<td>8.5 ± 3.7</td>
<td>F(2,49) = 10.5; P = .0002§</td>
</tr>
<tr>
<td>Range (4 ± 15)</td>
<td>10.5 ± 2.9</td>
<td>7.6 ± 2.9</td>
<td>5.7 ± 3.8</td>
<td>8.5 ± 3.7</td>
<td>F(2,49) = 10.5; P = .0002§</td>
</tr>
<tr>
<td>Visuospatial z score</td>
<td>0.4 ± 0.5</td>
<td>-0.1 ± 0.7</td>
<td>-0.9 ± 0.9</td>
<td>0.0 ± 0.8</td>
<td>F(2,49) = 14.3; P &lt; .0001‡</td>
</tr>
<tr>
<td>Range (0 ± 1.8)</td>
<td>0.4 ± 0.5</td>
<td>-0.1 ± 0.7</td>
<td>-0.9 ± 0.9</td>
<td>0.0 ± 0.8</td>
<td>F(2,49) = 14.3; P &lt; .0001‡</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer's disease; GCS, Global Cognitive Score; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NCI, no cognitive impairment; SCPMAT, Standard Progressive Matrices; SCLOPAIR, Standard Line Orientation; Visuospatial z score, composite z score of visuospatial ability based on SCPMAT and SCLOPAIR test scores.

*One-way analysis of variance.
†The AD case with an MMSE score = 5 was analyzed for primary visual cortex nerve growth factor protein levels but not for choline acetyltransferase or acetylcholinesterase activity.
‡Post hoc comparison by Tukey studentized range test, AD < (NCI, MCI).
§Post hoc comparison by Tukey studentized range test, AD < (NCI, MCI).
Despite a slightly weaker level of statistical significance, the above findings were maintained when the AD group was restricted to mild to moderate cases. The ChAT activity was lower in Braak neuropathological stages V/VI (mean ± SD=−1.45±1.62, log-transformed) compared with stages III/IV (−0.37±1.36) and I/II (−0.35±0.65), although statistical significance was not determined owing to sample size limitation.

**COMMENT**

The ChAT activity in the PVC was significantly reduced in AD, even when the analysis was restricted to mild to moderate AD (MMSE score range, 12-25), while remaining stable in MCI. This deficit could relate to some of the cognitive problems seen early in the course of AD, including difficulties in recognizing persons and objects. Furthermore, they may predispose to or precipitate more complex problems in higher visual cortex processing including visuospatial or naming deficits in patients with AD. Interestingly, the sensory visual input is mainly intact in AD, suggesting that changes in the PVC and association areas are more likely responsible for resulting cognitive abnormalities. The present study found only a trend for PVC ChAT activity to correlate with the measure of integrative visuospatial abilities while there were significant relationships with global cognitive abilities. Thus, a reduction in PVC ChAT activity may not only affect visuospatial functions but might interfere with the integration of higher visual processing into global cognitive performance. This finding provides a structural basis for the involvement of visual processing pathways during the clinical course of AD.

The ChAT activity appears to be specific for PVC, the only cortical area examined in which ChAT activity was reduced in patients with mild to moderate AD. Davis and colleagues reported a sig-

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**Table 3. Summary of ChAT and AChE Activities and NGF Protein, Levels (After Natural Logarithm Transformation) in the Primary Visual Cortex**

<table>
<thead>
<tr>
<th>ROS Clinical Diagnosis</th>
<th>NCI</th>
<th>MCI</th>
<th>AD</th>
<th>Total</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ChAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>18</td>
<td>18</td>
<td>11</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>−0.1±0.9</td>
<td>−0.5±1.4</td>
<td>−1.7±1.5</td>
<td>−0.6±1.4</td>
<td>F(2,44) = 6.01; P = .0049†</td>
</tr>
<tr>
<td>Range</td>
<td>(−1.6 to 1.6)</td>
<td>(−3.9 to 1.5)</td>
<td>(−3.9 to 0.9)</td>
<td>(−3.9 to 1.6)</td>
<td></td>
</tr>
<tr>
<td><strong>NGF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>21</td>
<td>17</td>
<td>12</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.5±0.9</td>
<td>2.5±1.0</td>
<td>1.8±1.4</td>
<td>2.3±1.1</td>
<td>F(2,47) = 1.87; P = .17</td>
</tr>
<tr>
<td>Range</td>
<td>(0.2 to 4.2)</td>
<td>(−1.3 to 3.3)</td>
<td>(−1.3 to 4.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AChE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of subjects</td>
<td>17</td>
<td>18</td>
<td>10</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>−0.8±0.3</td>
<td>−0.7±0.2</td>
<td>−0.8±0.2</td>
<td>−0.8±0.2</td>
<td>F(2,45) = 0.25; P = .78</td>
</tr>
<tr>
<td>Range</td>
<td>(−1.2 to −0.4)</td>
<td>(−1.4 to −0.4)</td>
<td>(−1.1 to −0.6)</td>
<td>(−1.4 to −0.4)</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Box-and-whiskers plots of choline acetyltransferase (ChAT) (A) and acetylcholinesterase (AChE) (B) activities and nerve growth factor (NGF) protein levels (C) in the primary visual cortex from the no cognitive impairment (NCI), mild cognitive impairment (MCI), and Alzheimer disease (AD) groups. The ChAT activity is decreased in the AD group.

**Abbreviations:** AChE, acetylcholinesterase; AD, Alzheimer’s disease; ChAT, choline acetyltransferase; MCI, mild cognitive impairment; NCI, no cognitive impairment; NGF, nerve growth factor; ROS, Religious Orders Study.

*One-way analysis of variance.
†Post hoc comparison by Tukey studentized range test, AD< (NCI, MCI).
significant loss of PVC ChAT and AChE activities only in severe dementia (Clinical Dementia Rating scale score = 5). This discordance could be due to differences in education, age, or neuropsychological testing information available. While our subjects were evaluated cognitively on the MMSE and GCS scales, Davis and colleagues used the Clinical Dementia Rating scale\(^{24}\) to define disease severity. Contrary to the synchronous loss of ChAT and AChE activity in severe AD,\(^{9}\) we found a selective ChAT activity deficit in the PVC in individuals with mild to moderate AD, suggesting that a reduction in ChAT activity precedes AChE loss. Thus, in early stages of the disease, there could be a deficit in the capacity of cholinergic neurons to produce and/or transport ChAT to PVC, thereby reducing the ability to synthesize acetylcholine in the PVC, while stable AChE activity could augment neurotransmitter deficit.

**CONCLUSIONS**

A reduction in PVC ChAT activity was found in mild to moderate AD but not in MCI. Such change might serve to distinguish between clinical and preclinical forms of the disease. A selective loss of the acetylcholine-synthesizing enzyme in PVC implies worsened cholinergic neurotransmission in this region of the brain, which could affect both higher visual processing and overall cognitive performance during the initial stages of the disease. A trend for a reduction in PVC ChAT activity in cases with Braak neuropathological stage V/VI suggests that cholinergic changes are not marked until the last (neocortical) stages of NFT pathology progression have occurred. Future studies should determine the extent of the visual cortex NFT and amyloid pathology and possible correlations with changes in the cholinergic projections and cholinergic enzyme activity in individuals with MCI and AD.

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**Author Contributions:** Study concept and design: Ikonomovic, Mufson, and DeKosky. Acquisition of data: Ikonomovic, Bennett, and DeKosky. Analysis and interpretation of data: Ikonomovic, Mufson, Wuu, Bennett, and DeKosky. Drafting of the manuscript: Ikonomovic, Mufson, Wuu, and DeKosky. Critical revision of the manuscript for important intellectual content: Ikonomovic, Mufson, Bennett, and DeKosky. Statistical analysis: Wuu and DeKosky. Obtained funding: Mufson, Bennett, and DeKosky. Administrative, technical, and material support: Ikonomovic and DeKosky. Study supervision: Ikonomovic, Mufson, Bennett, and DeKosky.

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**REFERENCES**


**Correction**

Error in Figure. In Figure 3 of the article titled “Pramipexole vs Levodopa as Initial Treatment for Parkinson Disease: A 4-Year Randomized Controlled Trial,” published in the July issue of the ARCHIVES (2004;61:1044-1053), the lines indicating mean change in Unified Parkinson’s Disease Rating Scale activities of daily living scores during pramipexole and levodopa treatments should be reversed. The top line should indicate pramipexole, and the bottom line should indicate levodopa.