Differential Expression of Synaptic Proteins in the Frontal and Temporal Cortex of Elderly Subjects With Mild Cognitive Impairment

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INTRODUCTION

Neocortical synapse loss is a prominent feature of Alzheimer disease (AD) (1–3). Decreases in cortical synaptic density correlate better than amyloid plaques or neurofibrillary tangles with the degree of cognitive impairment in AD, suggesting that synaptic dysfunction contributes to the clinical presentation of the disease (3, 4). The efficacy of synaptic transmission depends on the intricate coordination of multiple specialized proteins involved in synaptic vesicle trafficking (e.g., targeting and docking, membrane fusion/exocytosis, and endocytosis) and pre- and postsynaptic structure and plasticity (5, 6). Several studies have shown that the expression levels of select synaptic proteins such as synaptophysin are decreased in the neocortex in early- and/or late-stage AD (7–15). Therefore, perturbations in synaptic protein stoichiometry may play a role in synaptic dysfunction in AD. Whether similar alterations in synaptic protein levels occur in people clinically diagnosed with mild cognitive impairment (MCI), a putative prodromal stage of AD (16, 17), remain unclear. Alterations in specific cortical synaptic proteins during the prodromal stage of AD may offer mechanistic clues to the nature of synaptic dysfunction preceding the clinical expression of frank AD. To address this possibility, we performed quantitative immunoblotting experiments to measure the levels of synaptophysin (SYP, a presynaptic vesicle marker), synaptotagmin (SYT, a synaptic protein critical for Ca2+-dependent neurotransmitter release), and drebrin (DRB, a postsynaptic dendritic spine marker) in 5 cortical regions (anterior cingulate, superior frontal, superior temporal, inferior parietal, and visual) in tissue samples derived from the Religious Orders Study (ROS), an ongoing longitudinal study of aging and AD in elderly Catholic clergy (16, 18–20). The cognitive function of these individuals was classified antemortem as no cognitive impairment (NCI), MCI, or AD. We also examined whether changes in cortical synaptic protein levels were associated with performance on the Mini-Mental State Examination (MMSE) or with postmortem neuropathologic variables.

MATERIALS AND METHODS

Subjects

Cortical tissue samples were evaluated from 48 individuals who were participants in the ROS (Table 1) (16, 18–20). The Human Investigations Committee of Rush University Medical Center approved the study. Each participant agreed to an annual detailed clinical evaluation...
# TABLE 1. General Demographic Characteristics of Subject Population by Clinical Diagnostic Category

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>NCI (n = 18)</th>
<th>MCI (n = 16)</th>
<th>Mild/Moderate AD (n = 14)</th>
<th>Severe AD (n = 12)</th>
<th>Total (n = 60)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Age (years) at death:</td>
<td>Mean ± SD (Range)</td>
<td>83.1 ± 6.4 (67–92)</td>
<td>83.7 ± 5.6 (75–97)</td>
<td>85.7 ± 6.6 (70–95)</td>
<td>79.3 ± 4.1 (74–89)</td>
<td>83.1 ± 6.1 (67–97)</td>
</tr>
<tr>
<td>Number (%) of males:</td>
<td>10 (56%)</td>
<td>8 (50%)</td>
<td>8 (57%)</td>
<td>5 (42%)</td>
<td>5 (42%)</td>
<td>31 (52%)</td>
</tr>
<tr>
<td>Years of education:</td>
<td>Mean ± SD (Range)</td>
<td>17.5 ± 3.7 (8–23)</td>
<td>17.8 ± 3.6 (8–22)</td>
<td>15.8 ± 4.0 (6–21)</td>
<td>13.5 ± 2.2 (9–16)</td>
<td>16.4 ± 3.8 (6–23)</td>
</tr>
<tr>
<td>Number (%) with ApoE ε4 allele:</td>
<td>4 (22%)</td>
<td>4 (25%)</td>
<td>5 (36%)</td>
<td>NA</td>
<td>11 (27%)</td>
<td>p = 0.72†</td>
</tr>
<tr>
<td>Global cognitive score:</td>
<td>Mean ± SD (Range)</td>
<td>28.1 ± 1.5 (25–30)</td>
<td>26.0 ± 2.6 (20–30)</td>
<td>15.1 ± 8.3 (0–25)</td>
<td>5.4 ± 4.8 (0–13)</td>
<td>20.1 ± 10.0 (0–30)</td>
</tr>
<tr>
<td>Postmortem interval (hours):</td>
<td>Mean ± SD (Range)</td>
<td>7.3 ± 5.9 (2.2–24)</td>
<td>6.2 ± 3.8 (3–13.9)</td>
<td>6.2 ± 2.9 (3–12)</td>
<td>5.8 ± 2.5 (2–12)</td>
<td>6.4 ± 4.1 (2–24)</td>
</tr>
<tr>
<td>Distribution of Braak scores:</td>
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<td>0</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>I/II</td>
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<td>1</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>III/IV</td>
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<td>12</td>
<td>8</td>
<td>0</td>
<td>30</td>
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</tr>
<tr>
<td>V/VI</td>
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<td>2</td>
<td>5</td>
<td>12</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Pathological Reagan Dx (likelihood of AD):</td>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Low</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
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<td>10</td>
<td>6</td>
<td>0</td>
<td>23</td>
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</tr>
<tr>
<td>High</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

*, Analysis of variance (ANOVA).
†, Fisher exact test.
NCI, no cognitive impairment; MCI, mild cognitive impairment; AD, Alzheimer disease; Dx, diagnosis; SD, standard deviation; NA, not available.
and to brain donation at the time of death. For all but 2 subjects, cognitive testing scores were available within the last year of life; the average interval from last evaluation to time of death was 6.5 ± 3.5 months with no significant differences among the 3 diagnostic groups (p = 0.81). Subjects were categorized clinically as NCI, MCI insufficient to meet criteria for dementia, or mild/moderate AD (Table 1). Cortical samples were also obtained from 12 age-matched patients of the Rush Alzheimer’s Disease Core Center (RADCC; Table 1). These patients were evaluated within 12 months of death and clinically diagnosed with severe AD.

Clinical Evaluation: Religious Orders Study Population

Details of the ROS clinical evaluation have been published elsewhere (16, 18–20). Briefly, each subject received an annual uniform clinical evaluation that included a medical history, neurologic examination including assessments for stroke (21) and parkinsonism (22), neuropsychologic performance testing, review of brain scan when available, and diagnostic classification by the examining neurologist. Neuropsychologic performance tests were selected to assess a broad range of cognitive abilities commonly affected by aging and AD, including episodic memory (e.g. Word List Memory, Recall and Recognition 23), semantic memory (e.g. Verbal Fluency 23 and Boston Naming 24), working memory (e.g. Digit Span Forward and Backward 25), perceptual speed (e.g. Symbol Digit Modalities Test 26), and visuospatial ability (e.g. Standard Progressive Matrices 27). The MMSE was used as a measure of general cognitive function (28), and the previously described global cognitive score was used as a composite measure of individual test scores (16, 29) (Table 1). After neuropsychologic testing, a board-certified clinical neuropsychologist, blinded to the person’s age, sex, and race, reviewed the results of all cognitive tests, computer-generated impairment ratings and data on education, occupation, sensory and motor deficits, and effort. Based on review of these data, the neuropsychologist rendered a clinical judgment regarding the presence of impairment in memory and other cognitive domains (e.g. attention and language). After review of all clinical data from that year and examination of the participant, a board-certified neurologist with expertise in the evaluation of the elderly made a clinical diagnosis. The diagnosis of dementia and AD followed the recommendations of the joint working group of the National Institute of Neurological and Communicative Disorders and the Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS/ADRDA) (30). There are no consensus criteria for the clinical classification of MCI. Our MCI population was defined as those persons rated as impaired on neuropsychologic testing by the neuropsychologist but who were not found to have dementia by the examining neurologist (16, 18–20). The criteria used for the ROS are similar to, or compatible with, those used by others in the field to describe persons who are not cognitively normal but do not meet accepted criteria for dementia (31). At the time of death, an assessment was made to identify intercurrent medical conditions and therapeutic regimens that occurred during the interval between the last clinical evaluation and death as well as cause of death and potential agonal variables. Subsequently, all available clinical data were reviewed to render a summary clinical diagnosis. To ensure unbiased clinical pathologic correlations, the summary clinical diagnosis is made blinded to postmortem neuropathological data.

Clinical Evaluation: Rush Alzheimer’s Disease Center Population

Twelve end-stage AD cases from the RADCC Brain Bank were matched for age and postmortem interval (PMI) with the ROS diagnostic groups (Table 1). The clinical diagnosis of AD was made following a standardized ADC evaluation at a consensus conference using NINCDS/ADRDA (30) and the Diagnostic and Statistical Manual of Mental Disorders, Third Edition–Revised (DSM-III-R) (32) criteria.

Pathological Evaluation and Tissue Preparation

Brains from both ROS and RADCC cohorts were processed at autopsy as previously published (18–20, 33). The PMI did not differ across groups (p = 0.96; Table 1). Atrophy of cerebral structures, number and locations of hemorrhages and infarcts, degree of atherosclerosis, and presence and location of other abnormalities were recorded. Cases were excluded if they exhibited significant non-AD types of pathologic conditions (e.g. brain tumors, encephalitis, multiple lacunar infarctions). Brains were sectioned into 1-cm-thick slabs using a plastic cutting apparatus. Slabs

![FIGURE 1. Neuropathologic characteristics of Religious Orders Study (ROS) subjects evaluated for synaptic protein expression. Brains of ROS subjects were examined for pathologic evidence of Alzheimer disease (AD) using Braak stage (left) and NIA-Reagan (right) diagnostic criteria. Bar graphs show percentages of ROS subjects clinically diagnosed with no cognitive impairment (green), mild cognitive impairment (blue), or mild/moderate AD (red) represented within each of the four Braak stages (A) and within each of the 4 Reagan diagnostic groups (B).](http://jnen.oxfordjournals.org/)

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from one hemisphere were immersion-fixed in 4% paraformaldehyde. From the opposite hemisphere, slabs alternating around an anchor slab at the level of the crossing of the anterior commissure were either snap-frozen in liquid nitrogen or placed in 4% paraformaldehyde. Samples of gray matter from the anterior cingulate (Brodmann area [BA] 24), superior frontal (BA9), superior temporal (BA22), inferior parietal (BA39), and primary visual (BA17) cortex were dissected based on fiduciary landmarks. All frozen dissections were performed on dry ice to prevent tissue thawing. Samples were stored at −80°C until assayed.

Select brain regions from the remaining immersion-fixed slabs were dissected, paraffin-embedded, cut at 8 μm and stained with hematoxylin and eosin, modified Bielschowsky, thioflavin-S, and with an ubiquitin antibody for histopathologic analyses. A complete neuropathologic analysis was performed with special attention to pathologic lesions that may contribute to dementia, including brainstem and cortical Lewy bodies as well as strokes. A pathologic diagnosis was made for each case by a neuropathologist blinded to the clinical diagnosis. Designations of “normal” (with respect to AD or other dementing processes), “possible” or “probable AD,” or “definite AD” were based on Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) criteria, semiquantitative estimation of neuritic plaque density, an age-adjusted plaque score, and presence or absence of dementia (CERAD) (34). All cases also received Braak scores based on the staging of neurofibrillary tangle pathology (35), and subsequently each case was assigned a NIA-Reagan Diagnostic Criteria-based diagnosis (Table 1; Fig. 1) (36).

Quantitative Immunoblotting

Tissue samples from the anterior cingulate, superior frontal, superior temporal, inferior parietal, and visual cortex were sonicated in ice-cold homogenization buffer (20 mM Tris, 1 mM EGTA, 1 mM EDTA, 10% sucrose, pH 7.4) containing protease inhibitors (2 mg/mL leupeptin, 0.01 U/mL aprotinin, 1 mg/mL pepstatin A, 1 mg/mL antipain, 2.5 mg/mL chymostatin, 10 mM benzamidine, 0.1 mM PMSF, 0.4 mg/mL TPCK, 0.4 mg/mL TLCK, 0.4 mg/mL soybean trypsin inhibitor, 0.1 mM sodium fluoride, and 0.1 mM sodium orthovanadate). Samples were prepared by centrifugation at 1,000 rpm for 10 minutes at 4°C. Protein concentration of the resulting S1 supernatant was determined by the Bradford method (Bio-Rad, Hercules, CA) using bovine serum albumin as the protein standard. Sample proteins from the S1 fraction were denatured in sodium dodecyl sulfate (SDS) loading buffer to a final concentration of 5 mg/mL. Sample proteins (25 μg/sample) were separated by SDS polyacrylamide gel electrophoresis (10% acrylamide) and transferred to polyvinylidene fluoride membranes (Immobilon P; Millipore, Bedford, MA) electrophoretically. Membranes were blocked in TBS/0.1% Tween-20/5% milk for 30 minutes at room temperature (RT) and then incubated overnight at 4°C with mouse anti-DRB (clone M2F6,1:2000; MBL International, Woburn, MA), mouse anti-SYT (clone 41, 1:2000; BD Transduction Labs, San Diego, CA), and mouse anti-SYP (clone SY38, 1:1000; MP Biomedicals, Irvine, CA), mouse anti-SYT (clone 41, 1:2000; BD Transduction Labs, San Diego, CA), and mouse anti-DRB (clone M2F6,1:2000; MBL International, Woburn, MA) in blocking buffer. Each monoclonal antibody detected a single immunoreactive band at the correct antigen molecular weight (Fig 2A), thus precluding antibody cross-reactivity on the Western blots. Because cortical levels of β-tubulin are unchanged in AD brain relative to aged control brain (18, 37, 38), membranes were also incubated with mouse anti-β-tubulin (clone KMX-1, 1:50,000; Chemicon, Temecula, CA) at the same time as the synaptic monoclonal antibodies. β-tubulin-immunoreactive signals were used as an internal control for protein loading (18). After several rinses, the blots were incubated for 1 hour at RT with horse-radish peroxidase–conjugated goat antimouse IgG secondary antibody (1:8,000; Pierce, Rockford, IL). Immunoreactive proteins were visualized simultaneously by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ) on a Kodak Image Station 440CF (Perkin-Elmer, Wellesley, MA).

**FIGURE 2.** Quantitation of cortical synaptic protein immunoreactivity. (A) Representative immunoblot shows increasing antigen immunoreactivity with incremental amounts of loaded protein (10–100 μg) from anterior cingulate cortex of an individual with no cognitive impairment (NCI). (B) Bar graphs show relative drebrin, synaptotagmin, synaptophysin, and β-tubulin (TUB)-immunoreactive signal intensity (mean ± standard error of mean) with increasing amount of total protein. Densitometry was performed on samples of anterior cingulate, superior frontal, and superior temporal cortex, each selected from a different NCI case (n = 3). Signals from 25-μg loaded protein fell within a linear range of detection.
which provides linear data over a dynamic range of 4 orders of magnitude. Bands were quantified using Kodak 1D image analysis software. No immunoreactive bands were detected on blots probed with the antimouse secondary IgG alone (data not shown). Immunoreactive signals of the synaptic protein antigens were normalized to β-tubulin signals for quantitative analysis. Each sample was analyzed on 3 different Western blots in independent experiments.

**FIGURE 3.** Cortical levels of synaptic proteins are dynamically regulated during the progression of Alzheimer disease (AD). (A) Representative immunoblots of detergent lysates (25 μg) from superior frontal (left) and superior temporal (right) cortex were separated by SDS-PAGE and immunoblotted for synaptophysin (SYP), synaptotagmin (SYT), drebrin (DRB), and β-tubulin (TUB). (B) Cortical levels of SYP protein are reduced approximately 35% in the superior temporal and inferior parietal cortex of subjects with severe AD compared with those with no cognitive impairment. Quantitative analysis was performed by normalizing SYP-immunoreactive signals to TUB signals on the same blots. Box plots show relative SYP protein levels in each cortical area examined. (C) Cortical levels of SYT are unchanged across the clinical diagnostic groups. (D) DRB levels were reduced approximately 30% to 40% in the anterior cingulate, inferior parietal, and visual cortex of subjects with AD compared with no cognitive impairment (NCI). In the superior temporal cortex, DRB levels were reduced approximately 35% from NCI to mild cognitive impairment (MCI) and from MCI to AD. In the superior frontal cortex, DRB levels were increased approximately 30% in the MCI group compared with the NCI group and 50% compared to the AD groups. Statistical analysis of changes in cortical SYP and DRB levels among the diagnostic groups is summarized in Table 2.
TABLE 2. Synaptic Protein Levels in 5 Cortical Regions of the Clinical Diagnostic Groups

<table>
<thead>
<tr>
<th>Protein</th>
<th>Cortical Region</th>
<th>Clinical Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NCI</td>
</tr>
<tr>
<td>SYP</td>
<td>SF Mean ± SD</td>
<td>(range)</td>
</tr>
<tr>
<td></td>
<td>(0.70–1.13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>11</td>
</tr>
<tr>
<td>ST</td>
<td>Mean ± SD</td>
<td>(range)</td>
</tr>
<tr>
<td></td>
<td>(0.44–0.87)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>11</td>
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<tr>
<td>VC</td>
<td>Mean ± SD</td>
<td>(range)</td>
</tr>
<tr>
<td></td>
<td>(0.41–0.69)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>7</td>
</tr>
<tr>
<td>IP</td>
<td>Mean ± SD</td>
<td>(range)</td>
</tr>
<tr>
<td></td>
<td>(0.56–0.99)</td>
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</tr>
<tr>
<td></td>
<td>n</td>
<td>11</td>
</tr>
<tr>
<td>AC</td>
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<td></td>
<td>(0.39–1.68)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>10</td>
</tr>
<tr>
<td>DRB</td>
<td>SF Mean ± SD</td>
<td>(range)</td>
</tr>
<tr>
<td></td>
<td>(0.35–0.71)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>11</td>
</tr>
</tbody>
</table>

SYP, synaptophysin; SYT, syntaptotagmin; DRB, drebrin; NCI, no cognitive impairment; MCI, mild cognitive impairment; AD, Alzheimer disease; SF, superior frontal; ST, superior temporal; VC, visual cortex; IP, inferior parietal; AC, anterior cingulated; ANCOVA, analysis of covariance; SD, standard deviation.

Statistical Analysis

Variance component analysis showed good within-subject agreement, so the average value of triplicate measurements for each synaptic protein normalized in β-tubulin was used in subsequent analyses. Clinical, demographic, and pathologic variables were compared among the 4 diagnosis groups by one-way analysis of variance (ANOVA) or Fisher exact test. Synaptic protein levels were compared among groups by analysis of covariance (ANCOVA), adjusting for age and education. Tukey’s studentized range test or Bonferroni-type correction was used for post hoc comparisons as appropriate. The relationship between synaptic protein levels and clinical variables was assessed by Spearman partial correlation or ANCOVA, adjusting for age and education. To account for the large number of comparisons made, the level of statistical significance was set at 0.01 (2-tailed).

RESULTS

Case Demographics

Table 1 shows characteristics of the sample population by diagnostic group. The NCI, MCI, and mild/moderate AD individuals from the ROS and individuals with severe AD from the RADCC were similar in age, sex, apoE status, and PMI. The years of education were similar in the ROS cohort and somewhat shorter in the RADCC AD group. The subjects in the study ranged in their last MMSE scores from zero to 30. An ANOVA showed no significant difference in MMSE score between the NCI and MCI groups. The mild/moderate AD group had a significantly lower mean MMSE (5.4 ± 2.6) subjects, whereas the severe AD group had a significantly lower mean MMSE (1.18 ± 0.51) subjects. An ANOVA showed no significant difference in MMSE score between the NCI and MCI groups. The mild/moderate AD group had a significantly lower mean MMSE (5.4 ± 2.6) subjects, whereas the severe AD group had a significantly lower mean MMSE (1.18 ± 0.51) subjects. An ANOVA showed no significant difference in MMSE score between the NCI and MCI groups. The mild/moderate AD group had a significantly lower mean MMSE (5.4 ± 2.6) subjects, whereas the severe AD group had a significantly lower mean MMSE (1.18 ± 0.51) subjects.
Quantitative Immunoblotting

To ensure that densitometric measurements of SYP, SYT, DRB, and β-tubulin-immunoreactive signals on the blots were quantitative, serial dilutions of cortical detergent lysates were immunoblotted with the antibodies to create a standard curve for each antigen (Fig. 2A). Quantitative analysis demonstrated that the immunoreactive signal for each antigen fell along a linear range of detection when 25 μg of total protein was loaded (Fig. 2B).

Cortical Synaptophysin Levels in Mild Cognitive Impairment and Alzheimer Disease

Levels of SYP protein in the 5 cortical areas examined (anterior cingulate, superior frontal, superior temporal, inferior parietal, and visual) from the 4 clinical groups were evaluated by quantitative immunoblotting (Fig. 3A). Mean normalized SYP protein levels differed significantly among the diagnostic groups in the superior temporal (F(3,40) = 6.32, p = 0.0013) and inferior parietal (F(3,41) = 4.93, p = 0.0053) cortex (Fig. 3B; Table 2). Post hoc comparisons showed that these differences were most pronounced between the NCI and severe AD groups, in which the severe AD cases exhibited a significant approximately 35% decrease in SYP protein levels compared with NCI (Table 2).

Cortical Synaptotagmin Levels in Mild Cognitive Impairment and Alzheimer Disease

Levels of SYT were evaluated on the same blots as SYP (Fig. 3A). There were no significant differences in mean SYT levels among the NCI, MCI, and mild/moderate or severe AD groups in any of the cortical regions examined (Fig. 3C).

Cortical Drebrin Levels in Mild Cognitive Impairment and Alzheimer Disease

Levels of DRB were measured simultaneously with SYP and SYT (Fig. 3A). Mean DRB protein levels differed significantly among the clinical diagnostic groups in the anterior cingulate (F(3,39) = 6.25, p = 0.0015), superior frontal (F(3,39) = 14.7, p < 0.0001), superior temporal (F(3,40) = 34.9, p < 0.0001), inferior parietal (F(3,41) = 5.89, p = 0.0020), and visual (F(3,23) = 12.75, p < 0.0001) cortex (Fig. 3D, Table 2). Post hoc comparisons showed that DRB levels were decreased approximately 40% in the anterior cingulate cortex of subjects with mild/moderate AD compared with the NCI and MCI groups. Levels of DRB were reduced approximately 35% in subjects with severe AD relative to subjects with mild/moderate AD in the inferior parietal cortex, whereas DRB was reduced approximately 40% in the AD groups compared with NCI in the visual cortex.

In the superior temporal cortex, DRB levels exhibited a significant stepdown effect from NCI to MCI to AD. DRB levels in MCI subjects were decreased approximately 35% compared with NCI, whereas DRB levels in AD were decreased an additional approximately 35% compared with MCI (Table 2). In contrast, DRB levels in the superior frontal cortex were increased approximately 30% in subjects with MCI compared with subjects with NCI subjects and by approximately 50% compared with the AD groups (Table 2).

Cortical Synaptic Protein Levels versus Mini-Mental Status Examination Scores

Synaptic protein levels in each cortical region were tested for association with individual MMSE scores. Spearman partial correlations adjusting for age and education showed that MMSE scores were positively correlated with SYP levels in the superior temporal (r = 0.47, p = 0.0014; Fig. 4A) and inferior parietal (r = 0.51, p = 0.0005) cortex. MMSE scores were also positively correlated with DRB protein in the anterior cingulate (r = 0.55, p = 0.0002), superior temporal (r = 0.65, p < 0.0001; Fig. 4B), and visual cortex (r = 0.72, p < 0.0001).

Cortical Synaptic Protein Levels versus Postmortem Neuropathologic Classification

Synaptic protein levels in each cortical region were tested for associations with Braak stage and NIA-Reagan diagnosis. SYP levels in the superior temporal cortex were inversely correlated with Braak stage (r = −0.39,
DISCUSSION

Synapse loss in the neocortex and hippocampus correlates with the severity of clinical symptoms in AD (1–3), yet the pathogenic mechanisms underlying synaptic dysfunction remain unclear. Previous reports have demonstrated preferential reductions in specific synaptic proteins in early- or late-stage AD indicating that stoichiometric alterations in the expression of synaptic regulatory elements may contribute to synaptic impairment (7–15). The present quantitative immunoblotting study revealed that select synaptic proteins are differentially regulated in 5 different neocortical regions of subjects clinically diagnosed with MCI as well as those diagnosed with mild/moderate or severe AD. For example, protein levels of the presynaptic vesicle marker SYP were selectively decreased in the superior temporal and inferior parietal cortex of subjects with severe AD compared with subjects with NCI. These observations support previous studies demonstrating a moderate reduction in SYP immunoreactivity in these regions in cases defined as mild or severe AD based on clinical and pathologic criteria (3, 7, 9, 39, 40). Moreover, SYP levels in the superior temporal and inferior parietal cortex correlated with MMSE scores, suggesting that the loss of this presynaptic protein within select neocortical association areas marks the clinical progression of the disease. In contrast, cortical levels of SYT, another presynaptic vesicle protein, were unchanged across the clinical groups examined independent of the region evaluated and Braak stage. No correlations were detected between synaptic protein levels and NIA-Reagan criteria.

The most striking finding of this study was the differential expression of DRB found in the superior temporal and superior frontal cortex among the clinical groups. Whereas DRB protein levels were significantly decreased in all the cortical regions examined in AD compared with NCI, DRB levels were also downregulated in the superior temporal cortex of MCI subjects compared with NCI. On the other hand, DRB levels were upregulated in the superior frontal cortex in MCI compared with NCI and AD subjects. DRB is an F-actin-binding protein originally identified by its increased expression during synaptogenesis (44). Immunoelectron microscopic studies in the adult brain show that DRB is localized to postsynaptic dendritic spines at excitatory synapses (45–47), where it appears to play a role in synaptic plasticity by regulating spine morphogenesis and the formation of postsynaptic densities (46, 48). As such, a shift in DRB protein levels may represent changes in dendritic spine density in the cortical regions examined. Alternatively, as DRB is also known to mediate the synaptic targeting of receptors (49), increased DRB protein may signal a rise in receptor density on dendritic spines independent of morphologic alterations.

Previous immunoblotting studies have shown that DRB protein is decreased approximately 80% to 90% in the frontal (50) and temporal (10, 50) cortex and approximately 70% to 80% in the hippocampus of patients with end-stage AD (51). In contrast, the present immunoblotting study measured approximately 55% to 60% decreases in DRB protein in the superior temporal cortex and approximately 35% to 40% reductions in the other 4 regions examined in the severe AD group. Because all these studies used the same monoclonal antibody on crude homogenate (S1) fractions, these discrepancies in percent of DRB loss may be attributable to sampling differences such as the subject population (early-onset AD subjects [50] vs late-onset AD subjects [present study]), the precise cortical region examined (e.g., middle temporal [10] vs superior temporal cortex [present study]), or possibly to methodological differences related to immunoblotting protocols or image analysis. Regardless, the current findings support previous observations for a significant reduction in DRB immunoreactivity in late-stage AD cortex suggestive of a severe loss in dendritic spine density. Moreover, we show for the first time that DRB levels in the anterior cingulate, superior temporal, and visual cortex correlated with MMSE scores in an age-matched clinical cohort. Hence, the progression of cognitive deficits during the clinical course of AD may be related to a loss of excitatory postsynaptic contacts in the neocortex.

The finding that DRB levels were also decreased approximately 35% in the superior temporal cortex of subjects with MCI suggests that this cortical region is especially vulnerable to synaptic dysfunction. Previous neuropathologic studies have shown that the temporal lobe is an early site for the accumulation of AD-related neuropathology (35, 52, 53). In particular, the temporal neocortex is an early area for neurofibrillary tangle (NFT) formation (35) and select presynaptic markers (e.g., SYP) are reduced in NFT-bearing neurons (54, 55). Whether NFT formation plays a role in the reduction in postsynaptic markers remains an open question. The present observation that both SYP and DRB levels were inversely correlated with Braak stage in the superior temporal cortex supports the notion that NFT deposition is associated with decreased transsynaptic efficacy in this cortical region. Moreover, the positive correlation between SYP and DRB levels in the superior temporal cortex and MMSE scores suggest that pre- and postsynaptic protein reductions in this cortical region are related to cognitive impairment. Along these lines, an investigation of neuropathologic changes in AD cases of varying disease duration revealed that synaptic loss in the superior temporal cortex is significantly correlated with disease severity (56).

In striking contrast to our findings in the superior temporal cortex, DRB protein levels were significantly increased in the superior frontal cortex of subjects with MCI. Intriguingly, most of our MCI subjects were classified...
postmortem as Braak stage III–IV (high limbic stage). This finding is consistent with the observation by Mukaetova-Ladinska et al that protein levels of the dendritic protein microtubule-associated protein 2 (MAP2) is significantly elevated in the cortex of subjects classified as Braak stage IV, the majority of whom were clinically diagnosed with no dementia or mild dementia by the CAMDEX rating scale (11). At Braak stage III–IV, NFTs are relatively sparse in frontal cortical regions subserving executive function compared with relatively higher indices of this pathology in temporal cortical association areas (35). Therefore, increased DRB levels in the MCI frontal cortex may represent a postsynaptic dendritic structural reorganization aimed at maintaining frontal cortical neurotransmission in the face of mounting neuropathologic damage. Significantly, there is also an upregulation of choline acetyltransferase (ChAT, the synthetic enzyme for acetylcholine) activity in the superior frontal cortex of MCI cases from the ROS cohort (20). Perhaps the frontal cortex is capable of multiple plasticity responses that contribute to the relative functional preservation of this region during the early stages of AD. These putative compensatory mechanisms appear to be lost as the disease progresses and as NFTs accumulate in the frontal cortex, as evidenced by subsequent decreases in DRB protein (50, present study) and ChAT activity (20, 57, 58) in severe AD.

In summary, we show that synaptic regulatory proteins display discordant expression patterns in select neocortical regions during the clinical progression of AD. Reductions of SYP in the temporal and parietal cortex in subjects with severe AD and the association of lower SYP levels with reduced MMSE scores reinforce previous data for presynaptic dysfunction in AD (59). Furthermore, the reduction of DRB in the temporal cortex and increased DRB in the frontal cortex of people with MCI suggest a disparity of postsynaptic efficacy within these regions during the onset of AD. This differential expression of DRB in the temporal and frontal cortex in MCI may contribute mechanistically to the impairment of associative behaviors such as memory and language but relative preservation of executive function during the earliest stages of cognitive decline.

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