Dementia with Lewy Bodies Versus Pure Alzheimer Disease: Differences in Cognition, Neuropathology, Cholinergic Dysfunction, and Synapse Density

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Abstract. Dementia with Lewy bodies (DLB) is the second leading cause of cognitive impairment among the elderly. While it is usually accompanied by the neocortical neuritic plaques (NP) and entorhinal neurofibrillary tangles (NFT) characteristic of Alzheimer disease (AD), and so can be construed as a Lewy body variant of AD (LBV), it also occurs in pure form as diffuse Lewy body disease (DLBD). We assessed cognitive status in 17 DLB patients (12 with LBV and 5 with DLBD) and compared the results with 12 AD subjects and 5 controls. We then sought to determine which neuropathologic abnormalities correlate with cognitive impairment. Among DLB cases, neocortical Lewy body (LB) counts, modified Braak stages of NFT burden in the entorhinal cortex, neocortical NP counts, and loss of choline acetyltransferase (ChAT) activity all correlated with dementia severity. Unlike AD, neocortical NFT and anti-synaptophysin reactivity were uncorrelated with DLB dementia. Despite comparable LB counts and ChAT losses, the DLBD were significantly less demented than the LBV patients. We conclude that neocortical LB and ChAT depletion contribute to cognitive impairment in DLB and that concomitant AD pathology in LBV, represented by higher Braak stages and NP, promote increased dementia severity compared with that encountered in DLBD.

Key Words: Alzheimer disease; ChAT; Cholinergic; Dementia; Lewy bodies; Synapse density.

INTRODUCTION

Brainstem and neocortical Lewy bodies (LB) are found in about 20% of demented elderly patients at autopsy (1, 2). Most of these individuals carried clinical diagnoses of Alzheimer disease (AD), and indeed a majority of LB-bearing brains also have enough neocortical neuritic and diffuse plaques to meet widely accepted neuropathologic criteria for a diagnosis of AD (2–5). Our laboratory has labeled this group the Lewy body variant of AD, or LBV. A smaller percentage of dementia brains with LB, however, have no more AD pathology than age-matched nondemented controls (5), and we have described this group’s pathology as diffuse Lewy body disease, or DLBD (1). The percentage of patients falling into each of these disease categories is affected by the neuropathologic criteria one utilizes to arrive at a diagnosis. For example, if one requires that AD cases have neocortical neurofibrillary tangles (NFT) in addition to meeting plaque-based criteria, the proportion designated as LBV diminishes greatly, while the percentage called DLBD increases (6). An International Consortium on this topic has recommended “dementia with Lewy bodies” (DLB) as a generic term for all these cases, since it acknowledges the presence of LB without necessarily implying their relative importance in symptom formation with respect to other neuropathologic alterations, specifically the presence of concomitant AD pathology (7). A problem with this recommended rubric, however, is that it eliminates the distinction between the LBV and DLBD subgroups of LB. We have previously established that, while DLBD patients are globally demented, their dementia is less severe than the cognitive impairment exhibited by their LBV counterparts (8). The neocortical neuritic plaques (NP) and entorhinal NFT seen in LBV but not DLBD could account for greater dementia among the former.

Though dementia is the presenting complaint of LBV and DLBD patients, they typically display some features characteristic of idiopathic Parkinson’s disease (PD), including mask-like facies and rigidity (3, 5) and a fluctuating course that is often punctuated with hallucinations (9). We have previously described clinical features of LBV and DLBD patients that can serve to separate them from AD patients antemortem (10, 11). We have also reported the results of extensive neuropsychological testing in DLBD, which shows particularly severe impairments on visuospatial, visuconstructional, and psychomotor tasks (8).

The neuropathologic substrate underlying the cognitive impairment seen in DLB is complex and not thoroughly understood. These brains show subcortical LB and neuron loss in the locus ceruleus, substantia nigra, and nucleus basalis of Meynert (NBM), and any of these lesions could contribute to dementia (4, 12). They also have neocortical LB, which correlate with the degree of cognitive
impairment in LBV patients (10, 13). Neurochemical analyses have found marked deficiencies in necortical and neostriatal choline acetyltransferase (ChAT) levels exceeding those encountered in AD, and loss of dopamine in the putamen comparable to that found in PD (14). The concomitant AD pathology encountered in most, but not all, DLB brains also seems likely to impair cognition. Braak staging (15) of AD-type NFT pathology correlates with cognitive decline in the related condition of combined PD and AD, despite an initial presentation of parkinsonism rather than dementia (16).

Neocortical synapse loss is a major correlate of AD dementia (17), and estimates of neocortical and hippocampal synapse loss seem comparable in LBV and AD patients (18, 19). We were unable to estimate synapse density by immunocytochemistry among LBV cases in our previous report (10) and so could not assess its contribution to their cognitive impairment. Decreased synapse density has not been previously examined as a contributor to DLBD dementia. Accordingly, for the present study we selected patients and controls for whom neocortical synapses could be estimated by techniques previously applied in our laboratory (17, 20). We evaluated 17 DLB cases (12 LBV and 5 DLBD) along with 12 pure AD cases and 5 age-matched normal controls, all of whom had been cognitively assessed several months prior to death. Dependent measures included: (a) counts of midfrontal neocortical LB (for the DLB cases), NP, and NFT; (b) quantification of synapse density and ChAT; (c) modified Braak staging of AD-type neurofibrillary pathology; and (d) gross brain weight. The first 2 categories of variables were examined in the midfrontal cortex because previous work in our laboratory correlating synapse density with AD dementia used tissue from this brain region (17) and because our laboratory routinely obtains synapse and ChAT assay quantification data from midfrontal neocortical frozen tissue blocks. We analyzed these variables statistically so as to identify physical correlates of the qualitative and quantitative differences in dementia exhibited by LBV, DLBD, and AD subgroups.

MATERIALS AND METHODS

Subjects

We collected autopsy tissue from 29 demented patients (DLB = 17, AD = 12) and from 5 normal controls followed in the clinical series studied at the Alzheimer’s Disease Research Center (ADRC), University of California, San Diego. Some autopsies were performed within a few hours (h) of death and others after overnight refrigeration, but postmortem intervals never exceeded 24 h. Antemortem, the DLB and AD patients met NINCDS-ADRDA criteria for “probable AD” (21), and for each subject an estimate of disease duration in years was derived from detailed interviews with caregivers. Postmortem, those subsequently designated as LBV or AD all met neuropathological criteria for AD established by the National Institute on Aging (NIA) as well as by the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) for “probable” or “definite” AD (22, 23). Both sets of guidelines rely on the frequency of neocortical senile plaques for diagnosis (i.e. diffuse plaques or NP in the NIA criteria, and exclusively NP in the CERAD version), but neither requires the presence of NFT. Dementia brains with LB but insufficient neocortical plaque densities to warrant diagnosis of AD according to NIA and CERAD criteria were classified as DLBD. Both the LBV and DLBD cases would be designated DLB according to the recently published recommendations of an International Consortium on Dementia with Lewy Bodies (7). All of the LBV and DLBD cases had at least one LB in one or more subcortical nuclei—i.e. NBM, substantia nigra, or locus ceruleus—and at least one neocortical LB identified with hematoxylin and eosin (H&E) -stained preparations. We established these criteria (1, 3, 4) in order to separate LBV both from pure AD, which lacks LB, and from DLBD, which fails to meet NIA and CERAD neuropathological criteria for AD. In defining LBV and DLBD, we deliberately emphasized a qualitative distinction between the presence or absence of a neuropathologic feature (i.e. subcortical and neocortical LB) in order to avoid inevitably controversial judgments about “how many” LB might be required to make a diagnosis. These minimalist inclusion criteria understate the actual pathology, since typical cases have numerous neocortical and subcortical LB. Our definition of LBV and DLBD requires the presence of subcortical LB on H&E-stained sections, which obviates concern about overdiagnosis through misidentification of ubiquitin-positive globule neocortical NFT as LB.

Several intersecting criteria were used to select cases: (a) availability of data from a dot-blot assay for anti-synaptophysin reactivity with brain homogenates, a procedure performed on midfrontal cortical tissue from most dementia brains obtained through our ADRC autopsy program during the past two years; (b) neuropsychological testing performed within 2 years of death—testing which included not only the Mini-Mental State Examination (MMSE) and Information-Memory-Concentration test (IMC) discussed in this paper, but also additional measures to be used in other projects dealing with neuropsychological issues; and (c) availability of data from an enzymatic assay for midfrontal cortical ChAT activity, a procedure performed on tissue from fewer than half of all dementia brains coming to our laboratory during the past 2 years. We also preferred the most recently autopsied cases, which made the third criterion the most difficult to meet; of 12 LBV cases finally selected, 3 were lacking a ChAT score. The most recently autopsied 12 pure AD brains satisfying criteria 1 to 3 were selected for comparison. The DLBD group comprised all 5 examples of this neuropathological type accumulated in our ADRC brain bank over the past 8 years. Our brain bank also has a limited number of control brains, and application of criteria 1 to 3 yielded 5 cases that were reasonably well matched with the dementia patients in terms of age and sex.

Neuropsychological Tests

Results are reported for 3 commonly used assays of mental status: the memory-based IMC (24), the IMC as modified by Fuld (25) for American use, and the cognitively more
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Neuropathology

All brains were weighed at autopsy and then divided sagitally while fresh. The left hemispheres were fixed in 10% buffered formalin, and the right hemispheres were frozen at -70°C for chemical analysis. Following 10 to 14 days of fixation, the formalin-fixed left hemispheres were sectioned. Tissue blocks were taken from the midfrontal cortex, superior temporal gyrus, inferior parietal cortex, cingulate gyrus, basal ganglia/substantia innominata, mesencephalon, rostral pons, and other regions and paraffin embedded as previously described (27). Only midfrontal tissue was utilized in the present study. Seven-μm-thick sections were taken from the paraffin blocks and stained with H&E. Ten-μm-thick sections were stained with Thioflavin S and viewed with ultraviolet light and 400 nm band pass wavelength excitation filters to detect neuritic and diffuse plaques and NFT.

Quantification of NP and NFT: The NP count was the average number of Thioflavin S-positive NP seen in 3 separate fields of the cortical ribbon viewed under a 10X objective. The NFT count was the average number of NFT seen in 3 such fields viewed under a 40X objective. These quantifications are routinely done on all brain tissue coming to our laboratory, as previously described (27). In order to make standardized comparisons of the extent of AD neurofibrillary pathology between AD, LBV, DLBD, and non-AD control brains, our laboratory has adopted a modification of the neuropathological staging scheme for Alzheimer-related changes devised by Braak and Braak (15). We have described this procedure elsewhere (10) in some detail. Briefly, we count NFT in at least 5 neuron clusters in layer 2 of the entorhinal cortex and average the results for each brain. We also assess NFT in the midfrontal, inferior parietal, and superior temporal gyri for assigning cases to stages V or VI in those instances where neurofibrillary pathology has advanced beyond the confines of the medial temporal lobe. At what we call “Stage 0”, no NFT are seen in any of these sections. For brains assigned to Stage I, entorhinal cortex layer two average NFT counts range from 0 to 3, and there are scattered NFT in the adjacent transentorhinal cortex. In brains assigned to Stage II, entorhinal layer two NFT count averages range from 4 to 9. In Stage III, these averages are between 10 and 12, in Stage IV they range from 13 to 17, and in Stages V or VI they typically exceed 20. Distinctions between Stages V and VI are based on frequencies of neocortical NFT in the frontal, temporal, and parietal lobes. In Stage VI, all neocortical sections must have at least 3 NFT per 40X field in selected regions of the slide.

Quantification of LB: LB occurring in the brainstem nuclei were readily identifiable with H&E preparations. Neocortical LB were also identified on H&E-stained sections for all LBV and DLBD cases; they are more subtle than the subcortical ones—i.e. less eosinophilic, less well defined, and generally lacking a halo. Following established procedures for visualization of neocortical LB (28), we immunolabeled 10-μm-thick midfrontal paraffin sections with rabbit polyclonal affinity-purified anti-ubiquitin antibody (Chemicon, Temecula CA). This antibody has some nonspecific background and also labels NFT, but these fibrillary structures are generally distinguishable from labeled LB because the latter are round, nonfibrillar, intracellular structures that appear to peripherally displace the neuronal nucleus. However, experience has shown that NFT can sometimes have a clumped, spherical appearance within the neuronal cytoplasm (globose NFT) and may be indistinguishable from LB (10, 29).

We randomly intermixed anti-ubiquitin immunostained tissue slides from cases previously independently classified by a neuropathologist (LH) as LBV or DLBD so as to permit a blinded assessment of midfrontal neocortical LB frequency. Single sections from each neocortical area were scanned initially with a 10X objective to identify regions of the deeper layers of the cortical ribbon that had stained more densely with anti-ubiquitin. Promising regions were examined more closely with a 25X objective, and the first structure seen that approximated the above description of a LB was centered in the field. The objective was then switched to 40X with the structure still centered. A systematic search was made of the tissue within the span of one field diameter Immediately above or below or to the right or left of the target. The final field selected was one that included the target plus the maximum number of similar-appearing structures found within this area. If the initial lower-power scans of the cortical ribbon did not disclose any LB-appearing targets, then the region with the most dense staining was selected as the target and the above procedures applied. The count for a given 40X field was the number of LB contained within it. Counts were made in 5 separate regions per slide, and the average of all 5 counts was computed as the midfrontal LB score for each case. The code which concealed the identity of cases as LBV or DLBD was not broken until all LB counts were completed. Our tactic of zeroing in on the most promising targets of opportunity seen in initial low-power scans introduces an upward bias in LB counts per unit area, since unpromising regions are not sampled. We use this same tactic when counting NFT and NP, however, so counts of all these lesions are affected to a similar degree. Targeted counts combined across 5 separate fields should average out biases introduced by focal concentrations of lesions. The uniform application of the counting procedure to all cases also mitigates biasing influences. Our targeted counts could, in theory, be multiplied by a factor calculated to adjust for upward bias, but we doubt that such an adjustment would appropriately alter the results. Even so, the potential for targeted sampling to inflate lesion counts should be considered when interpreting the data reported here.

We applied the same techniques of immunostaining and LB quantification to samples of LBV and AD patients in our previous report (10) and to DLBD patients in this investigation, which permits comparison across studies. It happened that 4 LBV cases were included in both, with midfrontal LB blindly quantified on 2 separate occasions separated by many months. At the first assessment, mean LB counts per 0.1 square mm
TABLE 1
Mean Scores on Demographic and Neuropsychological Variables

<table>
<thead>
<tr>
<th></th>
<th>LBV (n = 12)</th>
<th>DLBD (n = 5)</th>
<th>AD (n = 12)</th>
<th>Controls (n = 5)</th>
<th>F (3, 30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
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</tr>
<tr>
<td>Age (years)</td>
<td>79.2 (5.9)</td>
<td>74.4 (1.7)</td>
<td>80.9 (9.1)</td>
<td>83.0 (3.8)</td>
<td>1.34</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/5</td>
<td>5/0</td>
<td>7/5</td>
<td>3/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>7.4 (3.2)</td>
<td>7.0 (2.7)</td>
<td>7.3 (3.1)</td>
<td>--</td>
<td>&lt;1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Test-death interval (months)</td>
<td>15.6 (12.6)</td>
<td>16.0 (10.6)</td>
<td>16.0 (11.0)</td>
<td>8.0 (4.8)</td>
<td>&lt;1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Neuropsychological tests</td>
<td></td>
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<tr>
<td>Mini-mental (MMSE)</td>
<td>6.4a (8.5)</td>
<td>16.8a (4.9)</td>
<td>10.5a (8.1)</td>
<td>28.2a (1.8)</td>
<td>10.80*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Blessed (IMC)</td>
<td>25.3a (9.8)</td>
<td>13.2b (9.4)</td>
<td>23.5a (8.6)</td>
<td>1.0c (0.0)</td>
<td>11.20</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

* For DLBD n = 4, so degrees of freedom were (3, 29).

Note: Post-hoc comparisons between means were performed when the one-way F test attained statistical significance. For these variables, means not sharing a common superscript differed significantly (p < 0.05) from one another. Thus, on the MMSE, the control mean differs from each of the other three (no shared superscripts); the DLBD mean differs from the LBV but not from the AD (shared b), while the AD and LBV means do not differ (shared c).

were 4.40, 3.20, 1.40, and 0.20 for these 4 cases; counts at the second assessment were 4.00, 4.00, 1.60, and 0.80, respectively. Four pure AD cases were also included in both samples and in the first study, their midfrontal neocortical "pseudo LB" or globule NFT were assessed using the same standardized procedure, yielding counts of 0.80, 0.20, 0, and 0. These observations reinforce several conclusions drawn previously: (a) our procedures for counting neocortical LB have good test-retest reliability, (b) pure AD patients have negligible numbers of globule NFT that could potentially be confused with LB, and (c) while there is a small overlap in the numbers of LB counted in LBV and of globule NFT counted in AD, the distributions are so obviously different that the presence of globule NFT cannot reasonably be invoked as an explanation for the correlations we have observed between LB counts and dementia severity in DLB patients.

Neurochemistry and immunocytochemistry: Midfrontal anti-synaptophysin reactivity in arbitrary units (au) per mg protein and ChAT activity in nmol/h/mg protein had been independently assessed by an experienced technician (MA) using dot-blot and enzymatic assay procedures, as described elsewhere (20, 27). The dot-blot synaptophysin assay was our main procedure for estimating synapse density. It actually measures the tissue concentration of a synaptic protein that could be affected in neurodegenerative disease due to changes in protein expression or metabolism rather than by direct synaptic destruction, but we have previously validated dot-blot measurements against a gold standard of direct observation and quantification of synapses using confocal microscopy (20). The dot-blot method is applicable to frozen brain tissue, which was available for all cases in our sample. The confocal method requires fixed tissue that has been exposed to formalin for less than 8 h, and tissue with this short fixation interval was not available for most of the specimens examined in this study.

For LBV and DLBD cases only, in addition to the dot-blot procedure, synapse density measurements were also derived from 10-μm midfrontal sections treated with anti-synaptophysin monoclonal antibody (SY38, Boehringer-Mannheim, Indianapolis, IN). The degree of binding was visualized using the immunoperoxidase method and quantified using a QuantiGem 970 optical densitometer (Leica, UK), as previously described (17). In this procedure, the average of 3 densitometry measurements taken from a white matter tract on the slide is subtracted from the average of 5 measurements taken from the cortical ribbon to yield a synapse density estimate that corrects for variations in background staining from one slide to the next.

Statistical Analyses
Data were analyzed with Statview (Abacus Concepts, Berkeley, CA) and SPSS Windows 6.1 program packages, which calculated Pearson product-moment correlations, or r values, and compared group mean scores by F values derived from one-way analysis of variance (ANOVA). Post-hoc comparisons of individual means were performed by the program package using a Fisher protected least square difference procedure (30). All significance levels were two-tailed (p < 0.05) except for tests of r values, which were one-tailed owing to the firmly directional nature of the predictions. The population distributions of scores underlying many of the neuropathological variables quantified in this study are not firmly established, including densitometry measurements that have the appearance of being distributed on a continuous linear scale. Since these variables could be regarded as somewhat semiquantitative and categorical, data were inspected by a statistician (CRH) for nonnormality, and tests using nonparametric equivalents were computed (Kendall tau and Kruskal-Wallis). Results supported the parametric computations reported here with minor differences in associated probabilities.

RESULTS
Subject Characteristics
Table 1 shows that the 3 patient groups and normal controls did not differ significantly in mean age (X = 74.4 to 83.0 years) or the number of months between the
TABLE 2

Mean Scores on Neuropathological and Neurochemical Variables

<table>
<thead>
<tr>
<th></th>
<th>LBV (n = 12)</th>
<th>DLBD (n = 5)</th>
<th>AD (n = 12)</th>
<th>Controls (n = 5)</th>
<th>F (3, 30)</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>X ± (SD)</td>
<td>X ± (SD)</td>
<td>X ± (SD)</td>
<td>X ± (SD)</td>
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<tr>
<td><strong>Neuropathology</strong></td>
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<tr>
<td>LB count (0.1 mm²)</td>
<td>2.72 (1.6)</td>
<td>1.44 (0.9)</td>
<td>—</td>
<td>2.78*</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>NFT count (0.1 mm²)</td>
<td>0.33 (0.5)</td>
<td>0.00* (0)</td>
<td>2.67* (3.4)</td>
<td>0.00* (0)</td>
<td>3.70</td>
<td>0.02</td>
</tr>
<tr>
<td>NP count (1.6 mm²)</td>
<td>39.67* (10.0)</td>
<td>2.60* (5.8)</td>
<td>41.50* (11.2)</td>
<td>3.80* (8.5)</td>
<td>34.27</td>
<td>0.0001</td>
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<td>Braak stage</td>
<td>3.08* (1.4)</td>
<td>0.80* (0.4)</td>
<td>5.08* (0.9)</td>
<td>0.80* (0.8)</td>
<td>29.94</td>
<td>0.0001</td>
</tr>
<tr>
<td>Brain weight (g)</td>
<td>1,133.0* (120.3)</td>
<td>1,386.6* (114.0)</td>
<td>1,101.3* (153.2)</td>
<td>1,288.4* (157.0)</td>
<td>6.56</td>
<td>0.002</td>
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<td><strong>Neurochemistry</strong></td>
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<tr>
<td>Synaptic density (au/µg)</td>
<td>75.2* (29.9)</td>
<td>96.7* (11.6)</td>
<td>80.1* (21.8)</td>
<td>108.1* (30.3)</td>
<td>2.48</td>
<td>0.08</td>
</tr>
<tr>
<td>ChAT (nmol/mg/hour)</td>
<td>49.78* (39.0)</td>
<td>40.50* (27.7)</td>
<td>147.4* (67.5)</td>
<td>211.6* (39.7)</td>
<td>14.63#</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

* LB counts were not performed for AD or controls, so degrees of freedom were (1, 15).
# For LBV n = 9 and for DLBD n = 4, so degrees of freedom were (3, 26).
Note: Post-hoc comparisons were performed for each variable with a significant or near-significant F value. Within a given row, means not sharing a common superscript are significantly (p < 0.05) different from one another, as described in Table 1. Quantification of cell counts is described in the text; LB = Lewy bodies, NFT = neurofibrillary tangles, NP = neuritic plagues, ChAT = choline acetyltransferase. The term "au" means "arbitrary units."

Date of last testing and death (Fs < 1). All 5 of the DLBD cases were male, whereas the other groups were fairly evenly split between males and females. Mean estimated duration of disease (7.0 to 7.4 years) did not differ appreciably between LBV, DLBD, and AD groups (F < 1). Thus, average age of onset ranged from 67.4 to 73.6 years and also did not differ significantly between groups (F < 1). Years of education were not known for every case, but the available data indicate no significant difference (F [3, 23] = 1.12) between groups (LBV = 12.9, n = 8; DLBD = 16.0, n = 4; AD = 13.7, n = 10; control = 14.0, n = 5).

On the MMSE, the controls (X = 28.2) performed significantly (p < 0.05) better than the AD (X = 10.5), DLBD (X = 16.8), or LBV (X = 6.4) patients. The LBV and AD groups were comparably demented, while the DLBD scored significantly higher than the LBV. The mean IMC score was minimal among the controls (X = 1.0), indicating absence of dementia as compared with the AD (X = 23.5), DLBD (X = 13.2), or LBV (X = 25.3) groups. The DLBD had a significantly lower IMC score than either the LBV or AD groups, indicating less severe impairment.

We described the clinical features that distinguish our LBV, DLBD, and AD patients in a previous report (8). Another feature of interest was their apolipoprotein E (apoE) genotype. These data were available for 9 of the 12 AD, 11 of the 12 LBV, 3 of the 5 DLBD, and 3 of the 5 control cases. Among the LBV, 55% (i.e. 6) were apoE 3/4 and 45% were 3/3. All 3 of the DLBD cases for whom apoE status was available were 3/3. Two of the 3 controls with known apoE status were 3/3 and the third was 2/3. Among the AD, 67% (i.e. 6) were apoE 3/4, 22% were apoE 4/4, and 11% were apoE 3/3. This strong association of one or more apoE4 alleles with AD is well recognized, and increased AD pathology is also linked with the apoE4 allele in LBV (31).

Neuropathological and Neurochemical Measures

Midfrontal neocortical LB counts per 40× field (0.1 square mm) were done only for the LBV (X = 2.72) and DLBD (X = 1.44) groups, who did not differ significantly on this index, as can be seen in Table 2. Other neuropathological and neurochemical features were assessed for AD and controls as well as LBV and DLBD. Midfrontal NFT counts per 40× field were significantly (p < 0.05) higher among the AD (X = 2.67) than the LBV (X = 0.33), DLBD (X = 0.0), or controls (X = 0.0). NP counts per 10× field were equally high among AD (X = 41.50) and LBV (X = 39.67) and, by definition, significantly higher than among the DLBD (X = 2.60) or controls (X = 3.80), who had virtually equivalent plaque counts. Brain weight was significantly greater for both DLBD (X = 1386.6 g) and controls (X = 1288.4 g) compared with AD (X = 1101.3 g) and LBV (X = 1133.0 g).

Braak staging of entorhinal neurofibrillary pathology also differed between groups, with AD (X = 5.08) cases at more advanced stages than LBV (X = 3.08), who were in turn at significantly higher stages than the DLBD (X = 0.80) or controls (X = 0.80). Our modified Braak staging system is a graded sequence of 7 semiquantitatively defined categories, and we have previously compared Braak stages between AD, LBV, and control groups using nonparametric chi-square and exact test statistics (6). When we included 58 AD, 58 LBV, and 10 controls in a frequency distribution, each of the 7 Braak stage categories contained cases, though the distribution for controls was skewed toward the low end.
and that for AD patients toward the high end (6). Because ANOVA F tests are statistically robust and can tolerate fairly wide departures from normality (skewness, specifically) in the underlying population distributions of scores (32) and because comparisons between means are clearer than a series of chi-square analyses, we here report parametric comparisons of group mean differences in Braak stages. A nonparametric Kruskal-Wallis analysis yielded the same results.

We predicted a priori that midfrontal neocortical dot-blot anti-synaptophysin reactivity would be higher among controls than among AD, LBV, or DLBD patients and so proceeded to test the significance of these predicted group mean differences when we analyzed our results. These planned comparisons (32) showed synapse density to be significantly (p < 0.05) higher among controls (X = 108.1 au) than among AD (X = 80.1 au) or LBV (X = 75.2 au), but not significantly different between controls and DLBD (X = 96.7 au).

Midfrontal ChAT activity by enzymatic assay was equivalent among LBV (X = 49.78 nmol/mg/h) and DLBD (X = 40.50). Both groups comprising the DLB contingent had significantly lower ChAT levels than the AD (X = 147.4), who in turn had significantly lower ChAT activity than controls (X = 211.6).

Correlations with Cognitive Status

Intercorrelations between neuropsychological, neuropathological, and neurochemical variables for DLB patients are shown in Table 3, and for AD patients in Table 4. Among the DLB, midfrontal LB frequency correlated significantly (p < 0.005) with MMSE (r = −0.75) and IMC (r = 0.61). Neither neocortical NFT counts nor synapse density as estimated by the dot-blot method correlated appreciably with cognitive status among the DLB, but NP counts correlated (p < 0.05) with MMSE (r = −0.46) and IMC (r = 0.50) scores. ChAT activity correlated (p < 0.005) with MMSE (r = 0.62). The LB count correlated negatively (p < 0.05) with midfrontal ChAT activity (r = −0.54). Braak stage correlated (p < 0.05) with dementia severity as indexed by the MMSE (r = −0.64) and IMC (r = 0.61) and with the frequency of neocortical LB (r = 0.55), NFT (r = 0.66), and NP (r = 0.72), but not with ChAT (r = −0.27) or synapse density (r = −0.09). Table 3 does not show correlations between cognitive status and midfrontal synapse density as visualized by immunocytochemistry and quantified by optical densitometry; these correlations were performed for DLB patients only and were also nonsignificant (ρ = 0.27 to 0.32).

When the analysis in Table 3 was limited to LBV cases, MMSE (r = −0.72) and IMC (r = 0.64) scores were still significantly (p < 0.025) associated with LB counts and were uncorrelated with all measures characteristic of neocortical AD pathology—i.e., NFT, NP, and synapse loss (r = −0.24 to 0.17). Midfrontal ChAT continued to correlate (p < 0.05) with MMSE (r = 0.67)

### Table 3

Intercorrelations Between Neuropsychological, Neuropathological and Neurochemical Variables: 
**DLB Patients Only (n = 17)**

<table>
<thead>
<tr>
<th></th>
<th>LB</th>
<th>NFT</th>
<th>NP</th>
<th>Synapses</th>
<th>ChAT</th>
<th>Braak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini-mental (MMSE; n = 16)</td>
<td>−0.75****</td>
<td>−0.22</td>
<td>−0.46*</td>
<td>−0.04</td>
<td>0.62**</td>
<td>−0.64****</td>
</tr>
<tr>
<td>Blessed (IMC)</td>
<td>0.61****</td>
<td>0.27</td>
<td>0.50**</td>
<td>−0.07</td>
<td>−0.33</td>
<td>0.61****</td>
</tr>
<tr>
<td>LB count</td>
<td>—</td>
<td>0.15</td>
<td>0.24</td>
<td>−0.29</td>
<td>−0.54*</td>
<td>0.55**</td>
</tr>
<tr>
<td>NFT count</td>
<td>—</td>
<td>—</td>
<td>−0.03</td>
<td>0.10</td>
<td>0.29</td>
<td>0.66****</td>
</tr>
<tr>
<td>NP count</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.18</td>
<td>−0.11</td>
<td>0.72****</td>
</tr>
<tr>
<td>Synapse density (Dot-blot)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ChAT activity (n = 13)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Braak stage</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note: Abbreviations are as in Table 2. **** p < 0.005; *** p < 0.01; ** p < 0.025; * p < 0.05.

### Table 4

Intercorrelations Between Neuropsychological, Neuropathological and Neurochemical Variables: 
**AD Patients Only (n = 12)**

<table>
<thead>
<tr>
<th></th>
<th>NFT</th>
<th>NP</th>
<th>Synapses</th>
<th>ChAT</th>
<th>Braak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini-mental (MMSE)</td>
<td>−0.58**</td>
<td>−0.62**</td>
<td>0.74****</td>
<td>0.64**</td>
<td>−0.78****</td>
</tr>
<tr>
<td>Blessed (IMC)</td>
<td>0.53*</td>
<td>0.63**</td>
<td>−0.70****</td>
<td>−0.78****</td>
<td>0.80****</td>
</tr>
<tr>
<td>NFT count</td>
<td>—</td>
<td>0.33</td>
<td>−0.54*</td>
<td>−0.75****</td>
<td>0.60**</td>
</tr>
<tr>
<td>NP count</td>
<td>—</td>
<td>—</td>
<td>−0.67****</td>
<td>−0.33</td>
<td>0.62**</td>
</tr>
<tr>
<td>Synapse density (Dot-blot)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ChAT activity</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.54*</td>
</tr>
<tr>
<td>Braak stage</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note: Abbreviations are as in Table 2. **** p < 0.005; *** p < 0.01; ** p < 0.025; * p < 0.05.
Fig. 1. Scatterplot showing the distribution of the last available scores on the Mini-Mental State Examination (MMSE) (maximum = 30 correct) among patients with diffuse Lewy body disease (i.e., LBV and DLBD [arrowheads]) as a function of LB count per 0.1 square mm in the midfrontal cortex. An MMSE score was missing for one DLBD patient.

Fig. 2. Scatterplot showing the distribution of the last available MMSE scores obtained from DLB patients (data points for DLBD marked with arrowheads) as a function of Braak stage scoring (maximum = 6) of neurofibrillary pathology in the entorhinal cortex. An MMSE score was missing for one DLBD patient.

Fig. 3. Scatterplot showing the distribution of the last available MMSE scores obtained from DLB patients (DLBD, arrowheads) as a function of midfrontal neocortical choline acetyltransferase (ChAT) activity in nmol/mg/hour. An MMSE score was missing for one DLBD patient, and ChAT scores were missing for 1 DLBD and 3 LBV patients.

and IMC \( (r = -0.60) \) scores and with LB counts \( (r = -0.66) \). For the DLBD considered separately, the correlation between MMSE and LB was weak, but was in the expected direction \( (r = -0.19) \); moreover, it is important to note that adding the small number of DLBD to the LBV cases slightly strengthened the negative correlation between MMSE and LB (from -0.72 to -0.75) in the combined DLB sample, which supports the inference that LB are associated with cognitive impairment in both subgroups. Also among the DLBD, ChAT correlated strongly with MMSE \( (r = 0.90) \) and midfrontal LB \( (r = -0.72) \), but these coefficients were not significant due to the small number of DLBD, one of which was lacking an MMSE and another a ChAT score.

Figures 1, 2, 3, and 4 are scatterplots of the distribution of MMSE scores related to midfrontal LB counts, ChAT activity, and Braak stages and of LB counts related to ChAT activity among DLB patients. Data points for the DLBD are marked by arrowheads. Figure 1 shows that, while several DLB patients, all of them LBV, had zero scores, the MMSE values were distributed throughout a broad range; one DLBD patient had no terminal MMSE, so \( n = 16 \). Figure 2 shows the correlation between MMSE and Braak stage scores, with the DLBD at all stages 0 or 1 and having generally higher MMSE scores than the LBV, though with considerable overlap between these 2 subgroups on the latter index. In Figure 2, the tendency for a higher Braak stage to be associated with a lower MMSE score was evident even when correlations were separately calculated for LBV \( (r = -0.45) \) and DLBD \( (r = -0.85) \) cases, though neither coefficient reached statistical significance. While LBV and DLBD cases did cluster at opposite ends of the regression line, there was some overlap in scores; combining these data in a single correlation therefore seems empirically justified, as will be further considered below. Figure 3 illustrates the correlation between MMSE and ChAT, with
Fig. 4. Scatterplot showing the inverse relationship between midfrontal neocortical LB counts and ChAT activity among DLB patients, with data points for the DLBD indicated by arrowheads. A ChAT score was missing for 1 DLBD patient and 3 LBV patients.

scores again spanning a broad range; 3 LBV and 1 DLBD lacked ChAT levels, so n = 12. Figure 4 shows the correlation between LB counts and ChAT activity, with a broad distribution on both axes (n = 13). In all 4 figures, the placement of the DLBD cases appears congruent with the overall pattern of data points and with the indicated correlations between variables.

Table 4 shows that among AD patients, dementia severity as assessed by the MMSE and IMC was significantly (p < 0.05) associated with higher NFT and plaque counts, lower synapse density and ChAT activity, and higher Braak stage score. NFT and plaque counts correlated negatively with synapse density (r = -0.54 to -0.67), and NFT counts correlated negatively with ChAT activity (r = -0.75, p < 0.01). A higher Braak stage score was significantly (p < 0.05) associated with higher midfrontal NFT and plaque counts (r = 0.60 to 0.62) and lower synapse density (r = -0.64) and ChAT activity (r = -0.77).

We performed stepwise multiple regressions to identify which neuropathological or neurochemical features were the strongest predictors (p < 0.05 required for entry into the regression equation) of MMSE and IMC when all variables shown in Tables 3 and 4 were included in separate analyses for DLB and AD patients, respectively. None of these regressions went past the first step, and the variable entered into the equation at that step was Braak stage (multiple r = 0.74 to 0.80) for IMC among DLB and for MMSE and IMC among AD patients but was LB count (r = 0.87) for MMSE among DLB cases. With Braak stage excluded, LB count was the strongest predictor of both MMSE and IMC among DLB patients (r = 0.87 and 0.68, respectively). Among AD cases, MMSE was best predicted by synapse density (r = 0.74), and IMC by ChAT (r = 0.78).

DISCUSSION

The results of this and previous investigations (4, 5, 9, 10, 14) imply that the physical basis of cognitive impairment in DLB is complex and multiply determined. Considering all cases of DLB together, including LBV and DLBD, we find that neocortical LB counts, NP, modified Braak stages of AD neurofibrillary pathology, and depletion of neocortical ChAT all correlate with dementia. We speculate that LB accumulation and ChAT depletion produce the moderate dementia (mean IMC = 13.2; mean MMSE = 16.8) encountered in DLBD. When neocortical NP and more advanced Braak stages of AD neurofibrillary pathology are added to the neurodegenerative mixture, dementia is more profound (mean IMC = 25.3; mean MMSE = 6.4)—similar to that seen in advanced severe AD (mean IMC = 23.5; mean MMSE = 10.5)—and gross brain weights reflect comparable atrophy.

Surprisingly, neocortical synapse loss does not correlate with cognitive impairment in DLB, and there is in fact no significant difference in dot-blotsynaptophysin measurements between DLBD brains and controls. Average midfrontal synaptophysin levels are low in LBV and comparable to those seen in pure AD, but synaptophysin depletion did not correlate with degree of cognitive impairment in LBV as it does in AD. It is possible that a correlation between loss of neocortical synaptophysin and dementia in DLB may yet exist if regions of the neocortex other than the midfrontal are examined. Although the midfrontal cortex does have LB, there is typically a greater accumulation of LB in the limbic regions (10, 18, 19), and synaptic loss in these areas—cingulate, entorhinal, insular, and superior temporal—may yet be shown to correlate with dementia. An additional caveat when evaluating correlations between neuropathologic and clinical variables is that our lesion counts came from the left cerebral hemisphere, while the neurochemical measures came from the right. We have not performed laterality studies in DLB, and this left-right, neuropathology-neurochemistry dichotomy may confound some interpretations of our results.

Of the other classic neocortical neuropathologic abnormalities of AD, only NP correlated with neuropsychological test scores in DLB, but neither neocortical NFT nor NP were related to cognitive decline in LBV considered separately. The failure of tangles to correlate with dementia in DLB was predictable, since most cases of LBV are "plaque-only" or "plaque-predominant" AD and hence lack neocortical NFT, or have very few. The lack of significant correlations between NP counts and cognitive impairment in LBV is perhaps harder to understand, but such correlations have been difficult to
demonstrate even among pure AD cases in prior work (33). Furthermore, while the distribution of amyloid plaques is similar in LBV and AD (34), most NP in LBV lack microtubule-associated protein tau positivity and thus reflect a less severe cytoskeletal disturbance than that seen in the tau-positive NP of AD (4, 5, 34).

Entorhinal neurofibrillary pathology, as reflected by Braak stages, has a major influence on cognitive status in DBL, as can be seen in Figure 2. This correlation is to some extent driven by the cluster of DLBD having low Braak and high MMSE scores, but we combined LBV and DLBD in our analysis precisely because we wanted to determine the extent to which AD pathology, ranging from very low (mostly DLBD) to very high (all LBV), could account for their dementia. We have long maintained the importance of distinguishing LBV from DLBD, but other investigators have lumped all dementia brains with Lewy bodies together under rubrics such as "senile dementia of the Lewy body type" (35) or "diffuse Lewy body disease" (36) without regard to the significance of concomitant neocortical "plaque only" AD pathology, or the Braak stages III-IV found in many of these cases. Even the recent consensus report on dementia with Lewy bodies defines DLB, which includes both LBV and DLBD, solely on the basis of neocortical and brainstem Lewy bodies, again disregarding or minimizing the possible significance of accompanying AD pathology (7). Among elderly subjects without brainstem and neocortical LB, the limbic Braak stages (III and IV) are weakly associated with declines in memory, and global declines in cognitive function characteristic of AD are not encountered until isocortical Braak stages (V and VI) are reached (37). In DBL, however, Braak stages III and IV found in LBV brains (10) are associated with severe dementia because of the synergistic influences of concomitant LB pathology and loss of neocortical ChAT. In LBV lacking higher Braak stages than age-matched controls (i.e. DLBD), dementia is far less severe than that seen in LBV and AD.

Table 2 shows that midfrontal ChAT was significantly and to an equal degree diminished in LBV and DLBD as compared with pure AD patients, who had less ChAT activity than controls. These data imply that the cholinergic neurotransmitter system is damaged in both DBL and AD, but that disproportionate damage occurs among the former and makes a more important contribution to dementia in DBL than in AD, where synapse loss is a dominant factor. NFT accumulation in the cholinergic magnocellular neurons of the NBM has been found to correlate significantly with the severity of AD dementia (38, 39). As others have suggested (5), the LB may play a role analogous to that of NFT in the basal forebrain. Such a hypothesis offers a neurochemical explanation for reports that LBV are more responsive than pure AD patients to therapeutic administration of the cholinesterase inhibitor tacrine (40–43), if one assumes that LB-mediated damage to magnocellular neurons is more inhibitory than terminally destructive. Neocortical LB could also be involved in suppressing basal forebrain cholinergic activity if, as has been suggested, NBM neurons are not so much "lost" in these diseases as they are shrunken due to reduced trophic feedback from damaged target neurons in the cortex (44).

The NFT of AD and the LB of DLB are both intraneuronal inclusion bodies that reflect disturbed cytoskeletal function, either of neurofilaments (LB) or of microtubule-associated protein tau (NFT). One study has demonstrated that these lesions can coexist within amygdala neurons in DBL, even in DLBD cases that otherwise lack NFT (45). The investigators described such coexistence as extremely rare in other brain regions, but their observations emphasize the need to apply histological techniques that unequivocally discriminate these neuronal inclusions (35, 45) so as to avoid the apparently inflated LB counts mentioned in some reports (46). We previously concluded that about 5% of neocortical LB counted in LBV cases using only antiubiquitin staining are really globular NFT (10).

Although neocortical LB, NP, cholinergic dysfunction, and Braak stages correlate well with dementia in DBL, we do not wish to dismiss other possibly contributory pathology. Specifically, lesions in the hippocampus can discriminate LBV from AD (5, 19, 36, 47), and synapse loss in the dentate gyrus correlates with dementia severity in LBV (19). Noncholinergic neurotransmitter systems, most notably the dopaminergic system, are also disrupted in LBV to a greater extent than in pure AD (14), and degeneration in the locus ceruleus probably contributes to dementia in DBL as it does in pure AD (48).

REFERENCES


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